School of Biological Sciences

# Characterising the influence of different wheat cultivar rhizospheres on variations in microbiome diversity and functionality

**Thesis submitted to the University of Reading**

**for the Degree of Doctor of Philosophy**

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**Declaration**

I confirm that this is my own work and the use of all materials from other sources has been properly and fully acknowledged.

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**Abstract**

In agricultural systems plant diseases caused by soil borne fungi are regarded as the most devastating. Wheat is recognized as an important crop worldwide, but it is highly susceptible to Take-all disease caused by the soil ascomycete fungus *Gaeumannomyces graminis* var*. tritici* (*Ggt*). Interest in biological control of Take-all has increased due to a lack of resistant wheat cultivars and chemical pesticides. Although this disease has been extensively studied it is still regarded as an excellent model for biological control of plant root diseases. *Pseudomonas* bacteria in the *P. fluorescens* complex are well recognized for their plant growth promoting and disease suppressive properties and they can often be found to be prevalent in controlling Take-all. In this work *Pseudomonas* isolates from the rhizosphere and endosphere of two wheat varieties, Hereward High Take-all Build up (H-TAB) and Cadenza Low Take-all Build up (L-TAB) were investigated. These isolates were screened for the presence or absence of two rhizosphere fitness loci, *wsm* and *fecB*, involved in host recognition and iron acquisition, respectively. It was found that these loci were significantly differentially associated with the two wheat cultivars where *wsm* was more abundant within the Hereward isolates while the *fecB* was found more within the Cadenza isolates. In addition, these isolates were tested for their *in vitro* inhibition of *Ggt*, which led to the identification of six strong *Ggt* growth inhibition isolates. Furthermore, testing these antagonistic isolates in the presence of the plant revealed that isolate 25R-7 was able to reduce the number of infected roots on Cadenza, while isolate 30R-11 reduced the number of infected roots on Hereward. Overall, the mixture of the six strong isolates reduced the number of infected roots in both cultivars more than that of individual strains. In addition the structure of bacterial communities associated with five wheat varieties (two L-TAB and three H-TAB) along with one barley (Unknown-

TAB) grown continually at the same site in three fields for a period of 5 years were investigated. The main difference in the structure of bacterial communities was based on field type and the rhizosphere samples separated well from those of bulk soil. Overall, no significant differences were found between the cultivars over time. The soil DNA concentrations of *Ggt* along with the soil DNA concentrations of bacteria, *Pseudomonas* and fungi were also studied from the same treatments. Year-to-year variation was the major factor in determining the amount of bacterial, *Pseudomonas*, fungal and *Ggt* DNA in the three fields.

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"My Success Can Only Come From Allah. In Him I Trust, And Unto Him I Return"

Quran (11:88).

**Table of contents**

**List of figures**

**List of tables**

## List of abbreviations

| | |
|---|---|
| ANOVA | Analysis of variance |
| ACC | 1-aminocyclopropane-1-carboxylate |
| BLAST | Basic Local Alignment Search Tool |
| cDNA | Complimentary deoxyribonucleic acid |
| CFU $ml^{-1}$ | Colony forming unit per ml |
| Ct | Cycle threshold |
| DAPG | 2,4-diacetylphloroglucinol |
| d.d. $H_2O$ | Double distilled water |
| d.f. | Degree of fredom |
| DGGE | Denaturing gradient gel electrophoresis |
| EF | Elongation factor |
| F | Field |
| h | Hour |
| IAA | Indole-3-acetate |
| JA | Jasmonic acid |
| KMB | King's medium B |
| LB | Luria-Bertani |
| LH 4 | Long Hoos 4 |
| LH 5 | Long Hoss 5 |
| mins | Minutes |
| NCBI | National centre for biotechnology information |
| $nH_2O$ | Nano pure water |
| NZ | New Zealand |
| O/N | Overnight |
| OD | Optical density |
| PGPR | Plant growth promoting rhizo-bacteria |
| PBS | Phosphate buffer saline |
| PCR | Polymerase chain reaction |
| qPCR | Quantitative/real-time PCR |
| rpm | Revolutions per minute |
| rRNA | Ribosomal RNA |
| s | seconds |
| SA | Salicylic acid |
| SAR | Systemic acquired resistance |
| s.e. | Standard error of the mean |
| s.e.d | Standard error of the difference |
| T3SS | Type III secretion system |
| TE | Tris-EDTA |
| Tm | Melting temperature |

## CHAPTER 1- Introduction

The major challenge of crop production in the twenty-first century is the sustainable production of enough food to feed the ever growing human population (Berg, 2009). Soil-borne plant pathogens, in particular fungi and oomycetes, are the main causes of yield reductions in agricultural ecosystems (Raaijmakers *et al.*, 2009). Also the current improper applications of agricultural pesticides and fertilizers lead to long term environmental and health effects (Berg, 2009; Lugtenberg & Kamilova, 2009).

Bread wheat, *Triticum aestivum*, is grown worldwide and is considered as one of the four main important crops. However, it is highly susceptible to Take-all disease of wheat, which challenges the successful cultivation and breeding of this crop (Schreiner *et al.*, 2010; Jenkyn *et al.*, 2014; McMillan *et al.*, 2014). The disease was reported in South Australia as early as 1852 (Kwak & Weller, 2013).

Take-all disease of wheat is caused by the soil-borne ascomycete fungus *Gaeumannomyces graminis* var. *tritici* (*Ggt*) leading to economically devastating losses in wheat crop yield and quality (Schreiner *et al.*, 2010; Jenkyn *et al.*, 2014; McMillan *et al.*, 2014). This fungus is able to infect wheat grown under both high and low precipitation, thus making it the most devastating wheat disease around the world (Yang *et al.*, 2011; Kwak & Weller, 2013). *Ggt* is a homothallic fungus, and its growth conditions in soil range from 4˚C - 30˚C and with pH of 3 – 10 (Kwak & Weller, 2013). The ability of *Ggt* to cause disease (pathogenicity) and the severity of this attack (virulence) depends on its colonization of the root and the production of enzymes and toxins that targets plant tissue for further invasion (Daval *et al.*, 2011). This fungus is especially damaging to graminaceous species like wheat and barley and other temperate cereals, with the exception of oats and some other grasses (Jenkyn *et al.*,

2014). In culture the optimum growth temperature for *Ggt* ranges between 20˚C - 25˚C with a colony growth rate of 6-10 mm per day (Kwak & Weller, 2013).

Although extensively studied, Take-all disease still poses a challenge for plant pathologists, due to the lack of effective fungicide treatments and resistant cultivars. Historically, crop rotation using non graminaceous species was used to manage Take-all disease (Cook, 2003). However, given the great demand for wheat and the lack of economically attractive break crops, wheat is commonly grown in short rotations where Take-all is a major issue. Soil fumigation with methyl bromide has been used for the complete control of Take- all disease, but this chemical is now banned as it causes ozone depletion. Moreover, chemical fungicides that act as microtubule assembly inhibitors or biosynthesis inhibitors, have also been used but were found to be effective on the seedling phase only resulting in inconsistent or economically invalid treatments (Kwak & Weller, 2013; Yang *et al.*, 2014). Recent governmental policies and consumer views tend to disfavour the use of agrochemicals due to their negative effects on the environment and human health (Lugtenberg & Kamilova, 2009). Thus, there is great interest in establishing alternative biological approaches for controlling plant diseases.

### 1.1 Plant root microbiome

Soils are recognized as one of the richest microbial ecosystems on earth (Bulgarelli *et al.*, 2013). In plants, bacterial communities can be found in different tissues; on leaves, roots or with in the plant as endophyte (Andreote *et al.*, 2009). Endophytic bacteria colonize the internal tissues of their host plant without having any negative effect (Ryan *et al.*, 2008). Such bacteria may be involved in plant growth promotion and disease suppression (Andreote *et al.*, 2009). The soil surrounding the roots can be

divided into bulk, rhizoplane and rhizosphere compartments (Bulgarelli *et al.*, 2013; Philippot *et al.*, 2013; Edwards *et al.*, 2015). In comparison to bulk soil, the rhizosphere harbours increased microbial numbers and activities. The rhizosphere is defined as the soil compartment under the influence of plant roots (Hirsch & Mauchline, 2012; Bakker *et al.*, 2013). This nutrient rich area attracts microbes from the surrounding bulk soil to carry out important functions for plant health (Berg & Smalla, 2009). The plant associated microorganisms facilitate nutrient uptake, stress tolerance and disease resistance (Berg, 2009). Many factors aid in shaping the rhizosphere microbiome. For instance, the soil chemical and physical properties, pH, particle size, texture, environmental factors (e.g. temperature and rainfall), plant species and genotype, and growth stage, have all been implicated (Berg & Smalla, 2009; McMillan *et al.*, 2011; Hirsch & Mauchline, 2012). The roots affect the soil architecture, pH and concentration of antimicrobials along with quorum sensing signals through rhizodeposition (Lundberg *et al.*, 2012; Haichar *et al.*, 2014). Out of the rhizodeposits, root exudates are carbon rich nutrient sources released into bulk soil (Berg & Smalla, 2009). Plants release 5-21% of their total photosynthetically fixed carbon through root exudation. Various abiotic and biotic factors, including plant species and growth stage, determine the quantity and quality of root exudates (Haichar *et al.*, 2014).The plants use their released exudates to attract beneficial microbes which in turn aid the host growth, help tolerate salt and drought stresses and provide protection from soil borne pathogens (Mendes *et al.*, 2013; Philippot *et al.*, 2013; Steinauer *et al.*, 2016; Mahoney *et al.*, 2017). Thus it is expected that a great plant diversity will lead to more diverse microbial communities through the variable range of exudate composition (Steinauer *et al.*, 2016). In addition, many studies have shown that plants exert a species- specific effect to determine the composition and the abundance of rhizosphere microbes

(Philippot *et al.*, 2013). For example, it was found that the fluorescent *Pseudomonas* spp. associated with the rhizosphere of flax and tomato were different from each other and that of bulk soil (Siciliano *et al.*, 1998). Also, it was reported that the cereal rhizospheres like oats and wheat are often enriched with cellulose degraders (Turner *et al.*, 2013a). Thus, implicating the importance of the presence of plant cell wall material in shaping the rhizosphere.

### 1.2 Exudates

Free living microorganisms in the soil are attracted by plant root secreted compounds (Lugtenberg *et al.*, 2001). The root exudates can be grouped into (a) low-molecular weight compounds like sugars, amino acids, phenolics, organic acids and other secondary metabolites, (b) high-molecular weight polysaccharides (mucilage) and proteins (Haichar *et al.*, 2014). The recruitment of microbes by the plant is evident to some extent by the ability of plant associated bacteria to degrade a range of plant released aromatics in the rhizosphere (Neal *et al.*, 2012). Also many bacterial strains were found to be positively chemotactic towards various components from root exudates (Brencic & Winans, 2005). For instance, flavonoids attract symbionts, such as *Bradyrhizobium japonicum*, they also stimulate mycorrhizal spore germination and hyphal branching, and influence quorum sensing (Philippot *et al.*, 2013; Haichar *et al.*, 2014). In legumes the concentration of flavonoids exuded increases in the presence of the compatible *Rhizobium* species which leads to the activation and expression of *nod* genes in rhizobia. The rhizobia then produce Nod factors which upon contact with receptors on host plants stimulate curling of root hairs around the invading rhizobia, entry of rhizobia into the plant through infection threads, and nodule development (Haichar *et al.*, 2014).

Strigolactones are root exudate signal molecules that stimulate extra-radical hyphae formation leading to arbuscular mycorrhiza fungi (AMF) symbiosis with plants (Haichar *et al.*, 2014; Haldar & Sengupta, 2015). The mutualistic associations formed between the plants and the AMF aid their survival in nutrient poor environments (Siciliano *et al.*, 1998). However, it was found that *Brassicaceae* like *Arabidopsis thaliana* are not well colonized by arbuscular mycorrhizal fungi (Lundberg *et al.*, 2012). Neal *et al.* (2012) showed that *Pseudomonas putida* strain KT2440 is recruited in maize rhizosphere by benzoxazinoid, 2,4-dihydroxy-7-methoxy-2H-1,4-benzoxazin-3(4H)-one (DIMBOA).

The plant hormones salicylic acid (SA), jasmonic acid (JA), and ethylene control systemic and induced plant immune responses (Vacheron *et al.*, 2013; Lebeis *et al.*, 2015). Lebeis *et al.* (2015) compared the endosphere and rhizosphere bacterial communities of wild type *Arabidopsis thaliana* Col-0 with a set of isogenic phytohormone mutants either lacking or dependent on one of SA, JA and ethylene signalling pathways. Overall their results showed that elimination of the three phytohormones resulted in an abnormal root microbiome compared to wild type. The leaf immune regulator, SA, was found to influence the composition of specific bacterial families in the root microbiome and was either used as a growth signal or carbon source by these groups. van de Mortel *et al.* (2012) showed that treating *A. thaliana* with *P. fluorescens* SS101 induced systemic resistance to *P. syringe* p *tomato* through SA signalling pathways rather than the JA/ethylene pathway. Thus, implicating an important role played by SA in plant and rhizobacteria interactions.

Notz *et al.* (2001) used a *lacZ* reporter gene linked with the *phlA* structural gene of *phlABCD* cluster to investigate factors influencing the expression of *phl* gene for the biosynthesis of 2,4-diacetylphloroglucinol , 2,4-DAPG, in *P. fluorescens* strain CHA0 in the rhizosphere of maize, wheat, bean and cucumber. It was found that the *phl* gene

expression was enhanced in maize and wheat rhizosphere in comparison to cucumber and was thought to be caused by inherented differences in root exudation between monocots and dicots. Also, host genotype, cultivar and plant age were important factors (Brencic & Winans, 2005).

de Weert *et al.* (2002) investigated *cheA* mutants derived from the efficient tomato root colonizer *P. fluorescens* strain WCS365. The *cheA* gene controls flagella-driven chemotaxis, and the mutant had impaired motility compared to the wild type. It was concluded that malic acid and citric acid are the main chemo-attractants in tomato exudate.

Taken together, the above examples show the diversity of molecules and signalling pathways that have been implicated in recruitment of microbes by plants.

### 1.3 Methods to study the rhizosphere microbiome

The rhizosphere is colonized by bacteria, fungi (including the arbuscular mycorrhizal fungi (AMF)), oomycetes, viruses, and archaea (Philippot *et al.*, 2013). Several factors including plant species, growth stage, type of exudation, and rhizo-deposition affect the bacterial diversity in this region of the soil (Gomes *et al.*, 2001). Classical community profiling is based on pure culture isolation, metabolic, morphologic, and physiological traits (Kent & Triplett, 2002; Lenc *et al.*, 2015). These included plate counts, microscopy, community level physiological profile (CLPP) and sole carbon source utilization (SCSU) patterns (Liu *et al.*, 2006; Andreote *et al.*, 2009).

Early studies on rhizosphere microbiomes utilized fatty acid methyl ester (FAME) profiles, which gave poor insight on the composition of microbial structure (Siciliano *et al.*, 1998). Methods based on nucleic acid extraction and amplification have been

proposed to overcome the limitations of culture dependent techniques and low resolution FAME (Andreote *et al.*, 2009). In addition fingerprinting techniques have been used for community analysis such as single strand conformation polymorphism (SSCP), denaturing gradient gel electrophoresis (DGGE), terminal restriction fragment length polymorphism (T-RFLP) where the differential banding patterns indicate differences in the microbial community but taxonomic assignment is achieved by sequencing of the band of interest except for T-RFLP (Kent & Triplett, 2002). Smalla *et al.* (2007) compared the 16S rRNA fingerprints of four soils generated by three methods DGGE, T-RFLP and SSCP. Although variations occurred between the three profiles the overall interpretation of the results were the same for the three fingerprinting methods. Also it was concluded that the T-RFLP is better for routine analysis due to automation availability unlike the other two methods which are subject to gel-gel variations. Yet the PCR based methods are preferentially used due to ease of analysing many samples and ability to tailor primers for organisms of interest (Kent & Triplett, 2002).

Bulgarelli *et al.* (2012) advanced the field by using pyrosequencing of 400 bp PCR amplicons of the bacterial 16S rRNA gene targeting the V5–V6 variable gene segments to analyse the root associated microbiome of two *A. thaliana* accessions, Shakdara and Landsberg, in two different soil types, silt-clay rich and sand rich. Lundberg *et al.* (2012) also used 454 pyrosequencing of the 16S rRNA gene to analyse the rhizosphere and endosphere of eight inbred *A. thaliana* accessions grown in two different soil types under controlled conditions. In both studies, the soil type was identified as the major factor determining the composition of the bacterial communities, whereas, the host genotype affected individual groups to a lesser extent.

Turner *et al.* (2013b) investigated the rhizosphere microbiome associated with wheat (*Triticum aestivum* var. Paragon), oat (*Avena strigose* accession S75) and pea (*Pisum sativum* var. Avola) grown in the same soil using RNA based comparative transcriptomics. Again, it was found that the rhizosphere microbiome of the different plants was different from bulk soil. Also, the effect of legume (pea) on its associated rhizosphere community was stronger than that of the cereals (oats and wheat). Mendes *et al.* (2014) used DNA shotgun sequencing to explore the soybean rhizosphere from the South-Eastern Brazilian Amazon. Similar to previous studies the rhizosphere bacterial communities clustered separately from the bulk soil. From these studies it was concluded that the plant species and soil characteristics were identified as the main influencing factors shaping the rhizosphere microbiome. In addition, although plant rhizosphere selection is being widely studied, the mechanisms deriving the selection are still poorly understood (Philippot *et al.*, 2013). Furthermore, determining the nature of the signalling molecules that move between the plants and the microbes remains an important question to be answered. Metabolomics can offer possible tools to explore the plant-microbe communication through exudates (Haichar *et al.*, 2014). Also, important functions were found to be enriched in the rhizosphere such as nitrogen, potassium, phosphorus metabolism systems and iron uptake and metabolism systems. These functions are thought to benefit the host plant (Fig.1).

Donn *et al.* (2015) used methods of selective microbial culturing, terminal restriction fragment length polymorphism (T-RLFP) and pyrosequencing of 16S rRNA V5-7 region to investigate the tight bond and lose bond microbial communities associated with wheat grown under filed conditions. Their results demonstrated that although the presence of the plant and its growth stage had an influence on the selection of cultured bacteria, there was no significant effect of plant genotype on the selection of microbial

communities using the other two methods. Thus, suggesting that no single method can provide a detailed view of a complex system such as the soil microbial communities (McSpadden Gardener & Weller, 2001).

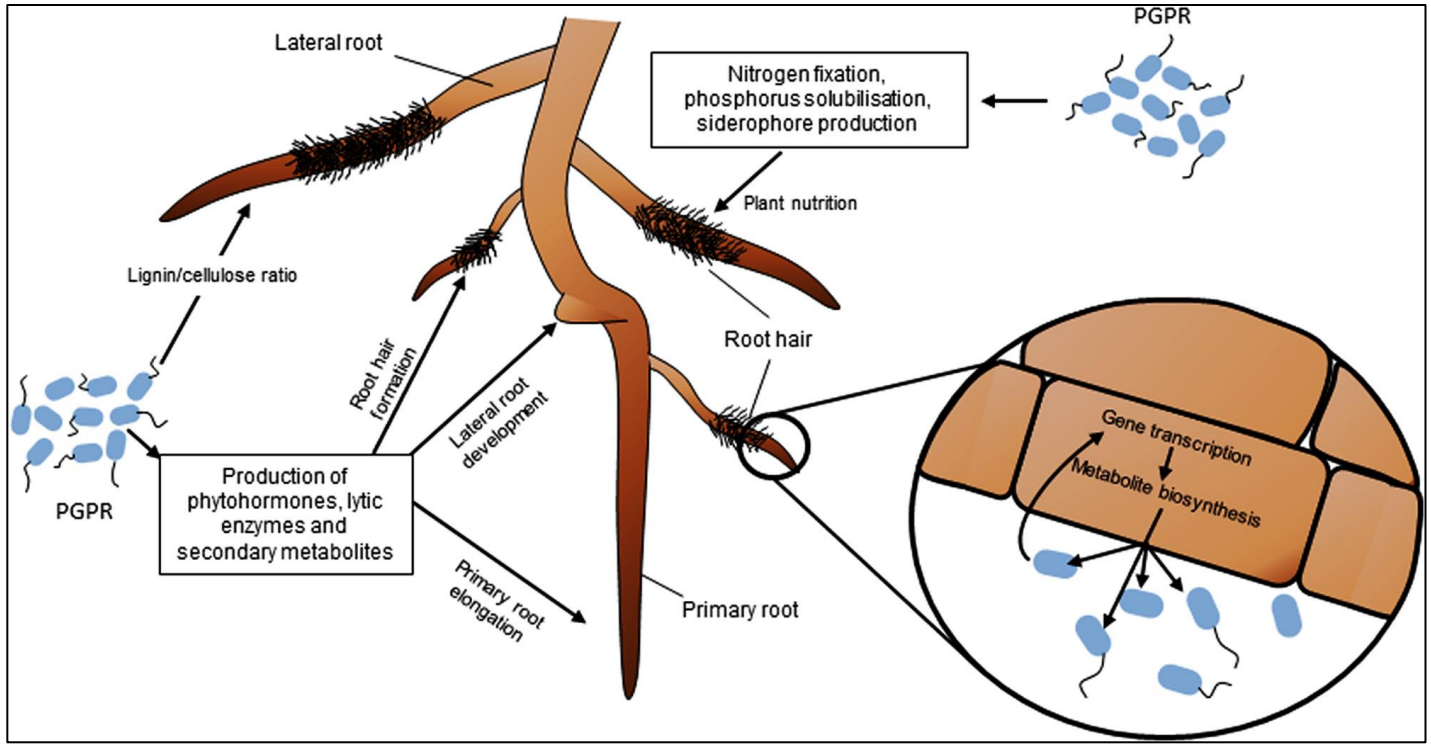



**Figure 1: Plant growth promoting rhizobacteria can modulate root development and influence plant nutrition.** PGPR: Plant Growth Promoing Rhizobacteria (Vacheron *et al*., 2013).

More recently, next generation sequencing has been widely used to examine the rhizosphere microbial communities with great resolving power (Philippot *et al.*, 2013). Using high throughput NGS, microbial specific databases and efficient clustering algorithms, it is now possible to classify the soil microbiome to operational taxonomic units (out) or even to species level (Mahoney *et al.*, 2017). Moreover, it is important to expand the knowledge from composition to functions using metatrascriptomics and proteomics (Turner *et al.*, 2013a).

It can be concluded that traditional physiological and biochemical methods depend on the ability to cultivate the isolates and test for their phenotypic features (respiration, enzyme production, catabolic potential) (Liu *et al.*, 2006). However, many microorganisms cannot be cultivated under laboratory conditions (McSpadden Gardener & Weller, 2001; Kirk *et al.*, 2004; Hirsch *et al.*, 2010). In addition many are present in low numbers (Liu *et al.*, 2006; Jousset *et al.*, 2017). Thus, soil microbiologist have shifted to molecular techniques to study the diversity of soil bacteria (Liu *et al.*, 2006). Although the PCR based methods might have their own bias based on primers used, other factors such as design, sampling, source, extraction protocols, choice of fragment DNA/RNA, availability of sequence information and the choice of statistical analysis all are important in determining the outcomes of soil microbial diversity study (Kent & Triplett, 2002; Hirsch *et al.*, 2010; Turner *et al.*, 2013b). Also the choice of technique used depends on the availability of equipment and expertise (Smalla *et al.*, 2007).

Overall despite the caveats associated with each method, the use of different techniques in combination can aid better understanding of a complex system such as the soil (Dunbar *et al.*, 2000; Kirk *et al.*, 2004).

## 1.4 Microbial Communities Associated with Plants

The proper functionality of the ecosystem depends on the microbial community which in turn play important roles in plant and animal health, including humans (Pfeiffer *et al.*, 2014). All plants are associated with microbes and depending on the type of this association, it can be defined as mutualistic or pathogenic or commensal (Knief *et al.*, 2011). Aerial parts of the plant termed as the phyllosphere are colonized by different bacteria, yeast, and fungi, though bacteria usually dominate. While only limited numbers of bacterial endophytes can be recovered from internal plant tissues, enormous numbers of epiphytic bacteria can be isolated from the surface of healthy plants (Lindow & Brandl, 2003; Bakker *et al.*, 2013). This suggests that the phyllosphere has a greater richness of bacterial species. However, the diversity of bacterial communities in the phyllosphere tends to be less than that of the rhizosphere probably due to the short life span of leaves, higher nutrient richness in the rhizosphere, and the ability of microorganisms to survive in soil in a dormant state for long periods of time (Vorholt, 2012). Moreover, the difference in the microbial community of the leaves from that of the roots might be due to the physiochemical variations between these two environments. For instance, pigmented bacteria are less frequently encountered in the rhizosphere, similarly *Azospirillum* fail to establish on leaves (Lindow & Brandl, 2003). Most of the microbiological work on the phyllosphere has focused on leaves, with some work on buds and flowers (Lindow & Brandl, 2003).

Innerebner *et al.* (2011) demonstrated the effects of two epiphytic leaf colonizing bacterial genera *Methylobacterium* and *Sphingomonas* spp. on the growth of plant pathogenic *Pseudomonas syringae* pv. *tomato* DC3000 on *Arabidopsis thaliana*. Their results showed that while the presence of *Methylobacterium* didn't affect the pathogen's growth, the presence of *Sphingomonas* spp. suppressed pathogen

population and prevented the onset of severe disease symptoms. This control was explained to be due to competition for macro elements and space, production of antimicrobial compounds, and the induction of systemic host resistance.

In addition, the plant's health depends on the composition of the rhizosphere microbial community. These microbes help the plants to acquire micro and macro nutrients from the soil (Siciliano & Germida, 1999). The plant tend to support and enrich the microbial density around the roots as it excretes up to 40% of its photosynthetic products into the rhizosphere (Berendsen *et al.*, 2012). The rhizosphere is defined as the narrow zone of soil surrounding roots where plant metabolic exudates are released. These exudates attract and stimulate the growth of plant growth promoting rhizobacteria (PGPR) (Compant *et al.*, 2005). According to Bergsma-Vlami *et al.* (2005) rhizosphere compatibility is an important feature for the success of biological control and this compatibility is determined by the fact that the host plant determines the genotype of its associated bacteria including the ones with antagonistic traits. For instance, Mauchline *et al.* (2015) found that the first year wheat genotype has great influence on the selection of the associated soil microbial communities with specific influence on *Pseudomonas* spp. Thus, exploring antagonistic bacteria associated with plants is important for many applications in biotechnology such as biological control of plant pathogens, and the isolation of bioactive compounds (Kowalchuk *et al.*, 2002).

### 1.5 Wheat Microbiota

In the past wheat breeders have focused on the above ground plant traits for increased yield but now the importance of belowground parts are becoming increasingly appreciated for disease resistance, stress tolerance and efficient water and nutrient

uptake (Corneo *et al.*, 2016; Mahoney *et al.*, 2017; Kavamura *et al.*, 2018). The plants characteristics are known to influence the endophytic and rhizosphere microbial communities (Siciliano & Germida, 1999). Donn *et al.* (2015) using culturing methods of selected microbial populations, found that the presence of wheat plant and its growth stage were the major factors influencing the rhizosphere microbial community in comparison to bulk soil.

Bergsma-Vlami *et al.* (2005) compared the effect of different host plants wheat, sugar beet, lily and potato, on rhizosphere bacteria with an interest in fluorescent *Pseudomonas* spp. in two types of Take-all soils, conducive and suppressive, respectively. They found that wheat influenced an increase in populations of fluorescent *Pseudomonas* spp. from 2 x $10^5$ to 6x$10^6$ CFU/g root in conducive soil and from 8x$10^5$ to 4x$10^6$ CFU/g root in suppressive soil. Similarly, the endophytic bacterial population can be influenced by the host plant genotype (Robinson *et al.*, 2015). Mauchline *et al.* (2015) compared the rhizosphere bacteria of two wheat cultivars (Hereward and Cadenza) under field conditions with different Take-all inoculum building properties.. Overall the 16S rRNA - gene amplicon analysis showed a highly complex microbiome, where high genetic diversity was encountered within the *P. fluorescens* group. In addition, more *Pseudomonas* were associated with Hereward than Cadenza. Thus it was concluded that the first year grown wheat variety had a selective pressure on *Pseudomonas* genomic diversity. Corneo *et al.* (2016), used T-RFLP of 16S rRNA - V3-V5 region to explore the rhizosphere microbiome of twenty four wheat genotypes. Analysis of alpha-diversity using Shannon index showed no significant difference in the associated rhizosphere bacterial diversity between the studied genotypes. However, there were significant difference in plant biomass and yield.

Recently, Mahoney *et al.* (2017) investigated the rhizosphere microbiome of nine wheat cultivars grown on two sites under field conditions and subject to minimum tillage management. Their analysis of 16S rDNA targeting the V1-V3 hypervariable region suggested that the wheat genotype had a minor but significant influence on the rhizosphere bacterial diversity with bacterial frequencies being different between the cultivars. However, no significant differences were observed for the *Pseudomonas* spp. between the cultivars. Kavamura *et al.* (2018), through 16S rRNA amplicon sequencing, showed the effect of long term nitrogen fertilization regime on the rhizosphere microbiome of the wheat cultivar Cadenza. Their results show that the application of organic nitrogen in the form of farm yard manure resulted in the highest bacterial diversity and richness and this microbiome appeared to be stable with time. In contract application of high levels of inorganic nitrogen negatively affected the bacterial community stability and showed reduced richness and diversity. Thus, indicating the role of biotic and abiotic factors in shaping plant associated microbiomes.

This suggest that in addition to the plant species, soil type, season, climate, agricultural managements, sampling and analysis are also important factors influencing the rhizosphere bacterial communities (Kavamura *et al.*, 2018).

Overall for a high demand crop such as wheat sustainable production can be achieved through breeding wheat cultivars that can recruit beneficial microbes and thus reduce input in agriculture (Mahoney *et al.*, 2017). This suggests the potential of microbiome manipulation for future sustainable agriculture (Corneo *et al.*, 2016).

## 1.6 Disease suppressive soils

Soils can be defined according to their ability to carry or suppress plant disease as conducive or suppressive soils, respectively (Lugtenberg *et al.*, 2013). Often suppressive soils have been associated with the presence of beneficial microbes (Raaijmakers & Weller, 2001; Mazzola *et al.*, 2004; Mavrodi *et al.*, 2012a,b; Philippot *et al.*, 2013). In addition, plants are able to defend themselves against soil borne diseases by stimulation of the growth of beneficial microflora (Mavrodi *et al.*, 2012b; Kwak & Weller, 2013). A comparison of the rhizosphere of both healthy and diseased wheat plants has shown that larger microbial populations are associated with Take-all diseased wheats (McSpadden Gardener & Weller, 2001).

Take-all decline is a natural form of disease suppression that occurs after a severe outbreak of the disease in very susceptible wheat or barley monoculture fields, which leads to higher yields and reduced disease severity in the consequently grown crops in the same field (McSpadden Gardener & Weller, 2001; Kwak & Weller, 2013; Jenkyn *et al.*, 2014). This disease supressiveness was thought to be achieved by a build-up in antagonistic microorganisms (McSpadden Gardener & Weller, 2001), such as populations of fluorescent *Pseudomonas* spp. (Liu *et al.*, 2009). Raaijmakers *et al.* (1997) stated that mixing small amounts of a Take-all suppressive soil with conducive soil is sufficient to transform the latter into a suppressive soil. In addition to Take-all decline, soil disease suppressiveness has been reported for other diseases including potato scab caused by *Streptomyces* spp., *Fusarium* wilt, and *Rhizoctonia* damping-off of sugar beet (Emmert & Handelsman, 1999; Berendsen *et al.*, 2012). The host plant also plays a role in this selection, for instance in Washington a site with wheat monoculture history was known to be suppressive to apple root rot caused by *Rhizoctonia*. However within three years of planting apple orchids on this site the soil

was conducive to *Rhizoctonia* with altered composition, with less *Pseudomonas putida* and more *P. syringae* and *P. fluorescens* bv. III (Mazzola & Gu, 2002).

In Washington state (USA) and the Netherlands it was found that Take-all decline was achieved after a period of wheat monoculture due to an increase in the antibiotic 2,4-diacetylphloroglucinol (2,4-DAPG) produced by fluorescent *Pseudomonas* spp. (Kwak & Weller, 2013). In Inland Pacific Northwest (PNW) it was found that seed treatments with biocontrol species of *Bacillus* and *Pseudomonas* increased resistance to *Ggt* and *Rhizoctonia solani* AG8 (Mavrodi *et al.*, 2012b; Yang *et al.*, 2014). In addition, biocontrol agents *Bacillus pumilus* 7 km and *P. fluorescens* CHAO were found to increase defence responses in wheat inoculated with *Ggt* through inducing the production of wheat peroxidases and glucanases (Daval *et al.*, 2011). Thus, the implementation of indigenous or introduced biocontrol agents tend to be a promising approach for sustainable management of plant root disease, like Take- all, caused by fungi (Cook, 2003; Liu *et al.*, 2009; Yang *et al.*, 2011; Kwak & Weller, 2013). Research on Take-all has focused on *Pseudomonas* spp., *Bacillus* spp., and *Actinomycete* spp. (Schreiner *et al.*, 2010; Malfanova *et al.*, 2011; Yang *et al.*, 2011).

## 1.7 Biological Control

### 1.7.1 Overview

Microorganisms can aid in plant disease suppression, this disease elimination can be specific due to the antagonistic action of a few microbes or it can be general due to a combination of biotic and abiotic factors and the total microbial community (Lenc *et al.*, 2015). Thus biological control can be defined as the use of microbial antagonists to impact pathogen growth or infectivity. An example is given by controlling soil borne

plant diseases using plant growth promoting bacteria (PGPB) (Raaijmakers & Weller, 1998; Lugtenberg & Kamilova, 2009; Daval *et al.*, 2011; Gao *et al.*, 2012; Chen *et al.*, 2013). These bacteria live in close association with the plants either in the rhizosphere (de Boer *et al.*, 2015), on the plant as epiphytes, or within the plants as endophytes (Berg *et al.*, 2005). PGPB benefit the plant using a single trait or a combination of mechanisms e.g: (1) increasing the availability of mineral nutrients, (2) production of plant growth stimulating compounds, (3) protection against pathogens using their antagonistic traits, (4) induction of host resistance (Innerebner *et al.*, 2011; de Boer *et al.*, 2015; Lenc *et al.*, 2015). Thus, understanding the mechanisms used by PGPB to enhance plant growth might lead to better utilization of these agents in plant disease suppression (Compant *et al.*, 2005).

Bacterial organic acids and siderophores can solubilize the poorly soluble inorganic nutrients in the soil (Berg, 2009). *Pseudomonas* spp. can solubilize the phosphate and make it available to the plants. This is achieved through acidification with gulconic acid to chelate the cations bound to phosphate and phosphatases or phytases that hydrolyse organic and inorganic phosphate (Vacheron *et al.*, 2013). The non-fluorescent *Pseudomonas stutzeri* has nitrogen fixing genes (*nif)*, and is able to fix nitrogen in rice paddies and has genes for ethylene inhibition which are thought to promote plant growth by reducing the impact of ethylene on root development (Rediers *et al.*, 2009; Silby *et al.*, 2011). Many PGPR bacteria including *Pseudomonas* spp. have the ACC deaminase coding gene (*acdS*), which degrades ACC into ammoniumand α-ketobutyrate. The ACC, 1-aminocyclopropane- 1-carboyclic acid, is an ethylene precursor in plants. Thus, by lowering the ethylene levels, the ACC deaminase producing bacteria reduce the biotic and abiotic stress on the plant and enhance root growth (Berg, 2009; Silby *et al.*, 2011; Vacheron *et al.*, 2013). Volatile

organic compounds (VOC) of *Bacillus subtilis* strain GB03 indirectly enhance iron uptake by *A. thaliana* through rhizosphere acidification and up-regulation of FRO2 and IRT1 genes, coding for $Fe^{3+}$ chelate reductase and $Fe^{2+}$ transporter; respectively (Vacheron *et al.*, 2013). *Pseudomonas* siderophores are well known for solubilizing iron thus making it available to the bacteria and plants (Rainey, 1999; Faraldo-Gómez & Sansom, 2003; Neal *et al.*, 2012). In dicots and non graminaceous monocots iron uptake is through surface reduction while grasses can produce phytosiderophores such as mugineic acid from barley, avenic acid A from oat, and 2' -deoxymugineic acid from wheat. Thus either through iron reduction or use of microbial siderophores these benefits are achieved (Leong, 1986).

Antagonistic effects of PGPB can be achieved through different mechanisms such as (i) competition for colonization sites, nutrients and minerals, (ii) inhibition of the pathogen by antibiotics, toxins and surface-active compounds called bio-surfactants, and (iii) parasitism, that may involve production of extracellular cell wall degrading enzymes such as chitinase and β -1,3 glucanase (Berg *et al.*, 2005). This suggests that the antagonistic effect is a combination of one or more of the above mentioned mechanisms (Shoda, 2000; Raaijmakers & Weller, 2001).

### 1.7.2 The ability of *Pseudomonas fluorescens* to suppress fungal diseases in soil

*Pseudomonas* spp. are saprophytic bacteria that actively colonize the rhizosphere of many different plants. They can enhance plant growth by pathogen exclusion (Rainey, 1999). This exclusion is achieved through their ability to secrete molecules such as iron scavenging siderophores, cyclic lipopepetides to aid motility, phenazines and anti-

fungal compounds pyoluteorin and pyrrolnitrin, along with hydrogen cyanide, which is a volatile metalloenzyme inhibitor (Fig. 2) (Rainey, 1999; Haas & Keel, 2003). Mutants with impaired growth rates or defects in LPS O-antigen synthesis showed sub-optimal colonization (Lugtenberg & Dekkers, 1999; Lugtenberg *et al.*, 2001). The antagonistic traits of fluorescent *Pseudomonas* spp. makes it an interesting target to study (Lenc *et al.*, 2015). For example, strains that synthesize the antifungal metabolite 2,4-diacetylphloroglucinol (2,4-DAPG) have the ability to suppress many soil borne fungal diseases like Take-all disease of wheat (Raaijmakers & Weller, 1998). In addition, different genotypes of DAPG-producing *Pseudomonas* were shown to differ in their ability to suppress Take-all disease of wheat (Bergsma-Vlami *et al.*, 2005). This suggests a selective pressure exerted by the wheat to select for specific genotypes of DAPG-producing *Pseudomonas* which are highly adapted to the wheat rhizosphere (Raaijmakers & Weller, 2001).

Motility seems to be another important trait for the initiation of successful colonization as evidenced by the findings that non-motile mutants of *P. fluorescens* lost their ability to colonize the roots (Capdevila *et al.*, 2004; Alsohim *et al.*, 2014). Root exudate derived chemotaxis often initiates this motility (Bais *et al.*, 2004).

Due to the complex nature of the rhizosphere, it is predicted that a diverse combination of genes is associated with its colonization. To date various genes involved in nutrient acquisition, motility, chemotaxis, adhesion, secretion and stress responses are found to be associated with *Pseudomonas* rhizosphere colonization ability (Walsh *et al.*, 2001; Jackson *et al.*, 2005; Silby *et al.*, 2009).

This ecological fitness of *Pseudomonas* spp. as a PGPB is defined by their performance and it is considered to be a sophisticated phenotype affected by various

environmental conditions (Rainey, 1999). For instance, the influence of the plant genotype on the selection of associated *Pseudomonas* spp. was highlighted by Mauchline *et al.* (2015) where the high Take-all inoculum builder wheat variety (Hereward) supported a population of *Pseudomonas* spp. with antagonistic traits. However, the low Take-all inoculum builder wheat variety (Cadenza) supported *Pseudomonas* spp. that were better adapted to host communication and nutrient acquisition. In addition, Mavrodi *et al.* (2012) have shown the effects of irrigation on the selection of antibiotic producing *Pseudomonas* spp. where phenazine-1-carboxylic acid (*Phz*+) producers dominated dryland wheat rhizosphere, while the 2,4-diacetylphloroglucinol producers (*Phl*+) were associated with wetland wheat rhizosphere.

The traditional way of introducing a biocontrol organism has in many cases failed to achieve desirable control under field conditions. Thus manipulating the rhizosphere to favour the growth of a specific genotype of the control organism seems to be a promising new approach in plant disease management (Thomashow & Weller, 1988; Mauchline *et al.*, 2015).

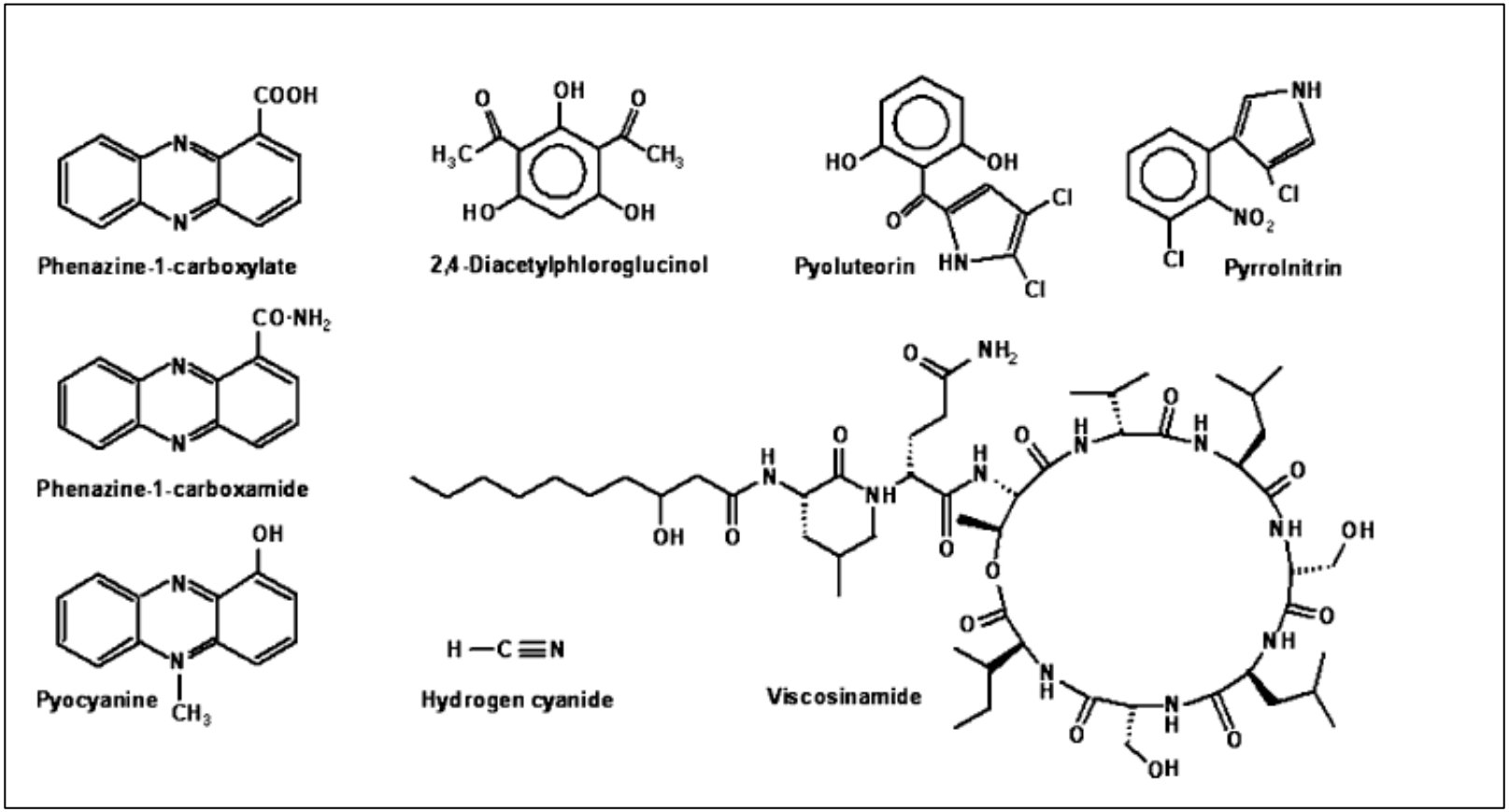



**Figure 2: Antibiotics produced by strains of *Pseudomonas fluorescens*** (Haas & Keel, 2003).

#### 1.7.2.1 *Pseudomonas* rhizosphere fitness genes

The rhizosphere is a nutrient rich area surrounding the roots and is influenced by its exudates (Lugtenberg & Dekkers, 1999). Many soil bacterial genes and traits are shown to be involved in root colonization (Capdevila *et al.*, 2004).

Several *Pseudomonas* species interact with the plants and some are known to contribute to plant health either directly by inducing the plant immune system or indirectly by antagonising pathogens (Silby & Levy, 2004; Silby *et al.*, 2011). Important environmental functions include motility, nutrient scavenging, stress responses, detoxification and regulation (Rainey, 1999; Gal *et al.*, 2003; Silby *et al.*, 2011). Niche specific genes are over expressed in a particular environment and thus are considered the main contributors to the colonization success in that environment in comparison to the regularly expressed genes in a range of environments (Rainey, 1999; Gal *et al.*, 2003). Silby & Levy (2004) found that 22 genes had elevated expression in soil in comparison to laboratory media. The use of promotor trapping strategy, in vivo expression technology (IVET), has enabled the assignment of rhizosphere induced genes (*rhi*) into six groups: attachment and surface colonization, nutrient acquisition, stress responses, antibiotic production, secretion and unknown (Jackson *et al.*, 2005; Rediers *et al*., 2003).

*Pseudomonas fluorescens* strains are commonly studied for their biocontrol and plant growth promotion. Unlike *P. aeruginosa* PAO1 *and P. syringe*, the saprophytic *P. putida* strain KT2440 and the *P. fluorescens* strains Pf01, Pf-5 and SBW25 lack pathogenesis genes (Silby *et al.*, 2011). Although the *rsp* gene cluster products of SBW25 resemble the type III secretion system (T3SS) of pathogenic bacteria, it does not seem to elicit plant defences suggesting that it probably targets another host, for

instance fungi (Jackson *et al.*, 2005; Silby *et al.*, 2011). *P. fluorescens* SBW25 protects sugar beet from damping-off disease caused by *Pythium ultimum* (Rainey, 1999; Alsohim *et al.*, 2014).

Rainey (1999) and Gal *et al.* (2003) used IVET to screen *P. fluorescens* SBW25 fitness genes in the rhizosphere of sugar beet. The former employed an expression trapping system with a promotor-less copy of *panB* while the latter used a *dapB* promotor-less system. The *panB* gene encodes for ketopantoate hydroxymethyltransferase required for the synthesis of pantothenate while the *dapB* gene encodes for diaminopimelate (DAP), an important peptidoglycan component, which is limited in soil. The *dapB* mutant phenotype is adequate for IVET selection since diaminopimelate auxotrophy is lethal to growing cells, while non-growing cells can remain viable for long periods (Silby & Levy, 2004).

In the first trapping system twenty rhizosphere induced genes were identified, six of which were unique while fourteen shared homology with [GenBank] sequences encoding genes in secretion, nutrient acquisition and stress responses. In the second IVET system twenty five rhizosphere induced genes were identified, of these a gene involved in cellulose synthesis *wssE* and a putative amidohydrolase gene were of interest. The *wss* operon is made of ten genes that encode acetylated cellulose polymer and is required for rhizosphere and phyllosphere fitness. The putative amidohydrolase gene was found to share some similarity with plant derived nitrilase which is required for the synthesis of the plant hormone indole acetic acid (IAA). Thus, suggests a role in plant growth promotion (Gal *et al.*, 2003).

The *dapB* system was also used by Jackson *et al.* (2005) to investigate the *rsp* gene cluster of SBW25. Rediers *et al.* (2009) also used *P. stutzeri dapB mutant* to screen for rice colonization and infection (*cii*) genes. Of the induced genes, induction of *bcp*

gene was explained as a response to oxidative stress. Proteins involved in the adaptation to various stresses in the rhizosphere, YhbH, Hfq, and MiaA were also induced. They also pointed to a possible role of *pta*-encoded phosphortransacetylase in providing energy form acetyl phosphate metabolism for nitrogen fixation in rice rhizosphere.

Silby & Levy (2004) found some overlap between their fusion products and those found by Gal *et al.* (2003), Rainey (1999) and Rediers *et al.* (2009) suggesting that these loci are important for the general soil colonization rather than the specific rhizosphere colonization.

Lipopolysaccharides (LPS) of Gram negative bacteria consist of Lipid-A, core, and O-antigen and act as receptors for bacteriophages. Bacterial LPS affects the colony morphology and plays an important role in determing cell surface charge, attachment and biofilm formation (Spiers & Rainey, 2005). *Pseudomonas* mutants with defect in the O-antigen had impaired colonization on potato roots implicating a possible role of LPS in root colonization (Lugtenberg *et al.*, 2001). In *P. fluorescens* SBW25 the wrinkly spreader (WS) morphotype is one of many different niche specialist genotypes that emerge following experimental propagation spatially structured microcosms (McDonald *et al.*, 2009). The fuzzy spreader is a type of WS with mutation in *fuzY* the fourth gene of the five-gene *fuzVWXYZ* operon. The gene *fuzY* encodes a β-glycosyltransferase that is predicted to modify surface lipopolysaccharide. This is implicated in suppressing the mat WS types and conferring resistance to SBW25ϕ2 phage (Ferguson *et al.*, 2013). Thus indicating the efficient ability of bacteria to adjust with specific niches. LPS in the outer membrane contains several heptoses which are phosphorylated. In addition, the LPS are strongly associated with porins and thus affect resistant to antibiotics. In the mutants with altered LPS, the space between the

pore protein and LPS becomes wider enabling the binding of polymyxin B. While the narrower pore opening makes the mutants less effective in competing for low level nutrients in the rhizosphere (De Weert *et al.*, 2006).

Phenazines also play a role in determining the colony morphology and play critical roles in biofilm formation. In *P. aeruginosa* the presence of phenazines results in smooth colonies while mutants start to wrinkle. This is because in smooth wild type, phenazines act as an alternate electron acceptors for the cells in the absence of oxygen. While in phenazine lacking mutants, wrinkeling is a starategy to increase the surface area and balance the intracelluar redox state of cells within the community (Dietrich *et al.*, 2013). Phenazines are naturally produced heterocyclic compounds with substitutions at different points around their rings. They serve as signal molecules for biofilm formation and promote redox homeostasis. Also, phenazines can enhance biofilm formation by increasing the availability of iron (Wang *et al.*, 2011). Their redox potential allows them to be reduced by bacterial cells and act as electron shuttles between the bacterium and an external substrate following reaction with extracellular higher potential oxidants like ferric iron and oxygen. This redox potential is mainly responsible for their antagonistic biological activity, where they act as antibiotics in soil and virulence factors during infection (Price-Whelan *et al.*, 2006; Dietrich *et al.*, 2013). Furthermore, phenazine production downregulates the genes for siderphore biosynthesis and transport (Wang *et al.*, 2011). In most phenazine producing pseudomonads, the phenazine encoding genes are arranged in one core operon; *phzABCDEFG.* Environmental conditions, population density and quorum sensing are among these and the levels of iron, oxygen and phosphate affect the expression of phenazine biosynthesis genes (Price-Whelan *et al.*, 2006). Mazzola *et al.*, (1992), has shown that phenazine producers; *P. aureofaciens* 30-84 and *P. fluorescens* 2-79 are

able to colonize wheat roots better than phenazine-lacking ($Phz^-$) mutants due to reduced competition with resident microflora.

The role of the biosurfactant viscosin from *P. fluorescens* SBW25 in aiding swarming motility, biofilm formation and plant protection was described by Alsohim *et al.* (2014). Viscosin is a cyclic lipopepetide (CLP) synthesized by large nonribosomal peptide synthases (NRPS), *viscA, viscB* and *viscC* (De Bruijn & Raaijmakers, 2009). Mauchline *et al.* (2015) analysis of *Pseudomonas* isolates from wheat endosphere and rhizosphere showed that phenotypic gene clusters of LPS, pili synthesis and viscosin operons were strongly associated with activities like microbial suppression, plant association/manipulation or scavenging and growing on plant material.

In Gram negative bacteria, iron uptake is regulated by Fur protein which acts as a repressor binding to DNA and preventing transcription. Under low intracellular iron concentration Fur loses its transcription repressor ability. In addition to this system, bacteria like *Escherichia coli* have genes for iron uptake through ferric citrate system *fecABCDE*. Binding of iron loaded siderophore to TonB- dependent FecA membrane receptor activates the expression of the corresponding gene cluster. These genes encode the periplasmic binding protein FecB, the cytoplasmic membrane proteins FecC and FecD and the ATPase FecE (Faraldo-Gómez & Sansom, 2003).

The toxin/antitoxin genes (TA) are made of stable toxin that can harm the cell and an unstable antitoxin that can inactivate it (Andersen *et al.*, 2017). Toxin-antitoxin (TA) are of three types in Type I and III antitoxins are RNAs while in type II they are proteins while the toxins are always proteins (Van Melderen, 2010). TA have been found to have roles in antibiotic resistance, phage inhibition, stress responses, biofilm formation and pathogenicity (Pandey & Gerdes, 2005; Van Melderen, 2010; Wood & Wood,

2016). The type II TA system are the most common. Ten toxin families have been identified of these, ParE targets DNA gyrase and thus affects replication by generating double strand breaks. While RelE and HigB affects translation by targeting the translating ribosome and inducing mRNAs cleavage (Van Melderen, 2010). For instance in *E. coli* the TA systems are stress-response element that aids cell survival under unfavourable growth conditions. The *relBE* locus encodes for RelE toxin and RelB antitoxin, where the latter counteracts the activity of the former by direct protein-protein interaction. That is during amino acid or glucose starvation, activation of RelE leads to translation inhibition by mRNA cleavage (Pandey & Gerdes, 2005). Clinical isolates of *P. aeruginosa* were found to harbour *higBA* TA (HigB/HigA) system in which the antitoxin HigA masks the toxicity of the toxin HigB (Wood & Wood, 2016). Deletion of the antitoxin gene *higA* results in phenotypes with impaired virulence such as those with reduced growth, swarming and biofilm formation, and with decreased production of pyocyanin and pyochelin (Wood & Wood, 2016; Andersen *et al.*, 2017). The TA system is still a subject of ongoing research (Andersen *et al.*, 2017).

From the above, it can be concluded that the wide range of identified rhizosphere induced genes provide a broad range of possibilities for use as markers to identify the loci under specific plant selection.

### 1.8 Aims and Objectives

The overall project objective was to explore the effect of wheat cultivars grown under field conditions on the associated microbiome under Take-all disease conditions. To address this five wheat cultivars were grown continuously in the same plot over three different fields at Rothamsted research. In addition, the isolated *Pseudomonas* spp.

were investigated for selective recruitment by the wheat and for ability to control Take-all *in vitro*. To achieve the previous objectives, the following specific questions were investigated:

- Do different wheat cultivars select for specific genotypes of the associated *Pseudomonas* spp.? This question was answered in chapter three through investigating some important rhizosphere colonization loci.

- Do the *Pseudomonas* isolates differ in their ability to inhibit the growth of Take-all fungi *in vitro*? The answer to this question was addressed in chapter four.

- Can we see the same *in vitro* inhibition when tested on the host plant? This question was addressed in chapter five.

- How do differential TAB (Take-all inoculum build-up) wheat cultivars shape their microbiome over time and in comparison between three different field sites? The answer was explored in chapter six.

## CHAPTER 2- Materials and Methods

### 2.1 Media

Analytical grade media supplied by Difco (Difco laboratories Ltd, Oxford), Merck (Merckserono, Middlesex, U.K), or Sigma (Sigma-Aldrich Company Ltd., Dorset, U.K) were used. The recipe of Maniatis *et al*. (1989) was used to prepare each medium. All the below listed media were prepared by adding the components to one litre of deionised water. For solid medium, Agar (Difco) was added to the prepared broth to a final concentration of 1.5% (15g $L^{-1}$). The media were sterilized by autoclaving at 121 °C for 20 mins. Filter sterilization through (0.22 µm) Millex$^{TM}$ Milipore® filter was used for heat labile substances which were added to the sterile media after cooling to 50°C. Pre-warmed medium (20 ml) was added to each Petri dish (SLS, Scientific Laboratory Supply Ltd).

**King's medium B (KMB)** (King *et al.*, 1954): To 1 L of water, proteose peptone (Difco) 20 g, $K_2HPO_4$ 1.5 g, $Mg_2SO_4.7H_2O$ 1.5 g, glycerol 10 ml, were added.

***Pseudomonas* Selective Agar (PSA):** *Pseudomonas* Agar Base (Oxoid$^{TM}$) was supplemented with antibiotics CFC (Cephalothin 25 mg, Fucidin 5 mg, and Cetrimide 5 mg).

**Potato Dextrose Agar (PDA)**: was purchased from Oxoid$^{TM}$.

**Phosphate Buffered Saline (PBS):** For 1 L of water NaCl 8 g, KCl 0.2 g, $Na_2HPO_4$ 1.15 g, $KH_2PO_4$ 0.2 g, adjust pH to 7.3.

**Full strength LB agar (Swarming motility):** For 1.2 L of water, tryptone (Fulka) 12g, Yeast extract (Oxoid) 6 g, NaCl (Sigma-Aldrich) 12 g were added, mixed and heated to dissolve. 400 ml of the dissolved mix were distributed into 500 ml Duran bottles. 1

g of Agar (Sigma-Aldrich) was added to each bottle and the medium was sterilized by autoclaving.

**Low strength (1/10) LB agar (Swimming motility):** For 1.2 L of water, tryptone (Fulka) 1.2 g, Yeast extract (Oxoid) 0.6 g, NaCl (Sigma-Aldrich) 1.2 g were added, mixed and heated to dissolve. 400 ml of the dissolved mix were distributed into 500 ml Duran bottles. 1 g of Agar (Sigma-Aldrich) was added to each bottle and the medium was sterilized by autoclaving.

### 2.2 Culture maintenance

All bacterial strains were grown on King's medium B (KA (agar) or KB (broth) at 27°C, shaking for 16 h or static (agar) for 48h).

From -80 °C frozen stocks, all *Pseudomonas* species were freshly grown on KB agar overnight at 27 °C. When liquid culture was needed, a single colony growing on an overnight plate of KMB agar was used to inoculate (10-30 ml) a KMB broth. The inoculated broth was then incubated overnight in an orbital shaker set at 27 °C and 200 rpm (Forma Scientific). The *Gaeumannonmyces graminis var. tritici (Ggt)* was grown by placing a 0.5 cm plug of growing hypha on PDA agar and the plates were incubated for 7 days at 24 °C. Plugs from the original plate were stored in sterile water at 4°C and were plated occasionally.

### 2.3 Genomic DNA extraction

For DNA extraction from *Pseudomonas spp,* a single colony grown on KA was used to inoculate 10 ml KB. The culture was incubated overnight on an orbital shaker at 27˚C (Forma Scientific). One millilitre (1 ml) of broth was used for DNA extraction using

a GeneJet Genomic DNA extraction kit following the manufacturer's instructions (Thermo Scientific). The quantity and quality of eluted DNA was determined using a NanoDrop® (ND-1000 UV-Vis Spectrophotometer) (LabTech). The eluted DNA was stored at -20°C until needed.

### 2.4 Gel electrophoresis

Bioline Molecular Grade Agarose powder was used to prepare gels for electrophoresis. Gels were made to a final concentration of 1% (w/v) depending on the volume used. Agarose powder was dissolved in 0.5X Ambion® TBE buffer (10X solution contains 0.89 M Tris, 0.89 M Borate, 0.02 M EDTA). Biotium Gel Red ™ (10,000X in water) was added to a final concentration of 0.1 mg $ml^{-1}$. The extracted DNA (4 µl) was mixed with (1 µl) of 5X DNA loading dye (200 mM Tris-HCl, 5 mM EDTA, 30% (v/v) glycerol, 0.1% (w/v in water) bromophenol blue, 0.1% (w/v in water) xylene cyanol) prior to loading in gel. BIOLINE HyperLadder ™ was used as a DNA band size marker. The gel was used in Alpha Laboratories Gel tanks at a voltage of 90 V for 40 mins. On completion of the run, DNA bands were visualized using a GBOX (Syngene) gel documentation system.

### 2.5 PCR

All polymerase chain reactions were carried out in bench-top Thermal Cyclers (Techne $P^3$ or BioRAD T100). PCRBIO Taq Mix Red was the PCR mix used in all the PCR reactions. Cycling conditions varied based on the primers. Primers used in this work were supplied by Eurofins (Eurofins Genomics, Ebensburg, Germany).

## 2.6 PCR purification

PCR products were purified using a QIAquick PCR Purification Kit (Qiagen) following the manufacturer instructions. The purified PCR products were eluted in 50 µl of supplied elution buffer. For Eurofins sequencing services, 15 µl of the purified PCR product were mixed with 2 µl of either forward or reverse primers.

## 2.7 Motility assay

For swarming motility, full-strength LB with 0.25% agar was used. Agar plates were freshly made by pipetting 30 ml of sterile molten agar into 88 mm Petri dishes. The plates were allowed to set at room temperature for up to 4 h. The plates were then allowed to dry for 30 mins with lids open in a class 2 safety cabinet. Using a sterile wire a single colony from an overnight culture was stabbed into the centre of LB plate, with the wire touching the bottom of the plate. For each isolate three replicas were made. The inoculated plates were then incubated without stacking in static incubator set at 27 °C (BINDER growth chamber). For swimming motility, 1/10 strength LB agar with 0.25% agar was used. The plates were prepared and the assay was conducted as described for swarming motility assay. Plates were monitored at 18, 24, and 42 h and images were taken using gel documentation system G:BOX (Syngene). The growth area were later measured using ImageJ software.

## 2.8 Seed sterilization validation

Wheat (*Triticum aestivum*) seeds of unknown variety were kindly provided by the University of Reading glass houses to validate the seed sterilization process and carry out initial plant assay. The seeds were surface sterilized by immersion in 2.5% (v/v

with with sterile n $H_2O$) solution of house-hold bleach for 3 mins followed by 3 rinses in sterile n $H_2O$. The seeds were then allowed to air dry for up to 3 h in class 2 safety cabinet (Labogene) (Weller & Cook, 1983). Air dried seeds were place in 25 ml sterile tubes with 5 ml of sterile PBS and 4 glass beads (3 mm). The tubes were vortexed for 1 min. Serial dilution was the prepared in a 96 well plate by mixing 20 µl of the seed/ PBS mixture with 180 µl of fresh sterile PBS. The drop spot method was used where 10 µl of each dilution was spotted on KA plate, with each dilution replicated 6 times. The plates were incubated at 27 °C and the plates were monitored for growth at 24 and 48 hrs. The process was replicated 3 times. In addition air dried seeds were placed on 20 ml water agar (WA) plates and incubated at room temperature for a week.

### 2.9 Soil core bioassay and Take-all index (TAI)

For the soil core bioassay, five soil cores, 5.5 cm diameter and 10 cm deep were taken in a zig-zag transect across each plot. Cores were inverted into plastic drinking cups (11 cm with water draining holes drilled in the bottom) which contained a basal layer of 30 $cm^3$ damp sand. The top of the inverted soil core was pressed to the sides of the cup. The soil was lightly watered and 10 wheat seeds (cv. Herewered) placed on the surface (originally the bottom of the core). Seeds were covered with a layer of horticultural grit, and the pots were transferred to a controlled environment room for 5 weeks (16 h day, 70% relative humidity, day/night temperatures 15/10 °C and watered twice weekly). After 5 weeks the plants were removed and the roots were washed with water. The roots were assessed for Take-all lesions in a white dish under water and the total numbers of plants and roots, along with the number of plants and infected roots were recorded. The percentage of plants and roots infected were calculated as a measure of the infectivity of the soil (McMillan *et al.*, 2011) . For Take-all index, adult-

plant samples (5 x 20 cm row length per plot) were taken from each field trial (Growth stage 71-73, milk development). Plant samples were transported back to the field laboratory, roots were washed from free soil, the tops chopped off and the remaining stem bases and root system air dried in a polytunnel for 4-5 days and then stored at room temperature before assessment for Take-all disease. Stored dried whole plant root systems were soaked in water for approximately 15-20 mins and then assessed in a white dish under water and scored for Take-all to calculate the Take-all index. The proportion of roots infected for each whole plant root system was estimated and graded into six categories: no symptoms, slight 1 (1-10% roots infected), slight 2 (11-25%), moderate 1 (26-50%), moderated 2 (51-75%) and severe (>75%). Using this the Take-all index was calculated for each plot; (1 x percentage plants in slight 1 category) + (2 x percentage plants in slight 2 category) + (3 x percentage plants in moderate 1 category) + (4 x percentage plants in moderate 2 category) + (5 x percentage plants in sever category); divided by the number of categories slight 1 to severe; with maximum TAI 100 (McMillan *et al.*, 2014).

## CHAPTER 3- Great Harpenden 2 Rhizosphere fitness loci

**Summary:**

Many studies have shown that plants are able to shape their microbiomes *Psuedomonas fluorescens* successfully colonizes the rhizosphere of many plants and is well recognized for its plant growth promoting and disease suppressive properties (Rainey, 1999; Spiers *et al.*, 2000; Silby *et al.*, 2009). It is also widely investigated for controlling Take-all disease of wheat caused by *Gaeumannomyces graminis var. tritici* (*Ggt)* (Raaijmakers & Weller, 1998; Mavrodi *et al.*, 2007; Yang *et al.*, 2014). Rhizosphere fitness is an important trait used to measure rhizosphere colonization. This fitness is encoded by a pool of rhizosphere fitness genes (*rhi)*. The *rhi* genes encode for many important colonization traits such as host recognition, motility and antagonism. An investigation by Mauchline *et al.* (2015) highlighted a possible selection exerted by sequential planting of wheat varieties, whereby wheat variety grown in the first year influenced the associated fluorescent *Pseudomonas* spp. regardless of which wheat variety was grown in the second year. To further investigate this finding, the same set of *Pseudomonas* spp. isolated by Mauchline *et al.* (2015) were screened for the differential harbouring of four putative *rhi* fitness loci; *wsm* , *viscB, tox* and *fecB*. The *wsm* locus is thought to aid in colonization while *fecB* likely plays a role in iron acquisition and probably antagonism. A Dot blot and polymerase chain reaction (PCR) approach was used to screen for the presence and absence of these loci. Statistical analysis on the outcomes of gene presence and absence showed a significant effect driven by first year wheat ($p= 0.046$) on the selection of either gene. The analysis also showed that the *wsm* locus was more associated with isolates of first year Hereward ($p < 0.001$) compared with the *fecB* gene ($p < 0.001$) which was

more associated with isolates from Cadenza background. Thus these results agree with earlier findings of Mauchline *et al.* (2015).

### 3.1 Introduction

The soil surrounding the roots can be divided into bulk soil and the closely attached rhizoplane and rhizosphere. The rhizosphere is densely populated with microbes due the presence of nutrient-rich exudates (Compant *et al.*, 2010). A wide range of interactions occur between the plant and the associated microorganisms in the rhizosphere. These interactions can be detrimental to plant health like in the case of pathogens or beneficial to the plant as seen with plant growth- promoting bacteria (PGPB) (Raaijmakers *et al.,* 2009; Mauchline *et al.*, 2015).

Due to the complex nature of the rhizosphere, it is predicted that a diverse combination of genes is associated with its colonization (Walsh *et al.*, 2001). *P fluorescens* is a Gram negative bacterium that colonizes the rhizosphere of many plants and is widely investigated for its disease control abilities (Keel *et al.*, 1992; Weller, 1988). To date various genes involved in nutrient acquisition, motility, chemotaxis, adhesion, secretion and stress responses are found to be associated with *Pseudomonas* rhizosphere colonization ability (Rainey, 1999; Walsh *et al.*, 2001; Gal *et al.*, 2003; Silby *et al.*, 2009; Mauchline *et al.*, 2015). These genes account for the ecological fitness of *Pseudomonas* spp. as a PGPB is defining their performance and adaptation to - various environmental conditions (Rainey, 1999). For instance, different genotypes of the phloroglucinol antibiotic, 2,4 Diacetylphloroglucinol (2,4–DAPG), producing fluorescent *Pseudomonas* have been shown to variably colonize the rhizosphere of

wheat, pea, and corn (Bergsma-Vlami *et al.,* 2005). In addition, they differed in their utilization of carbon sources and ability to produce other antibiotics.

The influence of the plant genotype on the selection of associated *Pseudomonas* spp. was highlighted by Mauchline *et al.* (2015) where the high Take-all inoculum builder wheat variety Hereward supported a population of *Pseudomonas* spp. with fungal antagonistic traits. However, the low Take-all inoculum builder wheat variety Cadenza supported *Pseudomonas* spp. that were better adapted to host communication and nutrient acquisition.

**Aims and objectives:**

To test the hypothesis that the wheat variety grown in the first year selects for the genotype of associated *Pseudomonas* spp., isolates from the same field experiment as Mauchline *et al.* (2015), were screened for *rhi* fitness genes. These loci were selected after identifying the rhizosphere fitness genes highlighted by Rainey (1999) as well as the most significant loci from Mauchline *et al.* (2015).Thus, genes involved in nutrient acquisition, host recognition, colonization, motility, and antagonism were selected as prime candidates for this investigation. Genes coding for these traits were searched for in the genome of *P. fluorescens* SBW25. The selected loci for this investigation were postulated to be involved in (i) host recognition like the lipopoly-saccharide (LPS), (ii) motility and antimicrobial surfactant (viscosin), (iii) nutrient acquisition (iron), and (v) toxin involved in stress responses and biofilm formation. These loci were labelled as *wsm*, *viscB*, *fecB*, and *tox* respectively.

## 3.2 Methods

### 3.2.1 Sample collection and bacterial isolation

Bacterial samples were kindly provided by Dr Tim Mauchline, Rothamsted Research, and were derived from a two-year field trial that ran in year 2010-2012. In this experiment there were two treatments as summarized in Table 1. In the first year, a high Take-all inoculum building (H-TAB) variety Hereward was grown in parallel with a low Take- all inoculum building (L-TAB) variety Cadenza. Following the harvest of first year plants the same plots were over sown in the second year with different wheat varieties. In this study only plots that were over-sown with either Hereward (H-TAB) or Xi-19 (L-TAB), another Low TAB variety, in the second year were sampled. Four-hundred and eleven (411) *Pseudomonas* spp. were selectively isolated by (Mauchline *et al.*, 2015). from the rhizosphere and endosphere of the second year sampled wheat.

The field design consisted of four main blocks divided into two main plots (Fig. 3) of Hereward and Cadenza in the first year. In the second year, these two main plots were divided into 8 split plots (Fig. 4) which were over-sown with other wheat varieties.

**Table 1:** Great Harpenden 2 (GH2) treatment structure.

| GH 2 | Year 1 | Year 2 | Sampled split plots in Year 2 |
|---|---|---|---|
| **Treatment (a)** | Hereward (H-TAB) | Hereward | 1, 32, 37, 44 |
| | Hereward (H-TAB) | Xi-19 (L-TAB) | 22, 28, 31, 34 |
| **Treatment (b)** | Cadenza (L-TAB) | Hereward | 8, 46, 52, 58 |
| | Cadenza (L-TAB) | Xi-19 (L-TAB) | 24, 25, 30, 35 |

The methods used for field sampling, rhizosphere harvesting, and the selective isolation of *P. fluorescens* were previously described in Mauchline *et al.* (2015)

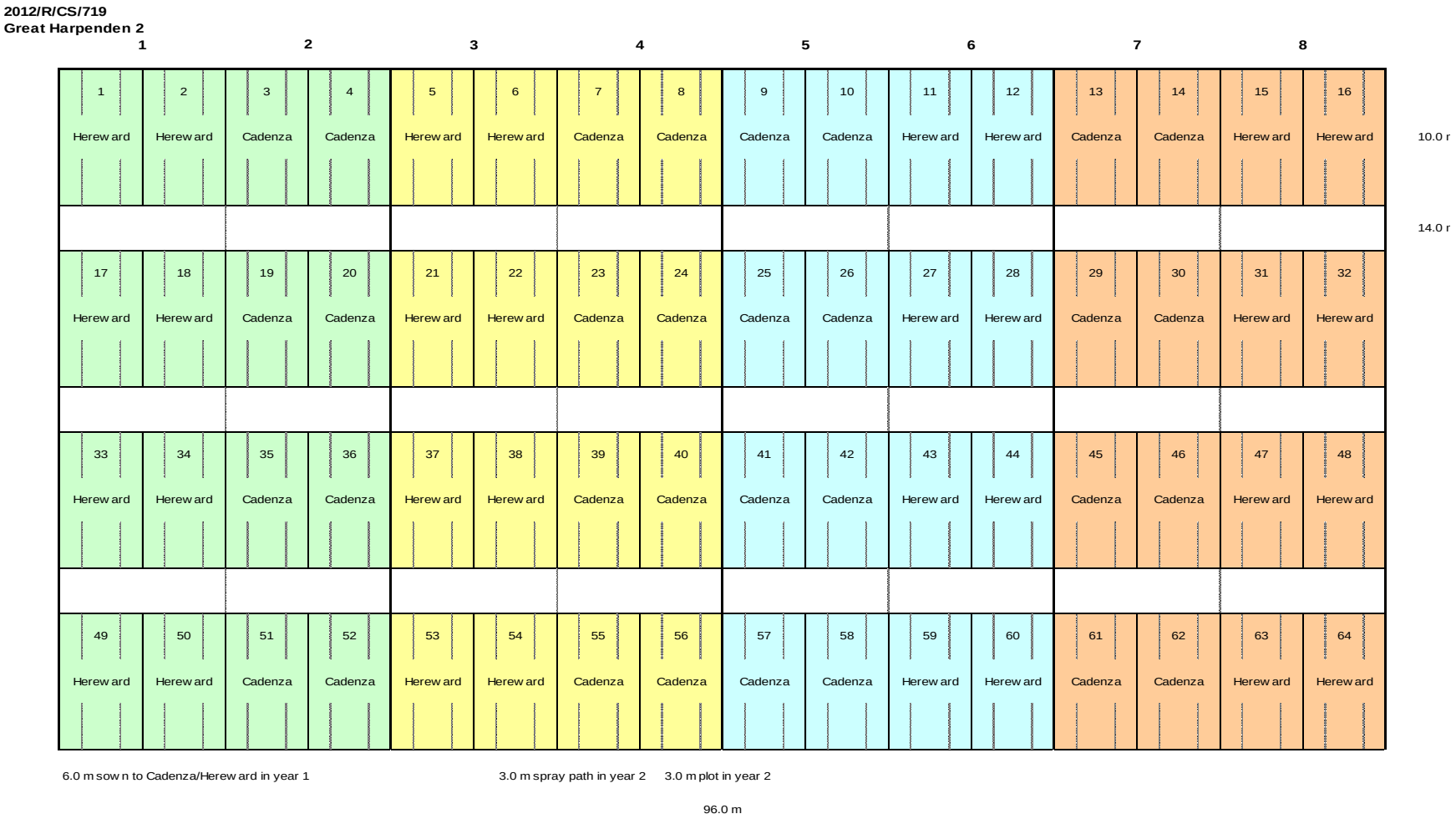



**Figure 3: Field layout of Great Harpenden 2 (GH2) Rothamsted in the first year**. The colours show the four main blocks with two main plots of either Hereward or Cadenza represented by the numbers outside (Source: Dr. Vanessa McMillan, Rothamsted Research).

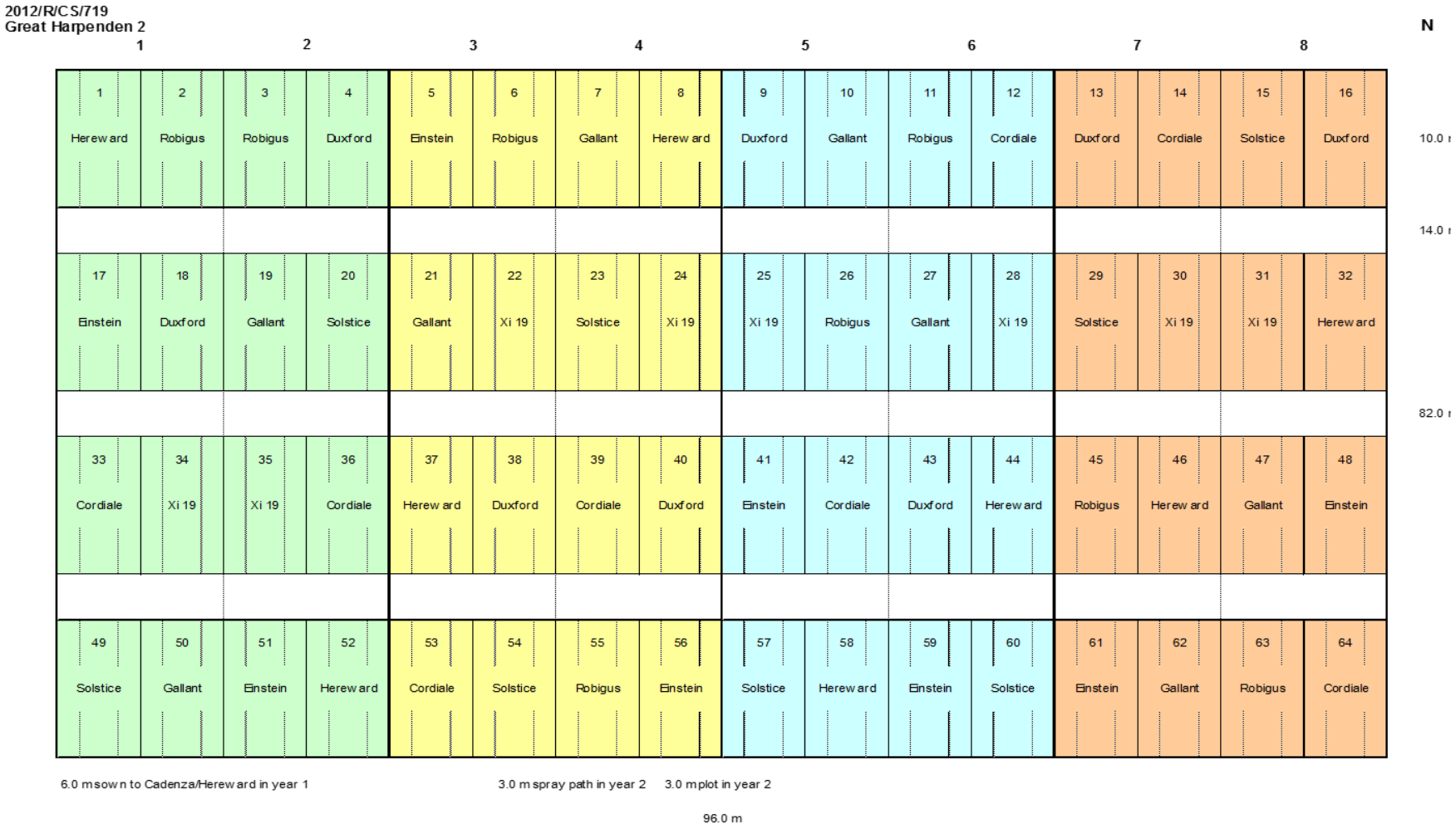



**Figure 4: Field layout of Great Harpenden 2 (GH2) Rothamsted in second year**. The two main plots numbered outside, were divided into two split-plots represented by the numbers inside, over-sown with other wheat cultivars. Only split-plots with second year Hereward and Xi-19 were sampled (Source: Dr. Vanessa McMillan, Rothamsted Research).

### 3.2.2 Bacterial growth and DNA extraction

Methods for bacterial growth and DNA extraction using a GeneJet kit (Thermo Scientific) are described in detail in 2.3. The quantity and quality of eluted DNA was determined using NanoDrop® (ND-1000 UV-Vis Spectrophotometer) (LabTech). Also, the integrity of extracted DNA was checked on 1% Agarose (Bioline) gel in 0.5X TBE buffer (Appendix II9.1.2) stained with gel red (Cambridge Bioscience). The DNA of SBW25 was then used as the template for PCR amplification and later probe synthesis.

### 3.2.3 Primer design and PCR

The genome of SBW25 was used as the model for primer design. Primers were designed using NCBI primer design tool (Table 2). First the sequences of the target genes were identified based on the loci showing significance in Mauchline *et al.* (2015). In total 9 primer pairs (5 for the *wsm* LPS operon components, 2 for viscosin operon components, one for *fecB* and one for *tox*) were synthesized by Eurofins Genomics. Gradient PCR was performed to allocate the optimum annealing temperature for each primer set. For primers MZ-1, MZ-5, MZ-7, MZ-15, and MZ-17 the optimum annealing temperature was 58.7 °C these were grouped as Set A, whereas primers MZ-3, MZ-9, and MZ-11 had an optimum temperature of 61.5 °C and were grouped as Set B. PCR with the primer pair MZ-13, which target the second component of the viscosin operon, resulted in multiple bands so was not analysed further.

A 50 µl PCR reaction was used for amplification and sequencing. For each reaction, 25 µl of 2X Taq mix (PCR Biosystems), 16 µl $nH_2O$, 2 µl of F primer (10 µM), 2 µl of R

primer (10 µM), and 5µl of SBW25 DNA (26.5 ng $\mu l^{-1}$) were used. The PCR amplification was performed using a Senso Quest gene flow thermal cycler and conditions were as follows: initial denaturation at 95 °C for 1 min, 30 cycles of denaturation at 95 °C for 30 seconds, annealing at 58.7 °C or 61.5 °C and extension at 72 °C for 1 min $kb^{-1}$, and a final extension step at 72 °C for 7 min for set A and 10 min for set B.

**Table 2:** Primers for Dot blot synthesis, the locus tags are from (Source: http://www.pseudomonas.com):

| No. | Primer pair | Operon | Set | Locus Tag | Gene (product) | F sequence | R sequence | Product size (bp) |
|---|---|---|---|---|---|---|---|---|
| 1 | MZ-1 | Wsm LPS | A | PFLU_0475 | Putative Carbamoyltransferase | TGACGATTCTTGGCCTGTCC | AGCCACTCGATCAGTTTGGG | 793 |
| 2 | MZ-3 | Wsm LPS | B | PFLU_0476 | Conserved hypothetical protein (*wsm*) | GGCAATGCCGAGATCATCCT | GGCGCTTTGCGTATTGAGAA | 519 |
| 3 | MZ-5 | Wsm LPS | A | PFLU_0477 | Putative deacetylase | GTTGATCCTCGCGATTGTGC | CAGGGTCACGATCCAGCTTT | 511 |
| 4 | MZ-7 | Wsm LPS | A | PFLU_0478 | Putative glycosyl transferase | TTCGAGTTTCCCGAGAAGGC | TCAAGCAAGGAAGGCATGGT | 668 |
| 5 | MZ-9 | Wsm LPS | B | PFLU_0479 | Putative glycosyl transferase | TGATTTCCAGGTGCAGCAGA | ATCGCTGAACTTGGCGTAGG | 523 |
| 6 | MZ-11 | Viscosin | B | PFLU_2553 | Putative non ribosomal peptide synthetase (*viscB*) | ACCGTACCGTGGAAAACCTC | GAATGCGATTAACCGGCACC | 1227 |
| 7 | MZ-13 | Viscosin | Didn't work | PFLU_2552 | Putative non ribosomal peptide synthetase (*viscC*) | GCGATGACGCCCAGGTATTA | ACCGATATACCGCTGCACTG | 1052 |
| 8 | MZ-15 | - | A | PFLU_4091 | Iron dicitrate –binding periplasmic protein (*fecB*) | TCCTGGCGTTCTCTTCAAGC | TCCAGCTGTCAACGATGCTC | 717 |
| 9 | MZ-17 | - | A | PFLU_3831 | Conserved hypothetical protein Toxin HigB-2 **(***tox)* | GAACAGGCGGTTTACGCAAG | TGTTTGCCGTACAGGGTGAA | 133 |

To visualize the PCR products a 1% agarose (Bioline) gel (w/v) with 3 µl Gel red (Cambridge Biosciences) was prepared using 0.5 X TBE buffer. The samples were run for 40 mins at 90 V. Once all PCR products were observed as single bands, these PCR products were cleaned up using QIAquick PCR purification kit following the manufacturer's instructions (Qiagen). Thirty microliters (30 µl) of each product were sent for sequencing to Eurofin genomics (15 µl DNA+ 2 µl F primer (10 µM), and 15 µl + 2 µl R primer (10 µM). The remaining 19 µl was stored at 4˚C for the following probe labelling reaction. Sequence alignment and comparison of sequences with genomic sequences was done using BLAST on NCBI.

### 3.2.4 Probe labelling

The DIG High Prime DNA labelling and detection starter kit II (Roche) was used for DNA labelling, hybridization and detection. The concentration of purified DNA was determined using NanoDrop® (ND-1000 UV-Vis Spectrophotometer) (LabTech) and 400 ng of DNA was required for the labelling process. In a 1.5 ml Eppendorf tube the DNA was added to sterile water ($nH_2O$) to make a final volume of 16 µl. The DNA was denatured by heating at 100 °C on a heating block for 10 mins, and then quickly chilled on ice. Then, 4 µl of well mixed DIG-high prime (Roche) were added to the denatured DNA. The mixture was centrifuged at 13,000 rpm for 10 s (Stuart microfuge I) before being incubated at 37 °C overnight. The labelling reaction was stopped by heating the tubes at 65 °C for 10 mins. Two (2 µl) of the labelled DNA was added to 50 µl sterile $nH_2O$ in a 1.5 ml Eppendorf tube. This mixture was denatured at 100 ˚C for 5 min then quickly chilled on ice. Finally, it was added to 5 ml DIG Easy Hyb buffer (Roche) pre-warmed at 42 ˚C.

Although 8 probes were labelled, the final analysis was carried out using 4 DIG-labelled probes; MZ-3 for *wsm*, MZ-11 for *viscB*, MZ-15 for *fecB*, and MZ-17 for *tox*.

### 3.2.5 Dot blot

#### 3.2.5.1 Bacterial DNA extraction and blot preparation

Overnight bacterial cultures from the -80 °C frozen stocks were grown on KA at 27 ˚C to be used for the screening process. In a 96-well microtiter plate 200 µl of 0.4 M NaOH-10 mM EDTA were loaded. A 48 tip multi blot replicator (70% ethanol dipped and flame sterilized) was used to touch the cells grown overnight and subsequently to inoculate the 96-well plate. For the positive control, SBW25, a sterile tip was used to touch a single bacterial colony to inoculate the plate. The negative control well had no bacterial inoculum. The plates were sealed with autoclave tape and incubated for 10 mins at 60°C in a Hybaid mini oven. Once the incubation was completed the plates were chilled on ice for 5 mins. In a clean tray, sufficient amount of 2X Saline Sodium Citrate (2X SSC, Appendix II 9.1.2) buffer was poured to wet the positively charged nylon membrane (Nylon membrane positively charged, Roche). Meanwhile, the sterile Bio-Rad blotter was un-screwed inside a -class 2 safety cabinet (Labogene). Using sterile forceps, the wetted membrane was transferred to the blotter and placed on to the rubber gasket. The upper part was placed back and the screws were alternatingly tightened. A vacuum was applied (by turning the tap on) and the screws were tightened further. The vacuum was then held and 180 µl of each sample were loaded using a multichannel pipette. The vacuum was then reapplied to draw the samples through. Once all the samples had passed through, the blotter was disassembled, and the nylon membrane was washed briefly in 2X SSC. The membrane was then left to air dry for 30 mins. Once dry, the membrane was wrapped in Saran wrap. Finally, to fix the DNA

to the membrane, the wrapped membrane was exposed to UV light (302 nm) for 2 mins using a UV trans-illuminator with the DNA side facing the UV light source.

### 3.2.5.2 Pre-hybridization and Hybridization reactions

The blot with fixed DNA was placed into a 110 ml glass Hybaid oven tube and 10 ml of DIG Easy Hyb buffer was added to the tube. For the pre-hybridization reaction, the tubes were incubated at 42 °C for up to 3 h inside the Hybaid oven with slow rotation. After this incubation the pre-hybridization buffer was discarded and replaced with a pre-warmed mixture of labelled probe and 5 ml of DIG Easy Hyb buffer. This reaction was incubated overnight at 42 °C with rotation in the Hybaid oven.

### 3.2.5.3 Stringency washes and Detection

Once the hybridization was concluded, the blot was removed from the Hybaid tube and the buffer was stored at -20 ˚C, as it can be reused up to 3 times. The blot was then subjected to a series of stringency washes. First it was placed in a tray with 150 ml of (2X SSC + 0.1% SDS) buffer (low stringency buffer; LSB) for 5 mins. This buffer was discarded, and the wash was repeated with fresh 150 ml of LSB. During this wash, 150 ml of (0.5X SSC+ 0.1%SDS) buffer (high stringency buffer; HSB) was pre-warmed at 65 °C in the oven. The blot was then transferred to a clean Hybaid glass tube where 75 ml of pre-warmed (HSB) was added, and it was left rotating in the Hybaid oven for 30 mins at 65 ˚C. This was repeated once more.

Once the stringency washes were complete the blot was placed in a clean plastic tray where 150 ml of washing buffer (0.1 M maleic acid buffer + 0.3 % Tween 20) was added. The blot was gently shaken for 5 mins at room temperature on a rocker. The

washing buffer was discarded. In the same tray 170 ml of the blocking solution was added to the blot, and left shaking for 1.5 h. After this the solution was discarded. Then 30 ml of antibody solution (750 U/ ml) was added to the blot, and left shaking for 30 mins. The blot was then washed in 150 ml of washing buffer for 30 mins and this was repeated with fresh buffer. Finally, the blot was equilibrated for 10 mins in 30 ml of detection buffer (0.1 M Tris-HCl, 0.1 M NaCl pH 9.5). The blot was then placed in a hybridization bag and 1 ml of disodium 3-(4-methoxyspiro {1,2- dioxetane-3,2'-(5'-chloro) tricycle [3.3.1.1$^{3,7}$] decan }-4-yl) phenyl phosphate (CSPD, Roche) was evenly pipetted on to the DNA side of the blot. The blot was left for 5 mins at room temperature and excess CSPD was drained off. The bag was resealed and incubated at 37°C for 10 mins to activate the CSPD. In a dark room, the blot was exposed to X-ray film in an X-ray cassette for 10 min with the DNA side facing the film. The film was then placed in a tray with developer (Carestream DENTAL). Once the dots appeared completely, the film was briefly washed in a second tray with water and then transferred to a third tray with the fixer (Carestream DENTAL). Once again, the film was briefly washed in fresh water and hung to air dry. Once dry the film was scanned using an Epson scanner connected to a computer (Epson Perfection V300 Photo) and the image was stored. Results were recorded using a scoring system based on spot intensity (Table 3). Alternatively, the blot was also visualised directly using the gel documentation system G:BOX (Syngene) using the blot option and CSPD as the detection system. The images were saved as JPG files. List of chemicals is in provided in Appendix II 9.1.2.

**Table 3:** The scoring system for Dot blot positive signals.

| Score | Observation |
|---|---|
| 0 | Negative control signal |
| 1 | Faint signal |
| 2 | Moderate signal |
| 3 | Strong signal |
| 4 | Positive control signal |

### 3.2.6 Dot blot confirmation via PCR

#### 3.2.6.1 Degenerate primers

The nucleotide sequences of the four loci (*wsm, viscB, fecB,* and *tox*) from *P. fluorescens* SBW25 were aligned against the 20-sequenced library from the same pool of isolates ("Jake. Available:https//streptomyces.org.uk/customers/jake/. [Accessed: 9 March 2019].") (http://sterptomyces.org.uk/cgi-bin/customers/jake/) (Mauchline *et al.*, 2015) using Mega (MEGA 7).

Two degenerate primers for *wsm*, and *fecB* were successfully designed (Table 4). The other two loci were highly variable, with a great mismatch. Thus, no further analysis was carried out.

#### 3.2.6.2 DNA template preparation

For DNA template preparation, 1 ml of overnight culture on (KB) incubated in a shaker at 27 °C was transferred into an Eppendorf tube. These tubes were centrifuged at 13,000 rpm for 3 mins (Microfuge, SCF2, Stuart). The supernatant was discarded and the pellets were re-suspended in 200 µl of TE buffer (1 M Tris HCl - 0.5 M EDTA pH 8). After vortexing, the cell suspension was heated at 100 °C for 10 mins using a heating block (Heat block, VWR Scientific). The tubes were then chilled on ice for 10 mins, followed by a second centrifugation (13,000 rpm for 3 mins). Carefully, the

supernatant was transferred to new clean tubes. This template DNA was either used immediately in a PCR reaction or stored at -20 °C for later use. Gradient PCR using SBW25 DNA was used to determine the optimum annealing temperatures for the degenerate primers.

### 3.2.6.3 PCR amplification

A 20 µl PCR reaction was carried out using 2 µl of the previous template DNA along with 10 µl 2X PCRBIO Taq Mix Red (PCR Biosystems), 6.4 µl $nH_2O$, 0.8 µl of each F and R primers (10 µM). The conditions for the PCR, 95 °C for (3 mins for *wsm*, 5 mins for *fecB*), followed by 30 cycles of denaturation at 95 °C for 30 s, annealing (63°C for *wsm* and 70 °C for *fecB*) for 30 s, extension at 72 °C for 30 s, and final extension at 72 °C for (7 mins *wsm*, 5 mins *fecB*). DNA template of SBW25 was used as the positive control, and $nH_2O$ as the negative control.

The *wsm* PCRs were carried out using T100 thermal cycler (BioRad), while the *fecB* PCRs were carried out using Prime$^3$ thermal cycler (Techne).

**Table 4:** Degenerate primers for *wsm* and *fecB*. (* just a label to differentiate from a non-successful *fecB* primer pair).

| No. | Loci | F primer | R primer | Tm (°C) | Product size (bp) |
|---|---|---|---|---|---|
| 1 | *wsm* | *wsm* F (GGCAAYGCCGARHTSATCC) | *wsm* R (GCACCARCGSTCYTTRTAYTCRCGGTC) | 63 | 603 |
| 2 | *fecB* | *fecB* F* (TGATCGTSGCCGACCTCAAYCG) | *fecB* R* (CCACARCGGCTGCTTGCTCCAG) | 70 | 455 |

### 3.2.7 Data analysis

Statistical analysis was done using the GenStat statistical package (2015, 18th edition, © VSN International, Hemel Hempstead, UK).

## 3.3 Results

### 3.3.1 Gene selection and probe synthesis

Rhizosphere fitness relies on genes involved in nutrient acquisition, host recognition, colonization, motility, and antagonism (Rainey, 1999). Mauchline *et al.* (2015), have found a strong positive correlation between the *Streptomyces* growth inhibiting phenotypes and genes involved in the biosynthesis of viscosin surfactant and *wsm* LPS operons. In addition, they focussed on genes for iron acquisition and toxin production. From their work it was hypothesised that a wider range of rhizosphere fitness loci will be present in the bacterial isolates taken from the plots. However, it is unknown which loci might be under selection for the different cultivar combinations. Therefore, the 411 isolates were screened for a range of different loci to determine if any correlations exist that might indicate specific loci under ecological selection. Genes coding for *wsm* LPS, viscosin, iron binding and toxin genes were searched for in the genome of *P. fluorescens* SBW25 (Fig. 5-8). The NCBI primer synthesis tool was used to synthesize the primers. Since the *wsm* LPS and viscosin operons had multiple genes (5 and 2 respectively), resulting in 9 primer pairs (one for each gene). In the process of identifying the optimum annealing temperature the *viscC* (MZ-13 primer pair) gave multiple bands unlike the others so was excluded from further work (Fig.9). Once single band were obtained (Fig. 10 and 11), these PCR products were then sent for sequencing (Eurofin genomics). Sequence alignment and comparison of the data on NCBI showed 99-100% sequence identity.

In addition, the dot blot screening was narrowed down by using one probe for each locus MZ-3 for *wsm*, MZ-11 for *viscB*, MZ-15 for *fecB* and MZ-17 for *tox* (Appendix I 9.1.1). MZ-3 was chosen out of the five *wsm* LPS operon components because it was giving consistent results in comparison to the other genes when initially tested. While for viscosin operon *viscB* primer pair gave specific binding than *viscC*.

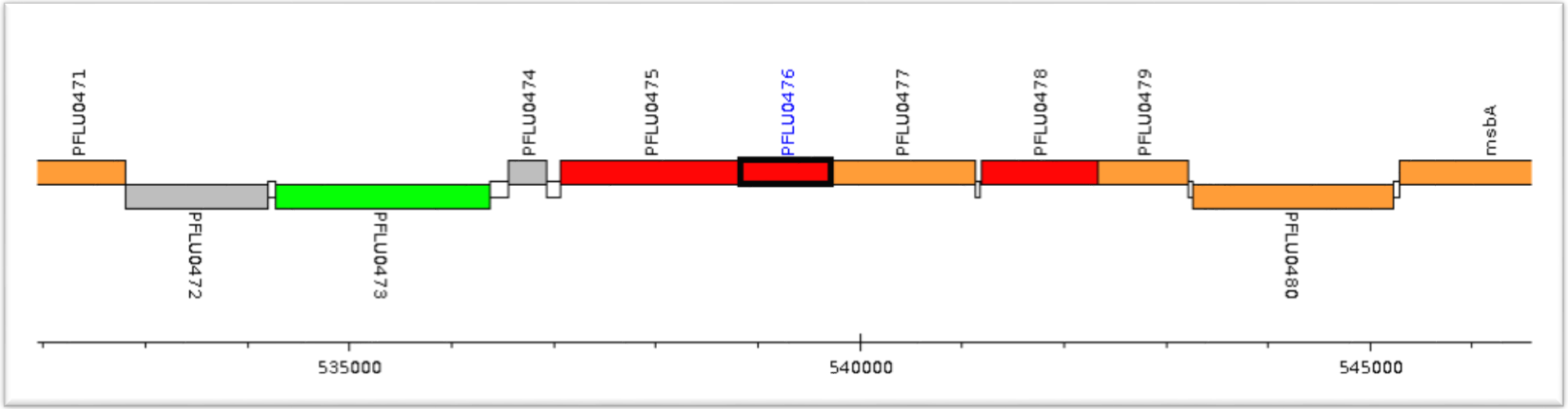



**Figure 5: The *wsm* LPS operon genes** PFLU_0476 SBW25 hypothetical protein (*wsm*). Red: cytoplasmic; Orange: cytoplasmic membrane; Green: outermembrane; Gray: unknown(Source: (Winsor *et al.*, 2016) http://www.pseudomonas.com).

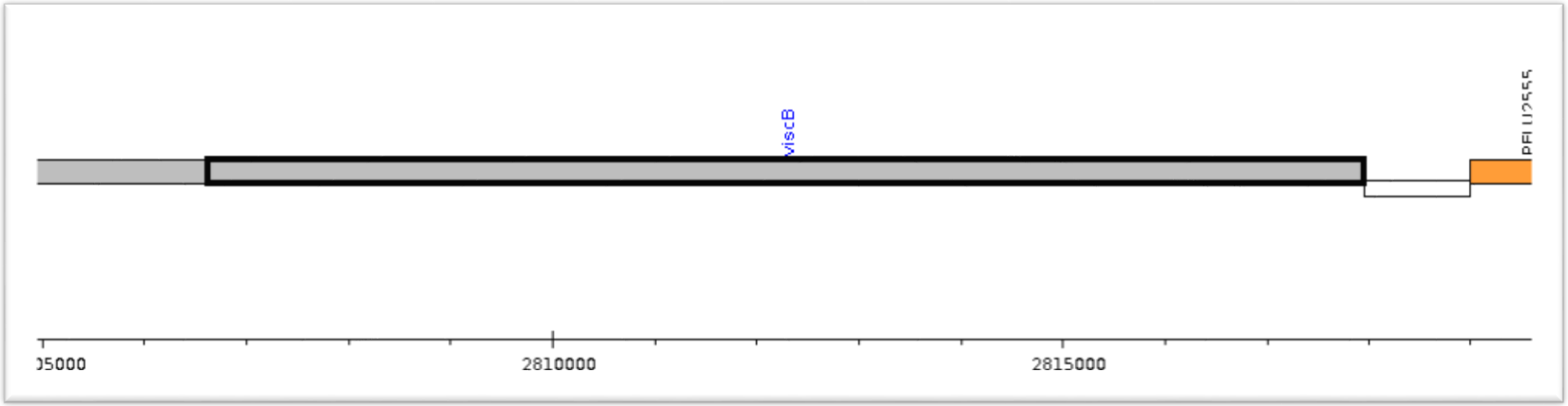



**Figure 6: The Viscosin operon** PFLU_2553 SBW25 *viscB* putative non-ribosomal peptide synthetase. Orange: cytoplasmic; Gray: unknown (Source: (Winsor *et al.*, 2016) http://www.pseudomonas.com).

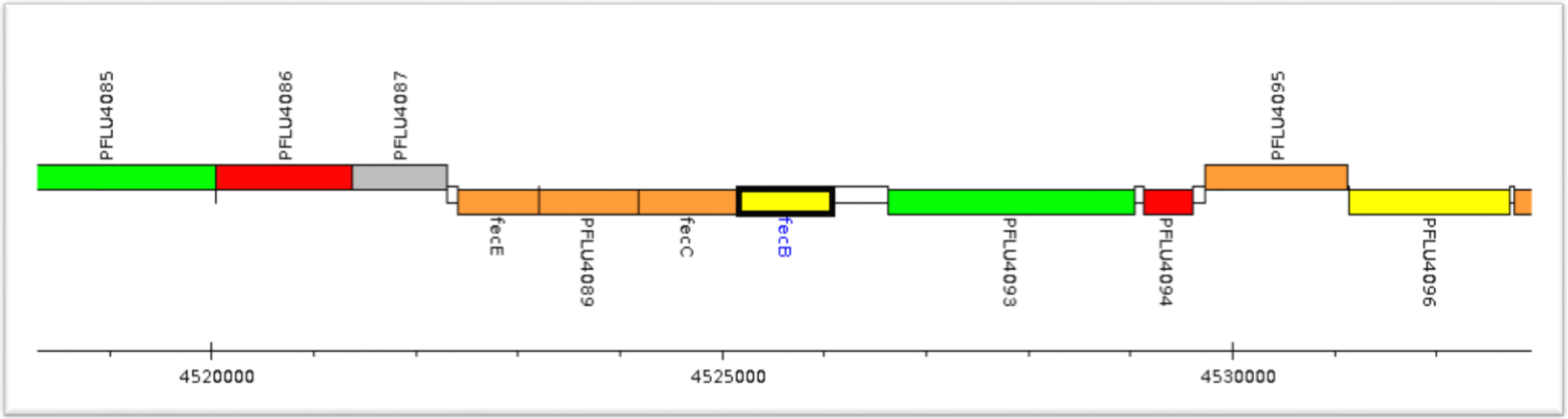



**Figure 7**: **The iron siderophore binding protein** PFLU_4091 SBW25 *fecB* iron-dicitrate transporter substrate-binding subunit. Red: cytoplasmic; Orange: cytoplasmic membrane; Green: outermembrane;Yellow: periplasmic; Gray: unknown (Source: (Winsor *et al.*, 2016) http://www.pseudomonas.com).

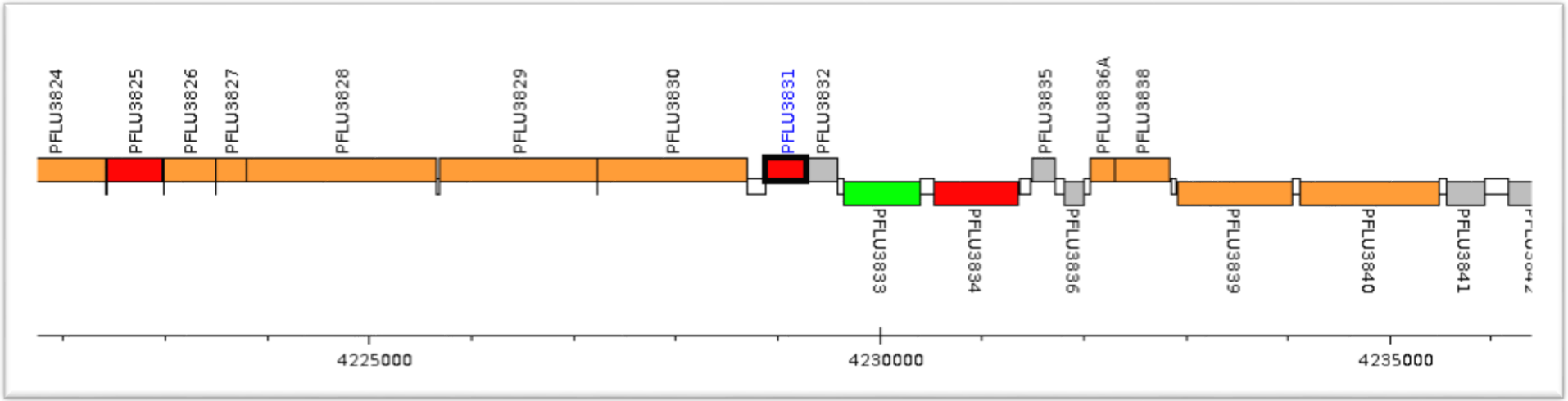



**Figure 8: The Toxin gene** PFLU_3831 gene of SBW25 Toxin HigB-2. Red: cytoplasmic; Orange: cytoplasmic membrane; Green: outermembrane; Gray: unknown (Source: (Winsor *et al.*, 2016) http://www.pseudomonas.com).

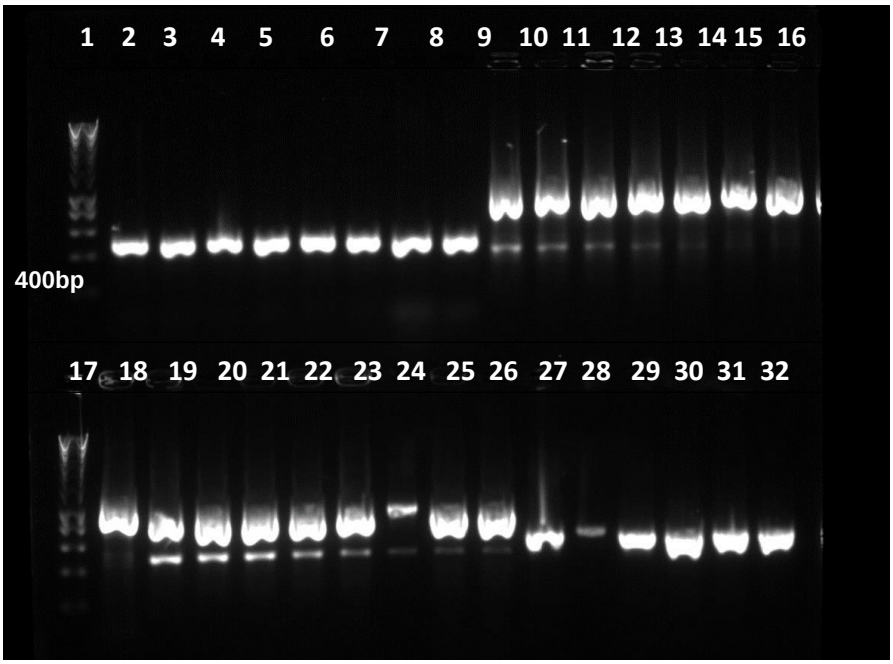



**Figure 9: Example of a gradient PCR for primer sets MZ-3, MZ-11 and MZ-15.** Well: 1 and 17 is the ladder (Bioline Hyper Ladder 1Kb); wells 2-8 PCR products of primer MZ-3 (519 bp); wells 9-16 and 18 PCR products of primer MZ-11 (1227 bp); wells 19-26 are PCR products of primer MZ-13 (1052 bp) and wells 27-32 are PCR products of primer MZ-15 (717 bp) (the last two temperatures were not loaded for this amplification), at temperatures are 54.9°C, 56.3°C, 57.7˚C, 59.1˚C, 60.3˚C, 61.5˚C, 62.4˚C, and 63.0˚C left to right; respectively.

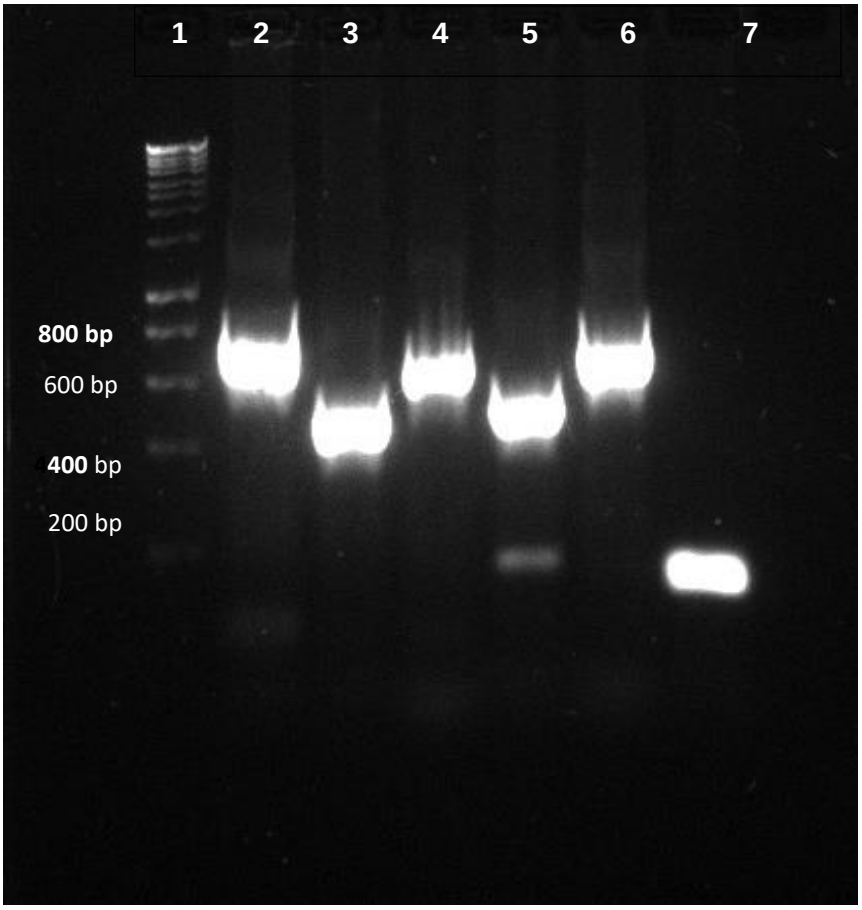



**Figure 10: DNA amplicons (probes for Dot blots) derived from PCR products at 58.7 °C.** Well: **1**, ladder (Bioline Hyper ladder 1Kb); 2, MZ-1 (*wsm* LPS) (793 bp); 3, MZ-5 (*wsm* LPS) (511 bp); 4, MZ-7 (*wsm* LPS) (668 bp); 5, MZ-9 (*wsm* LPS) (523 bp); 6, MZ-15 (*fecB*) (717 bp); 7, MZ-17 (*tox*) (133 bp).

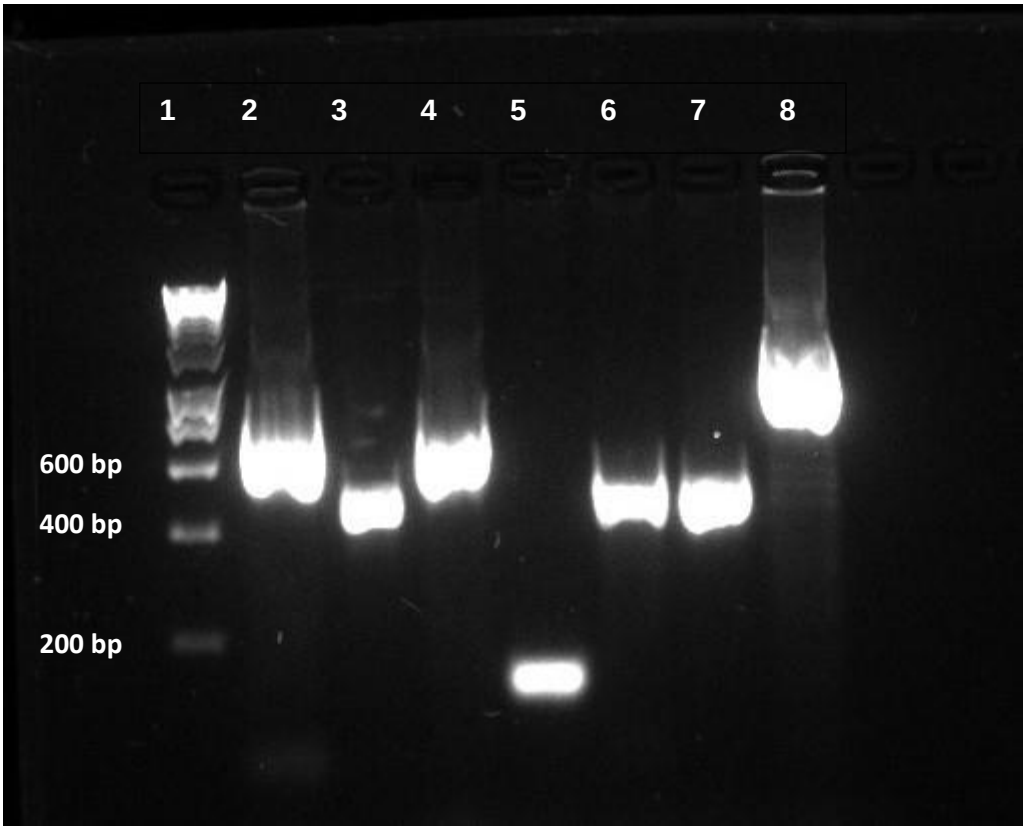



**Figure 11: DNA amplicons (probes for Dot blots) derived from Set A and set B PCR products.** Well: **1**, ladder (Bioline Hyper ladder 1Kb); **2**, MZ-1(*wsm LPS*) (793 bp); **3** MZ- 5 (*wsm LPS*) (511 bp); **4** MZ- 7 (*wsm LPS*) (668 bp); **5** MZ-17 (*tox*) (133 bp); **6** MZ-3 (*wsm LPS*) (519 bp); **7** MZ- 9 (*wsm LPS*) (523 bp); and **8** MZ- 11 (*viscB*) (1227 bp).

With the selection of the genes to be searched for in the 411 strains and optimisation of the PCR and dot blot conditions, the next step was to screen all the *Pseudomonas* spp. isolates to determine whether there are any significant correlations between genotype and wheat cultivar (Fig. 12). In an excel sheet the scores 3 and 4 were counted as 1 (gene is present), while 0, 1 and 2 were counted as zero (gene not present) (Appendix 9.1.6).

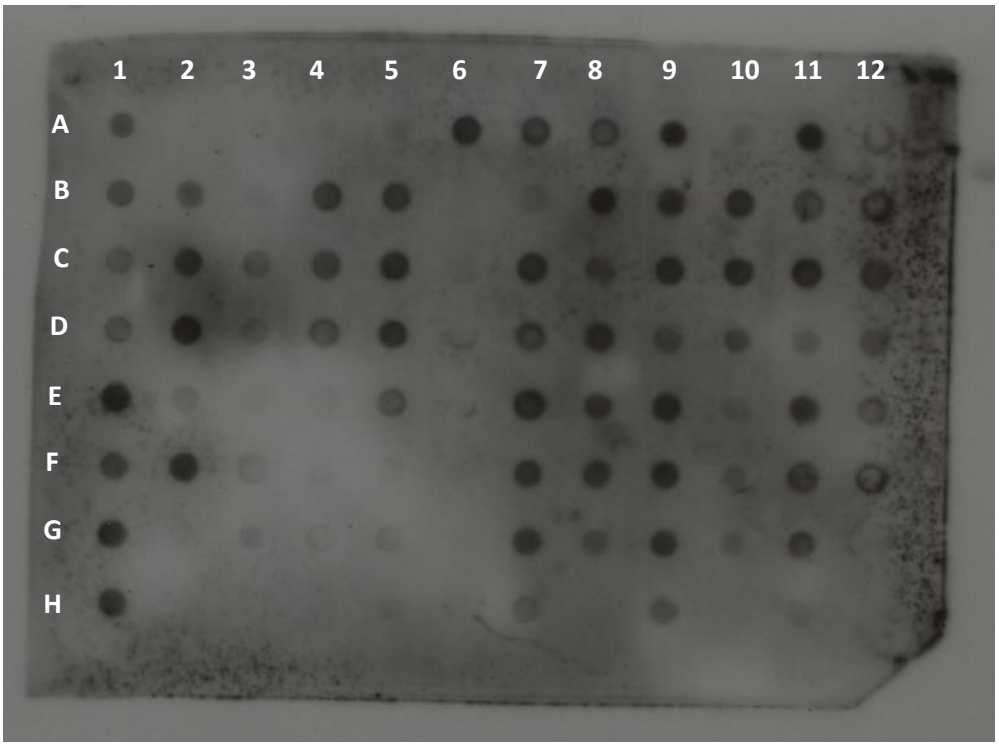



**Figure 12: Examples of Dot blot of 78 isolates from the 411 isolate collection**. The isolates are from plots 30R, 31R, 31E, 34R, 34E, and 35E. R: Rhizosphere, E: Endosphere. 6A is the positive control (SBW25). 12H is the negative control (non-bacterial). Numbers 31 and 34 refers to (H, Xi-19) planting combination. Numbers 30 and 35 refers to (C, Xi-19) planting combination. The blot was probed with (*wsm*) gene MZ-3 probe.

### 3.3.2 Dot blot screening for rhizosphere fitness loci

From Great Harpenden 2 field (GH2), four plots were sampled for each of the four main blocks, resulting in 16 sampled plots. These 16 plots covered the following planting schemes (H, H), (C, H), (H, Xi-19), (C, Xi-19). A total of 411 *Pseudomonas* isolates were selectively isolated from the rhizosphere (R) and endosphere (E) of year two wheat. The distribution of the isolates from the different plots was as follows: 84 from (H, H) plots, 102 from (C, H) plots, 108 from (H, Xi-19) plots, and 117 from (C, Xi-19) plots.

A total of 251/411 were successfully screened for the presence or absence of the four genes; *wsm*, *viscB*, *fecB*, and *tox* via dot blot. Where 108 isolates were from the (H, Xi-19), 102 isolates were from (C, Xi-19) planting scheme, 26 isolates from (H, H) planting scheme, and 15 isolates were from (C, H) planting scheme.

Since the 26 isolates of (H, H) and the 15 isolates of (C, H) were both obtained from single plots, they were not included in the analysis (Raw data in Appendix III 9.1.3).

Within the (H, Xi-19) screened isolates, 74/108 harboured either one, two, or three out of the four tested loci; no strains gave a signal for all four loci. The abundance of the four genes among these positive isolates is shown in Fig.13. Screening the 102 (C, Xi-19) isolates revealed 80 positive isolates having one, two, or three of the tested gene loci. Only one isolate was observed to have a signal for all four genes (Fig.14).

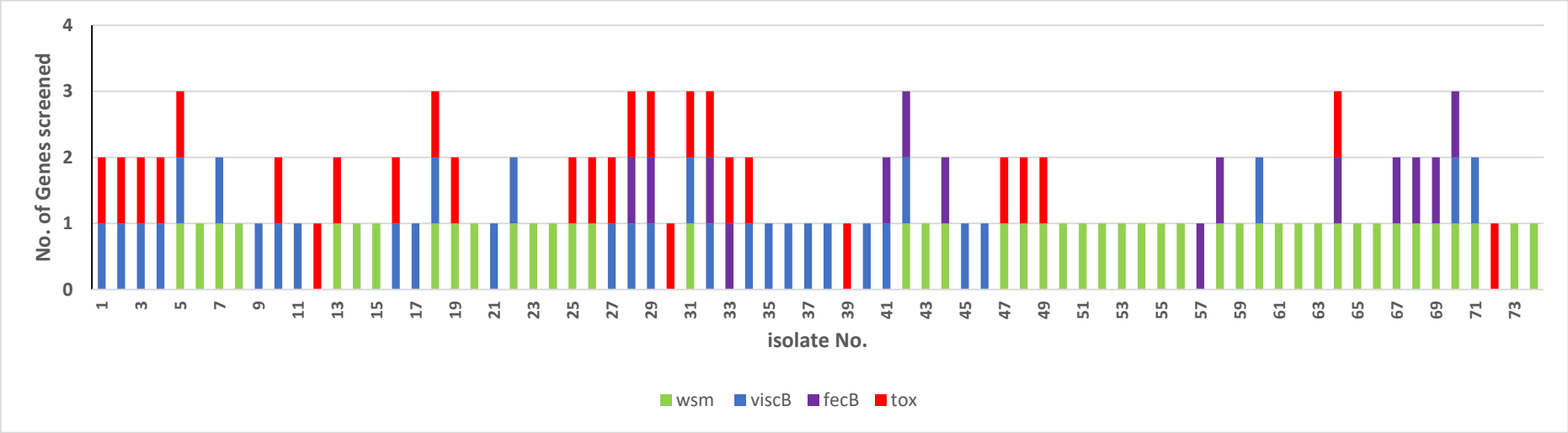



**Figure 13: The distribution of the four screened genes among the 74/108 positive isolates from (H, Xi-19) planting scheme.** The numbers on the Y axis refers to number of Gene 1 to 4. The colours represent (green) *wsm* (PFLU_RS02355), (blue) *viscB* (PFLU_RS12480), (purple) *fecB* (PFLU_RS19995), and (red) *tox* (PFLU_RS18680). None of the isolates had the four genes. H: Hereward, and Xi-19. Appendix IV shows Isolate number key.

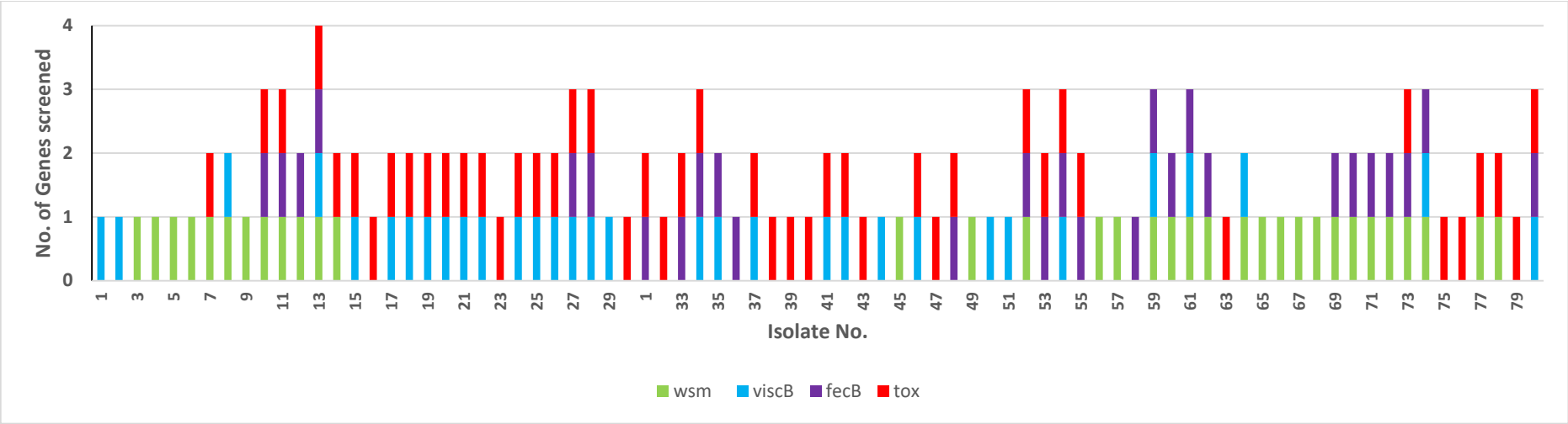



**Figure 14: The distribution of the four screened genes among the 80/102 positive isolates from (C, Xi-19) planting scheme.**The numbers on the Y axis refers to number of Gene 1 to 4. The colours represent (green) *wsm* (PFLU_RS02355), (blue) *viscB* (PFLU_RS12480), (purple) *fecB* (PFLU_RS19995), and (red) *tox* (PFLU_RS18680). Only one isolate, 25R/12,had all the four genes. C: Cadenza, and Xi-19. Appendix IV shows Isolate number key.

### 3.3.3 Statistical analysis of the rhizosphere fitness loci data

First using the raw data of Dot blot outcomes, the proportions of success for the presence of gene signal were calculated. That is the outcomes from the plots of the same planting schemes were averaged (Appendix IV 9.1.4). Analysis of variance (ANOVA) was performed on proportion data of the gene presence within Hereward rhizosphere (HR), Hereward endosphere (HE), Cadenza rhizosphere (CR), and Cadenza endosphere (CE) for the 251 *Pseudomonas* isolates for the four tested loci. The fitted model Treatment*Niche with block as Block/plot/niche (Tables 5- 8). For the *wsm* locus there was a significant difference between the treatments Hereward and Cadenza and the niches Rhizosphere and Endosphere (p= 0.042, d.f =1). This locus was more abundant among the Hereward isolates. In terms of *viscB* locus there was no significant difference between the treatments and the niches (p= 0.37, d.f =1). The *viscB* locus is thought to aid in colonization. The difference between the treatments Hereward and Cadenza was approaching significance (p= 0.067, d.f =1) for the *fecB* locus. This locus is involved in iron acquisition through the ferric citrate system. In terms of the *tox* locus there was a significant difference between the treatments Hereward and Cadenza (p= 0.026, d.f =1) and the niches Rhizosphere and Endosphere (p= 0.034, d.f =1).

**Table 5:** ANOVA summary for *wsm* locus proportion data. Block: main block of year 1 with Hereward or Cadenza; Plot: sampled plots of year 2 Hereward or Xi-19; Niche: endosphere or rhizosphere; Treatment: (year 1, year 2) planting combination; d.f: degrees of freedom; F pr.: F probability.

| **Source of variation** | **d.f.** | **(m.v.)** | **s.s.** | **m.s.** | **v.r.** | **F pr.** |
|---|---|---|---|---|---|---|
| block stratum | 3 | | 1.0074 | 0.3358 | 1.72 | |
| block.plot stratum | | | | | | |
| niche | 1 | | 0.0761 | 0.0761 | 0.39 | 0.596 |
| treatment | 1 | | 0.001 | 0.001 | 0 | 0.951 |
| Residual | 2 | -1 | 0.3902 | 0.1951 | 5.44 | |
| block.plot.niche stratum | | | | | | |
| niche | 1 | | 0.0355 | 0.0355 | 0.99 | 0.365 |
| niche.treatment | 1 | | 0.2633 | 0.2633 | 7.35 | 0.042 |
| Residual | 5 | | 0.1792 | 0.0358 | | |
| Total | 14 | -1 | 1.5614 | | | |

**Table 6:** ANOVA summary for *viscB* locus proportion data. Block: main block of year 1 with Hereward or Cadenza; Plot: sampled plots of year 2 Hereward or Xi-19; Niche: endosphere or rhizosphere; Treatment: (year 1, year 2) planting combination; d.f: degrees of freedom; F pr.: F probability.

| **Source of variation** | **d.f.** | **(m.v.)** | **s.s.** | **m.s.** | **v.r.** | **F pr.** |
|---|---|---|---|---|---|---|
| block stratum | 3 | | 0.2067 | 0.0689 | 6.13 | |
| block.plot stratum | | | | | | |
| niche | 1 | | 0 | 0 | 0 | 0.99 |
| treatment | 1 | | 0.0011 | 0.0011 | 0.1 | 0.786 |
| Residual | 2 | -1 | 0.0225 | 0.0112 | 0.29 | |
| block.plot.niche stratum | | | | | | |
| niche | 1 | | 0.0207 | 0.0207 | 0.53 | 0.498 |
| niche.treatment | 1 | | 0.0376 | 0.0376 | 0.97 | 0.37 |
| Residual | 5 | | 0.1939 | 0.0388 | | |
| Total | 14 | -1 | 0.4605 | | | |

**Table 7:** ANOVA summary for *fecB* locus proportion data. Block: main block of year 1 with Hereward or Cadenza; Plot: sampled plots of year 2 Hereward or Xi-19; Niche: endosphere or rhizosphere; Treatment: (year 1, year 2) planting combination; d.f: degrees of freedom; F pr.: F probability.

| **Source of variation** | **d.f.** | **(m.v.)** | **s.s.** | **m.s.** | **v.r.** | **F pr.** |
|---|---|---|---|---|---|---|
| block stratum | 3 | | 0.1999 | 0.0666 | 4.57 | |
| block.plot stratum | | | | | | |
| treatment | 1 | | 0.1163 | 0.1163 | 7.98 | 0.067 |
| Residual | 3 | | 0.0438 | 0.0146 | 0.5 | |
| block.plot.niche stratum | | | | | | |
| niche | 1 | | 0.0077 | 0.0077 | 0.26 | 0.632 |
| niche.treatment | 1 | | 0.0135 | 0.0135 | 0.46 | 0.529 |
| Residual | 5 | -1 | 0.1472 | 0.0295 | | |
| Total | 14 | -1 | 0.4357 | | | |

**Table 8:** ANOVA summary for *tox* locus proportion data. Block: main block of year 1 with Hereward or Cadenza; Plot: sampled plots of year 2 Hereward or Xi-19; Niche: endosphere or rhizosphere; Treatment: (year 1, year 2) planting combination; d.f: dgrees of freedom; F pr.: F probability.

| **Source of variation** | **d.f.** | **(m.v.)** | **s.s.** | **m.s.** | **v.r.** | **F pr.** |
|---|---|---|---|---|---|---|
| block stratum | 3 | | 0.2152 | 0.0717 | 11.54 | |
| block.plot stratum | | | | | | |
| treatment | 1 | | 0.1065 | 0.1065 | 17.13 | 0.026 |
| Residual | 3 | | 0.0187 | 0.0062 | 0.19 | |
| block.plot.niche stratum | | | | | | |
| niche | 1 | | 0.2742 | 0.2742 | 8.33 | 0.034 |
| niche.treatment | 1 | | 0.0093 | 0.0093 | 0.28 | 0.619 |
| Residual | 5 | -1 | 0.1647 | 0.0329 | | |
| Total | 14 | -1 | 0.7651 | | | |

Figure 15, compares the abundance of the screened loci between (H, Xi-19) and (C, Xi-19) planting schemes based on proportions of positive isolates from (H, Xi-19) and (C, Xi-19) rhizosphere and endosphere niches.

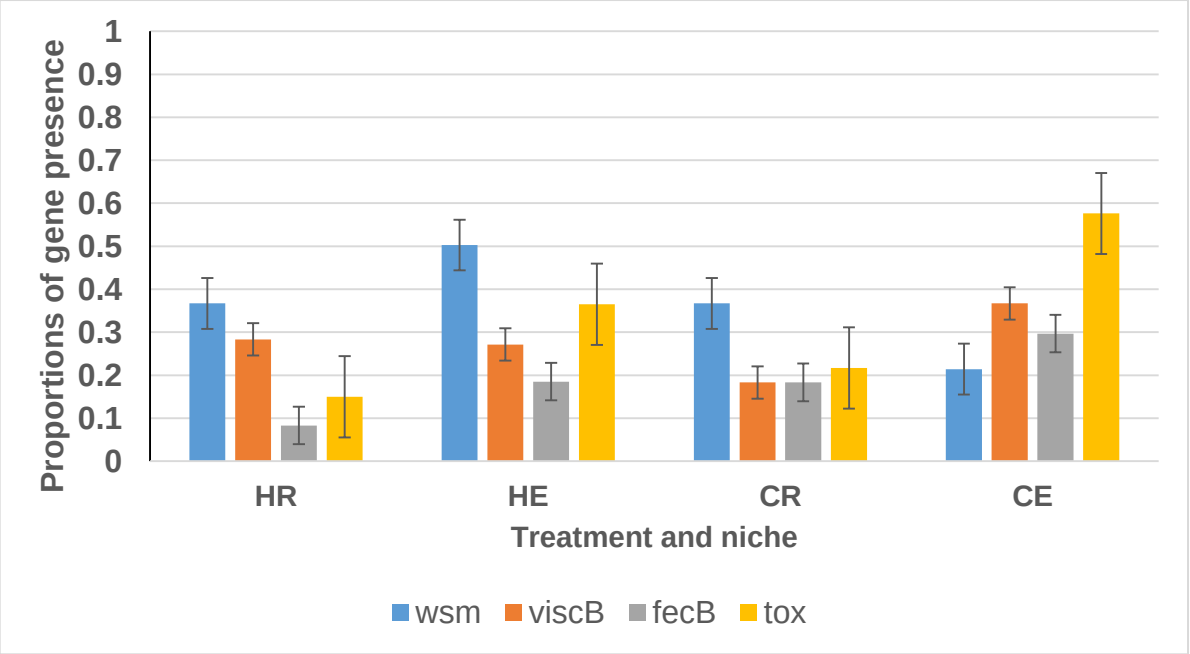



**Figure 15: Proportion of positive isolates for the tested genes (*wsm, viscB, fecB* and *tox*) from the rhizosphere and endosphere *Pseudomonas* isolates of *T. aestivum* cvs (H, Xi-19) and (C, Xi-19) planting schemes**. H: Hereward (Year1), C: Cadenza (Year1), R: Rhizosphere, E: Endosphere. The error bars are based on standard errors. For *wsm* (s.e.d= 0.2209) (d.f= 2), *viscB* (s.e.d= 0.053) (d.f= 2), *fecB* (s.e.d= 0.0989) (d.f= 7.94), and *tox* (s.e.d= 0.0989) (d.f= 6.67). The proportion for each locus were calculated as the number of success out of the total number of isolates for a given niche (E or R).

### 3.3.4 Dot blot vs. PCR

Given the large number of isolates (411), the Dot blot method was initially chosen as a cheap and fast method to screen the isolates. However, in practice it is a very time consuming and needs lots of optimization. Dealing with environmental samples is yet another challenge for probe design and procedure trouble shooting. Moreover, the isolates were screened for all 5 loci of the *wsm* LPS operon before choosing probe MZ-3. This probe was chosen due to consistent performance. Also in the beginning

the traditional method involving X-ray film was used which is more tedious than developing the signal in the G:Box which was subsequently found to be a much better alternative. Another issue with Dot blot was the background, which involved the preparation of alternative homemade recipe of Denhardts blocking solution (Appendix II 9.1.2). In many cases the process was repeated more than once to finally get the 251 isolates done. As such, the PCR method was used alternatively, the first step was to design degenerate primers, which were made for *wsm* and *fecB*. Thus, these two loci were the focuc of PCR screening. The second challenge was to get the template DNA for the 411 isolates. - Colony PCR didn't work and thus was replaced by a quick DNA template preparation method using TE buffer as described in section 3.2.6.2 which is a cheaper method than kit DNA extraction and a cleaner version of colony PCR. Once the DNA templates were prepared and the amplification conditions were optimized, the PCR screening proved to be an efficient and fast screening assay for the number of isolates in hand. Thus all isolates were screened for the presence or absence of *wsm* and *fecB* loci via PCR.

### 3.3.4.1 Comparing the outcomes of the Dot blot and PCR screens

To compare the match between the dot blot and the PCR outcomes for *wsm* and *fecB* loci of 251 isolates. Using a Binomial test for *wsm* and *fecB* loci separately, the proportion of matches between PCR and dot blot methods over all the isolates was significantly different ($p < 0.001$, Binomial test, $n=251$) from 0.75, as a benchmark proportion chosen (Table 9), and therefore also from 0.95 as a statistical requirement for the two methods to be the same. The two methods gave statistically significantly ($p < 0.001$, Binomial test, $n=251$) different proportions of presence of *wsm* and *fecB* genes (Table 10).

**Table 9:** One-sample Binomial test summary for success in match between Dot blot and PCR screening methods.

| **Sample size** | **Gene** | **Match success** | **Proportion of match** | **Bench mark proportion** | **Probability** |
|---|---|---|---|---|---|
| 251 | *wsm* | 108 | 0.430 | 0.75 | <.001 |
| 251 | *fecB* | 121 | 0.482 | 0.75 | <.001 |

**Table 10:** Two-sample Binomial test summary for proportions of gene presence comparing Dot blot to PCR, *n* = 251.

| **Gene** | **Dot blot signal** | **Dot blot proportion of gene presence** | **PCR signal** | **PCR proportion of gene presence** | **Difference in proportions** | **s.e.d** | **Probability** |
|---|---|---|---|---|---|---|---|
| *wsm* | 91 | 0.363 | 220 | 0.876 | 0.513 | 0.03 | < 0.001 |
| *fecB* | 45 | 0.179 | 159 | 0.633 | 0.454 | 0.03 | < 0.001 |

When the outcomes of both methods matched, i.e. both methods identified the presence or absence of test loci for an isolate, there was no problem. However, the proportion of outcomes of presence was much greater with PCR than with Dot blot (Table 10). This is thought to be due to the higher sensitivity of PCR compared to dot blot. The outcomes of presence for PCR but absence for dot blot might be explained by the biased judgement of spot intensity, as spots scoring 1 and 2 were taken as zeros for the final scoring in dot blot. The outcomes of presence for blot and absence for PCR might be due to the presence of PCR inhibitors (soluble fractions of the cell), as the DNA template was prepared using TE buffer protocol.

### 3.3.4.2 Statistical analysis of data to identify correlations in planting scheme, niche and bacterial genotypes.

To test for the main effects and interactions between the factors of wheat cultivar grown in the first year (Hereward or Cadenza), wheat cultivar grown in the second year (Hereward or Xi-19), and niche (endosphere or rhizosphere), the analysis was carried out at two steps. Firstly, Analysis of Variance (ANOVA) was performed on the isolate counts, the fitted model was Year1*Year2*Niche and the blocking structure Block/Main/Split/SplitSplit. The main effect of varieties grown in the first year of the experiment was significant on the abundance of isolates ($p = 0.046$) with greater abundance seen for Cadenza (means: Cadenza 13.75, Hereward 12.00 isolates per plot; s.e.d = 0.530 on d.f. = 3) (Table 11-13).

**Table 11:** ANOVA summary for PCR outcomes. Block: main block of year 1 with Hereward or Cadenza; Plot: sampled plots of year 2 Hereward or Xi-19; Niche: endosphere or rhizosphere; Treatment: (year 1, year 2) planting combination; d.f: degrees of freedom; F pr.: F probability.

| **Source of variation** | **d.f.** | **s.s.** | **m.s.** | **v.r.** | **F pr.** |
|---|---|---|---|---|---|
| Block stratum | 3 | 81.25 | 27.08 | 12.04 | |
| Block.Main stratum | | | | | |
| Year1 | 1 | 24.5 | 24.5 | 10.89 | 0.046 |
| Residual | 3 | 6.75 | 2.25 | 0.19 | |
| Block.Main.Split stratum | | | | | |
| Year2 | 1 | 40.5 | 40.5 | 3.42 | 0.114 |
| Year1.Year2 | 1 | 4.5 | 4.5 | 0.38 | 0.56 |
| Residual | 6 | 71 | 11.83 | 0.58 | |
| Block.Main.Split.SplitSplit stratum | | | | | |
| Niche | 1 | 6.12 | 6.12 | 0.3 | 0.594 |
| Year1.Niche | 1 | 6.12 | 6.12 | 0.3 | 0.594 |
| Year2.Niche | 1 | 6.12 | 6.12 | 0.3 | 0.594 |
| Year1.Year2.Niche | 1 | 1.12 | 1.12 | 0.05 | 0.819 |
| Residual | 12 | 245.5 | 20.46 | | |
| Total | 31 | 493.5 | | | |

**Table 12:** ANOVA table of means.

| **Year1** | **C** | **H** |
|---|---|---|
| | 13.75 | 12 |
| **Year2** | **H** | **Xi** |
| | 11.75 | 14 |
| **Niche** | **E** | **R** |
| | 12.44 | 13.31 |

**Table 13:** Standard error of differences, s.e.d in mean.

| **Table** | **Year1** | **Year2** | **Niche** | **Year1** |
|---|---|---|---|---|
| **rep.** | 16 | 16 | 16 | 8 |
| **s.e.d.** | 0.53 | 1.216 | 1.599 | 1.327 |
| **d.f.** | 3 | 6 | 12 | 7.93 |

Secondly, a generalized liner model (GLM) was fitted to PCR response data for each gene assuming a Binomial distribution for the proportion of gene presence and using a logit link function for the model. The fitted model was Block+ (Year1*Year2*Niche). There was no evidence of over-dispersion for the data given this model for either of the two genes. The outcomes of each locus were analysed separately.

For *wsm* loci, the wheat grown in the first year had the same strong effect ($p < 0.001$, F-test) as was shown by Mauchline *et al.* (2015) (Table 14). However, this effect was different for the two niches, there being a significant interaction between these two factors ($p < 0.001$, F-test). Presence of the *wsm* locus was associated more with Hereward than with Cadenza, but specifically more in the endosphere isolates for Hereward than for Cadenza (Table 15). Independently from this, there was a main effect of the wheat variety grown in the second year on presence of the gene ($p = 0.008$, F-test), with Xi-19 slightly favouring greater presence than Hereward (means (se): Hereward 1.639 (0.2063), Xi-19: 2.281 (0.2241), d.f. = 400) (Table 16).

**Table 14:** GLM table of accumulated deviance for *wsm* PCR outcome. Block: main block of year 1 with Hereward or Cadenza; Plot: sampled plots of year 2 Hereward or Xi-19; Niche: endosphere or rhizosphere; d.f: degrees of freedom; approx. F pr.: approximate F probability.

| Change | d.f. | deviance | mean deviance | deviance ratio | approx. F pr. |
|---|---|---|---|---|---|
| + Block | 3 | 23.414 | 7.8047 | 9.91 | <.001 |
| + Year1 | 1 | 22.85 | 22.8501 | 29.02 | <.001 |
| + Year2 | 1 | 5.9465 | 5.9465 | 7.55 | 0.006 |
| + Niche | 1 | 4.2758 | 4.2758 | 5.43 | 0.02 |
| + Year1.Year2 | 1 | 2.4907 | 2.4907 | 3.16 | 0.076 |
| + Year1.Niche | 1 | 9.1102 | 9.1102 | 11.57 | <.001 |
| + Year2.Niche | 1 | 1.4834 | 1.4834 | 1.88 | 0.171 |
| + Year1.Year2.Niche | 1 | 0.0002 | 0.0002 | 0 | 0.989 |
| Residual | 400 | 314.93 | 0.7873 | | |
| Total | 410 | 384.5 | 0.9378 | | |

**Table 15:** Predicted mean proportions from GLM for *wsm* pesence using PCR screening method exploring the effect of Year 1 and Niche, *n* = 411. C = Cadenza, H = Hereward, E = endosphere, R = rhizosphere.

| Niche | E Prediction | s.e. | R Prediction | s.e. |
|---|---|---|---|---|
| **Year1** | | | | |
| C | 0.764 | 0.2005 | 2.036 | 0.2816 |
| H | 3.009 | 0.3941 | 2.29 | 0.3009 |

**Table 16:** Predicted mean proportion from GLM of *wsm* presence using PCR screening method exploring the effect of Year 2, *n* = 411. C = Cadenza, H = Hereward, E = endosphere, R = rhizosphere.

| Year2 | Prediction | s.e. |
|---|---|---|
| H | 1.639 | 0.2063 |
| Xi | 2.281 | 0.2241 |

Presence of the *fecB* locus was found to be more associated with Cadenza overall, but there was again an interaction effect between the wheat variety grown in the first year and niche ($p < 0.001$, F-test) (Table 17). Specifically, there was an increase in presence of the gene within the Cadenza rhizosphere, compared to the endosphere isolates, but a decrease within the Hereward rhizosphere isolates, compared to the endosphere isolates, for *fecB* locus (Table 18).

**Table 17:** GLM table of accumulated deviance for *fecB* PCR outcome. Block: main block of year 1 with Hereward or Cadenza; Plot: sampled plots of year 2 Hereward or Xi-19; Niche: endosphere or rhizosphere; d.f: degrees of freedom; approx. F pr.: approximate F probability.

| Change | d.f. | deviance | mean deviance | deviance ratio | approx. F pr. |
|---|---|---|---|---|---|
| + Block | 3 | 2.34 | 0.78 | 0.59 | 0.622 |
| + Year1 | 1 | 7.312 | 7.312 | 5.53 | 0.019 |
| + Year2 | 1 | 3.105 | 3.105 | 2.35 | 0.126 |
| + Niche | 1 | 0.537 | 0.537 | 0.41 | 0.524 |
| + Year1.Year2 | 1 | 0.034 | 0.034 | 0.03 | 0.873 |
| + Year1.Niche | 1 | 14.589 | 14.589 | 11.04 | <.001 |
| + Year2.Niche | 1 | 1.719 | 1.719 | 1.3 | 0.255 |
| + Year1.Year2.Niche | 1 | 4.714 | 4.714 | 3.57 | 0.06 |
| Residual | 400 | 528.56 | 1.321 | | |
| Total | 410 | 562.91 | 1.373 | | |

**Table 18:** Predicted mean proportions from GLM for *fecB* presence using PCR screening method exploring the effects of Year 1 and niche, *n* = 411. C = Cadenza, H = Hereward, E = endosphere, R = rhizosphere.

| Niche | E Prediction | s.e. | R Prediction | s.e. |
|---|---|---|---|---|
| **Year1** | | | | |
| C | 0.1107 | 0.192 | 1.0049 | 0.2173 |
| H | 0.3308 | 0.2172 | -0.408 | 0.2131 |

### 3.4 Discussion:

Plants are known to influence the composition and dynamics in the rhizosphere (Germida & Siciliano, 2001).The ecological importance of fluorescent *Pseudomonas* spp. as plant growth promoting rhizo-bacteria (PGPR) has been at the centre of many studies; as their presence is often accompanied with pathogen exclusion and/or disease control (Haas & Keel 2003; Mauchline *et al.*, 2015; Rainey, 1999). They are highly adapted to the rhizosphere and effectively utilize root exudates such as sugars, amino acids, and carboxylic acids. Along with nutrient competition; niche exclusion is another trait responsible for their successful root colonization (Kwak & Weller, 2013). Root exudates are assumed to be responsible for the variability of bacterial communities carried on the roots of different plants. This is evident through total microbiome DNA analysis (Bakker *et al.*, 2013). Also root biomass is considered to be an important factor in shaping the interaction between rhizosphere bacteria and the pathogen (Jousset *et al.*, 2011). The correlation analysis of phenotypic loci by Mauchline *et al.* (2015) divided the studied *Pseudomonas* populations into two groups: group one were effective for actinomycete suppression, but produced few siderophores or plant-growth manipulation enzymes; this group had genes for viscosin, pili, *wsm* LPS etc, while group two had genes for hemophore, acetoin catabolism, and were able to produce and secrete siderophores and other small molecules, but had limited antibacterial capability.

McMillan *et al.* (2011) highlighted the differential ability of the different wheat cultivars in supporting the *Ggt* inoculum; Take-all build up (TAB). Thus, cultivars were classified as either L-TAB or H-TAB. Cadenza and Xi-19 are both L-TAB varieties while Hereward is an H-TAB variety. Also, wheat cultivars differ in their supportiveness of fluorescent *Pseudomonas* spp. colonization and the subsequent 2,4-DAPG production

(Kwak & Weller, 2013). Mavrodi *et al.* (2012) found that under irrigation conditions the wheat selects for 2,4-DAPG producing *Pseudomonas* spp. to over-come *Ggt* attack. While under dry conditions, when *Rhizoctonia solani* is the threat, it selects for phenazine producers. In a split root experiment carried out by Jousset *et al.* (2011), they were able to demonstrate the ability of barley to up-regulate the *phlA* gene in *Pseudomonas fluorescens* CHA0 on one root, due to infection by *Pythium ultimum* on the other root. Thus, the plant genotype, its exudates, and the presence of the pathogen, along with many other factors shapes the host associated microbiome. Mazzola & Gu (2002), investigated Eltan, Hill-81, Lewjain, Madsen, winter wheat cultivars and Penawawa spring wheat cultivar for establishment of *Rhizoctonia* suppressive apple orchid soils. They highlighted that host genotype is an important factor in establishing disease suppressive soils through supporting specific population of *Pseudomonas*, withthe quality of root exudation rather than the quantity being a possible factor.

In this study the effects of wheat (*T. aestivum*) varieties differing in their TAB traits on the associated fluorescent *Pseudomonas* spp. under Take-all disease conditions were investigated. Dot blot screening was comparing the outcomes of (H, Xi-19) and (C, Xi-19) planting schemes. Mauchline *et al*. (2015), highlighted that both bio-surfactants and lipopolysaccharides are important for root colonization, attachment and recognition. Also secreted molecules such as siderophores or antagonistic toxins play a role in plant growth promotion and pathogen exclusion (Leong, 1986). These loci were thus chosen for analysis based on gene identification and characteriazation in previous studies and because they were identifiable in a *P. fluorescens* genome for primer design (Faraldo-Gómez & Sansom, 2003; Alsohim *et al.*, 2014; Mauchline *et al.*, 2015).

In terms of target gene distribution, more *fecB* and *tox* loci were associated with (C,Xi-19). However, more *wsm* loci were associated with (H, Xi-19). The *viscB* loci had an equal abundance in both planting schemes. In the correlation analysis of phenotypic loci of Mauchline *et al.* (2015), they found that *Pseudomonas* isolates with genes for viscosin and *wsm* LPS produce few siderophores. They also highlighted that the *wsm* LPS biosynthesis operon and genes for toxin production were strongly correlated with first year Hereward. However, the *tox* gene screened here is different to that study, also the *fecB* locus is involved in iron acquisition but not siderophore production. Overall the results found here agree with the earlier findings of Mauchline *et al.* (2015) as more *fecB* indicates a role in nutrient acquisition.

For *tox* loci when searching the locus tag PFLU3831 in NCBI the result came out as ParE toxin of type II TA system under *P. fluorescens* SBW25 whole genome, while in Psedumonas.com under *P. fluorescens* SBW25 the same locus tag comes out as toxin HigB-2. Pandey & Gerdes (2005), showed that phylogenetic analysis of RelE, ParE, and HigB toxin super families from enteric isolates revealed weak but significant sequence similarity by which it was possible to see a clear separation of RelE and ParE but not between RelE and HigB. Thus, sequence similarity might explain the NCBI and Pseudomonas.com outcomes.

The PCR outcomes also showed that *wsm* loci were more highly associated with Hereward while *fecB* was associated with Cadenza. Also, as indicated by Mauchline *et al.* (2015) the first year wheat seems to be the driving factor in the selection of the associated *Pseudomonas* spp. gene loci.

Although the dot blot method seems to be adequate for processing large numbers of samples, there were several challenges associated with in: (1) probe preparation and

labelling, (2) two-day process of pre- and post- hybridization process, (3) long detection process if using the old x-ray film method. Another disadvantage of dot blot is the inaccuracy during the spot scoring method, which is subjective as it depends on human based determination of spot intensity. In comparison, designing degenerate primers for PCR to screen unknown isolates is also a tedious task. However, when appropriate sets of primers are successfully made, the PCR method offers a more consistent choice for screening the presence and absence of gene loci of interest. In general the results found in this chapter demonstrates the role of wheat cultivar in shaping the associated microbiome at least at the investigated *Pseudomonas* level.

## CHAPTER 4- Great Harpenden 2 *Ggt* phenotypic screening

**Summary:**

*Pseudomonas fluorescens* are known to enhance plant growth through increasing nutrient availability and suppressing the growth of fungal pathogens (Alsohim *et al.*, 2014). Great Harpenden 2 (GH2) *Pseudomonas* isolates (411) from the previous chapter were challenged for their ability to suppress the growth of the Take-all fungus *Gaemannomyces graminis* var. *tritici* (*Ggt*), using an isolate from field Long Hoos 4 (LH4). The *in vitro* inhibition assay revealed 6 highly antagonistic isolates which were further tested *in planta* in the next chapter. Analysis of variance (ANOVA) showed that there was a significant difference between isolates in their suppression of *Ggt* growth based on the *in vitro* inhibition zone data. This difference was mainly driven by the type of cultivar grown in year 1 (F statistics= 0.001). Although visually the isolates that had a large inhibition zone were from the Cadenza background, those from the Hereward background mainly isolates from the (H, H) planting combination had the smallest inhibition zones. These observations were statistically not significant however. The *gyrB* phylogeny of 25 GH2 isolates including the 6 antagonistic ones, showed evidence of clustering which separated the antagonistic isolates form non-antagonistic ones.

### 4.1 Introduction

*Pseudomonas* spp. are bacteria that colonize the rhizosphere of many different plants. They enhance plant growth mainly by pathogen exclusion (Rainey, 1999). This exclusion is achieved through their ability to secrete molecules such as iron scavenging siderophores, cyclic lipopeptides to aid motility, phenazines and anti-

fungal compounds pyoluteorin and pyrrolnitrin, along with hydrogen cyanide (HCN), which is a volatile metalloenzyme inhibitor (Rainey, 1999; Haas & Keel, 2003). The genus *Pseudomonas* consists of two main lineages *P. aeruginosa* and *P. fluorescens.* Within the later the *P. fluorescens* complex is further subdivided into nine subgroups of *P. protegens, P. chlororaphis, P. corrugata, P. koreensis, P. jessenii, P. mandelii, P. fragi, P. gessardii and P. fluorescens* (Vásquez-Ponce *et al.*, 2018).

Take-all disease, caused by *Gaeumannomyces graminis* var. *tritici* is the most important disease of wheat worldwide (Weller & Cook, 1983). Under continuous wheat monoculture the disease is most severe between the second to fourth year crops, then decreases. This later phenomenon is known as Take-all decline (TAD) (McMillan *et al.*, 2011). Investigations in Washington state reported TAD is associated with a build-up in populations of antibiotic-producing fluorescent *Pseudomonas* (McSpadden Gardener and Weller, 2001).

Studies on the biological control of Take-all using *Pseudomonas* spp. have implicated the role of iron chelating siderophores and antibiotics as mechanisms for disease suppression. Historically, mutants defective in antibiotic production have been used to study the role of antibiotics when the chemistry of the growth inhibition agent is unknown. *Pseudomonas fluorescens* strain 2-79, isolated from the rhizosphere of wheat, was found to supress *Ggt*. This strain produces the phenazine antibiotic; phenazine-1-carboxylate, which is active against Take-all fungi (Thomashow & Weller, 1988). Other fluorescent *Pseudomonas* are known to synthesize phloroglucinols like 2,4- diacetylphloroglucinol (2,4-DAPG) which is a broad spectrum antibiotic involved in Take-all suppression in addition to other fungal diseases (McSpadden Gardener and Weller, 2001). Production of hydrogen cyanide, HCN, is also considered as a disease suppressive mechanism by *Pseudomonas* (Hamdan *et al.*, 1991). Under iron

limiting conditions fluorescent *Pseudomonas* excrete iron scavenging molecules, siderophores, like the yellow green pyoverdine, pyochelin, pseudomonine, quinolobactin and the putative siderophore, pyridine-2,6-bis (thiocarboxylic acid) (Matthijs *et al.*, 2004; Alsohim *et al.*, 2014).

Matthijs *et al.* (2007) investigated the *in-vitro* growth inhibition ability of *P. fluorescence* ATCC 17400 on damping-off fungus *Pythium*. Their results showed that in addition to the involvement of high affinity iron-chelating siderophore, pyoverdine, the second low affinity iron binding siderophore, thioquinolobactin, was mainly responsible for this growth inhibition. Since thioquinolobactin rapidly degraded to quinolobactin, it was initially thought that the latter corresponds to the second siderophore (Matthijs *et al.*, 2004). However, the presence of sulphur genes within the biosynthesis operon along with the inhibitory activity of purified thioquinolobactin on *Pythium* have identified it as the main product (Matthijs *et al.*, 2007). Apart from iron level, factors like stress, quorum sensing and regulation of other secondary metabolites play roles in regulating siderophore production and uptake (Cornelis & Matthijs, 2002). Also, motility has been shown to be involved in competition, for instance wild type *P. aeruginosa* outcompetes its own non-motile variants for biofilm suitable sites (Hibbing *et al.*, 2010). In bacteria motility include flagellum-dependent swimming and swarming, or flagellum independent mechanisms like twitching/gliding using type IV pili, non-social gliding and sliding employing the reduced surface tension (Alsohim *et al.*, 2014).

Root colonization and increased population size are important features in successful bio-control using *Pseudomonas* spp. (Bull *et al.*, 1991). Studies on the *Pseudomonas* plant colonization mechanisms revealed a number of crucial interacting systems ranging from motility, secretion systems, and the production of extracellular

polysaccharides to specific nutrient uptake and metabolism (Rainey, 1999; Jackson *et al.*, 2005; Silby *et al.*, 2009; Alsohim *et al.*, 2014).

Given the importance of this group as potential biocontrol agents and the results from chapter 3 indicating that there was significant effect of cultivar on *Pseudomonas* genotype, the same *Pseudomonas* spp. isolates were investigated for Take-all fungus suppression *in vitro*.

**Aims and objectives:**

In chapter three a selection pressure of first year wheat on the associated *Pseudomonas* genotype was observed, where by more *fecB* locus was associated with isolates from first year Cadenza. Since this locus is related to iron-uptake it was hypothesized that these isolates might be antagonistic to *Ggt* through iron limitation or even by direct inhibition. Furthermore, *Ggt* antagonism was used as the phenotype to measure any kind of selection based on the cultivar type, planting combination, and niche.

## 4.2 Methods

### 4.2.1 *In vitro* inhibition of fungal pathogen:

All the *Pseudomonas* spp. were grown on King's B medium agar or broth (KA or KB) respectively as described in 2.2. Potato dextrose agar (PDA) was used to grow *Ggt*. The plate inhibition assay was performed on KA, where 5 µl of bacterial culture grown on KB overnight at 27 °C was spotted 1 cm from the edge of the plate. Three bacteria were used per plate and the fourth spot is non-inoculated KB used as a control. The

spots were allowed to soak into the agar before placing the fungal plug at the centre of the plate. A 0.5 cm plug from the leading edge of *Ggt* culture grown on PDA at 24 °C for 7 days was used as the fungal inoculum. Three replicates were prepared for each combination (Yang *et al.*, 2014). The plates were then incubated at 24°C and the distance between the edges of bacterial colonies and fungal mycelium were computed using imageJ software after 7 days (Fig. 16 A).

Using the average inhibition zone (cm) of the three treatment replicates, the isolates were classified into 3 classes. Class 1 inhibition zone = 0- 0.5 cm, class 2 = 0.5- 1 cm inhibition zone, and class 3 = >1 cm inhibition zone. The class 3 isolates (n=53) were then challenged in 1:1 setup with the fungus to validate their *Ggt* growth inhibiting action. Again, 5 µl of bacterial culture was spotted 1 cm away from the edge of the plate with the fungal plug placed at the centre. A non-inoculated 5 µl of KB was spotted as a control, and each treatment was replicated three times. The plates were incubated at 24°C for 7 days and the inhibition zone was measured using imageJ software (Fig.16 B).

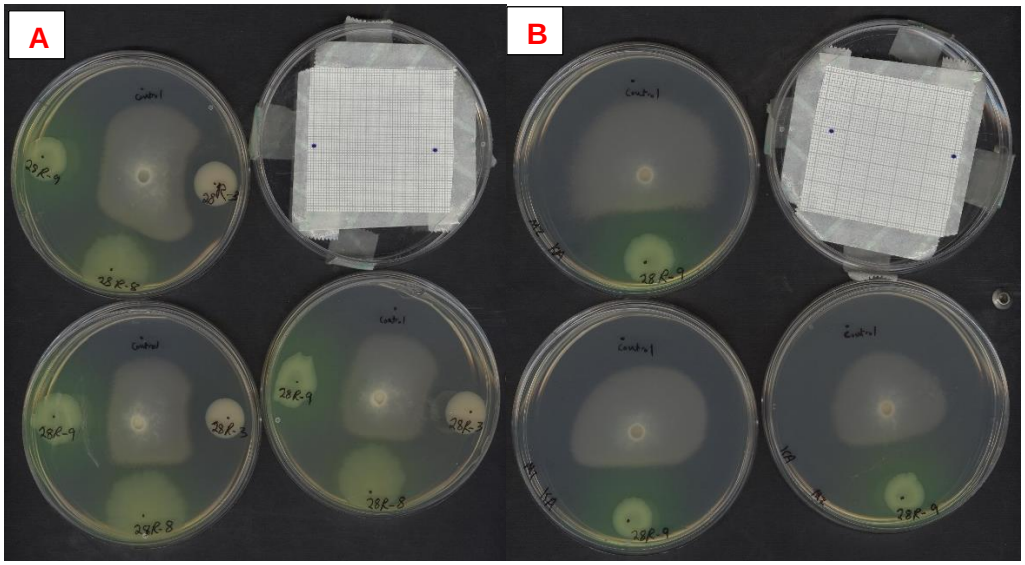



**Figure 16: *Ggt* antagonism assay**.The treatment was replicated three times and the fourth plate with the grid paper is used as a scale for ImageJ analysis. (A) Three isolate combination. (B) *Ggt* 1:1 screening for 53 class 3 isolates.

### 4.2.2 Phylogeny of GH2 *Pseudomonas* isolates

For the resulting six highly antagonistic *Ggt* isolates (Table 20), DNA template for *gyrB* (DNA gyrase B subunit) PCR was prepared using MicroLYSIS PLUS ™ (Microzone) following the manufacturer instructions. This method worked for five of the antagonistic isolates while for isolate 24E/2 genomic DNA extraction using GeneJet Extraction kit was used for DNA template preparation. An additional 19 GH2 isolates belonging to the different inhibition zone classes, field planting combinations and niches, were also sequenced for comparison. These included the two least antagonistic isolates; 37R/15 and 44R/4. For these, the DNA template was prepared using the TE buffer method described in 3.2.6.2 or with total DNA extraction as needed. Thus a total of 25 isolates were subjected to *gyrB* amplification and phylogenetic analysis. In addition, reference strains *Pseudomonas fluorescens* strains SBW25 and F113 from the lab were included for comparison and as a positive control for amplification. The DNA templates

for SBW25 and F113 were prepared using the TE buffer method. Phylogenetic analysis was done using *gyrB* PCR with primers *gyrB* F and *gyrB* R listed in (Table 19) (Yamamoto *et al.*, 2000). A 50 µl PCR reaction was prepared for amplification and sequencing. For each reaction 25 µl of 2X PCRBIO Taq Mix Red (PCR Biosystems), 16 µl $nH_2O$, 2 µl of F primer (10 µM), 2 µl of R primer (10 µM), and 5 µl of template DNA were used. The PCR amplification was performed using Bio Rad T100 thermal cycler and conditions were as follows: initial denaturation 94°C for 5mins, 35 cycles of denaturation at 94 °C 1 min, annealing at 63 °C for 30 s, extension at 72 °C for 2 mins, and a final extension step at 72 °C for 7mins. The annealing temperature for isolate 24E/2 was 58 °C. The expected amplified product size was 888-891 bp.

**Table 19:** *gyrB* primers for phylogeny of *Pseudomonas* isolates.

| **Name** | **Sequence** |
|---|---|
| *gyrB* F | CAGGAAACAGCTATGACCAYGSNGGNGGNAARTTYRA |
| *gyrB* R | TGTAAAACGACGGCCAGTGCNGGRTCYTTYTCYTGRCA |
| *gyrB* SF | CAGGAAACAGCTATGACC |
| *gyrB* SR | TGTAAAACGACGGCCAGT |

The amplified products were purified using PCR purification kit (Qiagen) and sent for sequencing (Eurofins Genomics) using the primers *gyrB* SF and *gyrB* SR (Table 19).

The resulting sequences were aligned in BioEdit software and the consensus sequence was blasted in NCBI. The phylogenetic tree was constructed using Neighbor Joining method (NJ) after alignment with MUSCLE using Geneious Prime 2019.0.4 (http://www.geneious.com). The node support was evaluated on 100 bootstrap

replication. The *gyrB* sequence of *Pseudomonas aeruginosa* downloaded from GenBank NCBI was used as the out-group. Also *gyrB* sequences of other members of the *P. fluorescens* complex were downloaded from [GenBank] NCBI (Appendix 9.2.3).

### 4.2.3 Phenotypic assays for the six *Ggt* antagonistic isolates

#### 4.2.3.1 Motility assay

Low (1/10) and Full strength LB agar plates were prepared as described in 2.7 for swimming and swarming motility assay; respectively. A single colony of an overnight culture on KA was used to stab the centre of the LB agar plate. The plates were monitored for colony growth and images were taken every 18, 24, and 42 h (Capdevila *et al.*, 2004). The images were taken using Gel documentation system G:BOX (Syngene). In addition, ImageJ software was later used to measure the growth area. The experiment consisted of three replicates per isolate. The less antagonistic isolates; 37R/15 and 44R/4, were included as negative controls.

#### 4.2.3.2 Fluorescence under UV light

Isolates were streaked on KA plates and incubated overnight at 27°C. Following the incubation the plates were visualized for fluorescence under bench top UV trans-illuminator. *Pseudomonas fluorescens* strains SBW25 and ATCC17400 were included as reference controls for fluorescence.

### 4.2.4 Statistical Analysis

The GENSTAT (17th edition, VSN International Ltd, Hemel Hempstead, UK) statistical package was used to analyse the inhibition zone, swimming and swarming motility data. For inhibition zone class 1-3 data a generalized linear model GLM was used, while for averaged inhibition zone data over plot analysis of variance ANOVA was used. Also ANOVA was used for motility assay data analysis.

## 4.3 Results

### 4.3.1 *Ggt* growth inhibition

The 411 isolates from GH2 were *in vitro* screened in a plate assay described in section 4.2.1 (Yang *et al.*, 2014). The first analysis consisted of three spots of individual isolates. This combination was tested in replicates of three. The inhibition zone was measured using ImageJ software and the data were recorded in an excel sheet (Appendix I 9.2.1). The isolates were grouped into 3 classes based on the size of inhibition zone; class 1: 0 - 0.5 cm, class 2: 0.5 - 1, and class 3: >1 cm. There were 172 class 1 isolates, 186 class 2 isolates and 53 class 3 isolates. The 53 class 3 isolates were further challenged in 1:1 setup with *Ggt* to confirm their antagonistic ability (Appendix I 9.2.1). From the 1:1 assay only six isolates (11.3%) maintained their *Ggt* inhibition of > 1cm (Table 20). While 19/53 were in class 1 and 28/53 were in class 2 after the 1:1 challenge.

The six antagonistic isolates were from a diverse range of field plots and 50:50 split of endosphere/rhizosphere niche. Four of the six isolates were from the Cadenza, Xi-19 planting combination with one isolate from each Hereward, Hereward and Hereward, Xi-19 planting combinations (Table 20).

**Table 20:** List of six most *Ggt* antagonistic isolates after 1:1 assay. Standard error s.e based on n= 3.

| No. | Isolate[1] | Planting Combination | Mean inhibition zone (cm) | s.e |
|---|---|---|---|---|
| 1 | 24E-2 | (C, Xi-19) | 1.24 | 0.055 |
| 2 | 24E-4 | (C, Xi-19) | 1.05 | 0.081 |
| 3 | 25R-7 | (C, Xi-19) | 1.10 | 0.078 |
| 4 | 28R-9 | (H, Xi-19) | 1.08 | 0.107 |
| 5 | 30R-11 | (C, Xi-19) | 1.23 | 0.343 |
| 6 | 44E-7 | (H, H) | 1.44 | 0.219 |

[1]Number = field plot, E= Endosphere, R = Rhizosphere

#### 4.3.1.1 Statistical analysis of inhibition zone data

The category (scores 1-3) (Appendix I 9.2.1) were analysed by fitting a generalized linear model (GLM) to account for the blocks in the experiment and test the main effects and interactions between the factors Year 1 (H or C cultivar), Year 2 (H or Xi-19 cultivar), and Niche (E or R). A Poisson distribution was assumed, and a log link function was incorporated. The fitted model was Block+ (Year1*Year2*Niche). There was no evidence of over-dispersion for this model. Predicted means on the log scale were output to enable comparisons using the standard error of the difference (SED) between them on the residual degrees of freedom from the model with the least significant difference (LSD) at the 5% level of significance. This regression analysis has shown some evidence of a 2-way interaction between Year 1 and Year 2 factors (F= 4.86 on 1 and 410 d.f.; p=0.028) (Tables 21 & 22), but no effect of niche.

**Table 21:** Accumulated analysis of deviance from GLM. Block: main block of year 1 Hereward or Cadenza; Year 2: Hereward or Xi-19; Niche: endosphere or rhizosphere; F pr.: F probability; d.f.= degrees of freedom.

| Change | d.f. | deviance | mean deviance | deviance ratio | approx. F pr. |
|---|---|---|---|---|---|
| + Block | 3 | 3.3602 | 1.1201 | 5.05 | 0.002 |
| + Year_1 | 1 | 5.7819 | 5.7819 | 26.08 | <.001 |
| + Year_2 | 1 | 2.8602 | 2.8602 | 12.9 | <.001 |
| + niche | 1 | 0.0205 | 0.0205 | 0.09 | 0.761 |
| + Year_1.Year_2 | 1 | 1.0784 | 1.0784 | 4.86 | 0.028 |
| + Year_1.niche | 1 | 0.6975 | 0.6975 | 3.15 | 0.077 |
| + Year_2.niche | 1 | 0.0409 | 0.0409 | 0.18 | 0.668 |
| + Year_1.Year_2.niche | 1 | 0.6643 | 0.6643 | 3 | 0.084 |
| Residual | 400 | 88.672 | 0.2217 | | |
| Total | 410 | 103.1759 | 0.2516 | | |

**Table 22:** Table of predicted means from GLM analysis. C: Cadenza; H: Hereward; XI: Xi-19; E: endosphere; R: rhizosphere.

| Niche | | E Prediction | s.e. | R Prediction | s.e. |
|---|---|---|---|---|---|
| **Year_1** | **Year_2** | | | | |
| **C** | **H** | 0.6799 | 0.04613 | 0.7571 | 0.0458 |
| | **XI** | 0.5223 | 0.04832 | 0.5272 | 0.047 |
| **H** | **H** | 0.5482 | 0.05643 | 0.36 | 0.0613 |
| | **XI** | 0.4261 | 0.05436 | 0.4184 | 0.0492 |

From Table 22 the greatest sized inhibition zone were more associated with (C, H) planting combinations in both endosphere (E) and rhizosphere (R ) ($P< 0.05$, LSD) in comparison with Year 2 Xi-19 planting combination but not from (H, H) endosphere (E) planting combination. The least sized inhibition zones were for (H, H) in the rhizosphere (R) niche.

Secondly analysis of variance (ANOVA) was performed on the inhibition zone data which were averaged per plot over the isolates (Appendix II 9.2.2). Again, the main effect was being driven by Year 1 cultivar ($F= 0.031$, d.f=1) regardless of what is grown in Year 2 and the Niche (Table 23). Also, the most antagonistic isolates (with big inhibition zone values cm) are favouring Cadenza background in Year 1 compared with Hereward (Table 24). This analysis is far more conservative than the former GLM.

**Table 23:** ANOVA of averaged inhibition zone data per plot. Block: main block of year 1 Hereward or Cadenza; Year 2: Hereward or Xi-19; Niche: endosphere or rhizosphere; F pr.: F probability; d.f.= degrees of freedom.

| Source of variation | d.f. | s.s. | m.s. | v.r. | F probability |
|---|---|---|---|---|---|
| **Block stratum** | 3 | 0.14855 | 0.04952 | 1.50 | |
| **Block. MainPlot stratum** | | | | | |
| **Year1** | 1 | 0.49178 | 0.49178 | 14.93 | 0.031 |
| **Residual** | 3 | 0.09884 | 0.03295 | 0.36 | |
| **Block. MainPlot. SplitPlot stratum** | | | | | |
| **Year2** | 1 | 0.04443 | 0.04443 | 0.48 | 0.513 |
| **Year1. Year2** | 1 | 0.20042 | 0.20042 | 2.18 | 0.191 |
| **Residual** | 6 | 0.55234 | 0.09206 | 1.85 | |
| **Block. MainPlot. SplitPlot. SplitSplit stratum** | | | | | |
| **Niche** | 1 | 0.00100 | 0.00100 | 0.02 | 0.889 |
| **Year1. Niche** | 1 | 0.02277 | 0.02277 | 0.46 | 0.511 |
| **Year2. Niche** | 1 | 0.00104 | 0.00104 | 0.02 | 0.887 |
| **Year1. Year2. Niche** | 1 | 0.04121 | 0.04121 | 0.83 | 0.381 |
| **Residual** | 12 | 0.59700 | 0.04975 | | |
| **Total** | 31 | 2.19939 | | | |

**Table 24:** Table of means from ANOVA. H: Hereward; Xi: Xi-19; E: endosphere; R: rhizosphere.

| | **Year2** | **H** | | **Xi** | |
|---|---|---|---|---|---|
| **Year1** | **Niche** | **E** | **R** | **E** | **R** |
| **C** | | 0.773 | 0.897 | 0.600 | 0.604 |
| **H** | | 0.491 | 0.366 | 0.492 | 0.533 |

Also, comparing the two full means tables from the two analyses (Tables 22 and 24) it can be seen that the E and R values for Year 1 Cadenza are still giving the greatest values.

However as mentioned earlier the GLM analysis took all the data from individual isolates as replicates per plot. This might be the reason that the 2-way interaction has been shown as significant. Thus, the findings can consider the findings visually rather than statistically. As isolates observed from the same plot may not be fully independent, this suggests that the average values per plot are more appropriate to analyse.

### 4.3.2 *gyrB* Phylogeny

Following *gyrB* amplification, the PCR products were purified and sent for sequencing to Eurofins genomics. The resulting forward and reverse sequences were aligned using Clustal W multiple alignment and the consensus sequence was built using (BioEdit). The consensus sequence was then blasted in NCBI. Blast results are summarized in Table 26. Phylogenetic analysis using *gyrB* gene was used to study 30 isolates including the six *Ggt* antagonistic ones. *P. aeruginosa* was included as outgroup and *P. fluorescens* SBW25 and F113 in addition to other members of the *P.*

*fluorescens* complex were included for comparison (Appendix 9.2.3). Following MUSCLE alignment a neighbour joining phylogenetic tree was constructed using Geneious Prime 2019.0.4 (http://www.geneious.com) (Fig.17). The node support was evaluated based on 100 bootstrap replications. The alignment is shown in Appendix 9.2.4.

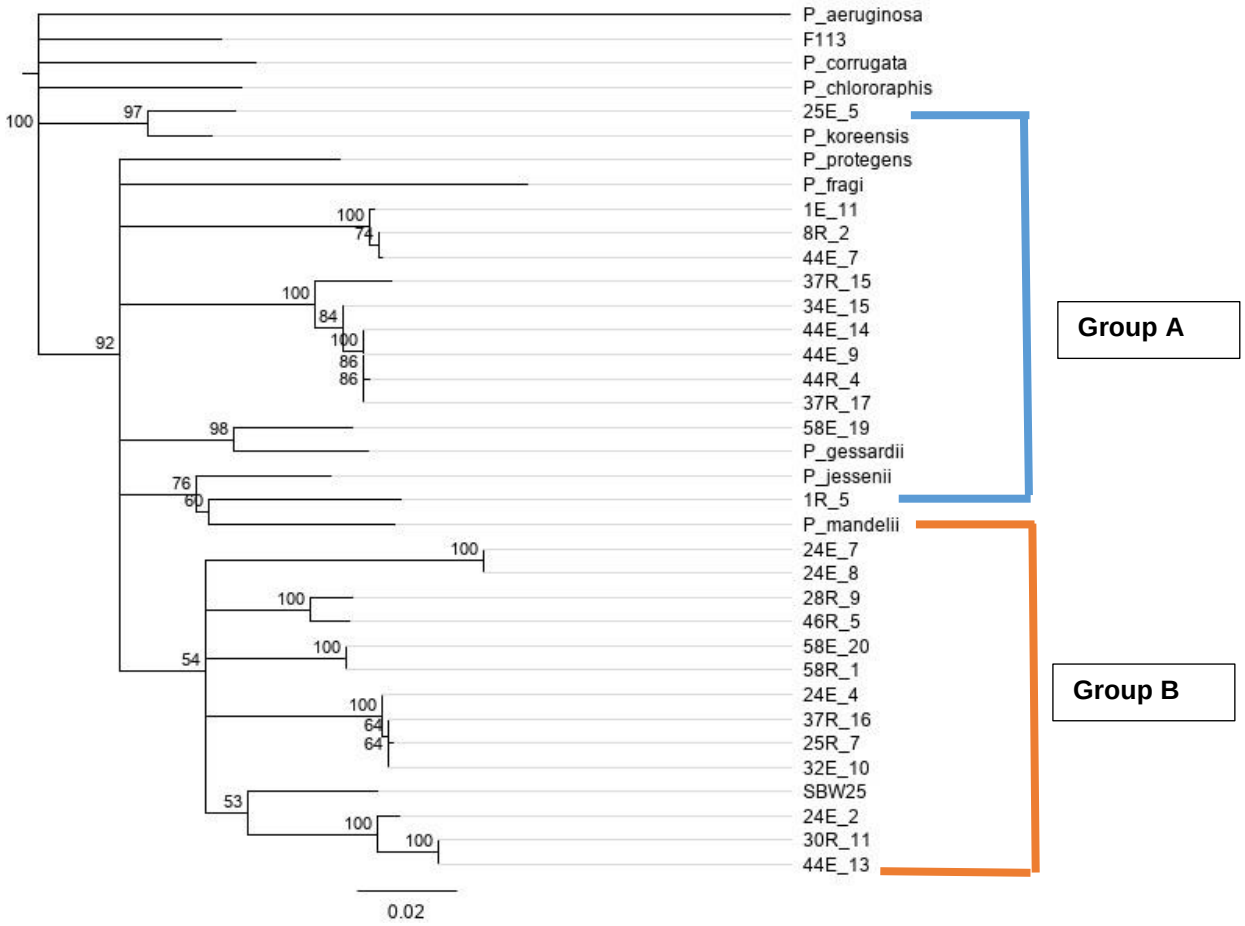



**Figure 17:*gyrB* phylogeny of 25 GH2 isolates in comparison to members of *P. fluorescens* complex.** *Pseudomonas aeruginosa* was used as outgroup. The tree was constructed using Neighbour Joining Method after alignment with MUSCLE using Geneious Prime. The numbers on the nodes indicate bootstrap probabilities. The bar is the nucleotide substitution per site.

**Table 25:** Description of the GH2 isolates used for *gyrB* phylogeny. The identification was based on BLAST outcomes in NCBI. Isolates are in the same order as they appear in Fig. 17. Class: inhibition zone class; Y1: year 1; Y2: year 2.

| no | Isolate | Class | Y1 | Y2 | *wsm* | *fecB* | Identified as |
|---|---|---|---|---|---|---|---|
| 1 | 25E/5 | 1 | C | Xi | 0 | 1 | *Pseudomonas* R-42020 |
| 2 | 1E/11 | 2 | H | H | 1 | 1 | *Pseudomonas fluorescens* |
| 3 | 8R/2 | 1 | C | H | 1 | 1 | *Pseudomonas orientalis* |
| 4 | 44E/7 | 3 | H | H | 1 | 1 | *Pseudomonas orientalis* |
| 5 | 37R/15 | 1 | H | H | 1 | 0 | *Pseudomonas* sp. GH1-PS70 |
| 6 | 34E/15 | 2 | H | Xi | 1 | 1 | *Pseudomonas* sp. RZ109 |
| 7 | 44E/14 | 1 | H | H | 1 | 0 | *Pseudomonas marginalis* |
| 8 | 44R/4 | 1 | H | H | 1 | 0 | Pseudomonas sp. Ra3 |
| 9 | 44E/9 | 3 | H | H | 1 | 0 | *Pseudomonas marginalis* |
| 10 | 37R/17 | 1 | H | H | 1 | 0 | *Pseudomonas* sp. RZ109 |
| 11 | 58E/19 | 1 | C | H | 0 | 0 | Pseudomonas sp. GH1-PS43 |
| 12 | 1R/5 | 1 | H | H | 0 | 0 | Pseudomonas sp. GH1-PS83 |
| 13 | 24E/7 | 3 | C | Xi | 1 | 1 | *Pseudomonas poae* |
| 14 | 24E/8 | 1 | C | Xi | 1 | 1 | *Pseudomonas poae* |
| 15 | 28R/9 | 3 | H | Xi | 1 | 1 | *Pseudomonas orientalis* |
| 16 | 46R/5 | 3 | C | H | 1 | 1 | *Pseudomonas orientalis* |
| 17 | 58E/20 | 2 | C | H | 1 | 1 | *Pseudomonas* sp. GH1-PS43 |
| 18 | 58R/1 | 1 | C | H | 1 | 1 | *Pseudomonas* sp. GH1-PS43 |
| 19 | 24E/4 | 3 | C | Xi | 1 | 1 | *Pseudomonas salmonii* |
| 20 | 37R/16 | 2 | H | H | 1 | 0 | *Pseudomonas fluorescens* |
| 21 | 25R/7 | 3 | C | Xi | 1 | 1 | *Pseudomonas* sp. |
| 22 | 32E/10 | 3 | H | H | 1 | 1 | *Pseudomonas poe* 36C8 |
| 23 | 24E/2 | 3 | C | Xi | 1 | 1 | *Pseudomonas* sp. R-41739 |
| 24 | 30R/11 | 3 | C | Xi | 0 | 0 | *Pseudomonas orientalis* |
| 25 | 44E/13 | 2 | H | H | 1 | 1 | *Pseudomonas fluorescens* |

Looking at the right side of the phylogenetic tree, two main clusters have been identified, with least antagonistic one group A separated from the antagonistic group B. In group A there are 12 isolates, 9 belong to year 1 Hereward and 3 belong to year 1 Cadenza. While in group B there were 13 isolates, 9 belong to year 1 Cadenza and 4 belong to year 1 Hereward (Table 25). These observations match the inhibition zone data.

### 4.3.3 Motility assay

Phenotypic analysis for the six antagonistic isolates along with two non-antagonists 37R/15 and 44R/4 (Table 26), included motility assay and fluorescence as described in section 4.2.3 . Motility is an important trait for root colonization by *Pseudomonas* (Alsohim *et al.*, 2014). *P. fluorescens* WCS365 mutants with modified LPS had lower growth rates on root exudates and impaired colonization ability compared to the wild type (Lugtenberg & Kamilova, 2009). Analysis of variance (ANOVA), on the growth area data ($cm^2$) for each swimming and swarming motility (Fig 18 - 19) shows significant difference between the 8 isolates ($F < 0.001$, d.f.= 14) (Table 27). Out of eight tested isolates, all were positive for *wsm* loci except for isolate 30R/11 and this might reflect its slow swimming and swarming behaviour in comparison to the other isolates (Fig. 20). Isolate 28R/9 is a fast swimmer and swarmer out of the 6 antagonistic isolates. While in general isolate 37R/15 is the fastest swarmer when comparing the total eight (antagonists and non-antagonists) (Fig. 20).

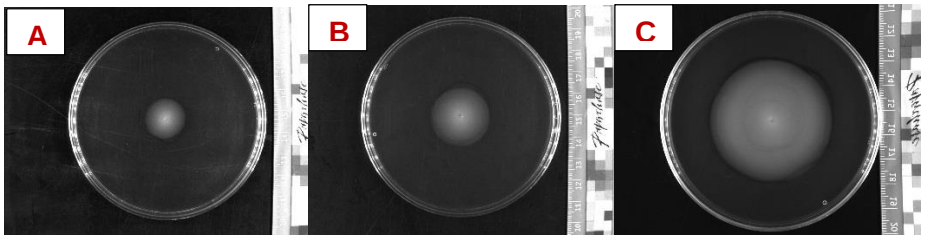



**Figure 18: Swimming motility on 1/10 strength LB of isolate 24E/2.** (a) After 18 h, (b) after 24 h, and (c) after 42 h.

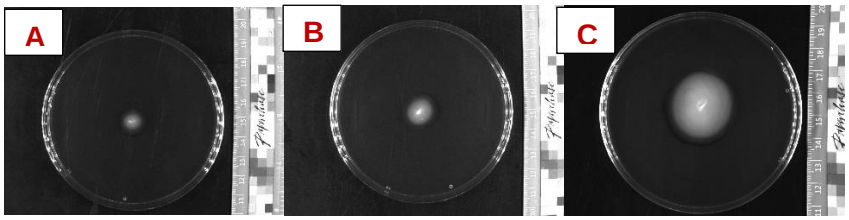



**Figure 19: Swarming motility on Full strength LB of isolate 24E/2**. (a) After 18 h, (b) after 24 h, and (c) after 42 h.

**Table 26:** Two of the least antagonistic isolates after 1:1 assay. Standard error s.e based on n= 3.

| **Isolate[1]** | **Planting Combination** | **Mean inhibition zone** | **s.e** |
|---|---|---|---|
| 44R-4 | (H, H) | 0.03 | 0.015 |
| 37R-15 | (H, H) | 0.05 | 0.029 |

[1]Number = field plot, R = Rhizosphere

**Table 27:** ANOVA of motility growth area data. Isolate_code: identifier (e.g.: 25R/7); Motility: swimming or swarming; Time: 18, 24 or 72 hr; F pr: F probability; d.f: degrees of freedom.

| Source of variation | d.f. | s.s. | m.s. | v.r. | F pr. |
|---|---|---|---|---|---|
| **Isolate_code** | 7 | 28253.45 | 4036.21 | 217.16 | <.001 |
| **motility** | 1 | 1411.66 | 1411.66 | 75.95 | <.001 |
| **time** | 2 | 15507.05 | 7753.53 | 417.16 | <.001 |
| **Isolate_code.motility** | 7 | 4847.24 | 692.46 | 37.26 | <.001 |
| **Isolate_code.time** | 14 | 3269.77 | 233.56 | 12.57 | <.001 |
| **motility.time** | 2 | 665.39 | 332.7 | 17.9 | <.001 |
| **Isolate_code.motility.time** | 14 | 1581.95 | 113 | 6.08 | <.001 |
| **Residual** | 96 | 1784.31 | 18.59 | | |
| **Total** | 143 | 57320.82 | | | |

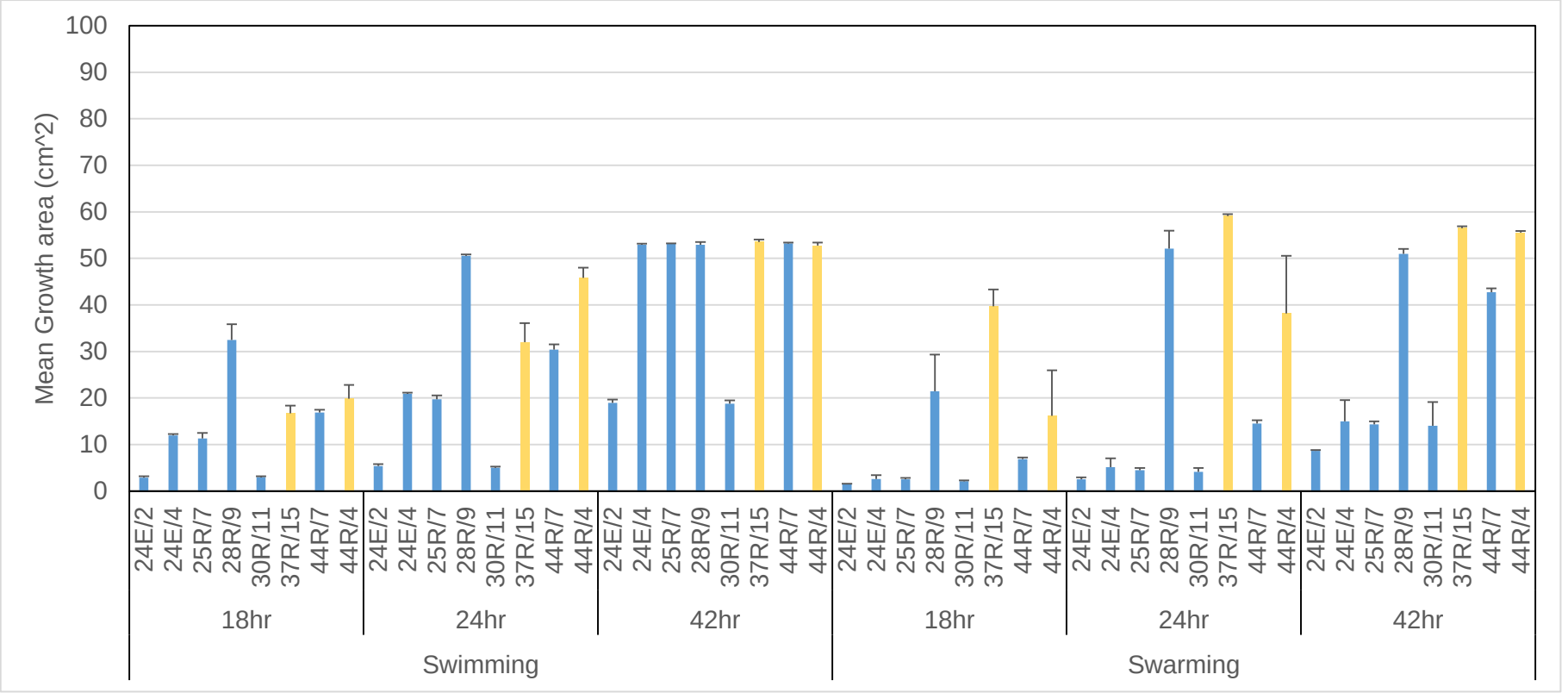



**Figure 20: Comparison of growth area of the six antagonistic isolates and two least antagonistic isolates for the swimming and swarming motility based on ANOVA table of means.** The bars are plotted form ANOVA table of means. Error bars are based on s.e (n=3). The six antagonistic isolates are in blue, the two least inhibitory are in yellow.

### 4.3.4 Fluorescence

Production of fluorescent siderophores has been often associated with plant growth promoting *Pseudomonas*. These molecules are thought to function either directly by making iron available for the plant or indirectly by depriving the pathogen (Rainey, 1999; Matthijs *et al.*, 2007; Mavrodi *et al.*, 2007). Out of the six antagonistic isolates, 24E/2 and 30R/11, had low or no fluorescence under UV light in comparison to the two reference strains used, SBW25 and ATCC 17400. The remaining four antagonists along with the other two least antagonists showed fluorescence (Table 28). Based on PCR screening five out of the six antagonistic isolates were positive for *fecB* loci except for 30R/11. While the two least antagonistic one were negative for *fecB*. Thus indicating that fluorescence in not dependent on this loci.

**Table 28:** Fluorescence data.

| **Isolate** | **Fluorescence** |
|---|---|
| *P. fluorescens* SBW25 | High |
| *P. fluorescens* ATCC17400 | High |
| 24E/2 | Low |
| 24E/4 | High |
| 25R/7 | High |
| 28R/9 | High |
| 30R/11 | Low |
| 44E/7 | High |
| 37R/15 | High |
| 44R/4 | High |

## 4.4 Discussion

*Pseudomonas fluorescens* have been shown to supress many soil borne plant diseases, including Take-all disease of wheat. Pathogen suppression is achieved through various mechanisms such as production of antibiotics, volatile organic compounds (VOCs), lytic enzymes, siderophores and HCN. In addition, they must be able to move and colonize the rhizosphere (Weller & Cook, 1983; Compant *et al.*, 2010; Lagzian *et al.*, 2013; Mendes *et al.*, 2013; Mauchline *et al.*, 2015). Here, out of the 411 *Pseudomonas* isolates, 6 *Ggt* growth inhibiting isolates *in vitro* were identified. Four out of the 6 strains were fluorescent under UV. The mode of action is still unknown.

In terms of Take-all disease control some studies have focused on the importance of antibiotic production while others considered that the production of siderophores is the main factor contributing to disease suppression. This variation in factors have been shown in the suppression of *Pythium* spp, causing damping off in cotton, by *P. fluorescens* strains 3551 and HV37a. While siderophore production is the potent agent of 3551 suppression, HV37a suppression is mediated by the production of oomycin A antibiotic (Hamdan *et al.*, 1991).

Phenazine antibiotics are products of aromatic amino acid synthesis pathway with chorismate acting as the branch point intermediate (Thomashow & Weller, 1988; Hamdan *et al.*, 1991). Also, *Pseudomonas* spp. are known to produce different types of phenazines under different growth conditions (Gurusiddaiah *et al.*, 1986). *Pseudomonas* spp. such as *P. aureofaciens* and *P. chlororaphis* produce phenazine-1-carboxylic acid (PCA) and its derivative such as 2-hydroxyphenazine-1-carboxylic acid and phenazine-1-carboxamide, respectively, because they have additional genes that modify PCA (Yang *et al.*, 2011). In the Pacific Northwest 2, 4-

diacetylphloroglucinol (2, 4-DAPG) producing *P. fluorescens* are responsible for natural Take-all disease suppression (Mavrodi *et al.*, 2012b). Yet, iron limitation can be a possible mode of action. Siderophores, including pyoverdins, facilitate biocontrol through ferric iron acquisition, and iron limitation thus are implicated as a suppression mechanism (Hamdan *et al.*, 1991). The role of pseudobactin, fluorescent siderophore of *P. fluorescens* strain B10, has been shown in Take-all disease suppression through limiting the iron to the pathogen (Leong, 1986). To test the hypothesis that *Ggt* antagonism of the isolates screened in this work is based on iron deprivation, the use of iron supplemented media provides a simple testing method. Where loss of antagonism in the presence of iron can be used as an indicator.

Yang *et al.*, (2011) compared a non-irrigated field to an irrigated one in China where wheat is rotated with corn and rice respectively. They postulated that these rotations prevent the development of TAD, but the *Ggt* antagonistic isolates from these fields did not produce any of the common known antibiotics: 2, 4-DAPG, pyrrolnitrin, or pyoluteorin. Similarly, Mavrodi *et al.* (2012) looked at the effect of crop management on indigenous antibiotic producing *Pseudomonas*. They found that Take-all disease dominates in irrigated fields compared to dry land. Furthermore, wheat grown under dry land or irrigated conditions differentially support and enrich for 2, 4-DAPG- and PCA- producing *Pseudomonas* spp. Yang *et al.* (2011), used a PCR method to screen the *Ggt* antagonistic isolates for antibiotic production. In chapter five the 6 antagonistic isolates were screened for growth *Ggt* inhibition in the presence of wheat plant and for antibiotic genes, PCA and 2,4-DAPG.

Here *in vitro Ggt* growth inhibition assay was used as a first step to identify potential biocontrol agents form the pool of 411 *Pseudomonas* isolates. In this assay the bacterial suspension is confronted with the *Ggt* plug. Likewise others have employed

similar method (Thomashow & Weller, 1988; Yang *et al.*, 2011; Lagzian *et al.*, 2013). Clear inhibition zones and in some cases discoloration of the clearing zone was seen, indicating possible chemistries produced. In the first assay a random combination of three isolates were spotted individually at approximately 1 cm from the edge of the Petri dish and evenly from each other (Fig16 A). Following this initial screening and based on inhibition zone data, isolates were classified into 172; class I, 186; class II and 53; class III. The 53 class III isolates were further tested in 1:1 setup with *Ggt* and 6 potential highly antagonistic isolates were identified. This suggests that the growth inhibiting ability of the other 47 isolates was influenced by interactions with the other *Pseudomonas* isolates in the first assay when combinations of isolates were tested together. Lagzian *et al.* (2013) screened 900 fluorescent *Pseudomonas* isolates for *Ggt* suppression *in vitro* and ended up with 27 isolates for greenhouse experiments. Interestingly when analysing the inhibition zone data it was found that the main effect was of cultivars grown in year 1 favouring Cadenza over Hereward but this was not statistically significant. Similarly, Yang *et al.* (2011) reported that no significant correlation were found between *in vitro Ggt* inhibition and the source of isolates (i) location wise, rain fed field in Jiangsu province *vs*. irrigated field in Hebei province China, (ii) from the plant part (leaf, stem and roots) (iii) niche, endosphere and rhizosphere prospective.Motility is an important trait for successful root colonization by *Pseudomonas*, where the presence of flagella and production of surfactants like, viscosin and viscosinamide were found to aid the process (Alsohim *et al.*, 2014). In addition, CLP (cyclic lipo-peptides) produced by *Pseudomonas* were found to aid motility and virulence (De Bruijn *et al.*, 2007). The polar flagellum in *Pseudomonas aeruginosa* is responsible for swimming motility in aqueous environments, flagella also mediate swarming motility (Deziel *et al.*, 2001). While swimming is the act of individual

cells, swarming involves multicellular movement on soil media (Calvio *et al.*, 2005). Colonization involves recognition, adherence, colonization, growth and different strategies for interaction (Berg, 2009). The process is initiated by exchanged signals between the plant roots and the soil microbes and thus motile bacteria are preferred (Berg, 2009; Lugtenberg & Kamilova, 2009). 365 *P. fluorescens* WCS mutants were investigated genetically and physiologically for tomato root tip colonization. It was shown that the major competitive traits required for colonization were motility, adhesion, enhanced growth rate on root exudates and the presence of O-antigenic side chain of lipopolysaccharide (Lugtenberg & Kamilova, 2009). Here, out of the six antagonistic isolates it was found that isolate 28R/9 to be a fast swimmer and swarmer. The other isolates were comparable to the least antagonistic control isolates. However, further investigation is needed to genetically and physiologically determine the best competitive root colonizers.

In this work phylogenetic analysis was carried out for 25 random *Pseudomonas* isolates covering a range of planting combinations, niches and antagonistic abilities including the 6 potential highly *Ggt* antagonists using single gene sequencing. Initially the *gyrB* phylogeny alone was compared with a combined phylogeny of 8 single copy genes (Mauchline *et al.*, 2015). Both methods agreed with each other, thus here the *gyrB* phylogenetic analysis was used. Also, compared with 16S rRNA this provides a better resolution (Yamamoto *et al.*, 2000). Following PCR amplification the resulting sequences were aligned using MUSCLE and a neighbor joining method was used to construct a *gyrB* phylogenetic tree on Geneious Prime 2019.0.4. The phylogenetic tree showed that the isolates with largest inhibition zones grouped separately from the one with small inhibition zones. The grouping agreed with the inhibition zone data which showed that isolates from first year Cadenza had larger inhibition zones than those

from first year Hereward. In addition the (H, H) planting scheme was the one with smallest *Ggt* inhibition zone. This suggests that the Cadenza wheat cultivar may be able to somehow select for antagonistic Pseudomonas isolates which results in its Low Take-all inoculum building ability (L-TAB). Silby *et al.* (2009) carried out phylogenetic analysis of fourteen *Pseudomonas* genomes which were compared all against all using reciprocal FASTA, aligned with gene-wise MUSCLE and analysed using Maximum Likelihood in RAxML version 7.0.0 with the JTT+gamma model. Their analysis have shown that the *P. fluorescens* SBW25, Pf01 and Pf-5 clustered separately form the other *Pseudomonas*. Furthermore within the *P. fluorescens,* two main lineages were identified with SBW25 belonging to the *P. fluorescens* lineage while Pf01 and Pf-5 belong to the *P. chlororaphis* lineages. These findings were in agreement with the *gyrB* and *rpoD* phylogenetic analysis carried out by Yamamoto *et al.* (2000).

Comparison of the isolates with other members of the *P. fluorescens* complex showed that they are distributed among the tested strains. In addition it is important to point out that the use of high fidelity Taq polymerase rather than the low fidelity Taq polymerase which was used here will provide more accurate results. Since the high fidelity Taq polymerase couples both low misincorporation rates with proofreading activity. However the use of reference strains of *P. fluorescens* SBW25 and F113 provided a positive control for the amplification.

Further work will be needed to identify the possible growth suppression agent which can be investigated through biochemical or genetic analysis. It is possible that different growth suppressing agents may be operating in the different isolates. Screening the isolates for production of HCN, VOCs, siderophores, antibiotics, proteases, chitinase and cellulase have been described by (Yang *et al.*, 2011; Lagzian *et al.*, 2013). In

addition, complete genome sequencing of the six antagonistic isolates is necessary to identify loci of plant growth promotion and or pathogen exclusion.

## CHAPTER 5- Great Harpenden 2 *In planta* assay

**Summary:**

In the previous chapter six potential *Ggt* growth inhibitors were identified. These isolates were further tested here for their *Ggt* inhibition in the presence of the host plant (wheat) cultivars Cadenza and Hereward. The shoot height, root length and fresh weights were used as measures for plant biomass. The number of blackened roots in comparison to the healthy roots was used to determine percent infected roots as a measure of disease severity. The two least antagonistic isolates were included for comparison. The initial screening showed that there was no significant difference in the shoot height, root length, and fresh weight of Cadenza and Hereward when comparing the treatments in the presence and absence of *Ggt*. However, there was a significant difference in the percent infected roots (F= 0.024), where inoculation of isolate 25R/7 led to less disease in Cadenza, while use of isolate 30R/11 led to less disease in Hereward. These two isolates were further investigated at different concentrations to validate the inhibitory effect on *Ggt in planta*.

### 5.1 Introduction

The difficulty of controlling soil borne plant diseases arises from the complexity and the dynamic nature of the rhizosphere (Handelsman & Stabb, 1996; Raaijmakers *et al.*, 2009). The rhizosphere is the narrow zone of soil surrounding the roots and influenced by its exudates (Rainey, 1999). Fungi and oomycetes are the most important soil-borne plant pathogens (Raaijmakers *et al.*, 2009). Interest in environmentally friendly plant disease control approaches are expanding, usually due to a lack of resistant plant genotypes and due to the deleterious effect of chemical

pesticides (Mauchline & Malone, 2017). For biocontrol agents to be effective they have to be present in the right place and time and in sufficient amounts. The Gram- negative *Pseudomonas* are among the most promising biocontrol rhizobacteria (Walker *et al.*, 2004). The ability of *Pseudomonas fluorescens* to suppress pathogens is related to its population density on the host (Haas *et al.*, 2000). In addition, certain traits like root colonization, production of antifungal compounds and induction of host resistance are crucial for the success of this class of rhizo-biocontrol bacteria (Haas *et al.*, 2000; Compant *et al.*, 2005). Nutrient competition is yet another important trait of *Pseudomonas* as an efficient rhizosphere colonizer and pathogen suppressor (Walsh *et al.*, 2001). Under iron limiting conditions, the yellow-green pigments, pyoverdines, form tight iron (III) complexes which are actively transported to bacterial cells, thus is recognized as an essential siderophore of many fluorescent *Pseudomonas* species (Meyer, 2000).

In many biocontrol studies, antibiotics have been identified as the agents of disease suppression (Handelsman & Stabb, 1996). For instance, control of Take-all disease caused by *Gaeumannomyces graminis* var. *tritici* was achieved by phenazine-1-carboxylic acid (PCA) in *Pseudomonas fluorescens* 2-79 and with the production of 2,4-diacetylphloroglucinol (Phl) by *P. fluorescens* CHAO (Raaijmakers *et al.*, 1997). In addition to phenazines and 2,4-diacetylphloroglucinol, the antimicrobial compounds produced by fluorescent *Pseudomonas* include HCN, pyoluteorin and pyrrolnitrin (Haas *et al.*, 2000; Liu *et al.*, 2009). Also, siderophores like thioquinolobactin (Matthijs *et al.*, 2007). Apart from disease resistance, the plant associated beneficial microbes aid plants health and growth through stress tolerance, enhanced nutrient uptake and availability (Berg, 2009). For instance ACC deaminase producing bacteria are able to degrade the ethylene precursor and thus enhance root elongation by lowering the

ethylene (Lugtenberg & Kamilova, 2009). In addition nitrogen fixation, phosphorus and iron solubilisation are well known examples of plant associated microbe nutrient supply (Berg, 2009; Vacheron *et al.*, 2013; Haichar *et al.*, 2014).

To date Take-all disease of wheat still constrains wheat productivity in the UK and worldwide (McMillan *et al.*, 2011; Yang *et al.*, 2014). Although many biocontrol agents have been identified, inconsistent field performance is still a major drawback of field and commercial application. This is largely due to the complex three compartment interaction: the plant, the microbes and the soil (Mauchline and Malone, 2017; Rainey, 1999). Therefore the quest to find and develop biocontrol agents continues for many soil-borne plant diseases such as Take-all disease of wheat.

In this chapter, the ability of six isolates that inhibited *Ggt in vitro* in controlling the disease on the host plant was further investigated. Since four of the identified isolates were from the first year Cadenza background, it was interesting to see their performance on Hereward and *vice versa.*

**Aims and objectives:**

1. To further investigate the six identified antagonistic isolates for their plant growth promotion and *Ggt* inhibition in the presence of the host plant.
2. To screen the isolates for antibiotic production using a PCR screening method.

## 5.2 Methods

### 5.2.1 Seed sterilization

Wheat (*Triticum aestivum*) seeds of cultivars Cadenza and Hereward were kindly provided by Vanessa McMillan (Rothamsted Research). The seeds were surface sterilized by immersion in a 2.5% (v/v with sterile Nano pure water n$H_2O$) solution of house-hold bleach for 3 mins followed by 3 rinses in sterile n$H_2O$. The seeds were then allowed to air dry for up to 3 h in class 2 safety cabinet (Labogene) (Weller & Cook, 1983). Methods for validation of seed sterilization were described in section 2.8.

### 5.2.2 Inoculum preparation and plant growth promotion assay

The six antagonistic isolates along with two poor *Ggt* inhibitors (negative control) (6 + 2) were grown on KA plates from -80 °C frozen stocks. A single colony was used to inoculate 30 ml of KB incubated at 27 °C in orbital shaker (200 rpm) (Forma Scientific).

1 ml of overnight culture was pelleted by centrifugation at 5000 rcf for 8 mins (bench top microfuge) (Micro Star 12, VWR). The pelleted cells were washed in 1ml PBS twice. 100 µl of washed cells were mixed with 900 µl of PBS and was used to measure the OD (OD600). A volume with a concentration equivalent to OD 1 was used to make a 10 ml washed cell stock in PBS to soak the above sterile seeds in a sterile 50 ml Falcon tube. For each isolate two tubes were prepared one for Cadenza seeds and one for Hereward seeds. Twelve seeds were placed in each tube, and the tubes with soaked seeds were placed in orbital shaker set at 27 °C for 1 h (Forma Scientific). After that the seeds were left to air dry in a class 2 safety cabinet (Labogene) for 2 h. First, to check the plant growth promoting effect of the isolates, in sterile 50 ml Falcon tube, 5 g of sterile fine vermiculite was placed. For each treatment, 3 replicates were

made, where one seed was placed 1 cm below the vermiculite surface. Each tube was watered with 25 ml sterile $nH_2O$ and covered with parafilm. The tubes were placed in a temperature-controlled room set at 21 °C with 16 h light-dark cycle and 70% humidity.

Non-bacterial soaked sterile seeds in PBS were used as a control. The parafilm was removed after 5 days. The seedlings were watered with 5-10 ml sterile $nH_2O$ on day 6 and day 13. The seedlings were ready for processing by day 21.

To check for inoculum recovery, air dried treated seeds were placed on KA plate. The plates were incubated overnight at 27 °C.

### 5.2.3 *In-planta Ggt* antagonistic assay

The growth chamber assay was slightly modified from the methods described by (McSpadden Gardener & Weller, 2001). Briefly, sterile seeds of Cadenza and Hereward soaked in bacterial cell suspensions were prepared as described in the previous section. Five *Ggt* plugs (0.5cm) from a 7-day old culture growing on PDA at 24 °C was placed 2 cm below the sown seed in sterile vermiculite. One seed was sown per 50 ml Falcon tube. The tubes were watered with 25 ml sterile $nH_2O$ and covered with parafilm. The tubes were placed in a temperature-controlled room set at 21°C with 16 h light-dark cycle and 70% humidity. Sterile seeds soaked in 10ml PBS grown in the presence of 5 *Ggt* plugs were used as a control.

### 5.2.4 Plant biomass

At day 21 the wheat seedlings were processed. First, the plants were gently pulled out of the tubes, excess vermiculite was shaken of, and the roots were dipped in 50 ml sterile $nH_2O$ placed in 100 ml sterile Duran to wash out any sticking vermiculite. The

roots were then blotted on lab roll. The shoot height and root lengths were measured with a ruler and recorded. Images of the three replicate plants per treatment were taken along with zoom-in images of the Take-all infected roots. The total number of roots and the infected roots was used to calculate percent infected roots for each seedling. The fresh weight of the seedlings was also recorded.

### 5.2.5 Investigating potential biocontrol agents

Following the initial plant assay, isolates 25R/7, 30R/11 and the mix of the 6 antagonistic isolates were screened at different concentrations for *Ggt* growth inhibition. For each treatment, three 30ml overnight broths were prepared in 50ml Falcon tubes. Then three concentrations were prepared for the washed cells in PBS at OD 1, OD 0.5, and OD 0.1. The procedure for inoculum preparation and seed soaking was described in 5.2.3. Sterile seeds soaked in PBS grown in the presence and absence of *Ggt* were used as controls. For the mix of six, the desired concentration was prepared for each of the six isolates separately then an equivalent volume was taken to make the total of 10ml stock for the seed soak stock suspension. Ten replicates were prepared per treatment. The seeds were grown and harvested as described in section 5.2.3 and 5.2.4. After 21 days, the shoot height, root length, fresh weight and the number of infected roots to healthy roots were determined.

### 5.2.6 Screening for antibiotic gene presence:

The six antagonistic isolates along with isolate 37R/15, least effective were screened for the presence of phenazine-1-carboxylic acid (PCA), and 2, 4-diacetylphloroglunicol (Phl).

### 5.2.6.1 PCR screening for PCA and Phl genes

Purified DNA templates for the six antagonistic isolates along with isolate 37R-15 were prepared using GeneJet genomic DNA purification kit (Thermo Scientific) following the manufacturer instructions. Primers used for PCA and Phl antibiotic screening along with the product size are listed in (Table 29). *Pseudomonas protegens* Pf-5 was included as a positive control for Phl antibiotic and negative for PCA. PCR amplification was carried out in a 20 µl reaction mixture, which contained 10 µl of 2X Taq mix red (PCR Biosystems), 0.8 µl of each primer at 10µM, 6.4 µl of n$H_2O$, and 2 µl of template DNA. The amplification cycle was performed using the BioRad T100 thermal cycler (BioRad) and consisted of an initial denaturation at 94°C for 2 mins, followed by 30 cycles of 94 °C for 1 min, 67 °C for 45 s, and 72 °C for 1 min, then a final extension at 72 °C for 5 mins. The PCR products were separated on a 1% agarose gel in 0.5X TBE buffer stained with gel red at 90V for 40 mins, and were visualized using the G-Box gel documentation system.

**Table 29:** Primers used for Phl and PCA antibiotic screening.

| primer | sequence | GC% | Tm | position | Reference | Product size |
|---|---|---|---|---|---|---|
| Phl2a | GAGGACGTCGAAGACCACCA | 60 | 73 | 1915 | (Raaijmakers *et al.*, 1997) | 745bp (*phlD*) |
| Phl2b | ACCGCAGCATCGTGTATGAG | 55 | 72 | 2660 | (Raaijmakers *et al.*, 1997) | |
| PCA2a | TTGCCAAGCCTCGCTCCAAC | 60 | 79 | 3191 | (Raaijmakers *et al.*, 1997) | 1150bp (*phzC* and *phzD)* |
| PCA3b | CCGCGTTGTTCCTCGTTCAT | 55 | 76 | 4341 | (Raaijmakers *et al.*, 1997) | |

### 5.2.7 Statistical analysis

The GENSTAT (17th edition, VSN International Ltd, Hemel Hempstead, UK) statistical package was used to analyse the data. For the initial plant biomass data analysis of variance, ANOVA, was used to analyse the data. While generalized linear model, GLM, was used to analyse the infected roots data. For the second plant assay mixed model using REML variance components was used to analyse the plant biomass data and GLM was used to analyse the infected roots data.

## 5.3 Results

In chapter four, out of the 411 *Pseudomonas* isolates tested, six maintained their *Ggt* inhibition when challenged in 1:1 *in vitro* setup. These six isolates are further investigated here for their plant growth promotion and *Ggt* control in presence of host plant. The wheat seeds of Cadenza and Hereward cultivars were surface sterilized and soaked in bacteria suspension. Methods for validation of seed sterilization were described in section 2.8 and no growth was recovered from the surface sterilized and air dried seeds on both KA and WA plates. The coated seeds were allowed to air dry in a class 2 safety cabinet prior to sowing. Bacterial suspensions consisted of individual isolates in addition to a mixture of the six antagonistic isolates. The two least effective isolates were included for comparison. Air dry coated seeds were grown in the presence and absence of five *Ggt* plugs. Initial analysis has showed that 5 plugs are sufficient to cause the disease while 3 plugs did not cause any disease (data not shown). In addition, as described in section 5.2.2 growth of fluorescent colony on the KA plate with bacterial coated and air dried seeds indicated the success of the treatment recovery.

### 5.3.1 Statistical analysis for plant growth promotion

Shoot height, root length and fresh weight data collected as described in 5.2.4 were used to check for plant growth promotion. Raw data are plotted in Figs 21-23. Analysis of variance (ANOVA) was performed on the shoot, root and fresh weight data (Appendix I 9.3.1) using the model Cultivar*(coating/ (Isolate+*Ggt*)). Shoot height shows that there was a significant difference between the cultivars Cadenza and Hereward ($F < 0.001$) (Fig.24). Also, the interaction between the coated seeds and the treatments where *Ggt* was present or absent was significant ($F < 0.001$). However, overall the interaction was not significant ($F=0.464$) (Table 30).

For root length there was a significant difference between the cultivars Cadenza and Hereward ($F = 0.004$) (Fig.25). While coating with bacteria had no significant effect on root length ($F = 0.771$). Overall there was no significant difference based on the three way interaction ($F= 0.097$) (Table 31). There was no significant difference between the cultivars in the fresh weight ($F = 0.185$) (Table 32). However, there was a significant difference between the sterile and coated seeds in interaction with the presence or absence of *Ggt* ($F < 0.001$). Overall there was no significant difference when taking into account the addition of bacteria into the system ($F = 0.312$) (Fig.26).

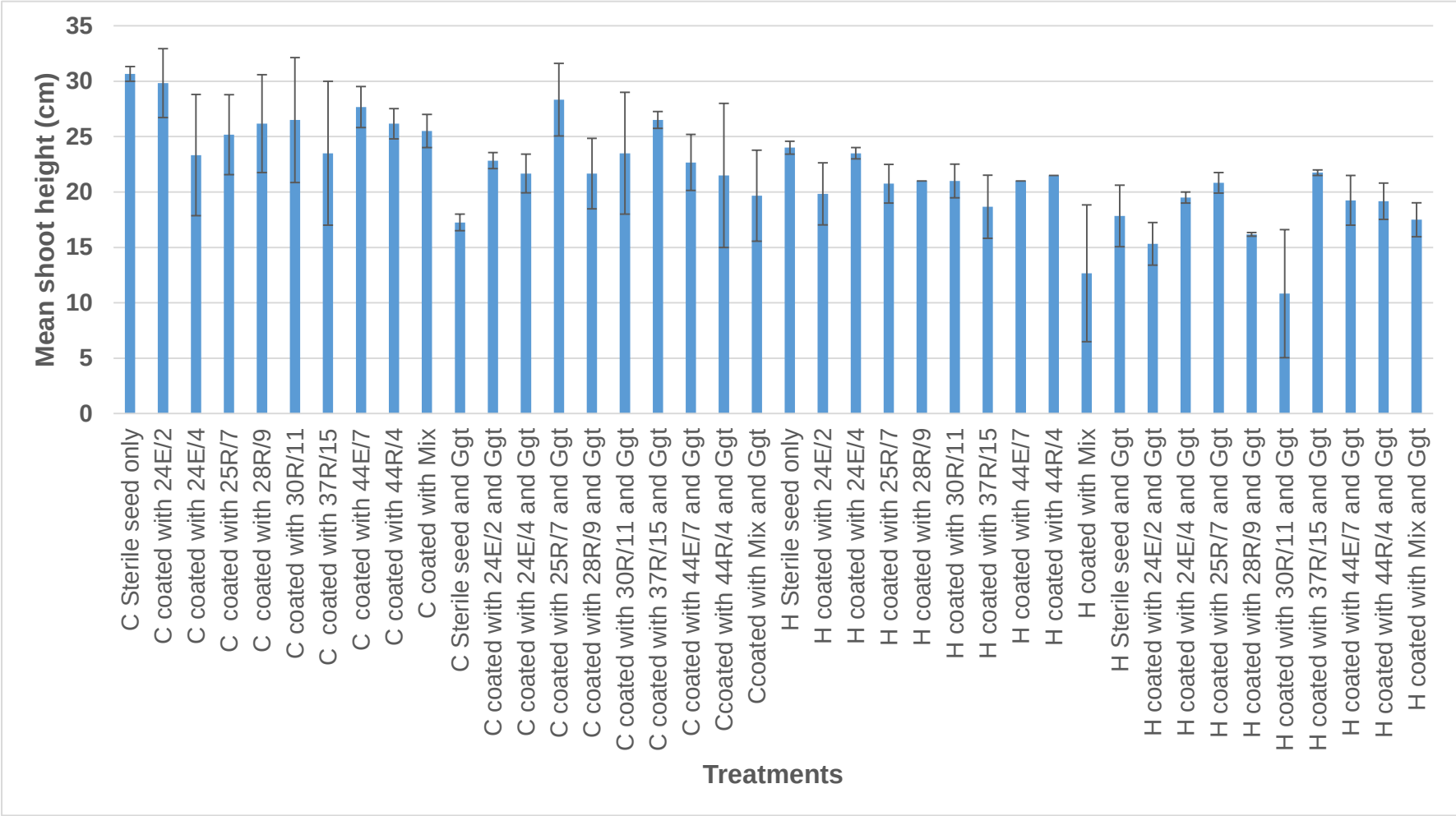
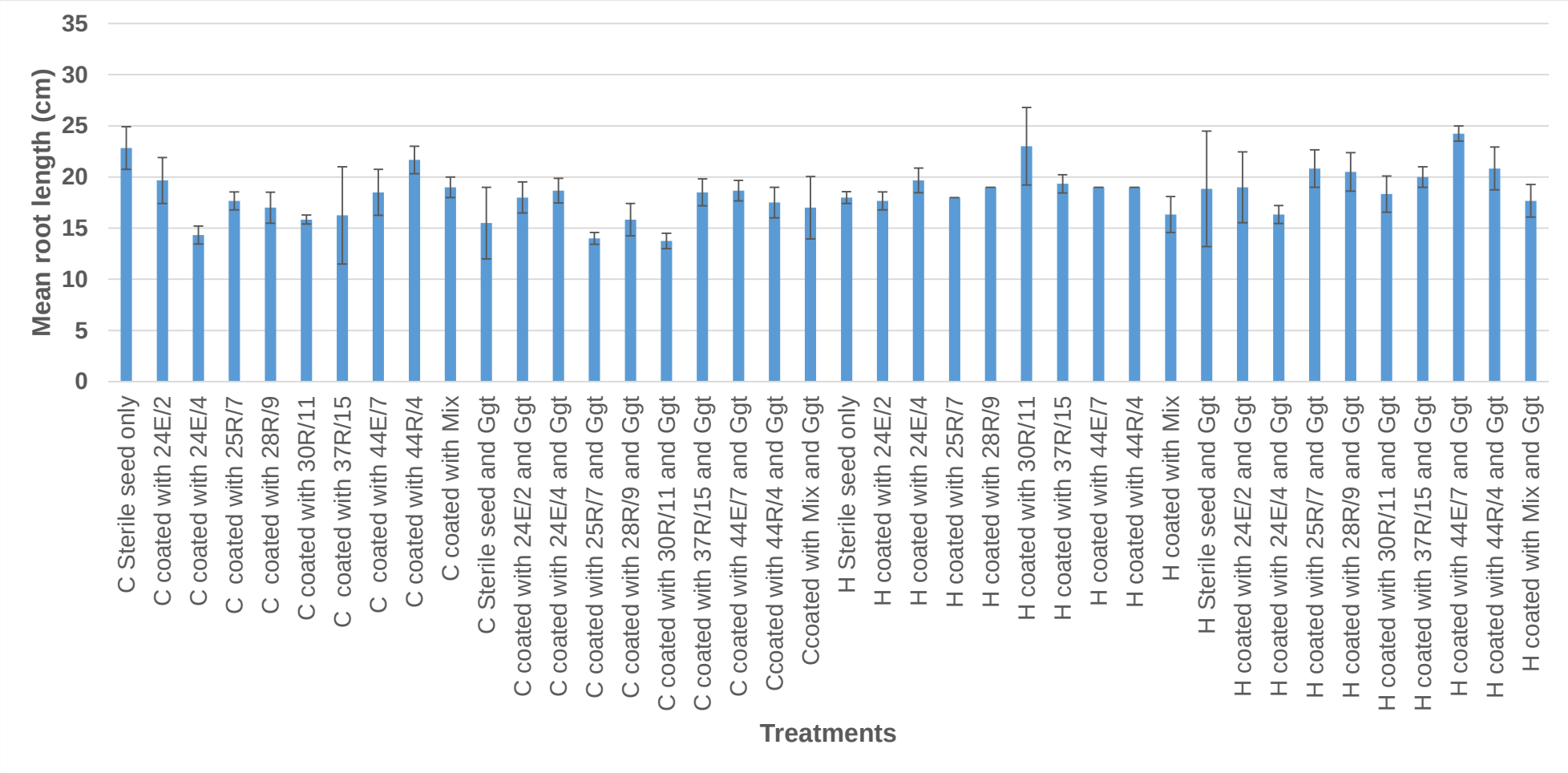



**Figure 21: Mean shoot height for Cadenza and Hereward 21 day old seedlings.** Error bars are based on s.e




**22: Mean root length for Cadenza and Hereward 21 day old seedlings**. Error bars are based on s.e.

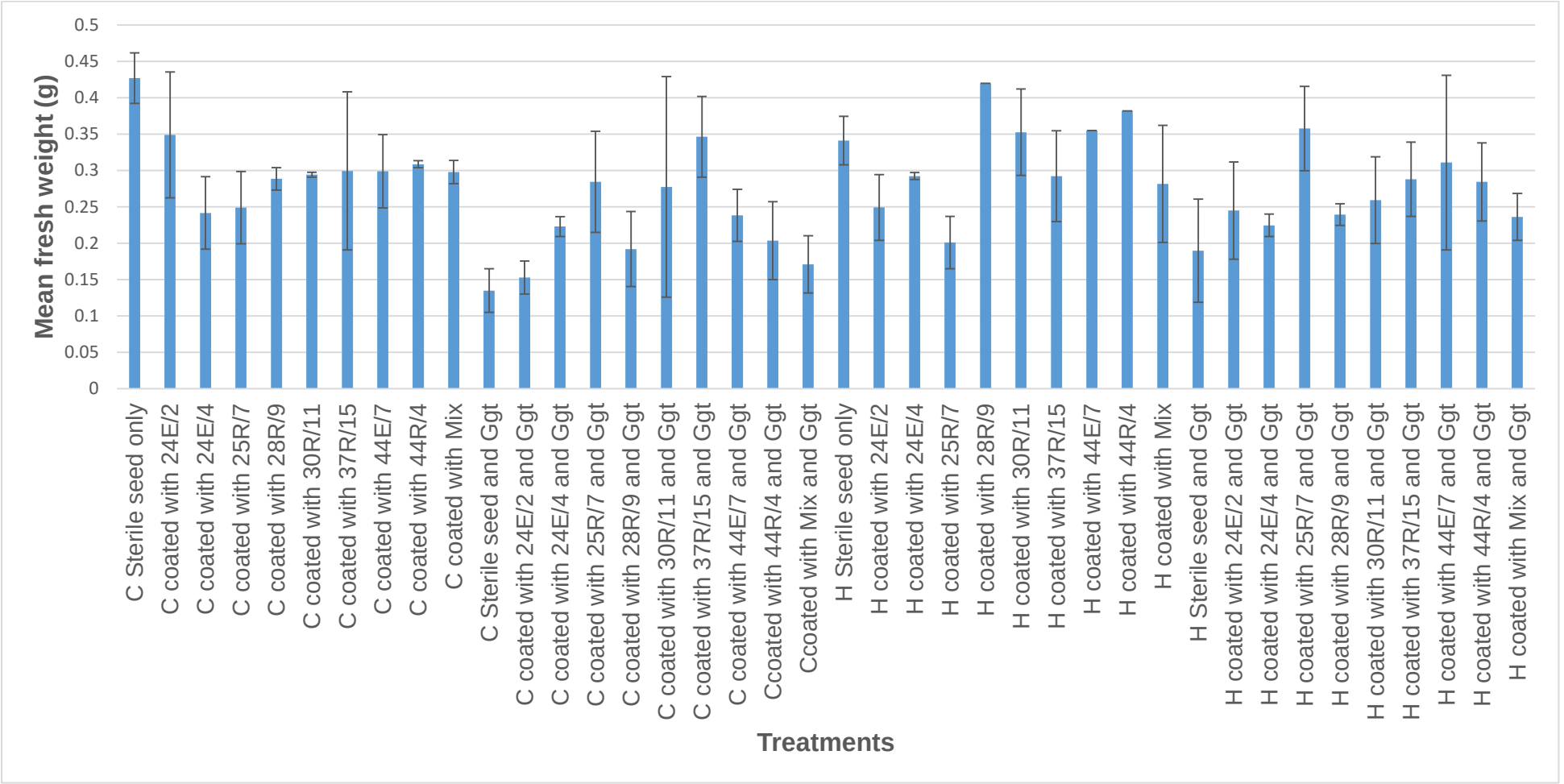



**Figure 23: Mean Fresh weight for Cadenza and Hereward 21 day old seedlings**. Error bars are based on s.e.

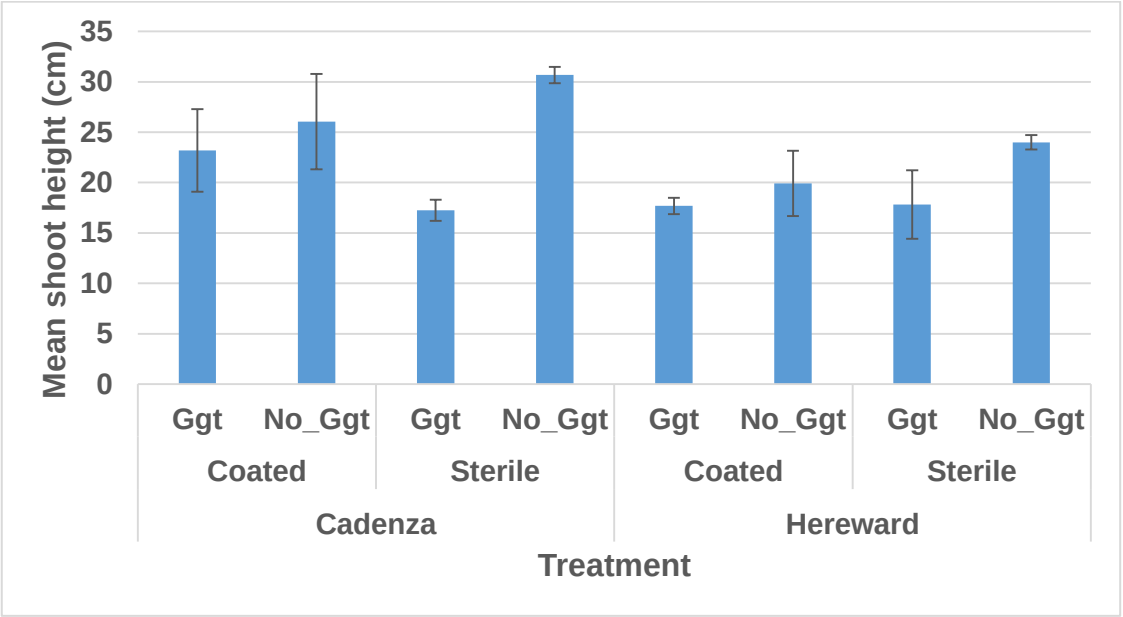



**Figure 24: ANOVA mean shoot height for Cadenza and Hereward 21 day old seedlings.**The bars are plotted form ANOVA mean table for the interaction of cultivar*coating*Ggt, combining the 10 treatments in the presence or absence of *Ggt* . s.e.d=1.417 and d.f.= 82. Error bars are based on s.e. (s.e.d: standard error of differences, d.f.: degrees of freedom, s.e.: standard error).

**Table 30:** ANOVA table for shoot height data. Cultivar: Cadenza or Hereward; Coating: soaked in bacterial suspension or sterile; Ggt: present or absent; Isolate: 24E/2, 24E/4, 25R/7, 28R/9, 30R/11, 44E/7, Mixture of the previous, 37R/15 and 44R/4; d.f: degrees of freedom; F pr.: F probability.

| **Source of variation** | **d.f.** | **(m.v.)** | **s.s.** | **m.s.** | **v.r.** | **F pr.** |
|---|---|---|---|---|---|---|
| Cultivar | 1 | | 919.41 | 919.41 | 33.92 | <.001 |
| Coating | 1 | | 5.69 | 5.69 | 0.21 | 0.648 |
| Cultivar.Coating | 1 | | 20.73 | 20.73 | 0.76 | 0.384 |
| Coating.Isolate | 8 | | 218.66 | 27.33 | 1.01 | 0.436 |
| Coating.Ggt | 2 | | 461.84 | 230.92 | 8.52 | <.001 |
| Cultivar.Coating.Isolate | 8 | | 149.26 | 18.66 | 0.69 | 0.701 |
| Cultivar.Coating.Ggt | 2 | | 42.06 | 21.03 | 0.78 | 0.464 |
| Residual | 82 | -14 | 2222.45 | 27.1 | | |
| Total | 105 | -14 | 3950.98 | | | |

**Table 31:** ANOVA table for root length data. Cultivar: Cadenza or Hereward; Coating: soaked in bacterial suspension or sterile; Ggt: present or absent; Isolate: 24E/2, 24E/4, 25R/7, 28R/9, 30R/11, 44E/7, Mixture of the previous, 37R/15 and 44R/4; d.f: degrees of freedom; F pr.: F probability.

| **Source of variation** | **d.f.** | **(m.v.)** | **s.s.** | **m.s.** | **v.r.** | **F pr.** |
|---|---|---|---|---|---|---|
| Cultivar | 1 | | 106.41 | 106.41 | 8.91 | 0.004 |
| Coating | 1 | | 1.02 | 1.02 | 0.09 | 0.771 |
| Cultivar.Coating | 1 | | 23.12 | 23.12 | 1.94 | 0.168 |
| Coating.Isolate | 8 | | 126.42 | 15.8 | 1.32 | 0.244 |
| Coating.Ggt | 2 | | 34.28 | 17.14 | 1.44 | 0.244 |
| Cultivar.Coating.Isolate | 8 | | 122.39 | 15.3 | 1.28 | 0.265 |
| Cultivar.Coating.Ggt | 2 | | 57.31 | 28.65 | 2.4 | 0.097 |
| Residual | 82 | -14 | 979.04 | 11.94 | | |
| Total | 105 | -14 | 1363.64 | | | |

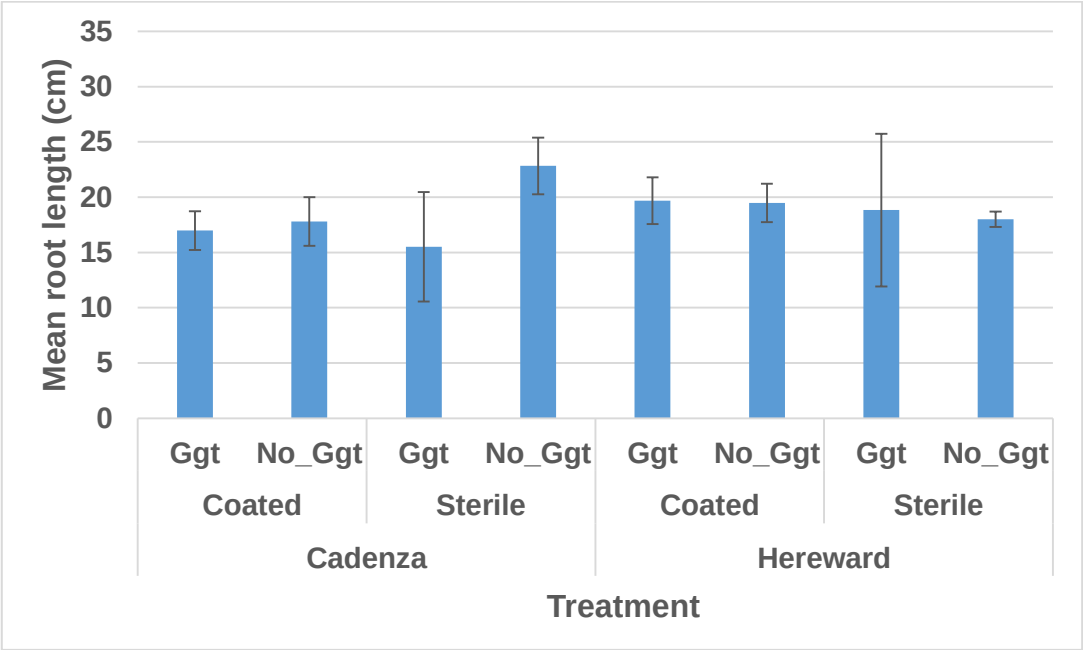



**Figure 25: ANOVA mean root length for Cadenza and Hereward 21 day old seedlings.**The bars are plotted form ANOVA mean table for the interaction of cultivar *coating*Ggt combining the 10 treatments in the presence or absence of Ggt. s.e.d=0.94 and d.f. = 82. Error bars are based on s.e. (s.e.d: standard error of differences, d.f.: degrees of freedom, s.e.: standard error).

**Table 32:** ANOVA of fresh weight data. Cultivar: Cadenza or Hereward; Coating: soaked in bacterial suspension or sterile; Ggt: present or absent; Isolate: 24E/2, 24E/4, 25R/7, 28R/9, 30R/11, 44E/7, Mixture of the previous, 37R/15 and 44R/4; d.f: degrees of freedom; F pr.: F probability.

| **Source of variation** | **d.f.** | **(m.v.)** | **s.s.** | **m.s.** | **v.r.** | **F pr.** |
|---|---|---|---|---|---|---|
| Cultivar | 1 | | 0.014518 | 0.014518 | 1.78 | 0.185 |
| Coating | 1 | | 0.000059 | 0.000059 | 0.01 | 0.932 |
| Cultivar.Coating | 1 | | 0.004688 | 0.004688 | 0.58 | 0.45 |
| Coating.Isolate | 8 | | 0.061032 | 0.007629 | 0.94 | 0.49 |
| Coating.Ggt | 2 | | 0.209625 | 0.104813 | 12.89 | <.001 |
| Cultivar.Coating.Isolate | 8 | | 0.027689 | 0.003461 | 0.43 | 0.903 |
| Cultivar.Coating.Ggt | 2 | | 0.019235 | 0.009617 | 1.18 | 0.312 |
| Residual | 82 | -14 | 0.667015 | 0.008134 | | |
| Total | 105 | -14 | 0.949346 | | | |

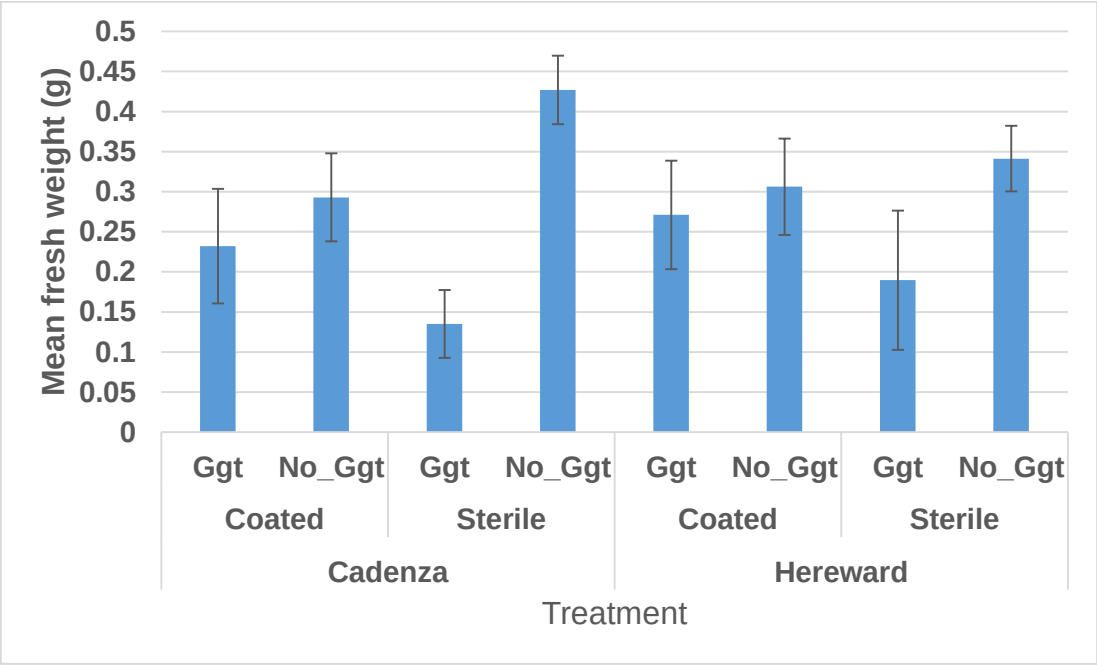



**Figure 26: ANOVA mean fresh weight for Cadenza and Hereward 21 day old seedlings**.The bars are plotted form ANOVA mean table for the interaction of cultivar*coating*Ggt combining the 10 treatments in the presence or absence of *Ggt* . s.e.d=0.02455 and d.f.= 82. Error bars are based on s.e. (s.e.d: standard error of differences, d.f.: degrees of freedom, s.e.: standard error).

### 5.3.2 Take-all disease control

When *Ggt* was absent the roots were healthy and no blacking was observed as described in section 5.2.4. Thus, treatments in the presence of *Ggt* plugs were used to compare disease control excluding the non-*Ggt* inoculated treatments. Raw data are plotted in Fig. 27.

Assessment of the number of infected roots to the total number of roots was used as a measure for disease control. Generalized linear model (GLM) assuming a Binomial distribution on logit scale was used to analyse the number of infected roots (Appendix I, table e). The model fitted was Cultivar*Isolate with the total number of roots as the binomial total. There was a significant difference in the number of infected roots between the two cultivars in the presence of the isolates (F= 0.031) (Table 33). When looking at the prediction from the regression model, inoculation of Cadenza with isolate number 4, 25R/7, led to less infected roots (mean= 0.095, s.e.=0.63). While inoculation of Hereward with isolate number 6, 30R/11, led to less infected roots (mean= -1.872, s.e. =1.09) (Table 34) (Fig. 28).

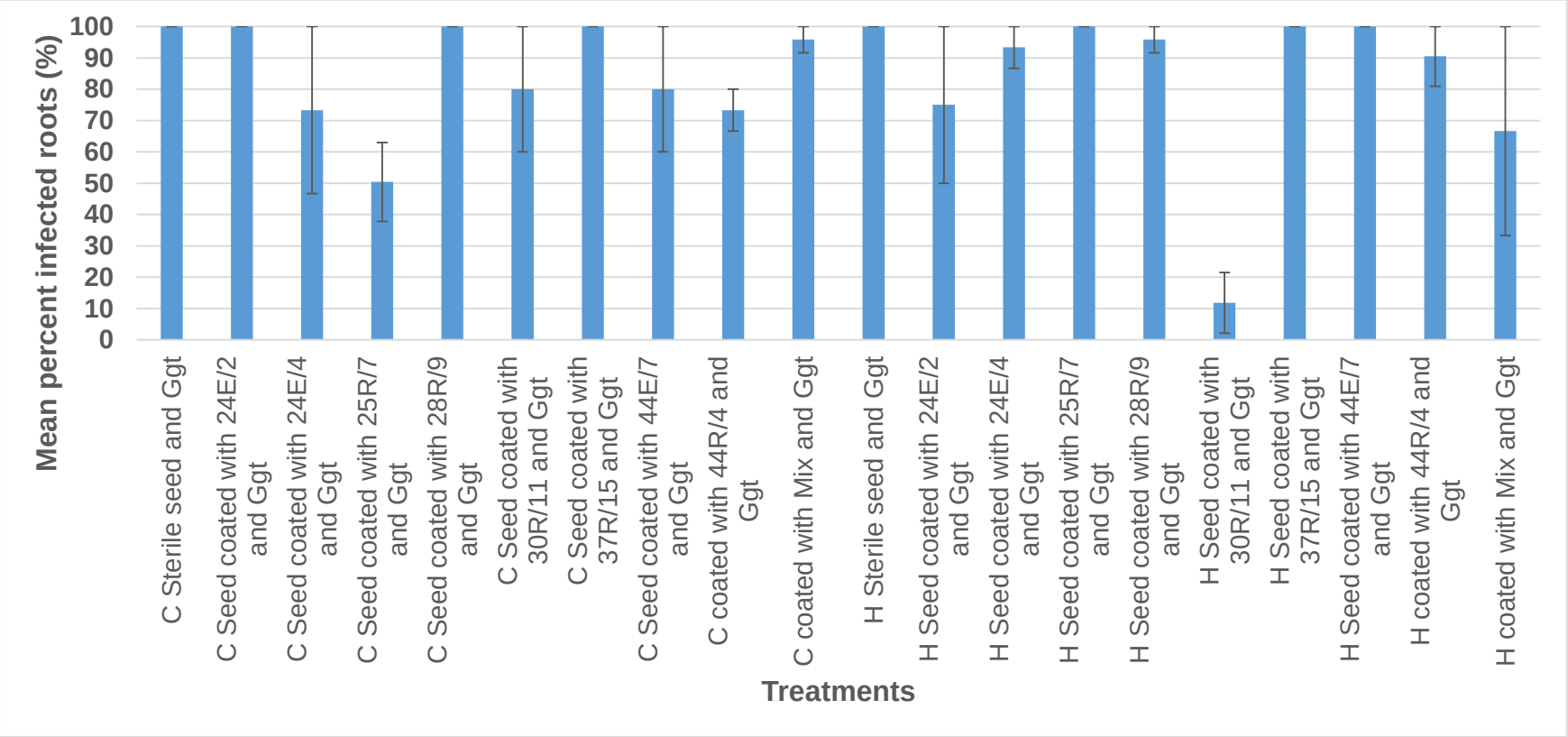



**Figure 27: Mean percent infected roots for Cadenza and Hereward 21 day old seedlings**. Error bars are based on s.e.

**Table 33:** Accumulated analysis of deviance from GLM on infected root data.

| Change | d.f. | deviance | mean deviance | deviance ratio | Approx. F pr. |
|---|---|---|---|---|---|
| + cultivar | 1 | 0.474 | 0.474 | 0.23 | 0.634 |
| + Isolate | 9 | 55.536 | 6.171 | 3 | 0.009 |
| + cultivar.Isolate | 9 | 44.512 | 4.946 | 2.4 | 0.031 |
| Residual | 35 | 72.025 | 2.058 | | |
| Total | 54 | 172.547 | 3.195 | | |

**Table 34:** Prediction from the regression model.

| Cultivar | C_Predictions | s.e. | H_Predictions | s.e. |
|---|---|---|---|---|
| **Isolate** | | | | |
| Sterile | 11.754 | 93.6 | 11.762 | 75.57 |
| 24E/2 | 11.879 | 73.87 | 1.466 | 0.92 |
| 24E/4 | 1.179 | 0.82 | 2.708 | 1.48 |
| 25R/7 | 0.095 | 0.63 | 11.721 | 76.34 |
| 28R/9 | 11.921 | 73.65 | 2.833 | 1.48 |
| 30R/11 | 1.504 | 1.12 | -1.872 | 1.09 |
| 37R/15 | 11.721 | 76.34 | 11.855 | 90.57 |
| 44E/7 | 1.466 | 0.92 | 11.6 | 95.78 |
| 44R/4 | 1.099 | 1.17 | 2.015 | 1.08 |
| Mix | 2.944 | 1.47 | 0.693 | 0.79 |

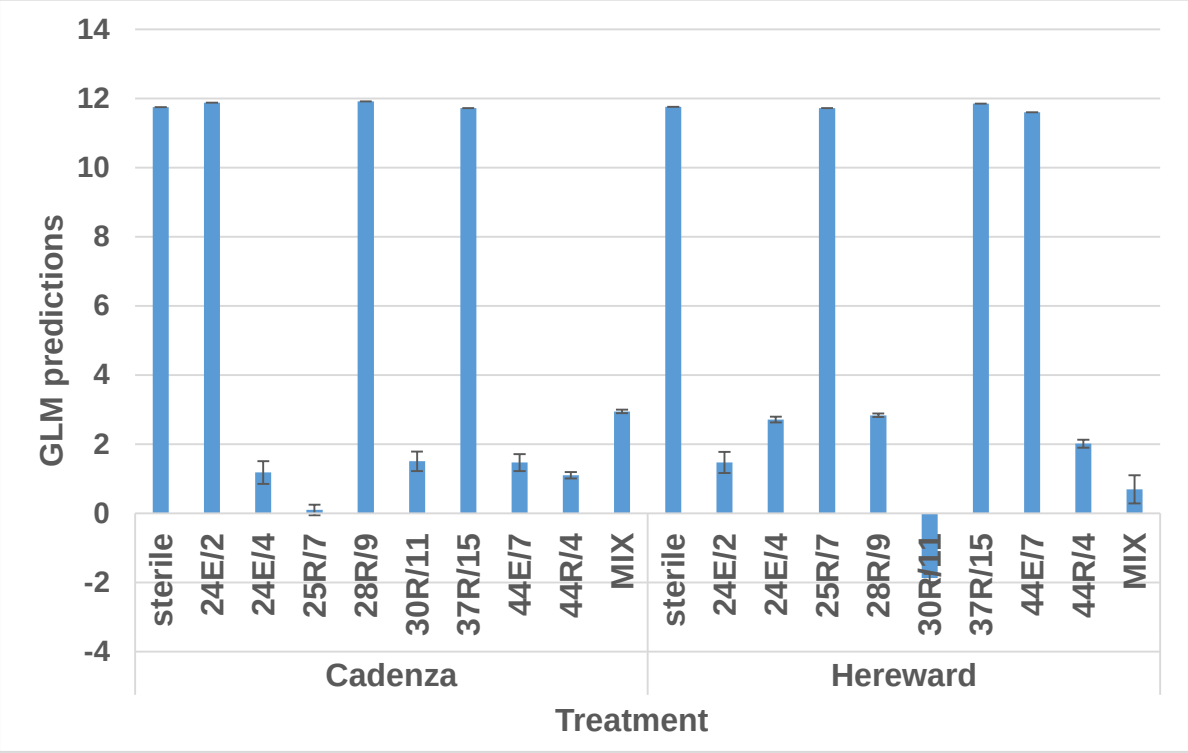



**Figure 28: Analysis of infected roots from Cadenza and Hereward 21 day old seedlings in the presence of *Ggt.*** Treatments included *Ggt* with sterile seeds and in combination with the isolates 24E/2, 24E/4, 25R/7, 30R/11, 28R/9, 44E/7, mixture of the six along with two least effective antagonists' 37R/15 and 44R/4. Bars are plotted form GLM prediction tables (Table 36). Error bars are based on s.e.

### 5.3.3 Potential biocontrol agents

Given strains 25R/7 and 30R/11 appeared the most promising biocontrol agents of *Ggt* (Fig. 28), these were selected for further analysis. The strains were analysed at different concentrations (100%, 50%, and 10% concentration of washed cells) equivalent to OD 1, OD 0.5, and OD 0.1 respectively. The mixture of the six strains was also tested at these concentrations. Seeds coated with sterile PBS growing in the presence and absence of *Ggt* were used as controls. Ten replicates were made for each treatment. The plants were harvested at day 21 and were analysed for shoot height, root length, fresh weight and the number of infected roots to the total number of roots as described in detail in section 5.2.5.

#### 5.3.3.3 Statistical analysis of plant biomass

Plant biomass data were collected as described in section 5.2.4. Raw data for shoot height, root length and fresh weight are shown in Figs. 29-31. Since the number of treatments was unbalanced, linear mixed model (REML) analysis of variance was used to analyse the shoot height, root length and fresh weight. While GLM was used to analyse the infected roots data.

Shoot height, root length and fresh weight were analysed using REML variance component and the model used was cultivar*(coating/ (isolate*conc+Ggt)). The non-significant terms were then gradually dropped off from the model to finally get all significant terms (Tables 35-37). In both shoot height and root length data there was a significant difference between the sterile and treated seeds from both cultivars (F= 0.019 and F=0.003, respectively).There was also a significant difference between the

sterile seed growing with/without *Ggt* and the treated seeds growing in the presence of *Ggt* (F= 0.038 and F= 0.032; respectively) (Figs. 32 and 33).

For fresh weight data, the interaction was significant at its highest level, where there was a significant difference between the cultivars in the used treatments (*i.e.:* sterile vs. treated with different bacteria at different concentrations) (F= 0.005) (Fig.34). Raw data are in (Appendix II 9.3.2).

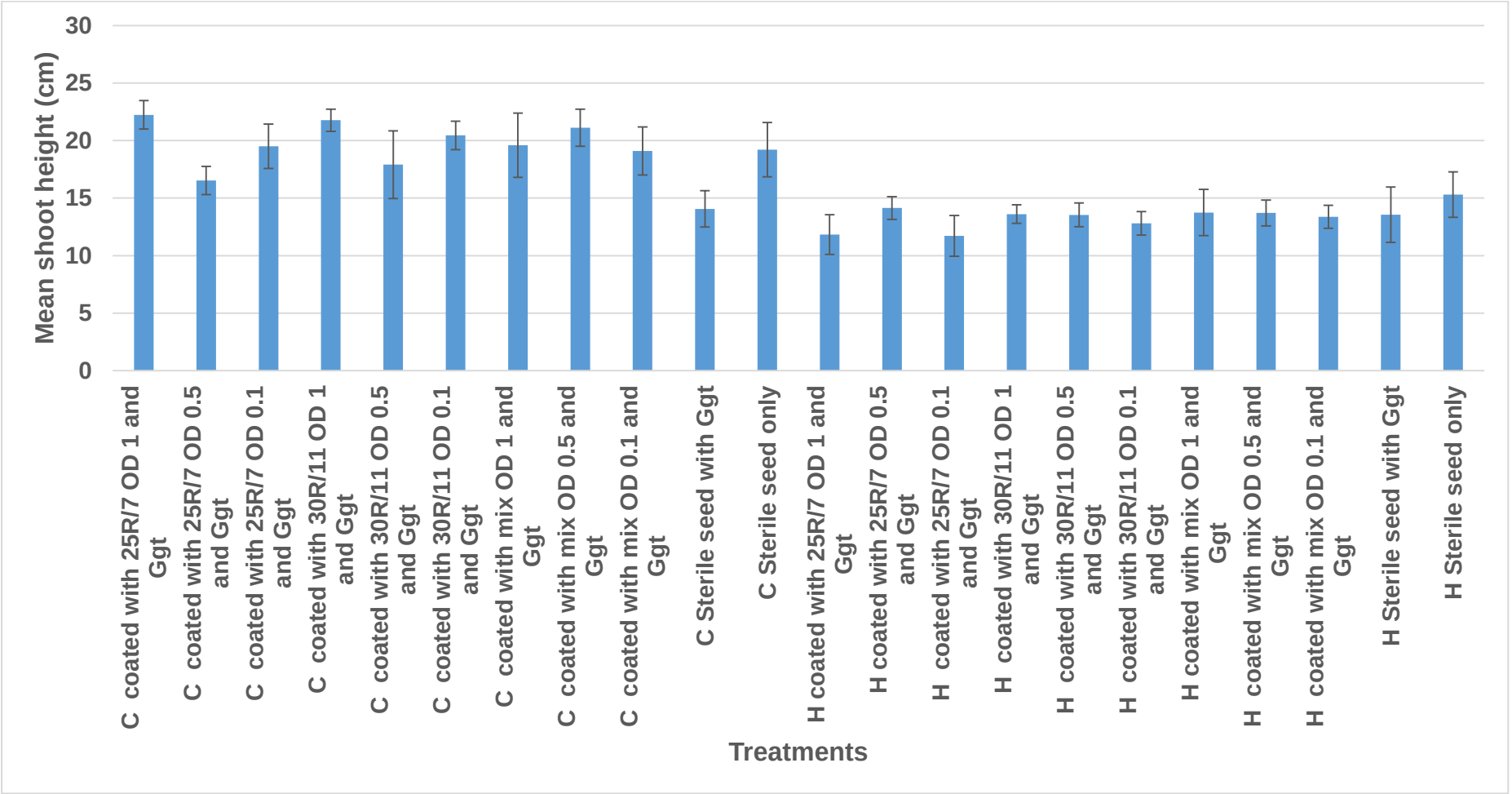



**Figure 29: Mean shoot height of Cadenza and Hereward 21 day old seedlings.** Using different concentrations of strains 25R/7, 30R/11, and the mixture of six. Error bars are based on s.e.

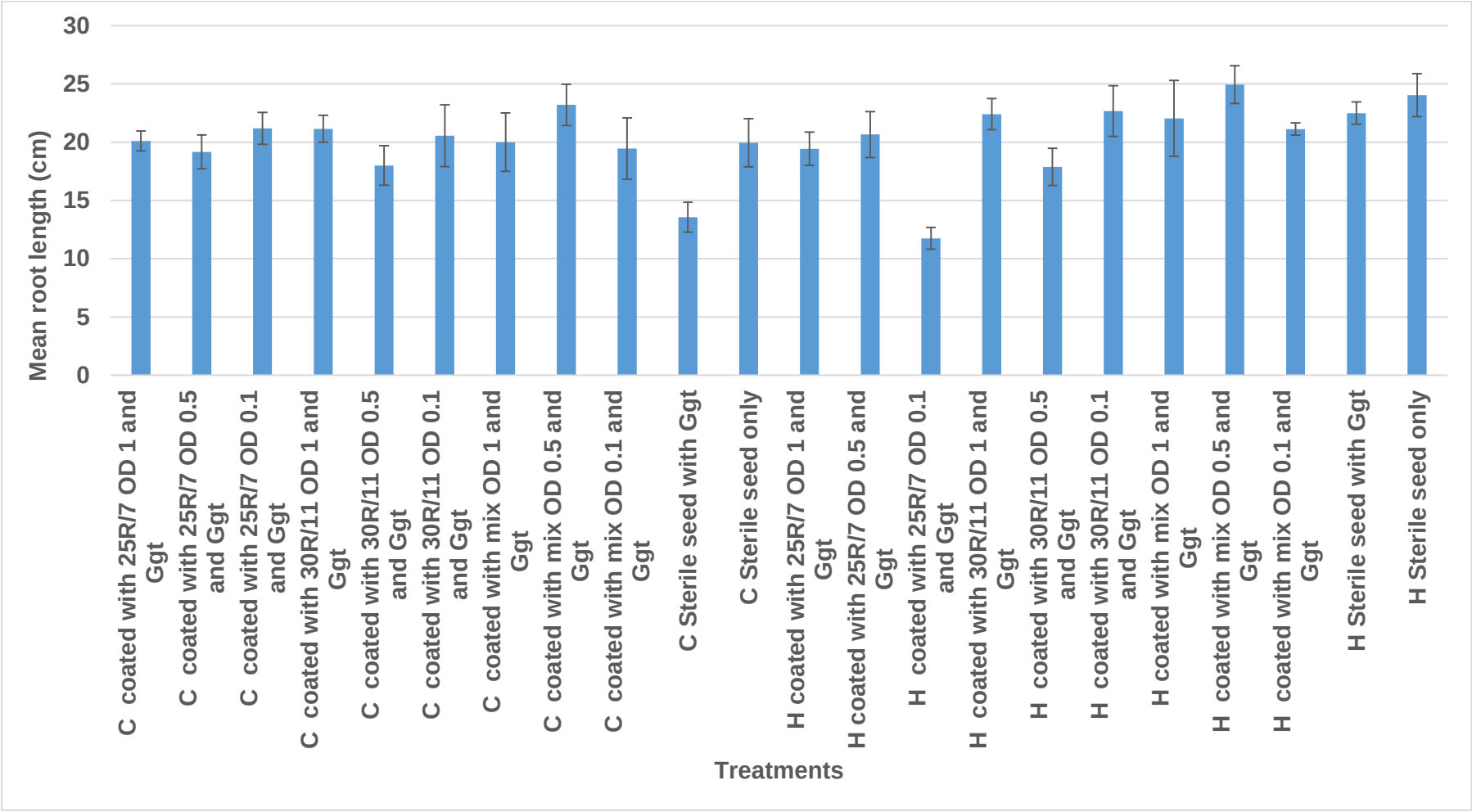



**Figure 29: Mean root length for Cadenza and Hereward 21 day old seedlings.** Using different concentrations of strains 25R/7, 30R/11, and the mixture of six. Error bars are based on s.e.

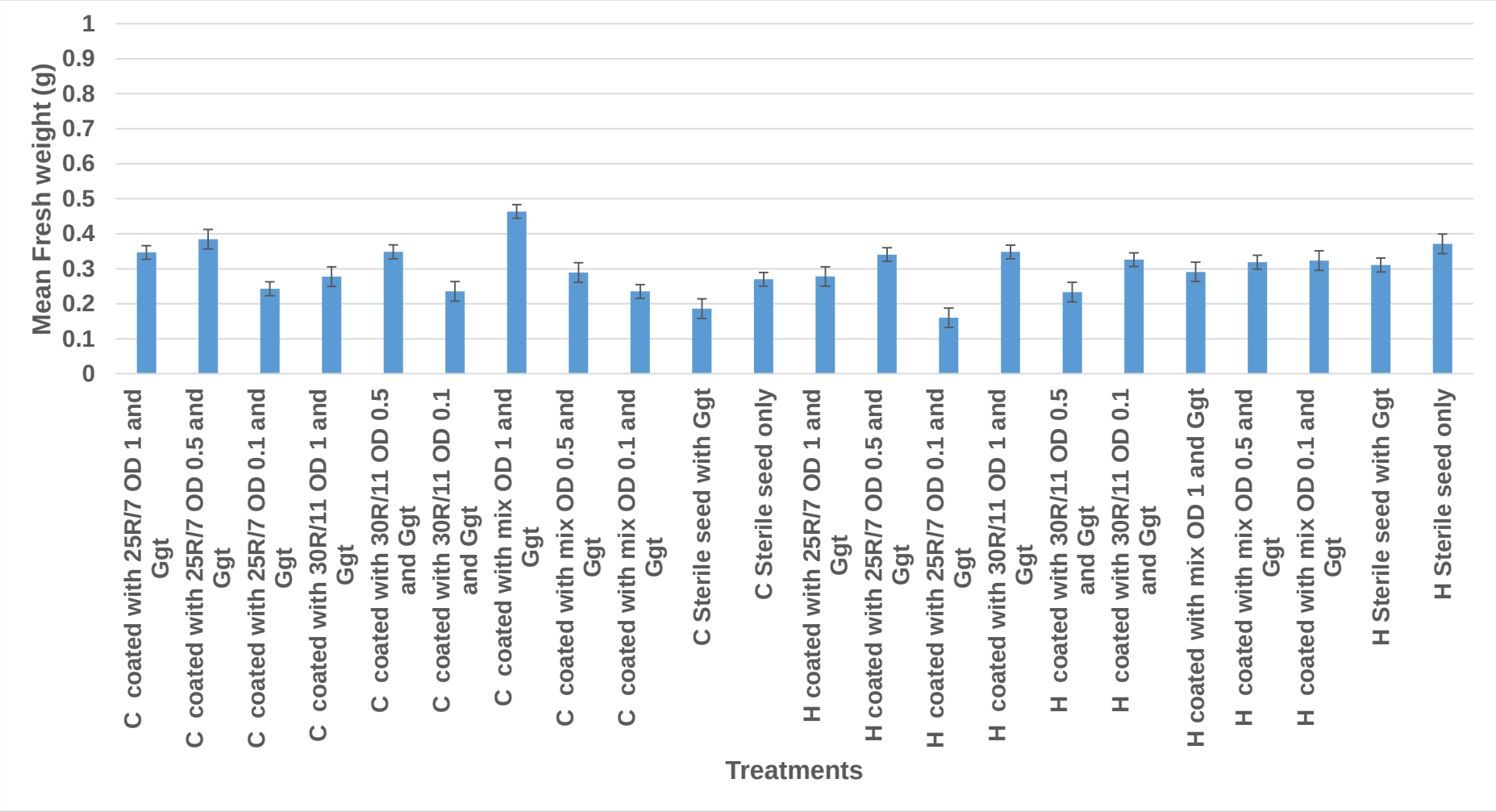



**Figure 30: Mean fresh weight for Cadenza and Hereward 21 day old seedlings**. Using different concentrations of strains 25R/7, 30R/11, and the mixture of six. Error bars are based on s.e.

**Table 35:** REML variance components for Shoot height data. Cultivar: Cadenza or Hereward; Coating: soaked in bacterial suspension or sterile; *Ggt*: present or absent.

| Fixed term | Wald statistic | n.d.f. | F statistic | d.d.f. | F pr. |
|---|---|---|---|---|---|
| Cultivar | 63.61 | 1 | 63.61 | 190 | <0.001 |
| Coating | 1.03 | 1 | 1.03 | 190 | 0.311 |
| Cultivar.Coating | 5.62 | 1 | 5.62 | 190 | 0.019 |
| Coating.Ggt | 4.35 | 1 | 4.35 | 190 | 0.038 |

**Table 36:** REML variance components for root length data. Cultivar: Cadenza or Hereward; Coating: soaked in bacterial suspension or sterile; *Ggt*: present or absent.

| Fixed term | Wald statistic | n.d.f. | F statistic | d.d.f. | F pr. |
|---|---|---|---|---|---|
| Cultivar | 3.31 | 1 | 3.31 | 190 | 0.07 |
| Coating | 0.15 | 1 | 0.15 | 190 | 0.697 |
| Cultivar.Coating | 8.83 | 1 | 8.83 | 190 | 0.003 |
| Coating.Ggt | 4.65 | 1 | 4.65 | 190 | 0.032 |

**Table 37:** REML variance components for fresh weight data. Cultivar: Cadenza or Hereward; Coating: soaked in bacterial suspension or sterile; Conc: concentration at OD 1, OD 0.5 and OD 0.1.

| Fixed term | Wald statistic | n.d.f. | F statistic | d.d.f. | F pr. |
|---|---|---|---|---|---|
| Cultivar | 0.04 | 1 | 0.04 | 175 | 0.838 |
| Coating | 0.79 | 1 | 0.79 | 175 | 0.375 |
| Cultivar.Coating | 9.42 | 1 | 9.42 | 175 | 0.002 |
| Coating.Isolate | 1.27 | 2 | 0.63 | 175 | 0.532 |
| Coating.Conc | 11.6 | 2 | 5.8 | 175 | 0.004 |
| Cultivar.Coating.Isolate | 2.99 | 2 | 1.5 | 175 | 0.227 |
| Cultivar.Coating.Conc | 5.39 | 2 | 2.7 | 175 | 0.07 |
| Coating.Isolate.Conc | 8.64 | 4 | 2.16 | 175 | 0.076 |
| Cultivar.Coating.Isolate.Conc | 15.41 | 4 | 3.85 | 175 | 0.005 |

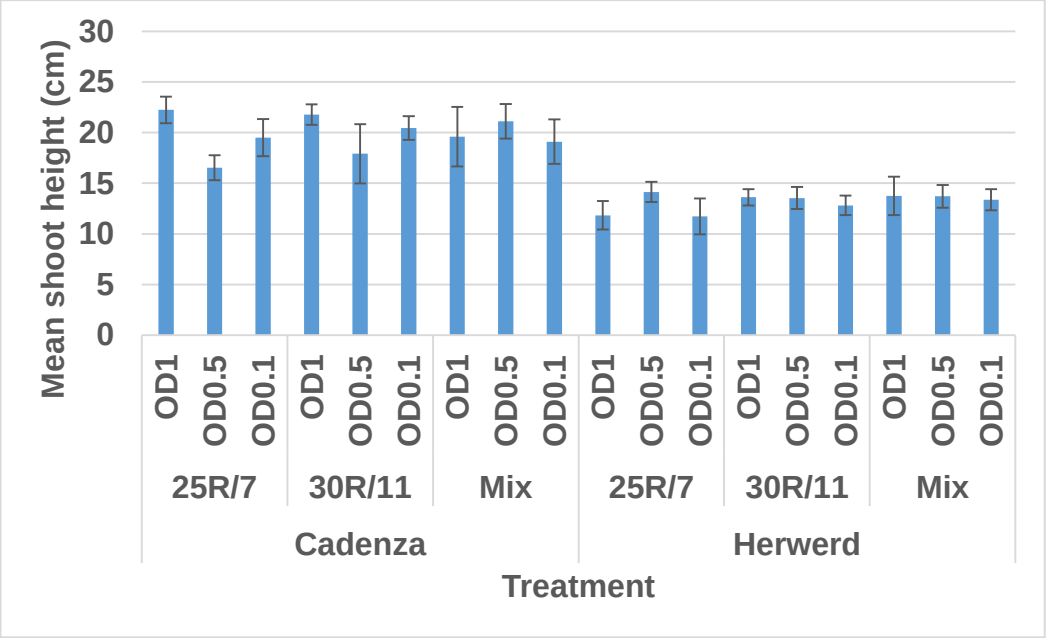



**Figure 31: ANOVA mean shoot height of Cadenza and Hereward 21 day old seedlings.** Using different concentrations of strains 25R/7, 30R/11, and the mix of six and in the presence of Ggt**.** The concentrations were equivalent to OD 1, 0.5, and 0.1. The bars are plotted from ANOVA table of means for the fitted model. Error bars are based on s.e.

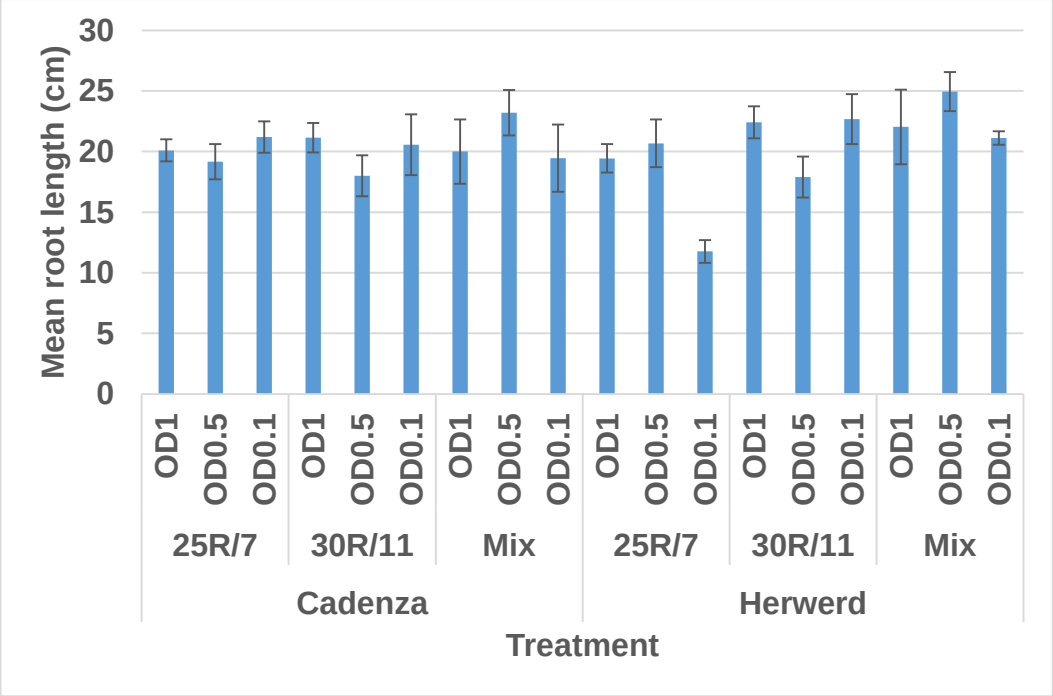



**Figure 32: ANOVA mean root length of Cadenza and Hereward 21 day old seedlings.** Using different concentrations of strains 25R/7, 30R/11, and the mix of six and in the presence of Ggt. The concentrations were equivalent to OD 1, 0.5, and 0.1. The bars are plotted from ANOVA table of means for the fitted model. Error bars are based on s.e.

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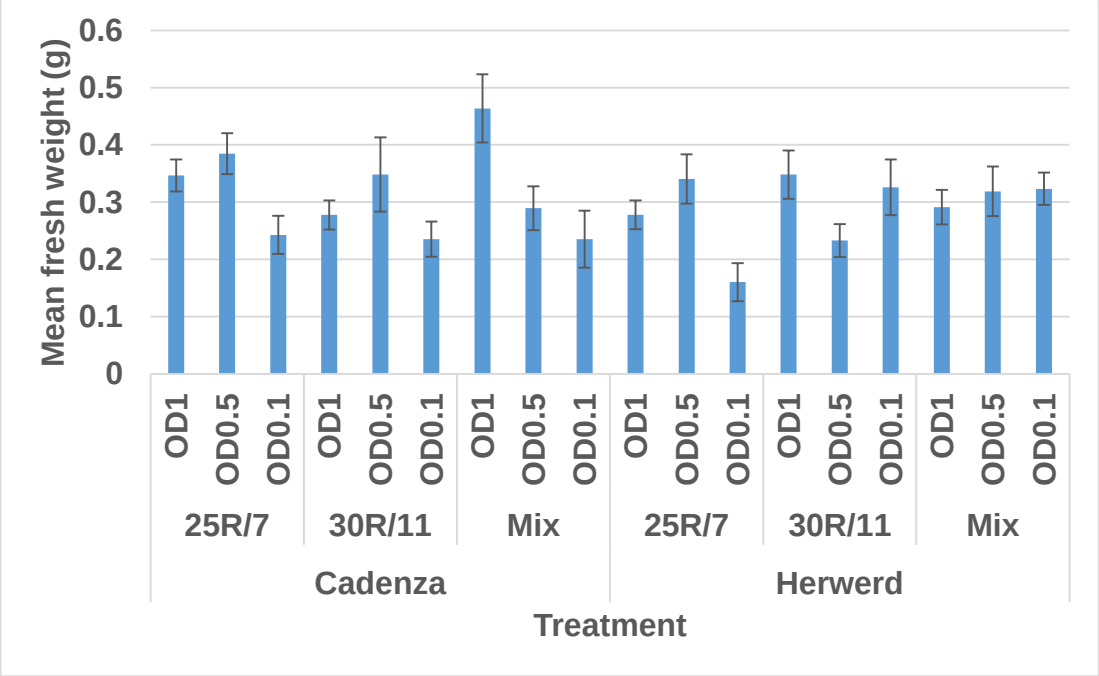


**Figure 33: ANOVA mean fresh weight of Cadenza and Hereward 21 day old seedlings.** Using different concentrations of strains 25R/7, 30R/11, and the mix of six and in the presence of Ggt. The concentrations were equivalent to OD 1, 0.5, and 0.1. The bars are plotted from ANOVA table of means for the fitted model. Error bars are based on s.e.

Raw data showing percent infected roots for all the treatments are shown in Fig. 35. For infected root data Appendix III 9.3.3, a generalized liner model (GLM) assuming a binomial distribution with logit link for the response infected roots was used. The total number of roots were used as the binomial totals to fit the model Cultivar*(Coating/(Isolate*Conc+*Ggt*)). However, few problems were encountered during fitting the model, which were then resolved by removing the sterile treatments (*i.e.*: controls). Once the 4-sterile treatment have been removed the model was reduced to Cultivar*Isolate*Conc. There was a significant difference between the cultivars coated with the different isolates at the used concentrations (F= 0.052) (Table 38) (Fig.36).

When looking at the predictions from the regression model in Table 39, for Cadenza at concentration equivalent to OD 1 the mixture of six had led to less infected roots (mean = -1.194, s.e.=0.746), followed by isolate 25R/7 (mean= 1.012, s.e.= 0.604). As the concentration decreased the effectiveness of the mixture of six decreased. This is shown by more infected roots at the concentration equivalent to OD 0.1 (mean =0.944, s.e. =0.651). While isolate 25R/7 had the least infected roots at concentration equivalent to OD 0.1. Overall, when ignoring the mixture of six, isolate 25R/7 had led to less infected roots than isolate 30R/11 in Cadenza except at concentration equivalent to OD 0.5. In Hereward when ignoring the mixture of six, isolate 30R/11 had led to less infected roots than 25R/7, except at concentration equivalent to O D0.1. In addition, as the concentration decreased the number of infected roots increased. This concentration dependent effect was true for 25R/7 and 30R/11 but not for the mix of six. Where at concentration equivalent to OD 0.5 the mix of six had led to the least number of infected roots.

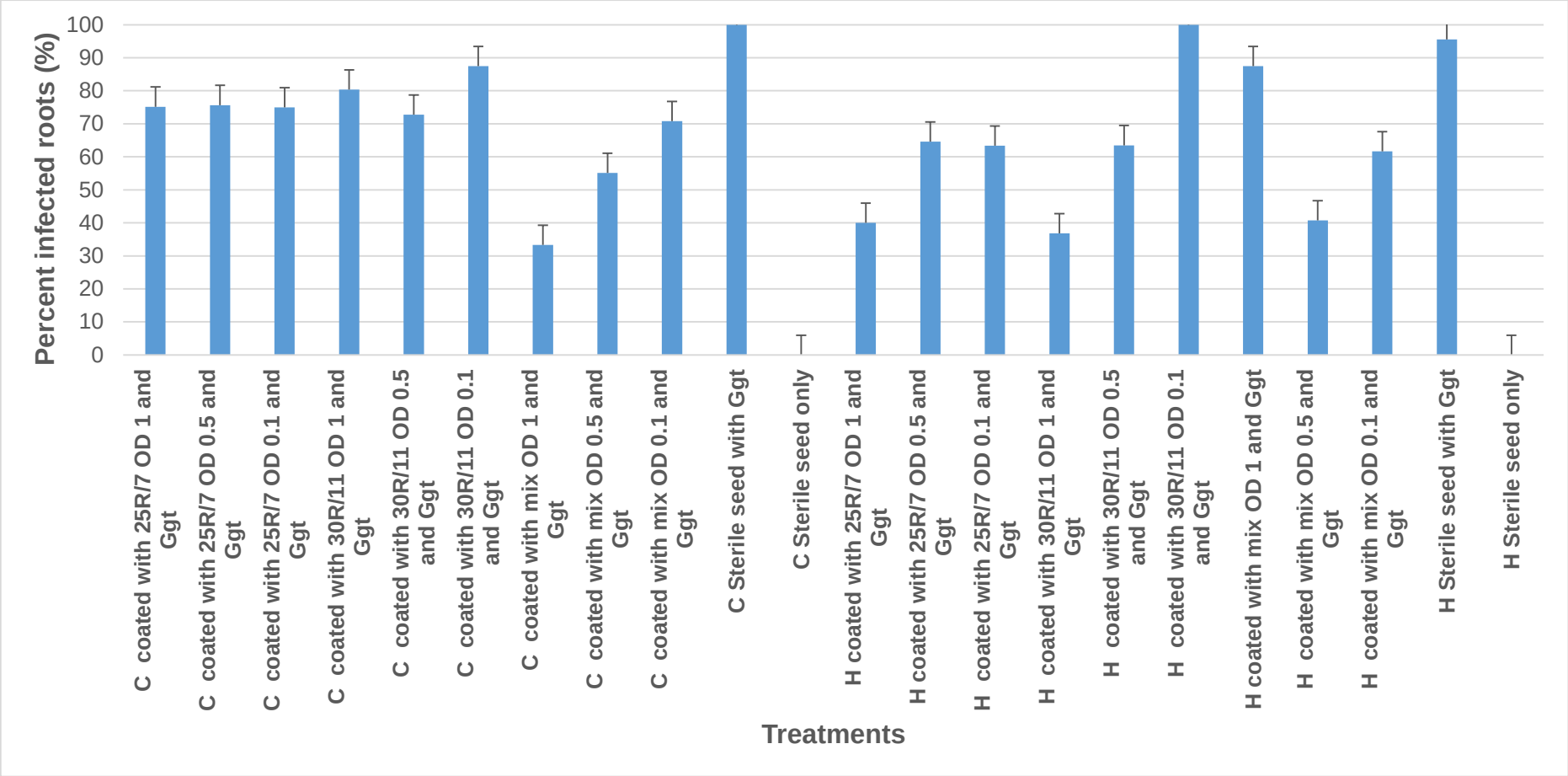


**Figure 34: Mean percent infected roots for Cadenza and Hereward 21 day old seedlings.** Using different concentrations of strains 25R/7, 30R/11, and the mixture of six. Error bars are based on s.e.

**Table 38:** Accumulated analysis of deviance for GLM. Cultivar: Cadenza or Hereward; Coating: soaked in bacterial suspension or sterile; Conc: concentration at OD 1, OD 0.5 and OD 0.1; d.f: degrees of freedom; Approx. F pr.: Approximate F probability.

| Change | d.f. | deviance | mean deviance | deviance ratio | Approx. F pr. |
|---|---|---|---|---|---|
| **+ Cultivar** | 1 | 5.655 | 5.655 | 1.32 | 0.252 |
| **+ Isolate** | 2 | 17.568 | 8.784 | 2.05 | 0.132 |
| **+ Conc** | 2 | 34.493 | 17.247 | 4.03 | 0.02 |
| **+ Cultivar.Isolate** | 2 | 9.051 | 4.526 | 1.06 | 0.35 |
| **+ Cultivar.Conc** | 2 | 1.993 | 0.997 | 0.23 | 0.792 |
| **+ Isolate.Conc** | 4 | 12.033 | 3.008 | 0.7 | 0.591 |
| **+ Cultivar.Isolate.Conc** | 4 | 41.259 | 10.315 | 2.41 | 0.052 |
| **Residual** | 139 | 594.202 | 4.275 | | |
| **Total** | 156 | 716.255 | 4.591 | | |

**Table 39:** Predictions from the regression model. C: Cadenza, H: Hereward.

| | | Cultivar | | | |
|---|---|---|---|---|---|
| **Conc.** | **isolate** | **C Prediction** | **s.e.** | **H Prediction** | **s.e.** |
| OD 1 | 25R/7 | 1.012 | 0.604 | -0.274 | 0.629 |
| | 30R/11 | 1.24 | 0.708 | -0.511 | 0.571 |
| | Mix | -1.194 | 0.746 | 0.693 | 0.702 |
| OD 0.5 | 25R/7 | 1.186 | 0.712 | 0.613 | 0.712 |
| | 30R/11 | 0.869 | 0.683 | 0.568 | 0.628 |
| | Mix | 0.372 | 0.601 | -0.234 | 0.635 |
| OD 0.1 | 25R/7 | 0.938 | 0.813 | 0.847 | 0.824 |
| | 30R/11 | 1.764 | 0.914 | 8.621 | 14.418 |
| | Mix | 0.944 | 0.651 | 0.405 | 0.771 |

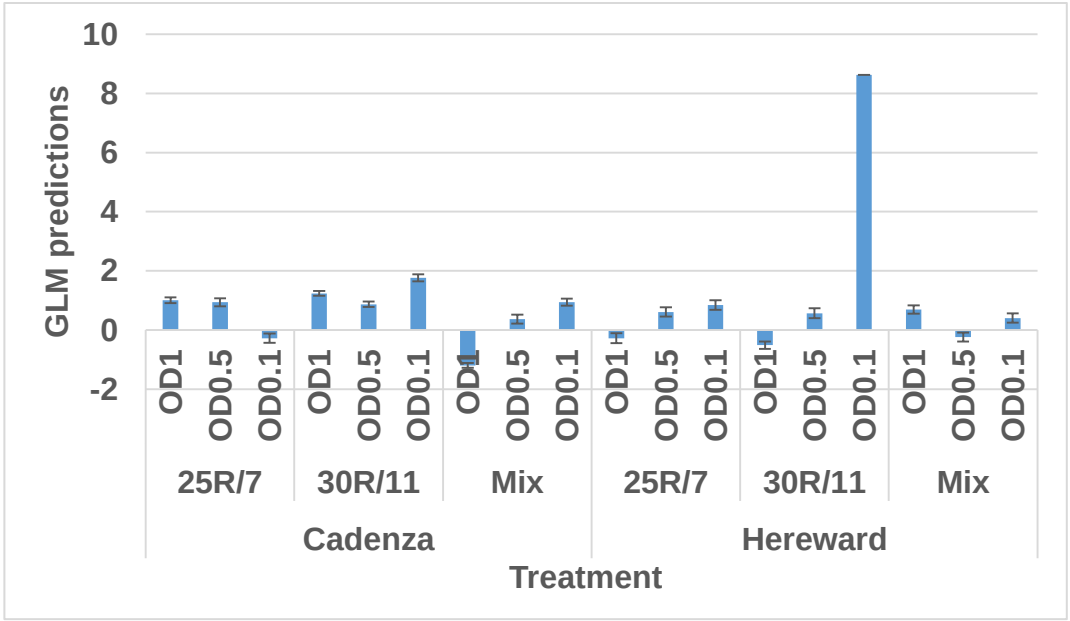


**Figure 35: Analysis of infected roots from Cadenza and Hereward 21 day old seedlings in the presence of *Ggt*. Using different concentrations of strains 25R/7, 30R/11, and the mix of six and in the presence of Ggt.** The concentrations were equivalent to OD 1, 0.5, and 0.1. The bars are plotted from GLM prediction (Table 41). Error bars based on s.e.

### 5.3.4 Antibiotic gene presence

In addition to the plant assay, the isolates tested here were screened for the presence of antibiotic biosynthesis genes as described in section 5.2.6. The two common antibiotics encountered in wheat rhizosphere being phenazine-1-carboxylic acid (*PCA*), and 2,4-diacetylphloroglunicol (*Phl*) (Mazzola *et al.*, 1995; Raaijmakers *et al.*, 1997; McSpadden Gardener *et al.*, 2001; Mavrodi *et al.*, 2012b).

PCR screening of the six antagonistic isolates along with isolate 37R/15 showed that none of the screened isolates produced a band for the Phl or PCA locus (Fig. 37 A & B). *P. protegens* strain Pf-5 (*Phl*$^{+}$) was included as a positive control for (2, 4-DAPG) antibiotic, the band is shown in well number 9 (Fig. 37 A). These findings suggest that the 2, 4-DAPG and the PCA antibiotics are not involved in the mechanism of *Ggt* growth inhibition by the six antagonistic isolates explored in this study.

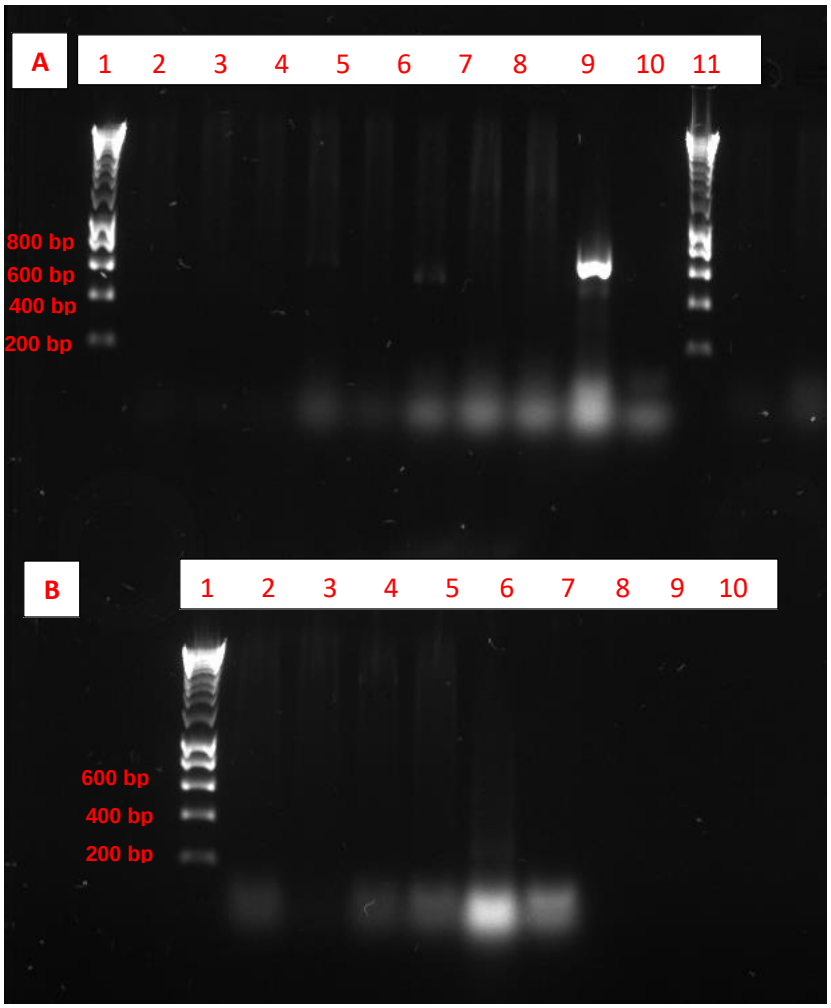


**Figure 36: Amplification products of Phl or PCA genes.** A is the Phl PCR products, wells: 1 and 11 is the Ladder (Bioline Hyper Ladder 1Kb); wells 2- 8 are PCR products of isolates 24E/2, 24E/4, 25R/7, 28R/9, 30R/11, 37R/15 and 44E/7 respectively; well 9 is the $Phl^{+}$ Pf-5 (745 bp); well 10 negative control. B is the PCA PCR products, wells: 1 is the Ladder; wells 2-10 are PCR products of isolates 24E/2, 24E/4, 25R/7, 28R/9, 30R/11, 37R/15, 44E/7, Pf-5 and negative control.

## 5.4 Discussion

In the field, Take-all disease is known to be patchy and often pathogens such *Rhizoctonia solani* and *Phythium* spp. co-occur on the same wheat roots as *Ggt* (Pierson & Weller, 1994). In addition, there is no effective chemical treatment nor are resistant wheat cultivars known for its control. Several factors had led to interest in the *Pseudomonas* group as a potential biocontrol target. For instance, a group of fluorescent *Pseudomonas* spp. are associated with the natural disease suppression, Take-all decline (TAD), seen after continuous wheat monoculture (Weller & Cook, 1983; Yang *et al.*, 2011, 2014). Unfortunately inconsistent field performance have always reduced the number of available, approved and marketed agents. Thus the hunt continues for more biocontrol agents along with interest in manipulating the microbiome as an alternative to single organism introduction (Mauchline *et al.*, 2015; Mauchline & Malone, 2017).

The use of indigenous rhizosphere *Pseudomonas fluorescens* as seed treatments to suppress Take-all in both green house and field experiments have been shown previously (Vrany *et al.*, 1981; Weller & Cook, 1983; Pierson & Weller, 1994). Also, mixtures have been found to be more effective in soil-borne disease control than the use of a single organism (Weller & Cook, 1983; Pierson & Weller, 1994; McSpadden Gardener & Weller, 2001).The *Pseudomonas* isolates tested *in planta* in this work were selected on the basis of *in vitro Ggt* inhibition. The initial screening had showed that there was a significant difference between the cultivars based on the treatment with different isolates. Isolate 25R/7 reduced the number of infected roots in Cadenza while isolate 30R/11 reduced the number of infected roots in Hereward more than the other isolates. Both isolates were originally isolated form the (C, Xi-19) planting combination and the rhizosphere niche. The mix of the six reduced more the number

of infected roots in Hereward in comparison to Cadenza (Table 36). These isolates were further investigated at different concentrations. However, in the first assay there was no significant effect on the plant biomass expressed as shoot height, root length and fresh weight. This suggested that the reduction in Take-all infected roots was not sufficient to alter overall plant health within the 3 week experimental conditions.

Isolates 25R/7, 30R/11 and a mix of the six antagonistic isolates were further tested at concentrations equivalent to OD 1, OD 0.5, and OD 0.1. This revealed complex interactions between concentrations and Take-all inhibition *in planta*. There was a significant difference between the cultivars based on the treatments at different concentrations. In Cadenza as the concentration of the mix decreased the number of infected roots increased as would be expected. This concentration dependent effect was true for 25R/7 and 30R/11 in Hereward but not for the mix. This might be due to the fact that four out of the six isolates in the mix were originally from the (C, Xi-19) while the other two were from (H, Xi-19) and (H, H) planting combination and thus competition for colonization might have affected the concentration effect. Bull *et al.* (1991) reported that variability in root colonization might affect Take-all disease control by the *Pseudomonas* spp. Furthermore for a given treatment there might be a threshold of effectiveness below or above which less or no disease suppression is obtained (Raaijmakers *et al.*, 1995).

In addition, when ignoring the mix of six, isolate 25R/7 reduced the number of infected roots in Cadenza at a given concentration more than 30R/11 except at the middle concentration equivalent to OD 0.5. Similarly, for a given concentration isolate 30R/11 reduced the number of infected roots in Hereward more than 25R/7 except at the lowest concentration, equivalent to OD 0.1. Likewise, Liu *et al.* (2009), using soil drench showed that for *Bacillus subtilis* strain E1R-j high cell densities of up to $10^{12}$

CFU $ml^{-1}$ had led to greater Take-all disease inhibition in comparison to low cell densities of $10^6$ CFU $ml^{-1}$. Here, concentrations equivalent to $10^8$ and $10^9$ CFU $ml^{-1}$ have been used. Similarly, Yang *et al.* (2011) and Lagzian *et al.* (2013) $10^8$ and $10^9$ CFU $ml^{-1}$ for controlled room and greenhouse experiments; respectively. Raaijmakers *et al.* (1995) investigated dose relationship in the efficacy of *Pseudomonas* to control Fusarium wilt in radish. Their results demonstrated that a non-linear asymptomatic relationship between disease suppression and population densities of the used *Pseudomonas* spp. Also, the ability of *Pseudomonas* to reduce the disease was dependent on the initial cell densities and the level of disease incidence. Thus, indicating the importance of initial inoculum application in disease control. However, it is important to note that the high initial concentration of $10^8$ and the very close range of $5 \times 10^7$ and $9 \times 10^7$; equivalent to OD 0.5 and 0.1 respectively, might have not been sufficient for showing any clear concentration based effect. Thus the use of a wider range might be more appropriate to further investigate the effects of the tested isolates on the seedlings. In addition, it is important to test pairwise combinations to determine the most effective combination in the mixture of six. As for now, it is not clear whether the effect of the mixture is based on individual antagonistic activity or that of two or more strains.

For both cultivars, overall the mix of six reduced the number of infected roots more than the individual isolates. This might be due to a combined or synergetic effect achieved by the mixture. Weller and Cook (1983) found that the seed application of combination of two *Ggt* inhibitory stains, *P. fluorescences* 2-79 and 13-79, was more effective in terms in disease suppression and increased yields than the application of each strain individually. Likewise, Pierson *et al.* (1994), showed that certain combinations of fluorescent *Pseudomonas* were more effective in Take-all disease

suppression under green house and field conditions than the individual strains. Factors favouring efficient disease reduction by the combination might include enhanced root colonization, involvement of multiple disease suppression traits and the diverse phenotypes can cover broad host range (Weller & Cook, 1983; Pierson *et al.*, 1994). Mehrabi *et al.* (2016) suggested that when using *Pseudomonas* mixtures of increased antagonism a reduction in Take-all inhibition was found under *in vitro* conditions and competition within the isolates was thought to cause this inverse relation. However, this was not the case in the *in planta* assay carried out here. Indicating that the behaviour of bacterial mixtures may vary *in vitro* and *in planta* when confronted with the pathogen. Under *in vitro* conditions there is a two way interaction: within the mixture and pathogen with the mixture, while in the case of *in planta* there is an additional influence of the host on deriving the complex interaction and the resulting behaviour. Interestingly isolates 24E/2, 24E/4, 25R/7 and 44E/7 gave positive signals for the PCR screening of *wsm* and *fecB* loci. While isolate 28R/9 and 30R/11 were positive for *wsm* and negative for *fecB*. Jousset *et al.* (2011) stated that genetically dissimilar microbial communities, better fight the invasion by efficient utilization of the resources and that increasing the genotypic richness in the form of toxin overproduction also affected the invasion success. Moreover, Hibbing *et al.* (2010) reported that the outcome of the antimicrobial production will depend on the context in which it is produced. This suggests that the mix might act as a group to fight the pathogen rather than competing with each other.

Also, as seen by Weller & Cook (1983), the biocontrol activity was disease suppressive rather than growth promoting as the effect on the plant biomass was not significant. However, the results discussed here were only based on a 21 day old seedling which was sufficient to monitor the disease, while further studies involving green house and

field trials maybe required to further assess the performance of the isolates in both *Ggt* inhibition and plant growth promotion at a different stages of the plant. Also the disease pressure was high in the tubes, which is not the case in the field where *Ggt* is very patchy. Also, Cadenza is inherently known to be taller than Hereward. Thus it is important not to confuse the inherent differences in the cultivars with effects due to growth promotion (Fig. 21). Lagzian *et al.* (2013) carried out a screening of 27 fluorescent *Pseudomonas* isolates with sufficient *in vitro Ggt* inhibition and observed that good inhibition in a Petri plate might not be enough for successful inhibition under greenhouse conditions. This suggests that successful colonization is also important along with antagonistic traits for a biocontrol agent.

The strong inhibition performance of isolate 25R/7 on Cadenza seedlings might reflect the fact that it was originally isolated from (C, Xi-19) and therefore is already better adapted to colonize it. However, the other isolates 24E/2, 24E/4 and 30R/11 although were originally isolated from the same planting combination did not have the expected inhibitory effect. Conversely, isolate 30R/11 which was originally isolated from (C, Xi-19) background, had better inhibition in Hereward. Thus, this implicates a complex system involving host colonization and Take-all control. For instance, Yang *et al.* (2011) reported that no significant correlation were found between *in vitro Ggt* inhibition and the source of isolates.

In many fluorescent *Pseudomonas* spp., the PCA and Phl antibiotics have been identified as the main determinants of biocontrol against soil borne plant pathogens. The Phl primer used here targets *phlD* while the PCA primers targets *phzC* and *phzD* (Raaijmakers *et al.*, 1997). Interestingly none of the isolates tested gave rise to a PCR product suggesting they lack these antibiotics thus indicating that the mode of action against *Ggt* was not based on production of these antimicrobials. Likewise Yang *et al.*

(2011) reported that that 13 fluorescent *Pseudomonas* isolates controlled *Ggt in vitro* and under greenhouse conditions. However, none amplified *phlD, prnC*, and *pltB* , genes involved in antibiotic DAPG, pyrrolnitrin and pyoluteorin biosynthesis; respectively, in comparison to Pf-5 which produces the three antibiotics. However, three were found to amplify the *phzF* gene and apparently it is highly conserved among phenazine producers. Weller & Cook (1983), reported that not all effective strains against *Ggt* produced antibiotics or siderophores *in vitro*. Yang *et al.* (2014) showed that a viscosin like cyclic lipopeptide (CLP) of *P. fluoresecens* strain HC1-07 was involved in the growth inhibition of *Ggt*. Thus, further analysis to point out the mode of action of isolates in hand against *Ggt* will be needed. These include lytic enzymes, siderophore, VOCs, antibiotics as investigated by (Yang *et al.*, 2011; Lagzian *et al.*, 2013). Monitoring of the populations of released biocontrol agents is also important and some PCR methods have been reported for this application (Martini *et al.*, 2015). The findings in this chapter represent step two out the eight sequential steps needed for the development and improvement of *Pseudomonas* based plant protection product described by Walsh *et al.* (2001). These start with (1) Isolation of indigenous *Pseudomonas* spp., (2) Assessment for antifungal activity, (3) Molecular characterisation of the antifungal compounds and modifications for enhanced efficacy, (4) Testing biocontrol efficacy using large scale field trails, (5) Development of delivery inoculants and formulations, (6) Approval by EU directive 90/220/EEC for GMOs release into the environment, (7) Approval for marketing as plant protection products PPPs under EU directive 91/414/EEC, (8) Commercial use of *Pseudomonas*. Thus, further molecular work for the characterization of disease suppression mechanism and the large scale efficacy and stabilization test are needed.

## CHAPTER 6- New Zealand and Long Hoos temporal field screening


### Summary

The rhizosphere microbiome plays an important role in plant health. Plants can selectively harbour a pool of beneficial microbes to aid processes like nutrient uptake. *Gaeumannomyces graminis var. tritici* (*Ggt*) constrains wheat productivity in the UK and worldwide. Recently McMillan *et al.* (2011) identified differential *Ggt* inoculum supportiveness across varieties within the wheat genetic improvement network (WGN). This trait was called Take-all build-up (TAB). Furthermore it was found that growing low TAB wheat variety in the first year will result in less disease and higher yield in the second year (Mauchline *et al.*, 2015; McMillan *et al.*, 2018). Thus, manipulating the soil microbiome through the use of specific cultivars may offer great potential for natural disease control of Take-all. In addition, recent developments in metagenome studies provide insights into rhizosphere microbiome structure. In this work methods of next generation sequencing and real time qPCR were used to compare bacterial communities and *Ggt* inoculum storage associated with five wheat varieties (two L-TAB and three H-TAB) in addition to barley (Unknown TAB) from three fields at different time lines. PERMANOVA analysis of all 16S rRNA gene amplicon data shows that the main factor separating the bacterial communities was based on field type ($p = 0.0001$). In addition there was a clear niche separation of bulk soil bacterial communities from those of the rhizosphere. Changes in *Ggt* populations were only based on year-to-year variations rather than being influenced by the cultivars ($p=0.001$).

## 6.1 Introduction

The rhizosphere is the area surrounding the roots where important functions for the plant occur (Berg & Smalla, 2009; Berendsen *et al.*, 2012; Bakker *et al.*, 2013)(Berg & Smalla, 2009; Berendsen *et al.*, 2012). Microbial communities in the rhizosphere affect plant immunity, pathogen abundance, nutrient acquisition and stress tolerance (Coats & Rumpho, 2014; Haichar *et al.*, 2014). Many biotic and abiotic factors aid in shaping the structural and functional diversity of the rhizosphere microbial communities (Berg and Smalla, 2009). To date various studies have shown the importance of the plant or the soil factors in structuring the rhizosphere microbiome (Haichar *et al.*, 2008; Berendsen *et al*., 2012).

For instance, the plants characteristics are known to influence the endophytic and rhizosphere microbial communities (Siciliano & Germida, 1999). Donn *et al.* (2015) found that the presence of wheat plant and its growth stage were the major factors influencing the rhizosphere microbial community when compared to bulk soil. The molecular basis of host specificity are mainly unknown (Berg and Smalla, 2009; Raaijmakers et al., 2009). Bergsma-Vlami *et al.* (2005) compared the effect of different host plants; wheat, sugar beet, lily and potato, on rhizosphere bacteria with an interest in fluorescent *Pseudomonas* spp. in two types of Take-all soils, conducive and suppressive, respectively. They found that wheat influenced an increase in populations of fluorescent *Pseudomonas* spp. from $2 \times 10^5$ to $6 \times 10^6$ CFU/g root in conducive soil and from $8 \times 10^5$ to $4 \times 10^6$ CFU/g root in suppressive soil. Similarly, the endophytic bacterial population was found to be determined by the host plant genotype (Robinson *et al.*, 2015). Thus, plants play an important role in the selection of certain microbial populations and in the development of suppressiveness (Schreiner *et al.*, 2010).

Recently Mauchline *et al.* (2015) compared the rhizosphere bacterial communities of two wheat cultivars with different Take-all inoculum building properties; Hereward and Cadenza (H-TAB and L-TAB, respectively). Overall the 16S rRNA gene amplicon analysis showed a highly complex microbiome, where high genetic diversity was encountered within the *P. fluorescens* group. In addition, more *Pseudomonas* were associated with Hereward than Cadenza. It was concluded that the first year grown wheat variety had a selective pressure on *Pseudomonas* genomic diversity. The concept of soil memory and immunity comes after extensive investigations of the microbial metagenome of plants (Lapsansky *et al.*, 2016). For instance, in suppressive soils, like Take-all decline (TAD) in which the disease decreases after several years of wheat monoculture due to build-up in populations of antagonistic, 2,4-DAPG producing fluorescent *Pseudomonas* spp. (Berendsen *et al.*, 2012). Manipulating the soil microbiome holds great promise in control of soil-borne plant diseases ( Mauchline *et al.*, 2015). Thus, it is important to understand the wheat rhizosphere microbiome composition of different TAB wheat and how they affect the *Ggt* inoculum in the field.

Apart from the plant, the effect of environmental factors on the selection of microbial communities have been highlighted. Mavrodi *et al*. (2012) found that soil moisture was the driving factor in the enrichment of antibiotic-producing Pseudomonads. They found the PCA ($Phz^+$) producers were mainly associated with dryland wheat, in comparison to 2, 4-DAPG ($Phl^+$) producers which dominated irrigated wheat.

The results here indicate that a complex bacterial structure is associated with wheat cultivars used in this work. Even for a single variety like, barley, the associated bacterial communities were highly variable at different timelines between samples from the same field. Factors like soil, and year-to-year variation might have masked the expected crop selective effects. In addition, as known with *Ggt,* disease patchiness

was evident between the plots in the same field and might have also contributed to the masking effect. Overall, the levels of *Ggt* were based on year-to-year variations rather being influenced by the cultivar.

**Aims and objectives:**

To better understand how wheat varieties differing in their TAB trait shape their rhizosphere microbiomes under *Ggt* disease conditions a long term temporal field experiment drilled in 2014 to 2018 was investigated to compare microbial communities and to assess changes in *Ggt* populations and inoculum storage over-time.

## 6.2 Methods

### 6.2.1 Structure of microbial communities

#### 6.2.1.1 Experimental design and field layout

This experiment is a 5 year long term experiment, located over three different fields at Rothamsted Research. It consists of 6 treatments (Tables 40 and 41) replicated 4 times resulting in 24 plots (Figs. 38-40). The plots were created using GenStat Randomization.

**Table 40:** The treatments in the 5 year experiment.

| Treatment | Variety | TAB trait |
|---|---|---|
| 1 | Cadenza | Low |
| 2 | Xi19 | Low |
| 3 | Hereward | High |
| 4 | Duxford | High |
| 5 | Hereford | High |
| 6 | Barley - KWS Cassia | Un-known |

**Table 41:** Allocated fields for each sample collection. The mineral content (P, K, Mg) are in mg $L^{-1}$, and the texture (Clay, Sand, Silt) are in percentages. P, phosphorus, K, potassium, Mg, magnesium. Data were generated by SoilQueist (Precision Agronomy services by Agrii™) 2016 report.

| Field No. | Field | P | K | Mg | pH | Clay | Sand | Silt | Texture |
|---|---|---|---|---|---|---|---|---|---|
| **1** | **New Zealand** | 18 | 262 | 96 | 6.8 | 18.65 | 35.63 | 45.72 | clay loam |
| **2** | **Long Hoos5** | 31 | 322 | 100 | 7 | 23.75 | 21.8 | 54.45 | clay loam |
| **3** | **Long Hoos4** | 36 | 319 | 82 | 6.65 | 18.97 | 35.145 | 45.885 | clay loam |

### 6.2.1.2 Rhizosphere soil sample collection

The field layouts of Long Hoos 5, New Zealand, and Long Hoos 4 are shown in Figs 30-32. From each plot, five samples of wheat plants at the late milk growth stage were sampled in a (W) formation across the plot to a depth of 30 cm approximately, with crown roots and a proportion of seminal roots attached (Table 42). The plants were placed into labelled plastic bags and transported back to the laboratory.

The bags were placed in the cold room 4 °C while being processed. The vegetative part, the leaves and the grains was chopped off, leaving approximately 15 cm of stem attached to the roots. Using sterile gloves, the bulk soil was shaken from each plant and discarded, in a way that only the portion of soil strongly attached to the roots (rhizosphere soil) was left. This rhizosphere soil was separated from the roots by physical messaging. Thecollected soil was divided into two portions in5 ml labelled tubes stored at -20˚C for amplicon analysis and 50 ml tubes stored at 4˚C for bacterial isolation.

**Table 42:** Description of soil samples collected from the three fields.

| Fields | New Zealand | Long Hoos5 | Long Hoos4 |
|---|---|---|---|
| Samples collected by Dr. Vanessa McMillan and Dr. Tim Mauchline | Year 1 (Rhizosphere) **(**2015) | Year 0 (bulk soil) (2015) | - |
| Samples collected by Mahira Al Zadjali | Year 2 (Rhizosphere) (2016) | Year 1 (Rhizosphere) (2016) | Year 0 (bulk soil) (2016) |
| Samples collected by Mahira Al Zadjali | Year 3 (Rhizosphere) (2017) | Year 2 (Rhizosphere) (2017) | Year 1 (Rhizosphere) (2017) |

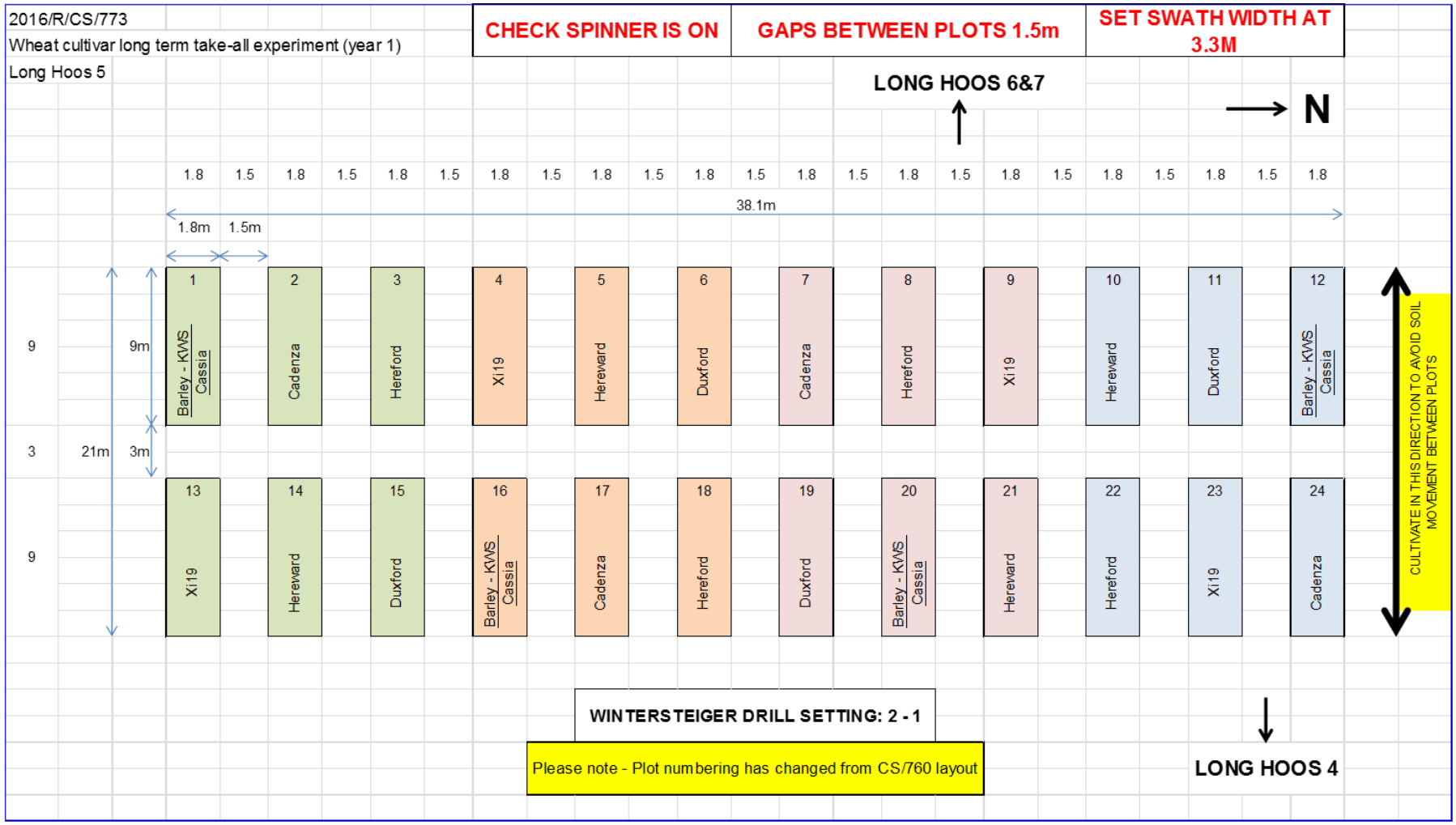


**Figure 37: Field layout at Long Hoos 5 at Rothamsted.** The four blocks are represented by different colours and the numbers 1-24 represent the plots. Source: Dr. Vanessa McMillan (Rothamsted Research).

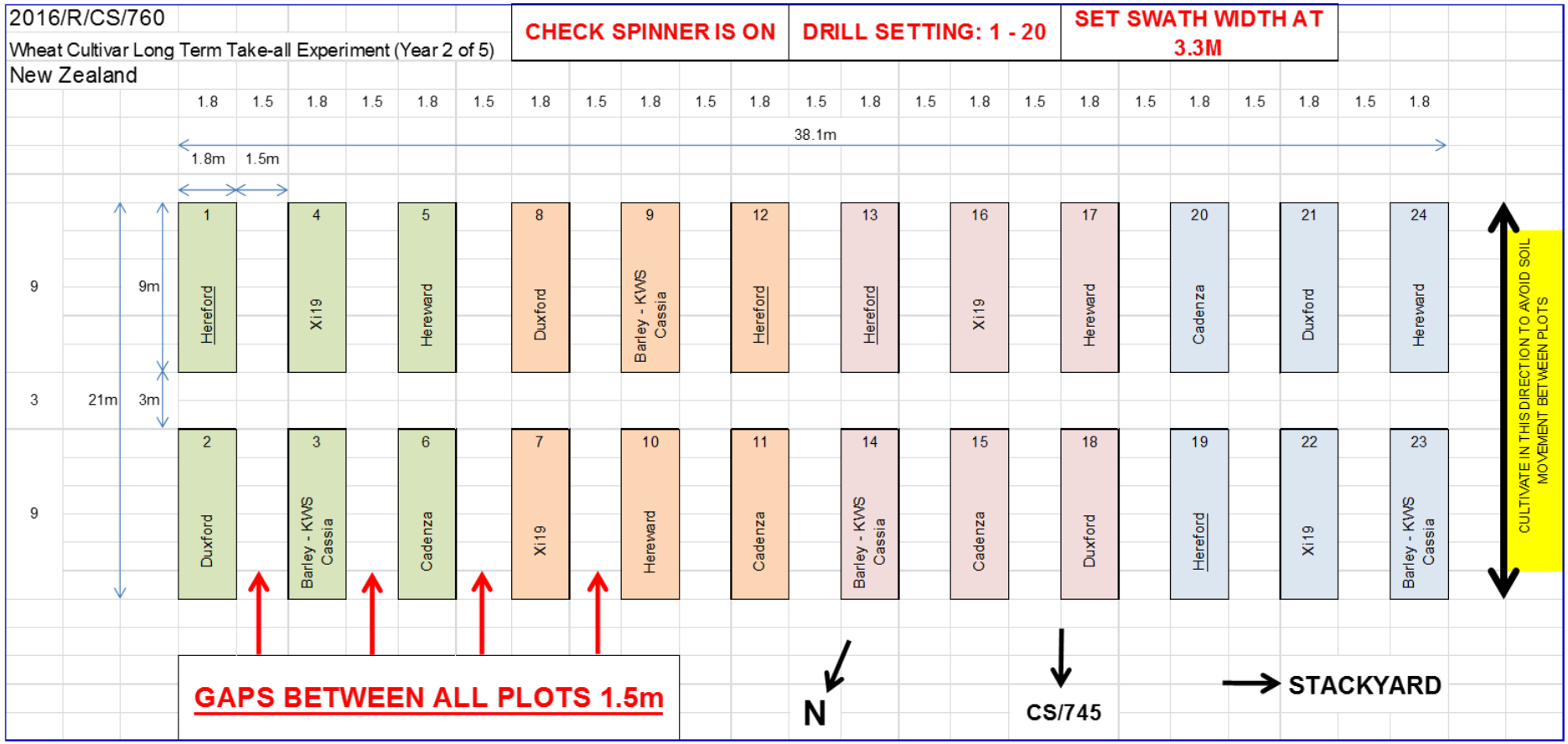


**Figure 38: Field layout at New Zealand at Rothamsted.** The four blocks are represented by different colours and the numbers 1-24 represent the plots. Source: Dr. Vanessa McMillan (Rothamsted Research).

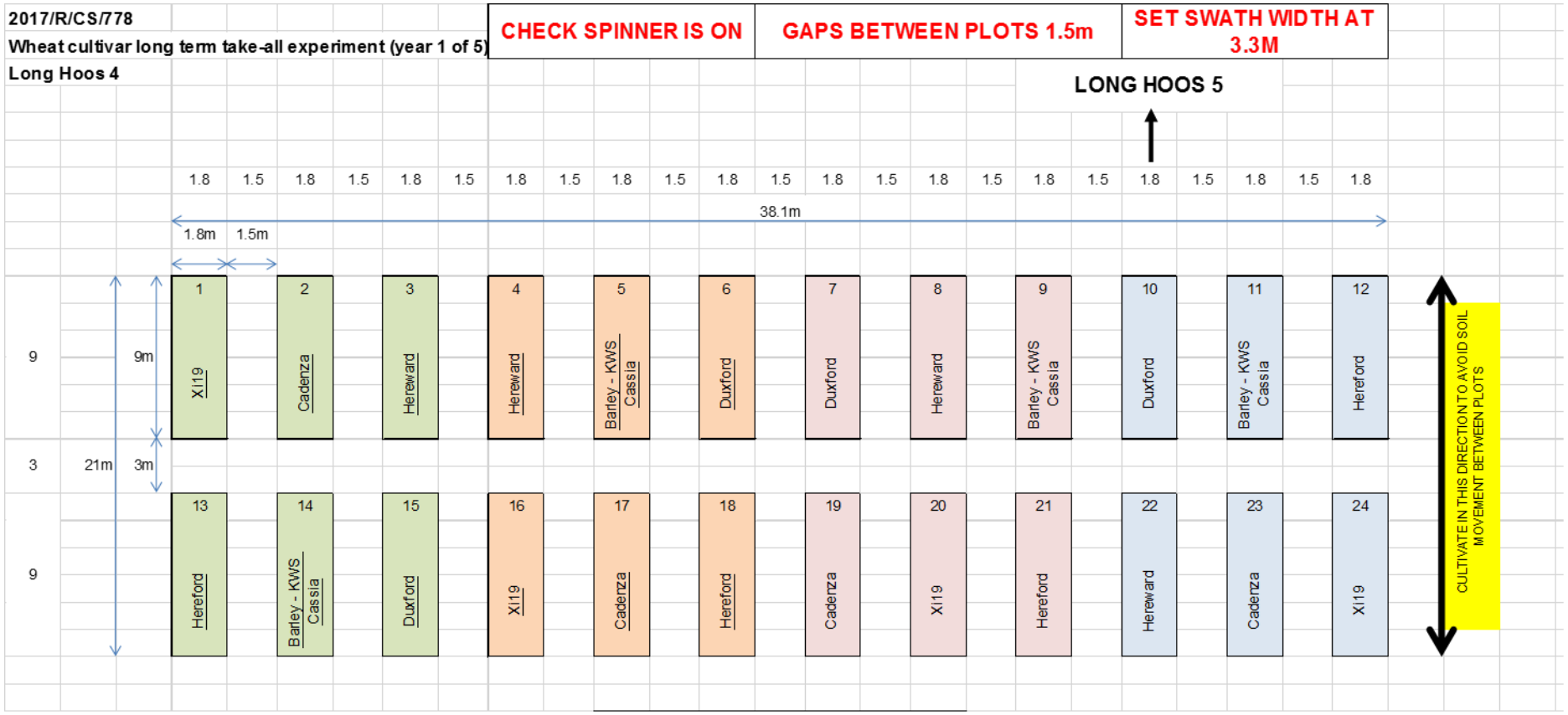


**Figure 39: Field layout at Long Hoos 4 at Rothamsted.** The four blocks are represented by different colours and the numbers 1-24 represent the plots. Source: Dr. Vanessa McMillan (Rothamsted Research).

### 6.2.1.3 Selective isolation of *Pseudomonas* spp.

1 g of bulk rhizosphere soil was weighed and placed in a 50 ml Falcon tube for each of the 24 plots, and 9ml of sterile distilled water was added. This mixture was vigorously shaken for 10 mins at 4°C. Next, four Eppendorf tubes were filled with 900 µl of sterile distilled water and were labelled as -1, -2, -3, and -4. A series of serial dilutions was made by placing 100 µl of the homogenised soil mixture into the -1 ($10^{-1}$) labelled Eppendorf tube. From the $10^{-1}$ dilution 100 µl was placed in the –2 labelled tube to get $10^{-2}$ dilution and the 10-fold dilution repeated to $10^{-4}$. 100 µl of $10^{-3}$ and $10^{-4}$ dilutions were plated onto *Pseudomonas* Selective Agar (PSA, Oxoid) supplemented with CFC (Cephalothin 25 mg, Fucidin 5 mg, and Cetrimide 5mg, Oxoid) as per manufacturer instructions. A lawn was made using sterile glass spreaders. The PSA plates were incubated overnight at 25°C. For each plot, a total of 20 colonies were picked from $10^{-3}$ and $10^{-4}$. The colonies were stabbed into 96 well plates containing 100 µl of King's B broth. The plates were incubated at 25 °C and then glycerol stocks were prepared from these plates.

### 6.2.1.4 Total soil DNA extraction

Total DNA extraction was performed using DNeasy power soil kit (Qiagen) following the manufacturers protocol. Approximately 0.25 g of rhizosphere soil was weighed and placed in the provided Power Bead tubes. For bead beating, the tissue lyser was used at 4 m/s for 40 s twice (MP BIO Fast Prep-24). During the elution step, the tubes were allowed to stand for 10mins before the final spin. The concentration of eluted DNA was quantified as using NanoDrop® (ND-1000 UV-Vis Spectrophotometer) (LabTech). The extracted DNA was stored at -20°C for further use.

#### 6.2.1.5 16S rRNA Microbial community analysis

Next generation sequencing targeting the bacterial 16S rRNA gene was performed using the Illumina MiSeq platform at the Centre for the Analysis of Genome Evolution and Function (CAGEF, University of Toronto, Canada). Briefly, the V4 hypervariable region of the 16S rRNA gene was amplified using a universal forward sequencing primer and a uniquely barcoded reverse sequencing primer to allow for multiplexing (Caporaso *et al.*, 2012). Amplification reactions were performed using 12.5 µl of KAPA2G Robust HotStart Ready Mix (KAPA Biosystems), 1.5 µl of 10 µM forward and reverse primers, 8.5 µl of sterile water and 1 µl of DNA. The V4 region was amplified by cycling the reaction at 95°C for 3 mins, 18 cycles of 95°C for 15 seconds, 50°C for 15 s and 72°C for 15 s, followed by a 5 min 72**°**C extension. The amplified amplicon size was ~ 390 bp ("Earth microbiome project. Available:www.earthmicrobiome.org/protocols-and-standards/16s/. [Accessed: 28 May 2019]."). All amplification reactions were done in triplicate, checked on a 1% agarose TBE gel, and then pooled to reduce amplification bias. Pooled triplicates were quantified using Quant-it PicoGreen dsDNA Assay (Thermo Fisher Scientific) and combined by even concentrations. The final library was purified using Ampure XP beads (Agencourt), selecting for the bacterial V4 amplified band. The purified library was quantified using Qubit dsDNA Assay (Thermo Fisher Scientific) and loaded on to the Illumina MiSeq for sequencing, according to manufacturer instructions (Illumina, San Diego, CA). Sequencing was performed using the V2 (150bp x 2) chemistry.

##### 6.2.1.5.1 Generation of OTU tables (Analysis of the bacterial microbiome)

The OTU tables were generated by (CAGEF) (Appendix V will be provided on request due large size). Briefly, the UNOISE pipeline, available through USEARCH version

10.0.240, was used for sequence analysis (Edgar, 2013, 2010; Edgar, 2016). The last base, typically error-prone, was removed from all the sequences. Sequences were assembled, and quality trimmed using –fastq_mergepairs and –fastq_filter, with a –fastq_maxee set at 1.0 and 0.5, respectively. Assembled sequences less than 233bp were removed. Following the UNOISE pipeline, unique sequences were identified from the merged pairs. Sequences were de-noised, and chimeras were removed using the unoise3 command in USEARCH. Assembled sequences were then mapped back to the chimera-free denoised sequences at 97% identity OTUs using the –otutab command. Taxonomy assignment was executed using SINTAX (Edgar, 2016), available through USEARCH, and the SINTAX compatible Ribosomal Database Project (RDP) database version 16, with the default minimum confidence cut-off of 0.8 (Wang *et al.*, 2007). OTU sequences were aligned using PyNast accessed through QIIME (Caporaso *et al.*, 2010). Sequences that did not align were removed from the dataset and a phylogenetic tree of the filtered aligned sequence data was made using FastTree (Price *et al.*, 2009). The average size of the OTU sequences were 253 bp.

#### 6.2.1.5.2 Structure of Bacterial communities

Differences in bacterial community structure were investigated by Permutational Analysis of Variance (PERMANOVA) (Anderson, 2001) in Paleontological Statistics Software Package for Education and Data Analysis (PAST) (Hammer *et al.*, 2001). PCoA plots were obtained using the same software. This analysis was kindly performed by Dr. Vanessa Nessner- Kavamura-Noguchi at Rothamsted Research.

#### 6.2.1.5.3 Analysis of differentially abundant taxa

The online tool for comprehensive statistical, visual and meta-analysis of microbiome data called MicrobiomeAnalyst (Dhariwal *et al.*, 2017) was used for detecting OTUs which were differentially abundant between samples. The OTU table was arranged as the required format and it was uploaded with the mapping and taxonomy files. Low abundance and low variance OTUs were removed using default values, where OTUs with less than 2 counts in less than 20% of the samples and 10% of the values below the determined inter-quantile range (IQR) were removed. The OTU table was normalised using the method of rarefying with replacement and relative log-expression (RLE) transformed. This analysis was kindly performed by Dr. Vanessa Nessner-Kavamura-Noguchi at Rothamsted Research due to software and server availability.

### 6.2.2 Quantitative real time PCR (qPCR)

#### 6.2.2.1 Determination of total soil DNA concentration using Qubit

The Qubit dsDNA BR assay kit (Invitrogen) was used to prepare the samples for quantification of DNA concentrations extracted in 6.2.1 following the manufacturers' protocol. A Qubit fluorometer 2.0 (Invitrogen) was used to determine the DNA concentrations. The DNA was then diluted to 10 ng $\mu l^{-1}$ with molecular grade double distilled water, d.d. $H_2O$, before use as template for qPCR.

#### 6.2.2.2 *Ggt* qPCR

##### 6.2.2.2.1 Preparation of *Ggt* standard DNA

The fungal DNA of *Ggt* was extracted using a Master Pure Yeast DNA purification kit (Epicenter) following the manufacturer instructions. This used either scraped fresh fungal mycelium or freeze dried mycelium.

##### 6.2.2.2.2 Taq-man probe *Ggt* qPCR

The method of Keenan *et al.* (2015) was used to measure the *Ggt* concentrations in the total soil DNA. This reaction targeted the translation elongation factor 1-alpha gene (*EF1*-α) to specifically detect *G. graminis* var*. tritici* resulting in a 106bp long product*.* The total reaction volume was 20 µl and consisted of: 10 µl of 2X KAPA Master Mix with ROX (BioRad Laboratories), 0.03 µl of EFPR1 probe at a final concentration of 0.15 µM, 0.06 µl of each primer GgtEFF1 and GgtEFR1 (Table 43) at a final concentration of 0.3 µM. The template DNA was pre-diluted to 10ng $\mu l^{-1}$ and 2 µl were used in each reaction. PCR amplifications were carried out in Mx3000 P qPCR machine (Agilent Technology) using the following thermal profile: initial denaturation at 95°C for 3 mins, followed by 40 cycles of amplification at 94°C for 15 s, 52°C for 20 s, and 72°C for 20 s. The standard DNA (SJ *Ggt* 12NZ66) 49.3ng $\mu l^{-1}$ was diluted to the concentration of 10ng $\mu l^{-1}$ and used to prepare 10-fold serial dilutions of genomic DNA.

The quantities of target DNA were estimated using a standard curve constructed by regressing Ct values onto $\log_{10}$ of the concentration of *Ggt* standards. The curve was automatically generated in the qPCR machine (Appendix I 9.4.1).

Each of the DNA samples and the standards were amplified in duplicate, and negative controls were included with each PCR assay. A test for PCR inhibitors was also performed by spiking some of the samples with known concentration of standard DNA.

**Table 43:** Elongation factor α primers and probe giving a 106bp product (Keenan *et al*., 2015).

| Primers and probes | 5'-- 3' sequence |
|---|---|
| GgtEFF1 | CCCTGCAAGCTCTTCCTCTTAG |
| GgtEFR1 | GCATGCGAGGTCCCAAAA |
| Taqman probe GgtEFPR1 | 6FAM-ACTGCACAGACCATC-MGB |

### 6.2.2.3 Quantification of fungal (ITS), bacterial (16S rRNA), and *Pseudomonas* (16S rRNA)

#### 6.2.2.3.1 Standard preparation

A mixture of soil (25% arable soil, 25% grassland, and 50% wilderness soil) total DNA was extracted using DNeasy Power Soil Kit (Qiagen) following the manufacturer instructions. This reference DNA was then diluted to 5 ng $\mu l^{-1}$. PCR amplification was performed using Quanti Fast SYBR Green PCR Kit (Qiagen). The primers used are listed in Table 44. The reaction mix consisted of 10 µl 2X Quanti Fast Master Mix, 2 µl of 10 µM Forward primer, 2 µl of 10 µM Reverse primer, 2 µl of water and 4 µl of 5ng $\mu l^{-1}$ reference DNA. The amplification program was carried using CFX96 thermal cycler (BioRad) and consisted of an initial enzyme activation at 95 °C for 5 mins, followed by 40 cycles of denaturation at 95 °C for 10 s, a combined annealing and extension at 60 °C for 30 s.

The PCR products were then visualized on 1% Agarose (Bioline) gel in TBE buffer (ran at 100V for 1h 20min). The target bands were excised and gel extraction was performed using a QIAquick PCR Purification Kit (Qiagen) following the manufacturer guidelines. The DNA was eluted in (3-50 µl) of TE buffer. The Qubit dsDNA BR Assay kit (Qubit) was used to prepare the DNA for determination of the concentrations in (ng $\mu l^{-1}$) on a Qubit fluorometer.

#### 6.2.2.3.2 Calculations to convert in copy number

$$\frac{\text{size of amplicon (bp)} \times \text{molecular weight of a 1 bp } (g.mol^{-1}.bp^{-1})}{\text{Avogadro number } (mol^{-1})} = \text{weight of the amplicon (g/copy)}$$

To convert the weight of the amplicon in ng (x $10^9$)

$$\frac{\text{standard concentration (ng/µl)}}{\text{weight of the amplicon } (\text{ng/copy})} = \text{concentration of the standard (copy/µl)}$$

#### 6.2.2.3.3 Standard dilutions

The stock obtained from gel extraction was diluted in TE buffer down to $10^9$ copy $\mu l^{-1}$ ($V_F$= 100 µl) (if needed, a first dilution to $10^{10}$ copy/µl is done). A dilution series was done in an 8-tube strip, from $10^8$ to $10^1$ copy/µl (10 µl of DNA + 90 µl TE Buffer). The standards were then distributed in 8 µl single use aliquots (6 µl are needed per qPCR, standard run in triplicate). The standards were then stored at -80 °C.

#### 6.2.2.3.4 qPCR

A quantitative PCR reaction mix was prepared using Quanti Fast SYBR Green PCR Kit (Qiagen). The reaction mix consisted of 5 µl of 2X Quanti Fast master mix, 0.1µl of 100 µM Forward primer, 0.1 µl of 100 µM Reverse primer, 2.8 µl of RNAse-free water

(Severn Biotech, Kidderminster, UK) and 2 µl of 5 ng $\mu l^{-1}$ template DNA. The BioRad CFX384 Touch Real-Time PCR Detection System was used to run the program. The amplification program consisted of an initial enzyme activation step at 95°C for 5mins, followed by 40 cycles of denaturation at 95 °C for 10 s, a combined annealing and extension at 60 °C for 30 s. Finally, a melt curve (fluorescence read) was detected between 60 °C- 95 °C every 0.5 °C. The primer sequences are listed in (Table 44).

**Table 44:** Primers used in qPCR targeting bacteria, *Pseudomonas* and fungi.

| Gene | Primer | Sequence | Reference | amplicon size (bp) |
|---|---|---|---|---|
| 16S rRNA *Pseudomonas* | 16S rRNA_PseuF1 | CTT CGG GCC TTG CGC TAT CA | (Clark & Hirsch, 2008) | 248 |
| | 16S rRNA_PseuR1 | GCCCTTCCTCCCAACTTAA | | |
| 16S rRNA Bacteria | 16S rRNA_bact_341F | CCTAYGGGRBGCASCAG | (Glaring *et al.*, 2015) | 465 |
| | 16S rRNA_bact_806R | GGACTACNNGGGTATCTAAT | (Glaring *et al.*, 2015) | |
| ITS fungi | ITS1f | TCC GTA GGT GAA CCT GCG G | (Gardes & Bruns, 1993) | ~ 300 |
| | 5.8s | CGC TGC GTT CTT CAT CG | (Vilgalys & Hester, 1990) | |

### 6.3 Take-all index and percent infected roots

Disease severity from each plot was assessed by soil-core bioassay and plant root assessment performed by Dr. Vanessa McMillan, Rothamsted research.

### 6.4 Statistical analysis

Statistical analysis was performed using GenStat (18$^{th}$ edition, VSN International Ltd, Hemel Hempstead, UK). Transformations were used to stabilize the residuals and aid the analysis as needed.

### 6.5 Results

McMillan *et al.* (2011), showed the differential ability of wheat cultivars to support the *Ggt* inoculum. These cultivars were classified as L-TAB or H-TAB. To further investigate this, a 5 year field experiment was designed to analyse the microbial communities associated with two L-TAB cultivars, Cadenza and Xi-19, and three H-TAB cultivars, Hereward, Hereford and Duxford, along with Barley of unknown-TAB. Thus adding a crop factor, wheat vs. barley, in addition to the cultivar factor. In addition, the experiment involved 3 fields, New Zealand, Long Hoos 5 and Long Hoos 4, to enable comparison between fields. Moreover, the start date for each field was a year ahead of the other, starting with New Zealand in 2014, followed by Long Hoos 5 then Long Hoos 4 (Table 42). Apart from field New Zealand, soil samples prior to planting, bulk soil (Year 0), were collected from the fields. Total soil DNA was extracted from bulk soil and the rhizosphere samples collected from the three field for different timelines. Methods of next generation sequencing were used to investigate the

bacterial communities from these fields. While qPCR was used to quantify the amount of *Ggt*, bacteria, *Pseudomonas*, and fungi.

Selective isolation of *Pseudomonas* spp. using methods described in section 6.2.1.3 were performed for field 1 year 2 and 3 (F1Y2), field 2 year 2 (F2Y2) and field 3 year 1 (F3Y1) resulting in 1868 isolates that can be investigated in future work.

### 6.5.1 16S rRNA Amplicon data

#### 6.5.1.1 Difference in bacterial communities

Total soil DNA was extracted from 192 soil samples, 0.25g each eluted to 100 µl. Quantification of the extracted DNA was done using NanoDrop and Qubit methods, however since the Quibt was much more accurate the DNA concentration data were used. Following DNA template quantification and quality check with gel electrophoresis 30 µl were sent to CAGEF, University of Toronto, Canada for 16S rRNA amplicon sequencing using methods described in section 6.2.1.5. The CAGEF service included the generation of operational taxonomic units; OTU table as described in section 6.2.1.5.1. The OTU table was then analysed kindly by Dr. Vanessa Nessner- Kavamura-Noguchi (Rothamsted Research), first multivariate analyses were performed primarily on all 16S rRNA gene amplicon data. The first axis in Principal Coordinates Analysis plot (PCoA) (Fig. 41) corresponds to 33.3% of the variation and clearly separated samples from the first field (F1) from samples collected in fields 2 and 3. PERMANOVA analysis corroborates the observed differences and shows that field type is mainly responsible for the observed differences in bacterial community structure (PERMANOVA, $F = 63.31$, $p = 0.0001$). The second axis,

corresponding to 12.15% of the variation, separated samples from the second and third fields based on niche; *i.e*. bulk soil vs. rhizosphere.

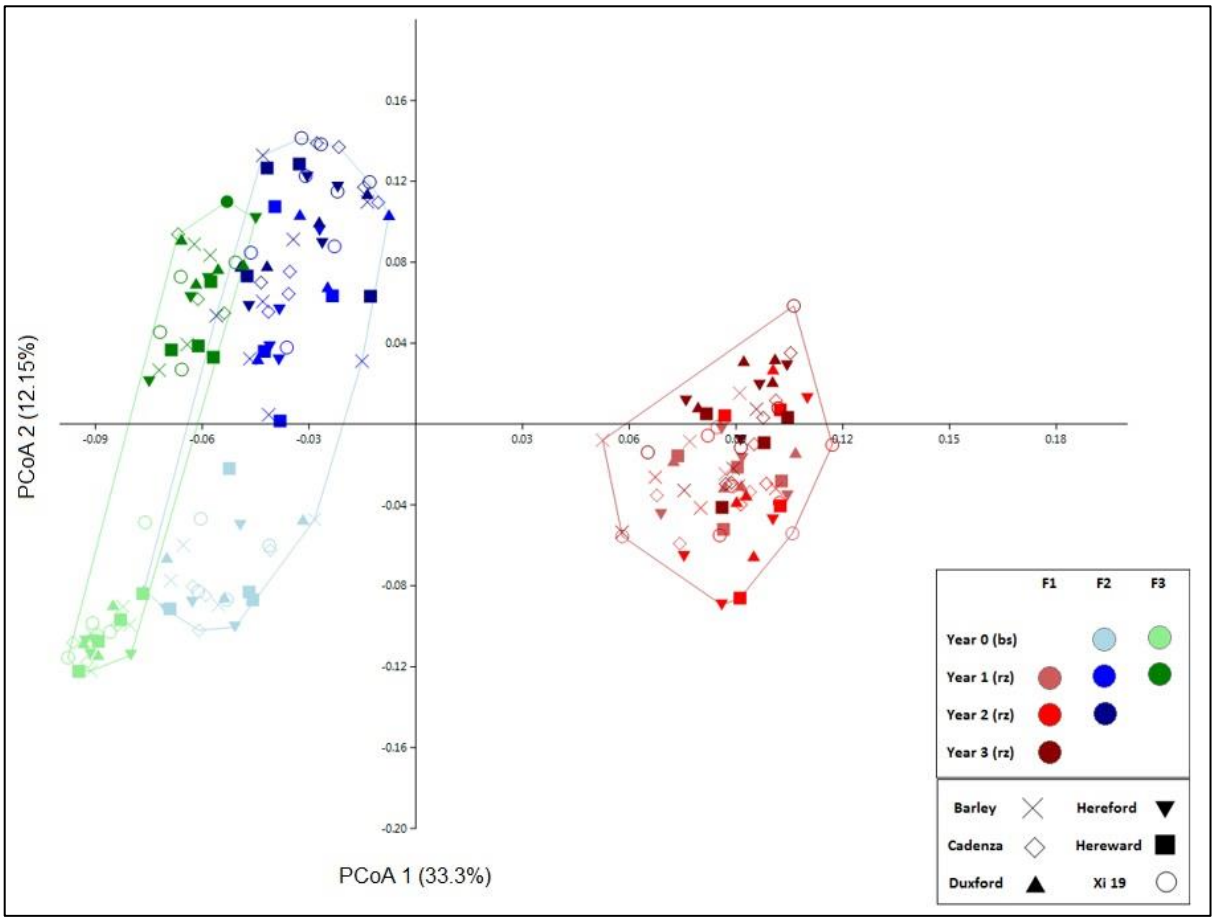


**Figure 40:** PCoA based on Bray-Curtis similarity distance matrix showing the structure of wheat bacterial communities from both bulk soil (light colours) and rhizosphere (dark colours), obtained from different wheat cultivars and one barley cultivar (represented by different symbols), collected from different fields: field 1 (New Zealand) (red colours), field 2 (Long Hoos 5) (blue colours) and field 3 (Long Hoos 4) (green colours) and from different years. Graph generated by Dr. Vanessa Nessner- Kavamura-Noguchi (Rothamsted Research).

Since the field type was identified as the main variation driving factor, further analysis was carried out for each field separately.

#### 6.5.1.1.1 Field 1 (New Zealand) Bacterial community structure

In Fig. 42, it is not possible to see a clear separation of rhizosphere samples collected from different years, and there is a small overlap, especially of samples from years 1 and 2. Also, there are no clear differences based on cultivar.

Analysing each year individually, during the first year of sampling, differences of the rhizosphere bacterial communities were not observed, and they are corroborated by non-significant statistical differences (PERMANOVA, $F = 0.9415$, $p = 0.7171$) (Fig.43). For the second (Fig.44) and third (Fig.45) years, the same trend is observed, and it can concluded that different cultivars did not influence the structure of rhizosphere bacterial communities even after three years in the first field.

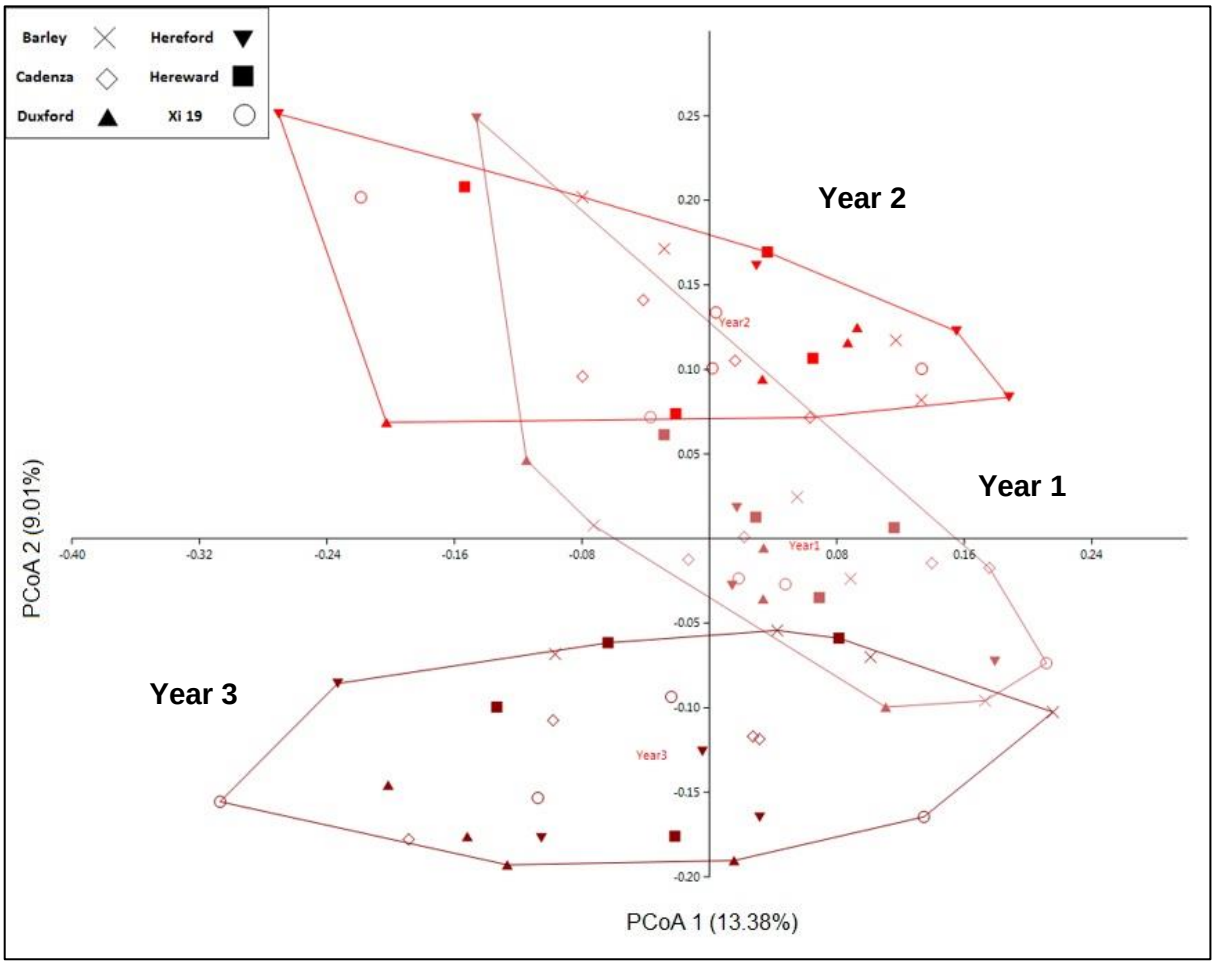


**Figure 41:** PCoA based on Bray-Curtis similarity distance matrix showing the structure of wheat bacterial communities from rhizosphere, obtained from different wheat cultivars and one barley cultivar (represented by different symbols), collected from field one (F1) across three years. (Grouped per year). Graph generated by Dr. Vanessa Nessner- Kavamura-Noguchi (Rothamsted Research).

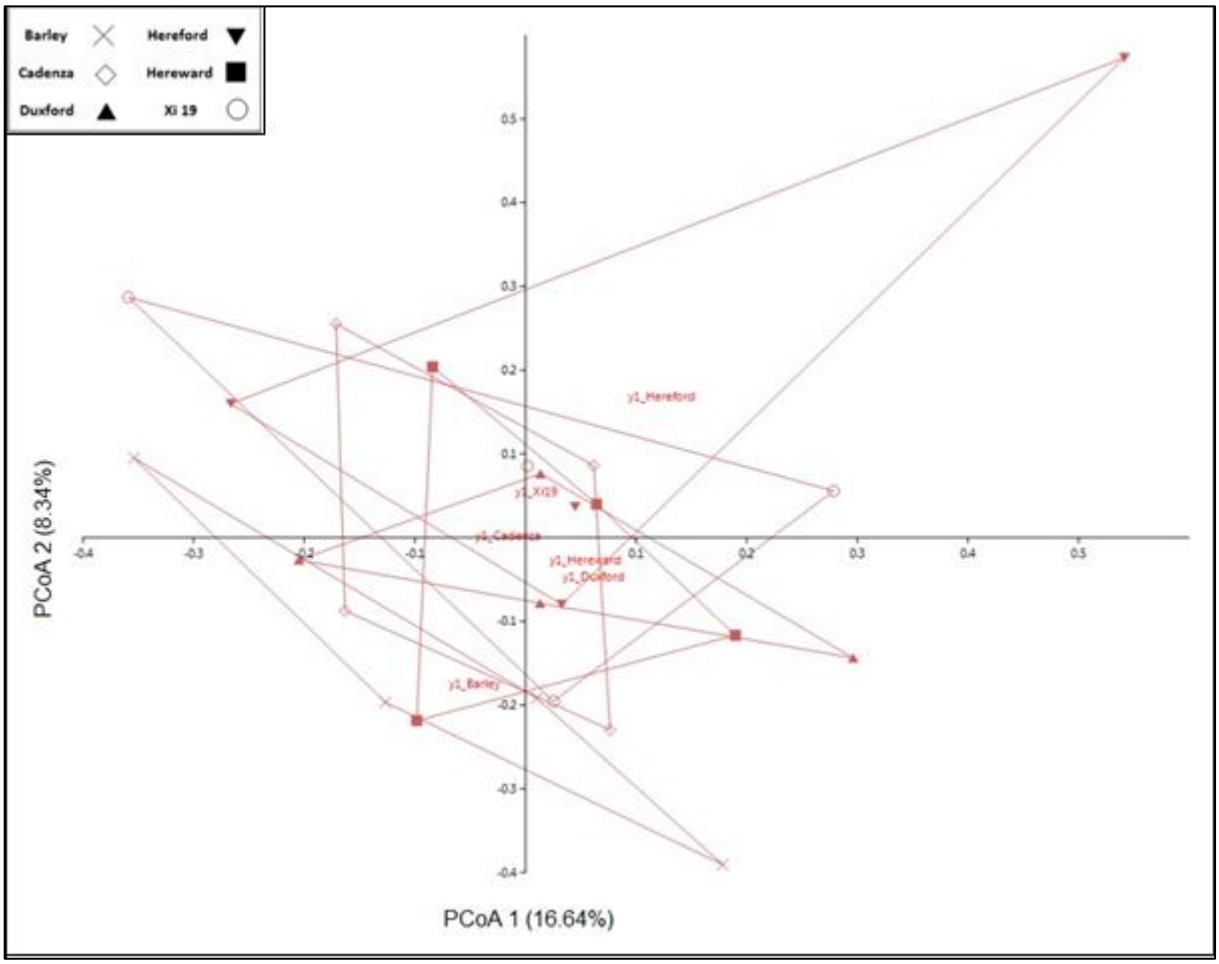


**Figure 42:** PCoA based on Bray-Curtis similarity distance matrix showing the structure of wheat bacterial communities from rhizosphere, obtained from different wheat cultivars and one barley cultivar (represented by different symbols), collected from field one (F1), year 1. Graph generated by Dr. Vanessa Nessner- Kavamura-Noguchi (Rothamsted Research).

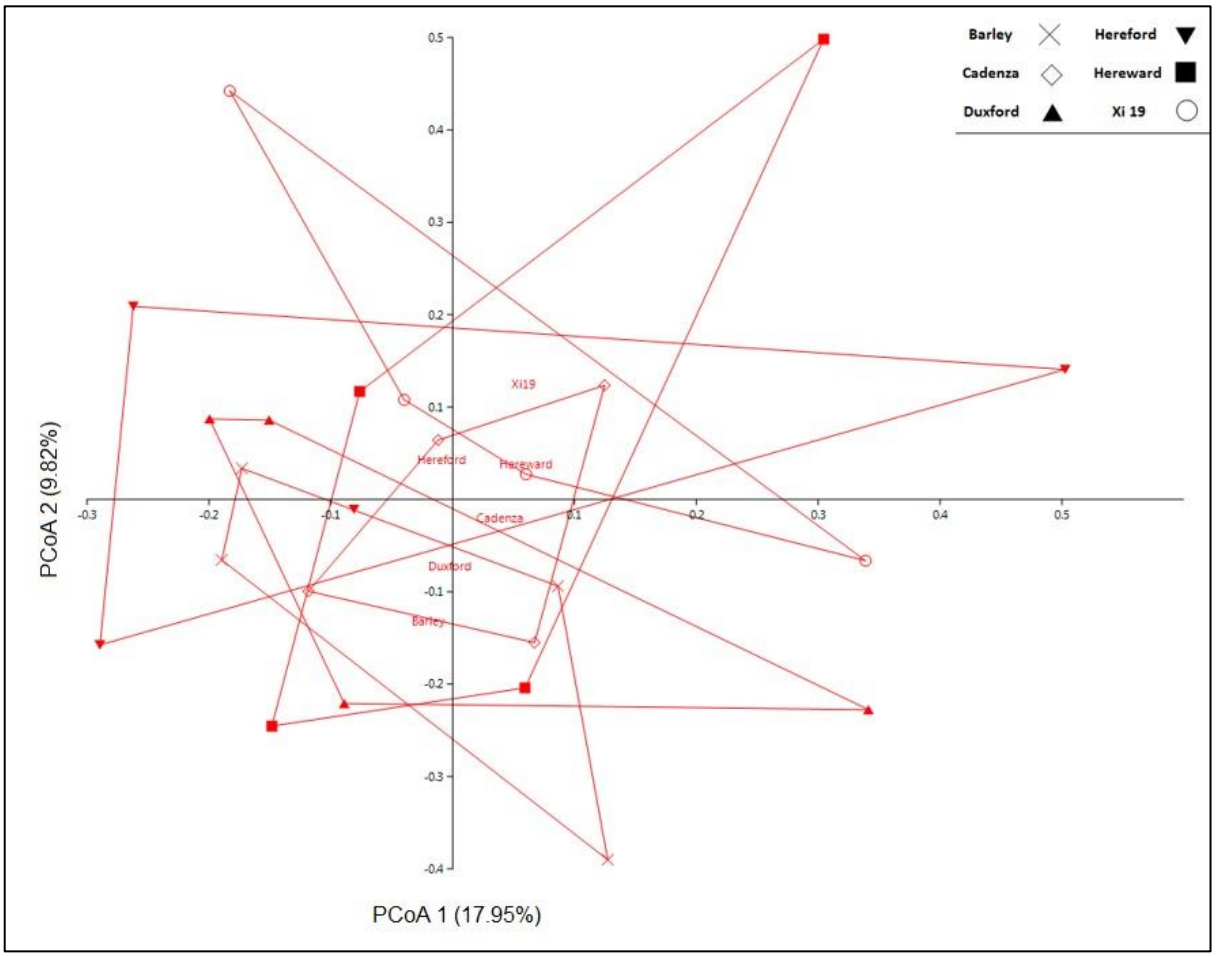


**Figure 43:** PCoA based on Bray-Curtis similarity distance matrix showing the structure of wheat bacterial communities from rhizosphere, obtained from different wheat cultivars and one barley cultivar (represented by different symbols), collected from field one (F1), year 2. Graph generated by Dr. Vanessa Nessner- Kavamura-Noguchi (Rothamsted Research).

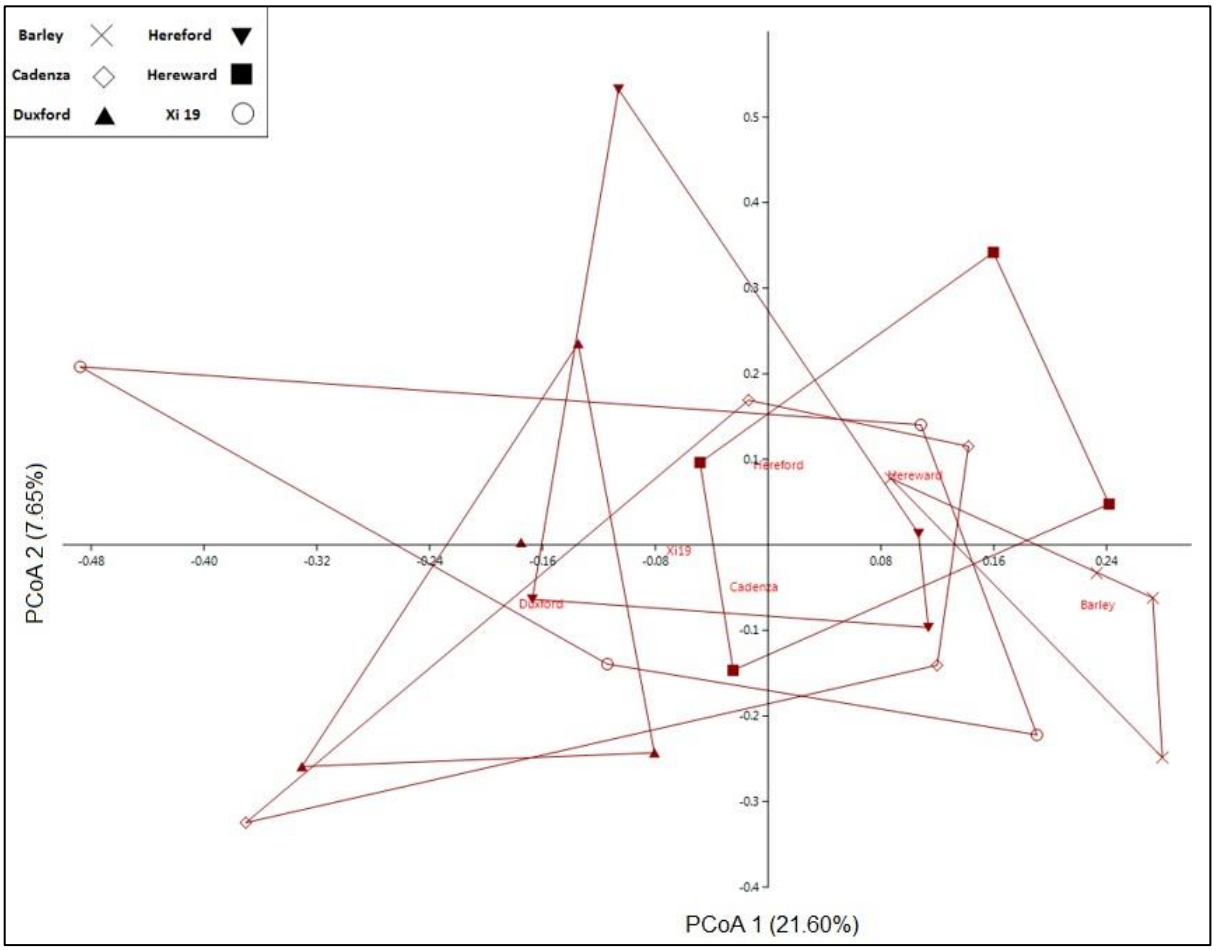


**Figure 44:** PCoA based on Bray-Curtis similarity distance matrix showing the structure of wheat bacterial communities from rhizosphere, obtained from different wheat cultivars and one barley cultivar (represented by different symbols), collected from field one (F1), year 3. Graph generated by Dr. Vanessa Nessner- Kavamura-Noguchi (Rothamsted Research).

#### 6.5.1.1.2 Field 2 (Long Hoos 5) Bacterial community structure

In the second field, a different correlation was observed (Fig.46). The first axis, representing 27.10% of the variation, shows a clear niche separation of bacterial communities, with samples on the right representing bacterial communities from bulk soil (year 0) and samples to the left representing rhizosphere bacterial communities

collected across two years. There is also a clear distinction of rhizosphere bacterial communities after one year (separation in the second axis 10.51%).

To check whether there were differences in bacterial communities from the rhizosphere, samples were analysed per year. In the first year, there is no clear separation based on cultivar (Fig. 47), however, barley communities tend to be slightly different from wheat communities (PERMANOVA, $F = 1.324$, $p = 0.0033$). This became particularly evident in the second year, with barley rhizosphere bacterial communities being completely different from wheat rhizosphere (PERMANOVA, $F = 1.245$, $p = 0.0106$). Conversely when comparing the bacterial community structure of the wheat rhizosphere from both years, after one year, it appeared that wheat cultivars tended to have more similar bacterial communities, as they were more closely clustered (Fig.48).

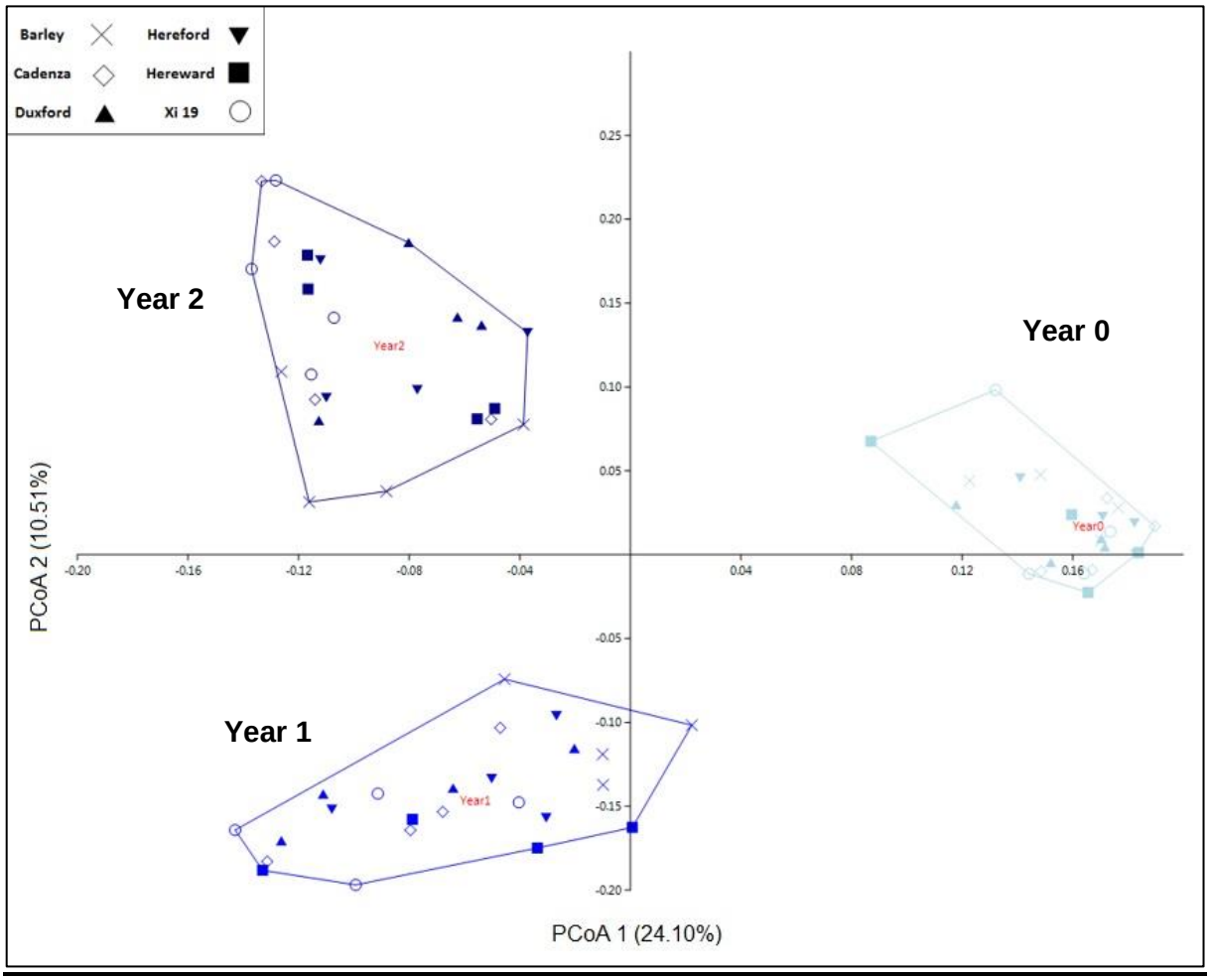


**Figure 45:** PCoA based on Bray-Curtis similarity distance matrix showing the structure of wheat bacterial communities from bulk soil and rhizosphere, obtained from different wheat cultivars and one barley cultivar (represented by different symbols), collected from field two (F2), across different years. Graph generated by Dr. Vanessa Nessner-Kavamura-Noguchi (Rothamsted Research).

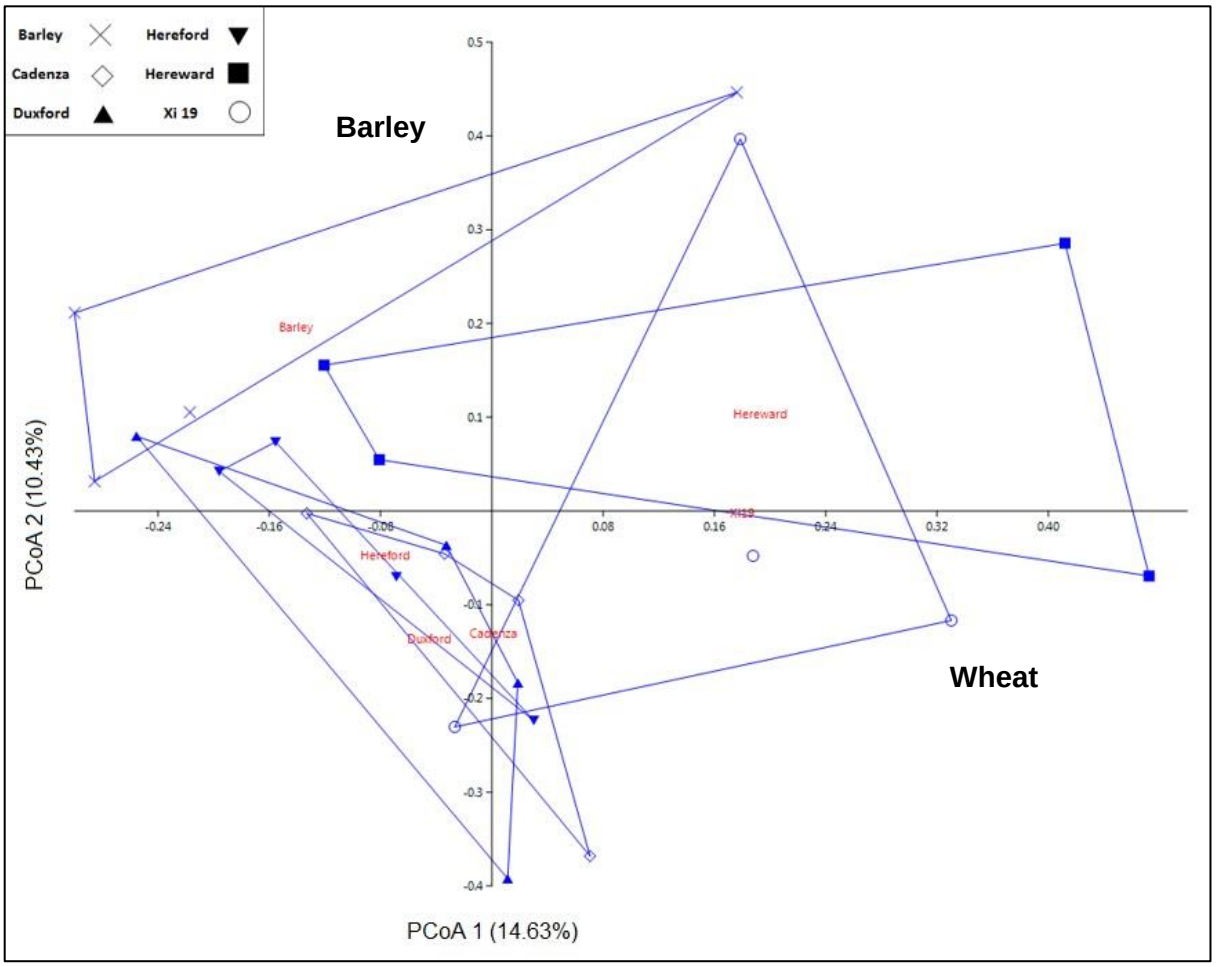


**Figure 46:** PCoA based on Bray-Curtis similarity distance matrix showing the structure of wheat bacterial communities from rhizosphere, obtained from different wheat cultivars and one barley cultivar (represented by different symbols), collected from field two (F2), year 1. Graph generated by Dr. Vanessa Nessner- Kavamura-Noguchi (Rothamsted Research).

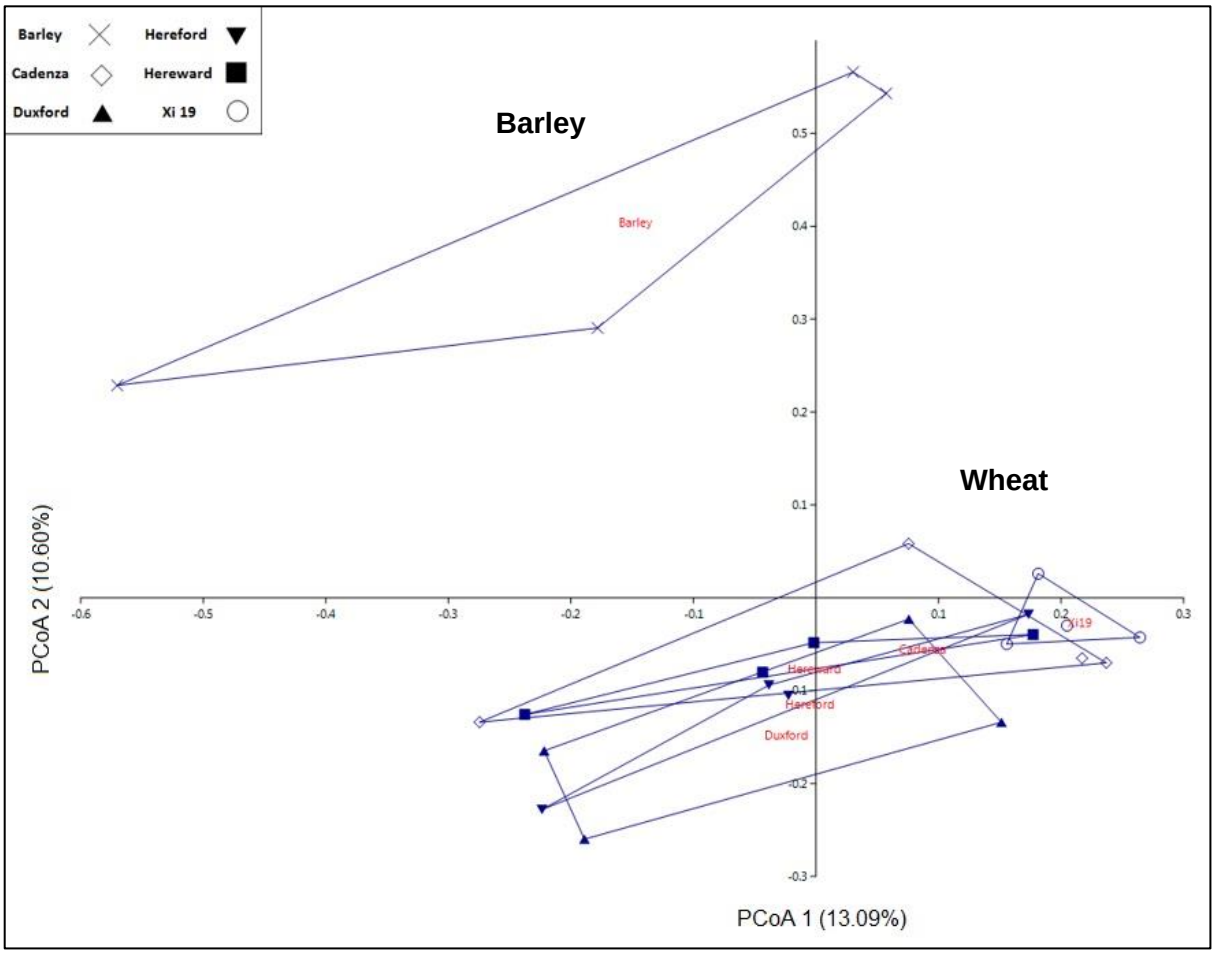


**Figure 47:** PCoA based on Bray-Curtis similarity distance matrix showing the structure of wheat bacterial communities from rhizosphere, obtained from different wheat cultivars and one barley cultivar (represented by different symbols), collected from field two (F2), year 2. Graph generated by Dr. Vanessa Nessner- Kavamura-Noguchi (Rothamsted Research).

#### 6.5.1.1.3 Field 3 (Long Hoos 3) Bacterial community structure

In the third field, similarly to field 2, there is a clear separation of samples based on niche (bulk vs. rhizosphere), with the first axis corresponding to 37.52% of the variation (Fig. 49) (PERMANOVA, $F = 25.63$, $p = 0.0001$).

During the first year, bacterial communities from the rhizosphere of different cultivars do not differ (PERMANOVA, $F = 1.117$, $p = 0.0914$) (Fig. 50).

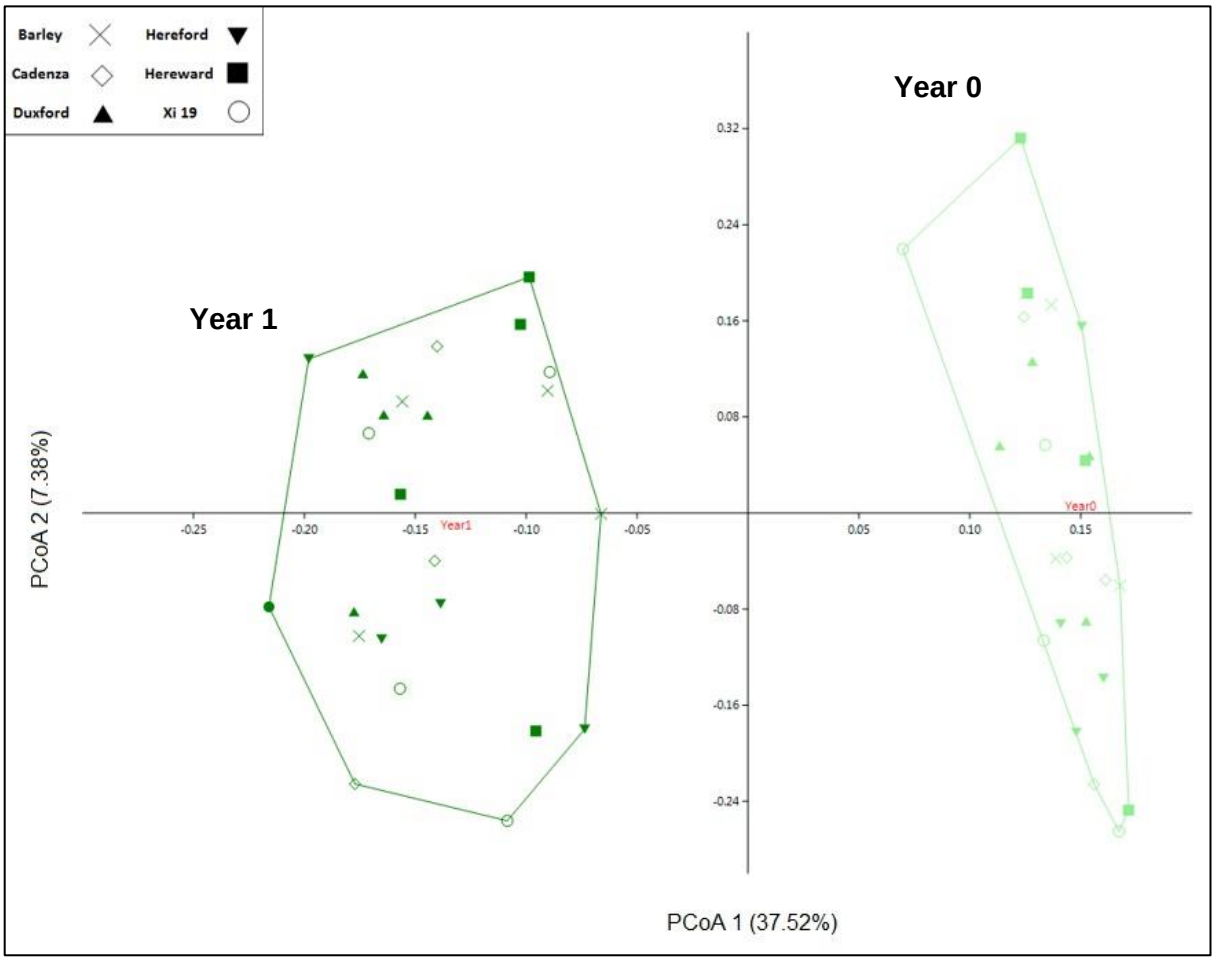


**Figure 48:** PCoA based on Bray-Curtis similarity distance matrix showing the structure of wheat bacterial communities from bulk soil (Year 0) and rhizosphere (Year 1), obtained from different wheat cultivars and one barley cultivar (represented by different symbols), collected from field three (F3). Graph generated by Dr. Vanessa Nessner- Kavamura-Noguchi (Rothamsted Research).

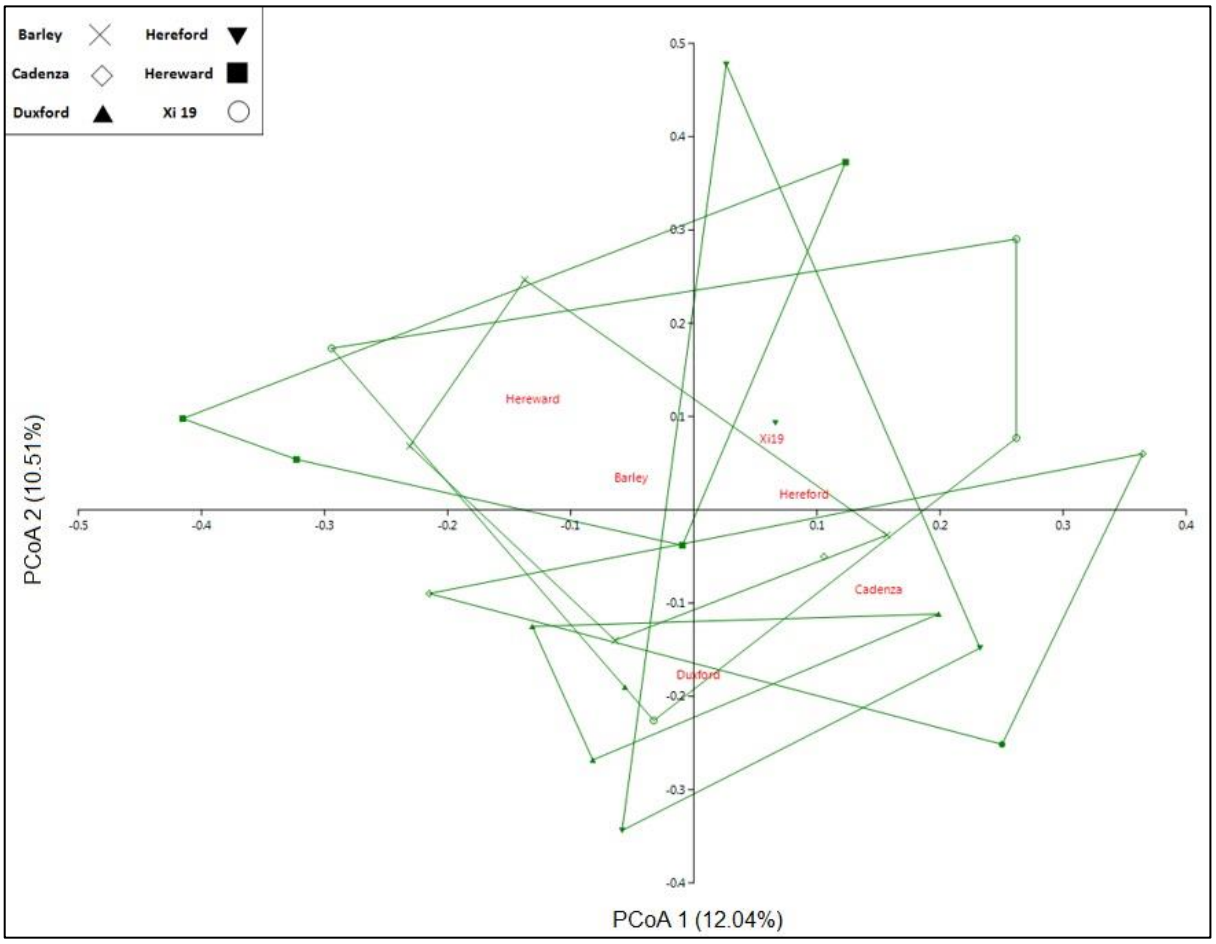


**Figure 49:** PCoA based on Bray-Curtis similarity distance matrix showing the structure of wheat bacterial communities from rhizosphere, obtained from different wheat cultivars and one barley cultivar (represented by different symbols), collected from field three (F3), year 1. Graph generated by Dr. Vanessa Nessner- Kavamura-Noguchi (Rothamsted Research).

### 6.5.2 Differentially abundant taxa

Secondly, Dr. Vanessa Nessner- Kavamura-Noguchi (Rothamsted Research) analysed the OTU table (Appendix V will be provided on request due large size) for differentially abundant taxa. Briefly, the online tool for comprehensive statistical, visual and meta-analysis of microbiome data called MicrobiomeAnalyst (Dhariwal et al., 2017) was used for detecting OTUs that were differentially abundant between samples. The OTU table was arranged as the required format and it was uploaded with the mapping and taxonomy files. Low abundance OTUs with less than 2 counts in less than 10% of the samples were removed. The OTU table was normalised using the method of rarefying with replacement and relative log-expression (RLE) transformed. The DESeq2 algorithm was used to check whether there were specific taxa enriched in specific wheat cultivars, whether they were related to a specific crop (wheat x barley) or whether they were related to Take-all level of susceptibility. Analyses were performed for rhizosphere samples only, for each field and year, separately (Table 45).

**Table 45:** Enriched genera for each crop (wheat vs. barley). Table generated by Dr. Vanessa Nessner- Kavamura-Noguchi (Rothamsted Research).

| Genera | F1Y1 | F1Y2 | F1Y3 | F2Y1 | F2Y2 | F3Y1 |
|---|---|---|---|---|---|---|
| *Aeromicrobium* | | | | | barley | |
| *Amycolatopsis* | | | wheat | | | |
| *Aquabacterium* | | | | | barley | |
| *Arthrobacter* | | | wheat | | | |
| *Asticcacaulis* | | | | | | barley |
| *Aureimonas* | | | barley | | | |
| *Brevundimonas* | | | barley | | barley | barley |
| *Burkholderia* | | | wheat | | wheat | |
| *Caulobacter* | | | | | barley | |
| *Cellvibrio* | | | | | | barley |
| *Chryseobacterium* | | | | | barley | |
| *Clavibacter* | | | | wheat | | |
| *Cytophaga* | barley | | | | barley | barley |
| *Dyadobacter* | | | | | barley | barley |
| *Dyella* | | | | | wheat | |
| *Emticicia* | | | | | barley | barley |
| *Flavitalea* | | | | barley | | |
| *Flavobacterium* | barley | barley | | | barley | barley |
| *Fluviicola* | | | | | | barley |
| *Gemmatimonas* | | | | | wheat | |
| *Haliangium* | | | barley | | | |
| *Herbaspirillum* | | | | | | barley |
| *Herminiimonas* | | | | | barley | |
| *Herpetosiphon* | | | | barley | | |
| *Hymenobacter* | | | | wheat | wheat | |
| *Kaistia* | | | | wheat | | |
| *Labilithrix* | | | barley | | | |
| *Lentzea* | | | | | wheat | |
| *Luteimonas* | | | | | wheat | |
| *Luteolibacter* | | | | | | barley |
| *Lysobacter* | | | | | wheat | |
| *Marmoricola* | | | | wheat | | |
| *Massilia* | | | wheat | | | |
| *Methylobacterium* | | | | wheat | | |
| *Methylotenera* | | barley | | | barley | |
| *Novosphingobium* | | | wheat | | wheat | |
| *Opitutus* | | | | wheat | | |
| *Peredibacter* | | | | | barley | |
| *Phycicoccus* | | | | | wheat | |
| *Promicromonospora* | | | wheat | | wheat | |
| *Porphyrobacter* | | | wheat | | wheat | |

| | | | | | | |
|---|---|---|---|---|---|---|
| *Prosthecobacter* | | | barley | barley | barley | barley |
| *Pseudomonas* | | | | barley | | |
| *Pseudorhodoferax* | | | | | | barley |
| *Pseudoxanthomonas* | | | | | barley | |
| *Rathayibacter* | | | barley | | barley | |
| *Rhizobia* | | | | barley | | |
| *Rhizoharbdus* | barley | barley | barley | | barley | barley |
| *Rhodanobacter* | | | | | | wheat |
| *Rhodococcus* | barley | | barley | | barley | barley |
| *Rubinisphaera* | | | | | barley | |
| *Rugamonas* | | | | barley | barley | |
| *Saccharothrix* | | | | | wheat | wheat |
| *Segetibacter* | | | | | wheat | wheat |
| *Serratia* | | | | | barley | |
| *Sphaerotilus* | | | wheat | | | |
| *Sphingobium* | | | | wheat | | wheat |
| *Sphingomonas* | | | wheat | barley | | |
| *Sphingopyxis* | | | | | barley | |
| *Spirosoma* | | | | wheat | | |
| *Taibaiella* | | | | | barley | barley |
| *Terrabacter* | | | wheat | | wheat | |
| *Vasilyevaea* | | | | wheat | | |
| *Verrucomicrobium* | | | | | | barley |
| *Virgisporangium* | | | | barley | wheat | |

#### 6.5.2.1 Field 1 (New Zealand) rhizosphere samples

When comparing barley vs. wheat of Year 1 rhizosphere, *Rhodoccoccus, Rhizoharbdus, Cytophaga* and *Flavobacterium* were enriched in the barley rhizosphere. When removing barley samples, no taxa were significantly differentially abundant between low and high disease susceptible wheat samples. In the Year 2 rhizosphere, when comparing barley vs. wheat, *Methylotenera, Rhizoharbdus* and *Flavobacterium* were enriched in barley rhizosphere. The same pattern as the first year was observed when removing barley samples, with no differentially abundant taxa. Finally, in Year 3 rhizosphere, *Rhodococcus, Prosthecobacter, Brevundimonas, Rhizoharbdus, Haliangium, Rathayibacter, Labilithrix* and *Aureimonas* were enriched

in the barley rhizosphere, whereas *Novosphingobium, Burkholderia, Porphyrobacter, Promicromonospora, Arthrobacter, Amycolatopsis, Dyella, Sphaerotilus, Sphingomonas, Terrabacter* and *Massilia* were significantly enriched in the wheat rhizosphere. No taxa were significantly differentially abundant between low and high disease susceptible wheat samples.

#### 6.5.2.2 Field 2 (Long Hoss5) rhizosphere samples

When comparing barley vs. wheat in the Year 1 rhizosphere of field 2, *Sphingomonas, Pesudomonas, Virgisporangium, Herpetosiphon, Prosthecobacter, Rhizobia, Rugamonas* and *Flavitalea,* were enriched in the barley rhizosphere. Conversely, *Hymenobacter, Sphingobium, Opitutus, Marmoricola, Methylobacterium, Kaistia, Clavibacter, Spirosoma* and *Vasilyevaea* were enriched in the wheat rhizosphere, regardless of disease incidence. When removing barley samples, one genus, *Tahibacter*, was found to be enriched in the rhizosphere of L-TAB wheat.

For the second year, the barley rhizosphere was enriched in the following genera: *Rhodococcus, Prosthecobacter, Methylotenera, Cytophaga, Rhizoharbdus, Brevundimonas, Sphinopyxis, Chryseobacterium, Aquabacterium, Serratia, Emticicia, Aeromicrobium, Rugamonas, Taibaiella, Peredibacter, Dyadobacter, Pseudoxanthomonas, Flavobacterium, Herminiimonas, Rubinisphaera, Caulobacter* and *Rathayibacter*. The wheat rhizosphere was enriched in the following genera: *Hymenobacter, Promicromonospora, Saccharothrix, Lysobacter, Porphyrobacter, Novosphingobium, Segetibacter, Phycicoccus, Dyella, Gemmatimonas, Luteimonas, Virgisporangium, Lentzea, Terrabacter* and *Burkholderia.* When removing barley samples, no taxa were significantly differentially abundant between low and high disease susceptible wheat samples.

### 6.5.2.3 Field 3 (Long Hoos 4) rhizosphere samples

In the rhizosphere of field 3, sixteen genera were enriched in the barley rhizosphere and they include *Rhizoharbdus, Cytophaga, Rhodococcus, Cellvibrio, Taibaiella, Dyadobacter, Asticcacaulis, Verrucomicrobium, Prosthecobacter, Emticicia, Herbaspirillum, Luteolibacter, Flavobacterium, Pseudorhodoferax, Brevundimonas* and *Fluviicola.* The wheat rhizosphere was enriched in *Saccharothrix, Segetibacter, Rhodanobacter* and *Sphingobium.* When removing the barley samples, no taxa were significantly differentially abundant between low and high disease susceptible wheat samples.

A summary with all the enriched genera per crop for each field with respective year are shown in Figs 51 and 52.

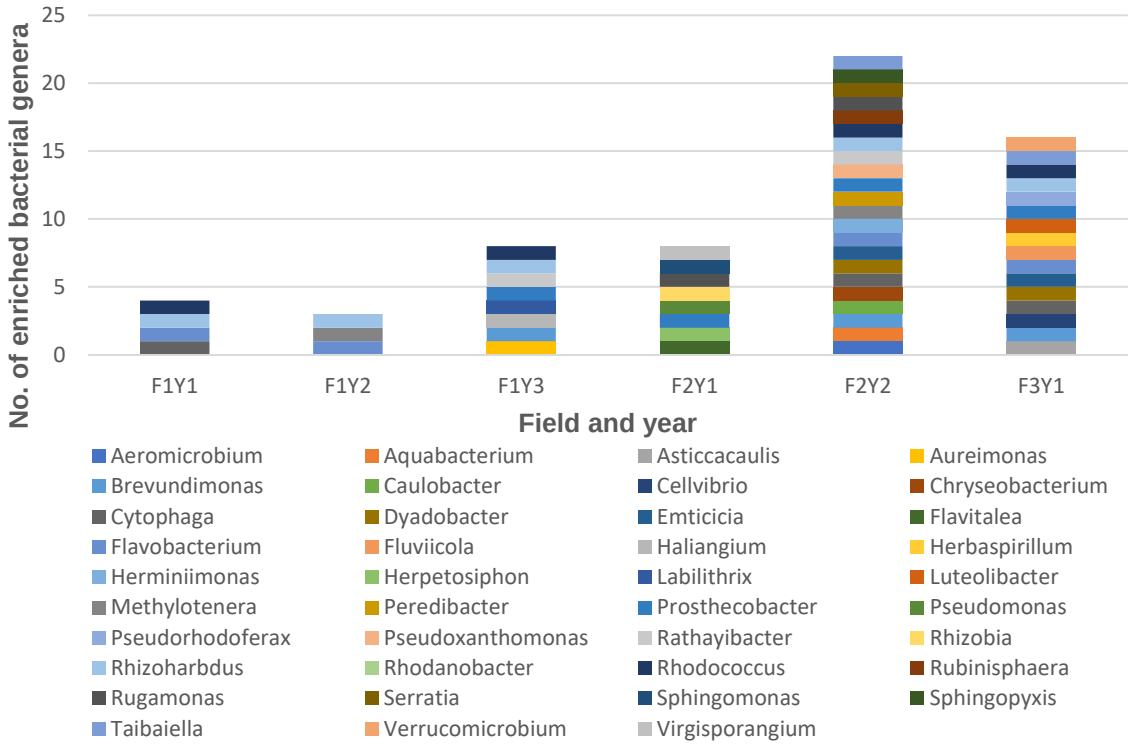


**Figure 50:** Enriched taxa in the Barley rhizosphere.

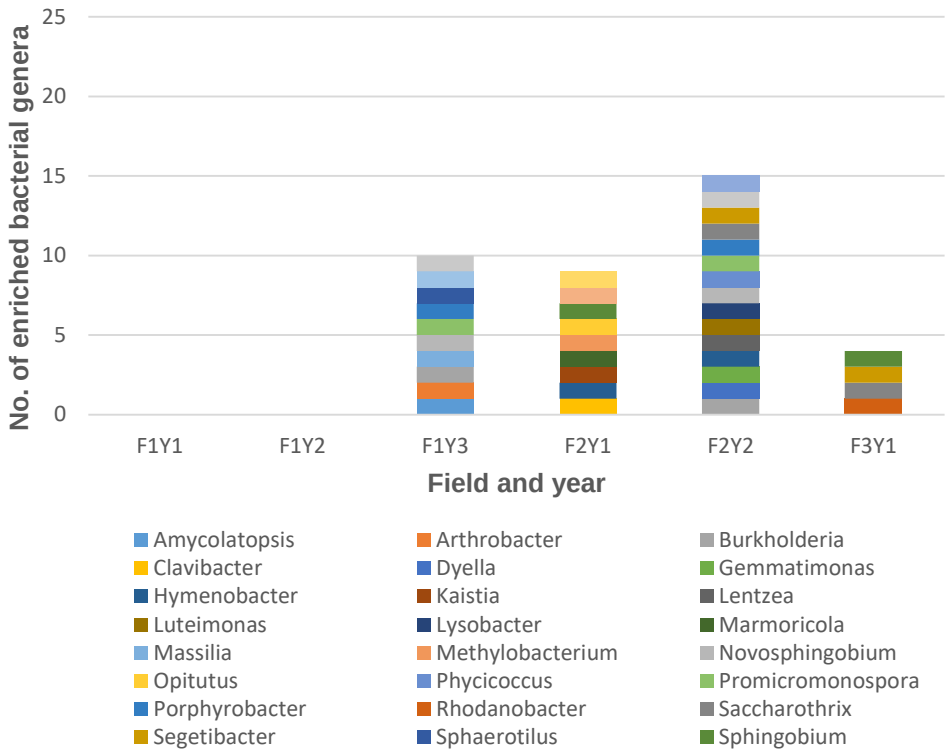


**Figure 51:** Enriched taxa in the wheat rhizosphere.

### 6.5.3 Quantitative real time PCR

The second aim of this field experiment was to monitor changes in *Ggt* soil DNA levels in comparison to bacteria, *Pseudomonas* and fungi. The fungi here, refers to total fungi excluding arbuscular mychorrhiza AMF, as the primers used didn't cover the *Glomeromycota* group.

For each of the 192 soil DNA, 30 µl were devoted towards *Ggt*, bacteria, *Pseudomonas* and fungi qPCR. Methods for Taqman and SYBR-Green qPCR were used as described in section 6.2.2. Both qPCRs were carried out at Rothamsted Research based on established assays.

#### 6.5.3.1 Statistical analysis of quantitative real time PCR (qPCR) data:

Analysis of qPCR data was performed using GenStat (GenStat, v17 and v18). Since the sampling seasons was not consistent over the three different fields *i.e*: bulk soil data was not available for field 1, no year 3 data for field 2, and no year 2 or year 3 for field 3, it was not possible to analyse the data of all fields in comparison to each other. Thus, each field was analysed individually. Furthermore, comparison might not be possible since the quantification of 16S rRNA bacterial, 16S rRNA *Pseudomonas*, and ITS were carried out using SYBR-Green, the standards used were obtained a mixture of soils (6.2.2.3.1) while Taqman qPCR was used for *Ggt* with standards from a pure *Ggt* culture (6.2.2.2.2). Thus, each set was analysed separately. The raw data are in Appendix II and III (9.4.2 and 9.4.3).

#### 6.5.3.1.1 Analysis of 16S rRNA bacterial, 16S rRNA *Pseudomonas*, and ITS qPCR data:

The outcomes of the two technical repeats were averaged and the data was reported as picograms per gram of dry soil (pg/ g of dry soil). Since samples were obtained from the same plots over time, ANOVA in Repeated Measurements menu was used for further analysis. First, both Generalized Linear Mixed Model (GLMM) and Analysis of Variance (ANOVA) in the repeated measurements menu were used on the data. Both gave similar outcomes (data not shown), however the ANOVA menu allowed incorporation of the time factor easily and therefore was used for further analysis. The term sampling season or sampling point was used to imply for the different time points where the soil samples were collected (Y0 bulk soil, Y1, Y2, Y3 rhizosphere soil). For each field the data were unstacked by the sampling season (years 0, 1, 2, 3) for the following data: averaged qPCR outcome (pg/g dry soil), crop, cultivar, block, and plot. The model to be fitted (crop / cultivar) using Block as the blocking term and for transformation of data square root or log base 10 were used as necessary. Data transformation was used to stabilize the residuals when needed.

##### 6.5.3.1.1.1 Field 1 (New Zealand):

For bacterial 16S rRNA levels there were significant differences across the three sampling seasons and between barley and wheat crops over time ($p < 0.001$, and $p = 0.049$ respectively). No significant difference in total bacterial levels were observed within the different wheat cultivars over time ($p = 0.508$). A close correlation was observed in the level of ITS for total fungi where there were significant differences across the three sampling seasons ($p < 0.001$) and approximating significance between the barley and wheat crops over time ($p = 0.063$). However, no significant

difference was observed in total fungal levels within the different wheat cultivars over time (p=0.298). The levels of *Pseudomonas* 16S rRNA were also significantly different across the growing seasons (p<0.001), but no differences were observed with the barley and wheat crops or with the wheat cultivars over time (p=0.151 and p=0.071, respectively) (Tables 46-48). These observations might imply that there are other factors that have not been taken into account while performing the analysis (like soil temperature, pH, conductivity, soil C and N content, plant variables).

**Table 46:** Field 1 ANOVA on repeated measurements for averaged bacterial DNA pg/ g soil. Block: the four main blocks; Crop: wheat or barley; Cultivar: Cadenza, Hereward, Hereford, Xi-19, Duxford and Barley; Time: year 2015-2017; d.f: Degrees of freedom; F pr.: F probability.

| **Source of variation** | **d.f.** | **s.s.** | **m.s.** | **v.r.** | **F pr.** |
|---|---|---|---|---|---|
| Block_1 stratum | 3 | 696470 | 232157 | 5 | |
| Block_1.Subject stratum | | | | | |
| Crop_1 | 1 | 4872 | 4872 | 0.1 | 0.75 |
| Crop_1.Cultivar_1 | 4 | 185689 | 46422 | 1 | 0.438 |
| Residual | 15 | 696398 | 46427 | 2.12 | |
| Block_1.Subject.Time stratum | | | | | |
| d.f. correction factor 0.8968 | | | | | |
| Time | 2 | 523412 | 261706 | 11.94 | <.001 |
| Time.Crop_1 | 2 | 151186 | 75593 | 3.45 | 0.049 |
| Time.Crop_1.Cultivar_1 | 8 | 160849 | 20106 | 0.92 | 0.508 |
| Residual | 36 | 789101 | 21919 | | |
| Total | 71 | 3207977 | | | |

**Table 47:** Field 1 ANOVA on repeated measurements for averaged *Pseudomonas* DNA pg/ g soil. Block: the four main blocks; Crop: wheat or barley; Cultivar: Cadenza, Hereward, Hereford, Xi-19, Duxford and Barley; Time: year 2015-2017; d.f: Degrees of freedom; F pr.: F probability.

| **Source of variation** | **d.f.** | **s.s.** | **m.s.** | **v.r.** | **F pr.** |
|---|---|---|---|---|---|
| Block_11 stratum | 3 | 18.883 | 6.294 | 0.75 | |
| Block_11.Subject stratum | | | | | |
| Crop_11 | 1 | 13.727 | 13.727 | 1.63 | 0.221 |
| Crop_11.Cultivar_11 | 4 | 36.435 | 9.109 | 1.08 | 0.401 |
| Residual | 15 | 126.361 | 8.424 | 2.03 | |
| Block_11.Subject.Time stratum | | | | | |
| d.f. correction factor 0.9653 | | | | | |
| Time | 2 | 81.707 | 40.853 | 9.84 | <.001 |
| Time.Crop_11 | 2 | 16.688 | 8.344 | 2.01 | 0.151 |
| Time.Crop_11.Cultivar_11 | 8 | 68.028 | 8.504 | 2.05 | 0.071 |
| Residual | 36 | 149.406 | 4.15 | | |
| Total | 71 | 511.236 | | | |

**Table 48:** Field 1 ANOVA on repeated measurements for averaged fungi DNA pg/ g soil. Block: the four main blocks; Crop: wheat or barley; Cultivar: Cadenza, Hereward, Hereford, Xi-19, Duxford and Barley; Time: year 2015-2017; d.f: Degrees of freedom; F pr.: F probability.

| **Source of variation** | **d.f.** | **s.s.** | **m.s.** | **v.r.** | **F pr.** |
|---|---|---|---|---|---|
| Block_21 stratum | 3 | 359768 | 119923 | 3.73 | |
| Block_21.Subject stratum | | | | | |
| Crop_21 | 1 | 116928 | 116928 | 3.64 | 0.076 |
| Crop_21.Cultivar_21 | 4 | 182282 | 45571 | 1.42 | 0.276 |
| Residual | 15 | 481904 | 32127 | 1.24 | |
| Block_21.Subject.Time stratum | | | | | |
| d.f. correction factor 0.8039 | | | | | |
| Time | 2 | 1190430 | 595215 | 22.99 | <.001 |
| Time.Crop_21 | 2 | 167791 | 83895 | 3.24 | 0.063 |
| Time.Crop_21.Cultivar_21 | 8 | 264034 | 33004 | 1.27 | 0.298 |
| Residual | 36 | 931910 | 25886 | | |
| Total | 71 | 3695047 | | | |

#### 6.5.3.1.1.2 Field 2 (Long Hoos 5)

The data of Bacterial 16S rRNA levels was transformed by taking the square root. The levels of bacterial 16S rRNA were significantly different across the three sampling points year 0 (bulk soil), year1 and year2 rhizospheres; ($p<0.001$). No significant differences were observed between the barley and wheat crops ($p=0.252$) or within the wheat cultivars ($p=0.644$). The data for *Pseudomonas* 16S rRNA were log10 transformed. There were no significant differences in *Pseudomonas* 16S rRNA levels between the three sampling points or between the barley and wheat crops or within the wheat cultivars ($p=0.153$, $p=0.504$, and $p=0.255$ respectively). The levels of ITS were significantly different across the three sampling points ($p<0.001$). However, no significant differences were observed in ITS levels between the barley and wheat crops or within the wheat cultivars over the three sampling points ($p=0.279$ and $p=0.780$, respectively) (Tables 49-51).

**Table 49:** Field 2 ANOVA on repeated measurements for averaged bacterial DNA pg/ g soil. The data was square root transformed. Block: the four main blocks; Crop: wheat or barley; Cultivar: Cadenza, Hereward, Hereford, Xi-19, Duxford and Barley; Time: year 2015-2017; d.f: Degrees of freedom; F pr.: F probability.

| Source of variation | d.f. | s.s. | m.s. | v.r. | F pr. |
|---|---|---|---|---|---|
| Block_1 stratum | 3 | 83.26 | 27.75 | 1.49 | |
| Block_1.Subject stratum | | | | | |
| Crop_1 | 1 | 0 | 0 | 0 | 0.999 |
| Crop_1.Cultivar_1 | 4 | 8.78 | 2.19 | 0.12 | 0.974 |
| Residual | 15 | 280.25 | 18.68 | 1.09 | |
| Block_1.Subject.Time stratum | | | | | |
| d.f. correction factor 0.7869 | | | | | |
| Time | 2 | 1263.4 | 631.71 | 36.74 | <.001 |
| Time.Crop_1 | 2 | 49.35 | 24.68 | 1.44 | 0.252 |
| Time.Crop_1.Cultivar_1 | 8 | 98.9 | 12.36 | 0.72 | 0.644 |
| Residual | 36 | 618.97 | 17.19 | | |
| Total | 71 | 2402.9 | | | |

**Table 50:** Field 2 ANOVA on repeated measurements for averaged *Pseudomonas* DNA pg/ g soil. The data were log transformed. Block: the four main blocks; Crop: wheat or barley; Cultivar: Cadenza, Hereward, Hereford, Xi-19, Duxford and Barley; Time: year 2015-2017; d.f: Degrees of freedom; F pr.: F probability.

| **Source of variation** | **d.f.** | **s.s.** | **m.s.** | **v.r.** | **F pr.** |
|---|---|---|---|---|---|
| Block_1 stratum | 3 | 0.00964 | 0.00321 | 0.08 | |
| Block_1.Subject stratum | | | | | |
| Crop_1 | 1 | 0.00919 | 0.00919 | 0.23 | 0.64 |
| Crop_1.Cultivar_1 | 4 | 0.12674 | 0.03169 | 0.79 | 0.551 |
| Residual | 15 | 0.60407 | 0.04027 | 0.68 | |
| Block_1.Subject.Time stratum | | | | | |
| d.f. correction factor 0.9513 | | | | | |
| Time | 2 | 0.23631 | 0.11815 | 2 | 0.153 |
| Time.Crop_1 | 2 | 0.08089 | 0.04045 | 0.68 | 0.504 |
| Time.Crop_1.Cultivar_1 | 8 | 0.6381 | 0.07976 | 1.35 | 0.255 |
| Residual | 36 | 2.12875 | 0.05913 | | |
| Total | 71 | 3.83368 | | | |

**Table 51:** Field 2 ANOVA on repeated measurements for averaged fungi DNA pg/ g soil. Block: the four main blocks; Crop: wheat or barley; Cultivar: Cadenza, Hereward, Hereford, Xi-19, Duxford and Barley; Time: year 2015-2017; d.f: Degrees of freedom; F pr.: F probability.

| **Source of variation** | **d.f.** | **s.s.** | **m.s.** | **v.r.** | **F pr.** |
|---|---|---|---|---|---|
| Block_11 stratum | 3 | 195720 | 65240 | 2.14 | |
| Block_11.Subject stratum | | | | | |
| Crop_11 | 1 | 43875 | 43875 | 1.44 | 0.249 |
| Crop_11.Cultivar_11 | 4 | 89425 | 22356 | 0.73 | 0.584 |
| Residual | 15 | 457825 | 30522 | 1.33 | |
| Block_11.Subject.Time stratum | | | | | |
| d.f. correction factor 0.9107 | | | | | |
| Time | 2 | 2524513 | 1262256 | 54.82 | <.001 |
| Time.Crop_11 | 2 | 60833 | 30417 | 1.32 | 0.279 |
| Time.Crop_11.Cultivar_11 | 8 | 105193 | 13149 | 0.57 | 0.78 |
| Residual | 36 | 828867 | 23024 | | |
| Total | 71 | 4306251 | | | |

#### 6.5.3.1.1.3 Field 3 (Long Hoos 4)

In Long Hoos 4 (LH4) the levels of bacterial 16S rRNA were significantly different between the two sampling seasons both bulk and rhizosphere soil ($p<0.001$). However, no significant difference in the levels were observed between the barley and wheat crops or within the different wheat cultivars overtime ($p= 0.219$ and $p=0.847$, respectively). The same trend was observed for 16S rRNA *Pseudomonas* levels with significant differences between the bulk soil and rhizosphere soil ($p<0.001$) and no significant difference was observed between crops or within the wheat cultivars overtime ($p= 0.219$ and $p=0.847$, respectively). The levels of *Pseudomonas* 16S rRNA were significantly different ($p<0.001$) between years zero and one (bulk vs. rhizosphere). However, no significant differences in *Pseudomonas* 16S rRNA levels were observed between the barley and wheat crops or within the wheat cultivars overtime ($p= 0.383$ and $p= 0.517$, respectively). Similarly, the levels of ITS were significantly different between the two sampling seasons ($p<0.001$). However, no significant differences were observed in ITS levels between the barley and wheat crops or within the wheat cultivars overtime ($p=0.818$ and $p= 0.092$, respectively) (Tables 52-54).

**Table 52:** Field 3 ANOVA on repeated measurements for averaged bacterial DNA pg/ g soil. Block: the four main blocks; Crop: wheat or barley; Cultivar: Cadenza, Hereward, Hereford, Xi-19, Duxford and Barley; Time: year 2015-2017; d.f: Degrees of freedom; F pr.: F probability.

| Source of variation | d.f. | s.s. | m.s. | v.r. | F pr. |
|---|---|---|---|---|---|
| Block_1 stratum | 3 | 33076 | 11025 | 0.21 | |
| Block_1.Subject stratum | | | | | |
| Crop_1 | 1 | 41873 | 41873 | 0.78 | 0.391 |
| Crop_1.Cultivar_1 | 4 | 92807 | 23202 | 0.43 | 0.784 |
| Residual | 15 | 805914 | 53728 | 1.25 | |
| Block_1.Subject.Time stratum | | | | | |
| d.f. correction factor 1.0000 | | | | | |
| Time | 1 | 1752298 | 1752298 | 40.63 | <.001 |
| Time.Crop_1 | 1 | 69971 | 69971 | 1.62 | 0.219 |
| Time.Crop_1.Cultivar_1 | 4 | 58878 | 14719 | 0.34 | 0.847 |
| Residual | 18 | 776335 | 43130 | | |
| Total | 47 | 3631151 | | | |

**Table 53:** Field 3 ANOVA on repeated measurements for averaged *Pseudomonas* DNA pg/ g soil. Block: the four main blocks; Crop: wheat or barley; Cultivar: Cadenza, Hereward, Hereford, Xi-19, Duxford and Barley; Time: year 2015-2017; d.f: Degrees of freedom; F pr.: F probability.

| Source of variation | d.f. | s.s. | m.s. | v.r. | F pr. |
|---|---|---|---|---|---|
| Block_11 stratum | 3 | 5.365 | 1.788 | 0.47 | |
| Block_11.Subject stratum | | | | | |
| Crop_11 | 1 | 9.883 | 9.883 | 2.62 | 0.126 |
| Crop_11.Cultivar_11 | 4 | 14.707 | 3.677 | 0.98 | 0.45 |
| Residual | 15 | 56.544 | 3.77 | 0.95 | |
| Block_11.Subject.Time stratum | | | | | |
| d.f. correction factor 1.0000 | | | | | |
| Time | 1 | 156.973 | 156.973 | 39.66 | <.001 |
| Time.Crop_11 | 1 | 3.165 | 3.165 | 0.8 | 0.383 |
| Time.Crop_11.Cultivar_11 | 4 | 13.312 | 3.328 | 0.84 | 0.517 |
| Residual | 18 | 71.252 | 3.958 | | |
| Total | 47 | 331.201 | | | |

**Table 54:** Field 3 ANOVA on repeated measurements for averaged fungi DNA pg/ g soil. Block: the four main blocks; Crop: wheat or barley; Cultivar: Cadenza, Hereward, Hereford, Xi-19, Duxford and Barley; Time: year 2015-2017; d.f: Degrees of freedom; F pr.: F probability.

| Source of variation | d.f. | s.s. | m.s. | v.r. | F pr. |
|---|---|---|---|---|---|
| Block_21 stratum | 3 | 274987 | 91662 | 0.76 | |
| Block_21.Subject stratum | | | | | |
| Crop_21 | 1 | 12981 | 12981 | 0.11 | 0.747 |
| Crop_21.Cultivar_21 | 4 | 1095848 | 273962 | 2.28 | 0.109 |
| Residual | 15 | 1804579 | 120305 | 0.94 | |
| Block_21.Subject.Time stratum | | | | | |
| d.f. correction factor 1.0000 | | | | | |
| Time | 1 | 3869395 | 3869395 | 30.09 | <.001 |
| Time.Crop_21 | 1 | 7034 | 7034 | 0.05 | 0.818 |
| Time.Crop_21.Cultivar_21 | 4 | 1215087 | 303772 | 2.36 | 0.092 |
| Residual | 18 | 2314926 | 128607 | | |
| Total | 47 | 10594836 | | | |

### 6.5.3.2 Analysis of *Ggt* qPCR data:

The *Ggt* data were analysed at two levels. First to test the differential probability of *Ggt* presence within the six treatments (1 barley and 5 wheat cultivars), the data were converted to (0, 1) for presence and absence. The data were then analysed at the level of each field and each sampling point within a given field individually. These data were analysed under a regression menu Generalized Linear Mixed Model (GLMM) using a Binomial distribution on logit scale. However, this analysis failed when there was not much variation like in the case of New Zealand data and LH5 year 0 where all the values were zero. Thus, the analysis was replaced by a simple count (Appendix III 9.4.3).

#### 6.5.3.2.1 Field 1 New Zealand

In year 1 *Ggt* was present in three plots (Duxford, Barley, and Cadenza) out of the 24 plots. The amounts were too low to appear in the graph (Fig. 53). In year 2 *Ggt* was present in all plots except for one plot (Hereward). In year 3 it was present in all 24 plots. For all the treatments the amount of *Ggt* DNA was increasing from year 1 to year 2 and then was decreasing in year 3, except for Cadenza and Hereford, which exhibited an increase in Year 3.

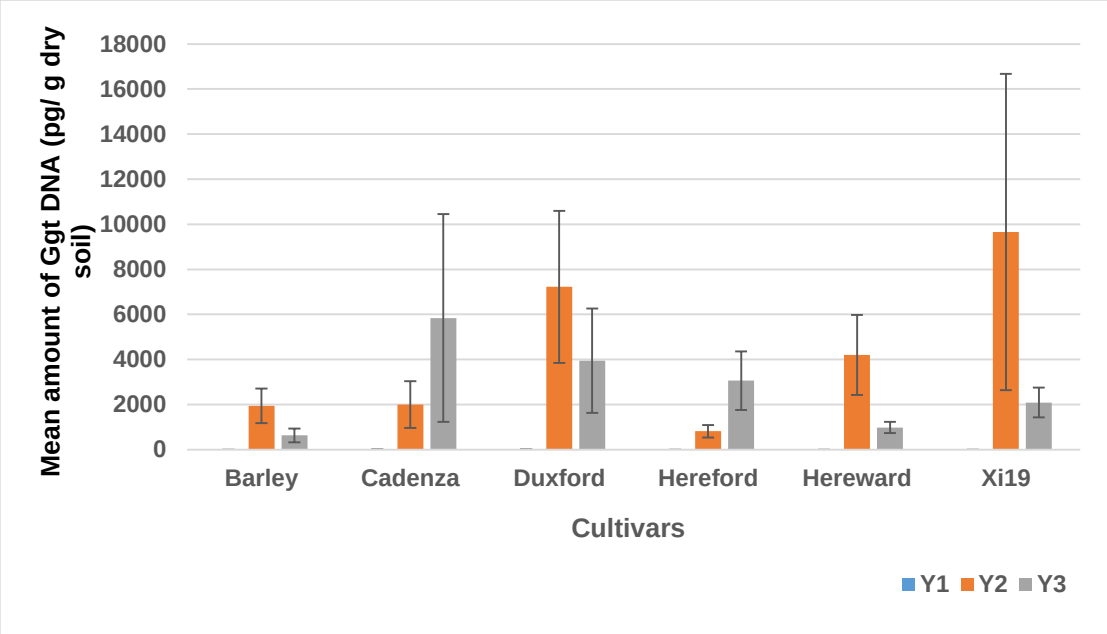


**Figure 52: Concentration of *Ggt* DNA (pg/ g dry soil) from field 1 New Zealand Y 1, Y 2 and Y 3.** The bars are the averaged amount of DNA over the four replicates. Error bars are based on s.e.

#### 6.5.3.2.2 Field 2 Long Hoos 5

In year 0 (bulk soil) no *Ggt* was detected in all 24 plots (Fig. 54). In year 1 there was no significant difference in the presence and absence of *Ggt* between the barley and wheat crops (p= 0.913) or within the wheat cultivars (p=0.532) (Table 55). Again, in year 2 there was no significant difference in the presence and absence of *Ggt* between

the barley and wheat crops and within the wheat cultivars (p= 0.962 and p=1, respectively) (Table 56). There was an increase in the amount of *Ggt* DNA from year 0 to year 1, with the highest levels recorded for year 2.

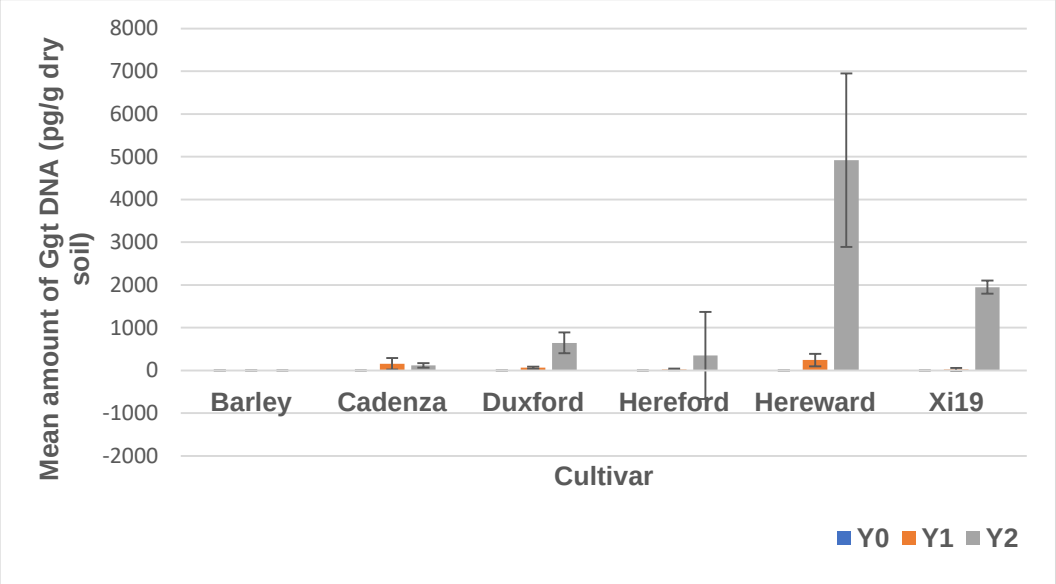


**Figure 53: Concentration of *Ggt* DNA (pg/ g dry soil) from field 2 Long Hoos 5 Y 0, Y 1 and Y 2.** The bars are the averaged amount of DNA over the four replicates. Error bars are based on s.e.

**Table 55:** Test for fixed effects from GLMM for Long Hoos 5 year 1.

| Fixed term | Wald statistic | n.d.f. | F statistic | d.d.f. | F pr. |
|---|---|---|---|---|---|
| Crop | 0.01 | 1 | 0.01 | 18 | 0.913 |
| Crop.Cultivar | 3.26 | 4 | 0.81 | 18 | 0.532 |

**Table 56:** Test for fixed effects from GLMM for Long Hoos 5 year 2.

| Fixed term | Wald statistic | n.d.f. | F statistic | d.d.f. | F pr. |
|---|---|---|---|---|---|
| Crop | 0 | 1 | 0 | 18 | 0.962 |
| Crop.Cultivar | 0.02 | 4 | 0.01 | 18 | 1 |

#### 6.5.3.2.3 Field 3 Long Hoos 4

In year 0 there was no significant differences in *Ggt* presence and absence between the barley and wheat crops or within the wheat cultivars (p= 0.646 and p= 0.915, respectively) (Table 57). In year 1, again there were no significant differences in *Ggt* presence and absence between the barley and wheat crops or within the wheat cultivars (p= 0.539 and p= 0.751, respectively) (Table 58).

**Table 57:** Test for fixed effects from GLMM for Long Hoos 4 year 0.

| Fixed term | Wald statistic | n.d.f. | F statistic | d.d.f. | F pr. |
|---|---|---|---|---|---|
| Crop | 0.22 | 1 | 0.22 | 18 | 0.646 |
| Crop.Cultivar | 0.94 | 4 | 0.24 | 18 | 0.915 |

**Table 58:** Test for fixed effects from GLMM for Long Hoos 4 year 1.

| Fixed term | Wald statistic | n.d.f. | F statistic | d.d.f. | F pr. |
|---|---|---|---|---|---|
| Crop | 0.39 | 1 | 0.39 | 18 | 0.539 |
| Crop.Cultivar | 1.92 | 4 | 0.48 | 18 | 0.751 |

Again, in field Long Hoos 4 a similar pattern as Long Hoos 5 in the amount of *Ggt* DNA. An increase from year 0 to year 1, with Hereford harbouring the highest concentration of *Ggt* DNA (pg/g dry soil) (Fig. 55).

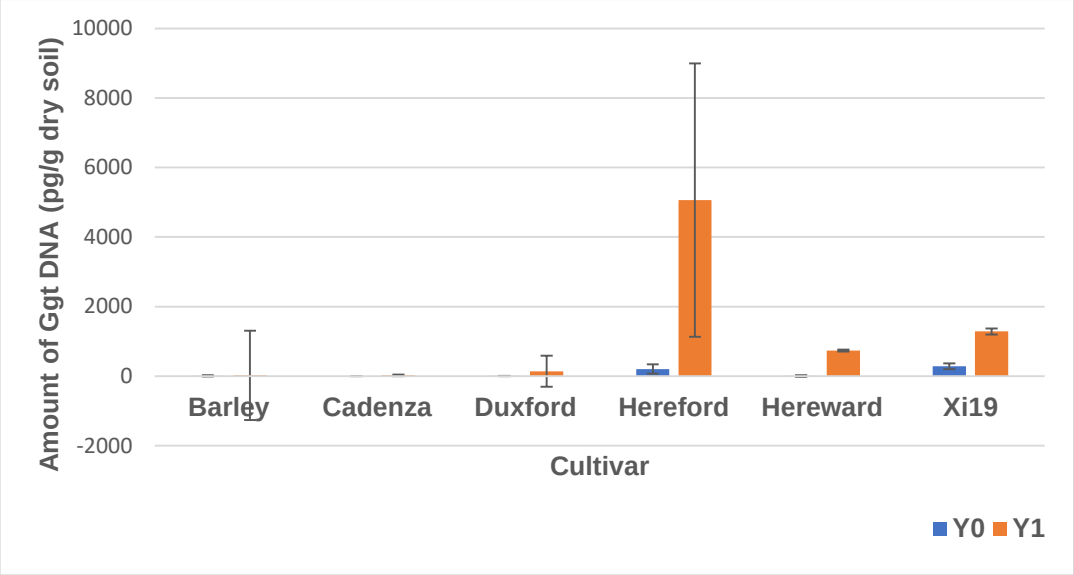


**Figure 54: Concentration of Ggt DNA (pg/ g dry soil) from field 3 Long Hoos 4 Y 0 and Y 1.** The bars are the averaged amount of DNA over the four replicates. Error bars are based on s.e.

This patchiness of Take-all in the field was reported previously by McMillan *et al*. (2011) and is believed to constrain field trials targeting the study of this fungus.

Given that *Ggt* was detected, it was important to determine if there was any difference in their levels within the cultivars. To do this all the zeros were converted into missing data (*) then the amount of *Ggt* was log10 transformed. Again, each field was analysed individually. Using the mixed model menu Residual Maximum Likelihood (REML) variance was used to analyse the data under linear mixed model with Fixed model (Cultivar * Time) and Random model (Block + Plot. Sampling season). In field 1 (New Zealand), there was a significant difference in the abundance of *Ggt* over time ($p<0.001$), but no significant difference was reported between cultivars ($p= 0.244$). There was a close to significant interaction between cultivars at the three sampling points ($p=0.070$) (Table 59). In field 2 (Long Hoos 5) there was a significant difference in *Ggt* abundance over time and between cultivars ($p= 0.004$ and $p= 0.001$,

respectively), but no significant interaction between cultivars at different time points (p=0.233) (Table 60). In field 3 (Long Hoos 4), there was a significant difference in the abundance of *Ggt* between the sampling points (p<0.001) and within the cultivars (p=0.006) however, there was no significant interaction detected for the cultivars over time (p=0.556) (Table 61).

**Table 59:** Test for fixed effects from REML variance components for *Ggt* presence data from New Zealand.

| Fixed term | Wald statistic | n.d.f. | F statistic | d.d.f. | F pr. |
|---|---|---|---|---|---|
| Cultivar | 7.6 | 5 | 1.52 | 14.4 | 0.244 |
| Time | 37.08 | 2 | 18.51 | 20.8 | <0.001 |
| Cultivar.Time | 16.12 | 7 | 2.3 | 19.1 | 0.07 |

**Table 60:** Test for fixed effects from REML variance components for *Ggt* presence data from Long Hoos 5.

| Fixed term | Wald statistic | n.d.f. | F statistic | d.d.f. | F pr. |
|---|---|---|---|---|---|
| Cultivar | 45.8 | 5 | 9.07 | 10.7 | 0.001 |
| Time | 26.22 | 1 | 26.22 | 4.8 | 0.004 |
| Cultivar.Time | 7.84 | 4 | 1.95 | 5.4 | 0.233 |

**Table 61:** Test for fixed effects from REML variance components for *Ggt* presence data from Long Hoos 4.

| Fixed term | Wald statistic | n.d.f. | F statistic | d.d.f. | F pr. |
|---|---|---|---|---|---|
| Cultivar | 206.16 | 5 | 41.22 | 3 | 0.006 |
| Time | 183.86 | 1 | 183.86 | 3 | <0.001 |
| Cultivar.Time | 3.62 | 4 | 0.91 | 3 | 0.556 |

The significant differences in *Ggt* by year were also evident in the soil core bioassay data targeting Take-all inoculum levels measured by McMillan *et al*. (2011). This might be due to year-by-year variations in environmental conditions such as rainfall and temperature, where warm and moist conditions favour the presence of *Ggt.*

### 6.5.4 The relationship between Take-all indexes (TAI), percent infected roots and the amount of *Ggt* DNA:

For New Zealand and Long Hoos (LH 5 and LH 4) fields, Dr. Vanessa McMillan at Rothamsted Research has collected soil cores to determine soil inoculum infectivety (percent infected roots in seedling soil core bioassay) while adult plant roots were assessed for disease incidence and severity (Take-all index (TAI)) (Table 62). Methods for soil core bioassay and Take-all index were described by (McMillan *et al.*, 2011, 2014) and are listed in methods section 2.8. The available data was used to look at correlations between the *Ggt* DNA concentrations, TAI and percent infected roots. The raw data are available in appendix IV 9.4.4.

**Table 62:** Data available per field and per year for TAI and percent infected roots. Where F 1 is New Zealand, F 2 is LH 5, F 3 is LH 4, while Y 1,Y 2, Y 3 are year 1, 2 and 3 respectively.

| Field/ Year | Data available for % infected roots | Data available for Take-all Index |
|---|---|---|
| F1 Y1 | YES | YES |
| F1 Y2 | YES | NO |
| F1 Y3 | NO | YES |
| F2 Y1 | YES | NO |
| F2 Y2 | NO | YES |
| F3 Y1 | NO | YES |

#### 6.5.4.1 Field 1 NZ:

In Year 1 there was a weak negative correlation between the amount of *Ggt* DNA (pg/ g of dry soil) and the percent infected roots in the soil core bioassay ($R^2$= 0.1904; Fig.56). In Year 2, there was a weak positive correlation between the amount of *Ggt*

DNA (pg/ g of dry soil) and the percent infected roots in the soil core bioassay ($R^2$= 0.1493; Fig.57). Both in Year 1 and 3 there was a moderate to weak positive correlation between the amount of *Ggt* DNA (pg/ g of dry soil) and the Take-all index ($R^2$= 0.4842 and 0.1038, respectively; Figs 58 and 59).

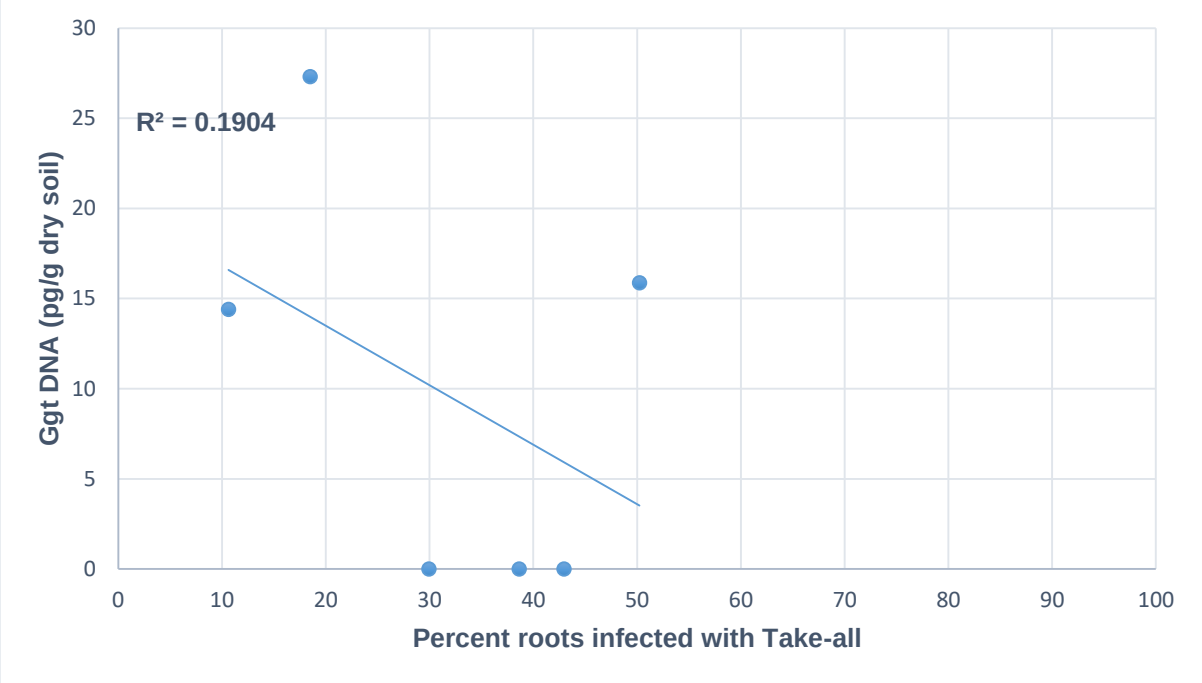


**Figure 55:** Correlation between amount of *Ggt* DNA (pg/g dry soil) and the percent infected roots from field 1(New Zealand) Year 1.

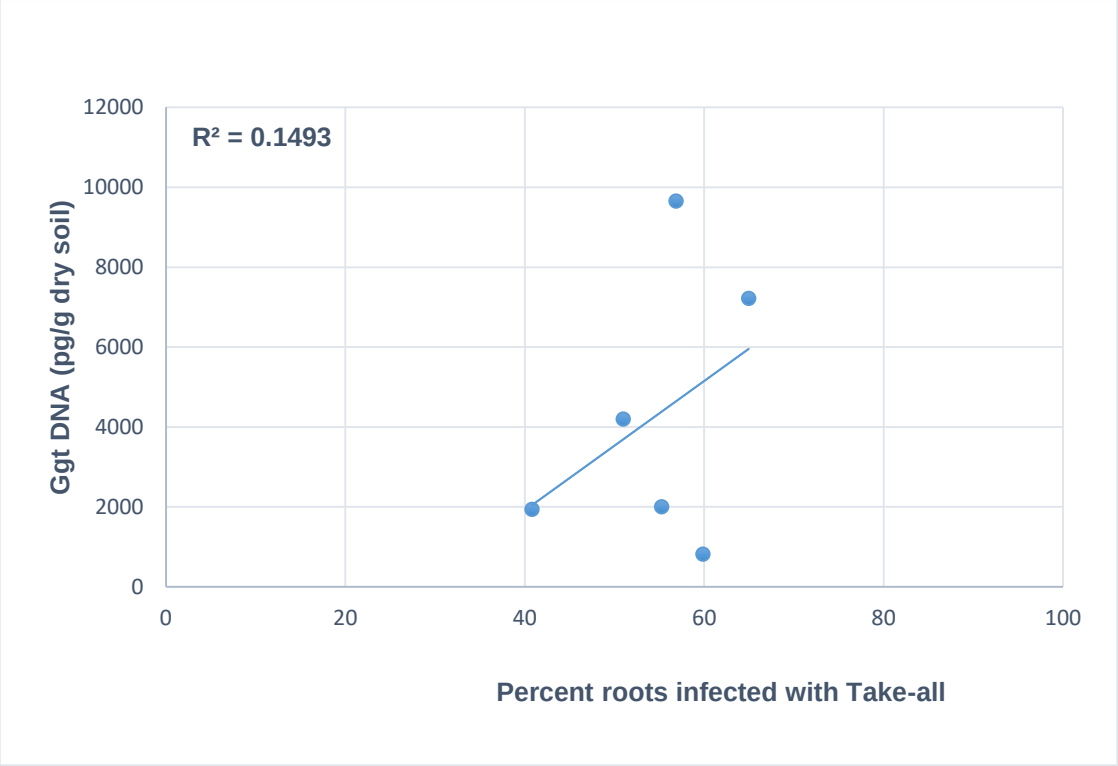


**Figure 56:** Correlation between amount of *Ggt* DNA (pg/g dry soil) and the percent infected roots from field 1 (New Zealand) Year 2.

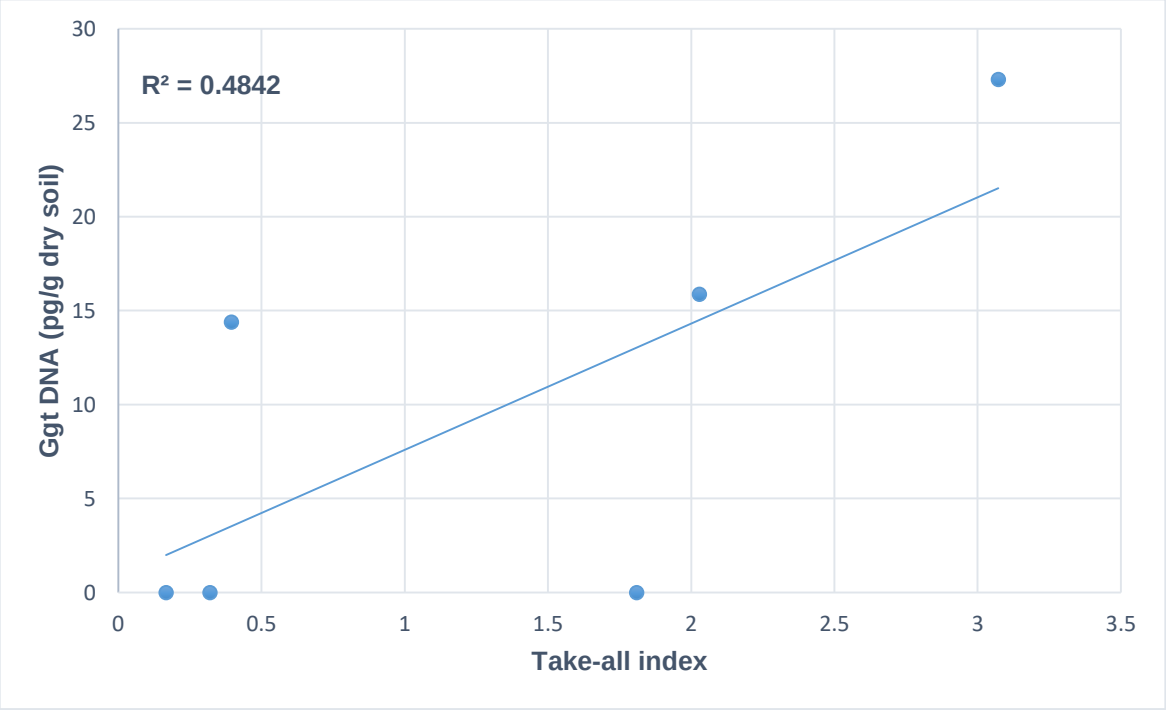


**Figure 57:** Correlation between amount of Ggt DNA (pg/g dry soil) and Take-all index (TAI) from field 1 (New Zealand) Year 1.

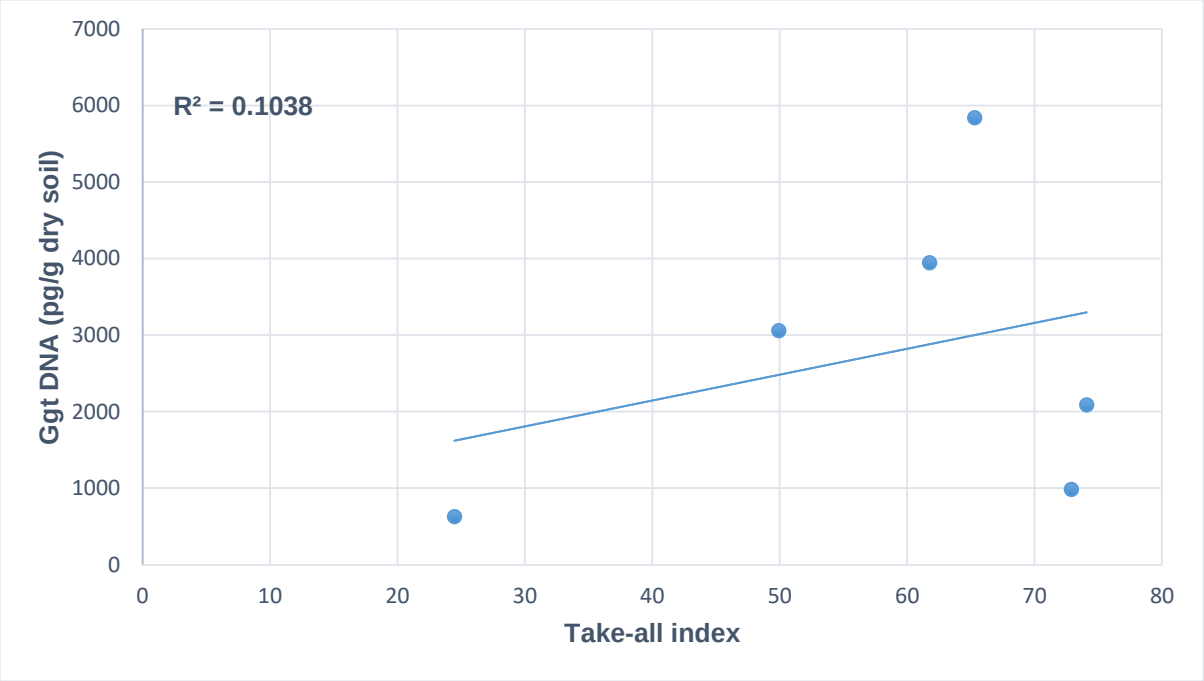


**Figure 58:** Correlation between amount of *Ggt* DNA (pg/g dry soil) and Take-all index from field 1 (New Zealand) Year 3.

### 6.5.4.2 Field 2 LH5:

In Year 1 there was a strong positive correlation between the amount of *Ggt* DNA (pg/ g of dry soil) and the percent infected roots in the soil core bioassy ($R^2$= 0.64559; Fig.60). Also, in Year 2 there was a strong positive correlation between the amount of *Ggt* DNA (pg/ g of dry soil) and Take-all index ($R^2$= 0.9221; Fig.61).

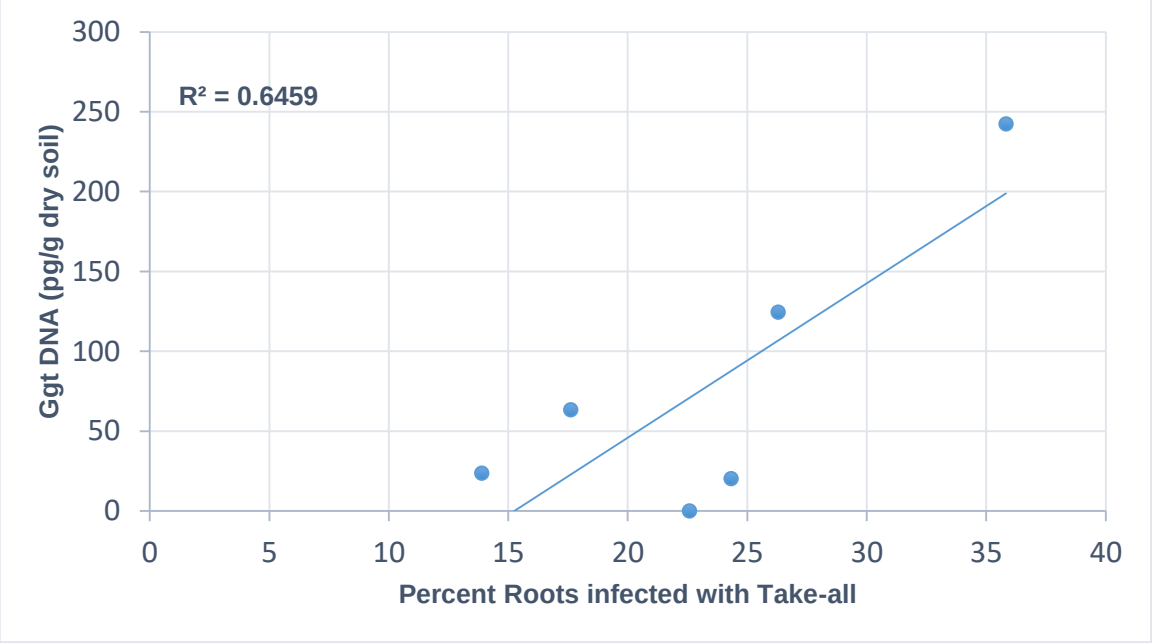


**Figure 59:** Correlation between amount of *Ggt* DNA (pg/g dry soil) and percent infected roots from field 2 (Long Hoos 5) Year 1.

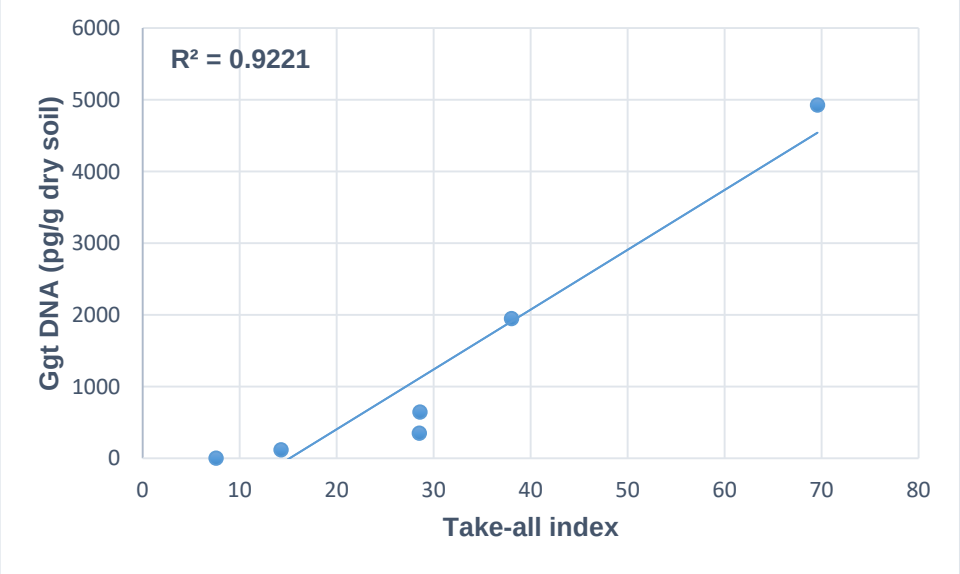


**Figure 60:** Correlation between amount of *Ggt* DNA (pg/g dry soil) and Take-all index for samples from field 2 (Long Hoos 5) Year 2.

### 6.5.4.3 Field3 LH4:

In Year 1 there was a moderate positive correlation between the amount of *Ggt* DNA (pg/ g of dry soil) and Take-all index of adult plant samples ($R^2$= 0.62; Fig.62).

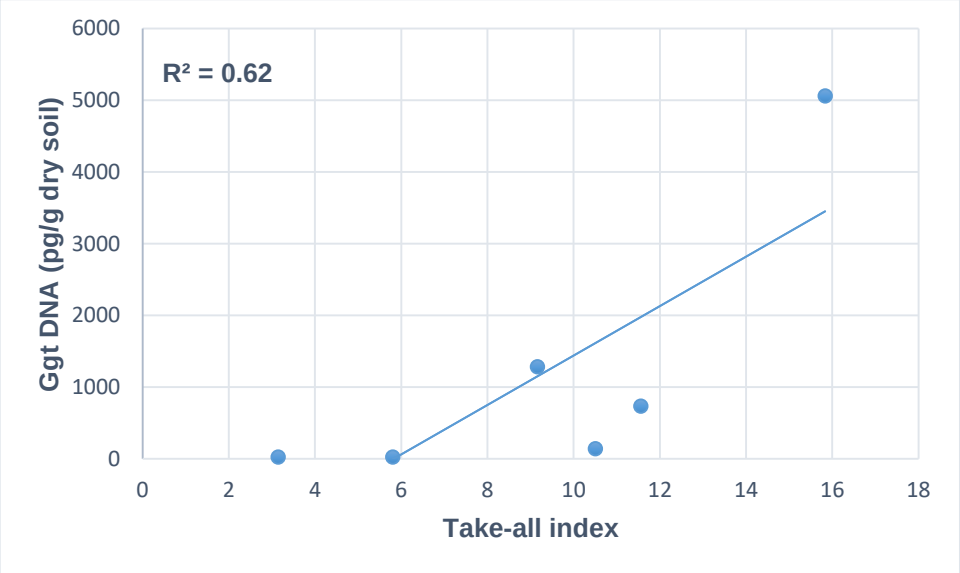


**Figure 61:** Correlation between the amount of *Ggt* DNA (pg/g dry soil) and Take-all index for samples from field 3 (Long Hoos 4) Year 1.

## 6.4 Discussion

Although extensively studied, Take-all disease of wheat caused by *Gaeumannomyces graminis* var *tritici* (*Ggt*), remains the most important disease of wheat. Lack of effective fungicide treatments and resistant cultivars still constrains the effective disease control (Cook, 2003; McMillan *et al*., 2014). Many fluorescent *Pseudomonas* species have been found to suppress Take-all *in vitro* but inconsistent performance is still an unresolved issue for field application (Pierson & Weller, 1994). McMillan *et al*. (2011) showed that wheat varieties differed in their Take-all build up (TAB).

Understanding the plant microbiome composition and functioning is important for developing sustainable disease control measures (Andreote & Pereira e Silva, 2017).

The beneficial effects of rhizobacteria range from nitrogen fixation, phosphorous and iron solubilisation, pathogen exclusion to induced systemic resistance (Neal *et al.*, 2012). Well studied plant beneficial bacteria include *Pseudomonas, Burkholderia,* and *Bacillus* (Raaijmakers *et al.*, 2009). The soil microbial communities are affected by both plant and soil factors (McSpadden Gardener & Weller, 2001). Take-all disease suppressive soils (TAD), were found to be associated with an increase in 2,4-DAPG producing *Pseudomonas* (Raaijmakers & Weller, 1998). In addition, Sanguin *et al.* (2009) through a combination of 16S rRNA microarray, cloning and sequencing showed a possible involvement of non *Pseudomonas* bacterial groups in TAD. These findings suggest a combined role of different bacteria rather than the involvement of single group.

Community based approaches are important in identifying potential microbial populations involved in disease suppression. For instance, repeated wheat cropping can lead to specific taxa enrichment, while a disease out-break can provide a more nutrient rich environment. Two important factors influence TAD: (1) monoculture of a susceptible host; (2) the presence of a virulent pathogen (Schreiner *et al.*, 2010).

In general, the rhizosphere microbiome of agricultural systems is less diverse than a natural ecosystem (Andreote & Pereira e Silva, 2017). Lloyd-Jones *et al.* (2005), found that the amount of *Pseudomonas* populations were significantly more abundant in forest soil than cropping soil. Driven by high nutrient availability from root exudates; the rhizosphere also contains far more large and complex microbial populations than the surrounding bulk soil (Bakker et al., 2013; Berg and Smalla, 2009). Similarly, in this study it was found that the bacterial communities of the bulk soil were different from that of the rhizosphere soil (Fig. 33). Also Berg and Smalla (2009) stated that the rhizosphere of old wheat cultivars were found to be colonized by a more complex

population of microbes in comparison to the rhizosphere of modern wheat which is mainly colonized by *Proteobacteria*. This was not evident in this study where the older varities Cadenza and Hereward (1980s/1990s) were not significantly different from Xi-19 (2000s) of Duxford and Hereford (2010s).

Lebeis *et al.* (2015) investigated the rhizosphere microbiome of *Arabidopsis thaliana,* comparing both wild type and a range of mutants deficient in plant defence phyto-hormones, salicylic acid (SA), jasmonic acid (JA) and ethylene. Their results demonstrated a role of SA in modulating root associated bacteria. Whether the SA effect is direct at the level of microbe interaction or by altering the root physiology, is still unclear. The role of root exudates in recruiting beneficial plant microbes have been widely demonstrated (Bakker *et al.*, 2013). For instance, using green fluorescent reporter protein (GFP) technology, Neal *et al.* (2012) was able to show that benzoxazinoids, such as DIMBOA (2,4-dihydroxy-7-methoxy-2H-1,4-benzoxazin-3(4H)-one), is involved in attracting *Pseudomonas putida* KT2440 in the maize rhizosphere. Also legumes, under nitrogen limiting conditions, attract rhizobia via release of certain flavonoids. While *Arabidopsis* uses malic acid to recruit microbes from the soil to activate host defences in response to *Pseudomonas syringe* pv. *tomato* foliar attack (Chaparro *et al.*, 2013).

In this study it was found that the field was the major factor separating the bacterial communities from each other. These differences might be due to the soil type, with soil type from the first field F1 being different from the soil type from F 2 and F 3 The major difference between the three field soils was in the percentage of phosphorous (P) as shown in the chemical and physical analysis (Table 43). For plants, phosphorous is a key element for energy, photosynthesis and sugar transformation. In addition, most soils have naturally low phosphorous content or components that

bind elemental phosphorous making it less available for the plants (Gumiere *et al.*, 2019). Soil bacterial species play key roles in P mobilization into plant available forms and arbuscular mycorrhizea fungi (AMF) enhance plants P uptake (Mander *et al.*, 2012; Gumiere *et al.*, 2019).

Liu *et al.* (2013) investigated the effect of N and P additions on the microbial structure of old-growth tropical forest in southern China. Their phosopholipid fatty acid analysis (PFLA) demonstrated that addition of P increased the relative abundance of AMF PFLAs and soil microbial biomass. While, Gumiere *et al.* (2019) showed that there was a high correlation between phosphate sources and the structure of bacterial and fungal communities. However, the effects of P source on soil bacterial diversity are likely to be variable and site dependent (Silva *et al.*,2017). Mander *et al.* (2012) reported that in New Zealand pasture soils with low P levels had the highest numbers of P solubilizing bacteria like, Proteobacteria, Actinobacteria, Firmicutes, and Bacteroidetes. However, in this study the previous trend was not evident.

The influence of soil type on shaping the microbiome is already known (Bakker *et al.*, 2013; Haichar *et al.*, 2014). Also Clark & Hirsch (2008) found that the bacterial diversity, abundance and survival in archived soils was influenced by the soil organic matter and clay content. In this study it was also found that there was no significant difference in the overall bacterial communities between the treatments (i.e: cultivars). Schreiner *et al.* (2010) likewise found no significant differences between the rhizobacterial communities between inoculated microcosms and non-inoculated microcosms with *Ggt.* They also reported that the presence of naturally occurring *Ggt* in the non-inoculated microcosms was not sufficient to establish disease or Take-all decline (TAD). Here, for enriched taxa, the *Pseudomonas* group was of interest however there was no evidence of their enrichment among the wheat cultivars.

Similarly, Mehrabi *et al.* (2016) based on 16S rRNA reads found no significant difference in the diversity and richness of *Pseudomonas* communities from low and high Take-all plots. Mahoney *et al.* (2017) reported that the wheat cultivars tested by them didn't show any selection for *Pseudomonas* spp. unlike Mazzola & Gu, (2002) who found that same wheat cultivars under greenhouse conditions selectively supported populations of *Pseudomonas* that were antagonistic to *Rhizoctonita solani* AG-5 and AG-8 when grown in apple orchid soils. Thus suggesting that the differences in experimental design, sampling and analysis plays an important role in determining the results. In addition, when comparing the enriched taxa between barley and wheat, 38 bacterial taxa were enriched within the barley rhizosphere and 29 bacterial taxa were enriched within the wheat rhizosphere. Interestingly, only two taxa were common in both barley and wheat rhizospheres, *Sphingomonas* and *Virgisporangium*. Other studies have assigned Sphingobacteriaceae group as a dominant taxon in the wheat rhizosphere (Donn *et al.*, 2015; Mahoney *et al.*, 2017). When barley was removed from the analysis to remove any masking effect, the genus *Tahibacter* was found to be enriched in the rhizosphere of L-TAB wheat. The relevance of this finding is still unknown. *Tahibacter* falls into the family *Xanthomonadaceae,* along with *Lysobacter*. Recently Gadhave *et al.* (2018) investigated the endophytic microbial communities of sprouting broccoli roots following external application of individual and mix *Bacillus* using 16S rRNA 454 pyrosequencing. Their results have shown that external application of *B. amyloliquefaciens* was associated with a large decrease in the relative abundance of the most common endophyte, *Pseudomonas* along with loss of *Rhizobium*, and these changes were accompanied by an increase in the relative abundance of a wide range of genera, particularly *Dyadobacter, Variovorax, Tahibacter*, and *Sphingomonas*. Suggesting that these genera fail to establish when

*Pseudomonas* and *Rhizobium* are present due to antagonistic interactions. In this study *Pseudomonas* and *Rhizobia* were enriched within the barley rhizosphere while *Burkholderia*, *Lysobacter* and *Sphingobium* were enriched in wheat rhizosphere. Thus from Gadhave *et al.* (2018), it can be suggested that there was an unknown factor in wheat rhizosphere which counteracted against *Pseudomonas* establishment in greater abundance.

Also, it was shown that either soil type, plant species or a combination of both were identified as major factors driving the rhizosphere microbial communities (Berg and Smalla, 2009; Raaijmakers et al., 2009). The clear niche effect of bulk soil versus the rhizosphere was very clear in the results here and the same trend was reported previously by others e.g. Donn *et al.* (2015). Apparently, the plants, through their root exudates, are able to select for specific microbial groups present in the bulk soil to colonize their rhizosphere. Thus, the microbial communities in the rhizosphere are less diverse than those found in bulk soil (Berendsen *et al.*, 2012). When investigating the rhizosphere bacteria of the graminoid *Avena fatua* it was found that the relative abundances of 147 of the 1,917 of the detected bacterial taxa were significantly different from those of the bulk soil, with most of the rhizosphere species belonging to the phyla Firmicutes or Actinobacteria or to the class Alphaproteobacteria (Philippot *et al.*, 2013). Other studies have indicated Proteobacteria (*Pseudomonadaceae* or *Burkholderiaceae* family) as dominant members of rhizosphere microbiome. Bulgarelli *et al.* (2012) investigated the microbiome of *A. thaliana* grown under controlled conditions and in different types of soil. They found that roots were favourably colonized by specific members of Proteobacteria, Bacteroidetes and Actinobacteria. Mahoney *et al.* (2017) found that the most abundant OTUs within the wheat rhizosphere were Acidobacteria (Gp1), Actinobacteria, Bacteriodetes (Flavobacteria

and Sphingobacteria), Verrucomicrobia (Opitutae), Proteobacteria (Alpha/beta/gamma/proteobacteria), and Gemmatimonadetes. These OTUs were also identified in this study. Overall the OTUs found in this study were in agreement with previously identified rhizosphere OTUs (Bulgarelli *et al.*, 2012; Philippot *et al.*, 2013; Donn *et al.*, 2015; Mahoney *et al.*, 2017).

Lundberg *et al*. (2012) used 454 pyrosequencing of 16S rRNA to analyse the rhizosphere and endosphere of eight inbred *A. thaliana* accessions grown in two different soil types under controlled conditions. They found that the soil was the main factor deriving the rhizosphere microbiome composition of *Arabidopsis thaliana*. Also the niche, bulk soil, was separated from the endosphere. The growth stage at harvesting and the host genotype didn't have a quantifiable enrichment on one bacterial group over the other. Similarly, Bulgarelli *et al*. (2012) stated that the soil type defined the composition of root-inhabiting bacterial communities while the host genotype had little effect in determining individual profiles. Likewise, here no clear cultivar based enrichment was observed, but there was however a crop (wheat vs. barley) related enrichment of certain taxa. In addition it was found that the rhizosphere of modern wheat is colonized by fast growing Proteobacteria unlike the old wheat cultivars which were colonized by phylogenetically diverse groups of bacteria (Berg & Smalla, 2009). However this difference was not statistically significant and the explanation for these differences might be due to changes in root morphology or chemical exudation between old and modern wheat (Germida & Siciliano, 2001).

For the amount of *Ggt* DNA, the results of this study show an uneven distribution of *Ggt* inoculum even between plots from the same field. This patchiness of Take-all even within the same field was reported previously by Bithell *et al.* 2012 and McMillan *et al.* 2011 and is believed to constrain field trials targeting the study of this fungus. Adding

to this was the annual variation in the inoculum level within the same field. The significant differences in *Ggt* levels by year were also evident in soil core bioassay data targeting Take-all inoculum levels by McMillan *et al.* (2011). These annual differences can be explained by year-to-year variations in environmental conditions such as rainfall and temperature; where warm and moist conditions favours the presence of *Ggt*. Furthermore, the approaching significance figures might be due to the small number of observations (24 sampled plots per year per field), which might have reduced the statistical power necessary to achieve significance. In addition, lack of the same number of sampling years made it very difficult to compare the OTU of the fields with each other in the same analysis. Other factors might have an influence on the analysis, but rather were missing from the analysis. These include the plant variables such as height, grain size, chlorophyll content, and root exudates (Haichar *et al.*, 2008, 2014; Bakker *et al.*, 2013). Overall it is usually difficult to compare the wheat rhizosphere representative OTUs from this study with published data due to the differences in sequenced regions, platforms, and the analysis techniques used (Mahoney *et al.*, 2017). For instance Mahoney *et al.* (2017) used amplicon sequencing using V1- V3 region of 16S rDNA along with Network correlation analysis and Phylogenetic Investigation of Communities by Reconstruction of Unobserved States (PICRUSt) to assign potential OTU functions associated with nine winter wheat cultivars.

In addition, in this study, the beta diversity analysis using ordination has been used which enables the description of microbial community patterns over fields and by host genotype. However, newer methods such as co-occurrence networking provide insights of the positive or negative species co-occurrence and their functional roles in a given habitat (Mahoney *et al.*, 2017). This suggests that the depth of the analysis

method might influence the data outcomes. Although metagenome studies can cover for the missed information from culture based techniques, gene expression and transcriptomic studies offer insights into microbial activities (Haichar *et al.*, 2008; Bakker *et al.*, 2013). An important challenge associated with next generation sequencing is applying the appropriate statistical analyses to answer the question in hand (Coats & Rumpho, 2014). In addition fortifying sequencing data with selective isolation and culturing of potential biocontrol agents offer more useful approaches for bio-control of plant diseases (Mauchline *et al.*, 2015). Studying changes at the single taxon level can aid better understanding of underlying plant-microbe interactions (Mauchline & Malone, 2017). For instance, barley monoculture was found to have an effect on population gene expression and/or enrichment within the community rather than the between the populations (Schreiner *et al.*, 2010).

In addition to 16S rRNA amplicon analysis, the amount of bacterial, *Pseudomonas*, fungi and *Ggt* DNA was quatified from the extracted total soil DNA. The qPCR protocols adopted here were based on established by Rothamsted Research for their routine research analysis. A SYBR Green qPCR approach was used for determining bacterial, *Pseudomonas*, fungi soil DNA concentrations while a Taq-man probe qPCR approach was used for determining soil *Ggt* concentrations. The initial aim was to compare the data with each other, however due to the difference in the standards used, pure culture for *Ggt* while soil mixture DNA for the others, the quantification was at different scale. Martini *et al.* (2015), stated that for qPCR it is important to establish the standard curve with samples of known target DNA quantities resembling as much as possible to natural samples. For instance, when aiming to determine endophytic concentrations of applied rhizobacteria biocontrol agents, a solution of total genomic DNA extracted from roots of untreated plants is recommended. Therefore, analysis of

bacterial, *Pseudomonas*, fungi soil DNA was carried out separately from those of *Ggt* soil DNA concentrations. In addition this analysis was carried out for each field separately again due to time line inconsistency. That is no bulk soil sample was available from field 1, no year 3 soil sample for field 2, and no year 2 and 3 soil samples for field 3. For the concentration of bacterial *Pseudomonas*, fungi soil DNA concentrations from the three fields the main difference was based on year to year variations. Exceptions included the bacterial DNA concentration in field 1 that showed a significant difference between the crops (wheat vs. barley), and in field 2, soil DNA concentrations of *Pseudomonas* showed no significant difference for any factor including the year-to year variation.

For bacteria the DNA encoding 16S ribosomal RNA gene is a common target in qPCR amplification. While for *Pseudomonas* spp. in addition to 16S rRNA, *phlD* (a key gene in the biosynthesis of 2,4-DAPG) and *hcnBC* (hydrogen cyanide synthesis gene), and *gacA* (response regulator gene) have been also used for specific quantification of fluorescent *Pseudomonas* spp. Also, the use of housekeeping genes such as *gyrB*, *rpoD* and *rpoB* are evolving based on their success in the phylogenetic analysis of *Pseudomonas* spp (Martini *et al.*, 2015). Therefore, here the choice of using 16S rRNA for determining the *Pseudomonas* soil DNA concentration might not have been the best one. However, Lloyd-Jones *et al.* (2005) used 16S rRNA Taq-man PCR to quantify *Pseudomonas* spp. from six New Zealand soils in comparison to selective cultivation methods. Their results show that cultivated *Pseudomonas* represent <1% of the total quantifiable *Pseudomonas* population and this total *Pseudomonas* population is in turn <1% of the total bacterial population in the tested soils. This suggests that the cultivated *Pseudomonas* isolates from soil are not numerically the dominant ones. Thus our knowledge is still restricted and relies on advances in

establishing more sensitive detection and quantification approaches. Again the large number of samples, 192, which required duplication per run and the amplification of four targets was both an expensive and tricky task. Overall, the quantity and the structure of bacterial community were not influenced by the cultivar. Also it is important to keep in mind that the qPCR accounts for culturable, unculturable and dead cells, plus any free target DNA, without any possible discrimination (Lloyd-Jones *et al.*, 2005).

In this work the amount of *Ggt* DNA showed high variation even within plots of the same cultivar. However, this is not surprising for a patchy disease like Take-all (Bithell *et al.*, 2012; McMillan *et al.*, 2014). No *Ggt* was found in bulk soil form field 2, while in field 3 bulk soil *Ggt* was present in 7 plots out of the 24. Again, as seen with bacterial, *Pseudomonas*, fungi soil DNA, the significant difference in the amount of *Ggt* DNA was mainly based on year to year variation rather than being influenced by the cultivar. Fluctuations in annual temperature and rainfall are known to influence field experiments. There is a complex relationship between Take-all fungus and environmental conditions. Conditions that favour Take-all are moderate temperatures and high precipitation, while high temperatures and dry conditions limit inoculum build-up (McMillan *et al.*, 2011). Also, cold weather restricts mycelial growth and increases the rate of inoculum decay. The main period of inoculum build-up in first wheat is from May to harvest. When environmental conditions favour Take-all inoculum build-up, high amounts can be found even with the low TAB wheat variety, thus, masking the cultivar effect to some extent. Moreover, Take-all risk prediction is influenced by differences in the growing season and number of years planted to wheat. The use of break crops prior to first year wheat sowing explains the low Take-all inoculum in first year, as the break crops minimizes Take-all inoculum carry over (Bithell *et al.*, 2012).

Also, the presence of other parasitic root colonizing fungi, *Phialophora graminicola* and *Phialophora* sp. can mask cultivar effects by preventing Take-all inoculum build-up (McMillan *et al.*, 2011). The greatest potential to estimate Take-all severity is in second year wheat (Bithell *et al.*, 2012) and this might explain the results of (Mauchline et al., 2015) where cultivar selection was evident. Also, high *Ggt* concentrations in second and third year wheat with low Take-all index (TAI) can be explained by the development of Take-all suppression (Bithell *et al.*, 2012).Take-all decline interferes with Take-all disease severity predictions.

It is important to point out that the ITS primers I used in qPCR to determine the fungal soil DNA concentrations did not cover the arbuscular mycorrhiza fungi, AMF. However, our aim was to compare the amount of *Ggt* DNA with those of fungi in general rather than investigating the structure of the fungal community in the wheat rhizosphere. Mycorrhiza and rhizobia assist the plants with uptake of phosphorus and nitrogen (Berendsen *et al.*, 2012). They also help the plants by carrying molecular messages from stressed to neighbouring plants as an alert system to activate defence mechanisms (Lapsansky et al., 2016). Fungi can influence the bacterial communities through modifying the amount and the composition of the root exudate. The fungi can also affect the growth and the chemotaxis of bacteria in the soil (Barret *et al.*, 2009). Plant beneficial fungi include *Trichoderma, Gliocladium* (Raaijmakers *et al.*, 2009). Here, - investigating the wheat associated fungal community was not possible due to limited funding. Thus, further investigation is needed to better explore this important component of the microbial system.

The results in hand along with future root exudate analysis and gene expression can aid in better understanding of wheat rhizosphere and *Ggt* interactions.

## CHAPTER 7- General Discussion

**Summary:**

The rhizosphere microbiome is crucial for plant health and environmental functioning. Among the rhizosphere inhabitants *Pseudomonas* spp. are well recognized for their plant growth promoting and disease control. Many biotic and abiotic factors aid in shaping the structure and composition of the rhizosphere microbiome. Take-all disease of wheat to date is a major challenge for wheat growers. This disease also serves as an excellent model to study plant root diseases. Research done by McMillan *et al.* (2011) has shown that wheat cultivars differ in their supportiveness to Take-all inoculum. Therefore these wheat varieties were classified as low Take-all builders or high Take-all builders (L-TAB, H-TAB; respectively). In this work Take-all disease of wheat both at individual isolate and population level was investigated. Methods of simple culture, dot blot and PCR were employed along with more sophisticated next generation sequencing and qPCR techniques.

### 7.1 Characterization of *Pseudomonas* isolates

The rhizosphere is one of the most complex ecosystems on earth. It is the narrow zone surrounding the roots and is under the influence of root exudates (Mendes *et al.*, 2013). The microbial communities associated with roots are concentrated in the rhizosphere and rhizoplane. It is generally assumed that rhizosphere communities are recruited from the surrounding pool of bulk soil (Sasse *et al.*, 2018). For instance *Pseudomonas putida* strain KT2440 is chemotactically attracted and recruited by DIMBOA in the maize rhizosphere (Neal *et al.*, 2012). Legumes use flavonoids to attract rhizobia, L-malic acid was also found to recruit *Bacillus subtilis* strain FB17

(Badri *et al.*, 2009). In addition the root structure, shape, exudation, border cells and mucilage play an important role in the rhizosphere recruitment process (Haichar *et al.*, 2014; Sasse *et al.*, 2018). Siciliano *et al.* (1998) found that there was a cultivar effect on the microbial composition associated with canola but not in wheat, probably because wheat has a dense fibrous root system compared the coarse roots of canola. Thus the competition for microsites is greater on wheat than on canola.

Durán *et al.* (2017) stated that the rhizosphere community was highly related to the soil chemical and physical properties. The beneficial activity on plant health of rhizosphere residents is well recognized for bacteria like *Pseudomonas* and *Burkholderia* and fungi like *Trichoderma* and *Gliocladium* (Badri *et al.*, 2009). The rhizosphere shows greater microbial clustering than the bulk soil (Kirk *et al.*, 2004).

Certain stains of fluorescent *Pseudomonas* spp. are known to colonize the plant rhizosphere and promote plant health (Thomashow & Weller, 1988). Plant growth promotion can be achieved by enhanced nutrient uptake, stress tolerance, and pathogen inhibition (Walsh *et al.*, 2001). The use of a promotor trapping strategy led to the identification of six classes of rhizosphere induced loci (*rhi*). These included important functions such as nutrient acquisition, stress response, attachment and surface colonization, antibiotic production, secretion, as well as unknown genes (Rainey, 1999; Jackson *et al.*, 2005). Several studies have shown rhizosphere induced genes that account for *Pseudomonas* successful colonization and pathogen exclusion in plant rhizosphere (Rainey, 1999; Gal *et al.*, 2003; Jackson *et al.*, 2005; Silby *et al.*, 2009).

Production of iron-scavenging siderophores and antibiotics are possible mechanisms of pathogen suppression. Phenazine-1-carboxylate produced by *Pseudomonas*

*fluorescens* 2-79 isolated from wheat rhizosphere was found to inhibit the growth of *Ggt* (Thomashow & Weller, 1988) while, Raaijmakers *et al*. (1997), found a high proportion of 2,4-DAPG producing *Pseudomonas* to be associated with natural Take-all disease suppressive soils. Moreover, Mavrodi *et al.* (2012) has demonstrated the impact of irrigation on the distribution of Phenazine-1-carboxylate (*Phz*$^{+}$) and 2,4-DAPG (*Phl*$^{+}$) producing *Pseudomonas* in the wheat rhizosphere. *Phl*$^{+}$ strains were enriched in irrigated fields under conditions that favour the presence of *Ggt* while *Phz*$^{+}$ isolates were dominant in non-irrigated where the main pathogen is *Rhizoctonia*. Thus, there has been an increasing interest in biological control of Take-all using *P. fluorescens* (Mauchline *et al.*, 2015).

In this study, the effect of wheat cultivars differing in their Take-all building trait (TAB) on the selection of specific *Pseudomonas* genotypes was investigated. The selectively isolated *Pseudomonas* spp. from the rhizosphere and endosphere of the following wheat planting combinations (C,H), (C,Xi-19), (H,H), and (H,Xi-19) were analysed. Screening these isolates for rhizosphere fitness genes involved in host recognition (*wsm*) and nutrient acquisition (*fecB*), showed a strong selection exerted by the wheat variety grown in the first year (i.e: Cadenza or Hereward). With higher scores for *wsm* locus presence was observed with Hereward isolates in comparison to Cadenza. On the other hand the *fecB* locus was more abundant with Cadenza isolates than in those of Hereward. These findings are in agreement with Mauchline *et al.*, (2015), who highlighted a selective pressure expressed by the wheat cultivars on the genotypes of associated *Pseudomonas*.

Furthermore, the ability of these isolates to antagonize *Ggt* was screened to test the hypothesis that iron deprivation was the possible mode of action against the pathogen. The *in vitro* inhibition assay which showed that isolates from first year Cadenza

background were more antagonistic to *Ggt in vitro,* in comparison to isolates from first year Herewerd background. Again supporting that the main effect on the selection of *Pseudomonas* spp. was based on wheat varieties grown in the first year (Mauchline *et al.*, 2015; Mauchline & Malone, 2017). The finding that isolates associated with Cadenza had more *fecB* loci and were more antagonistic to *Ggt* might imply that iron limitation can be a possible antagonistic strategy used by the tested *Pseudomonas* isolates for inhibiting the growth of *Ggt*. From the *in vitro* inhibition assay six isolates maintained their high *Ggt* inhibition and these were further tested for their growth promotion and *Ggt* inhibition *in vivo* in the presence of the plant under controlled conditions.

From the *in planta* assay, overall the mixture of six isolates (rather than individual isolates) was more effective in reducing the number of infected roots in both cultivars. Although Mehrabi *et al.* (2016), reported that competition with in a given mixture of biocontrol agents might reduce their efficiency. The situation was different in the mixture tested here where the synergistic effect was leading to better disease control. Moreover, screening of these six *Ggt* antagonistic isolates for presence of Phl and PCA antibiotic genes showed that none harboured the tested antibiotic loci. This suggests that the mode of action against *Ggt* does not involve antibiosis at least by the tested antibiotics. In conclusion, the recruitment of *Ggt* antagonistic *Pseudomonas* isolates by Cadenza might explain the L-TAB trait. Although the basis of this selection are still not clear.

## 7.2 Temporal and Spatial analysis of wheat microbiome

To date, most of the studies on the rhizosphere focused on 'who is there and what are they doing?' (Mendes *et al.*, 2013). For diversity studies typically the 16S rRNA, 18S rRNA, ITS are targeted as these regions are not affected by horizontal gene transfer and the their sequence data are available (Kirk *et al.*, 2004). Sequencing of the 16S rRNA gene hyper variable regions allows taxonomic identification to species and strain levels for prokaryotes. However, such level of discrimination is not provided by sequencing the 18S RNA for eukaryotes like fungi, and thus is replaced by the internally transcribed spacer ITS (Turner *et al.*, 2013b). In a metagenomics study total DNA from the rhizosphere is extracted and sequenced leading to taxonomic assignments, while in meta-transcriptomics, where total RNA from the environment is sequenced, active community members and metabolic pathways are revealed. Furthermore, the use of mRNA in functional transcriptomics will enable determination of the various biochemical activities carried out in the rhizosphere (Turner *et al.*, 2013b). It is now possible to classify the soil microbiome to operational taxonomic units OTU or even to species using the high throughput NGS, microbial specific databases and efficient clustering algorithms (Mahoney *et al.*, 2017). *Pseudomonas* are a diverse group that occupy a wide range of niches. It is thought that this ability is due to their diverse genomes and the subsequent metabolic versatility (Silby *et al.*, 2011). Yamamoto *et al.* (2000), used a combined *rpoD* and *gyrB* analysis to phylogenetically analyse members of the genus *Pseudomonas*. Since, Mauchline *et al.* (2015) demonstrated that a phylogenetic analysis using 8 single copy housekeeping genes (*aroE, atpD, dnaE, guaA, gyrB, mutL, pyrC* and *recA*) agreed with the extensive *gyrB* phylogeny of *Pseudomonas* isolates, thus, here the *gyrB* phylogeny was used to classify the isolates in hand.

Take-all decline (TAD) is a natural disease suppression that occurs after many years of wheat monoculture (Chng *et al.*, 2015). This decline in the Take-all disease can be general or specific, with the former being attributed to total soil components which disfavours the growth of the fungi while the specific suppression is caused by one or a group of antagonistic organisms (Cook, 2003; Chng *et al.*, 2015).

Here, the long term effect of five wheat varieties (two L-TAB, and three H-TAB) and one Barley (Unknown-TAB) on the bacterial community structure and *Ggt* inoculum storage under field conditions was studied. Three fields from Rothamsted Research were investigated. Each field trial was drilled at a different year, starting with New Zealand followed by Long Hoos 5 and finally Long Hoos 4. Each field had four main blocks. These blocks were further divided into 24 plots made of the 6 treatments (five wheat + one barley) replicated four times. After harvest each plot was over-sown with the same cultivar. The field trial was initiated in autumn 2014 till 2018. Methods of next generation 16S rRNA gene amplicon sequencing were used to investigate the bacterial community structure and qPCR to quantify *Ggt* soil DNA concentration along with total soil DNA concentrations of total bacteria, *Pseudomonas*, and fungi (excluding AMF). No significant differences in the diversity and abundance of microbial communities were observed between the wheat cultivars overtime. Changes in *Ggt* populations were only based on year-to-year variations rather than being influenced by the cultivars. Difficulties arose from the inherent patchiness of Take-all within replicated plots of the same wheat cultivar. In addition it is known that Take-all build-up trait cannot be simulated in pot or laboratory trials (McMillan *et al.*, 2014). These findings in general agree to some extent with earlier published data (Bulgarelli *et al.*, 2012; Philippot *et al.*, 2013; Donn *et al.*, 2015; Mahoney *et al.*, 2017). In addition, it is important to point out that the outcomes of field based trials are different for pot trials

using field soil (Gu & Mazzola, 2003; Mahoney *et al.*, 2017). To our knowledge this is a first long term field based trial, sampling more than two years, in the context of assessing wheat cultivars under Take-all disease conditions at least at UK level. Although external factors might have masked the influence of cultivars on the overall microbial community, a deeper investigation using newer network approaches and functional analysis can further shed some light on the situation.

### 7.3 Take-all fungi & determining soil DNA concentrations

*Gaeumannomyces graminis* var*. tritici* (*Ggt*) is a soil borne fungus that causes Take-all of wheat. It survives saprophytically in the soil on plant debris in the absence of its host. Techniques to estimate the amount of *Ggt* in soil are important to predict disease severity and subsequent crop yields (Herdina & Roget, 2000; Herdina *et al.*, 2004). Seasonal influences in disease severity in Take-all was reported by (Hardwick *et al.*, 2001). *Ggt* requires high soil water potential for growth and the amount of rainfall directly affects inoculum carry over to the following season. The winter and spring rainfall predicts disease severity while summer rainfall outcomes predicts inoculum survival saprophytically. *Ggt* can survive prolonged dry conditions however it has poor ability to compete with other microbes after rainfall in the absence of its host (Roget, 2001). In terms of Take-all control, no resistant wheat cultivars are available and no fully effective fungicide treatment is known (Bithell et al., 2012; Cook, 2003; McMillan et al., 2011). Pre-sowing knowledge of Take-all risk is important for predicting disease severity and crop loses (Bithell et al., 2012). In addition, the distribution, amount, viability and the metabolic status of the mycelium, along with the virulence of the strain are important factors in determining the relationship between the amount of *Ggt* in soil and disease severity (Herdina *et al.*, 2004). Traditionally, the soil core bioassay, where

wheat seedlings are grown in soil samples, was used to predict Take-all inoculum in the field after harvest of 1st wheat crops and the potential risk of severe disease developing on a following second wheat (McMillan *et al.*, 2011; Bithell *et al.*, 2012). Overall, it was found that when postharvest soil core bioassay shows 20% infected roots, then the disease severity is likely to be high in the subsequent wheat. This finding was based on long term data from the United Kingdom (Bithell *et al.*, 2012). In a system where wheat is grown continuously, high *Ggt* concentrations in second and third wheat with yet low Take-all index (TAI) can be explained by the development of Take-all suppression (Bithell *et al.*, 2012; Chng *et al.*, 2015).Take-all suppression interferes with Take-all disease severity predictions.

In Australia a molecular based method to quantify *Ggt* DNA directly from field samples have been developed, however this method is currently not available in the UK for farmers (McMillan *et al.*, 2011). Furthermore, based on this method four risk categories have been identified in Australia for risk of Take-all (1) below detection limit (BDL)(<5), (2) low(5 to <130), (3) medium (130 to <325), and (4) high (>325) where the numbers refer to picograms of *Ggt* DNA per gram of soil (pg *Ggt* DNA/ g soil) (Bithell et al., 2012).

When using DNA based techniques to quantify the *Ggt* fungi in soil, careful measures must be taken so that DNA from dead fungi do not overestimate the quantification. However it was found that the DNA degrades very quickly in the environment eliminating the overestimation due to dead material (Herdina *et al.*, 2004). Bithell *et al*. (2012) stated that the Australian risk categories didn't fully apply to Take-all conditions in New Zealand fields. In general Take-all epidemics are affected by climatic conditions with soil moisture being more important factor than temperature (Bithell *et*

*al.*, 2012). This annual difference were evident in this work and were the main contributors to the variation found in the soil DNA concentrations of *Ggt*.

Moreover, the stages of disease cycle are the essence of plant disease severity prediction models (De Wolf & Isard, 2007). Plant disease prediction models are less common for plant soil borne disease than for plant leaf disease (Roget, 2001). The greatest potential to estimate Take-all severity is in second year wheat (Bithell *et al.*, 2012) and this might explain the results of (Mauchline *et al.*, 2015) where the wheat variety selection pressure was evident. Given the above, in this work it was found that *Ggt* was very patchy with concentrations varing even between the plots of the same field. *Ggt* was either absent or had the lowest concentrations in the first year. Also the main effect on the levels of *Ggt* soil DNA concentrations were based on year-to-year variations and the number of years that wheat had been grown. These findings agree with previous work on patchiness of Take-all and the effect of annual variations in temperatures and rainfall in determing Take-all severity and persistence (Bithell *et al.*, 2012; McMillan *et al.*, 2014; Keenan *et al.*, 2015).

### 7.4 Conclusions and future work

Plant diseases account for 10% of crop loses globally (De Wolf & Isard, 2007). Take-all disease of wheat, although extensively studied, is still regarded as an important soil borne disease (Pierson & Weller, 1994). Apart from Take-all, other important wheat head, stem and foliar diseases include Fusarium, Eyespot, Yellow dwarf virus, yellow rust, brown rust and Septoria tritici in winter wheat which cause substantial yield loses (“AHDB Cereals & Oilseeds : Wheat disease management. Available:https://cereals.ahdb.org.uk/crop-managment/wheat-disease-

management.aspx. [Accessed: 9 March 2019]"). Take-all risk prediction is influenced by differences in the growing season and number of years planted to wheat (Bithell *et al.*, 2012).The use of break crops prior to first year wheat sowing explains the low Take-all inoculum in first year as the break crops minimizes Take-all inoculum carry over (Bithell *et al.*, 2012). Knowledge of the effects of different soil types on Take-all and data from long term weather forecasts are crucial for disease severity predictions (Bithell *et al.*, 2012). The complexity of the interacting biological, chemical and physical factors in the plant-microbe-soil interaction are yet to be completely resolved (Kirk *et al.*, 2004). The role of any specific plant signalling molecule in recruiting a particular group of microbes is still poorly understood (Badri *et al.*, 2009).

Here potential *Ggt* antagonistic *Pseudomonas* that can be further explored for their disease control mechanism and possible use as Take-all biocontrol agents have been identified. The expected wheat cultivar selection on the microbiome from the long term field trials was not evident at the amplicon sequencing level tested here. The main differences were based on field and year-to-year variations based on the number of years wheat had been planted. However, it can anticipated that the presence of an unknown factor that might have masked the cultivar driven effect.

Further investigations will be needed to test and apply the biocontrol isolates. In addition examination of root exudates from the different wheat cultivars might shed some light on potential signalling compounds. In addition the plants produce their exudates during the growth stage, thus sampling the rhizosphere at different stages of plant growth might provide better information about the on-going interaction. The more sophisticated functional anlysis can also support our knowelege. Also, the recent trends in ecological moduling, cross link and network studies might be useful if applied properly to fit the system under study.

Finally by recalling a quote by Leonardo da Vinci that '*We know better the mechanics of celestial bodies than the functioning of the soil below our feet*' (Badri *et al.*, 2009), something like this shows not only how complex the problem is to study, but for how long this issue has been recognized. This work provides a foundation for unravelling this complexity.

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# CHAPTER 9- ANNEX

## 9.1 Chapter 3 Appendix

### 9.1.1 Appendix I

**PFLU_0476 (*wsm*)**
The complete gene is >NC_012660.1: 538831-539727

The amplified fragment:

>539101 **MZ-3F**

GGCAATGCCGAGATCATCCTCCGGCCGATCGCCGCCGATGCGCAGTTGCCGGTGCGCCATCGTGGGCGCTACTTGTCAGCGCTGAACGA
GGGCCGCATCAGCACCCTCAAGCCGCAGGCCGAGCAGTTGGCCATGGCGCGCACCCCCGAAGACCTGTCGAAGAAATTTCGCTACAACC
AGCGCCGCGAATTGCGTCTGCTGGAAGAGGCGGGTGGCGTGGTGCGGGCGGTGAATGAGTTTTCCAGTACGGAACTGGCGGCCATCTAC
TGCGATCTGTTCCAGCGCCGCTGGGGTTTCCCCGCCACCGGCGCCGAGCGCCTGGCTGAGGTGCTGGCGTTGCTCAAGGATTTCCTGAT
GGGCTCGGTGCTGTTTCTCAACGATGCGCCGA <539485 **MZ-3R**

Red: Forward Green: Reverse

**PFLU_2553 *(viscB)***
The complete gene is >NC_012660.1: 2806627-2817948

The amplified fragment:

>2815085 **MZ-11F**

ACCGTACCGTGGAAAACCTCGTGAATTGGCACTGCGAAGCCTTTGGCCTGGGTGCCACGGGCCACACCAGCAGCGTCGCCG
GGTTCGGTTTTGATGCGATGGCCTGGGAGGTGTGGCCGGCACTGTGCGTGGGGGCCACCTTGCACCTGCCGCCGGCCAACG
TCGGCAATGAAAACATCGATGAGCTGCTGGCCTGGTGGCTGGCGCAGCCGCTGGACGTCAGCTTCCTGCCGACGCCGGTGG
CCGAATACGCCTTCAGCCAGCAGCTGCAACACCCTACGTTGCGCATCCTGCTGATCGGTGGCGATCGCCTGCGTCAGTTCAC
TCACGAGCGGCGCTTTGCGGTGATCAACAACTACGGTCCCACCGAGGCCACCGTGGTTGCCACCTCCGGCCGCGTGCGCGC
CGGGCAGGTGTTGCATATCGGTCGGCCGATCGCCAATGCCAGCGTCTACTTGCTGGACGCGCAATTGCGCCCGGTGCCGGT
GGGGGTGACGGGCGAGCTGTATGTGGGCGGCAGCGGTGTGGCGCGGGGTTACCTGAACCGGCCGGACCTGACTGCCGAGC
GCTTCCTGCAAGACCCGTTCAACGCCGGGCGCATGTACCGTACCGGCGACCTGGCGCGCTGGCTGCCCGATGGCAACATCG
AGTACCTGGGGCGTAACGATGACCAGGTCAAACTGCGCGGCGTACGAGTGGAACTGGGGGAAATCGAAAGCCGCCTGGCCG
CCCTGGACGGCGTCGGCGAAGCGGTGGTACTGGTGCGCGAAGGTCGCTTGATTGCCTGGTTCACCGCACAGCAACCGCTGG
ACATCGACACCCTGCGCACGCAGCTGCAAGCCCAATTGCCCGATGCCCTGGTCCCGGTCGCCTATGTGAAGCTGCACGCATT
ACCGCTGACCGCCAACGGCAAGCTCGACCGCAAGGCGCTGCCGGAACCCGATCACGCCGCGCTGCTGACCCGTGTATACGA
AGCGCCCCAAGGCGAAGTTGAAACCACCTTGGCGCGCATCTGGGCCGAGGTCTTGCACGTCGAACAGGTCGGGCGCCATGA
CCACTTCTTCGAGCTGGGCGGCCATTCGTTGCTGGCCGTCAGCCTGATCGAACGCATGCGCCAGGTCGGCCTGAGTGCCGAT
GTGCGCGTGCTGTTCAGCCAGCCGACCCTGGCCGCACTGGCCGCCGCCGTCGGCAGTGGCCGCGAAGTGCAGGTGCCGGT
TAATCGCATTC <2814772 **MZ-11R**

Red: Forward Green: Reverse

**PFLU_4091 *(fecB)***
The complete gene >NC_012660.1: 4526090-4525170
The amplified fragment:
>4526130 **MZ-15F**
TCCTGGCGTTCTCTTCAAGCCTGCTGAGCGCCGCCCCCATCGACCTCAACGACGGCCAGCACGCCGTGCATCTGCCGGACG
CACCCAAGCGCGTGGTGGTGTTGGAGTTCTCGTTTCTCGACAGCCTCGCCGCGGTTGACGTGACCCCCGTCGGCGCCGCCG
ACGATGGCGACGCCAACCGTGTGTTGCCCCGTGTGCGCCAGGCCATCGGCCAGTGGACGTCCGTGGGCCTGCGCTCGCAGC
CGAGCATCGAGGAAATCGCGCGTCTCAAGCCGGACCTGATCGTCGCCGACCTCAACCGCCATCAGGCGCTGTACAACGACCT
GTCGAGCATTGCACCGACCCTGTTGCTGCCGTCGCGTGGCGAGGATTATGAAGGCAGCCTCAAGTCCGCCGAGCTGATCGG
CAAGGCCCTGGGCAAAAGCCCGCAGATGACCGCGCGCATCGCGCAAAACCGTGAAAACCTGAAAAACATCGCCCAACAGATC
CCCGCCGGCGCCAGCGTGCTGTTCGGTGTGGCGCGGGAAGACAGCTTCTCCGTACACGGCCCGGACTCCTACGCCGGCAG
CGTGCTGCAAGCCATTGGCCTGAAAGTCCCGTCGGTACGTGCCAACGCCGCGCCCACCGAGTTCGTCAGCCTGGAGCAACT
GCTTGCCCTCGACCCGGGCTGGTTGCTGGTCGGCCATTACCGTCGCCCGAGCATCGTTGACAGCTGGA <4525006 **MZ-15R**

Red: Forward Green: Reverse

**PFLU_3831 *(tox)***
The complete gene >NC_012660.1:4228933-4229283
The amplified fragment:
>4229060 **MZ-17F**
GAACAGGCGGTTTACGCAAGGTTCGCTTTGTCGACGAACGACGCAACAAAGGCAAGCGCGGTGGCCTGCGGGTCATTGACTA
CTGGTGGTCGGGCGGCACGCAATTCTGGTTATTCACCCTGTACGGCAAACA <4229192 **MZ-17R**

Red: Forward Green: Reverse

### 9.1.2 Appendix II

**List of Chemicals:**

| |
|---|
| Electrophoresis 5X TBE buffer |
| 54 g Tris-base + 27.5 g Boric acid + 20 ml 0.5 M EDTA (pH 8) make up volume to 1 L n $H_2O$ |
| 0.5 X TBE buffer |
| 100 ml of 5X TBE buffer in 900 ml n $H_2O$ |

| **Stock solutions** |
|---|
| **20X SSC:**<br>176 g NaCl + 88 g Sodium citrate make up volume to 1 L n $H_2O$ (adjust the pH 7 with 1M HCl). Autoclave. |
| **10X (1M) Maleic acid solution:**<br>116 g Maleic acid + 88 g NaCl make up volume to 1 L n $H_2O$ (adjust the pH 7.5 with solid NaOH). Autoclave. |
| **100X Denhardts solution:**<br>1 g Ficoll 400 + 1 g Bovine serum albumin +1 g Polyvinyl pyrrolidone make up volume to 50 ml n $H_2O$ |

| **Working solutions** |
|---|
| **2X SSC**<br>80 ml of 20 X SSC + 720ml n$H_2O$, autoclave. |
| **2X SSC (low stringency buffer)**<br>80 ml of 20 X SSC + 720 ml n$H_2O$, autoclave then replace 8 ml with 10%SDS to get a 0.1%SDS final concentration. |
| **0.5X SSC (high stringency buffer)**<br>20 ml 20X SSC +780 ml n$H_2O$, autoclave then replace 8 ml with 10%SDS to get a 0.1%SDS final concentration. |
| **0.1M Maleic acid solution**<br>80 ml of 10X Maleic acid solution + 720 ml n$H_2O$, autoclave. |
| **Washing buffer**<br>80 ml of 10X Maleic acid solution + 720 ml n$H_2O$, autoclave then replace 24 ml with 10% Tween 20 to get a 0.3% Tween 20 final concentration. |
| **Blocking solution (200ml)**<br>20 ml of 10X Blocking solution (Roche)+ 180 ml of sterile 0.1 M Maleic acid solution. (Prepare fresh). Use 30 ml of this for Anti-DIG antibody preparation. Store at 4°C till time to use. |
| **Anti-DIG antibody solution**<br>30 ml of fresh prepared Blocking solution + 3 µl of anti-DIG AP (Roche). Store at 4°C till time to use. Note: centrifuge Anti-DIG AP for 5 min at 13,000 rpm before taking the 3 µl. |
| **Detection buffer**<br>0.1 M Tris-HCl, 0.1 M NaCl, pH 9.5. |

**Homemade blocking solution**
5 ml of 100X Denhardts solution + 5 ml 10% SDS + 20 ml 20XSSC + 200 µl Salmon testis DNA (2mg/ ml) make up volume to 100 ml n $H_2O$.

### 9.1.3 Appendix III: Dot blot screening outcomes

| No. | Isolate | Year1 | Year2 | Niche | Block | MZ-3 | MZ-11 | MZ-15 | MZ-17 |
|---|---|---|---|---|---|---|---|---|---|
| 1 | 1R-5 | H | H | R | 1 | 0 | 0 | 0 | 0 |
| 2 | 1R-7 | H | H | R | 1 | 0 | 0 | 0 | 0 |
| 3 | 1R-4 | H | H | R | 1 | 0 | 0 | 0 | 0 |
| 4 | 1R-19 | H | H | R | 1 | 0 | 0 | 0 | 0 |
| 5 | 1R-10 | H | H | R | 1 | 0 | 0 | 0 | 0 |
| 6 | 1R-12 | H | H | R | 1 | 0 | 0 | 0 | 0 |
| 7 | 1R-16 | H | H | R | 1 | 1 | 0 | 0 | 1 |
| 8 | 1R-13 | H | H | R | 1 | 1 | 0 | 0 | 1 |
| 9 | 1R-9 | H | H | R | 1 | 1 | 0 | 1 | 0 |
| 10 | 1R-11 | H | H | R | 1 | 1 | 1 | 0 | 1 |
| 11 | 1R-1 | H | H | R | 1 | 1 | 0 | 0 | 0 |
| 12 | 1R-17 | H | H | R | 1 | 0 | 0 | 0 | 0 |
| 13 | 1R-18 | H | H | R | 1 | 0 | 0 | 0 | 0 |
| 14 | 1E - 5 | H | H | E | 1 | 1 | 1 | 0 | 0 |
| 15 | 1E -10 | H | H | E | 1 | 0 | 1 | 0 | 0 |
| 16 | 1E -11 | H | H | E | 1 | 1 | 0 | 0 | 0 |
| 17 | 1E -1 | H | H | E | 1 | 0 | 0 | 0 | 0 |
| 18 | 1E -15 | H | H | E | 1 | 0 | 0 | 0 | 0 |
| 19 | 1E -14 | H | H | E | 1 | 1 | 0 | 0 | 0 |
| 20 | 1E -17 | H | H | E | 1 | 1 | 0 | 0 | 0 |
| 21 | 1E -19 | H | H | E | 1 | 0 | 0 | 0 | 0 |
| 22 | 1E -13 | H | H | E | 1 | 0 | 0 | 0 | 0 |
| 23 | 1E -6 | H | H | E | 1 | 0 | 0 | 0 | 0 |
| 24 | 1E -7 | H | H | E | 1 | 1 | 0 | 0 | 1 |
| 25 | 1E -8 | H | H | E | 1 | 0 | 0 | 0 | 0 |
| 26 | 1E -4 | H | H | E | 1 | 0 | 0 | 0 | 0 |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| 27 | 8R -7a | C | H | R | 2 | 0 | 1 | 0 | 1 |
| 28 | 8R -11 | C | H | R | 2 | 0 | 1 | 0 | 0 |
| 29 | 8R -13 | C | H | R | 2 | 0 | 1 | 0 | 0 |
| 30 | 8R -14 | C | H | R | 2 | 0 | 1 | 0 | 1 |
| 31 | 8R -15 | C | H | R | 2 | 0 | 0 | 0 | 0 |
| 32 | 8R -2 | C | H | R | 2 | 1 | 0 | 0 | 1 |
| 33 | 8R -5 | C | H | R | 2 | 1 | 0 | 0 | 0 |
| 34 | 8R -6 | C | H | R | 2 | 0 | 0 | 0 | 0 |
| 35 | 8R -7b | C | H | R | 2 | 0 | 1 | 1 | 0 |
| 36 | 8R -9 | C | H | R | 2 | 0 | 1 | 0 | 0 |
| 37 | 8R -12 | C | H | R | 2 | 0 | 1 | 0 | 1 |
| 38 | 8R -17 | C | H | R | 2 | 0 | 1 | 0 | 1 |
| 39 | 8R -18 | C | H | R | 2 | 0 | 1 | 0 | 1 |
| 40 | 8R -20 | C | H | R | 2 | 0 | 0 | 1 | 1 |
| 41 | 8R -8 | C | H | R | 2 | 0 | 1 | 0 | 0 |
| 42 | 22R - 19 | H | Xi | R | 2 | 0 | 1 | 0 | 1 |
| 43 | 22R -14 | H | Xi | R | 2 | 0 | 1 | 0 | 1 |
| 44 | 22R -16 | H | Xi | R | 2 | 0 | 1 | 0 | 1 |
| 45 | 22R -13 | H | Xi | R | 2 | 0 | 1 | 0 | 1 |
| 46 | 22R -15 | H | Xi | R | 2 | 1 | 1 | 0 | 1 |
| 47 | 22R -18a | H | Xi | R | 2 | 1 | 0 | 0 | 0 |
| 48 | 22R -3 | H | Xi | R | 2 | 1 | 1 | 0 | 0 |
| 49 | 22R -4 | H | Xi | R | 2 | 1 | 0 | 0 | 0 |
| 50 | 22R - 9 | H | Xi | R | 2 | 0 | 1 | 0 | 0 |
| 51 | 22R -11 | H | Xi | R | 2 | 0 | 1 | 0 | 1 |
| 52 | 22R -10 | H | Xi | R | 2 | 0 | 1 | 0 | 0 |
| 53 | 22R -12 | H | Xi | R | 2 | 0 | 0 | 0 | 1 |
| 54 | 22R -17 | H | Xi | R | 2 | 1 | 0 | 0 | 1 |
| 55 | 22R -18b | H | Xi | R | 2 | 1 | 0 | 0 | 0 |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| 56 | 22R -20 | H | Xi | R | 2 | 1 | 0 | 0 | 0 |
| 57 | 22E -5 | H | Xi | E | 2 | 0 | 0 | 0 | 0 |
| 58 | 22E -6 | H | Xi | E | 2 | 0 | 1 | 0 | 1 |
| 59 | 22E -7 | H | Xi | E | 2 | 0 | 1 | 0 | 0 |
| 60 | 22E -8 | H | Xi | E | 2 | 0 | 0 | 0 | 0 |
| 61 | 22E -1 | H | Xi | E | 2 | 1 | 1 | 0 | 1 |
| 62 | 22E -3 | H | Xi | E | 2 | 0 | 0 | 0 | 0 |
| 63 | 22E -9 | H | Xi | E | 2 | 1 | 0 | 0 | 1 |
| 64 | 22E -10 | H | Xi | E | 2 | 0 | 0 | 0 | 0 |
| 65 | 22E -11 | H | Xi | E | 2 | 1 | 0 | 0 | 0 |
| 66 | 22E -12 | H | Xi | E | 2 | 0 | 1 | 0 | 0 |
| 67 | 22E -18 | H | Xi | E | 2 | 1 | 1 | 0 | 0 |
| 68 | 22E -19 | H | Xi | E | 2 | 1 | 0 | 0 | 0 |
| 69 | 22E -20 | H | Xi | E | 2 | 1 | 0 | 0 | 0 |
| 70 | 22E -13a | H | Xi | E | 2 | 1 | 0 | 0 | 1 |
| 71 | 22E -13b | H | Xi | E | 2 | 1 | 0 | 0 | 1 |
| 72 | 25R -14 | C | Xi | R | 3 | 0 | 1 | 0 | 0 |
| 73 | 25R -13 | C | Xi | R | 3 | 0 | 1 | 0 | 0 |
| 74 | 25R -5 | C | Xi | R | 3 | 1 | 0 | 0 | 0 |
| 75 | 25R -7 | C | Xi | R | 3 | 1 | 0 | 0 | 0 |
| 76 | 25R -20 | C | Xi | R | 3 | 1 | 0 | 0 | 0 |
| 77 | 25R -16 | C | Xi | R | 3 | 1 | 0 | 0 | 0 |
| 78 | 25R -6 | C | Xi | R | 3 | 1 | 0 | 0 | 1 |
| 79 | 25R -8 | C | Xi | R | 3 | 1 | 1 | 0 | 0 |
| 80 | 25R -4 | C | Xi | R | 3 | 0 | 0 | 0 | 0 |
| 81 | 25R -10 | C | Xi | R | 3 | 1 | 0 | 0 | 0 |
| 82 | 25R -11 | C | Xi | R | 3 | 1 | 0 | 1 | 1 |
| 83 | 25R -17 | C | Xi | R | 3 | 1 | 0 | 1 | 1 |
| 84 | 25R -9 | C | Xi | R | 3 | 1 | 0 | 1 | 0 |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| 85 | 25R -12 | C | Xi | R | 3 | 1 | 1 | 1 | 1 |
| 86 | 25R -18 | C | Xi | R | 3 | 1 | 0 | 0 | 1 |
| 87 | 25E -14 | C | Xi | E | 3 | 0 | 0 | 0 | 0 |
| 88 | 25E -15 | C | Xi | E | 3 | 0 | 0 | 0 | 0 |
| 89 | 25E -10 | C | Xi | E | 3 | 0 | 0 | 0 | 0 |
| 90 | 25E -5 | C | Xi | E | 3 | 0 | 0 | 0 | 0 |
| 91 | 25E -7 | C | Xi | E | 3 | 0 | 0 | 0 | 0 |
| 92 | 25E -8 | C | Xi | E | 3 | 0 | 1 | 0 | 1 |
| 93 | 25E -1 | C | Xi | E | 3 | 0 | 0 | 0 | 1 |
| 94 | 25E -3 | C | Xi | E | 3 | 0 | 0 | 0 | 0 |
| 95 | 25E -4 | C | Xi | E | 3 | 0 | 1 | 0 | 1 |
| 96 | 25E -17 | C | Xi | E | 3 | 0 | 1 | 0 | 1 |
| 97 | 25E -18 | C | Xi | E | 3 | 0 | 1 | 0 | 1 |
| 98 | 25E -19 | C | Xi | E | 3 | 0 | 1 | 0 | 1 |
| 99 | 25E -20 | C | Xi | E | 3 | 0 | 1 | 0 | 1 |
| 100 | 25E -56 | C | Xi | E | 3 | 0 | 1 | 0 | 1 |
| 101 | 24E -1 | C | Xi | E | 2 | 0 | 0 | 0 | 1 |
| 102 | 24E -2 | C | Xi | E | 2 | 0 | 1 | 0 | 1 |
| 103 | 24E -3 | C | Xi | E | 2 | 0 | 1 | 0 | 1 |
| 104 | 24E -4 | C | Xi | E | 2 | 0 | 1 | 0 | 1 |
| 105 | 24E -5 | C | Xi | E | 2 | 0 | 1 | 1 | 1 |
| 106 | 24E -6 | C | Xi | E | 2 | 0 | 1 | 1 | 1 |
| 107 | 24E -7 | C | Xi | E | 2 | 0 | 1 | 0 | 0 |
| 108 | 24E -8 | C | Xi | E | 2 | 0 | 0 | 0 | 0 |
| 109 | 24E -9 | C | Xi | E | 2 | 0 | 0 | 0 | 0 |
| 110 | 24E -11 | C | Xi | E | 2 | 0 | 0 | 0 | 1 |
| 111 | 24E -15 | C | Xi | E | 2 | 0 | 0 | 1 | 1 |
| 112 | 24E -16 | C | Xi | E | 2 | 0 | 0 | 0 | 1 |
| 113 | 24E -14 | C | Xi | E | 2 | 0 | 0 | 1 | 1 |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| 114 | 24E -13 | C | Xi | E | 2 | 0 | 1 | 1 | 1 |
| 115 | 24E -18 | C | Xi | E | 2 | 0 | 0 | 0 | 0 |
| 116 | 24R -13 | C | Xi | R | 2 | 0 | 0 | 0 | 0 |
| 117 | 24R -6 | C | Xi | R | 2 | 0 | 0 | 0 | 0 |
| 118 | 24R- 2 | C | Xi | R | 2 | 0 | 0 | 0 | 0 |
| 119 | 24R -3 | C | Xi | R | 2 | 0 | 0 | 0 | 0 |
| 120 | 24R -4a | C | Xi | R | 2 | 0 | 1 | 1 | 0 |
| 121 | 24R -17 | C | Xi | R | 2 | 0 | 0 | 1 | 0 |
| 122 | 24R -18 | C | Xi | R | 2 | 0 | 0 | 0 | 0 |
| 123 | 24R -19 | C | Xi | R | 2 | 0 | 0 | 0 | 0 |
| 124 | 24R -20 | C | Xi | R | 2 | 0 | 1 | 0 | 1 |
| 125 | 24R -15 | C | Xi | R | 2 | 0 | 0 | 0 | 1 |
| 126 | 24R -11 | C | Xi | R | 2 | 0 | 0 | 0 | 1 |
| 127 | 24R -8 | C | Xi | R | 2 | 0 | 0 | 0 | 1 |
| 128 | 24R -12 | C | Xi | R | 2 | 0 | 1 | 0 | 1 |
| 129 | 24R -4b | C | Xi | R | 2 | 0 | 1 | 0 | 1 |
| 130 | 24R -16 | C | Xi | R | 2 | 0 | 0 | 0 | 1 |
| 131 | 28E -18 | H | Xi | E | 3 | 0 | 1 | 0 | 1 |
| 132 | 28E -19 | H | Xi | E | 3 | 0 | 1 | 1 | 1 |
| 133 | 28E -20 | H | Xi | E | 3 | 0 | 1 | 1 | 1 |
| 134 | 28E -2 | H | Xi | E | 3 | 0 | 0 | 0 | 1 |
| 135 | 28E -3 | H | Xi | E | 3 | 1 | 1 | 0 | 1 |
| 136 | 28E -15 | H | Xi | E | 3 | 0 | 1 | 1 | 1 |
| 137 | 28E -14 | H | Xi | E | 3 | 0 | 0 | 1 | 1 |
| 138 | 28E -9 | H | Xi | E | 3 | 0 | 1 | 0 | 1 |
| 139 | 28E -10 | H | Xi | E | 3 | 0 | 1 | 0 | 0 |
| 140 | 28E -11 | H | Xi | E | 3 | 0 | 1 | 0 | 0 |
| 141 | 28E -12 | H | Xi | E | 3 | 0 | 0 | 0 | 0 |
| 142 | 28E -5 | H | Xi | E | 3 | 0 | 0 | 0 | 0 |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| 143 | 28E -8 | H | Xi | E | 3 | 0 | 0 | 0 | 0 |
| 144 | 28E -1 | H | Xi | E | 3 | 0 | 0 | 0 | 0 |
| 145 | 28R -8 | H | Xi | R | 3 | 0 | 0 | 0 | 0 |
| 146 | 28R -12 | H | Xi | R | 3 | 0 | 1 | 0 | 0 |
| 147 | 28R -9 | H | Xi | R | 3 | 0 | 1 | 0 | 0 |
| 148 | 28R -17 | H | Xi | R | 3 | 0 | 0 | 0 | 0 |
| 149 | 28R -18 | H | Xi | R | 3 | 0 | 0 | 0 | 0 |
| 150 | 28R -19 | H | Xi | R | 3 | 0 | 0 | 0 | 0 |
| 151 | 28R -20 | H | Xi | R | 3 | 0 | 0 | 0 | 0 |
| 152 | 28R -14 | H | Xi | R | 3 | 0 | 0 | 0 | 0 |
| 153 | 28R -16 | H | Xi | R | 3 | 0 | 0 | 0 | 0 |
| 154 | 28R -1 | H | Xi | R | 3 | 0 | 0 | 0 | 0 |
| 155 | 28R -6 | H | Xi | R | 3 | 0 | 0 | 0 | 0 |
| 156 | 28R -5 | H | Xi | R | 3 | 0 | 0 | 0 | 0 |
| 157 | 28R -7 | H | Xi | R | 3 | 0 | 0 | 0 | 0 |
| 158 | 28R -13 | H | Xi | R | 3 | 0 | 0 | 0 | 0 |
| 159 | 28R -3 | H | Xi | R | 3 | 0 | 0 | 0 | 0 |
| 160 | 30E -17 | C | Xi | E | 4 | 0 | 1 | 0 | 0 |
| 161 | 30E -18 | C | Xi | E | 4 | 0 | 0 | 0 | 0 |
| 162 | 30E -20 | C | Xi | E | 4 | 1 | 0 | 0 | 0 |
| 163 | 30E -13 | C | Xi | E | 4 | 0 | 1 | 0 | 1 |
| 164 | 30E -14 | C | Xi | E | 4 | 0 | 0 | 0 | 1 |
| 165 | 30E -15 | C | Xi | E | 4 | 0 | 0 | 1 | 1 |
| 166 | 30E -16 | C | Xi | E | 4 | 0 | 0 | 0 | 0 |
| 167 | 30E -9 | C | Xi | E | 4 | 1 | 0 | 0 | 0 |
| 168 | 30E -11 | C | Xi | E | 4 | 0 | 1 | 0 | 0 |
| 169 | 30E -12 | C | Xi | E | 4 | 0 | 1 | 0 | 0 |
| 170 | 30E -5 | C | Xi | E | 4 | 1 | 0 | 1 | 1 |
| 171 | 30E -7 | C | Xi | E | 4 | 0 | 0 | 1 | 1 |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| 172 | 30E -8 | C | Xi | E | 4 | 0 | 1 | 1 | 1 |
| 173 | 30E -4 | C | Xi | E | 4 | 0 | 0 | 1 | 1 |
| 174 | 30R-15 | C | Xi | R | 4 | 1 | 0 | 0 | 0 |
| 175 | 30R-11 | C | Xi | R | 4 | 1 | 0 | 0 | 0 |
| 176 | 30R-5 | C | Xi | R | 4 | 0 | 0 | 0 | 0 |
| 177 | 30R-17 | C | Xi | R | 4 | 0 | 0 | 1 | 0 |
| 178 | 30R-18 | C | Xi | R | 4 | 1 | 1 | 1 | 0 |
| 179 | 30R/19a | C | Xi | R | 4 | 1 | 0 | 1 | 0 |
| 180 | 30R/1 | C | Xi | R | 4 | 1 | 1 | 1 | 0 |
| 181 | 30R/4a | C | Xi | R | 4 | 1 | 0 | 1 | 0 |
| 182 | 30R/9 | C | Xi | R | 4 | 0 | 0 | 0 | 1 |
| 183 | 30R/10 | C | Xi | R | 4 | 1 | 1 | 0 | 0 |
| 184 | 30R/6a | C | Xi | R | 4 | 1 | 0 | 0 | 0 |
| 185 | 30R/6b | C | Xi | R | 4 | 1 | 0 | 0 | 0 |
| 186 | 30R/12 | C | Xi | R | 4 | 0 | 0 | 0 | 0 |
| 187 | 30R/4b | C | Xi | R | 4 | 1 | 0 | 0 | 0 |
| 188 | 30R/19b | C | Xi | R | 4 | 0 | 0 | 0 | 0 |
| 189 | 31R/11 | H | Xi | R | 4 | 0 | 0 | 0 | 1 |
| 190 | 31R/12 | H | Xi | R | 4 | 0 | 0 | 0 | 0 |
| 191 | 31R/13 | H | Xi | R | 4 | 0 | 0 | 0 | 0 |
| 192 | 31R/18 | H | Xi | R | 4 | 0 | 0 | 0 | 0 |
| 193 | 31R/14 | H | Xi | R | 4 | 0 | 0 | 0 | 0 |
| 194 | 31R/15 | H | Xi | R | 4 | 0 | 1 | 0 | 0 |
| 195 | 31R/16 | H | Xi | R | 4 | 0 | 0 | 0 | 0 |
| 196 | 31R/5 | H | Xi | R | 4 | 0 | 1 | 1 | 0 |
| 197 | 31R/6 | H | Xi | R | 4 | 0 | 0 | 0 | 0 |
| 198 | 31R/7 | H | Xi | R | 4 | 1 | 1 | 1 | 0 |
| 199 | 31R/8 | H | Xi | R | 4 | 1 | 0 | 0 | 0 |
| 200 | 31R/1 | H | Xi | R | 4 | 1 | 0 | 1 | 0 |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| 201 | 31R/2 | H | Xi | R | 4 | 0 | 0 | 0 | 0 |
| 202 | 31R/4 | H | Xi | R | 4 | 0 | 1 | 0 | 0 |
| 203 | 31R/17 | H | Xi | R | 4 | 0 | 1 | 0 | 0 |
| 204 | 31E/1 | H | Xi | E | 4 | 0 | 0 | 0 | 0 |
| 205 | 31E/2 | H | Xi | E | 4 | 1 | 0 | 0 | 1 |
| 206 | 31E/3 | H | Xi | E | 4 | 1 | 0 | 0 | 1 |
| 207 | 31E/4 | H | Xi | E | 4 | 1 | 0 | 0 | 1 |
| 208 | 31E/5 | H | Xi | E | 4 | 1 | 0 | 0 | 0 |
| 209 | 31E/6 | H | Xi | E | 4 | 0 | 0 | 0 | 0 |
| 210 | 31E/7 | H | Xi | E | 4 | 0 | 0 | 0 | 0 |
| 211 | 31E/8 | H | Xi | E | 4 | 0 | 0 | 0 | 0 |
| 212 | 34R/11a | H | Xi | R | 1 | 1 | 0 | 0 | 0 |
| 213 | 34R/10 | H | Xi | R | 1 | 0 | 0 | 0 | 0 |
| 214 | 34R/11b | H | Xi | R | 1 | 1 | 0 | 0 | 0 |
| 215 | 34R/9b | H | Xi | R | 1 | 1 | 0 | 0 | 0 |
| 216 | 34R/9a | H | Xi | R | 1 | 1 | 0 | 0 | 0 |
| 217 | 34R/12 | H | Xi | R | 1 | 1 | 0 | 0 | 0 |
| 218 | 34R/5 | H | Xi | R | 1 | 1 | 0 | 0 | 0 |
| 219 | 34R/6 | H | Xi | R | 1 | 0 | 0 | 1 | 0 |
| 220 | 34R/8 | H | Xi | R | 1 | 1 | 0 | 1 | 0 |
| 221 | 34R/15 | H | Xi | R | 1 | 1 | 0 | 0 | 0 |
| 222 | 34R/16 | H | Xi | R | 1 | 1 | 1 | 0 | 0 |
| 223 | 34R/20 | H | Xi | R | 1 | 1 | 0 | 0 | 0 |
| 224 | 34R/2 | H | Xi | R | 1 | 1 | 0 | 0 | 0 |
| 225 | 34R/3 | H | Xi | R | 1 | 1 | 0 | 0 | 0 |
| 226 | 34R/4 | H | Xi | R | 1 | 0 | 0 | 0 | 0 |
| 227 | 34E/19 | H | Xi | E | 1 | 1 | 0 | 1 | 1 |
| 228 | 34E/20 | H | Xi | E | 1 | 1 | 0 | 0 | 0 |
| 229 | 34E/9 | H | Xi | E | 1 | 1 | 0 | 0 | 0 |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| 230 | 34E/10 | H | Xi | E | 1 | 1 | 0 | 1 | 0 |
| 231 | 34E/11 | H | Xi | E | 1 | 1 | 0 | 1 | 0 |
| 232 | 34E/7 | H | Xi | E | 1 | 1 | 0 | 1 | 0 |
| 233 | 34E/13 | H | Xi | E | 1 | 1 | 1 | 1 | 0 |
| 234 | 34E/14 | H | Xi | E | 1 | 1 | 1 | 0 | 0 |
| 235 | 34E/15 | H | Xi | E | 1 | 0 | 0 | 0 | 1 |
| 236 | 34E/2 | H | Xi | E | 1 | 1 | 0 | 0 | 0 |
| 237 | 34E/4 | H | Xi | E | 1 | 1 | 0 | 0 | 0 |
| 238 | 35E/1 | C | Xi | E | 1 | 1 | 0 | 0 | 0 |
| 239 | 35E/3 | C | Xi | E | 1 | 1 | 0 | 1 | 0 |
| 240 | 35E/4 | C | Xi | E | 1 | 1 | 0 | 1 | 0 |
| 241 | 35E/5 | C | Xi | E | 1 | 1 | 0 | 1 | 0 |
| 242 | 35E/7 | C | Xi | E | 1 | 1 | 0 | 1 | 0 |
| 243 | 35E/8 | C | Xi | E | 1 | 1 | 0 | 1 | 1 |
| 244 | 35E/9 | C | Xi | E | 1 | 1 | 1 | 1 | 0 |
| 245 | 35E/11 | C | Xi | E | 1 | 0 | 0 | 0 | 0 |
| 246 | 35E/12 | C | Xi | E | 1 | 0 | 0 | 0 | 1 |
| 247 | 35E/13 | C | Xi | E | 1 | 0 | 0 | 0 | 1 |
| 248 | 35E/14 | C | Xi | E | 1 | 1 | 0 | 0 | 1 |
| 249 | 35E/16 | C | Xi | E | 1 | 1 | 0 | 0 | 1 |
| 250 | 35E/17 | C | Xi | E | 1 | 0 | 0 | 0 | 1 |
| 251 | 35E/19 | C | Xi | E | 1 | 0 | 1 | 1 | 1 |

**9.1.4 Appendix IV: Proportion data for dot blot.** (* no isolates are available for this plot)

| treatment | wheat | R or E | niche | block | proportion | plot | Locus |
|---|---|---|---|---|---|---|---|
| 1 | H | R | 1 | 2 | 0.466667 | 22 | wsm |
| 1 | H | E | 2 | 2 | 0.533333 | 22 | wsm |
| 1 | H | E | 2 | 3 | 0.071429 | 28 | wsm |
| 1 | H | R | 1 | 3 | 0 | 28 | wsm |
| 1 | H | R | 1 | 4 | 0.2 | 31 | wsm |
| 1 | H | E | 2 | 4 | 0.5 | 31 | wsm |
| 1 | H | R | 1 | 1 | 0.8 | 34 | wsm |
| 1 | H | E | 2 | 1 | 0.909091 | 34 | wsm |
| 2 | C | R | 1 | 3 | 0.8 | 25 | wsm |
| 2 | C | E | 2 | 3 | 0 | 25 | wsm |
| 2 | C | E | 2 | 2 | 0 | 24 | wsm |
| 2 | C | R | 1 | 2 | 0 | 24 | wsm |
| 2 | C | E | 2 | 1 | 0.642857 | 35 | wsm |
| 2 | C | R | 1 | 1 | * | 35 | wsm |
| 2 | C | E | 2 | 4 | 0.214286 | 30 | wsm |
| 2 | C | R | 1 | 4 | 0.666667 | 30 | wsm |
| 1 | H | R | 1 | 2 | 0.6 | 22 | visB |
| 1 | H | E | 2 | 2 | 0.3333 | 22 | visB |
| 1 | H | E | 2 | 3 | 0.5714 | 28 | visB |
| 1 | H | R | 1 | 3 | 0.1333 | 28 | visB |
| 1 | H | R | 1 | 4 | 0.333 | 31 | visB |
| 1 | H | E | 2 | 4 | 0 | 31 | visB |
| 1 | H | R | 1 | 1 | 0.07 | 34 | visB |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| 1 | H | E | 2 | 1 | 0.18 | 34 | visB |
| 2 | C | R | 1 | 3 | 0.27 | 25 | visB |
| 2 | C | E | 2 | 3 | 0.5 | 25 | visB |
| 2 | C | E | 2 | 2 | 0.47 | 24 | visB |
| 2 | C | R | 1 | 2 | 0.27 | 24 | visB |
| 2 | C | E | 2 | 1 | 0.14 | 35 | visB |
| 2 | C | R | 1 | 1 | * | 35 | visB |
| 2 | C | E | 2 | 4 | 0.36 | 30 | visB |
| 2 | C | R | 1 | 4 | 0.2 | 30 | visB |
| 1 | H | R | 1 | 2 | 0 | 22 | fecB |
| 1 | H | E | 2 | 2 | 0 | 22 | fecB |
| 1 | H | E | 2 | 3 | 0.285714 | 28 | fecB |
| 1 | H | R | 1 | 3 | 0 | 28 | fecB |
| 1 | H | R | 1 | 4 | 0.2 | 31 | fecB |
| 1 | H | E | 2 | 4 | 0 | 31 | fecB |
| 1 | H | R | 1 | 1 | 0.133333 | 34 | fecB |
| 1 | H | E | 2 | 1 | 0.454545 | 34 | fecB |
| 2 | C | R | 1 | 3 | 0.266667 | 25 | fecB |
| 2 | C | E | 2 | 3 | 0 | 25 | fecB |
| 2 | C | E | 2 | 2 | 0.333333 | 24 | fecB |
| 2 | C | R | 1 | 2 | 0.133333 | 24 | fecB |
| 2 | C | E | 2 | 1 | 0.5 | 35 | fecB |
| 2 | C | R | 1 | 1 | * | 35 | fecB |
| 2 | C | E | 2 | 4 | 0.357143 | 30 | fecB |
| 2 | C | R | 1 | 4 | 0.333333 | 30 | fecB |
| 1 | H | R | 1 | 2 | 0.53 | 22 | tox |
| 1 | H | E | 2 | 2 | 0.33 | 22 | tox |
| 1 | H | E | 2 | 3 | 0.57 | 28 | tox |
| 1 | H | R | 1 | 3 | 0 | 28 | tox |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| 1 | H | R | 1 | 4 | 0.07 | 31 | tox |
| 1 | H | E | 2 | 4 | 0.375 | 31 | tox |
| 1 | H | R | 1 | 1 | 0 | 34 | tox |
| 1 | H | E | 2 | 1 | 0.18 | 34 | tox |
| 2 | C | R | 1 | 3 | 0.33 | 25 | tox |
| 2 | C | E | 2 | 3 | 0.57 | 25 | tox |
| 2 | C | E | 2 | 2 | 0.73 | 24 | tox |
| 2 | C | R | 1 | 2 | 0.47 | 24 | tox |
| 2 | C | E | 2 | 1 | 0.5 | 35 | tox |
| 2 | C | R | 1 | 1 | * | 35 | tox |
| 2 | C | E | 2 | 4 | 0.5 | 30 | tox |
| 2 | C | R | 1 | 4 | 0.07 | 30 | tox |

**9.1.5 Appendix V:** PCR outcomes of GH2 isolate screening for *wsm* and *fecB* loci.

| No. | Isolate | Regime | Niche | wsm_PCR | fecB_PCR |
|---|---|---|---|---|---|
| 1 | 1E/1 | h,h | E | 1 | 1 |
| 2 | 1E/10 | h,h | E | 1 | 0 |
| 3 | 1E/11 | h,h | E | 1 | 1 |
| 4 | 1E/13 | h,h | E | 1 | 1 |
| 5 | 1E/14 | h,h | E | 1 | 0 |
| 6 | 1E/15 | h,h | E | 1 | 0 |
| 7 | 1E/17 | h,h | E | 1 | 1 |
| 8 | 1E/19 | h,h | E | 1 | 1 |
| 9 | 1E/4 | h,h | E | 1 | 1 |
| 10 | 1E/5 | h,h | E | 1 | 1 |

| | | | | | |
|---|---|---|---|---|---|
| 11 | 1E/6 | h,h | E | 1 | 1 |
| 12 | 1E/7 | h,h | E | 1 | 1 |
| 13 | 1E/8 | h,h | E | 1 | 1 |
| 14 | 1R/1 | h,h | R | 1 | 1 |
| 15 | 1R/10 | h,h | R | 1 | 0 |
| 16 | 1R/11 | h,h | R | 0 | 0 |
| 17 | 1R/12 | h,h | R | 1 | 1 |
| 18 | 1R/13 | h,h | R | 1 | 1 |
| 19 | 1R/16 | h,h | R | 1 | 1 |
| 20 | 1R/17 | h,h | R | 1 | 0 |
| 21 | 1R/18 | h,h | R | 1 | 1 |
| 22 | 1R/19 | h,h | R | 1 | 1 |
| 23 | 1R/4 | h,h | R | 0 | 0 |
| 24 | 1R/5 | h,h | R | 0 | 0 |
| 25 | 1R/7 | h,h | R | 0 | 0 |
| 26 | 1R/9 | h,h | R | 1 | 0 |
| 27 | 22E/1 | h,xi | E | 1 | 0 |
| 28 | 22E/10 | h,xi | E | 1 | 0 |
| 29 | 22E/11 | h,xi | E | 1 | 1 |
| 30 | 22E/12 | h,xi | E | 1 | 1 |
| 31 | 22E/13a | h,xi | E | 1 | 0 |
| 32 | 22E/13b | h,xi | E | 1 | 0 |
| 33 | 22E/18 | h,xi | E | 1 | 0 |
| 34 | 22E/19 | h,xi | E | 1 | 1 |
| 35 | 22E/20 | h,xi | E | 1 | 1 |
| 36 | 22E/3 | h,xi | E | 0 | 0 |
| 37 | 22E/5 | h,xi | E | 1 | 1 |
| 38 | 22E/6 | h,xi | E | 1 | 1 |
| 39 | 22E/7 | h,xi | E | 1 | 0 |

| | | | | | |
|---|---|---|---|---|---|
| 40 | 22E/8 | h,xi | E | 1 | 0 |
| 41 | 22E/9 | h,xi | E | 1 | 0 |
| 42 | 22R/10 | h,xi | R | 1 | 1 |
| 43 | 22R/11 | h,xi | R | 1 | 1 |
| 44 | 22R/12 | h,xi | R | 1 | 1 |
| 45 | 22R/13 | h,xi | R | 1 | 0 |
| 46 | 22R/14 | h,xi | R | 1 | 1 |
| 47 | 22R/15 | h,xi | R | 1 | 0 |
| 48 | 22R/16 | h,xi | R | 1 | 0 |
| 49 | 22R/17 | h,xi | R | 1 | 0 |
| 50 | 22R/18a | h,xi | R | 1 | 0 |
| 51 | 22R/18b | h,xi | R | 1 | 1 |
| 52 | 22R/19 | h,xi | R | 1 | 0 |
| 53 | 22R/20 | h,xi | R | 1 | 1 |
| 54 | 22R/3 | h,xi | R | 1 | 1 |
| 55 | 22R/4 | h,xi | R | 1 | 1 |
| 56 | 22R/9 | h,xi | R | 1 | 1 |
| 57 | 24E/1 | c,xi | E | 1 | 1 |
| 58 | 24E/11 | c,xi | E | 1 | 0 |
| 59 | 24E/13 | c,xi | E | 1 | 0 |
| 60 | 24E/16 | c,xi | E | 1 | 1 |
| 61 | 24E/18 | c,xi | E | 0 | 1 |
| 62 | 24E/2 | c,xi | E | 1 | 1 |
| 63 | 24E/3 | c,xi | E | 1 | 1 |
| 64 | 24E/4 | c,xi | E | 1 | 1 |
| 65 | 24E/5 | c,xi | E | 1 | 0 |
| 66 | 24E/6 | c,xi | E | 0 | 1 |
| 67 | 24E/7 | c,xi | E | 1 | 1 |
| 68 | 24E/8 | c,xi | E | 1 | 1 |

| | | | | | |
|---|---|---|---|---|---|
| 69 | 24E/9 | c,xi | E | 1 | 1 |
| 70 | 24R/11 | c,xi | R | 1 | 1 |
| 71 | 24R/12 | c,xi | R | 1 | 1 |
| 72 | 24R/13 | c,xi | R | 1 | 0 |
| 73 | 24R/15 | c,xi | R | 1 | 1 |
| 74 | 24R/16 | c,xi | R | 1 | 0 |
| 75 | 24R/17 | c,xi | R | 1 | 1 |
| 76 | 24R/18 | c,xi | R | 1 | 1 |
| 77 | 24R/19 | c,xi | R | 1 | 1 |
| 78 | 24R/2 | c,xi | R | 1 | 1 |
| 79 | 24R/20 | c,xi | R | 1 | 1 |
| 80 | 24R/3 | c,xi | R | 1 | 1 |
| 81 | 24R/4a | c,xi | R | 1 | 1 |
| 82 | 24R/4b | c,xi | R | 1 | 1 |
| 83 | 24R/6 | c,xi | R | 1 | 1 |
| 84 | 24R/8 | c,xi | R | 1 | 1 |
| 85 | 25E/1 | c,xi | E | 1 | 1 |
| 86 | 25E/10 | c,xi | E | 1 | 1 |
| 87 | 25E/14 | c,xi | E | 0 | 1 |
| 88 | 25E/14 | c,xi | E | 0 | 0 |
| 89 | 25E/15 | c,xi | E | 0 | 0 |
| 90 | 25E/15 | c,xi | E | 1 | 0 |
| 91 | 25E/17 | c,xi | E | 1 | 0 |
| 92 | 25E/18 | c,xi | E | 0 | 1 |
| 93 | 25E/19 | c,xi | E | 0 | 1 |
| 94 | 25E/20 | c,xi | E | 0 | 0 |
| 95 | 25E/3 | c,xi | E | 1 | 1 |
| 96 | 25E/4 | c,xi | E | 1 | 0 |
| 97 | 25E/5 | c,xi | E | 0 | 1 |

| | | | | | |
|---|---|---|---|---|---|
| 98 | 25E/56 | c,xi | E | 0 | 1 |
| 99 | 25E/7 | c,xi | E | 0 | 0 |
| 100 | 25E/8 | c,xi | E | 1 | 1 |
| 101 | 25R/10 | c,xi | R | 1 | 1 |
| 102 | 25R/11 | c,xi | R | 1 | 1 |
| 103 | 25R/12 | c,xi | R | 1 | 1 |
| 104 | 25R/13 | c,xi | R | 1 | 1 |
| 105 | 25R/14 | c,xi | R | 1 | 0 |
| 106 | 25R/16 | c,xi | R | 1 | 1 |
| 107 | 25R/17 | c,xi | R | 1 | 1 |
| 108 | 25R/18 | c,xi | R | 1 | 1 |
| 109 | 25R/20 | c,xi | R | 1 | 1 |
| 110 | 25R/4 | c,xi | R | 1 | 1 |
| 111 | 25R/5 | c,xi | R | 1 | 1 |
| 112 | 25R/6 | c,xi | R | 1 | 1 |
| 113 | 25R/7 | c,xi | R | 1 | 1 |
| 114 | 25R/8 | c,xi | R | 1 | 1 |
| 115 | 25R/9 | c,xi | R | 1 | 1 |
| 116 | 28E/1 | h,xi | E | 0 | 1 |
| 117 | 28E/10 | h,xi | E | 1 | 1 |
| 118 | 28E/11 | h,xi | E | 1 | 1 |
| 119 | 28E/12 | h,xi | E | 1 | 1 |
| 120 | 28E/14 | h,xi | E | 1 | 1 |
| 121 | 28E/15 | h,xi | E | 1 | 1 |
| 122 | 28E/18 | h,xi | E | 1 | 0 |
| 123 | 28E/19 | h,xi | E | 1 | 1 |
| 124 | 28E/2 | h,xi | E | 1 | 1 |
| 125 | 28E/20 | h,xi | E | 1 | 1 |
| 126 | 28E/3 | h,xi | E | 1 | 1 |

| | | | | | |
|---|---|---|---|---|---|
| 127 | 28E/5 | h,xi | E | 1 | 0 |
| 128 | 28E/8 | h,xi | E | 1 | 1 |
| 129 | 28E/9 | h,xi | E | 1 | 0 |
| 130 | 28R/1 | h,xi | R | 1 | 1 |
| 131 | 28R/12 | h,xi | R | 1 | 0 |
| 132 | 28R/13 | h,xi | R | 0 | 0 |
| 133 | 28R/14 | h,xi | R | 1 | 0 |
| 134 | 28R/16 | h,xi | R | 1 | 1 |
| 135 | 28R/17 | h,xi | R | 1 | 1 |
| 136 | 28R/18 | h,xi | R | 1 | 1 |
| 137 | 28R/19 | h,xi | R | 1 | 1 |
| 138 | 28R/20 | h,xi | R | 0 | 0 |
| 139 | 28R/3 | h,xi | R | 0 | 0 |
| 140 | 28R/5 | h,xi | R | 1 | 0 |
| 141 | 28R/6 | h,xi | R | 1 | 1 |
| 142 | 28R/7 | h,xi | R | 1 | 0 |
| 143 | 28R/8 | h,xi | R | 1 | 0 |
| 144 | 28R/9 | h,xi | R | 1 | 1 |
| 145 | 30E/11 | c,xi | E | 1 | 0 |
| 146 | 30E/12 | c,xi | E | 1 | 1 |
| 147 | 30E/13 | c,xi | E | 0 | 0 |
| 148 | 30E/14 | c,xi | E | 1 | 1 |
| 149 | 30E/15 | c,xi | E | 1 | 1 |
| 150 | 30E/16 | c,xi | E | 0 | 0 |
| 151 | 30E/17 | c,xi | E | 0 | 0 |
| 152 | 30E/18 | c,xi | E | 1 | 1 |
| 153 | 30E/20 | c,xi | E | 0 | 0 |
| 154 | 30E/4 | c,xi | E | 1 | 1 |
| 155 | 30E/5 | c,xi | E | 0 | 1 |

| | | | | | |
|---|---|---|---|---|---|
| 156 | 30E/7 | c,xi | E | 1 | 1 |
| 157 | 30E/8 | c,xi | E | 1 | 0 |
| 158 | 30E/9 | c,xi | E | 1 | 1 |
| 159 | 30R/1 | c,xi | R | 1 | 1 |
| 160 | 30R/10 | c,xi | R | 1 | 1 |
| 161 | 30R/11 | c,xi | R | 0 | 0 |
| 162 | 30R/12 | c,xi | R | 1 | 1 |
| 163 | 30R/15 | c,xi | R | 1 | 1 |
| 164 | 30R/17 | c,xi | R | 1 | 0 |
| 165 | 30R/18 | c,xi | R | 1 | 1 |
| 166 | 30R/19a | c,xi | R | 1 | 1 |
| 167 | 30R/19b | c,xi | R | 1 | 1 |
| 168 | 30R/4a | c,xi | R | 1 | 1 |
| 169 | 30R/4b | c,xi | R | 1 | 1 |
| 170 | 30R/5 | c,xi | R | 1 | 1 |
| 171 | 30R/6a | c,xi | R | 1 | 1 |
| 172 | 30R/6b | c,xi | R | 1 | 0 |
| 173 | 30R/9 | c,xi | R | 1 | 1 |
| 174 | 31E/1 | h,xi | E | 1 | 1 |
| 175 | 31E/2 | h,xi | E | 1 | 0 |
| 176 | 31E/3 | h,xi | E | 1 | 0 |
| 177 | 31E/4 | h,xi | E | 0 | 0 |
| 178 | 31E/5 | h,xi | E | 1 | 0 |
| 179 | 31E/6 | h,xi | E | 1 | 0 |
| 180 | 31E/7 | h,xi | E | 1 | 0 |
| 181 | 31E/8 | h,xi | E | 1 | 0 |
| 182 | 31R/1 | h,xi | R | 1 | 1 |
| 183 | 31R/11 | h,xi | R | 1 | 1 |
| 184 | 31R/12 | h,xi | R | 1 | 1 |

| | | | | | |
|---|---|---|---|---|---|
| 185 | 31R/13 | h,xi | R | 0 | 0 |
| 186 | 31R/14 | h,xi | R | 1 | 1 |
| 187 | 31R/15 | h,xi | R | 1 | 1 |
| 188 | 31R/16 | h,xi | R | 1 | 1 |
| 189 | 31R/17 | h,xi | R | 1 | 0 |
| 190 | 31R/18 | h,xi | R | 1 | 1 |
| 191 | 31R/2 | h,xi | R | 1 | 1 |
| 192 | 31R/4 | h,xi | R | 1 | 0 |
| 193 | 31R/5 | h,xi | R | 1 | 1 |
| 194 | 31R/6 | h,xi | R | 1 | 1 |
| 195 | 31R/7 | h,xi | R | 1 | 1 |
| 196 | 31R/8 | h,xi | R | 1 | 1 |
| 197 | 32E/1 | h,h | E | 1 | 1 |
| 198 | 32E/10 | h,h | E | 1 | 1 |
| 199 | 32E/12 | h,h | E | 1 | 1 |
| 200 | 32E/13 | h,h | E | 1 | 1 |
| 201 | 32E/14 | h,h | E | 1 | 1 |
| 202 | 32E/16 | h,h | E | 0 | 1 |
| 203 | 32E/2 | h,h | E | 1 | 0 |
| 204 | 32E/20 | h,h | E | 0 | 0 |
| 205 | 32E/3 | h,h | E | 0 | 1 |
| 206 | 32E/4 | h,h | E | 1 | 1 |
| 207 | 32E/5 | h,h | E | 1 | 1 |
| 208 | 32E/6 | h,h | E | 1 | 1 |
| 209 | 32E/7 | h,h | E | 1 | 1 |
| 210 | 32E/8 | h,h | E | 1 | 1 |
| 211 | 32E/9 | h,h | E | 1 | 1 |
| 212 | 34E/10 | h,xi | E | 1 | 1 |
| 213 | 34E/11 | h,xi | E | 1 | 1 |

| | | | | | |
|---|---|---|---|---|---|
| 214 | 34E/13 | h,xi | E | 1 | 0 |
| 215 | 34E/14 | h,xi | E | 1 | 1 |
| 216 | 34E/15 | h,xi | E | 1 | 1 |
| 217 | 34E/19 | h,xi | E | 1 | 1 |
| 218 | 34E/2 | h,xi | E | 1 | 1 |
| 219 | 34E/20 | h,xi | E | 1 | 1 |
| 220 | 34E/4 | h,xi | E | 1 | 0 |
| 221 | 34E/7 | h,xi | E | 1 | 1 |
| 222 | 34E/9 | h,xi | E | 1 | 0 |
| 223 | 34R/10 | h,xi | R | 0 | 0 |
| 224 | 34R/11a | h,xi | R | 1 | 0 |
| 225 | 34R/11b | h,xi | R | 1 | 0 |
| 226 | 34R/12 | h,xi | R | 1 | 0 |
| 227 | 34R/15 | h,xi | R | 1 | 0 |
| 228 | 34R/16 | h,xi | R | 0 | 0 |
| 229 | 34R/2 | h,xi | R | 1 | 1 |
| 230 | 34R/20 | h,xi | R | 1 | 0 |
| 231 | 34R/3 | h,xi | R | 1 | 1 |
| 232 | 34R/4 | h,xi | R | 1 | 0 |
| 233 | 34R/5 | h,xi | R | 1 | 0 |
| 234 | 34R/6 | h,xi | R | 1 | 1 |
| 235 | 34R/8 | h,xi | R | 1 | 1 |
| 236 | 34R/9a | h,xi | R | 1 | 0 |
| 237 | 34R/9b | h,xi | R | 1 | 0 |
| 238 | 35E/1 | c,xi | E | 1 | 0 |
| 239 | 35E/11 | c,xi | E | 1 | 1 |
| 240 | 35E/12 | c,xi | E | 1 | 1 |
| 241 | 35E/13 | c,xi | E | 1 | 0 |
| 242 | 35E/14 | c,xi | E | 1 | 0 |

| | | | | | |
|---|---|---|---|---|---|
| 243 | 35E/16 | c,xi | E | 1 | 0 |
| 244 | 35E/17 | c,xi | E | 1 | 0 |
| 245 | 35E/19 | c,xi | E | 1 | 0 |
| 246 | 35E/3 | c,xi | E | 1 | 1 |
| 247 | 35E/4 | c,xi | E | 0 | 1 |
| 248 | 35E/5 | c,xi | E | 1 | 1 |
| 249 | 35E/7 | c,xi | E | 1 | 0 |
| 250 | 35E/8 | c,xi | E | 1 | 1 |
| 251 | 35E/9 | c,xi | E | 1 | 0 |
| 252 | 35R/1 | c,xi | R | 1 | 1 |
| 253 | 35R/10 | c,xi | R | 1 | 1 |
| 254 | 35R/11 | c,xi | R | 0 | 0 |
| 255 | 35R/12 | c,xi | R | 1 | 1 |
| 256 | 35R/13 | c,xi | R | 1 | 0 |
| 257 | 35R/14 | c,xi | R | 0 | 0 |
| 258 | 35R/18 | c,xi | R | 0 | 0 |
| 259 | 35R/19 | c,xi | R | 0 | 0 |
| 260 | 35R/2 | c,xi | R | 1 | 0 |
| 261 | 35R/20 | c,xi | R | 1 | 0 |
| 262 | 35R/5 | c,xi | R | 1 | 0 |
| 263 | 35R/6 | c,xi | R | 1 | 1 |
| 264 | 35R/7 | c,xi | R | 1 | 1 |
| 265 | 35R/9 | c,xi | R | 1 | 0 |
| 266 | 37R/1 | h,h | R | 1 | 0 |
| 267 | 37R/10 | h,h | R | 1 | 1 |
| 268 | 37R/11 | h,h | R | 1 | 0 |
| 269 | 37R/12 | h,h | R | 1 | 0 |
| 270 | 37R/13 | h,h | R | 1 | 0 |
| 271 | 37R/14 | h,h | R | 1 | 0 |

| | | | | | |
|---|---|---|---|---|---|
| 272 | 37R/15 | h,h | R | 1 | 0 |
| 273 | 37R/16 | h,h | R | 1 | 0 |
| 274 | 37R/17 | h,h | R | 1 | 0 |
| 275 | 37R/18 | h,h | R | 1 | 0 |
| 276 | 37R/2 | h,h | R | 1 | 0 |
| 277 | 37R/3 | h,h | R | 1 | 0 |
| 278 | 37R/6 | h,h | R | 1 | 1 |
| 279 | 37R/7 | h,h | R | 1 | 0 |
| 280 | 37R/9 | h,h | R | 1 | 0 |
| 281 | 44E/1 | h,h | E | 1 | 0 |
| 282 | 44E/10 | h,h | E | 1 | 0 |
| 283 | 44E/13 | h,h | E | 1 | 1 |
| 284 | 44E/14 | h,h | E | 1 | 0 |
| 285 | 44E/15 | h,h | E | 1 | 0 |
| 286 | 44E/16 | h,h | E | 1 | 0 |
| 287 | 44E/17 | h,h | E | 1 | 0 |
| 288 | 44E/2 | h,h | E | 1 | 0 |
| 289 | 44E/20 | h,h | E | 1 | 1 |
| 290 | 44E/3 | h,h | E | 1 | 0 |
| 291 | 44E/5 | h,h | E | 1 | 0 |
| 292 | 44E/7 | h,h | E | 1 | 1 |
| 293 | 44E/9 | h,h | E | 1 | 0 |
| 294 | 44R/1 | h,h | R | 1 | 0 |
| 295 | 44R/10 | h,h | R | 1 | 1 |
| 296 | 44R/11 | h,h | R | 1 | 0 |
| 297 | 44R/12 | h,h | R | 1 | 0 |
| 298 | 44R/13 | h,h | R | 1 | 0 |
| 299 | 44R/14 | h,h | R | 0 | 0 |
| 300 | 44R/15 | h,h | R | 1 | 0 |

| | | | | | |
|---|---|---|---|---|---|
| 301 | 44R/16 | h,h | R | 1 | 1 |
| 302 | 44R/18 | h,h | R | 1 | 0 |
| 303 | 44R/19 | h,h | R | 1 | 1 |
| 304 | 44R/2 | h,h | R | 1 | 0 |
| 305 | 44R/3 | h,h | R | 1 | 0 |
| 306 | 44R/4 | h,h | R | 1 | 0 |
| 307 | 44R/5 | h,h | R | 1 | 0 |
| 308 | 44R/6 | h,h | R | 1 | 0 |
| 309 | 46E/1 | c,h | E | 0 | 0 |
| 310 | 46E/2 | c,h | E | 0 | 0 |
| 311 | 46E/3 | c,h | E | 0 | 0 |
| 312 | 46E/4 | c,h | E | 0 | 0 |
| 313 | 46E/5 | c,h | E | 0 | 0 |
| 314 | 46E/6 | c,h | E | 0 | 0 |
| 315 | 46E/7 | c,h | E | 0 | 0 |
| 316 | 46E/8 | c,h | E | 0 | 0 |
| 317 | 46R/1 | c,h | R | 1 | 1 |
| 318 | 46R/2 | c,h | R | 0 | 0 |
| 319 | 46R/3 | c,h | R | 0 | 0 |
| 320 | 46R/5 | c,h | R | 1 | 1 |
| 321 | 46R/6 | c,h | R | 1 | 1 |
| 322 | 46R/7 | c,h | R | 1 | 1 |
| 323 | 46R/8 | c,h | R | 1 | 1 |
| 324 | 52E/1 | c,h | E | 0 | 0 |
| 325 | 52E/11 | c,h | E | 0 | 1 |
| 326 | 52E/12 | c,h | E | 0 | 1 |
| 327 | 52E/15 | c,h | E | 0 | 1 |
| 328 | 52E/16 | c,h | E | 1 | 0 |
| 329 | 52E/17 | c,h | E | 1 | 1 |

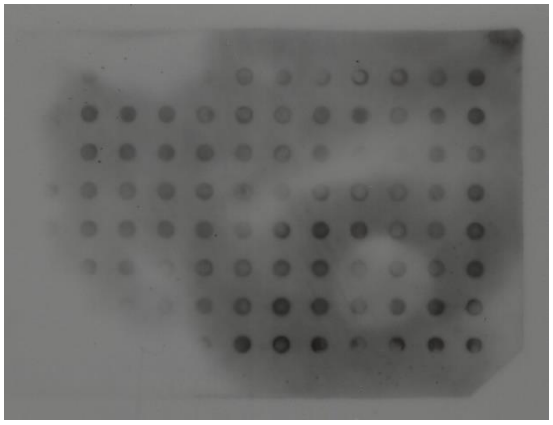
| | | | | | |
|---|---|---|---|---|---|
| 330 | 52E/19 | c,h | E | 0 | 1 |
| 331 | 52E/2 | c,h | E | 0 | 0 |
| 332 | 52E/20 | c,h | E | 1 | 0 |
| 333 | 52E/3 | c,h | E | 0 | 1 |
| 334 | 52E/5 | c,h | E | 1 | 0 |
| 335 | 52E/6 | c,h | E | 0 | 1 |
| 336 | 52E/7 | c,h | E | 0 | 1 |
| 337 | 52E/8 | c,h | E | 0 | 1 |
| 338 | 52E/9 | c,h | E | 0 | 1 |
| 339 | 52R/10 | c,h | R | 0 | 0 |
| 340 | 52R/12 | c,h | R | 0 | 1 |
| 341 | 52R/13 | c,h | R | 1 | 1 |
| 342 | 52R/14 | c,h | R | 1 | 1 |
| 343 | 52R/15 | c,h | R | 1 | 1 |
| 344 | 52R/17 | c,h | R | 1 | 1 |
| 345 | 52R/2 | c,h | R | 0 | 1 |
| 346 | 52R/20 | c,h | R | 0 | 1 |
| 347 | 52R/4 | c,h | R | 1 | 0 |
| 348 | 52R/5 | c,h | R | 1 | 1 |
| 349 | 52R/7 | c,h | R | 1 | 0 |
| 350 | 52R/8 | c,h | R | 0 | 0 |
| 351 | 52R/9 | c,h | R | 0 | 0 |
| 352 | 58E/1 | c,h | E | 1 | 1 |
| 353 | 58E/10 | c,h | E | 1 | 1 |
| 354 | 58E/11 | c,h | E | 1 | 1 |
| 355 | 58E/12 | c,h | E | 1 | 1 |
| 356 | 58E/13 | c,h | E | 1 | 1 |
| 357 | 58E/15 | c,h | E | 1 | 1 |
| 358 | 58E/16 | c,h | E | 1 | 1 |

| | | | | | |
|---|---|---|---|---|---|
| 359 | 58E/19 | c,h | E | 0 | 0 |
| 360 | 58E/2 | c,h | E | 1 | 1 |
| 361 | 58E/20 | c,h | E | 1 | 1 |
| 362 | 58E/3 | c,h | E | 1 | 1 |
| 363 | 58E/4 | c,h | E | 1 | 1 |
| 364 | 58E/5 | c,h | E | 1 | 1 |
| 365 | 58E/6 | c,h | E | 1 | 1 |
| 366 | 58E/9 | c,h | E | 1 | 1 |
| 367 | 58R/1 | c,h | R | 1 | 1 |
| 368 | 58R/10 | c,h | R | 1 | 1 |
| 369 | 58R/11 | c,h | R | 1 | 1 |
| 370 | 58R/12 | c,h | R | 1 | 1 |
| 371 | 58R/2 | c,h | R | 0 | 0 |
| 372 | 58R/3a | c,h | R | 1 | 1 |
| 373 | 58R/3b | c,h | R | 0 | 0 |
| 374 | 58R/4 | c,h | R | 1 | 1 |
| 375 | 58R/5a | c,h | R | 0 | 0 |
| 376 | 58R/5b | c,h | R | 1 | 1 |
| 377 | 58R/7 | c,h | R | 1 | 0 |
| 378 | 58R/8a | c,h | R | 1 | 0 |
| 379 | 58R/8b | c,h | R | 0 | 0 |
| 380 | 58R/9a | c,h | R | 1 | 1 |
| 381 | 58R/9b | c,h | R | 1 | 1 |
| 382 | 8E/1 | c,h | E | 0 | 0 |
| 383 | 8E/10 | c,h | E | 1 | 0 |
| 384 | 8E/11 | c,h | E | 1 | 1 |
| 385 | 8E/13 | c,h | E | 1 | 0 |
| 386 | 8E/14 | c,h | E | 1 | 0 |
| 387 | 8E/16 | c,h | E | 0 | 0 |

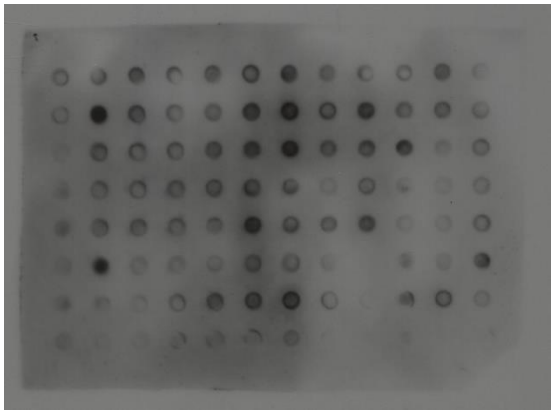
| | | | | | |
|---|---|---|---|---|---|
| 388 | 8E/18 | c,h | E | 1 | 0 |
| 389 | 8E/19 | c,h | E | 1 | 0 |
| 390 | 8E/2 | c,h | E | 1 | 0 |
| 391 | 8E/20 | c,h | E | 1 | 0 |
| 392 | 8E/3 | c,h | E | 1 | 0 |
| 393 | 8E/4 | c,h | E | 1 | 0 |
| 394 | 8E/5 | c,h | E | 1 | 0 |
| 395 | 8E/6 | c,h | E | 1 | 0 |
| 396 | 8E/7 | c,h | E | 1 | 0 |
| 397 | 8R/11 | c,h | R | 1 | 0 |
| 398 | 8R/12 | c,h | R | 1 | 1 |
| 399 | 8R/13 | c,h | R | 1 | 1 |
| 400 | 8R/14 | c,h | R | 1 | 1 |
| 401 | 8R/15 | c,h | R | 1 | 1 |
| 402 | 8R/17 | c,h | R | 1 | 1 |
| 403 | 8R/18 | c,h | R | 1 | 1 |
| 404 | 8R/2 | c,h | R | 1 | 1 |
| 405 | 8R/20 | c,h | R | 1 | 1 |
| 406 | 8R/5 | c,h | R | 1 | 1 |
| 407 | 8R/6 | c,h | R | 1 | 1 |
| 408 | 8R/7a | c,h | R | 1 | 1 |
| 409 | 8R/7b | c,h | R | 1 | 1 |
| 410 | 8R/8 | c,h | R | 1 | 1 |
| 411 | 8R/9 | c,h | R | 1 | 0 |

### 9.1.6 Appendix VI: Dot blots

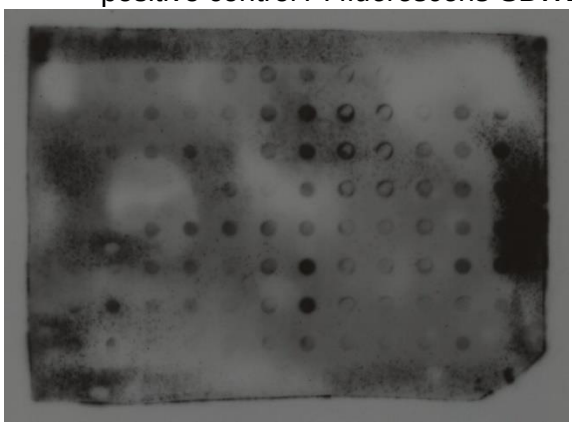
### A. Film developed and G:BOX developed blots.



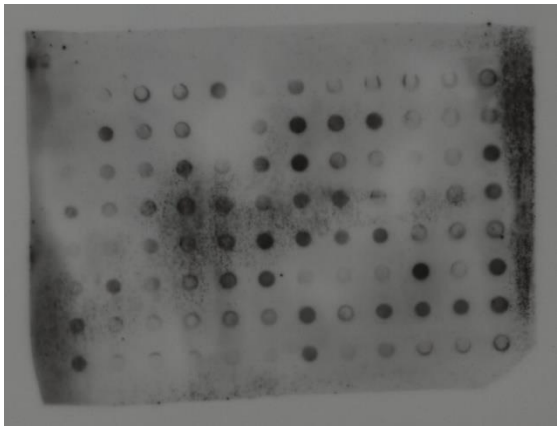
1. Film developed Plate 1 probe 3 (MZ3), Left to right 1-12, Top to bottom A-H, 2F positive control *P. fluorescens* SBW25, 4H negative control non inoculated.



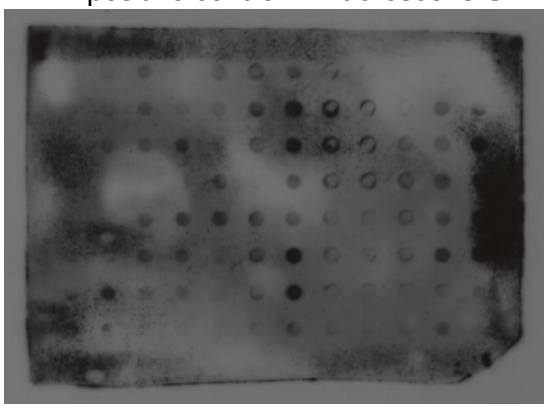
2. Film developed Plate 1 probe 11 (MZ11), Left to right 1-12, Top to bottom A-H, 2F positive control *P. fluorescens* SBW25, 4H negative control non inoculated.



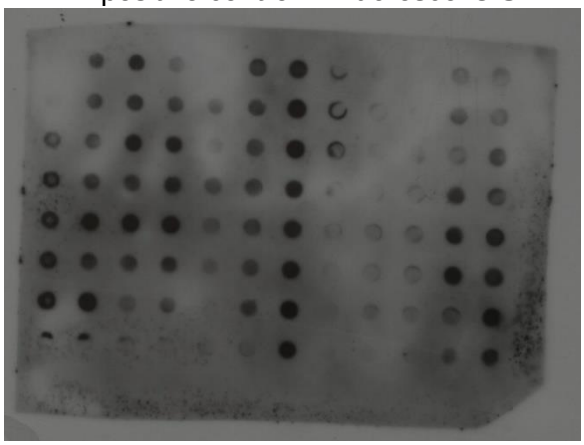
3. Film developed Plate 1 probe 15 (MZ15), Left to right 1-12, Top to bottom A-H, 2F positive control *P. fluorescens* SBW25, 4H negative control non inoculated.



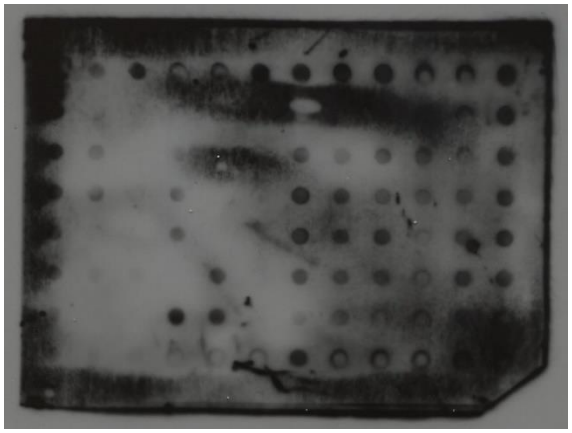
4. Film developed Plate 1 probe 17 (MZ17), Left to right 1-12, Top to bottom A-H, 2F positive control *P. fluorescens* SBW25, 4H negative control non inoculated.



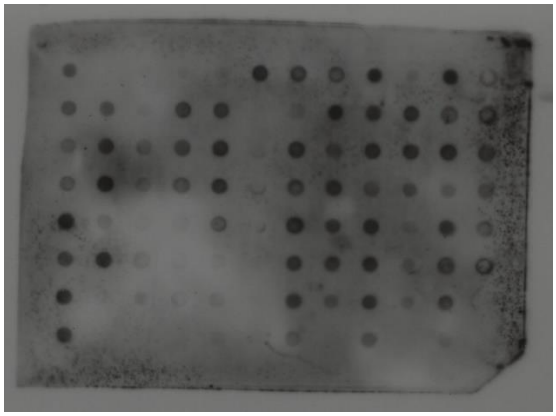
5. Film developed Plate 2 probe 15 (MZ15), Left to right 1-12, Top to bottom A-H, 2G positive control *P. fluorescens* SBW25, 2H negative control non inoculated.



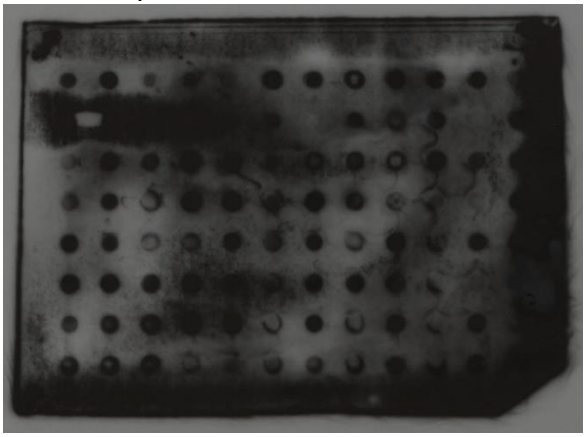
6. Film developed Plate 2 probe 17 (MZ17), Left to right 1-12, Top to bottom A-H, 2G positive control *P. fluorescens* SBW25, 2H negative control non inoculated.



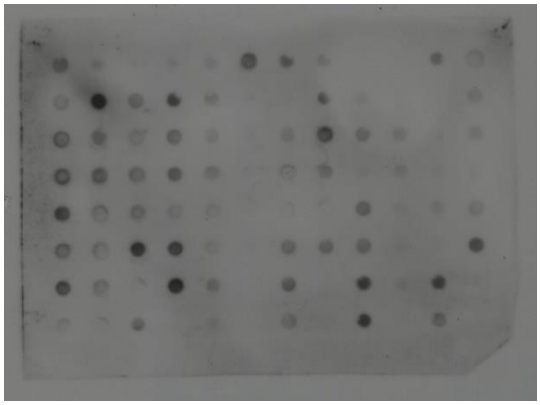
7. Film developed Plate 3 probe 3 (MZ3), Left to right 1-12, Top to bottom A-H, 6A positive control *P. fluorescens* SBW25, 12H negative control non inoculated.



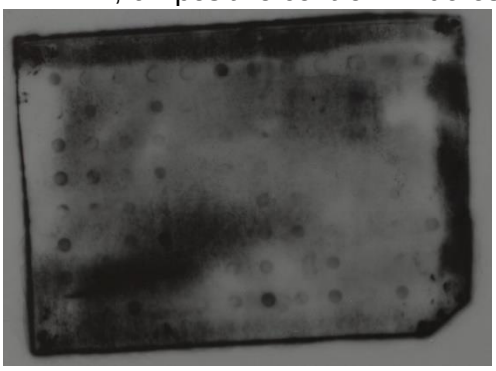
8. Film developed Plate 3 probe 3 (MZ3) Repeated, Left to right 1-12, Top to bottom A-H, 6A positive control *P. fluorescens* SBW25, 12H negative control non inoculated.



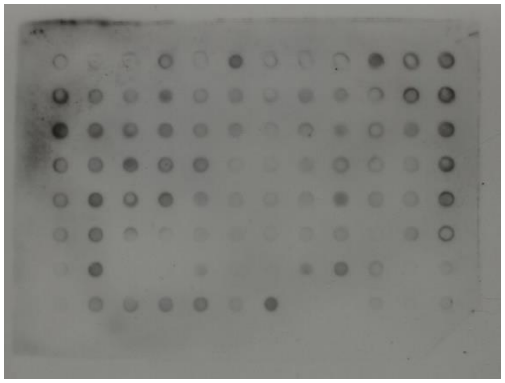
9. Film developed Plate 3 probe 11 (MZ11), Left to right 1-12, Top to bottom A-H, 6A positive control *P. fluorescens* SBW25, 12H negative control non inoculated.



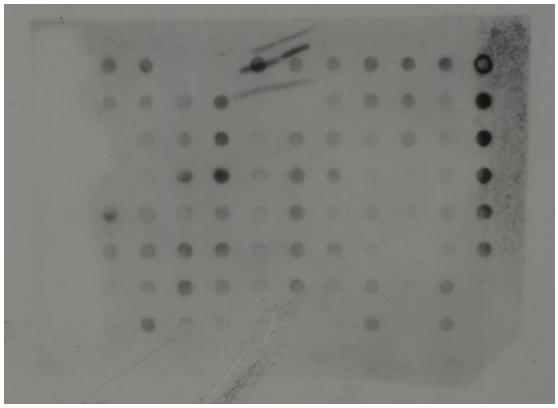
10. Film developed Plate 3 probe 11 (MZ11) Repeated, Left to right 1-12, Top to bottom A-H, 6A positive control *P. fluorescens* SBW25, 12H negative control non inoculated.



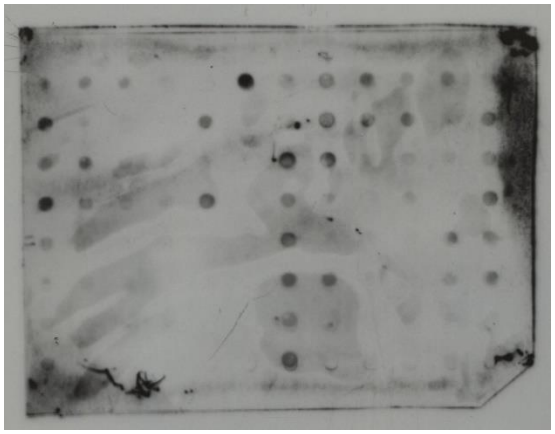
11. Film developed Plate 3 probe 15 (MZ15), Left to right 1-12, Top to bottom A-H, 6A positive control *P. fluorescens* SBW25, 12H negative control non inoculated.



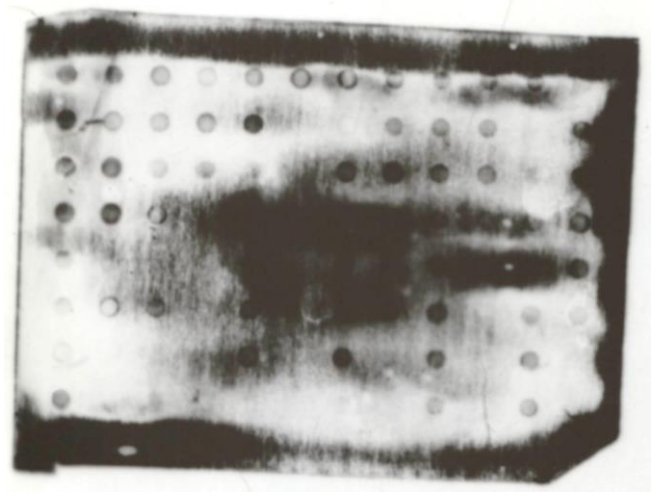
12. Film developed Plate 3 probe 15 (MZ15) Repeated, Left to right 1-12, Top to bottom A-H, 6A positive control *P. fluorescens* SBW25, 12H negative control non inoculated.



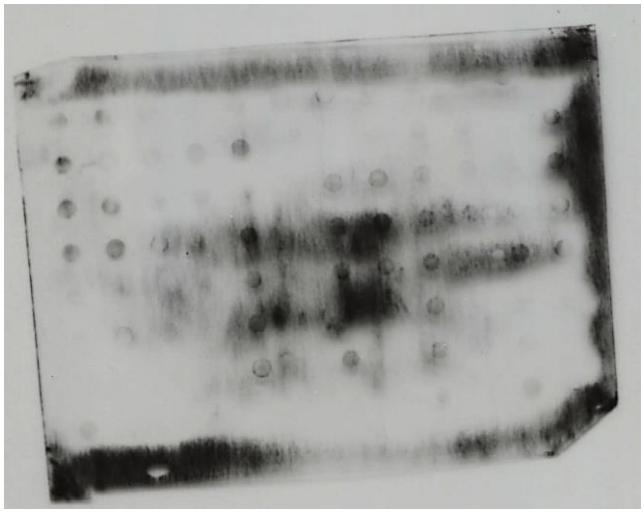
13. Film developed Plate 3 probe 17 (MZ17), Left to right 1-12, Top to bottom A-H, 6A positive control *P. fluorescens* SBW25, 12H negative control non inoculated.



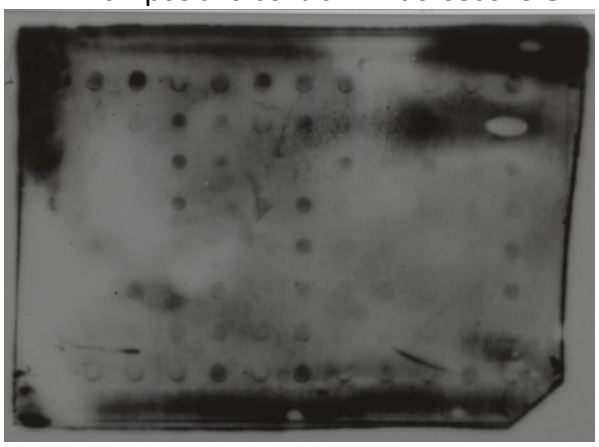
14. Film developed Plate 3 probe 1 (MZ1), Left to right 1-12, Top to bottom A-H, 6A positive control *P. fluorescens* SBW25, 12H negative control non inoculated.



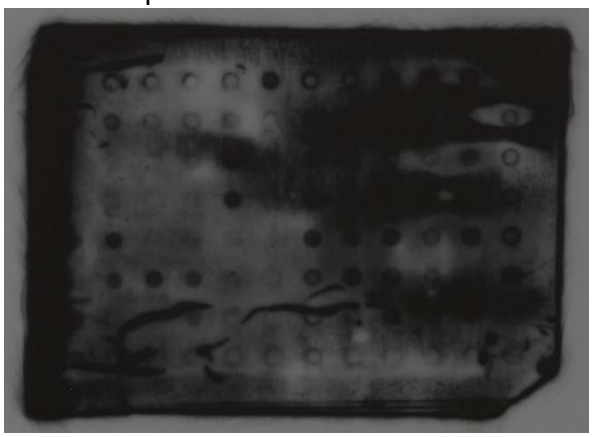
15. Film developed Plate 3 probe 5 (MZ5), Left to right 1-12, Top to bottom A-H, 6A positive control *P. fluorescens* SBW25, 12H negative control non inoculated.



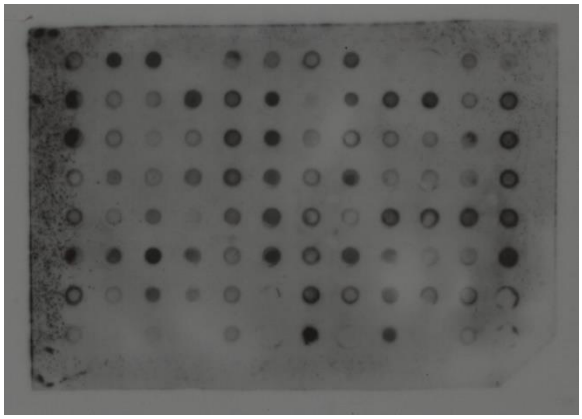
16. Film developed Plate 3 probe 5 (MZ5) Repeated, Left to right 1-12, Top to bottom A-H, 6A positive control *P. fluorescens* SBW25, 12H negative control non inoculated.



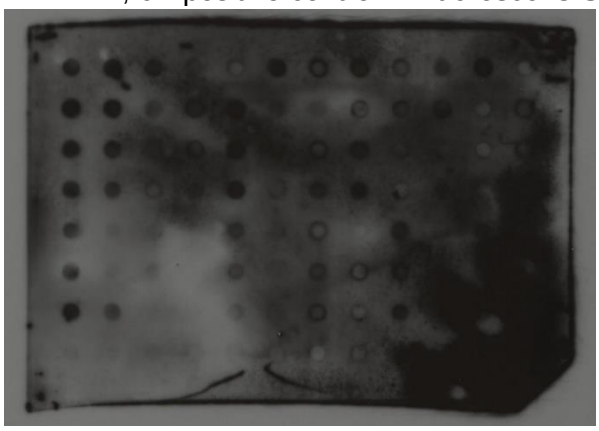
17. Film developed Plate 3 probe 5 (MZ5) Repeated, Left to right 1-12, Top to bottom A-H, 6A positive control *P. fluorescens* SBW25, 12H negative control non inoculated.



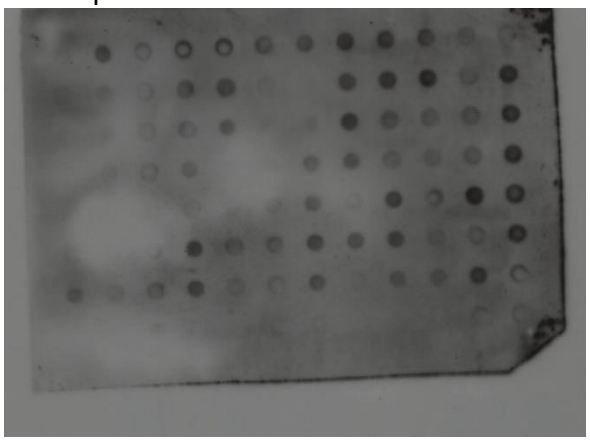
18. Film developed Plate 3 probe 7 (MZ7), Left to right 1-12, Top to bottom A-H, 6A positive control P. fluorescens SBW25, 12H negative control non inoculated.



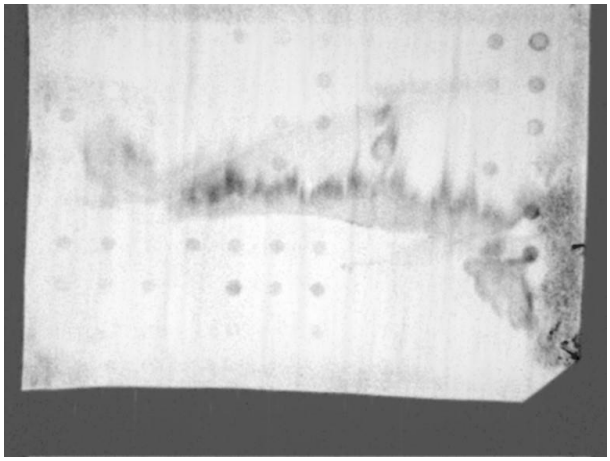
19. Film developed Plate 3 probe 7 (MZ7) Repeated, Left to right 1-12, Top to bottom A-H, 6A positive control *P. fluorescens* SBW25, 12H negative control non inoculated.



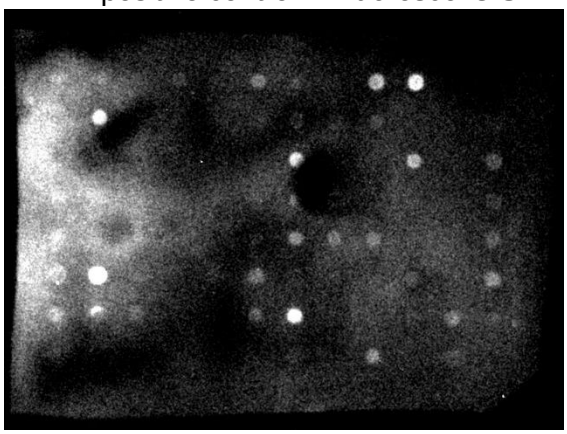
20. Film developed Plate 3 probe 9 (MZ9), Left to right 1-12, Top to bottom A-H, 6A positive control *P. fluorescens* SBW25, 12H negative control non inoculated.



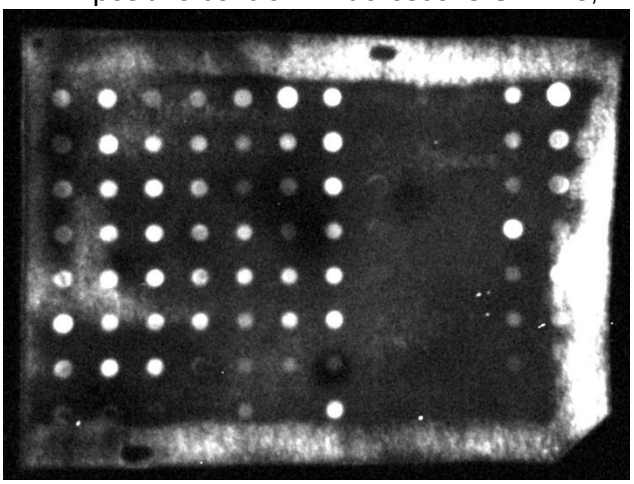
21. Film developed Plate 3 probe 9 (MZ9), Left to right 1-12, Top to bottom A-H, 6A positive control *P. fluorescens* SBW25, 12H negative control non inoculated.



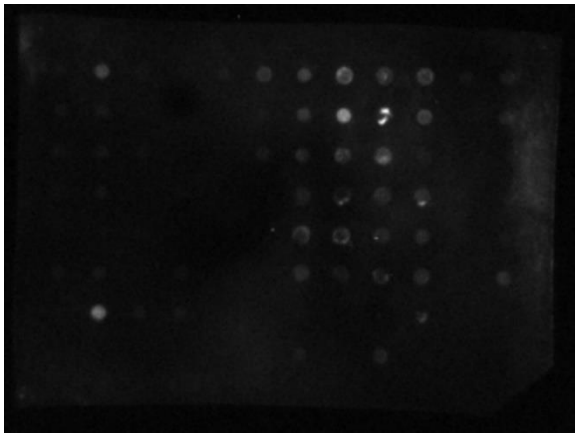
22. G:Box developed Plate 2 probe 11 (MZ11), Left to right 1-12, Top to bottom A-H, 2G positive control *P. fluorescens* SBW25, 2H negative control non inoculated.



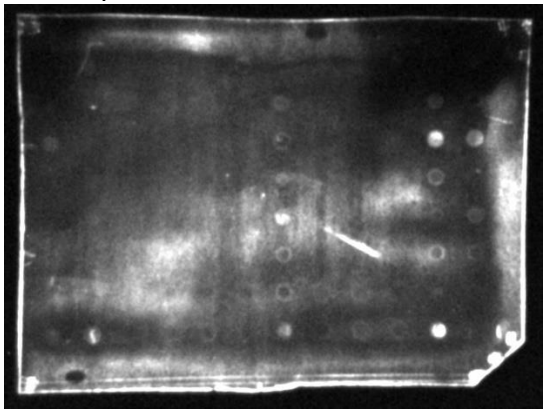
23. G:Box developed Plate 1 probe 9 (MZ9), Left to right 1-12, Top to bottom A-H, 2F positive control *P. fluorescens* SBW25, 4H negative control non inoculated.



24. G:Box developed Plate 1 probe 11 (MZ11), Left to right 1-12, Top to bottom A-H, 2F positive control *P. fluorescens* SBW25, 4H negative control non inoculated.



25. G:Box developed Plate 2 probe 3 (MZ3), Left to right 1-12, Top to bottom A-H, 2G positive control *P. fluorescens SBW25*, 2H negative control non inoculated.



26. G:Box developed Plate 2 probe 3 (MZ3) Repeated, Left to right 1-12, Top to bottom A-H, 2G positive control P. fluorescens SBW25, 2H negative control non inoculated.

**B. 96 well format description of the dot blots.** *Plot numbers indication planting combination (1: (H,H), 8: (C,H), 22, 28, 31, 34: (H,Xi-19), 24, 25, 30, 35: (C,Xi-19); R: rhizosphere, E: endosphere; Green boxes: empty wells.

**Plate No.1**

| Isolates from* | 1 R | 1 R | 1 E | 1 E | 8 R | 8 R | 22 R | 22 R | 22 E | 22 E | 25 R | 25 R |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
| A | 5 | 9 | 5 | 13 | 7a | 7b | 19 | 9 | 5 | 11 | 14 | 4 |
| B | 7 | 11 | 10 | 6 | 11 | 9 | 14 | 11 | 6 | 12 | 13 | 10 |
| C | 4 | 1 | 11 | 7 | 13 | 12 | 16 | 10 | 7 | 18 | 5 | 11 |
| D | 19 | 17 | 1 | 8 | 14 | 17 | 13 | 12 | 8 | 19 | 7 | 17 |
| E | 10 | 18 | 15 | 4 | 15 | 18 | 15 | 17 | 1 | 20 | 20 | 9 |
| F | 12 | | 14 | | 2 | 20 | 18a | 18b | 3 | 13a | 16 | 12 |
| G | 16 | | 17 | | 5 | 8 | 3 | 20 | 9 | 13b | 6 | 18 |
| H | 13 | | 19 | | 6 | | 4 | | 10 | | 8 | |

**Plate No. 2**

| Isolates from | 25 E | 25 E | 24 E | 24 E | 24 R | 24 R | 28 E | 28 E | 28 R | 28 R | 30 E | 30 E |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
| A | 14 | 4 | 1 | 9 | 13 | 20 | 18 | 10 | 8 | 16 | 17 | 11 |
| B | 15 | 17 | 2 | 11 | 6 | 15 | 19 | 11 | 12 | 1 | 18 | 12 |
| C | 10 | 18 | 3 | 15 | 2 | 11 | 20 | 12 | 9 | 6 | 20 | 5 |
| D | 5 | 19 | 4 | 16 | 3 | 8 | 2 | 5 | 17 | 5 | 13 | 7 |
| E | 7 | 20 | 5 | 14 | 4a | 12 | 3 | 8 | 18 | 7 | 14 | 8 |
| F | 8 | 56 | 6 | 13 | 17 | 4b | 15 | 1 | 19 | 13 | 15 | 4 |
| G | 1 | | 7 | 18 | 18 | 16 | 14 | | 20 | 3 | 16 | |
| H | 3 | | 8 | | 19 | | 9 | | 14 | | 9 | |

**Plate No. 3**

| Isolates from | 30 R | 30 R | 31 R | 31 R | 31 E | 31 E | 34 R | 34 R | 34 E | 34 E | 35 E | 35 E |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
| A | 15 | 9 | 11 | 6 | 1 | | 11a | 8 | 19 | 15 | 1 | 12 |
| B | 11 | 10 | 12 | 7 | 2 | | 10 | 15 | 20 | 2 | 3 | 13 |
| C | 5 | 6a | 13 | 8 | 3 | | 11b | 16 | 9 | 4 | 4 | 14 |
| D | 17 | 6b | 18 | 1 | 4 | | 9b | 20 | 10 | | 5 | 16 |
| E | 18 | 12 | 14 | 2 | 5 | | 9a | 2 | 11 | | 7 | 17 |
| F | 19a | 4b | 15 | 4 | 6 | | 12 | 3 | 7 | | 8 | 19 |
| G | 1 | 19b | 16 | 17 | 7 | | 5 | 4 | 13 | | 9 | |
| H | 4a | | 5 | | 8 | | 6 | | 14 | | 11 | |

## 9.2 Chapter 4 Appendix

### 9.2.1 Appendix I: Inhibition zone class data for the 411 isolates.

| no. | Isolate | Year 1 | Year 2 | Block | Main plot no. | niche | Average | Inhibition zone class |
|---|---|---|---|---|---|---|---|---|
| 1 | 1E/1 | H | H | 1 | 1 | E | 0.693667 | 2 |
| 2 | 1E/10 | H | H | 1 | 1 | E | 0.987667 | 2 |
| 3 | 1E/11 | H | H | 1 | 1 | E | 1.022 | 2 |
| 4 | 1E/13 | H | H | 1 | 1 | E | 0.740667 | 2 |
| 5 | 1E/14 | H | H | 1 | 1 | E | 1.019667 | 2 |
| 6 | 1E/15 | H | H | 1 | 1 | E | 0.823333 | 2 |
| 7 | 1E/17 | H | H | 1 | 1 | E | 0.760333 | 2 |
| 8 | 1E/19 | H | H | 1 | 1 | E | 0.179667 | 1 |
| 9 | 1E/4 | H | H | 1 | 1 | E | 0.816333 | 2 |
| 10 | 1E/5 | H | H | 1 | 1 | E | 0.760333 | 2 |
| 11 | 1E/6 | H | H | 1 | 1 | E | 0.715667 | 2 |
| 12 | 1E/7 | H | H | 1 | 1 | E | 0.673 | 2 |
| 13 | 1E/8 | H | H | 1 | 1 | E | 0.406333 | 1 |
| 14 | 1R/1 | H | H | 1 | 1 | R | 0.699667 | 2 |
| 15 | 1R/10 | H | H | 1 | 1 | R | 0.659 | 2 |
| 16 | 1R/11 | H | H | 1 | 1 | R | 0.159 | 1 |
| 17 | 1R/12 | H | H | 1 | 1 | R | 0.76 | 2 |
| 18 | 1R/13 | H | H | 1 | 1 | R | 0.779 | 2 |
| 19 | 1R/16 | H | H | 1 | 1 | R | 0.572333 | 2 |
| 20 | 1R/17 | H | H | 1 | 1 | R | 0.928333 | 2 |
| 21 | 1R/18 | H | H | 1 | 1 | R | 0.485667 | 1 |
| 22 | 1R/19 | H | H | 1 | 1 | R | 0.923333 | 2 |
| 23 | 1R/4 | H | H | 1 | 1 | R | 0.594333 | 2 |
| 24 | 1R/5 | H | H | 1 | 1 | R | 0.361 | 1 |

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| 25 | 1R/7 | H | H | 1 | 1 | R | 0.643333 | 2 |
| 26 | 1R/9 | H | H | 1 | 1 | R | 0.823333 | 2 |
| 27 | 22E/1 | H | XI | 2 | 3 | E | 0.185 | 1 |
| 28 | 22E/10 | H | XI | 2 | 3 | E | 0.212 | 1 |
| 29 | 22E/11 | H | XI | 2 | 3 | E | 0.453 | 1 |
| 30 | 22E/12 | H | XI | 2 | 3 | E | 1.185667 | 3 |
| 31 | 22E/13a | H | XI | 2 | 3 | E | 0.563 | 2 |
| 32 | 22E/13b | H | XI | 2 | 3 | E | 0.383 | 1 |
| 33 | 22E/18 | H | XI | 2 | 3 | E | 0.163 | 1 |
| 34 | 22E/19 | H | XI | 2 | 3 | E | 0.616333 | 2 |
| 35 | 22E/20 | H | XI | 2 | 3 | E | 0.527667 | 2 |
| 36 | 22E/3 | H | XI | 2 | 3 | E | 0.688 | 2 |
| 37 | 22E/5 | H | XI | 2 | 3 | E | 0.644667 | 2 |
| 38 | 22E/6 | H | XI | 2 | 3 | E | 0.805333 | 2 |
| 39 | 22E/7 | H | XI | 2 | 3 | E | 0.653333 | 2 |
| 40 | 22E/8 | H | XI | 2 | 3 | E | 0.814333 | 2 |
| 41 | 22E/9 | H | XI | 2 | 3 | E | 0.815667 | 2 |
| 42 | 22R/10 | H | XI | 2 | 3 | R | 0.494333 | 1 |
| 43 | 22R/11 | H | XI | 2 | 3 | R | 0.805667 | 2 |
| 44 | 22R/12 | H | XI | 2 | 3 | R | 0.404667 | 1 |
| 45 | 22R/13 | H | XI | 2 | 3 | R | 0.305 | 1 |
| 46 | 22R/14 | H | XI | 2 | 3 | R | 0.228 | 1 |
| 47 | 22R/15 | H | XI | 2 | 3 | R | 0.106333 | 1 |
| 48 | 22R/16 | H | XI | 2 | 3 | R | 0.289 | 1 |
| 49 | 22R/17 | H | XI | 2 | 3 | R | 0.6 | 2 |
| 50 | 22R/18a | H | XI | 2 | 3 | R | 0.865333 | 2 |
| 51 | 22R/18b | H | XI | 2 | 3 | R | 0.850333 | 2 |
| 52 | 22R/19 | H | XI | 2 | 3 | R | 0.604 | 2 |
| 53 | 22R/20 | H | XI | 2 | 3 | R | 0.803333 | 2 |

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| 54 | 22R/3 | H | XI | 2 | 3 | R | 0.767667 | 2 |
| 55 | 22R/4 | H | XI | 2 | 3 | R | 0.879 | 2 |
| 56 | 22R/9 | H | XI | 2 | 3 | R | 0.764 | 2 |
| 57 | 24E/1 | C | XI | 2 | 4 | E | 0.760333 | 2 |
| 58 | 24E/11 | C | XI | 2 | 4 | E | 0.653 | 2 |
| 59 | 24E/13 | C | XI | 2 | 4 | E | 0.159333 | 1 |
| 60 | 24E/14 | C | XI | 2 | 4 | E | 0.650333 | 2 |
| 61 | 24E/15 | C | XI | 2 | 4 | E | 0.745333 | 2 |
| 62 | 24E/16 | C | XI | 2 | 4 | E | 0.732333 | 2 |
| 63 | 24E/18 | C | XI | 2 | 4 | E | 0.712667 | 2 |
| 64 | 24E/2 | C | XI | 2 | 4 | E | 1.104667 | 3 |
| 65 | 24E/3 | C | XI | 2 | 4 | E | 0.530333 | 2 |
| 66 | 24E/4 | C | XI | 2 | 4 | E | 1.197667 | 3 |
| 67 | 24E/5 | C | XI | 2 | 4 | E | 0.580667 | 2 |
| 68 | 24E/6 | C | XI | 2 | 4 | E | 0.901667 | 2 |
| 69 | 24E/7 | C | XI | 2 | 4 | E | 1.347 | 3 |
| 70 | 24E/8 | C | XI | 2 | 4 | E | 0.395667 | 1 |
| 71 | 24E/9 | C | XI | 2 | 4 | E | 0.867333 | 2 |
| 72 | 24R/11 | C | XI | 2 | 4 | R | 0.504333 | 2 |
| 73 | 24R/12 | C | XI | 2 | 4 | R | 0.622333 | 2 |
| 74 | 24R/13 | C | XI | 2 | 4 | R | 0.132333 | 1 |
| 75 | 24R/15 | C | XI | 2 | 4 | R | 0.633667 | 2 |
| 76 | 24R/16 | C | XI | 2 | 4 | R | 0.339333 | 1 |
| 77 | 24R/17 | C | XI | 2 | 4 | R | 0.503 | 1 |
| 78 | 24R/18 | C | XI | 2 | 4 | R | 0.52 | 1 |
| 79 | 24R/19 | C | XI | 2 | 4 | R | 0.892667 | 2 |
| 80 | 24R/2 | C | XI | 2 | 4 | R | 0.599333 | 2 |
| 81 | 24R/20 | C | XI | 2 | 4 | R | 0.163333 | 1 |
| 82 | 24R/3 | C | XI | 2 | 4 | R | 0.489333 | 2 |

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| 83 | 24R/4a | C | XI | 2 | 4 | R | 0.617 | 2 |
| 84 | 24R/4b | C | XI | 2 | 4 | R | 0.680333 | 2 |
| 85 | 24R/6 | C | XI | 2 | 4 | R | 0.664 | 2 |
| 86 | 24R/8 | C | XI | 2 | 4 | R | 0.664667 | 2 |
| 87 | 25E/1 | C | XI | 3 | 3 | E | 1.286 | 3 |
| 88 | 25E/10 | C | XI | 3 | 3 | E | 0.186667 | 1 |
| 89 | 25E/14 | C | XI | 3 | 3 | E | 0.636 | 2 |
| 90 | 25E/15 | C | XI | 3 | 3 | E | 1.473667 | 3 |
| 91 | 25E/17 | C | XI | 3 | 3 | E | 1.246 | 3 |
| 92 | 25E/18 | C | XI | 3 | 3 | E | 0.702 | 2 |
| 93 | 25E/19 | C | XI | 3 | 3 | E | 0.384333 | 1 |
| 94 | 25E/20 | C | XI | 3 | 3 | E | 0.959 | 2 |
| 95 | 25E/3 | C | XI | 3 | 3 | E | 0.688667 | 2 |
| 96 | 25E/4 | C | XI | 3 | 3 | E | 0.385 | 1 |
| 97 | 25E/5 | C | XI | 3 | 3 | E | 0.429333 | 1 |
| 98 | 25E/56 | C | XI | 3 | 3 | E | 0.818333 | 2 |
| 99 | 25E/7 | C | XI | 3 | 3 | E | 1.001667 | 2 |
| 100 | 25E/8 | C | XI | 3 | 3 | E | 0.602667 | 2 |
| 101 | 25R/10 | C | XI | 3 | 3 | R | 0.812667 | 2 |
| 102 | 25R/11 | C | XI | 3 | 3 | R | 0.743333 | 2 |
| 103 | 25R/12 | C | XI | 3 | 3 | R | 0.941667 | 2 |
| 104 | 25R/13 | C | XI | 3 | 3 | R | 0.931 | 2 |
| 105 | 25R/14 | C | XI | 3 | 3 | R | 0.488 | 1 |
| 106 | 25R/16 | C | XI | 3 | 3 | R | 1.01 | 2 |
| 107 | 25R/17 | C | XI | 3 | 3 | R | 0.712333 | 2 |
| 108 | 25R/18 | C | XI | 3 | 3 | R | 0.994 | 2 |
| 109 | 25R/20 | C | XI | 3 | 3 | R | 1.126667 | 3 |
| 110 | 25R/4 | C | XI | 3 | 3 | R | 0.918 | 2 |
| 111 | 25R/5 | C | XI | 3 | 3 | R | 0.917 | 2 |

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| 112 | 25R/6 | C | XI | 3 | 3 | R | 0.810333 | 2 |
| 113 | 25R/7 | C | XI | 3 | 3 | R | 1.155667 | 3 |
| 114 | 25R/8 | C | XI | 3 | 3 | R | 0.771333 | 2 |
| 115 | 25R/9 | C | XI | 3 | 3 | R | 0.635333 | 2 |
| 116 | 28E/1 | H | XI | 3 | 4 | E | 0.356333 | 1 |
| 117 | 28E/10 | H | XI | 3 | 4 | E | 1.094667 | 3 |
| 118 | 28E/11 | H | XI | 3 | 4 | E | 0.408667 | 1 |
| 119 | 28E/12 | H | XI | 3 | 4 | E | 0.294 | 1 |
| 120 | 28E/14 | H | XI | 3 | 4 | E | 0.588 | 2 |
| 121 | 28E/15 | H | XI | 3 | 4 | E | 0.615 | 2 |
| 122 | 28E/18 | H | XI | 3 | 4 | E | 0.429667 | 1 |
| 123 | 28E/19 | H | XI | 3 | 4 | E | 0.646667 | 2 |
| 124 | 28E/2 | H | XI | 3 | 4 | E | 0.525333 | 1 |
| 125 | 28E/20 | H | XI | 3 | 4 | E | 0.732 | 2 |
| 126 | 28E/3 | H | XI | 3 | 4 | E | 0.172 | 1 |
| 127 | 28E/4 | H | XI | 3 | 4 | E | 0.161333 | 2 |
| 128 | 28E/5 | H | XI | 3 | 4 | E | 0.980333 | 1 |
| 129 | 28E/8 | H | XI | 3 | 4 | E | 0.282333 | 2 |
| 130 | 28E/9 | H | XI | 3 | 4 | E | 0.402333 | 1 |
| 131 | 28R/1 | H | XI | 3 | 4 | R | 0.234333 | 1 |
| 132 | 28R/12 | H | XI | 3 | 4 | R | 0.547333 | 1 |
| 133 | 28R/13 | H | XI | 3 | 4 | R | 0.517333 | 2 |
| 134 | 28R/14 | H | XI | 3 | 4 | R | 0.304333 | 2 |
| 135 | 28R/16 | H | XI | 3 | 4 | R | 0.388667 | 1 |
| 136 | 28R/17 | H | XI | 3 | 4 | R | 0.450333 | 1 |
| 137 | 28R/18 | H | XI | 3 | 4 | R | 0.502667 | 1 |
| 138 | 28R/19 | H | XI | 3 | 4 | R | 0.608333 | 1 |
| 139 | 28R/20 | H | XI | 3 | 4 | R | 0.611 | 2 |
| 140 | 28R/3 | H | XI | 3 | 4 | R | 0.758333 | 2 |

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| 141 | 28R/5 | H | XI | 3 | 4 | R | 0.92 | 2 |
| 142 | 28R/6 | H | XI | 3 | 4 | R | 0.238333 | 2 |
| 143 | 28R/7 | H | XI | 3 | 4 | R | 0.255333 | 1 |
| 144 | 28R/8 | H | XI | 3 | 4 | R | 1.082667 | 1 |
| 145 | 28R/9 | H | XI | 3 | 4 | R | 0.254667 | 3 |
| 146 | 30E/11 | C | XI | 4 | 3 | E | 0.707 | 1 |
| 147 | 30E/12 | C | XI | 4 | 3 | E | 0.737667 | 2 |
| 148 | 30E/13 | C | XI | 4 | 3 | E | 0.413333 | 2 |
| 149 | 30E/14 | C | XI | 4 | 3 | E | 0.934667 | 1 |
| 150 | 30E/15 | C | XI | 4 | 3 | E | 0.693333 | 2 |
| 151 | 30E/16 | C | XI | 4 | 3 | E | 0.141333 | 2 |
| 152 | 30E/17 | C | XI | 4 | 3 | E | 0.691333 | 1 |
| 153 | 30E/18 | C | XI | 4 | 3 | E | 0.662333 | 2 |
| 154 | 30E/20 | C | XI | 4 | 3 | E | 0.929 | 2 |
| 155 | 30E/5 | C | XI | 4 | 3 | E | 0.904333 | 2 |
| 156 | 30E/7 | C | XI | 4 | 3 | E | 0.91 | 2 |
| 157 | 30E/8 | C | XI | 4 | 3 | E | 0.432667 | 1 |
| 158 | 30E/9 | C | XI | 4 | 3 | E | 0.368667 | 1 |
| 159 | 30R/1 | C | XI | 4 | 3 | R | 0.245667 | 1 |
| 160 | 30R/10 | C | XI | 4 | 3 | R | 0.507333 | 2 |
| 161 | 30R/11 | C | XI | 4 | 3 | R | 1.112333 | 3 |
| 162 | 30R/12 | C | XI | 4 | 3 | R | 0.164 | 1 |
| 163 | 30R/15 | C | XI | 4 | 3 | R | 0.669667 | 2 |
| 164 | 30R/17 | C | XI | 4 | 3 | R | 0.804 | 2 |
| 165 | 30R/18 | C | XI | 4 | 3 | R | 0.282667 | 1 |
| 166 | 30R/19a | C | XI | 4 | 3 | R | 0.669333 | 2 |
| 167 | 30R/19b | C | XI | 4 | 3 | R | 0.715333 | 2 |
| 168 | 30R/4a | C | XI | 4 | 3 | R | 0.883 | 2 |
| 169 | 30R/4b | C | XI | 4 | 3 | R | 0.718333 | 2 |

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| 170 | 30R/5 | C | XI | 4 | 3 | R | 0.648 | 2 |
| 171 | 30R/6a | C | XI | 4 | 3 | R | 0.394 | 1 |
| 172 | 30R/6b | C | XI | 4 | 3 | R | 0.762333 | 2 |
| 173 | 30R/9 | C | XI | 4 | 3 | R | 0.226667 | 1 |
| 174 | 31E/1 | H | XI | 4 | 2 | E | 0.444667 | 1 |
| 175 | 31E/2 | H | XI | 4 | 2 | E | 0.881667 | 2 |
| 176 | 31E/3 | H | XI | 4 | 2 | E | 0.857333 | 2 |
| 177 | 31E/4 | H | XI | 4 | 2 | E | 0.149667 | 1 |
| 178 | 31E/5 | H | XI | 4 | 2 | E | 0.783667 | 2 |
| 179 | 31E/6 | H | XI | 4 | 2 | E | 0.456333 | 1 |
| 180 | 31E/7 | H | XI | 4 | 2 | E | 0.854 | 2 |
| 181 | 31E/8 | H | XI | 4 | 2 | E | 0.555667 | 2 |
| 182 | 31R/1 | H | XI | 4 | 2 | R | 0.576333 | 2 |
| 183 | 31R/11 | H | XI | 4 | 2 | R | 0.681333 | 2 |
| 184 | 31R/12 | H | XI | 4 | 2 | R | 0.112333 | 1 |
| 185 | 31R/13 | H | XI | 4 | 2 | R | 0.883667 | 2 |
| 186 | 31R/14 | H | XI | 4 | 2 | R | 0.988333 | 2 |
| 187 | 31R/15 | H | XI | 4 | 2 | R | 0.888333 | 2 |
| 188 | 31R/16 | H | XI | 4 | 2 | R | 0.121333 | 1 |
| 189 | 31R/17 | H | XI | 4 | 2 | R | 0.905667 | 2 |
| 190 | 31R/18 | H | XI | 4 | 2 | R | 0.652 | 2 |
| 191 | 31R/2 | H | XI | 4 | 2 | R | 0.905333 | 2 |
| 192 | 31R/4 | H | XI | 4 | 2 | R | 0.129333 | 1 |
| 193 | 31R/5 | H | XI | 4 | 2 | R | 0.896667 | 2 |
| 194 | 31R/6 | H | XI | 4 | 2 | R | 0.814667 | 2 |
| 195 | 31R/7 | H | XI | 4 | 2 | R | 0.951333 | 2 |
| 196 | 31R/8 | H | XI | 4 | 2 | R | 1.126 | 3 |
| 197 | 32E/1 | H | H | 4 | 4 | E | 0.728333 | 2 |
| 198 | 32E/10 | H | H | 4 | 4 | E | 1.093667 | 3 |

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| 199 | 32E/12 | H | H | 4 | 4 | E | 0.708 | 2 |
| 200 | 32E/13 | H | H | 4 | 4 | E | 0.972333 | 2 |
| 201 | 32E/14 | H | H | 4 | 4 | E | 0.839 | 2 |
| 202 | 32E/16 | H | H | 4 | 4 | E | 0.713 | 2 |
| 203 | 32E/2 | H | H | 4 | 4 | E | 0.786667 | 2 |
| 204 | 32E/20 | H | H | 4 | 4 | E | 0.904 | 2 |
| 205 | 32E/3 | H | H | 4 | 4 | E | 0.842333 | 2 |
| 206 | 32E/4 | H | H | 4 | 4 | E | 0.968333 | 2 |
| 207 | 32E/5 | H | H | 4 | 4 | E | 0.828667 | 2 |
| 208 | 32E/6 | H | H | 4 | 4 | E | 0.898667 | 2 |
| 209 | 32E/7 | H | H | 4 | 4 | E | 0.924667 | 2 |
| 210 | 32E/8 | H | H | 4 | 4 | E | 0.766667 | 2 |
| 211 | 32E/9 | H | H | 4 | 4 | E | 1.066333 | 3 |
| 212 | 34E/10 | H | XI | 1 | 5 | E | 0.275667 | 1 |
| 213 | 34E/11 | H | XI | 1 | 5 | E | 0.095333 | 1 |
| 214 | 34E/13 | H | XI | 1 | 5 | E | 0.266 | 1 |
| 215 | 34E/14 | H | XI | 1 | 5 | E | 0.112 | 1 |
| 216 | 34E/15 | H | Xi | 1 | 5 | E | | 2 |
| 217 | 34E/19 | H | XI | 1 | 5 | E | 0.250333 | 1 |
| 218 | 34E/2 | H | XI | 1 | 5 | E | 0.139667 | 1 |
| 219 | 34E/20 | H | XI | 1 | 5 | E | 0.139667 | 1 |
| 220 | 34E/4 | H | XI | 1 | 5 | E | 0.514333 | 1 |
| 221 | 34E/7 | H | XI | 1 | 5 | E | 0.095667 | 1 |
| 222 | 34E/9 | H | XI | 1 | 5 | E | 0.551 | 2 |
| 223 | 34R/10 | H | XI | 1 | 5 | R | 0.524 | 1 |
| 224 | 34R/11a | H | XI | 1 | 5 | R | 0.143667 | 1 |
| 225 | 34R/11b | H | XI | 1 | 5 | R | 0.504667 | 1 |
| 226 | 34R/12 | H | XI | 1 | 5 | R | 0.005667 | 1 |
| 227 | 34R/15 | H | XI | 1 | 5 | R | 0.128667 | 1 |

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|---|---|---|---|---|---|---|---|---|
| 228 | 34R/16 | H | XI | 1 | 5 | R | 0.510667 | 1 |
| 229 | 34R/2 | H | XI | 1 | 5 | R | 0.632333 | 2 |
| 230 | 34R/20 | H | XI | 1 | 5 | R | 0.437 | 1 |
| 231 | 34R/3 | H | XI | 1 | 5 | R | 0.206333 | 1 |
| 232 | 34R/4 | H | XI | 1 | 5 | R | 0.550333 | 2 |
| 233 | 34R/5 | H | XI | 1 | 5 | R | 0.213 | 1 |
| 234 | 34R/6 | H | XI | 1 | 5 | R | 0.337 | 1 |
| 235 | 34R/8 | H | XI | 1 | 5 | R | 0.139 | 1 |
| 236 | 34R/9a | H | XI | 1 | 5 | R | 0.196667 | 1 |
| 237 | 34R/9b | H | XI | 1 | 5 | R | 0.232 | 1 |
| 238 | 35E/1 | C | XI | 1 | 6 | E | 0.159333 | 1 |
| 239 | 35E/11 | C | XI | 1 | 6 | E | 0.095667 | 1 |
| 240 | 35E/12 | C | XI | 1 | 6 | E | 0.286 | 1 |
| 241 | 35E/13 | C | XI | 1 | 6 | E | 0.186333 | 1 |
| 242 | 35E/14 | C | XI | 1 | 6 | E | 0.125 | 1 |
| 243 | 35E/16 | C | XI | 1 | 6 | E | 0.101667 | 1 |
| 244 | 35E/17 | C | XI | 1 | 6 | E | 0.140667 | 1 |
| 245 | 35E/19 | C | XI | 1 | 6 | E | 0.294 | 1 |
| 246 | 35E/3 | C | XI | 1 | 6 | E | 0.591333 | 2 |
| 247 | 35E/4 | C | XI | 1 | 6 | E | 0.690667 | 2 |
| 248 | 35E/5 | C | XI | 1 | 6 | E | 0.191 | 1 |
| 249 | 35E/7 | C | XI | 1 | 6 | E | 0.135 | 1 |
| 250 | 35E/8 | C | XI | 1 | 6 | E | 0.275333 | 1 |
| 251 | 35E/9 | C | XI | 1 | 6 | E | 0.169333 | 1 |
| 252 | 35R/1 | C | XI | 1 | 6 | R | 0.755667 | 2 |
| 253 | 35R/10 | C | XI | 1 | 6 | R | 0.185333 | 1 |
| 254 | 35R/11 | C | XI | 1 | 6 | R | 0.583667 | 2 |
| 255 | 35R/12 | C | XI | 1 | 6 | R | 0.371 | 1 |
| 256 | 35R/13 | C | XI | 1 | 6 | R | 0.492333 | 1 |

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| 257 | 35R/14 | C | XI | 1 | 6 | R | 0.667667 | 2 |
| 258 | 35R/18 | C | XI | 1 | 6 | R | 0.586333 | 2 |
| 259 | 35R/19 | C | XI | 1 | 6 | R | 0.629667 | 2 |
| 260 | 35R/2 | C | XI | 1 | 6 | R | 0.112333 | 1 |
| 261 | 35R/20 | C | XI | 1 | 6 | R | 0.270667 | 1 |
| 262 | 35R/5 | C | XI | 1 | 6 | R | 0.387333 | 1 |
| 263 | 35R/6 | C | XI | 1 | 6 | R | 0.408 | 1 |
| 264 | 35R/7 | C | XI | 1 | 6 | R | 0.366 | 1 |
| 265 | 35R/9 | C | XI | 1 | 6 | R | 0.213667 | 1 |
| 266 | 37R/1 | H | H | 2 | 5 | R | 0.861333 | 2 |
| 267 | 37R/10 | H | H | 2 | 5 | R | 0.459333 | 1 |
| 268 | 37R/11 | H | H | 2 | 5 | R | 0.567333 | 2 |
| 269 | 37R/12 | H | H | 2 | 5 | R | 0.418667 | 1 |
| 270 | 37R/13 | H | H | 2 | 5 | R | 0.138667 | 1 |
| 271 | 37R/14 | H | H | 2 | 5 | R | 0.511667 | 1 |
| 272 | 37R/15 | H | H | 2 | 5 | R | 0.049667 | 1 |
| 273 | 37R/16 | H | H | 2 | 5 | R | 0.914333 | 2 |
| 274 | 37R/17 | H | H | 2 | 5 | R | 0.421 | 1 |
| 275 | 37R/18 | H | H | 2 | 5 | R | 0.140667 | 1 |
| 276 | 37R/2 | H | H | 2 | 5 | R | 0.870667 | 2 |
| 277 | 37R/3 | H | H | 2 | 5 | R | 0.439 | 1 |
| 278 | 37R/6 | H | H | 2 | 5 | R | 0.541667 | 1 |
| 279 | 37R/7 | H | H | 2 | 5 | R | 0.511 | 1 |
| 280 | 37R/9 | H | H | 2 | 5 | R | 0.225 | 1 |
| 281 | 44E/1 | H | H | 3 | 6 | E | 0.137667 | 1 |
| 282 | 44E/10 | H | H | 3 | 6 | E | 0.474333 | 1 |
| 283 | 44E/13 | H | H | 3 | 6 | E | 0.826667 | 2 |
| 284 | 44E/14 | H | H | 3 | 6 | E | 0.446333 | 1 |
| 285 | 44E/15 | H | H | 3 | 6 | E | 0.094333 | 1 |

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|---|---|---|---|---|---|---|---|---|
| 286 | 44E/16 | H | H | 3 | 6 | E | 0.028333 | 1 |
| 287 | 44E/17 | H | H | 3 | 6 | E | 0.245667 | 1 |
| 288 | 44E/2 | H | H | 3 | 6 | E | 0.352 | 1 |
| 289 | 44E/20 | H | H | 3 | 6 | E | 0.557 | 2 |
| 290 | 44E/3 | H | H | 3 | 6 | E | 0.165667 | 1 |
| 291 | 44E/5 | H | H | 3 | 6 | E | 0.098667 | 1 |
| 292 | 44E/7 | H | H | 3 | 6 | E | 1.071333 | 3 |
| 293 | 44E/9 | H | H | 3 | 6 | E | 0.225333 | 1 |
| 294 | 44R/1 | H | H | 3 | 6 | R | 0.560333 | 2 |
| 295 | 44R/10 | H | H | 3 | 6 | R | 0.621 | 2 |
| 296 | 44R/11 | H | H | 3 | 6 | R | 0.678 | 2 |
| 297 | 44R/12 | H | H | 3 | 6 | R | 0.468333 | 1 |
| 298 | 44R/13 | H | H | 3 | 6 | R | 0.052333 | 1 |
| 299 | 44R/14 | H | H | 3 | 6 | R | 0.176333 | 1 |
| 300 | 44R/15 | H | H | 3 | 6 | R | 0.36 | 1 |
| 301 | 44R/16 | H | H | 3 | 6 | R | 0.301333 | 1 |
| 302 | 44R/18 | H | H | 3 | 6 | R | 0.313333 | 1 |
| 303 | 44R/19 | H | H | 3 | 6 | R | 0.439667 | 1 |
| 304 | 44R/2 | H | H | 3 | 6 | R | 0.244333 | 1 |
| 305 | 44R/3 | H | H | 3 | 6 | R | 0.335667 | 1 |
| 306 | 44R/4 | H | H | 3 | 6 | R | 0.029667 | 1 |
| 307 | 44R/5 | H | H | 3 | 6 | R | 0.322667 | 1 |
| 308 | 44R/6 | H | H | 3 | 6 | R | 0.313 | 1 |
| 309 | 46E/1 | C | H | 4 | 5 | E | 0.795 | 2 |
| 310 | 46E/2 | C | H | 4 | 5 | E | 0.702 | 2 |
| 311 | 46E/3 | C | H | 4 | 5 | E | 0.818667 | 2 |
| 312 | 46E/4 | C | H | 4 | 5 | E | 0.869667 | 2 |
| 313 | 46E/5 | C | H | 4 | 5 | E | 0.820667 | 2 |
| 314 | 46E/6 | C | H | 4 | 5 | E | 1.051667 | 3 |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| 315 | 46E/7 | C | H | 4 | 5 | E | 1.102667 | 3 |
| 316 | 46E/8 | C | H | 4 | 5 | E | 0.729 | 2 |
| 317 | 46R/1 | C | H | 4 | 5 | R | 1.062 | 3 |
| 318 | 46R/2 | C | H | 4 | 5 | R | 1.399 | 3 |
| 319 | 46R/3 | C | H | 4 | 5 | R | 1.373 | 3 |
| 320 | 46R/5 | C | H | 4 | 5 | R | 1.084333 | 3 |
| 321 | 46R/6 | C | H | 4 | 5 | R | 1.001667 | 2 |
| 322 | 46R/7 | C | H | 4 | 5 | R | 1.007333 | 2 |
| 323 | 46R/8 | C | H | 4 | 5 | R | 1.007 | 2 |
| 324 | 52E/1 | C | H | 1 | 8 | E | 1.608333 | 3 |
| 325 | 52E/11 | C | H | 1 | 8 | E | 1.127333 | 3 |
| 326 | 52E/12 | C | H | 1 | 8 | E | 0.666 | 2 |
| 327 | 52E/15 | C | H | 1 | 8 | E | 1.009 | 2 |
| 328 | 52E/16 | C | H | 1 | 8 | E | 0.632 | 2 |
| 329 | 52E/17 | C | H | 1 | 8 | E | 0.685333 | 2 |
| 330 | 52E/19 | C | H | 1 | 8 | E | 0.763333 | 2 |
| 331 | 52E/2 | C | H | 1 | 8 | E | 1.525 | 3 |
| 332 | 52E/20 | C | H | 1 | 8 | E | 0.600333 | 2 |
| 333 | 52E/3 | C | H | 1 | 8 | E | 0.888 | 2 |
| 334 | 52E/5 | C | H | 1 | 8 | E | 0.355 | 1 |
| 335 | 52E/6 | C | H | 1 | 8 | E | 0.559 | 2 |
| 336 | 52E/7 | C | H | 1 | 8 | E | 0.790333 | 2 |
| 337 | 52E/8 | C | H | 1 | 8 | E | 1.293667 | 3 |
| 338 | 52E/9 | C | H | 1 | 8 | E | 0.688 | 2 |
| 339 | 52R/10 | C | H | 1 | 8 | R | 0.210333 | 1 |
| 340 | 52R/12 | C | H | 1 | 8 | R | 0.713333 | 2 |
| 341 | 52R/13 | C | H | 1 | 8 | R | 0.79 | 2 |
| 342 | 52R/14 | C | H | 1 | 8 | R | 1.167333 | 3 |
| 343 | 52R/15 | C | H | 1 | 8 | R | 1.585667 | 3 |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| 344 | 52R/17 | C | H | 1 | 8 | R | 1.634667 | 3 |
| 345 | 52R/2 | C | H | 1 | 8 | R | 1.257667 | 3 |
| 346 | 52R/20 | C | H | 1 | 8 | R | 1.23 | 3 |
| 347 | 52R/4 | C | H | 1 | 8 | R | 0.233667 | 1 |
| 348 | 52R/5 | C | H | 1 | 8 | R | 0.813333 | 2 |
| 349 | 52R/7 | C | H | 1 | 8 | R | 0.234 | 1 |
| 350 | 52R/8 | C | H | 1 | 8 | R | 0.669 | 2 |
| 351 | 52R/9 | C | H | 1 | 8 | R | 0.210333 | 1 |
| 352 | 58E/1 | C | H | 3 | 7 | E | 0.039667 | 1 |
| 353 | 58E/10 | C | H | 3 | 7 | E | 0.434667 | 1 |
| 354 | 58E/11 | C | H | 3 | 7 | E | 0.881 | 2 |
| 355 | 58E/12 | C | H | 3 | 7 | E | 1.064 | 3 |
| 356 | 58E/13 | C | H | 3 | 7 | E | 1.179 | 3 |
| 357 | 58E/15 | C | H | 3 | 7 | E | 1.662667 | 3 |
| 358 | 58E/16 | C | H | 3 | 7 | E | 1.583667 | 3 |
| 359 | 58E/19 | C | H | 3 | 7 | E | 0 | 1 |
| 360 | 58E/2 | C | H | 3 | 7 | E | 0.775333 | 2 |
| 361 | 58E/20 | C | H | 3 | 7 | E | 0.743 | 2 |
| 362 | 58E/3 | C | H | 3 | 7 | E | 0.643 | 2 |
| 363 | 58E/4 | C | H | 3 | 7 | E | 1.054333 | 2 |
| 364 | 58E/5 | C | H | 3 | 7 | E | 1.409333 | 3 |
| 365 | 58E/6 | C | H | 3 | 7 | E | 1.299333 | 3 |
| 366 | 58E/9 | C | H | 3 | 7 | E | 1.48 | 3 |
| 367 | 58R/1 | C | H | 3 | 7 | R | 0.423 | 1 |
| 368 | 58R/10 | C | H | 3 | 7 | R | 1.302667 | 3 |
| 369 | 58R/11 | C | H | 3 | 7 | R | 0.127 | 1 |
| 370 | 58R/12 | C | H | 3 | 7 | R | 1.5 | 3 |
| 371 | 58R/2 | C | H | 3 | 7 | R | 0.813333 | 2 |
| 372 | 58R/3a | C | H | 3 | 7 | R | 0.005667 | 1 |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| 373 | 58R/3b | C | H | 3 | 7 | R | 0.896667 | 2 |
| 374 | 58R/4 | C | H | 3 | 7 | R | 1.027 | 2 |
| 375 | 58R/5a | C | H | 3 | 7 | R | 0.582 | 2 |
| 376 | 58R/5b | C | H | 3 | 7 | R | 1.159667 | 3 |
| 377 | 58R/7 | C | H | 3 | 7 | R | 0.39 | 1 |
| 378 | 58R/8a | C | H | 3 | 7 | R | 1.15 | 3 |
| 379 | 58R/8b | C | H | 3 | 7 | R | 1.304333 | 3 |
| 380 | 58R/9a | C | H | 3 | 7 | R | 1.248667 | 3 |
| 381 | 58R/9b | C | H | 3 | 7 | R | 0.646667 | 2 |
| 382 | 8E/1 | C | H | 2 | 2 | E | 0.179667 | 1 |
| 383 | 8E/10 | C | H | 2 | 2 | E | 0.391 | 1 |
| 384 | 8E/11 | C | H | 2 | 2 | E | 0.427333 | 1 |
| 385 | 8E/13 | C | H | 2 | 2 | E | 0.510333 | 1 |
| 386 | 8E/14 | C | H | 2 | 2 | E | 0.051333 | 1 |
| 387 | 8E/16 | C | H | 2 | 2 | E | 1.581667 | 3 |
| 388 | 8E/18 | C | H | 2 | 2 | E | 0.197 | 1 |
| 389 | 8E/19 | C | H | 2 | 2 | E | 0.061667 | 1 |
| 390 | 8E/2 | C | H | 2 | 2 | E | 1.040333 | 2 |
| 391 | 8E/20 | C | H | 2 | 2 | E | 0.278 | 1 |
| 392 | 8E/3 | C | H | 2 | 2 | E | 0.108333 | 1 |
| 393 | 8E/4 | C | H | 2 | 2 | E | 0.097 | 1 |
| 394 | 8E/5 | C | H | 2 | 2 | E | 0.069667 | 1 |
| 395 | 8E/6 | C | H | 2 | 2 | E | 0.408333 | 1 |
| 396 | 8E/7 | C | H | 2 | 2 | E | 0.591667 | 2 |
| 397 | 8R/11 | C | H | 2 | 2 | R | 0.696333 | 2 |
| 398 | 8R/12 | C | H | 2 | 2 | R | 0.999 | 2 |
| 399 | 8R/13 | C | H | 2 | 2 | R | 0.64 | 2 |
| 400 | 8R/14 | C | H | 2 | 2 | R | 0.757667 | 2 |
| 401 | 8R/15 | C | H | 2 | 2 | R | 0.818333 | 2 |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| 402 | 8R/17 | C | H | 2 | 2 | R | 1.554667 | 3 |
| 403 | 8R/18 | C | H | 2 | 2 | R | 0.457 | 1 |
| 404 | 8R/2 | C | H | 2 | 2 | R | 0.366667 | 1 |
| 405 | 8R/20 | C | H | 2 | 2 | R | 0.661 | 2 |
| 406 | 8R/5 | C | H | 2 | 2 | R | 0.976 | 2 |
| 407 | 8R/6 | C | H | 2 | 2 | R | 0.032333 | 1 |
| 408 | 8R/7a | C | H | 2 | 2 | R | 0.953667 | 2 |
| 409 | 8R/7b | C | H | 2 | 2 | R | 0.707 | 2 |
| 410 | 8R/8 | C | H | 2 | 2 | R | 1.294 | 3 |
| 411 | 8R/9 | C | H | 2 | 2 | R | 0.95 | 2 |

**9.2.2 Appendix II: Averaged inhibition zone data by block. (*** No samples are available for this planting combination).

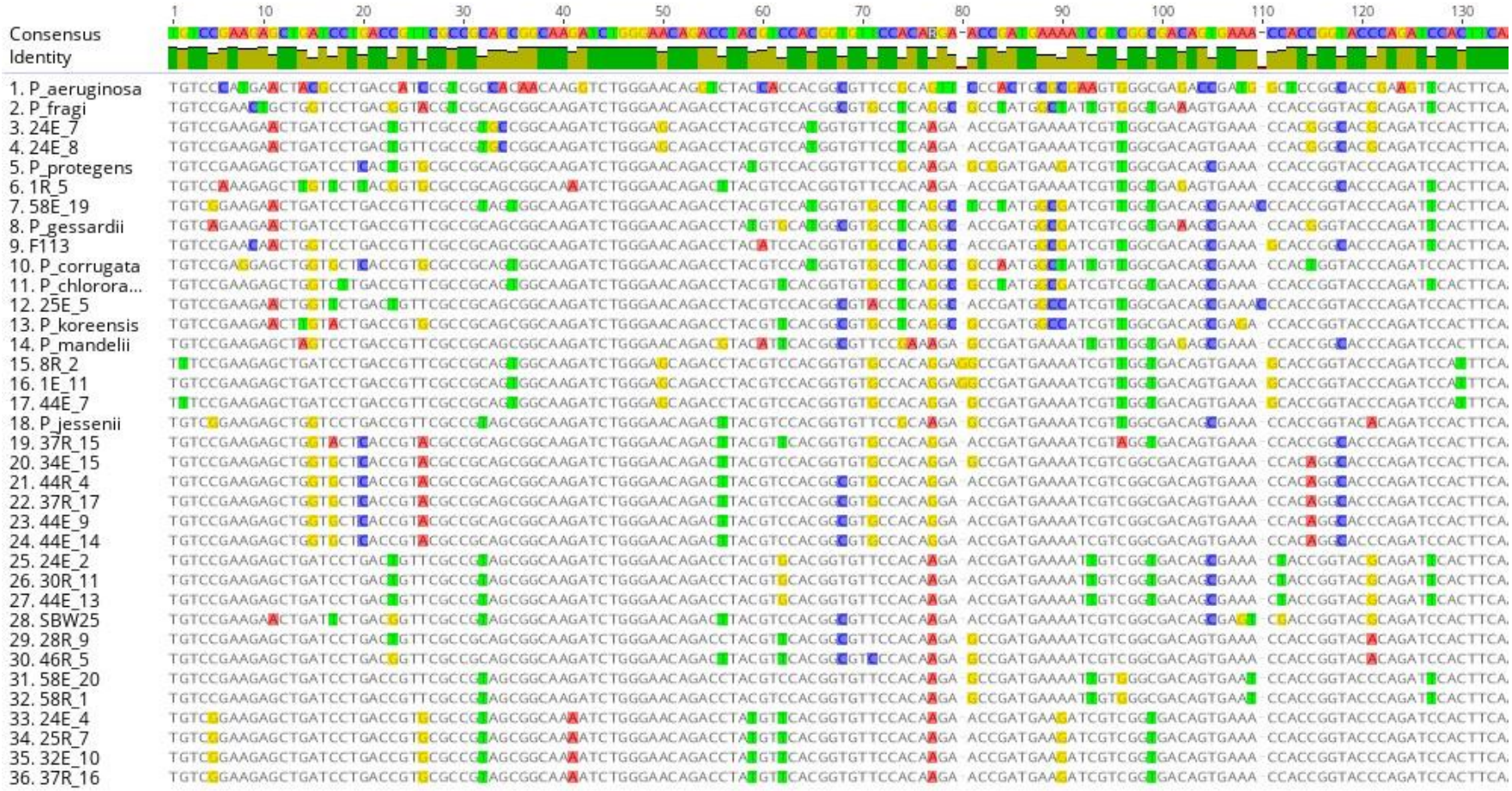
| block no. | Plot no. | Year1 | Year2 | niche | average |
|---|---|---|---|---|---|
| 1 | 1 | H | H | E | 0.738359 |
| 1 | 1 | H | H | R | 0.645256 |
| 1 | 2 | H | Xi | E | 0.243967 |
| 1 | 2 | H | Xi | R | 0.3174 |
| 1 | 3 | C | Xi | E | 0.24581 |
| 1 | 3 | C | Xi | R | 0.43069 |
| 1 | 4 | C | H | E | 0.879378 |
| 1 | 4 | C | H | R | 0.826872 |
| 2 | 1 | C | H | E | 0.399556 |
| 2 | 1 | C | H | R | 0.790911 |
| 2 | 2 | H | Xi | E | 0.580667 |

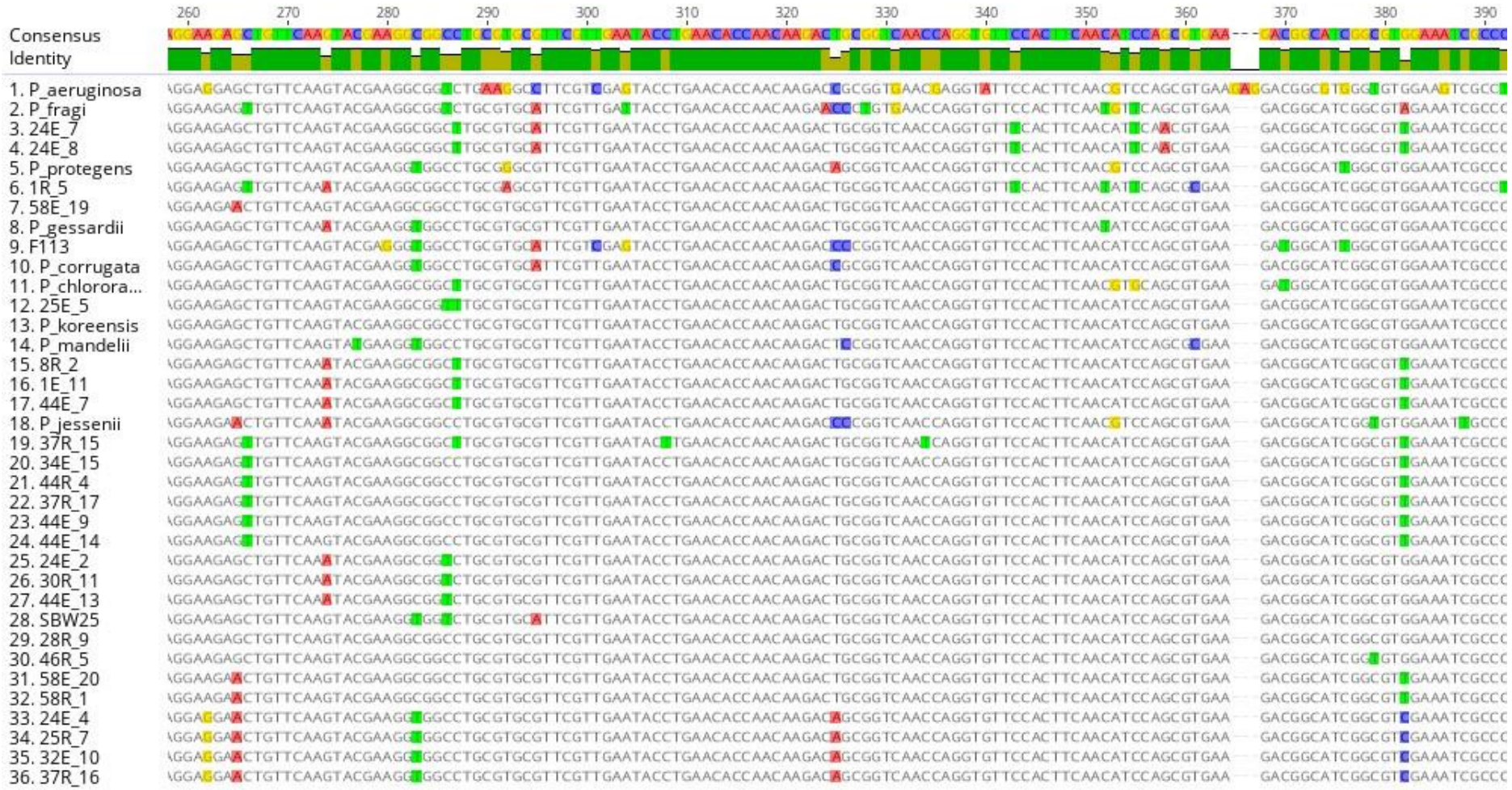
| | | | | | |
|---|---|---|---|---|---|
| 2 | 2 | H | Xi | R | 0.584444 |
| 2 | 3 | C | Xi | E | 0.755889 |
| 2 | 3 | C | Xi | R | 0.535044 |
| 2 | 4 | H | H | E | * |
| 2 | 4 | H | H | R | 0.471333 |
| 3 | 1 | C | Xi | E | 0.771381 |
| 3 | 1 | C | Xi | R | 0.864489 |
| 3 | 2 | H | Xi | E | 0.520452 |
| 3 | 2 | H | Xi | R | 0.521422 |
| 3 | 3 | H | H | E | 0.363333 |
| 3 | 3 | H | H | R | 0.347733 |
| 3 | 4 | C | H | E | 0.949933 |
| 3 | 4 | C | H | R | 0.838444 |
| 4 | 1 | C | Xi | E | 0.627167 |
| 4 | 1 | C | Xi | R | 0.586844 |
| 4 | 2 | H | Xi | E | 0.622875 |
| 4 | 2 | H | Xi | R | 0.708844 |
| 4 | 3 | H | H | E | 0.863978 |
| 4 | 3 | H | H | R | * |
| 4 | 4 | C | H | E | 0.861167 |
| 4 | 4 | C | H | R | 1.133476 |

### 9.2.3: *Pseudomonas fluorescens* complex GenBank IDs.

| no | GenBank ID | Strain |
|---|---|---|
| 1 | KX696671.1 | *Pseudomonas chlororaphis* strain 48G9 DNA gyrase subunit B (gyrB) |
| 2 | KY307842.1 | *Pseudomonas corrugata* strain RS-C DNA gyrase subunit B (gyrB) |
| 3 | DQ882266.1 | *Pseudomonas fragi* strain ATCC 27363 DNA gyrase subunit B (gyrB) |
| 4 | KJ475047.1 | *Pseudomonas gessardii* strain IARI-CL14 DNA gyrase subunit B (gyrB) |
| 5 | KJ475044.1 | *Pseudomonas jessenii* strain IARI-BR2 DNA gyrase subunit B (gyrB) |
| 6 | AM293563..1 | *Pseudomonas koreensis* partial gyrB gene for DNA gyrase subunit B |
| 7 | FN554200.1 | *Pseudomonas mandelii* partial gyrB gene for DNA gyrase subunit B |
| 8 | KU0525859.1 | *Pseudomonas protegens* strain Pf5 DNA gyrase subunit B (gyrB) |

**9.2.4:** Alignment of 25 GH2 isolates with members of *Pseudomonas fluorescens* complex. *P. aeuroginosa* was the outgroup. The alignment was made with MUSCLE in Geneious Prime.

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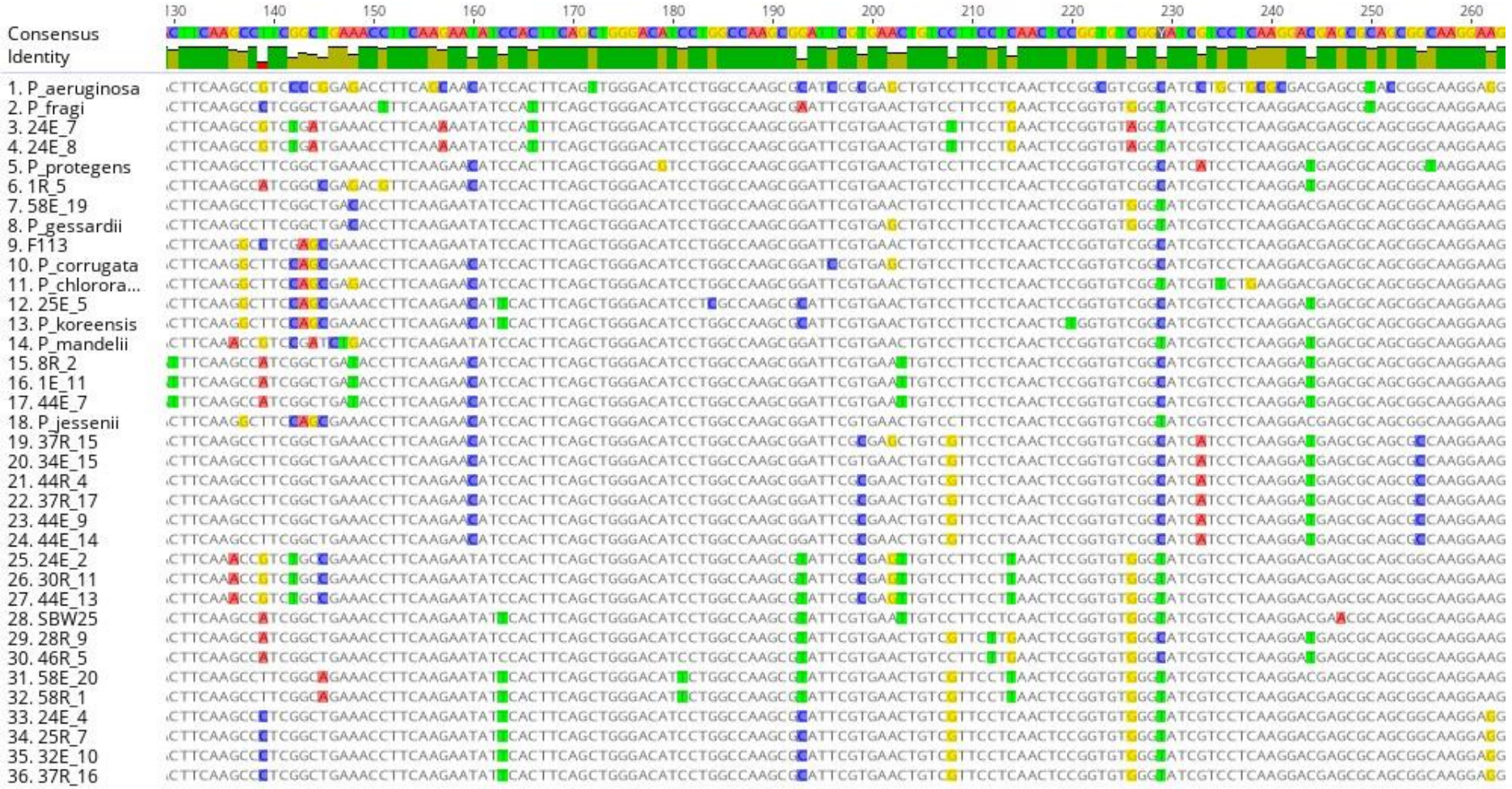
290
300
310
320
330
340
350
360
370
380
390
Consensus
Identity
1. P_aeruginosa
2. P_fragi
3. 24E_7
4. 24E_8
5. P_protegens
6. 1R_5
7. 58E_19
8. P_gessardii
9. F113
10. P_corrugata
11. P_chlorora...
12. 25E_5
13. P_koreensis
14. P_mandelii
15. 8R_2
16. 1E_11
17. 44E_7
18. P_jessenii
19. 37R_15
20. 34E_15
21. 44R_4
22. 37R_17
23. 44E_9
24. 44E_14
25. 24E_2
26. 30R_11
27. 44E_13
28. SBW25
29. 28R_9
30. 46R_5
31. 58E_20
32. 58R_1
33. 24E_4
34. 25R_7
35. 32E_10
36. 37R_16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140
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190
200
210
220
230
240
250
260
Consensus
Identity
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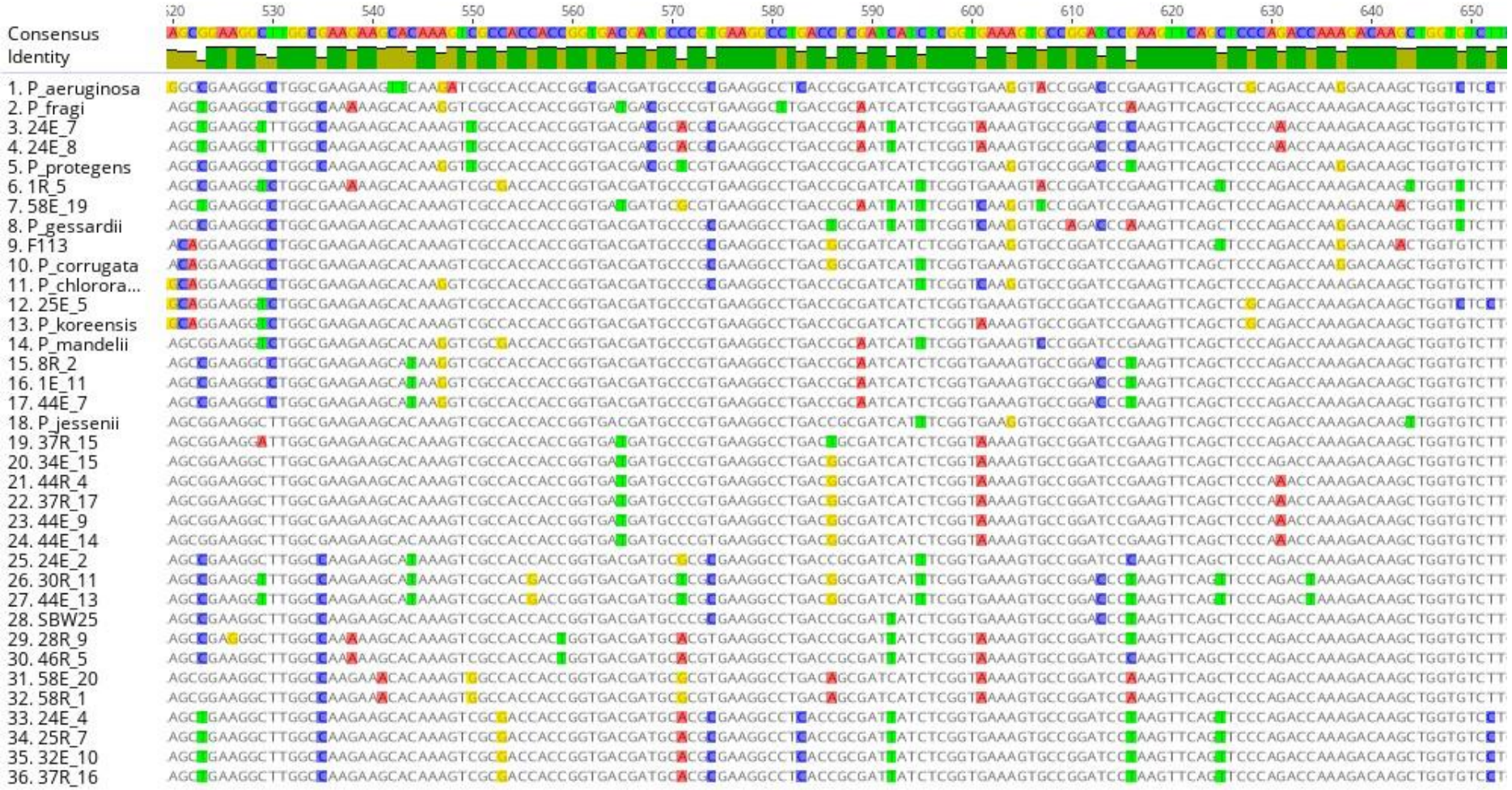
2. P_fragi
3. 24E_7
4. 24E_8
5. P_protegens
6. 1R_5
7. 58E_19
8. P_gessardii
9. F113
10. P_corrugata
11. P_chlorora...
12. 25E_5
13. P_koreensis
14. P_mandelii
15. 8R_2
16. 1E_11
17. 44E_7
18. P_jessenii
19. 37R_15
20. 34E_15
21. 44R_4
22. 37R_17
23. 44E_9
24. 44E_14
25. 24E_2
26. 30R_11
27. 44E_13
28. SBW25
29. 28R_9
30. 46R_5
31. 58E_20
32. 58R_1
33. 24E_4
34. 25R_7
35. 32E_10
36. 37R_16


390
400
410
420
430
440
450
460
470
480
490
500
510
520
Consensus
Identity
1. P_aeruginosa
2. P_fragi
3. 24E_7
4. 24E_8
5. P_protegens
6. 1R_5
7. 58E_19
8. P_gessardii
9. F113
10. P_corrugata
11. P_chlorora...
12. 25E_5
13. P_koreensis
14. P_mandelii
15. 8R_2
16. 1E_11
17. 44E_7
18. P_jessenii
19. 37R_15
20. 34E_15
21. 44R_4
22. 37R_17
23. 44E_9
24. 44E_14
25. 24E_2
26. 30R_11
27. 44E_13
28. SBW25
29. 28R_9
30. 46R_5
31. 58E_20
32. 58R_1
33. 24E_4
34. 25R_7
35. 32E_10
36. 37R_16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580
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640
650
Consensus
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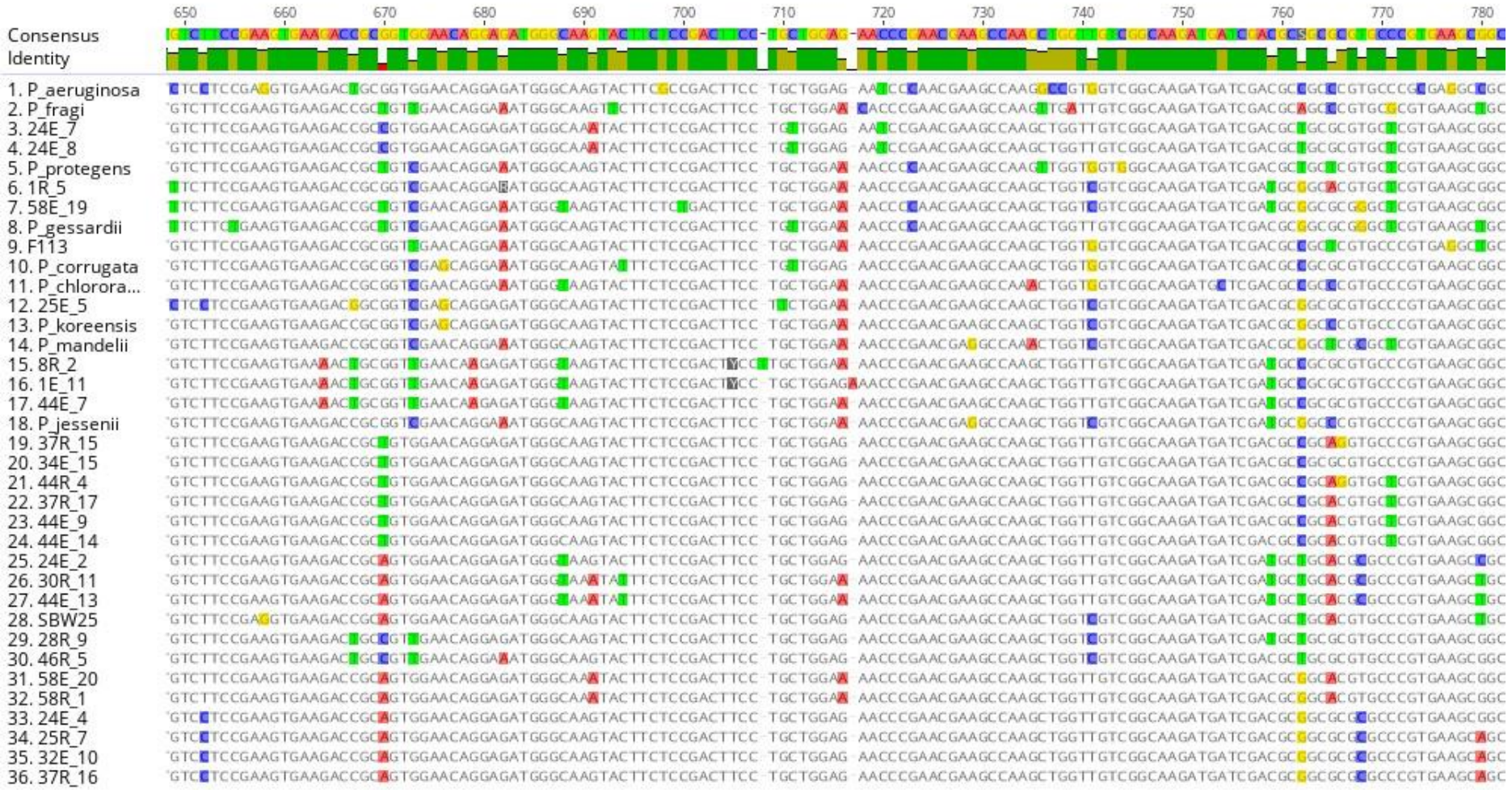
2. P_fragi
3. 24E_7
4. 24E_8
5. P_protegens
6. 1R_5
7. 58E_19
8. P_gessardii
9. F113
10. P_corrugata
11. P_chlorora...
12. 25E_5
13. P_koreensis
14. P_mandelii
15. 8R_2
16. 1E_11
17. 44E_7
18. P_jessenii
19. 37R_15
20. 34E_15
21. 44R_4
22. 37R_17
23. 44E_9
24. 44E_14
25. 24E_2
26. 30R_11
27. 44E_13
28. SBW25
29. 28R_9
30. 46R_5
31. 58E_20
32. 58R_1
33. 24E_4
34. 25R_7
35. 32E_10
36. 37R_16


650
660
670
680
690
700
710
720
730
740
750
760
770
780
Consensus
Identity
1. P_aeruginosa
2. P_fragi
3. 24E_7
4. 24E_8
5. P_protegens
6. 1R_5
7. 58E_19
8. P_gessardii
9. F113
10. P_corrugata
11. P_chlorora...
12. 25E_5
13. P_koreensis
14. P_mandelii
15. 8R_2
16. 1E_11
17. 44E_7
18. P_jessenii
19. 37R_15
20. 34E_15
21. 44R_4
22. 37R_17
23. 44E_9
24. 44E_14
25. 24E_2
26. 30R_11
27. 44E_13
28. SBW25
29. 28R_9
30. 46R_5
31. 58E_20
32. 58R_1
33. 24E_4
34. 25R_7
35. 32E_10
36. 37R_16


660
670
680
690
700
710
720
730
740
750
760
770
780
785
Consensus
Identity
1. P_aeruginosa
2. P_fragi
3. 24E_7
4. 24E_8
5. P_protegens
6. 1R_5
7. 58E_19
8. P_gessardii
9. F113
10. P_corrugata
11. P_chlorora...
12. 25E_5
13. P_koreensis
14. P_mandelii
15. 8R_2
16. 1E_11
17. 44E_7
18. P_jessenii
19. 37R_15
20. 34E_15
21. 44R_4
22. 37R_17
23. 44E_9
24. 44E_14
25. 24E_2
26. 30R_11
27. 44E_13
28. SBW25
29. 28R_9
30. 46R_5
31. 58E_20
32. 58R_1
33. 24E_4
34. 25R_7
35. 32E_10
36. 37R_16

## 9.3 Chapter 5 Appendix

### 9.3.1 Appendix I:

**Table a:** Shoot height data. (*) data not available, refer to table d for isolate code. Coating: 1; coated, 2; sterile. Cultivar: H; Hereward, C; Cadenza. Rep: 3 replicates per treatment. Trt: 40 identified treatments.

| No | !Isolate | !Coating | !Ggt | !Trt | !Cultivar | !rep | shoot_length |
|---|---|---|---|---|---|---|---|
| 1 | **1** | **2** | **2** | **1** | **H** | 1 | 25 |
| 2 | 1 | 2 | **2** | 1 | **H** | 2 | 24 |
| 3 | 1 | 2 | **2** | 1 | **H** | 3 | 23 |
| 4 | 2 | 1 | **2** | 2 | **H** | 1 | 24 |
| 5 | 2 | 1 | **2** | 2 | **H** | 2 | 14.5 |
| 6 | 2 | 1 | **2** | 2 | **H** | 3 | 21 |
| 7 | 3 | 1 | **2** | 3 | **H** | 1 | 24.5 |
| 8 | 3 | 1 | **2** | 3 | **H** | 2 | 23 |
| 9 | 3 | 1 | **2** | 3 | **H** | 3 | 23 |
| 10 | 4 | 1 | **2** | 4 | **H** | 1 | * |
| 11 | 4 | 1 | **2** | 4 | **H** | 2 | 19 |
| 12 | 4 | 1 | **2** | 4 | **H** | 3 | 22.5 |
| 13 | 5 | 1 | **2** | 5 | **H** | 1 | * |
| 14 | 5 | 1 | **2** | 5 | **H** | 2 | * |
| 15 | 5 | 1 | **2** | 5 | **H** | 3 | 21 |
| 16 | 6 | 1 | **2** | 6 | **H** | 1 | 23 |
| 17 | 6 | 1 | **2** | 6 | **H** | 2 | 22 |
| 18 | 6 | 1 | **2** | 6 | **H** | 3 | 18 |
| 19 | 7 | 1 | **2** | 7 | **H** | 1 | 22 |
| 20 | 7 | 1 | **2** | 7 | **H** | 2 | 21 |
| 21 | 7 | 1 | **2** | 7 | **H** | 3 | 13 |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| 22 | 8 | 1 | **2** | 8 | **H** | 1 | * |
| 23 | 8 | 1 | **2** | 8 | **H** | 2 | * |
| 24 | 8 | 1 | **2** | 8 | **H** | 3 | 21 |
| 25 | 9 | 1 | **2** | 9 | **H** | 1 | * |
| 26 | 9 | 1 | **2** | 9 | **H** | 2 | * |
| 27 | 9 | 1 | **2** | 9 | **H** | 3 | 21.5 |
| 28 | 10 | 1 | **2** | 10 | **H** | 1 | 1 |
| 29 | 10 | 1 | **2** | 10 | **H** | 2 | 15 |
| 30 | 10 | 1 | **2** | 10 | **H** | 3 | 22 |
| 31 | **1** | **2** | **1** | **11** | **H** | 1 | 13.5 |
| 32 | 1 | 2 | **1** | 11 | **H** | 2 | 17 |
| 33 | 1 | 2 | **1** | 11 | **H** | 3 | 23 |
| 34 | 2 | 1 | **1** | 12 | **H** | 1 | 17.5 |
| 35 | 2 | 1 | **1** | 12 | **H** | 2 | 17 |
| 36 | 2 | 1 | **1** | 12 | **H** | 3 | 11.5 |
| 37 | 3 | 1 | **1** | 13 | **H** | 1 | 20.5 |
| 38 | 3 | 1 | **1** | 13 | **H** | 2 | 19 |
| 39 | 3 | 1 | **1** | 13 | **H** | 3 | 19 |
| 40 | 4 | 1 | **1** | 14 | **H** | 1 | 19 |
| 41 | 4 | 1 | **1** | 14 | **H** | 2 | 21.5 |
| 42 | 4 | 1 | **1** | 14 | **H** | 3 | 22 |
| 43 | 5 | 1 | **1** | 15 | **H** | 1 | 16 |
| 44 | 5 | 1 | **1** | 15 | **H** | 2 | 16.5 |
| 45 | 5 | 1 | **1** | 15 | **H** | 3 | 16 |
| 46 | 6 | 1 | **1** | 16 | **H** | 1 | 0.5 |
| 47 | 6 | 1 | **1** | 16 | **H** | 2 | 11.5 |
| 48 | 6 | 1 | **1** | 16 | **H** | 3 | 20.5 |
| 49 | 7 | 1 | **1** | 17 | **H** | 1 | * |
| 50 | 7 | 1 | **1** | 17 | **H** | 2 | 21.5 |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| 51 | 7 | 1 | **1** | 17 | **H** | 3 | 22 |
| 52 | 8 | 1 | **1** | 18 | **H** | 1 | * |
| 53 | 8 | 1 | **1** | 18 | **H** | 2 | 21.5 |
| 54 | 8 | 1 | **1** | 18 | **H** | 3 | 17 |
| 55 | 9 | 1 | **1** | 19 | **H** | 1 | 16 |
| 56 | 9 | 1 | **1** | 19 | **H** | 2 | 21.5 |
| 57 | 9 | 1 | **1** | 19 | **H** | 3 | 20 |
| 58 | 10 | 1 | **1** | 20 | **H** | 1 | 20.5 |
| 59 | 10 | 1 | **1** | 20 | **H** | 2 | 16.5 |
| 60 | 10 | 1 | **1** | 20 | **H** | 3 | 15.5 |
| 61 | **1** | **2** | **2** | **21** | **C** | 1 | 30 |
| 62 | 1 | 2 | **2** | 21 | **C** | 2 | 32 |
| 63 | 1 | 2 | **2** | 21 | **C** | 3 | 30 |
| 64 | 2 | 1 | **2** | 22 | **C** | 1 | 27.5 |
| 65 | 2 | 1 | **2** | 22 | **C** | 2 | 26 |
| 66 | 2 | 1 | **2** | 22 | **C** | 3 | 36 |
| 67 | 3 | 1 | **2** | 23 | **C** | 1 | 12.5 |
| 68 | 3 | 1 | **2** | 23 | **C** | 2 | 27.5 |
| 69 | 3 | 1 | **2** | 23 | **C** | 3 | 30 |
| 70 | 4 | 1 | **2** | 24 | **C** | 1 | 19 |
| 71 | 4 | 1 | **2** | 24 | **C** | 2 | 25 |
| 72 | 4 | 1 | **2** | 24 | **C** | 3 | 31.5 |
| 73 | 5 | 1 | **2** | 25 | **C** | 1 | 29 |
| 74 | 5 | 1 | **2** | 25 | **C** | 2 | 17.5 |
| 75 | 5 | 1 | **2** | 25 | **C** | 3 | 32 |
| 76 | 6 | 1 | **2** | 26 | **C** | 1 | 16.5 |
| 77 | 6 | 1 | **2** | 26 | **C** | 2 | 36 |
| 78 | 6 | 1 | **2** | 26 | **C** | 3 | 27 |
| 79 | 7 | 1 | **2** | 27 | **C** | 1 | 17 |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| 80 | 7 | 1 | **2** | 27 | **C** | 2 | 30 |
| 81 | 7 | 1 | **2** | 27 | **C** | 3 | * |
| 82 | 8 | 1 | **2** | 28 | **C** | 1 | 29 |
| 83 | 8 | 1 | **2** | 28 | **C** | 2 | 24 |
| 84 | 8 | 1 | **2** | 28 | **C** | 3 | 30 |
| 85 | 9 | 1 | **2** | 29 | **C** | 1 | 27 |
| 86 | 9 | 1 | **2** | 29 | **C** | 2 | 28 |
| 87 | 9 | 1 | **2** | 29 | **C** | 3 | 23.5 |
| 88 | 10 | 1 | **2** | 30 | **C** | 1 | * |
| 89 | 10 | 1 | **2** | 30 | **C** | 2 | 27 |
| 90 | 10 | 1 | **2** | 30 | **C** | 3 | 24 |
| 91 | **1** | 2 | **1** | 31 | **C** | 1 | * |
| 92 | 1 | 2 | **1** | 31 | **C** | 2 | 16.5 |
| 93 | 1 | 2 | **1** | 31 | **C** | 3 | 18 |
| 94 | 2 | 1 | **1** | 32 | **C** | 1 | 24 |
| 95 | 2 | 1 | **1** | 32 | **C** | 2 | 23 |
| 96 | 2 | 1 | **1** | 32 | **C** | 3 | 21.5 |
| 97 | 3 | 1 | **1** | 33 | **C** | 1 | 18.5 |
| 98 | 3 | 1 | **1** | 33 | **C** | 2 | 24.5 |
| 99 | 3 | 1 | **1** | 33 | **C** | 3 | 22 |
| 100 | 4 | 1 | **1** | 34 | **C** | 1 | 30 |
| 101 | 4 | 1 | **1** | 34 | **C** | 2 | 22 |
| 102 | 4 | 1 | **1** | 34 | **C** | 3 | 33 |
| 103 | 5 | 1 | **1** | 35 | **C** | 1 | 28 |
| 104 | 5 | 1 | **1** | 35 | **C** | 2 | 19 |
| 105 | 5 | 1 | **1** | 35 | **C** | 3 | 18 |
| 106 | 6 | 1 | **1** | 36 | **C** | 1 | * |
| 107 | 6 | 1 | **1** | 36 | **C** | 2 | 18 |
| 108 | 6 | 1 | **1** | 36 | **C** | 3 | 29 |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| 109 | 7 | 1 | **1** | 37 | **C** | 1 | 26 |
| 110 | 7 | 1 | **1** | 37 | **C** | 2 | 28 |
| 111 | 7 | 1 | **1** | 37 | **C** | 3 | 25.5 |
| 112 | 8 | 1 | **1** | 38 | **C** | 1 | 19 |
| 113 | 8 | 1 | **1** | 38 | **C** | 2 | 27.5 |
| 114 | 8 | 1 | **1** | 38 | **C** | 3 | 21.5 |
| 115 | 9 | 1 | **1** | 39 | **C** | 1 | * |
| 116 | 9 | 1 | **1** | 39 | **C** | 2 | 15 |
| 117 | 9 | 1 | **1** | 39 | **C** | 3 | 28 |
| 118 | 10 | 1 | **1** | 40 | **C** | 1 | 21 |
| 119 | 10 | 1 | **1** | 40 | **C** | 2 | 26 |
| 120 | 10 | 1 | **1** | 40 | **C** | 3 | 12 |

**Table b:** Root length data. (*) data not available, refer to table d for isolate code. Coating: 1; coated, 2; sterile. Cultivar: H; Hereward, C; Cadenza. Rep: 3 replicates per treatment. Trt: 40 identified treatments.

| No | **Isolate** | **Coating** | **Ggt** | **Trt** | **Cultivar** | **rep** | **root_length** |
|---|---|---|---|---|---|---|---|
| 1 | **1** | **2** | **2** | **1** | **H** | 1 | 17 |
| 2 | 1 | 2 | **2** | 1 | **H** | 2 | 19 |
| 3 | 1 | 2 | **2** | 1 | **H** | 3 | 18 |
| 4 | 2 | 1 | **2** | 2 | **H** | 1 | 19 |
| 5 | 2 | 1 | **2** | 2 | **H** | 2 | 16 |
| 6 | 2 | 1 | **2** | 2 | **H** | 3 | 18 |
| 7 | 3 | 1 | **2** | 3 | **H** | 1 | 22 |
| 8 | 3 | 1 | **2** | 3 | **H** | 2 | 19 |
| 9 | 3 | 1 | **2** | 3 | **H** | 3 | 18 |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| 10 | 4 | 1 | **2** | 4 | **H** | 1 | * |
| 11 | 4 | 1 | **2** | 4 | **H** | 2 | 18 |
| 12 | 4 | 1 | **2** | 4 | **H** | 3 | 18 |
| 13 | 5 | 1 | **2** | 5 | **H** | 1 | * |
| 14 | 5 | 1 | **2** | 5 | **H** | 2 | * |
| 15 | 5 | 1 | **2** | 5 | **H** | 3 | 19 |
| 16 | 6 | 1 | **2** | 6 | **H** | 1 | 30 |
| 17 | 6 | 1 | **2** | 6 | **H** | 2 | 22 |
| 18 | 6 | 1 | **2** | 6 | **H** | 3 | 17 |
| 19 | 7 | 1 | **2** | 7 | **H** | 1 | 21 |
| 20 | 7 | 1 | **2** | 7 | **H** | 2 | 19 |
| 21 | 7 | 1 | **2** | 7 | **H** | 3 | 18 |
| 22 | 8 | 1 | **2** | 8 | **H** | 1 | * |
| 23 | 8 | 1 | **2** | 8 | **H** | 2 | * |
| 24 | 8 | 1 | **2** | 8 | **H** | 3 | 19 |
| 25 | 9 | 1 | **2** | 9 | **H** | 1 | * |
| 26 | 9 | 1 | **2** | 9 | **H** | 2 | * |
| 27 | 9 | 1 | **2** | 9 | **H** | 3 | 19 |
| 28 | 10 | 1 | **2** | 10 | **H** | 1 | 17 |
| 29 | 10 | 1 | **2** | 10 | **H** | 2 | 13 |
| 30 | 10 | 1 | **2** | 10 | **H** | 3 | 19 |
| 31 | **1** | **2** | **1** | **11** | **H** | 1 | 9.5 |
| 32 | 1 | 2 | **1** | 11 | **H** | 2 | 18 |
| 33 | 1 | 2 | **1** | 11 | **H** | 3 | 29 |
| 34 | 2 | 1 | **1** | 12 | **H** | 1 | 13 |
| 35 | 2 | 1 | **1** | 12 | **H** | 2 | 19 |
| 36 | 2 | 1 | **1** | 12 | **H** | 3 | 25 |
| 37 | 3 | 1 | **1** | 13 | **H** | 1 | 15 |
| 38 | 3 | 1 | **1** | 13 | **H** | 2 | 16 |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| 39 | 3 | 1 | **1** | 13 | **H** | 3 | 18 |
| 40 | 4 | 1 | **1** | 14 | **H** | 1 | 19 |
| 41 | 4 | 1 | **1** | 14 | **H** | 2 | 24.5 |
| 42 | 4 | 1 | **1** | 14 | **H** | 3 | 19 |
| 43 | 5 | 1 | **1** | 15 | **H** | 1 | 17.5 |
| 44 | 5 | 1 | **1** | 15 | **H** | 2 | 24 |
| 45 | 5 | 1 | **1** | 15 | **H** | 3 | 20 |
| 46 | 6 | 1 | **1** | 16 | **H** | 1 | 15 |
| 47 | 6 | 1 | **1** | 16 | **H** | 2 | 21 |
| 48 | 6 | 1 | **1** | 16 | **H** | 3 | 19 |
| 49 | 7 | 1 | **1** | 17 | **H** | 1 | * |
| 50 | 7 | 1 | **1** | 17 | **H** | 2 | 21 |
| 51 | 7 | 1 | **1** | 17 | **H** | 3 | 19 |
| 52 | 8 | 1 | **1** | 18 | **H** | 1 | * |
| 53 | 8 | 1 | **1** | 18 | **H** | 2 | 25 |
| 54 | 8 | 1 | **1** | 18 | **H** | 3 | 23.5 |
| 55 | 9 | 1 | **1** | 19 | **H** | 1 | 19 |
| 56 | 9 | 1 | **1** | 19 | **H** | 2 | 25 |
| 57 | 9 | 1 | **1** | 19 | **H** | 3 | 18.5 |
| 58 | 10 | 1 | **1** | 20 | **H** | 1 | 20.5 |
| 59 | 10 | 1 | **1** | 20 | **H** | 2 | 17.5 |
| 60 | 10 | 1 | **1** | 20 | **H** | 3 | 15 |
| 61 | **1** | **2** | **2** | **21** | **C** | 1 | 20.5 |
| 62 | 1 | 2 | **2** | 21 | **C** | 2 | 21 |
| 63 | 1 | 2 | **2** | 21 | **C** | 3 | 27 |
| 64 | 2 | 1 | **2** | 22 | **C** | 1 | 18.5 |
| 65 | 2 | 1 | **2** | 22 | **C** | 2 | 16.5 |
| 66 | 2 | 1 | **2** | 22 | **C** | 3 | 24 |
| 67 | 3 | 1 | **2** | 23 | **C** | 1 | 16 |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| 68 | 3 | 1 | **2** | 23 | **C** | 2 | 13 |
| 69 | 3 | 1 | **2** | 23 | **C** | 3 | 14 |
| 70 | 4 | 1 | **2** | 24 | **C** | 1 | 16 |
| 71 | 4 | 1 | **2** | 24 | **C** | 2 | 19 |
| 72 | 4 | 1 | **2** | 24 | **C** | 3 | 18 |
| 73 | 5 | 1 | **2** | 25 | **C** | 1 | 16 |
| 74 | 5 | 1 | **2** | 25 | **C** | 2 | 15 |
| 75 | 5 | 1 | **2** | 25 | **C** | 3 | 20 |
| 76 | 6 | 1 | **2** | 26 | **C** | 1 | 15 |
| 77 | 6 | 1 | **2** | 26 | **C** | 2 | 16 |
| 78 | 6 | 1 | **2** | 26 | **C** | 3 | 16.5 |
| 79 | 7 | 1 | **2** | 27 | **C** | 1 | 11.5 |
| 80 | 7 | 1 | **2** | 27 | **C** | 2 | 21 |
| 81 | 7 | 1 | **2** | 27 | **C** | 3 | * |
| 82 | 8 | 1 | **2** | 28 | **C** | 1 | 20.5 |
| 83 | 8 | 1 | **2** | 28 | **C** | 2 | 14 |
| 84 | 8 | 1 | **2** | 28 | **C** | 3 | 21 |
| 85 | 9 | 1 | **2** | 29 | **C** | 1 | 19 |
| 86 | 9 | 1 | **2** | 29 | **C** | 2 | 23 |
| 87 | 9 | 1 | **2** | 29 | **C** | 3 | 23 |
| 88 | 10 | 1 | **2** | 30 | **C** | 1 | * |
| 89 | 10 | 1 | **2** | 30 | **C** | 2 | 18 |
| 90 | 10 | 1 | **2** | 30 | **C** | 3 | 20 |
| 91 | **1** | 2 | **1** | 31 | **C** | 1 | * |
| 92 | 1 | 2 | **1** | 31 | **C** | 2 | 19 |
| 93 | 1 | 2 | **1** | 31 | **C** | 3 | 12 |
| 94 | 2 | 1 | **1** | 32 | **C** | 1 | 15 |
| 95 | 2 | 1 | **1** | 32 | **C** | 2 | 20 |
| 96 | 2 | 1 | **1** | 32 | **C** | 3 | 19 |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| 97 | 3 | 1 | **1** | 33 | **C** | 1 | 18 |
| 98 | 3 | 1 | **1** | 33 | **C** | 2 | 21 |
| 99 | 3 | 1 | **1** | 33 | **C** | 3 | 17 |
| 100 | 4 | 1 | **1** | 34 | **C** | 1 | 14 |
| 101 | 4 | 1 | **1** | 34 | **C** | 2 | 13 |
| 102 | 4 | 1 | **1** | 34 | **C** | 3 | 15 |
| 103 | 5 | 1 | **1** | 35 | **C** | 1 | 14 |
| 104 | 5 | 1 | **1** | 35 | **C** | 2 | 19 |
| 105 | 5 | 1 | **1** | 35 | **C** | 3 | 14.5 |
| 106 | 6 | 1 | **1** | 36 | **C** | 1 | * |
| 107 | 6 | 1 | **1** | 36 | **C** | 2 | 13 |
| 108 | 6 | 1 | **1** | 36 | **C** | 3 | 14.5 |
| 109 | 7 | 1 | **1** | 37 | **C** | 1 | 16.5 |
| 110 | 7 | 1 | **1** | 37 | **C** | 2 | 21 |
| 111 | 7 | 1 | **1** | 37 | **C** | 3 | 18 |
| 112 | 8 | 1 | **1** | 38 | **C** | 1 | 18.5 |
| 113 | 8 | 1 | **1** | 38 | **C** | 2 | 20.5 |
| 114 | 8 | 1 | **1** | 38 | **C** | 3 | 17 |
| 115 | 9 | 1 | **1** | 39 | **C** | 1 | * |
| 116 | 9 | 1 | **1** | 39 | **C** | 2 | 16 |
| 117 | 9 | 1 | **1** | 39 | **C** | 3 | 19 |
| 118 | 10 | 1 | **1** | 40 | **C** | 1 | 13 |
| 119 | 10 | 1 | **1** | 40 | **C** | 2 | 23 |
| 120 | 10 | 1 | **1** | 40 | **C** | 3 | 15 |

**Table c:** Fresh weight data. (*) data not available, refer to table d for isolate code. Coating: 1; coated, 2; sterile. Cultivar: H; Hereward, C; Cadenza. Rep: 3 replicates per treatment. Trt: 40 identified treatments.

| No | !Isolate | !Coating | !Ggt | !Trt | !Cultivar | !rep | plant_weight |
|---|---|---|---|---|---|---|---|
| 1 | **1** | **2** | **2** | **1** | **H** | 1 | 0.275 |
| 2 | 1 | 2 | **2** | 1 | **H** | 2 | 0.369 |
| 3 | 1 | 2 | **2** | 1 | **H** | 3 | 0.38 |
| 4 | 2 | 1 | **2** | 2 | **H** | 1 | 0.298 |
| 5 | 2 | 1 | **2** | 2 | **H** | 2 | 0.159 |
| 6 | 2 | 1 | **2** | 2 | **H** | 3 | 0.291 |
| 7 | 3 | 1 | **2** | 3 | **H** | 1 | 0.283 |
| 8 | 3 | 1 | **2** | 3 | **H** | 2 | 0.296 |
| 9 | 3 | 1 | **2** | 3 | **H** | 3 | 0.298 |
| 10 | 4 | 1 | **2** | 4 | **H** | 1 | * |
| 11 | 4 | 1 | **2** | 4 | **H** | 2 | 0.165 |
| 12 | 4 | 1 | **2** | 4 | **H** | 3 | 0.237 |
| 13 | 5 | 1 | **2** | 5 | **H** | 1 | * |
| 14 | 5 | 1 | **2** | 5 | **H** | 2 | * |
| 15 | 5 | 1 | **2** | 5 | **H** | 3 | 0.42 |
| 16 | 6 | 1 | **2** | 6 | **H** | 1 | 0.416 |
| 17 | 6 | 1 | **2** | 6 | **H** | 2 | 0.408 |
| 18 | 6 | 1 | **2** | 6 | **H** | 3 | 0.234 |
| 19 | 7 | 1 | **2** | 7 | **H** | 1 | 0.416 |
| 20 | 7 | 1 | **2** | 7 | **H** | 2 | 0.246 |
| 21 | 7 | 1 | **2** | 7 | **H** | 3 | 0.215 |
| 22 | 8 | 1 | **2** | 8 | **H** | 1 | * |
| 23 | 8 | 1 | **2** | 8 | **H** | 2 | * |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| 24 | 8 | 1 | **2** | 8 | **H** | 3 | 0.355 |
| 25 | 9 | 1 | **2** | 9 | **H** | 1 | * |
| 26 | 9 | 1 | **2** | 9 | **H** | 2 | * |
| 27 | 9 | 1 | **2** | 9 | **H** | 3 | 0.382 |
| 28 | 10 | 1 | **2** | 10 | **H** | 1 | 0.121 |
| 29 | 10 | 1 | **2** | 10 | **H** | 2 | 0.365 |
| 30 | 10 | 1 | **2** | 10 | **H** | 3 | 0.359 |
| 31 | **1** | **2** | **1** | **11** | **H** | 1 | 0.102 |
| 32 | 1 | 2 | **1** | 11 | **H** | 2 | 0.137 |
| 33 | 1 | 2 | **1** | 11 | **H** | 3 | 0.33 |
| 34 | 2 | 1 | **1** | 12 | **H** | 1 | 0.156 |
| 35 | 2 | 1 | **1** | 12 | **H** | 2 | 0.203 |
| 36 | 2 | 1 | **1** | 12 | **H** | 3 | 0.376 |
| 37 | 3 | 1 | **1** | 13 | **H** | 1 | 0.194 |
| 38 | 3 | 1 | **1** | 13 | **H** | 2 | 0.236 |
| 39 | 3 | 1 | **1** | 13 | **H** | 3 | 0.244 |
| 40 | 4 | 1 | **1** | 14 | **H** | 1 | 0.407 |
| 41 | 4 | 1 | **1** | 14 | **H** | 2 | 0.424 |
| 42 | 4 | 1 | **1** | 14 | **H** | 3 | 0.242 |
| 43 | 5 | 1 | **1** | 15 | **H** | 1 | 0.225 |
| 44 | 5 | 1 | **1** | 15 | **H** | 2 | 0.224 |
| 45 | 5 | 1 | **1** | 15 | **H** | 3 | 0.269 |
| 46 | 6 | 1 | **1** | 16 | **H** | 1 | 0.17 |
| 47 | 6 | 1 | **1** | 16 | **H** | 2 | 0.372 |
| 48 | 6 | 1 | **1** | 16 | **H** | 3 | 0.236 |
| 49 | 7 | 1 | **1** | 17 | **H** | 1 | * |
| 50 | 7 | 1 | **1** | 17 | **H** | 2 | 0.237 |
| 51 | 7 | 1 | **1** | 17 | **H** | 3 | 0.339 |
| 52 | 8 | 1 | **1** | 18 | **H** | 1 | * |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| 53 | 8 | 1 | **1** | 18 | **H** | 2 | 0.191 |
| 54 | 8 | 1 | **1** | 18 | **H** | 3 | 0.431 |
| 55 | 9 | 1 | **1** | 19 | **H** | 1 | 0.178 |
| 56 | 9 | 1 | **1** | 19 | **H** | 2 | 0.35 |
| 57 | 9 | 1 | **1** | 19 | **H** | 3 | 0.325 |
| 58 | 10 | 1 | **1** | 20 | **H** | 1 | 0.293 |
| 59 | 10 | 1 | **1** | 20 | **H** | 2 | 0.181 |
| 60 | 10 | 1 | **1** | 20 | **H** | 3 | 0.235 |
| 61 | **1** | **2** | **2** | **21** | **C** | 1 | 0.385 |
| 62 | 1 | 2 | **2** | 21 | **C** | 2 | 0.4 |
| 63 | 1 | 2 | **2** | 21 | **C** | 3 | 0.496 |
| 64 | 2 | 1 | **2** | 22 | **C** | 1 | 0.197 |
| 65 | 2 | 1 | **2** | 22 | **C** | 2 | 0.497 |
| 66 | 2 | 1 | **2** | 22 | **C** | 3 | 0.353 |
| 67 | 3 | 1 | **2** | 23 | **C** | 1 | 0.273 |
| 68 | 3 | 1 | **2** | 23 | **C** | 2 | 0.144 |
| 69 | 3 | 1 | **2** | 23 | **C** | 3 | 0.308 |
| 70 | 4 | 1 | **2** | 24 | **C** | 1 | 0.253 |
| 71 | 4 | 1 | **2** | 24 | **C** | 2 | 0.161 |
| 72 | 4 | 1 | **2** | 24 | **C** | 3 | 0.333 |
| 73 | 5 | 1 | **2** | 25 | **C** | 1 | 0.307 |
| 74 | 5 | 1 | **2** | 25 | **C** | 2 | 0.301 |
| 75 | 5 | 1 | **2** | 25 | **C** | 3 | 0.258 |
| 76 | 6 | 1 | **2** | 26 | **C** | 1 | 0.301 |
| 77 | 6 | 1 | **2** | 26 | **C** | 2 | 0.291 |
| 78 | 6 | 1 | **2** | 26 | **C** | 3 | 0.291 |
| 79 | 7 | 1 | **2** | 27 | **C** | 1 | 0.408 |
| 80 | 7 | 1 | **2** | 27 | **C** | 2 | 0.191 |
| 81 | 7 | 1 | **2** | 27 | **C** | 3 | * |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| 82 | 8 | 1 | **2** | 28 | **C** | 1 | 0.337 |
| 83 | 8 | 1 | **2** | 28 | **C** | 2 | 0.361 |
| 84 | 8 | 1 | **2** | 28 | **C** | 3 | 0.199 |
| 85 | 9 | 1 | **2** | 29 | **C** | 1 | 0.299 |
| 86 | 9 | 1 | **2** | 29 | **C** | 2 | 0.314 |
| 87 | 9 | 1 | **2** | 29 | **C** | 3 | 0.313 |
| 88 | 10 | 1 | **2** | 30 | **C** | 1 | * |
| 89 | 10 | 1 | **2** | 30 | **C** | 2 | 0.314 |
| 90 | 10 | 1 | **2** | 30 | **C** | 3 | 0.282 |
| 91 | **1** | 2 | **1** | 31 | **C** | 1 | * |
| 92 | 1 | 2 | **1** | 31 | **C** | 2 | 0.165 |
| 93 | 1 | 2 | **1** | 31 | **C** | 3 | 0.105 |
| 94 | 2 | 1 | **1** | 32 | **C** | 1 | 0.141 |
| 95 | 2 | 1 | **1** | 32 | **C** | 2 | 0.197 |
| 96 | 2 | 1 | **1** | 32 | **C** | 3 | 0.121 |
| 97 | 3 | 1 | **1** | 33 | **C** | 1 | 0.205 |
| 98 | 3 | 1 | **1** | 33 | **C** | 2 | 0.25 |
| 99 | 3 | 1 | **1** | 33 | **C** | 3 | 0.214 |
| 100 | 4 | 1 | **1** | 34 | **C** | 1 | 0.311 |
| 101 | 4 | 1 | **1** | 34 | **C** | 2 | 0.153 |
| 102 | 4 | 1 | **1** | 34 | **C** | 3 | 0.389 |
| 103 | 5 | 1 | **1** | 35 | **C** | 1 | 0.134 |
| 104 | 5 | 1 | **1** | 35 | **C** | 2 | 0.147 |
| 105 | 5 | 1 | **1** | 35 | **C** | 3 | 0.295 |
| 106 | 6 | 1 | **1** | 36 | **C** | 1 | * |
| 107 | 6 | 1 | **1** | 36 | **C** | 2 | 0.126 |
| 108 | 6 | 1 | **1** | 36 | **C** | 3 | 0.429 |
| 109 | 7 | 1 | **1** | 37 | **C** | 1 | 0.282 |
| 110 | 7 | 1 | **1** | 37 | **C** | 2 | 0.457 |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| 111 | 7 | 1 | **1** | 37 | **C** | 3 | 0.3 |
| 112 | 8 | 1 | **1** | 38 | **C** | 1 | 0.243 |
| 113 | 8 | 1 | **1** | 38 | **C** | 2 | 0.298 |
| 114 | 8 | 1 | **1** | 38 | **C** | 3 | 0.174 |
| 115 | 9 | 1 | **1** | 39 | **C** | 1 | * |
| 116 | 9 | 1 | **1** | 39 | **C** | 2 | 0.15 |
| 117 | 9 | 1 | **1** | 39 | **C** | 3 | 0.257 |
| 118 | 10 | 1 | **1** | 40 | **C** | 1 | 0.155 |
| 119 | 10 | 1 | **1** | 40 | **C** | 2 | 0.112 |
| 120 | 10 | 1 | **1** | 40 | **C** | 3 | 0.246 |

**Table d:** Isolate Key.

| **isolate** | **CODE** |
|---|---|
| sterile | **1** |
| 24E/2 | 2 |
| 24E/4 | 3 |
| 25R/7 | 4 |
| 28R/9 | 5 |
| 30R/11 | **6** |
| 37R/15 | 7 |
| 44E/7 | 8 |
| 44R/4 | 9 |
| MIX of six | 10 |

**Table e:** Infected roots to the total number of roots. (*) data not available refer to table d for isolate code. Trt: 20 identified. Rep: 3 replicates per treatment. Cultivar: H; Hereward, C; Cadenza treatments. Ggt: 1;present.

| no | !Trt | !rep | !Isolate | !cultivar | !Ggt | total_roots | no_infected | infected_present | !inf_p |
|---|---|---|---|---|---|---|---|---|---|
| 1 | 1 | 1 | 1 | H | 1 | 4 | 4 | 1 | 1 |
| 2 | 1 | 2 | 1 | H | 1 | 6 | 6 | 1 | 1 |
| 3 | 1 | 3 | 1 | H | 1 | 7 | 7 | 1 | 1 |
| 4 | 2 | 1 | 2 | H | 1 | 7 | 7 | 1 | 1 |
| 5 | 2 | 2 | 2 | H | 1 | 5 | 5 | 1 | 1 |
| 6 | 2 | 3 | 2 | H | 1 | 4 | 1 | 1 | 1 |
| 7 | 3 | 1 | 3 | H | 1 | 6 | 6 | 1 | 1 |
| 8 | 3 | 2 | 3 | H | 1 | 5 | 5 | 1 | 1 |
| 9 | 3 | 3 | 3 | H | 1 | 5 | 4 | 1 | 1 |
| 10 | 4 | 1 | 4 | H | 1 | 4 | 4 | 1 | 1 |
| 11 | 4 | 2 | 4 | H | 1 | 5 | 5 | 1 | 1 |
| 12 | 4 | 3 | 4 | H | 1 | 7 | 7 | 1 | 1 |
| 13 | 5 | 1 | 5 | H | 1 | 8 | 7 | 1 | 1 |
| 14 | 5 | 2 | 5 | H | 1 | 5 | 5 | 1 | 1 |
| 15 | 5 | 3 | 5 | H | 1 | 5 | 5 | 1 | 1 |
| 16 | 6 | 1 | 6 | H | 1 | 3 | 1 | 1 | 1 |
| 17 | 6 | 2 | 6 | H | 1 | 7 | 0 | 0 | 0 |
| 18 | 6 | 3 | 6 | H | 1 | 5 | 1 | 1 | 1 |
| 19 | 7 | 1 | 7 | H | 1 | * | * | * | * |
| 20 | 7 | 2 | 7 | H | 1 | 5 | 5 | 1 | 1 |
| 21 | 7 | 3 | 7 | H | 1 | 8 | 8 | 1 | 1 |
| 22 | 8 | 1 | 8 | H | 1 | * | * | * | * |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| 23 | 8 | 2 | 8 | H | 1 | 6 | 6 | 1 | 1 |
| 24 | 8 | 3 | 8 | H | 1 | 3 | 3 | 1 | 1 |
| 25 | 9 | 1 | 9 | H | 1 | 5 | 5 | 1 | 1 |
| 26 | 9 | 2 | 9 | H | 1 | 7 | 5 | 1 | 1 |
| 27 | 9 | 3 | 9 | H | 1 | 5 | 5 | 1 | 1 |
| 28 | 10 | 1 | 10 | H | 1 | 5 | 5 | 1 | 1 |
| 29 | 10 | 2 | 10 | H | 1 | 5 | 5 | 1 | 1 |
| 30 | 10 | 3 | 10 | H | 1 | 5 | 0 | 0 | 0 |
| 31 | 11 | 1 | 1 | C | 1 | * | * | * | * |
| 32 | 11 | 2 | 1 | C | 1 | 5 | 5 | 1 | 1 |
| 33 | 11 | 3 | 1 | C | 1 | 6 | 6 | 1 | 1 |
| 34 | 12 | 1 | 2 | C | 1 | 8 | 8 | 1 | 1 |
| 35 | 12 | 2 | 2 | C | 1 | 7 | 7 | 1 | 1 |
| 36 | 12 | 3 | 2 | C | 1 | 5 | 5 | 1 | 1 |
| 37 | 13 | 1 | 3 | C | 1 | 5 | 1 | 1 | 1 |
| 38 | 13 | 2 | 3 | C | 1 | 5 | 5 | 1 | 1 |
| 39 | 13 | 3 | 3 | C | 1 | 7 | 7 | 1 | 1 |
| 40 | 14 | 1 | 4 | C | 1 | 7 | 3 | 1 | 1 |
| 41 | 14 | 2 | 4 | C | 1 | 8 | 6 | 1 | 1 |
| 42 | 14 | 3 | 4 | C | 1 | 6 | 2 | 1 | 1 |
| 43 | 15 | 1 | 5 | C | 1 | 7 | 7 | 1 | 1 |
| 44 | 15 | 2 | 5 | C | 1 | 6 | 6 | 1 | 1 |
| 45 | 15 | 3 | 5 | C | 1 | 8 | 8 | 1 | 1 |
| 46 | 16 | 1 | 6 | C | 1 | * | * | * | * |
| 47 | 16 | 2 | 6 | C | 1 | 6 | 6 | 1 | 1 |
| 48 | 16 | 3 | 6 | C | 1 | 5 | 3 | 1 | 1 |
| 49 | 17 | 1 | 7 | C | 1 | 4 | 4 | 1 | 1 |
| 50 | 17 | 2 | 7 | C | 1 | 7 | 7 | 1 | 1 |
| 51 | 17 | 3 | 7 | C | 1 | 5 | 5 | 1 | 1 |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| 52 | 18 | 1 | 8 | C | 1 | 4 | 4 | 1 | 1 |
| 53 | 18 | 2 | 8 | C | 1 | 5 | 2 | 1 | 1 |
| 54 | 18 | 3 | 8 | C | 1 | 7 | 7 | 1 | 1 |
| 55 | 19 | 1 | 9 | C | 1 | * | * | * | * |
| 56 | 19 | 2 | 9 | C | 1 | 3 | 2 | 1 | 1 |
| 57 | 19 | 3 | 9 | C | 1 | 5 | 4 | 1 | 1 |
| 58 | 20 | 1 | 10 | C | 1 | 7 | 7 | 1 | 1 |
| 59 | 20 | 2 | 10 | C | 1 | 8 | 7 | 1 | 1 |
| 60 | 20 | 3 | 10 | C | 1 | 5 | 5 | 1 | 1 |

### 9.3.2 Appendix II:

**Table a: shoot raw data**. Isolate 1 is 25R/7, isolate 2 is 30R/11, isolate 3 is the mix of 6 antagonistic isolates, isolate 4 sterile; coating 1 is seeds coated, coating 2 non-bacterial coated seeds; conc.1 is OD1, conc.2 is OD0.5, conc. 3 is OD 0.1; *Ggt* 1: present, 2: absent; and rep are the 10 replicates per treatment. (*) refers to missing values (no data).

| No. | Cultivar | Isolate | Coating | Conc | Rep | Ggt | average shoot (cm) |
|---|---|---|---|---|---|---|---|
| 1 | C | 1 | 1 | 1 | 1 | 1 | 23.5 |
| 2 | C | 1 | 1 | 1 | 2 | 1 | 18.75 |
| 3 | C | 1 | 1 | 1 | 3 | 1 | 19.75 |
| 4 | C | 1 | 1 | 1 | 4 | 1 | 26.65 |
| 5 | C | 1 | 1 | 1 | 5 | 1 | 20.5 |
| 6 | C | 1 | 1 | 1 | 6 | 1 | 14.83333333 |
| 7 | C | 1 | 1 | 1 | 7 | 1 | 24.25 |
| 8 | C | 1 | 1 | 1 | 8 | 1 | 27.75 |
| 9 | C | 1 | 1 | 1 | 9 | 1 | 25 |
| 10 | C | 1 | 1 | 1 | 10 | 1 | 21.45 |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| 11 | C | 1 | 1 | 2 | 1 | 1 | 17.66666667 |
| 12 | C | 1 | 1 | 2 | 2 | 1 | 19.33333333 |
| 13 | C | 1 | 1 | 2 | 3 | 1 | 15.15 |
| 14 | C | 1 | 1 | 2 | 4 | 1 | 17 |
| 15 | C | 1 | 1 | 2 | 5 | 1 | 16.9 |
| 16 | C | 1 | 1 | 2 | 6 | 1 | 20.5 |
| 17 | C | 1 | 1 | 2 | 7 | 1 | 16.75 |
| 18 | C | 1 | 1 | 2 | 8 | 1 | 17.9 |
| 19 | C | 1 | 1 | 2 | 9 | 1 | 7.625 |
| 20 | C | 1 | 1 | 2 | 10 | 1 | * |
| 21 | C | 1 | 1 | 3 | 1 | 1 | 20.75 |
| 22 | C | 1 | 1 | 3 | 2 | 1 | 24.5 |
| 23 | C | 1 | 1 | 3 | 3 | 1 | 24 |
| 24 | C | 1 | 1 | 3 | 4 | 1 | 21.75 |
| 25 | C | 1 | 1 | 3 | 5 | 1 | 21.25 |
| 26 | C | 1 | 1 | 3 | 6 | 1 | 11 |
| 27 | C | 1 | 1 | 3 | 7 | 1 | 22 |
| 28 | C | 1 | 1 | 3 | 8 | 1 | 10.775 |
| 29 | C | 1 | 1 | 3 | 9 | 1 | * |
| 30 | C | 1 | 1 | 3 | 10 | 1 | * |
| 31 | C | 2 | 1 | 1 | 1 | 1 | 26.45 |
| 32 | C | 2 | 1 | 1 | 2 | 1 | 24.25 |
| 33 | C | 2 | 1 | 1 | 3 | 1 | 24.25 |
| 34 | C | 2 | 1 | 1 | 4 | 1 | 19.75 |
| 35 | C | 2 | 1 | 1 | 5 | 1 | 25 |
| 36 | C | 2 | 1 | 1 | 6 | 1 | 7 |
| 37 | C | 2 | 1 | 1 | 7 | 1 | 21.5 |
| 38 | C | 2 | 1 | 1 | 8 | 1 | 20.25 |
| 39 | C | 2 | 1 | 1 | 9 | 1 | 23.25 |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| 40 | C | 2 | 1 | 1 | 10 | 1 | 26 |
| 41 | C | 2 | 1 | 2 | 1 | 1 | 27.25 |
| 42 | C | 2 | 1 | 2 | 2 | 1 | 20.75 |
| 43 | C | 2 | 1 | 2 | 3 | 1 | 3.5 |
| 44 | C | 2 | 1 | 2 | 4 | 1 | 17 |
| 45 | C | 2 | 1 | 2 | 5 | 1 | 8.666666667 |
| 46 | C | 2 | 1 | 2 | 6 | 1 | 9.666666667 |
| 47 | C | 2 | 1 | 2 | 7 | 1 | 23.33333333 |
| 48 | C | 2 | 1 | 2 | 8 | 1 | 28.5 |
| 49 | C | 2 | 1 | 2 | 9 | 1 | 22.5 |
| 50 | C | 2 | 1 | 2 | 10 | 1 | * |
| 51 | C | 2 | 1 | 3 | 1 | 1 | 25.4 |
| 52 | C | 2 | 1 | 3 | 2 | 1 | 18.4 |
| 53 | C | 2 | 1 | 3 | 3 | 1 | 26 |
| 54 | C | 2 | 1 | 3 | 4 | 1 | 18.25 |
| 55 | C | 2 | 1 | 3 | 5 | 1 | 17.6 |
| 56 | C | 2 | 1 | 3 | 6 | 1 | 17.5 |
| 57 | C | 2 | 1 | 3 | 7 | 1 | 21.75 |
| 58 | C | 2 | 1 | 3 | 8 | 1 | 18.75 |
| 59 | C | 2 | 1 | 3 | 9 | 1 | * |
| 60 | C | 2 | 1 | 3 | 10 | 1 | * |
| 61 | C | 3 | 1 | 1 | 1 | 1 | 0.5 |
| 62 | C | 3 | 1 | 1 | 2 | 1 | 23.75 |
| 63 | C | 3 | 1 | 1 | 3 | 1 | 23 |
| 64 | C | 3 | 1 | 1 | 4 | 1 | 15.83333333 |
| 65 | C | 3 | 1 | 1 | 5 | 1 | 20.5 |
| 66 | C | 3 | 1 | 1 | 6 | 1 | 28 |
| 67 | C | 3 | 1 | 1 | 7 | 1 | 8.75 |
| 68 | C | 3 | 1 | 1 | 8 | 1 | 26 |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| 69 | C | 3 | 1 | 1 | 9 | 1 | 23.16666667 |
| 70 | C | 3 | 1 | 1 | 10 | 1 | 26.5 |
| 71 | C | 3 | 1 | 2 | 1 | 1 | 24.75 |
| 72 | C | 3 | 1 | 2 | 2 | 1 | 25.75 |
| 73 | C | 3 | 1 | 2 | 3 | 1 | 15.25 |
| 74 | C | 3 | 1 | 2 | 4 | 1 | 15 |
| 75 | C | 3 | 1 | 2 | 5 | 1 | 21 |
| 76 | C | 3 | 1 | 2 | 6 | 1 | 14.5 |
| 77 | C | 3 | 1 | 2 | 7 | 1 | 25.65 |
| 78 | C | 3 | 1 | 2 | 8 | 1 | 19.5 |
| 79 | C | 3 | 1 | 2 | 9 | 1 | 20.83333333 |
| 80 | C | 3 | 1 | 2 | 10 | 1 | 29 |
| 81 | C | 3 | 1 | 3 | 1 | 1 | 5.5 |
| 82 | C | 3 | 1 | 3 | 2 | 1 | 25.8 |
| 83 | C | 3 | 1 | 3 | 3 | 1 | 22.25 |
| 84 | C | 3 | 1 | 3 | 4 | 1 | 12.5 |
| 85 | C | 3 | 1 | 3 | 5 | 1 | 13.75 |
| 86 | C | 3 | 1 | 3 | 6 | 1 | 21.85 |
| 87 | C | 3 | 1 | 3 | 7 | 1 | 23.85 |
| 88 | C | 3 | 1 | 3 | 8 | 1 | 18.76666667 |
| 89 | C | 3 | 1 | 3 | 9 | 1 | 25.75 |
| 90 | C | 3 | 1 | 3 | 10 | 1 | 21 |
| 91 | C | 4 | 2 | 4 | 1 | 1 | 16.5 |
| 92 | C | 4 | 2 | 4 | 2 | 1 | 3.666666667 |
| 93 | C | 4 | 2 | 4 | 3 | 1 | 12 |
| 94 | C | 4 | 2 | 4 | 4 | 1 | 21 |
| 95 | C | 4 | 2 | 4 | 5 | 1 | 17.25 |
| 96 | C | 4 | 2 | 4 | 6 | 1 | 14.66666667 |
| 97 | C | 4 | 2 | 4 | 7 | 1 | 14.5 |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| 98 | C | 4 | 2 | 4 | 8 | 1 | 12.75 |
| 99 | C | 4 | 2 | 4 | 9 | 1 | 14.2 |
| 100 | C | 4 | 2 | 4 | 10 | 1 | * |
| 101 | C | 4 | 2 | 4 | 1 | 2 | 24 |
| 102 | C | 4 | 2 | 4 | 2 | 2 | 1.5 |
| 103 | C | 4 | 2 | 4 | 3 | 2 | 11.16666667 |
| 104 | C | 4 | 2 | 4 | 4 | 2 | 23.16666667 |
| 105 | C | 4 | 2 | 4 | 5 | 2 | 23.25 |
| 106 | C | 4 | 2 | 4 | 6 | 2 | 24.75 |
| 107 | C | 4 | 2 | 4 | 7 | 2 | 23.25 |
| 108 | C | 4 | 2 | 4 | 8 | 2 | 23.5 |
| 109 | C | 4 | 2 | 4 | 9 | 2 | 20 |
| 110 | C | 4 | 2 | 4 | 10 | 2 | 17.5 |
| 111 | H | 1 | 1 | 1 | 1 | 1 | 5.5 |
| 112 | H | 1 | 1 | 1 | 2 | 1 | 9.25 |
| 113 | H | 1 | 1 | 1 | 3 | 1 | 13 |
| 114 | H | 1 | 1 | 1 | 4 | 1 | 12.16666667 |
| 115 | H | 1 | 1 | 1 | 5 | 1 | 14.66666667 |
| 116 | H | 1 | 1 | 1 | 6 | 1 | 16 |
| 117 | H | 1 | 1 | 1 | 7 | 1 | 6.833333333 |
| 118 | H | 1 | 1 | 1 | 8 | 1 | 17.25 |
| 119 | H | 1 | 1 | 1 | 9 | 1 | * |
| 120 | H | 1 | 1 | 1 | 10 | 1 | * |
| 121 | H | 1 | 1 | 2 | 1 | 1 | 13.5 |
| 122 | H | 1 | 1 | 2 | 2 | 1 | 13 |
| 123 | H | 1 | 1 | 2 | 3 | 1 | 18.33333333 |
| 124 | H | 1 | 1 | 2 | 4 | 1 | 13.25 |
| 125 | H | 1 | 1 | 2 | 5 | 1 | 12.5 |
| 126 | H | 1 | 1 | 2 | 6 | 1 | 14.25 |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| 127 | H | 1 | 1 | 2 | 7 | 1 | * |
| 128 | H | 1 | 1 | 2 | 8 | 1 | * |
| 129 | H | 1 | 1 | 2 | 9 | 1 | * |
| 130 | H | 1 | 1 | 2 | 10 | 1 | * |
| 131 | H | 1 | 1 | 3 | 1 | 1 | 10.25 |
| 132 | H | 1 | 1 | 3 | 2 | 1 | 4.5 |
| 133 | H | 1 | 1 | 3 | 3 | 1 | 13.66666667 |
| 134 | H | 1 | 1 | 3 | 4 | 1 | 10.66666667 |
| 135 | H | 1 | 1 | 3 | 5 | 1 | 15.16666667 |
| 136 | H | 1 | 1 | 3 | 6 | 1 | 16 |
| 137 | H | 1 | 1 | 3 | 7 | 1 | * |
| 138 | H | 1 | 1 | 3 | 8 | 1 | * |
| 139 | H | 1 | 1 | 3 | 9 | 1 | * |
| 140 | H | 1 | 1 | 3 | 10 | 1 | * |
| 141 | H | 2 | 1 | 1 | 1 | 1 | 16.75 |
| 142 | H | 2 | 1 | 1 | 2 | 1 | 11 |
| 143 | H | 2 | 1 | 1 | 3 | 1 | 14 |
| 144 | H | 2 | 1 | 1 | 4 | 1 | 11.66666667 |
| 145 | H | 2 | 1 | 1 | 5 | 1 | 12.66666667 |
| 146 | H | 2 | 1 | 1 | 6 | 1 | 17.75 |
| 147 | H | 2 | 1 | 1 | 7 | 1 | 15.43333333 |
| 148 | H | 2 | 1 | 1 | 8 | 1 | 8.433333333 |
| 149 | H | 2 | 1 | 1 | 9 | 1 | 14.75 |
| 150 | H | 2 | 1 | 1 | 10 | 1 | * |
| 151 | H | 2 | 1 | 2 | 1 | 1 | 10.16666667 |
| 152 | H | 2 | 1 | 2 | 2 | 1 | 14.73333333 |
| 153 | H | 2 | 1 | 2 | 3 | 1 | 13 |
| 154 | H | 2 | 1 | 2 | 4 | 1 | 16.66666667 |
| 155 | H | 2 | 1 | 2 | 5 | 1 | 19 |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| 156 | H | 2 | 1 | 2 | 6 | 1 | 16.75 |
| 157 | H | 2 | 1 | 2 | 7 | 1 | 15.5 |
| 158 | H | 2 | 1 | 2 | 8 | 1 | 15.03333333 |
| 159 | H | 2 | 1 | 2 | 9 | 1 | 1 |
| 160 | H | 2 | 1 | 2 | 10 | 1 | * |
| 161 | H | 2 | 1 | 3 | 1 | 1 | 13 |
| 162 | H | 2 | 1 | 3 | 2 | 1 | 8.833333333 |
| 163 | H | 2 | 1 | 3 | 3 | 1 | 13.66666667 |
| 164 | H | 2 | 1 | 3 | 4 | 1 | 16.25 |
| 165 | H | 2 | 1 | 3 | 5 | 1 | 13.25 |
| 166 | H | 2 | 1 | 3 | 6 | 1 | 9 |
| 167 | H | 2 | 1 | 3 | 7 | 1 | 13.5 |
| 168 | H | 2 | 1 | 3 | 8 | 1 | 14.5 |
| 169 | H | 2 | 1 | 3 | 9 | 1 | 13.25 |
| 170 | H | 2 | 1 | 3 | 10 | 1 | * |
| 171 | H | 3 | 1 | 1 | 1 | 1 | 13 |
| 172 | H | 3 | 1 | 1 | 2 | 1 | 10.5 |
| 173 | H | 3 | 1 | 1 | 3 | 1 | 13.4 |
| 174 | H | 3 | 1 | 1 | 4 | 1 | 7.325 |
| 175 | H | 3 | 1 | 1 | 5 | 1 | 18.1 |
| 176 | H | 3 | 1 | 1 | 6 | 1 | 15.25 |
| 177 | H | 3 | 1 | 1 | 7 | 1 | 12.75 |
| 178 | H | 3 | 1 | 1 | 8 | 1 | 13.15 |
| 179 | H | 3 | 1 | 1 | 9 | 1 | 16.75 |
| 180 | H | 3 | 1 | 1 | 10 | 1 | 17.25 |
| 181 | H | 3 | 1 | 2 | 1 | 1 | 14.5 |
| 182 | H | 3 | 1 | 2 | 2 | 1 | 12.75 |
| 183 | H | 3 | 1 | 2 | 3 | 1 | 17.25 |
| 184 | H | 3 | 1 | 2 | 4 | 1 | 7.45 |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| 185 | H | 3 | 1 | 2 | 5 | 1 | 14.5 |
| 186 | H | 3 | 1 | 2 | 6 | 1 | 14.5 |
| 187 | H | 3 | 1 | 2 | 7 | 1 | 13 |
| 188 | H | 3 | 1 | 2 | 8 | 1 | 15.75 |
| 189 | H | 3 | 1 | 2 | 9 | 1 | * |
| 190 | H | 3 | 1 | 2 | 10 | 1 | * |
| 191 | H | 3 | 1 | 3 | 1 | 1 | 0.5 |
| 192 | H | 3 | 1 | 3 | 2 | 1 | 15.5 |
| 193 | H | 3 | 1 | 3 | 3 | 1 | 14 |
| 194 | H | 3 | 1 | 3 | 4 | 1 | 17.5 |
| 195 | H | 3 | 1 | 3 | 5 | 1 | 10.75 |
| 196 | H | 3 | 1 | 3 | 6 | 1 | 17.25 |
| 197 | H | 3 | 1 | 3 | 7 | 1 | 14 |
| 198 | H | 3 | 1 | 3 | 8 | 1 | 17.5 |
| 199 | H | 3 | 1 | 3 | 9 | 1 | * |
| 200 | H | 3 | 1 | 3 | 10 | 1 | * |
| 201 | H | 4 | 2 | 4 | 1 | 1 | 12.25 |
| 202 | H | 4 | 2 | 4 | 2 | 1 | 11.33333333 |
| 203 | H | 4 | 2 | 4 | 3 | 1 | 14.5 |
| 204 | H | 4 | 2 | 4 | 4 | 1 | 6.75 |
| 205 | H | 4 | 2 | 4 | 5 | 1 | 14.66666667 |
| 206 | H | 4 | 2 | 4 | 6 | 1 | 12.63333333 |
| 207 | H | 4 | 2 | 4 | 7 | 1 | 17.9 |
| 208 | H | 4 | 2 | 4 | 8 | 1 | 14.45 |
| 209 | H | 4 | 2 | 4 | 9 | 1 | 17.5 |
| 210 | H | 4 | 2 | 4 | 10 | 1 | * |
| 211 | H | 4 | 2 | 4 | 1 | 2 | 20 |
| 212 | H | 4 | 2 | 4 | 2 | 2 | 16.33333333 |
| 213 | H | 4 | 2 | 4 | 3 | 2 | 15.75 |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| 214 | H | 4 | 2 | 4 | 4 | 2 | 20 |
| 215 | H | 4 | 2 | 4 | 5 | 2 | 15.75 |
| 216 | H | 4 | 2 | 4 | 6 | 2 | 12.75 |
| 217 | H | 4 | 2 | 4 | 7 | 2 | 14.56666667 |
| 218 | H | 4 | 2 | 4 | 8 | 2 | 12.83333333 |
| 219 | H | 4 | 2 | 4 | 9 | 2 | 15.25 |
| 220 | H | 4 | 2 | 4 | 10 | 2 | 9.833333333 |

**Table b: root length raw data.** Isolate 1 is 25R/7, isolate 2 is 30R/11, isolate 3 is the mix of 6 antagonistic isolates, isolate 4 sterile; coating 1 is seeds coated, coating 2 non-bacterial coated seeds; conc.1 is OD1, conc.2 is OD0.5, conc. 3 is OD 0.1; Ggt 1: present, 2: absent; and rep are the 10 replicates per treatment. * refers to missing values (no data).

| No. | Cultivar | Isolate | Coating | Conc | Rep | Ggt | root (cm) |
|---|---|---|---|---|---|---|---|
| 1 | C | 1 | 1 | 1 | 1 | 1 | 22 |
| 2 | C | 1 | 1 | 1 | 2 | 1 | 21.5 |
| 3 | C | 1 | 1 | 1 | 3 | 1 | 18 |
| 4 | C | 1 | 1 | 1 | 4 | 1 | 24 |
| 5 | C | 1 | 1 | 1 | 5 | 1 | 23 |
| 6 | C | 1 | 1 | 1 | 6 | 1 | 16 |
| 7 | C | 1 | 1 | 1 | 7 | 1 | 17 |
| 8 | C | 1 | 1 | 1 | 8 | 1 | 21.5 |
| 9 | C | 1 | 1 | 1 | 9 | 1 | 20 |
| 10 | C | 1 | 1 | 1 | 10 | 1 | 18 |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| 11 | C | 1 | 1 | 2 | 1 | 1 | 30 |
| 12 | C | 1 | 1 | 2 | 2 | 1 | 18 |
| 13 | C | 1 | 1 | 2 | 3 | 1 | 19 |
| 14 | C | 1 | 1 | 2 | 4 | 1 | 17.5 |
| 15 | C | 1 | 1 | 2 | 5 | 1 | 19 |
| 16 | C | 1 | 1 | 2 | 6 | 1 | 20 |
| 17 | C | 1 | 1 | 2 | 7 | 1 | 16 |
| 18 | C | 1 | 1 | 2 | 8 | 1 | 15 |
| 19 | C | 1 | 1 | 2 | 9 | 1 | 18 |
| 20 | C | 1 | 1 | 2 | 10 | 1 | * |
| 21 | C | 1 | 1 | 3 | 1 | 1 | 22 |
| 22 | C | 1 | 1 | 3 | 2 | 1 | 18 |
| 23 | C | 1 | 1 | 3 | 3 | 1 | 20 |
| 24 | C | 1 | 1 | 3 | 4 | 1 | 20 |
| 25 | C | 1 | 1 | 3 | 5 | 1 | 27 |
| 26 | C | 1 | 1 | 3 | 6 | 1 | 18 |
| 27 | C | 1 | 1 | 3 | 7 | 1 | 17.5 |
| 28 | C | 1 | 1 | 3 | 8 | 1 | 27 |
| 29 | C | 1 | 1 | 3 | 9 | 1 | * |
| 30 | C | 1 | 1 | 3 | 10 | 1 | * |
| 31 | C | 2 | 1 | 1 | 1 | 1 | 25 |
| 32 | C | 2 | 1 | 1 | 2 | 1 | 16 |
| 33 | C | 2 | 1 | 1 | 3 | 1 | 24 |
| 34 | C | 2 | 1 | 1 | 4 | 1 | 19 |
| 35 | C | 2 | 1 | 1 | 5 | 1 | 26.5 |
| 36 | C | 2 | 1 | 1 | 6 | 1 | 17 |
| 37 | C | 2 | 1 | 1 | 7 | 1 | 22 |
| 38 | C | 2 | 1 | 1 | 8 | 1 | 20 |
| 39 | C | 2 | 1 | 1 | 9 | 1 | 24 |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| 40 | C | 2 | 1 | 1 | 10 | 1 | 18 |
| 41 | C | 2 | 1 | 2 | 1 | 1 | 22.5 |
| 42 | C | 2 | 1 | 2 | 2 | 1 | 21 |
| 43 | C | 2 | 1 | 2 | 3 | 1 | 7 |
| 44 | C | 2 | 1 | 2 | 4 | 1 | 17 |
| 45 | C | 2 | 1 | 2 | 5 | 1 | 18 |
| 46 | C | 2 | 1 | 2 | 6 | 1 | 13.5 |
| 47 | C | 2 | 1 | 2 | 7 | 1 | 21 |
| 48 | C | 2 | 1 | 2 | 8 | 1 | 23 |
| 49 | C | 2 | 1 | 2 | 9 | 1 | 19 |
| 50 | C | 2 | 1 | 2 | 10 | 1 | * |
| 51 | C | 2 | 1 | 3 | 1 | 1 | 17 |
| 52 | C | 2 | 1 | 3 | 2 | 1 | 16 |
| 53 | C | 2 | 1 | 3 | 3 | 1 | 37 |
| 54 | C | 2 | 1 | 3 | 4 | 1 | 16 |
| 55 | C | 2 | 1 | 3 | 5 | 1 | 15 |
| 56 | C | 2 | 1 | 3 | 6 | 1 | 25 |
| 57 | C | 2 | 1 | 3 | 7 | 1 | 22 |
| 58 | C | 2 | 1 | 3 | 8 | 1 | 16.5 |
| 59 | C | 2 | 1 | 3 | 9 | 1 | * |
| 60 | C | 2 | 1 | 3 | 10 | 1 | * |
| 61 | C | 3 | 1 | 1 | 1 | 1 | 0.5 |
| 62 | C | 3 | 1 | 1 | 2 | 1 | 25 |
| 63 | C | 3 | 1 | 1 | 3 | 1 | 26 |
| 64 | C | 3 | 1 | 1 | 4 | 1 | 24 |
| 65 | C | 3 | 1 | 1 | 5 | 1 | 19 |
| 66 | C | 3 | 1 | 1 | 6 | 1 | 27 |
| 67 | C | 3 | 1 | 1 | 7 | 1 | 14.5 |
| 68 | C | 3 | 1 | 1 | 8 | 1 | 18 |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| 69 | C | 3 | 1 | 1 | 9 | 1 | 25 |
| 70 | C | 3 | 1 | 1 | 10 | 1 | 21 |
| 71 | C | 3 | 1 | 2 | 1 | 1 | 32 |
| 72 | C | 3 | 1 | 2 | 2 | 1 | 28 |
| 73 | C | 3 | 1 | 2 | 3 | 1 | 30 |
| 74 | C | 3 | 1 | 2 | 4 | 1 | 18 |
| 75 | C | 3 | 1 | 2 | 5 | 1 | 21.5 |
| 76 | C | 3 | 1 | 2 | 6 | 1 | 15 |
| 77 | C | 3 | 1 | 2 | 7 | 1 | 23 |
| 78 | C | 3 | 1 | 2 | 8 | 1 | 17 |
| 79 | C | 3 | 1 | 2 | 9 | 1 | 24 |
| 80 | C | 3 | 1 | 2 | 10 | 1 | 23.5 |
| 81 | C | 3 | 1 | 3 | 1 | 1 | 7 |
| 82 | C | 3 | 1 | 3 | 2 | 1 | 29 |
| 83 | C | 3 | 1 | 3 | 3 | 1 | 29 |
| 84 | C | 3 | 1 | 3 | 4 | 1 | 11.5 |
| 85 | C | 3 | 1 | 3 | 5 | 1 | 21 |
| 86 | C | 3 | 1 | 3 | 6 | 1 | 16 |
| 87 | C | 3 | 1 | 3 | 7 | 1 | 12 |
| 88 | C | 3 | 1 | 3 | 8 | 1 | 16 |
| 89 | C | 3 | 1 | 3 | 9 | 1 | 22 |
| 90 | C | 3 | 1 | 3 | 10 | 1 | 31 |
| 91 | C | 4 | 2 | 4 | 1 | 1 | 13 |
| 92 | C | 4 | 2 | 4 | 2 | 1 | 12 |
| 93 | C | 4 | 2 | 4 | 3 | 1 | 13 |
| 94 | C | 4 | 2 | 4 | 4 | 1 | 13 |
| 95 | C | 4 | 2 | 4 | 5 | 1 | 19 |
| 96 | C | 4 | 2 | 4 | 6 | 1 | 18 |
| 97 | C | 4 | 2 | 4 | 7 | 1 | 16 |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| 98 | C | 4 | 2 | 4 | 8 | 1 | 6 |
| 99 | C | 4 | 2 | 4 | 9 | 1 | 12 |
| 100 | C | 4 | 2 | 4 | 10 | 1 | * |
| 101 | C | 4 | 2 | 4 | 1 | 2 | 30 |
| 102 | C | 4 | 2 | 4 | 2 | 2 | 8 |
| 103 | C | 4 | 2 | 4 | 3 | 2 | 13 |
| 104 | C | 4 | 2 | 4 | 4 | 2 | 17 |
| 105 | C | 4 | 2 | 4 | 5 | 2 | 24 |
| 106 | C | 4 | 2 | 4 | 6 | 2 | 25 |
| 107 | C | 4 | 2 | 4 | 7 | 2 | 18 |
| 108 | C | 4 | 2 | 4 | 8 | 2 | 23.5 |
| 109 | C | 4 | 2 | 4 | 9 | 2 | 24 |
| 110 | C | 4 | 2 | 4 | 10 | 2 | 17 |
| 111 | H | 1 | 1 | 1 | 1 | 1 | 25 |
| 112 | H | 1 | 1 | 1 | 2 | 1 | 15 |
| 113 | H | 1 | 1 | 1 | 3 | 1 | 20 |
| 114 | H | 1 | 1 | 1 | 4 | 1 | 19 |
| 115 | H | 1 | 1 | 1 | 5 | 1 | 21.5 |
| 116 | H | 1 | 1 | 1 | 6 | 1 | 14 |
| 117 | H | 1 | 1 | 1 | 7 | 1 | 19.5 |
| 118 | H | 1 | 1 | 1 | 8 | 1 | 21.5 |
| 119 | H | 1 | 1 | 1 | 9 | 1 | * |
| 120 | H | 1 | 1 | 1 | 10 | 1 | * |
| 121 | H | 1 | 1 | 2 | 1 | 1 | 24 |
| 122 | H | 1 | 1 | 2 | 2 | 1 | 16 |
| 123 | H | 1 | 1 | 2 | 3 | 1 | 30 |
| 124 | H | 1 | 1 | 2 | 4 | 1 | 15 |
| 125 | H | 1 | 1 | 2 | 5 | 1 | 21 |
| 126 | H | 1 | 1 | 2 | 6 | 1 | 18 |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| 127 | H | 1 | 1 | 2 | 7 | 1 | * |
| 128 | H | 1 | 1 | 2 | 8 | 1 | * |
| 129 | H | 1 | 1 | 2 | 9 | 1 | * |
| 130 | H | 1 | 1 | 2 | 10 | 1 | * |
| 131 | H | 1 | 1 | 3 | 1 | 1 | 12 |
| 132 | H | 1 | 1 | 3 | 2 | 1 | 6 |
| 133 | H | 1 | 1 | 3 | 3 | 1 | 11.5 |
| 134 | H | 1 | 1 | 3 | 4 | 1 | 12 |
| 135 | H | 1 | 1 | 3 | 5 | 1 | 17 |
| 136 | H | 1 | 1 | 3 | 6 | 1 | 12 |
| 137 | H | 1 | 1 | 3 | 7 | 1 | * |
| 138 | H | 1 | 1 | 3 | 8 | 1 | * |
| 139 | H | 1 | 1 | 3 | 9 | 1 | * |
| 140 | H | 1 | 1 | 3 | 10 | 1 | * |
| 141 | H | 2 | 1 | 1 | 1 | 1 | 24 |
| 142 | H | 2 | 1 | 1 | 2 | 1 | 19 |
| 143 | H | 2 | 1 | 1 | 3 | 1 | 23 |
| 144 | H | 2 | 1 | 1 | 4 | 1 | 20 |
| 145 | H | 2 | 1 | 1 | 5 | 1 | 30.2 |
| 146 | H | 2 | 1 | 1 | 6 | 1 | 31 |
| 147 | H | 2 | 1 | 1 | 7 | 1 | 25 |
| 148 | H | 2 | 1 | 1 | 8 | 1 | 14.5 |
| 149 | H | 2 | 1 | 1 | 9 | 1 | 15 |
| 150 | H | 2 | 1 | 1 | 10 | 1 | * |
| 151 | H | 2 | 1 | 2 | 1 | 1 | 18.5 |
| 152 | H | 2 | 1 | 2 | 2 | 1 | 15 |
| 153 | H | 2 | 1 | 2 | 3 | 1 | 15 |
| 154 | H | 2 | 1 | 2 | 4 | 1 | 16 |
| 155 | H | 2 | 1 | 2 | 5 | 1 | 22.5 |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| 156 | H | 2 | 1 | 2 | 6 | 1 | 16.5 |
| 157 | H | 2 | 1 | 2 | 7 | 1 | 21 |
| 158 | H | 2 | 1 | 2 | 8 | 1 | 20.5 |
| 159 | H | 2 | 1 | 2 | 9 | 1 | 16 |
| 160 | H | 2 | 1 | 2 | 10 | 1 | * |
| 161 | H | 2 | 1 | 3 | 1 | 1 | 17.5 |
| 162 | H | 2 | 1 | 3 | 2 | 1 | 26 |
| 163 | H | 2 | 1 | 3 | 3 | 1 | 20 |
| 164 | H | 2 | 1 | 3 | 4 | 1 | 23 |
| 165 | H | 2 | 1 | 3 | 5 | 1 | 24 |
| 166 | H | 2 | 1 | 3 | 6 | 1 | 30 |
| 167 | H | 2 | 1 | 3 | 7 | 1 | 23 |
| 168 | H | 2 | 1 | 3 | 8 | 1 | 23 |
| 169 | H | 2 | 1 | 3 | 9 | 1 | 17.5 |
| 170 | H | 2 | 1 | 3 | 10 | 1 | * |
| 171 | H | 3 | 1 | 1 | 1 | 1 | 21 |
| 172 | H | 3 | 1 | 1 | 2 | 1 | 26 |
| 173 | H | 3 | 1 | 1 | 3 | 1 | 12 |
| 174 | H | 3 | 1 | 1 | 4 | 1 | 15 |
| 175 | H | 3 | 1 | 1 | 5 | 1 | 26 |
| 176 | H | 3 | 1 | 1 | 6 | 1 | 21.4 |
| 177 | H | 3 | 1 | 1 | 7 | 1 | 23 |
| 178 | H | 3 | 1 | 1 | 8 | 1 | 28 |
| 179 | H | 3 | 1 | 1 | 9 | 1 | 25 |
| 180 | H | 3 | 1 | 1 | 10 | 1 | 23 |
| 181 | H | 3 | 1 | 2 | 1 | 1 | 24 |
| 182 | H | 3 | 1 | 2 | 2 | 1 | 30 |
| 183 | H | 3 | 1 | 2 | 3 | 1 | 28 |
| 184 | H | 3 | 1 | 2 | 4 | 1 | 15.5 |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| 185 | H | 3 | 1 | 2 | 5 | 1 | 31 |
| 186 | H | 3 | 1 | 2 | 6 | 1 | 25 |
| 187 | H | 3 | 1 | 2 | 7 | 1 | 30 |
| 188 | H | 3 | 1 | 2 | 8 | 1 | 16 |
| 189 | H | 3 | 1 | 2 | 9 | 1 | * |
| 190 | H | 3 | 1 | 2 | 10 | 1 | * |
| 191 | H | 3 | 1 | 3 | 1 | 1 | 1.5 |
| 192 | H | 3 | 1 | 3 | 2 | 1 | 22 |
| 193 | H | 3 | 1 | 3 | 3 | 1 | 30 |
| 194 | H | 3 | 1 | 3 | 4 | 1 | 23 |
| 195 | H | 3 | 1 | 3 | 5 | 1 | 23 |
| 196 | H | 3 | 1 | 3 | 6 | 1 | 31.5 |
| 197 | H | 3 | 1 | 3 | 7 | 1 | 21 |
| 198 | H | 3 | 1 | 3 | 8 | 1 | 17 |
| 199 | H | 3 | 1 | 3 | 9 | 1 | * |
| 200 | H | 3 | 1 | 3 | 10 | 1 | * |
| 201 | H | 4 | 2 | 4 | 1 | 1 | 22.5 |
| 202 | H | 4 | 2 | 4 | 2 | 1 | 24 |
| 203 | H | 4 | 2 | 4 | 3 | 1 | 15.5 |
| 204 | H | 4 | 2 | 4 | 4 | 1 | 15 |
| 205 | H | 4 | 2 | 4 | 5 | 1 | 24 |
| 206 | H | 4 | 2 | 4 | 6 | 1 | 30 |
| 207 | H | 4 | 2 | 4 | 7 | 1 | 21.5 |
| 208 | H | 4 | 2 | 4 | 8 | 1 | 27 |
| 209 | H | 4 | 2 | 4 | 9 | 1 | 23 |
| 210 | H | 4 | 2 | 4 | 10 | 1 | * |
| 211 | H | 4 | 2 | 4 | 1 | 2 | 21 |
| 212 | H | 4 | 2 | 4 | 2 | 2 | 23.5 |
| 213 | H | 4 | 2 | 4 | 3 | 2 | 24 |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| 214 | H | 4 | 2 | 4 | 4 | 2 | 27 |
| 215 | H | 4 | 2 | 4 | 5 | 2 | 24 |
| 216 | H | 4 | 2 | 4 | 6 | 2 | 24 |
| 217 | H | 4 | 2 | 4 | 7 | 2 | 22 |
| 218 | H | 4 | 2 | 4 | 8 | 2 | 25 |
| 219 | H | 4 | 2 | 4 | 9 | 2 | 25 |
| 220 | H | 4 | 2 | 4 | 10 | 2 | 25 |

**Table c: fresh weight raw data.** Isolate 1 is 25R/7, isolate 2 is 30R/11, isolate 3 is the mix of 6 antagonistic isolates, isolate 4 sterile; coating 1 is seeds coated, coating 2 non-bacterial coated seeds; conc.1 is OD1, conc.2 is OD0.5, conc. 3 is OD 0.1; Ggt 1:present, 2: absent; and rep are the 10 replicates per treatment. * refers to missing values (no data).

| No. | Cultivar | Isolate | Coating | Conc | Rep | Ggt | Fresh weight (g) |
|---|---|---|---|---|---|---|---|
| 1 | C | 1 | 1 | 1 | 1 | 1 | 0.3063 |
| 2 | C | 1 | 1 | 1 | 2 | 1 | 0.4205 |
| 3 | C | 1 | 1 | 1 | 3 | 1 | 0.3393 |
| 4 | C | 1 | 1 | 1 | 4 | 1 | 0.2672 |
| 5 | C | 1 | 1 | 1 | 5 | 1 | 0.438 |
| 6 | C | 1 | 1 | 1 | 6 | 1 | 0.397 |
| 7 | C | 1 | 1 | 1 | 7 | 1 | 0.3105 |
| 8 | C | 1 | 1 | 1 | 8 | 1 | 0.1718 |
| 9 | C | 1 | 1 | 1 | 9 | 1 | 0.4085 |
| 10 | C | 1 | 1 | 1 | 10 | 1 | 0.4073 |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| 11 | C | 1 | 1 | 2 | 1 | 1 | 0.2243 |
| 12 | C | 1 | 1 | 2 | 2 | 1 | 0.371 |
| 13 | C | 1 | 1 | 2 | 3 | 1 | 0.5545 |
| 14 | C | 1 | 1 | 2 | 4 | 1 | 0.2752 |
| 15 | C | 1 | 1 | 2 | 5 | 1 | 0.4626 |
| 16 | C | 1 | 1 | 2 | 6 | 1 | 0.4554 |
| 17 | C | 1 | 1 | 2 | 7 | 1 | 0.2808 |
| 18 | C | 1 | 1 | 2 | 8 | 1 | 0.448 |
| 19 | C | 1 | 1 | 2 | 9 | 1 | 0.3888 |
| 20 | C | 1 | 1 | 2 | 10 | 1 | * |
| 21 | C | 1 | 1 | 3 | 1 | 1 | 0.1541 |
| 22 | C | 1 | 1 | 3 | 2 | 1 | 0.1918 |
| 23 | C | 1 | 1 | 3 | 3 | 1 | 0.2351 |
| 24 | C | 1 | 1 | 3 | 4 | 1 | 0.3754 |
| 25 | C | 1 | 1 | 3 | 5 | 1 | 0.3398 |
| 26 | C | 1 | 1 | 3 | 6 | 1 | 0.348 |
| 27 | C | 1 | 1 | 3 | 7 | 1 | 0.1914 |
| 28 | C | 1 | 1 | 3 | 8 | 1 | 0.107 |
| 29 | C | 1 | 1 | 3 | 9 | 1 | * |
| 30 | C | 1 | 1 | 3 | 10 | 1 | * |
| 31 | C | 2 | 1 | 1 | 1 | 1 | 0.3606 |
| 32 | C | 2 | 1 | 1 | 2 | 1 | 0.3346 |
| 33 | C | 2 | 1 | 1 | 3 | 1 | 0.3866 |
| 34 | C | 2 | 1 | 1 | 4 | 1 | 0.2797 |
| 35 | C | 2 | 1 | 1 | 5 | 1 | 0.251 |
| 36 | C | 2 | 1 | 1 | 6 | 1 | 0.1846 |
| 37 | C | 2 | 1 | 1 | 7 | 1 | 0.1381 |
| 38 | C | 2 | 1 | 1 | 8 | 1 | 0.3149 |
| 39 | C | 2 | 1 | 1 | 9 | 1 | 0.2644 |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| 40 | C | 2 | 1 | 1 | 10 | 1 | 0.263 |
| 41 | C | 2 | 1 | 2 | 1 | 1 | 0.1532 |
| 42 | C | 2 | 1 | 2 | 2 | 1 | 0.4557 |
| 43 | C | 2 | 1 | 2 | 3 | 1 | 0.0914 |
| 44 | C | 2 | 1 | 2 | 4 | 1 | 0.288 |
| 45 | C | 2 | 1 | 2 | 5 | 1 | 0.2503 |
| 46 | C | 2 | 1 | 2 | 6 | 1 | 0.1908 |
| 47 | C | 2 | 1 | 2 | 7 | 1 | 0.5353 |
| 48 | C | 2 | 1 | 2 | 8 | 1 | 0.6175 |
| 49 | C | 2 | 1 | 2 | 9 | 1 | 0.5538 |
| 50 | C | 2 | 1 | 2 | 10 | 1 | * |
| 51 | C | 2 | 1 | 3 | 1 | 1 | 0.1424 |
| 52 | C | 2 | 1 | 3 | 2 | 1 | 0.1756 |
| 53 | C | 2 | 1 | 3 | 3 | 1 | 0.328 |
| 54 | C | 2 | 1 | 3 | 4 | 1 | 0.152 |
| 55 | C | 2 | 1 | 3 | 5 | 1 | 0.2775 |
| 56 | C | 2 | 1 | 3 | 6 | 1 | 0.2174 |
| 57 | C | 2 | 1 | 3 | 7 | 1 | 0.1886 |
| 58 | C | 2 | 1 | 3 | 8 | 1 | 0.4015 |
| 59 | C | 2 | 1 | 3 | 9 | 1 | * |
| 60 | C | 2 | 1 | 3 | 10 | 1 | * |
| 61 | C | 3 | 1 | 1 | 1 | 1 | 0.1146 |
| 62 | C | 3 | 1 | 1 | 2 | 1 | 0.4293 |
| 63 | C | 3 | 1 | 1 | 3 | 1 | 0.4591 |
| 64 | C | 3 | 1 | 1 | 4 | 1 | 0.4863 |
| 65 | C | 3 | 1 | 1 | 5 | 1 | 0.2421 |
| 66 | C | 3 | 1 | 1 | 6 | 1 | 0.537 |
| 67 | C | 3 | 1 | 1 | 7 | 1 | 0.4112 |
| 68 | C | 3 | 1 | 1 | 8 | 1 | 0.6492 |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| 69 | C | 3 | 1 | 1 | 9 | 1 | 0.6649 |
| 70 | C | 3 | 1 | 1 | 10 | 1 | 0.6423 |
| 71 | C | 3 | 1 | 2 | 1 | 1 | 0.4968 |
| 72 | C | 3 | 1 | 2 | 2 | 1 | 0.3248 |
| 73 | C | 3 | 1 | 2 | 3 | 1 | 0.2948 |
| 74 | C | 3 | 1 | 2 | 4 | 1 | 0.1698 |
| 75 | C | 3 | 1 | 2 | 5 | 1 | 0.1554 |
| 76 | C | 3 | 1 | 2 | 6 | 1 | 0.3155 |
| 77 | C | 3 | 1 | 2 | 7 | 1 | 0.4445 |
| 78 | C | 3 | 1 | 2 | 8 | 1 | 0.1901 |
| 79 | C | 3 | 1 | 2 | 9 | 1 | 0.2969 |
| 80 | C | 3 | 1 | 2 | 10 | 1 | 0.2051 |
| 81 | C | 3 | 1 | 3 | 1 | 1 | 0.3724 |
| 82 | C | 3 | 1 | 3 | 2 | 1 | 0.5204 |
| 83 | C | 3 | 1 | 3 | 3 | 1 | 0.1531 |
| 84 | C | 3 | 1 | 3 | 4 | 1 | 0.4189 |
| 85 | C | 3 | 1 | 3 | 5 | 1 | 0.2183 |
| 86 | C | 3 | 1 | 3 | 6 | 1 | 0.1599 |
| 87 | C | 3 | 1 | 3 | 7 | 1 | 0.1617 |
| 88 | C | 3 | 1 | 3 | 8 | 1 | 0.0832 |
| 89 | C | 3 | 1 | 3 | 9 | 1 | 0.0854 |
| 90 | C | 3 | 1 | 3 | 10 | 1 | 0.1793 |
| 91 | C | 4 | 2 | 4 | 1 | 1 | 0.1464 |
| 92 | C | 4 | 2 | 4 | 2 | 1 | 0.1835 |
| 93 | C | 4 | 2 | 4 | 3 | 1 | 0.1416 |
| 94 | C | 4 | 2 | 4 | 4 | 1 | 0.0647 |
| 95 | C | 4 | 2 | 4 | 5 | 1 | 0.165 |
| 96 | C | 4 | 2 | 4 | 6 | 1 | 0.1809 |
| 97 | C | 4 | 2 | 4 | 7 | 1 | 0.2489 |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| 98 | C | 4 | 2 | 4 | 8 | 1 | 0.3174 |
| 99 | C | 4 | 2 | 4 | 9 | 1 | 0.2265 |
| 100 | C | 4 | 2 | 4 | 10 | 1 | * |
| 101 | C | 4 | 2 | 4 | 1 | 2 | 0.2676 |
| 102 | C | 4 | 2 | 4 | 2 | 2 | 0.088 |
| 103 | C | 4 | 2 | 4 | 3 | 2 | 0.2316 |
| 104 | C | 4 | 2 | 4 | 4 | 2 | 0.2593 |
| 105 | C | 4 | 2 | 4 | 5 | 2 | 0.2748 |
| 106 | C | 4 | 2 | 4 | 6 | 2 | 0.2255 |
| 107 | C | 4 | 2 | 4 | 7 | 2 | 0.2388 |
| 108 | C | 4 | 2 | 4 | 8 | 2 | 0.3132 |
| 109 | C | 4 | 2 | 4 | 9 | 2 | 0.4161 |
| 110 | C | 4 | 2 | 4 | 10 | 2 | 0.3847 |
| 111 | H | 1 | 1 | 1 | 1 | 1 | 0.2163 |
| 112 | H | 1 | 1 | 1 | 2 | 1 | 0.3055 |
| 113 | H | 1 | 1 | 1 | 3 | 1 | 0.1503 |
| 114 | H | 1 | 1 | 1 | 4 | 1 | 0.154 |
| 115 | H | 1 | 1 | 1 | 5 | 1 | 0.2006 |
| 116 | H | 1 | 1 | 1 | 6 | 1 | 0.3307 |
| 117 | H | 1 | 1 | 1 | 7 | 1 | 0.4788 |
| 118 | H | 1 | 1 | 1 | 8 | 1 | 0.3873 |
| 119 | H | 1 | 1 | 1 | 9 | 1 | * |
| 120 | H | 1 | 1 | 1 | 10 | 1 | * |
| 121 | H | 1 | 1 | 2 | 1 | 1 | 0.1327 |
| 122 | H | 1 | 1 | 2 | 2 | 1 | 0.3974 |
| 123 | H | 1 | 1 | 2 | 3 | 1 | 0.2243 |
| 124 | H | 1 | 1 | 2 | 4 | 1 | 0.3257 |
| 125 | H | 1 | 1 | 2 | 5 | 1 | 0.7162 |
| 126 | H | 1 | 1 | 2 | 6 | 1 | 0.247 |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| 127 | H | 1 | 1 | 2 | 7 | 1 | * |
| 128 | H | 1 | 1 | 2 | 8 | 1 | * |
| 129 | H | 1 | 1 | 2 | 9 | 1 | * |
| 130 | H | 1 | 1 | 2 | 10 | 1 | * |
| 131 | H | 1 | 1 | 3 | 1 | 1 | 0.1242 |
| 132 | H | 1 | 1 | 3 | 2 | 1 | 0.075 |
| 133 | H | 1 | 1 | 3 | 3 | 1 | 0.1455 |
| 134 | H | 1 | 1 | 3 | 4 | 1 | 0.2249 |
| 135 | H | 1 | 1 | 3 | 5 | 1 | 0.2762 |
| 136 | H | 1 | 1 | 3 | 6 | 1 | 0.1145 |
| 137 | H | 1 | 1 | 3 | 7 | 1 | * |
| 138 | H | 1 | 1 | 3 | 8 | 1 | * |
| 139 | H | 1 | 1 | 3 | 9 | 1 | * |
| 140 | H | 1 | 1 | 3 | 10 | 1 | * |
| 141 | H | 2 | 1 | 1 | 1 | 1 | 0.1277 |
| 142 | H | 2 | 1 | 1 | 2 | 1 | 0.5251 |
| 143 | H | 2 | 1 | 1 | 3 | 1 | 0.1785 |
| 144 | H | 2 | 1 | 1 | 4 | 1 | 0.42 |
| 145 | H | 2 | 1 | 1 | 5 | 1 | 0.4403 |
| 146 | H | 2 | 1 | 1 | 6 | 1 | 0.2914 |
| 147 | H | 2 | 1 | 1 | 7 | 1 | 0.4172 |
| 148 | H | 2 | 1 | 1 | 8 | 1 | 0.4087 |
| 149 | H | 2 | 1 | 1 | 9 | 1 | 0.3244 |
| 150 | H | 2 | 1 | 1 | 10 | 1 | * |
| 151 | H | 2 | 1 | 2 | 1 | 1 | 0.3518 |
| 152 | H | 2 | 1 | 2 | 2 | 1 | 0.1989 |
| 153 | H | 2 | 1 | 2 | 3 | 1 | 0.1663 |
| 154 | H | 2 | 1 | 2 | 4 | 1 | 0.1906 |
| 155 | H | 2 | 1 | 2 | 5 | 1 | 0.3658 |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| 156 | H | 2 | 1 | 2 | 6 | 1 | 0.2481 |
| 157 | H | 2 | 1 | 2 | 7 | 1 | 0.2995 |
| 158 | H | 2 | 1 | 2 | 8 | 1 | 0.2312 |
| 159 | H | 2 | 1 | 2 | 9 | 1 | 0.0457 |
| 160 | H | 2 | 1 | 2 | 10 | 1 | * |
| 161 | H | 2 | 1 | 3 | 1 | 1 | 0.4162 |
| 162 | H | 2 | 1 | 3 | 2 | 1 | 0.25 |
| 163 | H | 2 | 1 | 3 | 3 | 1 | 0.5149 |
| 164 | H | 2 | 1 | 3 | 4 | 1 | 0.1584 |
| 165 | H | 2 | 1 | 3 | 5 | 1 | 0.2104 |
| 166 | H | 2 | 1 | 3 | 6 | 1 | 0.4718 |
| 167 | H | 2 | 1 | 3 | 7 | 1 | 0.2272 |
| 168 | H | 2 | 1 | 3 | 8 | 1 | 0.2872 |
| 169 | H | 2 | 1 | 3 | 9 | 1 | 0.3982 |
| 170 | H | 2 | 1 | 3 | 10 | 1 | * |
| 171 | H | 3 | 1 | 1 | 1 | 1 | 0.2331 |
| 172 | H | 3 | 1 | 1 | 2 | 1 | 0.2005 |
| 173 | H | 3 | 1 | 1 | 3 | 1 | 0.225 |
| 174 | H | 3 | 1 | 1 | 4 | 1 | 0.2245 |
| 175 | H | 3 | 1 | 1 | 5 | 1 | 0.3627 |
| 176 | H | 3 | 1 | 1 | 6 | 1 | 0.2217 |
| 177 | H | 3 | 1 | 1 | 7 | 1 | 0.3812 |
| 178 | H | 3 | 1 | 1 | 8 | 1 | 0.3886 |
| 179 | H | 3 | 1 | 1 | 9 | 1 | 0.2503 |
| 180 | H | 3 | 1 | 1 | 10 | 1 | 0.4241 |
| 181 | H | 3 | 1 | 2 | 1 | 1 | 0.1688 |
| 182 | H | 3 | 1 | 2 | 2 | 1 | 0.2308 |
| 183 | H | 3 | 1 | 2 | 3 | 1 | 0.1464 |
| 184 | H | 3 | 1 | 2 | 4 | 1 | 0.3306 |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| 185 | H | 3 | 1 | 2 | 5 | 1 | 0.4216 |
| 186 | H | 3 | 1 | 2 | 6 | 1 | 0.5585 |
| 187 | H | 3 | 1 | 2 | 7 | 1 | 0.2473 |
| 188 | H | 3 | 1 | 2 | 8 | 1 | 0.4472 |
| 189 | H | 3 | 1 | 2 | 9 | 1 | * |
| 190 | H | 3 | 1 | 2 | 10 | 1 | * |
| 191 | H | 3 | 1 | 3 | 1 | 1 | 0.4211 |
| 192 | H | 3 | 1 | 3 | 2 | 1 | 0.438 |
| 193 | H | 3 | 1 | 3 | 3 | 1 | 0.2375 |
| 194 | H | 3 | 1 | 3 | 4 | 1 | 0.3671 |
| 195 | H | 3 | 1 | 3 | 5 | 1 | 0.318 |
| 196 | H | 3 | 1 | 3 | 6 | 1 | 0.3243 |
| 197 | H | 3 | 1 | 3 | 7 | 1 | 0.3183 |
| 198 | H | 3 | 1 | 3 | 8 | 1 | 0.1627 |
| 199 | H | 3 | 1 | 3 | 9 | 1 | * |
| 200 | H | 3 | 1 | 3 | 10 | 1 | * |
| 201 | H | 4 | 2 | 4 | 1 | 1 | 0.1161 |
| 202 | H | 4 | 2 | 4 | 2 | 1 | 0.2399 |
| 203 | H | 4 | 2 | 4 | 3 | 1 | 0.3139 |
| 204 | H | 4 | 2 | 4 | 4 | 1 | 0.2312 |
| 205 | H | 4 | 2 | 4 | 5 | 1 | 0.4065 |
| 206 | H | 4 | 2 | 4 | 6 | 1 | 0.2443 |
| 207 | H | 4 | 2 | 4 | 7 | 1 | 0.259 |
| 208 | H | 4 | 2 | 4 | 8 | 1 | 0.5288 |
| 209 | H | 4 | 2 | 4 | 9 | 1 | 0.4558 |
| 210 | H | 4 | 2 | 4 | 10 | 1 | * |
| 211 | H | 4 | 2 | 4 | 1 | 2 | 0.3041 |
| 212 | H | 4 | 2 | 4 | 2 | 2 | 0.4613 |
| 213 | H | 4 | 2 | 4 | 3 | 2 | 0.5143 |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| 214 | H | 4 | 2 | 4 | 4 | 2 | 0.2885 |
| 215 | H | 4 | 2 | 4 | 5 | 2 | 0.4528 |
| 216 | H | 4 | 2 | 4 | 6 | 2 | 0.2955 |
| 217 | H | 4 | 2 | 4 | 7 | 2 | 0.3257 |
| 218 | H | 4 | 2 | 4 | 8 | 2 | 0.4256 |
| 219 | H | 4 | 2 | 4 | 9 | 2 | 0.2842 |
| 220 | H | 4 | 2 | 4 | 10 | 2 | 0.3581 |

**9.3.3 Appendix III: Infected roots data.** Isolate 1 is 25R/7, isolate 2 is 30R/11, isolate 3 is the mix of 6 antagonistic isolates, isolate 4 sterile; conc.1 is OD1, conc.2 is OD0.5, conc. 3 is OD 0.1; Ggt 1:present, Ggt 2: absent; Trt 22 identified treatments; and rep are the 10 replicates per treatment. * refers to missing values (no data).

| No. | !Cultivar | !isolate | !conc | !Rep | !Ggt | !Trt | %infected_roots | infected_present | !inf_p | Total no. of roots | infected roots |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | C | 1 | 1 | 1 | 1 | 1 | 50 | 1 | 1 | 6 | 3 |
| 2 | C | 1 | 1 | 2 | 1 | 1 | 60 | 1 | 1 | 5 | 3 |
| 3 | C | 1 | 1 | 3 | 1 | 1 | 100 | 1 | 1 | 6 | 6 |
| 4 | C | 1 | 1 | 4 | 1 | 1 | 100 | 1 | 1 | 6 | 6 |
| 5 | C | 1 | 1 | 5 | 1 | 1 | 100 | 1 | 1 | 5 | 5 |
| 6 | C | 1 | 1 | 6 | 1 | 1 | 33.33333333 | 1 | 1 | 9 | 3 |
| 7 | C | 1 | 1 | 7 | 1 | 1 | 100 | 1 | 1 | 6 | 6 |
| 8 | C | 1 | 1 | 8 | 1 | 1 | 33.33333333 | 1 | 1 | 6 | 2 |
| 9 | C | 1 | 1 | 9 | 1 | 1 | 100 | 1 | 1 | 7 | 7 |
| 10 | C | 1 | 1 | 10 | 1 | 1 | 75 | 1 | 1 | 4 | 3 |
| 11 | C | 1 | 2 | 1 | 1 | 2 | 100 | 1 | 1 | 3 | 3 |
| 12 | C | 1 | 2 | 2 | 1 | 2 | 100 | 1 | 1 | 7 | 7 |
| 13 | C | 1 | 2 | 3 | 1 | 2 | 100 | 1 | 1 | 4 | 4 |
| 14 | C | 1 | 2 | 4 | 1 | 2 | 100 | 1 | 1 | 7 | 7 |
| 15 | C | 1 | 2 | 5 | 1 | 2 | 66.66666667 | 1 | 1 | 3 | 2 |
| 16 | C | 1 | 2 | 6 | 1 | 2 | 100 | 1 | 1 | 7 | 7 |
| 17 | C | 1 | 2 | 7 | 1 | 2 | 14.28571429 | 1 | 1 | 7 | 1 |
| 18 | C | 1 | 2 | 8 | 1 | 2 | 100 | 1 | 1 | 5 | 5 |
| 19 | C | 1 | 2 | 9 | 1 | 2 | 0 | 0 | 0 | 4 | 0 |
| 20 | C | 1 | 2 | 10 | 1 | 2 | * | * | * | * | * |
| 21 | C | 1 | 3 | 1 | 1 | 3 | 100 | 1 | 1 | 5 | 5 |

| | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 22 | C | 1 | 3 | 2 | 1 | 3 | 100 | 1 | 1 | 6 | 6 |
| 23 | C | 1 | 3 | 3 | 1 | 3 | 100 | 1 | 1 | 5 | 5 |
| 24 | C | 1 | 3 | 4 | 1 | 3 | 0 | 0 | 0 | 5 | 0 |
| 25 | C | 1 | 3 | 5 | 1 | 3 | 100 | 1 | 1 | 1 | 1 |
| 26 | C | 1 | 3 | 6 | 1 | 3 | 100 | 1 | 1 | 3 | 3 |
| 27 | C | 1 | 3 | 7 | 1 | 3 | 100 | 1 | 1 | 3 | 3 |
| 28 | C | 1 | 3 | 8 | 1 | 3 | 0 | 0 | 0 | 4 | 0 |
| 29 | C | 1 | 3 | 9 | 1 | 3 | * | * | * | * | * |
| 30 | C | 1 | 3 | 10 | 1 | 3 | * | * | * | * | * |
| 31 | C | 2 | 1 | 1 | 1 | 4 | 100 | 1 | 1 | 5 | 5 |
| 32 | C | 2 | 1 | 2 | 1 | 4 | 100 | 1 | 1 | 5 | 5 |
| 33 | C | 2 | 1 | 3 | 1 | 4 | 50 | 1 | 1 | 6 | 3 |
| 34 | C | 2 | 1 | 4 | 1 | 4 | 60 | 1 | 1 | 5 | 3 |
| 35 | C | 2 | 1 | 5 | 1 | 4 | 33.33333333 | 1 | 1 | 6 | 2 |
| 36 | C | 2 | 1 | 6 | 1 | 4 | 80 | 1 | 1 | 5 | 4 |
| 37 | C | 2 | 1 | 7 | 1 | 4 | 100 | 1 | 1 | 4 | 4 |
| 38 | C | 2 | 1 | 8 | 1 | 4 | 100 | 1 | 1 | 3 | 3 |
| 39 | C | 2 | 1 | 9 | 1 | 4 | 100 | 1 | 1 | 5 | 5 |
| 40 | C | 2 | 1 | 10 | 1 | 4 | 80 | 1 | 1 | 5 | 4 |
| 41 | C | 2 | 2 | 1 | 1 | 5 | 40 | 1 | 1 | 5 | 2 |
| 42 | C | 2 | 2 | 2 | 1 | 5 | 100 | 1 | 1 | 8 | 8 |
| 43 | C | 2 | 2 | 3 | 1 | 5 | 66.66666667 | 1 | 1 | 3 | 2 |
| 44 | C | 2 | 2 | 4 | 1 | 5 | 40 | 1 | 1 | 5 | 2 |
| 45 | C | 2 | 2 | 5 | 1 | 5 | 100 | 1 | 1 | 4 | 4 |
| 46 | C | 2 | 2 | 6 | 1 | 5 | 100 | 1 | 1 | 3 | 3 |
| 47 | C | 2 | 2 | 7 | 1 | 5 | 42.85714286 | 1 | 1 | 7 | 3 |
| 48 | C | 2 | 2 | 8 | 1 | 5 | 66.66666667 | 1 | 1 | 6 | 4 |
| 49 | C | 2 | 2 | 9 | 1 | 5 | 100 | 1 | 1 | 3 | 3 |
| 50 | C | 2 | 2 | 10 | 1 | 5 | * | * | * | * | * |

| | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 51 | C | 2 | 3 | 1 | 1 | 6 | 100 | 1 | 1 | 5 | 5 |
| 52 | C | 2 | 3 | 2 | 1 | 6 | 100 | 1 | 1 | 5 | 5 |
| 53 | C | 2 | 3 | 3 | 1 | 6 | 100 | 1 | 1 | 5 | 5 |
| 54 | C | 2 | 3 | 4 | 1 | 6 | 100 | 1 | 1 | 5 | 5 |
| 55 | C | 2 | 3 | 5 | 1 | 6 | 100 | 1 | 1 | 5 | 5 |
| 56 | C | 2 | 3 | 6 | 1 | 6 | 0 | 0 | 0 | 6 | 0 |
| 57 | C | 2 | 3 | 7 | 1 | 6 | 100 | 1 | 1 | 5 | 5 |
| 58 | C | 2 | 3 | 8 | 1 | 6 | 100 | 1 | 1 | 5 | 5 |
| 59 | C | 2 | 3 | 9 | 1 | 6 | * | * | * | * | * |
| 60 | C | 2 | 3 | 10 | 1 | 6 | * | * | * | * | * |
| 61 | C | 3 | 1 | 1 | 1 | 7 | 0 | 0 | 0 | 2 | 0 |
| 62 | C | 3 | 1 | 2 | 1 | 7 | 25 | 1 | 1 | 4 | 1 |
| 63 | C | 3 | 1 | 3 | 1 | 7 | 0 | 0 | 0 | 6 | 0 |
| 64 | C | 3 | 1 | 4 | 1 | 7 | 50 | 1 | 1 | 4 | 2 |
| 65 | C | 3 | 1 | 5 | 1 | 7 | 0 | 0 | 0 | 6 | 0 |
| 66 | C | 3 | 1 | 6 | 1 | 7 | 60 | 1 | 1 | 5 | 3 |
| 67 | C | 3 | 1 | 7 | 1 | 7 | 0 | 0 | 0 | 2 | 0 |
| 68 | C | 3 | 1 | 8 | 1 | 7 | 0 | 0 | 0 | 5 | 0 |
| 69 | C | 3 | 1 | 9 | 1 | 7 | 40 | 1 | 1 | 5 | 2 |
| 70 | C | 3 | 1 | 10 | 1 | 7 | 50 | 1 | 1 | 4 | 2 |
| 71 | C | 3 | 2 | 1 | 1 | 8 | 60 | 1 | 1 | 5 | 3 |
| 72 | C | 3 | 2 | 2 | 1 | 8 | 100 | 1 | 1 | 5 | 5 |
| 73 | C | 3 | 2 | 3 | 1 | 8 | 0 | 0 | 0 | 3 | 0 |
| 74 | C | 3 | 2 | 4 | 1 | 8 | 100 | 1 | 1 | 5 | 5 |
| 75 | C | 3 | 2 | 5 | 1 | 8 | 100 | 1 | 1 | 5 | 5 |
| 76 | C | 3 | 2 | 6 | 1 | 8 | 0 | 0 | 0 | 5 | 0 |
| 77 | C | 3 | 2 | 7 | 1 | 8 | 0 | 0 | 0 | 4 | 0 |
| 78 | C | 3 | 2 | 8 | 1 | 8 | 100 | 1 | 1 | 5 | 5 |
| 79 | C | 3 | 2 | 9 | 1 | 8 | 71.42857143 | 1 | 1 | 7 | 5 |

| | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 80 | C | 3 | 2 | 10 | 1 | 8 | 20 | 1 | 1 | 5 | 1 |
| 81 | C | 3 | 3 | 1 | 1 | 9 | 0 | 0 | 0 | 3 | 0 |
| 82 | C | 3 | 3 | 2 | 1 | 9 | 100 | 1 | 1 | 4 | 4 |
| 83 | C | 3 | 3 | 3 | 1 | 9 | 100 | 1 | 1 | 4 | 4 |
| 84 | C | 3 | 3 | 4 | 1 | 9 | 100 | 1 | 1 | 5 | 5 |
| 85 | C | 3 | 3 | 5 | 1 | 9 | 75 | 1 | 1 | 4 | 3 |
| 86 | C | 3 | 3 | 6 | 1 | 9 | 100 | 1 | 1 | 5 | 5 |
| 87 | C | 3 | 3 | 7 | 1 | 9 | 100 | 1 | 1 | 7 | 7 |
| 88 | C | 3 | 3 | 8 | 1 | 9 | 33.33333333 | 1 | 1 | 6 | 2 |
| 89 | C | 3 | 3 | 9 | 1 | 9 | 50 | 1 | 1 | 6 | 3 |
| 90 | C | 3 | 3 | 10 | 1 | 9 | 50 | 1 | 1 | 6 | 3 |
| 91 | C | 4 | 1 | 1 | 1 | 10 | 100 | 1 | 1 | 5 | 5 |
| 92 | C | 4 | 1 | 2 | 1 | 10 | 100 | 1 | 1 | 4 | 4 |
| 93 | C | 4 | 1 | 3 | 1 | 10 | 100 | 1 | 1 | 5 | 5 |
| 94 | C | 4 | 1 | 4 | 1 | 10 | 100 | 1 | 1 | 6 | 6 |
| 95 | C | 4 | 1 | 5 | 1 | 10 | 100 | 1 | 1 | 5 | 5 |
| 96 | C | 4 | 1 | 6 | 1 | 10 | 100 | 1 | 1 | 6 | 6 |
| 97 | C | 4 | 1 | 7 | 1 | 10 | 100 | 1 | 1 | 5 | 5 |
| 98 | C | 4 | 1 | 8 | 1 | 10 | 100 | 1 | 1 | 5 | 5 |
| 99 | C | 4 | 1 | 9 | 1 | 10 | 100 | 1 | 1 | 4 | 4 |
| 100 | C | 4 | 1 | 10 | 1 | 10 | * | * | * | * | * |
| 101 | C | 4 | 1 | 1 | 2 | 11 | 0 | 0 | 0 | 5 | 0 |
| 102 | C | 4 | 1 | 2 | 2 | 11 | 0 | 0 | 0 | 4 | 0 |
| 103 | C | 4 | 1 | 3 | 2 | 11 | 0 | 0 | 0 | 3 | 0 |
| 104 | C | 4 | 1 | 4 | 2 | 11 | 0 | 0 | 0 | 6 | 0 |
| 105 | C | 4 | 1 | 5 | 2 | 11 | 0 | 0 | 0 | 3 | 0 |
| 106 | C | 4 | 1 | 6 | 2 | 11 | 0 | 0 | 0 | 4 | 0 |
| 107 | C | 4 | 1 | 7 | 2 | 11 | 0 | 0 | 0 | 6 | 0 |
| 108 | C | 4 | 1 | 8 | 2 | 11 | 0 | 0 | 0 | 5 | 0 |

| | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 109 | C | 4 | 1 | 9 | 2 | 11 | 0 | 0 | 0 | 5 | 0 |
| 110 | C | 4 | 1 | 10 | 2 | 11 | 0 | 0 | 0 | 4 | 0 |
| 111 | H | 1 | 1 | 1 | 1 | 12 | 0 | 0 | 0 | 6 | 0 |
| 112 | H | 1 | 1 | 2 | 1 | 12 | 0 | 0 | 0 | 3 | 0 |
| 113 | H | 1 | 1 | 3 | 1 | 12 | 100 | 1 | 1 | 5 | 5 |
| 114 | H | 1 | 1 | 4 | 1 | 12 | 0 | 0 | 0 | 9 | 0 |
| 115 | H | 1 | 1 | 5 | 1 | 12 | 20 | 1 | 1 | 5 | 1 |
| 116 | H | 1 | 1 | 6 | 1 | 12 | 100 | 1 | 1 | 7 | 7 |
| 117 | H | 1 | 1 | 7 | 1 | 12 | 0 | 0 | 0 | 3 | 0 |
| 118 | H | 1 | 1 | 8 | 1 | 12 | 100 | 1 | 1 | 6 | 6 |
| 119 | H | 1 | 1 | 9 | 1 | 12 | * | * | * | * | * |
| 120 | H | 1 | 1 | 10 | 1 | 12 | * | * | * | * | * |
| 121 | H | 1 | 2 | 1 | 1 | 13 | 100 | 1 | 1 | 6 | 6 |
| 122 | H | 1 | 2 | 2 | 1 | 13 | 0 | 0 | 0 | 4 | 0 |
| 123 | H | 1 | 2 | 3 | 1 | 13 | 62.5 | 1 | 1 | 8 | 5 |
| 124 | H | 1 | 2 | 4 | 1 | 13 | 100 | 1 | 1 | 6 | 6 |
| 125 | H | 1 | 2 | 5 | 1 | 13 | 14.28571429 | 1 | 1 | 7 | 1 |
| 126 | H | 1 | 2 | 6 | 1 | 13 | 100 | 1 | 1 | 6 | 6 |
| 127 | H | 1 | 2 | 7 | 1 | 13 | * | * | * | * | * |
| 128 | H | 1 | 2 | 8 | 1 | 13 | * | * | * | * | * |
| 129 | H | 1 | 2 | 9 | 1 | 13 | * | * | * | * | * |
| 130 | H | 1 | 2 | 10 | 1 | 13 | * | * | * | * | * |
| 131 | H | 1 | 3 | 1 | 1 | 14 | 80 | 1 | 1 | 5 | 4 |
| 132 | H | 1 | 3 | 2 | 1 | 14 | 0 | 0 | 0 | 2 | 0 |
| 133 | H | 1 | 3 | 3 | 1 | 14 | 0 | 0 | 0 | 6 | 0 |
| 134 | H | 1 | 3 | 4 | 1 | 14 | 100 | 1 | 1 | 6 | 6 |
| 135 | H | 1 | 3 | 5 | 1 | 14 | 100 | 1 | 1 | 7 | 7 |
| 136 | H | 1 | 3 | 6 | 1 | 14 | 100 | 1 | 1 | 4 | 4 |
| 137 | H | 1 | 3 | 7 | 1 | 14 | * | * | * | * | * |

| | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 138 | H | 1 | 3 | 8 | 1 | 14 | * | * | * | * | * |
| 139 | H | 1 | 3 | 9 | 1 | 14 | * | * | * | * | * |
| 140 | H | 1 | 3 | 10 | 1 | 14 | * | * | * | * | * |
| 141 | H | 2 | 1 | 1 | 1 | 15 | 50 | 1 | 1 | 6 | 3 |
| 142 | H | 2 | 1 | 2 | 1 | 15 | 0 | 0 | 0 | 9 | 0 |
| 143 | H | 2 | 1 | 3 | 1 | 15 | 0 | 0 | 0 | 6 | 0 |
| 144 | H | 2 | 1 | 4 | 1 | 15 | 77.77777778 | 1 | 1 | 9 | 7 |
| 145 | H | 2 | 1 | 5 | 1 | 15 | 20 | 1 | 1 | 5 | 1 |
| 146 | H | 2 | 1 | 6 | 1 | 15 | 33.33333333 | 1 | 1 | 6 | 2 |
| 147 | H | 2 | 1 | 7 | 1 | 15 | 0 | 0 | 0 | 5 | 0 |
| 148 | H | 2 | 1 | 8 | 1 | 15 | 50 | 1 | 1 | 4 | 2 |
| 149 | H | 2 | 1 | 9 | 1 | 15 | 100 | 1 | 1 | 6 | 6 |
| 150 | H | 2 | 1 | 10 | 1 | 15 | * | * | * | * | * |
| 151 | H | 2 | 2 | 1 | 1 | 16 | 0 | 0 | 0 | 6 | 0 |
| 152 | H | 2 | 2 | 2 | 1 | 16 | 28.57142857 | 1 | 1 | 7 | 2 |
| 153 | H | 2 | 2 | 3 | 1 | 16 | 100 | 1 | 1 | 7 | 7 |
| 154 | H | 2 | 2 | 4 | 1 | 16 | 0 | 0 | 0 | 5 | 0 |
| 155 | H | 2 | 2 | 5 | 1 | 16 | 100 | 1 | 1 | 5 | 5 |
| 156 | H | 2 | 2 | 6 | 1 | 16 | 100 | 1 | 1 | 5 | 5 |
| 157 | H | 2 | 2 | 7 | 1 | 16 | 100 | 1 | 1 | 6 | 6 |
| 158 | H | 2 | 2 | 8 | 1 | 16 | 100 | 1 | 1 | 5 | 5 |
| 159 | H | 2 | 2 | 9 | 1 | 16 | 0 | 0 | 0 | 1 | 0 |
| 160 | H | 2 | 2 | 10 | 1 | 16 | * | * | * | * | * |
| 161 | H | 2 | 3 | 1 | 1 | 17 | 100 | 1 | 1 | 6 | 6 |
| 162 | H | 2 | 3 | 2 | 1 | 17 | 100 | 1 | 1 | 4 | 4 |
| 163 | H | 2 | 3 | 3 | 1 | 17 | 100 | 1 | 1 | 3 | 3 |
| 164 | H | 2 | 3 | 4 | 1 | 17 | 100 | 1 | 1 | 5 | 5 |
| 165 | H | 2 | 3 | 5 | 1 | 17 | 100 | 1 | 1 | 5 | 5 |
| 166 | H | 2 | 3 | 6 | 1 | 17 | 100 | 1 | 1 | 2 | 2 |

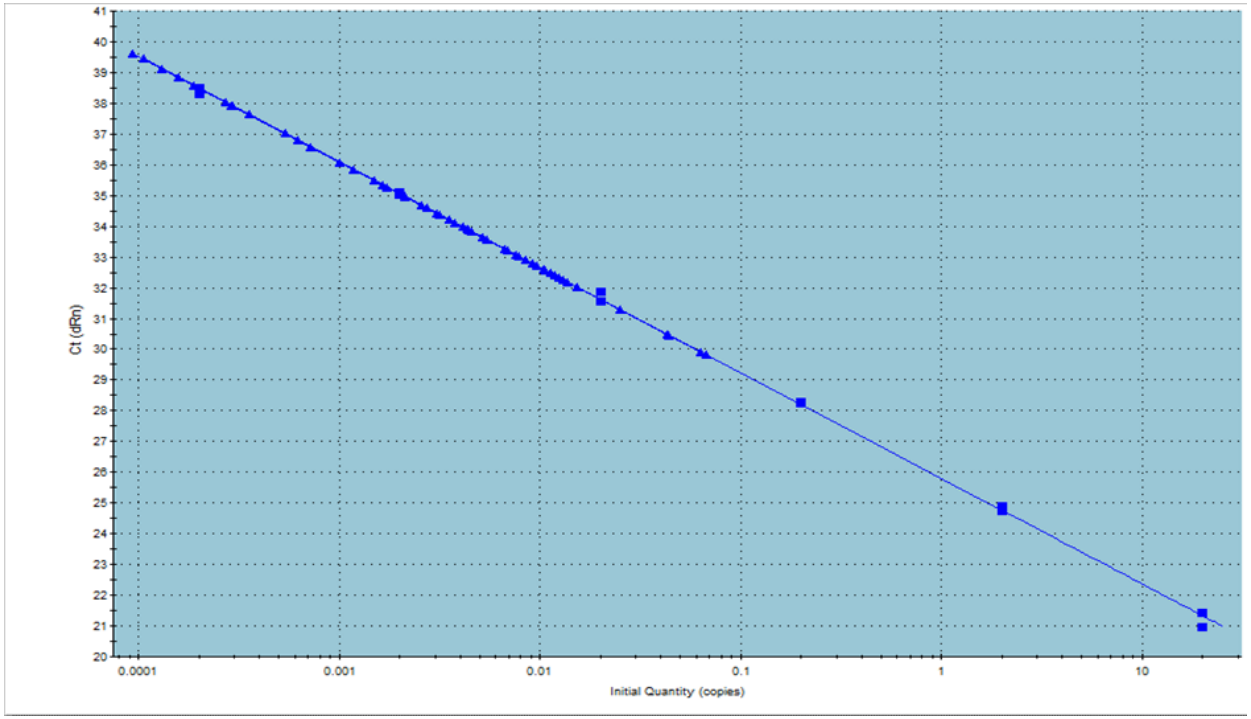
| | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 167 | H | 2 | 3 | 7 | 1 | 17 | 100 | 1 | 1 | 7 | 7 |
| 168 | H | 2 | 3 | 8 | 1 | 17 | 100 | 1 | 1 | 4 | 4 |
| 169 | H | 2 | 3 | 9 | 1 | 17 | 100 | 1 | 1 | 6 | 6 |
| 170 | H | 2 | 3 | 10 | 1 | 17 | * | * | * | * | * |
| 171 | H | 3 | 1 | 1 | 1 | 18 | 0 | 0 | 0 | 4 | 0 |
| 172 | H | 3 | 1 | 2 | 1 | 18 | 100 | 1 | 1 | 3 | 3 |
| 173 | H | 3 | 1 | 3 | 1 | 18 | 100 | 1 | 1 | 3 | 3 |
| 174 | H | 3 | 1 | 4 | 1 | 18 | 75 | 1 | 1 | 4 | 3 |
| 175 | H | 3 | 1 | 5 | 1 | 18 | 0 | 0 | 0 | 4 | 0 |
| 176 | H | 3 | 1 | 6 | 1 | 18 | 100 | 1 | 1 | 5 | 5 |
| 177 | H | 3 | 1 | 7 | 1 | 18 | 100 | 1 | 1 | 2 | 2 |
| 178 | H | 3 | 1 | 8 | 1 | 18 | 75 | 1 | 1 | 4 | 3 |
| 179 | H | 3 | 1 | 9 | 1 | 18 | 100 | 1 | 1 | 6 | 6 |
| 180 | H | 3 | 1 | 10 | 1 | 18 | 25 | 1 | 1 | 4 | 1 |
| 181 | H | 3 | 2 | 1 | 1 | 19 | 100 | 1 | 1 | 3 | 3 |
| 182 | H | 3 | 2 | 2 | 1 | 19 | 0 | 0 | 0 | 4 | 0 |
| 183 | H | 3 | 2 | 3 | 1 | 19 | 40 | 1 | 1 | 5 | 2 |
| 184 | H | 3 | 2 | 4 | 1 | 19 | 16.66666667 | 1 | 1 | 6 | 1 |
| 185 | H | 3 | 2 | 5 | 1 | 19 | 0 | 0 | 0 | 5 | 0 |
| 186 | H | 3 | 2 | 6 | 1 | 19 | 0 | 0 | 0 | 6 | 0 |
| 187 | H | 3 | 2 | 7 | 1 | 19 | 100 | 1 | 1 | 7 | 7 |
| 188 | H | 3 | 2 | 8 | 1 | 19 | 85.71428571 | 1 | 1 | 7 | 6 |
| 189 | H | 3 | 2 | 9 | 1 | 19 | * | * | * | * | * |
| 190 | H | 3 | 2 | 10 | 1 | 19 | * | * | * | * | * |
| 191 | H | 3 | 3 | 1 | 1 | 20 | 0 | 0 | 0 | 1 | 0 |
| 192 | H | 3 | 3 | 2 | 1 | 20 | 100 | 1 | 1 | 5 | 5 |
| 193 | H | 3 | 3 | 3 | 1 | 20 | 100 | 1 | 1 | 4 | 4 |
| 194 | H | 3 | 3 | 4 | 1 | 20 | 60 | 1 | 1 | 5 | 3 |
| 195 | H | 3 | 3 | 5 | 1 | 20 | 33.33333333 | 1 | 1 | 3 | 1 |

| | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 196 | H | 3 | 3 | 6 | 1 | 20 | 0 | 0 | 0 | 2 | 0 |
| 197 | H | 3 | 3 | 7 | 1 | 20 | 0 | 0 | 0 | 5 | 0 |
| 198 | H | 3 | 3 | 8 | 1 | 20 | 100 | 1 | 1 | 5 | 5 |
| 199 | H | 3 | 3 | 9 | 1 | 20 | * | * | * | * | * |
| 200 | H | 3 | 3 | 10 | 1 | 20 | * | * | * | * | * |
| 201 | H | 4 | 1 | 1 | 1 | 21 | 100 | 1 | 1 | 3 | 3 |
| 202 | H | 4 | 1 | 2 | 1 | 21 | 100 | 1 | 1 | 5 | 5 |
| 203 | H | 4 | 1 | 3 | 1 | 21 | 100 | 1 | 1 | 5 | 5 |
| 204 | H | 4 | 1 | 4 | 1 | 21 | 100 | 1 | 1 | 1 | 1 |
| 205 | H | 4 | 1 | 5 | 1 | 21 | 66.66666667 | 1 | 1 | 3 | 2 |
| 206 | H | 4 | 1 | 6 | 1 | 21 | 100 | 1 | 1 | 5 | 5 |
| 207 | H | 4 | 1 | 7 | 1 | 21 | 100 | 1 | 1 | 5 | 5 |
| 208 | H | 4 | 1 | 8 | 1 | 21 | 0 | 0 | 0 | 5 | 0 |
| 209 | H | 4 | 1 | 9 | 1 | 21 | 60 | 1 | 1 | 5 | 3 |
| 210 | H | 4 | 1 | 10 | 1 | 21 | * | * | * | * | * |
| 211 | H | 4 | 1 | 1 | 2 | 22 | 0 | 0 | 0 | 5 | 0 |
| 212 | H | 4 | 1 | 2 | 2 | 22 | 0 | 0 | 0 | 4 | 0 |
| 213 | H | 4 | 1 | 3 | 2 | 22 | 0 | 0 | 0 | 5 | 0 |
| 214 | H | 4 | 1 | 4 | 2 | 22 | 0 | 0 | 0 | 5 | 0 |
| 215 | H | 4 | 1 | 5 | 2 | 22 | 0 | 0 | 0 | 5 | 0 |
| 216 | H | 4 | 1 | 6 | 2 | 22 | 0 | 0 | 0 | 3 | 0 |
| 217 | H | 4 | 1 | 7 | 2 | 22 | 0 | 0 | 0 | 1 | 0 |
| 218 | H | 4 | 1 | 8 | 2 | 22 | 0 | 0 | 0 | 3 | 0 |
| 219 | H | 4 | 1 | 9 | 2 | 22 | 0 | 0 | 0 | 4 | 0 |
| 220 | H | 4 | 1 | 10 | 2 | 22 | 0 | 0 | 0 | 3 | 0 |

## 9.4 Chapter 6 Appendix

### 9.4.1 Appendix I: Standard curve for the quantitative detection of *Gaemanonnomyces graminis* var. *tritici* DNA using primers GgtEFF1 and GgtEFR1 in conjugation with Taqman probe GgtEFPR1

. Square dots are the standards and the triangles are the samples with $R^2$= 0.999. Results of qPCR are given as Cycle threshold (Ct).



**9.4.2 Appendix II: 16S rRNA bacteria, 16S rRNA *Pseudomonas* and ITS fungi qPCR metadata.** The values are the average of the two technical replicates and the units are in pg/ g dry soil.

| Sample | Field | Year | Block | Plot | Replicate | Cultivar | Crop | Qubit | Average.Bact.16S rRNA | Average.Pseudo.16S rRNA | Average.Fungi.ITS |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | NZ | 2015 | 1 | 1 | 1 | Hereford | wheat | 8.06 | 132.188047 | 1.618742821 | 214.1446112 |
| 2 | NZ | 2015 | 1 | 2 | 1 | Duxford | wheat | 11.3 | 281.0613882 | 1.613051017 | 262.0904618 |
| 3 | NZ | 2015 | 1 | 3 | 1 | Barley | Barley | 12.1 | 316.5392684 | 3.943751205 | 704.7093261 |
| 4 | NZ | 2015 | 1 | 4 | 1 | Xi19 | wheat | 12.5 | 301.697385 | 1.362053488 | 306.1469033 |
| 5 | NZ | 2015 | 1 | 5 | 1 | Hereward | wheat | 15.9 | 536.1395185 | 3.707825792 | 436.0346207 |
| 6 | NZ | 2015 | 1 | 6 | 1 | Cadenza | wheat | 16.8 | 580.136551 | 2.997712439 | 496.9396757 |
| 7 | NZ | 2015 | 2 | 7 | 2 | Xi19 | wheat | 7.96 | 319.9224508 | 1.824589537 | 246.7655427 |
| 8 | NZ | 2015 | 2 | 8 | 2 | Duxford | wheat | 6.69 | 204.6679971 | 2.037940006 | 154.4169271 |
| 9 | NZ | 2015 | 2 | 9 | 2 | Barley | Barley | 11.7 | 260.1073792 | 3.119935193 | 430.324396 |
| 10 | NZ | 2015 | 2 | 10 | 2 | Hereward | wheat | 10.4 | 373.7966666 | 1.447211706 | 247.7437816 |
| 11 | NZ | 2015 | 2 | 11 | 2 | Cadenza | wheat | 15.3 | 465.8463622 | 1.241744086 | 268.253805 |
| 12 | NZ | 2015 | 2 | 12 | 2 | Hereford | wheat | 9.18 | 255.4816795 | 5.721509549 | 233.6182212 |
| 13 | NZ | 2015 | 3 | 13 | 3 | Hereford | wheat | 11 | 212.4355355 | 4.894657647 | 286.7177808 |
| 14 | NZ | 2015 | 3 | 14 | 3 | Barley | Barley | 14.8 | 363.1652327 | 6.956021247 | 571.546217 |
| 15 | NZ | 2015 | 3 | 15 | 3 | Cadenza | wheat | 11.5 | 230.0322856 | 2.637331486 | 232.8462 |
| 16 | NZ | 2015 | 3 | 16 | 3 | Xi19 | wheat | 12.5 | 350.2768803 | 2.223075911 | 271.9219696 |
| 17 | NZ | 2015 | 3 | 17 | 3 | Hereward | wheat | 9.75 | 195.9700154 | 3.554313316 | 202.0891299 |
| 18 | NZ | 2015 | 3 | 18 | 3 | Duxford | wheat | 16.2 | 613.4533302 | 5.26113112 | 526.8840706 |
| 19 | NZ | 2015 | 4 | 19 | 4 | Hereford | wheat | 11.9 | 296.2008297 | 2.995464687 | 190.5435162 |
| 20 | NZ | 2015 | 4 | 20 | 4 | Cadenza | wheat | 13.4 | 363.8609462 | 4.151136946 | 336.9508956 |
| 21 | NZ | 2015 | 4 | 21 | 4 | Duxford | wheat | 14.3 | 481.141639 | 2.094363822 | 269.4296726 |

| | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 22 | NZ | 2015 | 4 | 22 | 4 | Xi19 | wheat | 11.1 | 384.9094477 | 2.489693219 | 184.9018332 |
| 23 | NZ | 2015 | 4 | 23 | 4 | Barley | Barley | 14.8 | 613.2220752 | 7.458125012 | 484.4738326 |
| 24 | NZ | 2015 | 4 | 24 | 4 | Hereward | wheat | 10.2 | 386.6726888 | 1.813629318 | 208.7304145 |
| 25 | NZ | 2016 | 1 | 1 | 1 | Hereford | wheat | 7.45 | 135.4720628 | 1.354004101 | 244.7492461 |
| 26 | NZ | 2016 | 1 | 2 | 1 | Duxford | wheat | 22.1 | 520.9570355 | 10.12397309 | 616.5661803 |
| 27 | NZ | 2016 | 1 | 3 | 1 | Barley | Barley | 13.5 | 346.8791589 | 4.547214132 | 456.965843 |
| 28 | NZ | 2016 | 1 | 4 | 1 | Xi19 | wheat | 14.2 | 528.0882432 | 5.843503717 | 948.5177172 |
| 29 | NZ | 2016 | 1 | 5 | 1 | Hereward | wheat | 15.3 | 620.6414431 | 2.918644741 | 385.0500948 |
| 30 | NZ | 2016 | 1 | 6 | 1 | Cadenza | wheat | 15.5 | 510.1313739 | 2.669309978 | 580.9582232 |
| 31 | NZ | 2016 | 2 | 7 | 2 | Xi19 | wheat | 19.2 | 457.2252686 | 8.683755452 | 584.3975301 |
| 32 | NZ | 2016 | 2 | 8 | 2 | Duxford | wheat | 20.7 | 691.2837741 | 10.71950613 | 622.8239963 |
| 33 | NZ | 2016 | 2 | 9 | 2 | Barley | Barley | 11 | 112.6665295 | 3.280561058 | 215.0430941 |
| 34 | NZ | 2016 | 2 | 10 | 2 | Hereward | wheat | 17.2 | 667.2895353 | 5.014608397 | 490.9268379 |
| 35 | NZ | 2016 | 2 | 11 | 2 | Cadenza | wheat | 19 | 761.4004347 | 7.306855422 | 675.432198 |
| 36 | NZ | 2016 | 2 | 12 | 2 | Hereford | wheat | 16.7 | 631.32406 | 1.936133577 | 398.0731673 |
| 37 | NZ | 2016 | 3 | 13 | 3 | Hereford | wheat | 17.1 | 256.8606822 | 3.737839051 | 428.6584776 |
| 38 | NZ | 2016 | 3 | 14 | 3 | Barley | Barley | 16.8 | 334.4706436 | 8.992517904 | 596.8295821 |
| 39 | NZ | 2016 | 3 | 15 | 3 | Cadenza | wheat | 16.3 | 527.0642583 | 5.271105851 | 1083.297501 |
| 40 | NZ | 2016 | 3 | 16 | 3 | Xi19 | wheat | 17.9 | 440.9685405 | 3.867231037 | 451.2392783 |
| 41 | NZ | 2016 | 3 | 17 | 3 | Hereward | wheat | 6.86 | 119.194467 | 1.726078918 | 314.4102154 |
| 42 | NZ | 2016 | 3 | 18 | 3 | Duxford | wheat | 16.9 | 780.3399968 | 6.097509794 | 531.0038419 |
| 43 | NZ | 2016 | 4 | 19 | 4 | Hereford | wheat | 21.1 | 794.8341341 | 5.789966384 | 782.6014992 |
| 44 | NZ | 2016 | 4 | 20 | 4 | Cadenza | wheat | 23.1 | 1025.127421 | 10.10894982 | 900.4382635 |
| 45 | NZ | 2016 | 4 | 21 | 4 | Duxford | wheat | 19.8 | 673.2364687 | 6.454393322 | 564.5790376 |
| 46 | NZ | 2016 | 4 | 22 | 4 | Xi19 | wheat | 16.3 | 503.0872379 | 2.980948092 | 911.9177074 |
| 47 | NZ | 2016 | 4 | 23 | 4 | Barley | Barley | 18 | 768.5464586 | 10.31487772 | 999.203666 |
| 48 | NZ | 2016 | 4 | 24 | 4 | Hereward | wheat | 23 | 1043.736978 | 3.725635069 | 790.5409145 |
| 49 | NZ | 2017 | 1 | 1 | 1 | Hereford | wheat | 11.4 | 154.1457852 | 6.198797294 | 192.6674752 |
| 50 | NZ | 2017 | 1 | 2 | 1 | Duxford | wheat | 10.7 | 192.55211 | 3.909298485 | 231.3817312 |

| | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 51 | NZ | 2017 | 1 | 3 | 1 | Barley | Barley | 13.2 | 222.1332451 | 5.011819539 | 223.8269079 |
| 52 | NZ | 2017 | 1 | 4 | 1 | Xi19 | wheat | 15 | 373.4209719 | 10.53719271 | 425.0035449 |
| 53 | NZ | 2017 | 1 | 5 | 1 | Hereward | wheat | 15.4 | 414.7017818 | 5.115993846 | 304.9102179 |
| 54 | NZ | 2017 | 1 | 6 | 1 | Cadenza | wheat | 10.7 | 301.8822106 | 2.773840425 | 199.2482518 |
| 55 | NZ | 2017 | 2 | 7 | 2 | Xi19 | wheat | 16.4 | 531.7121876 | 3.553813633 | 371.9010949 |
| 56 | NZ | 2017 | 2 | 8 | 2 | Duxford | wheat | 16.6 | 510.2569251 | 9.617418961 | 407.3653912 |
| 57 | NZ | 2017 | 2 | 9 | 2 | Barley | Barley | 13.1 | 312.1457502 | 3.751597122 | 223.4917383 |
| 58 | NZ | 2017 | 2 | 10 | 2 | Hereward | wheat | 10.9 | 297.357926 | 4.526409907 | 336.8378474 |
| 59 | NZ | 2017 | 2 | 11 | 2 | Cadenza | wheat | 11.1 | 336.0983048 | 1.756664038 | 212.4436321 |
| 60 | NZ | 2017 | 2 | 12 | 2 | Hereford | wheat | 15.4 | 251.4541373 | 4.971590539 | 297.5571465 |
| 61 | NZ | 2017 | 3 | 13 | 3 | Hereford | wheat | 17.4 | 379.3288396 | 6.041340593 | 381.7675868 |
| 62 | NZ | 2017 | 3 | 14 | 3 | Barley | Barley | 14.4 | 331.8215615 | 5.219977702 | 339.4232812 |
| 63 | NZ | 2017 | 3 | 15 | 3 | Cadenza | wheat | 15.1 | 397.3145985 | 3.042030092 | 323.3997647 |
| 64 | NZ | 2017 | 3 | 16 | 3 | Xi19 | wheat | 9.92 | 248.1275308 | 2.891109829 | 199.6094542 |
| 65 | NZ | 2017 | 3 | 17 | 3 | Hereward | wheat | 10.7 | 319.3402685 | 3.116931565 | 215.1929026 |
| 66 | NZ | 2017 | 3 | 18 | 3 | Duxford | wheat | 12.5 | 416.1522672 | 7.010460559 | 266.4213569 |
| 67 | NZ | 2017 | 4 | 19 | 4 | Hereford | wheat | 15.5 | 492.1194501 | 8.424596968 | 521.9948351 |
| 68 | NZ | 2017 | 4 | 20 | 4 | Cadenza | wheat | 13.6 | 404.283942 | 10.84775203 | 369.0837725 |
| 69 | NZ | 2017 | 4 | 21 | 4 | Duxford | wheat | 10.2 | 277.44475 | 3.645973104 | 330.2667619 |
| 70 | NZ | 2017 | 4 | 22 | 4 | Xi19 | wheat | 19.1 | 763.1519795 | 8.955503057 | 736.5841739 |
| 71 | NZ | 2017 | 4 | 23 | 4 | Barley | Barley | 20.9 | 1001.407553 | 5.736130686 | 946.3485941 |
| 72 | NZ | 2017 | 4 | 24 | 4 | Hereward | wheat | 13.8 | 524.8490637 | 2.411011204 | 265.9460571 |
| 73 | LH5 | 2015 | 1 | 1 | 1 | Barley | Barley | 6.62 | 168.8561731 | 5.551433432 | 106.2256834 |
| 74 | LH5 | 2015 | 1 | 2 | 1 | Cadenza | wheat | 3.8 | 101.6890999 | 4.365091111 | 95.97168851 |
| 75 | LH5 | 2015 | 1 | 3 | 1 | Hereford | wheat | 6.5 | 174.5725817 | 8.653267758 | 218.0143123 |
| 76 | LH5 | 2015 | 2 | 4 | 1 | Xi19 | wheat | 2.3 | 79.75568908 | 2.965791189 | 78.9145396 |
| 77 | LH5 | 2015 | 2 | 5 | 1 | Hereward | wheat | 4.9 | 253.0587843 | 4.27263108 | 117.9062642 |
| 78 | LH5 | 2015 | 2 | 6 | 1 | Duxford | wheat | 4.09 | 162.7054573 | 4.632388193 | 193.687847 |
| 79 | LH5 | 2015 | 3 | 7 | 2 | Cadenza | wheat | 3.9 | 167.1198067 | 3.61123775 | 69.79286151 |

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|---|---|---|---|---|---|---|---|---|---|---|---|
| 80 | LH5 | 2015 | 3 | 8 | 2 | Hereford | wheat | 4.6 | 222.8733072 | 6.570751051 | 115.2562538 |
| 81 | LH5 | 2015 | 3 | 9 | 2 | Xi19 | wheat | 10.6 | 448.7375892 | 8.591993983 | 165.0650067 |
| 82 | LH5 | 2015 | 4 | 10 | 2 | Hereward | wheat | 12.1 | 489.6862092 | 7.913846401 | 226.9541933 |
| 83 | LH5 | 2015 | 4 | 11 | 2 | Duxford | wheat | 6.54 | 215.3301116 | 5.649746315 | 108.7374249 |
| 84 | LH5 | 2015 | 4 | 12 | 2 | Barley | Barley | 6.04 | 249.8170781 | 6.347469274 | 137.8210601 |
| 85 | LH5 | 2015 | 1 | 13 | 3 | Xi19 | wheat | 4.05 | 95.42737815 | 4.978135569 | 132.3954925 |
| 86 | LH5 | 2015 | 1 | 14 | 3 | Hereward | wheat | 4.99 | 147.7163935 | 9.427334679 | 100.5180426 |
| 87 | LH5 | 2015 | 1 | 15 | 3 | Duxford | wheat | 5.42 | 210.6038418 | 6.33595613 | 139.6024246 |
| 88 | LH5 | 2015 | 2 | 16 | 3 | Barley | Barley | 6.3 | 249.4589412 | 4.226351538 | 204.1653623 |
| 89 | LH5 | 2015 | 2 | 17 | 3 | Cadenza | wheat | 6.73 | 324.8353265 | 4.060975621 | 222.7383542 |
| 90 | LH5 | 2015 | 2 | 18 | 3 | Hereford | wheat | 6.5 | 278.8452078 | 8.49601972 | 146.4782278 |
| 91 | LH5 | 2015 | 3 | 19 | 4 | Duxford | wheat | 7.75 | 579.156704 | 17.02562098 | 153.7515687 |
| 92 | LH5 | 2015 | 3 | 20 | 4 | Barley | Barley | 5.1 | 330.6151818 | 6.540648803 | 269.5800251 |
| 93 | LH5 | 2015 | 3 | 21 | 4 | Hereward | wheat | 3.37 | 118.1530921 | 10.22568964 | 74.82333003 |
| 94 | LH5 | 2015 | 4 | 22 | 4 | Hereford | wheat | 2.14 | 58.00243212 | 1.784482733 | 37.27715345 |
| 95 | LH5 | 2015 | 4 | 23 | 4 | Xi19 | wheat | 6.67 | 619.3661109 | 8.70760665 | 204.2304795 |
| 96 | LH5 | 2015 | 4 | 24 | 4 | Cadenza | wheat | 5.19 | 190.4719381 | 8.793252585 | 138.5491618 |
| 97 | LH5 | 2016 | 1 | 1 | 1 | Barley | Barley | 8.04 | 267.0199151 | 6.158505018 | 535.90489 |
| 98 | LH5 | 2016 | 1 | 2 | 1 | Cadenza | wheat | 10.7 | 410.3433581 | 9.528794486 | 518.4686801 |
| 99 | LH5 | 2016 | 1 | 3 | 1 | Hereford | wheat | 10.7 | 380.9174459 | 9.239742319 | 608.3956459 |
| 100 | LH5 | 2016 | 2 | 4 | 1 | Xi19 | wheat | 7.4 | 305.6145746 | 8.601447878 | 382.6180558 |
| 101 | LH5 | 2016 | 2 | 5 | 1 | Hereward | wheat | 10.7 | 655.4852345 | 5.863788778 | 381.6742775 |
| 102 | LH5 | 2016 | 2 | 6 | 1 | Duxford | wheat | 10.3 | 468.8027877 | 8.121676251 | 579.812087 |
| 103 | LH5 | 2016 | 3 | 7 | 2 | Cadenza | wheat | 11.7 | 510.626601 | 7.219396942 | 502.7497514 |
| 104 | LH5 | 2016 | 3 | 8 | 2 | Hereford | wheat | 12.5 | 553.4315857 | 8.39785919 | 484.1738514 |
| 105 | LH5 | 2016 | 3 | 9 | 2 | Xi19 | wheat | 4.64 | 207.1123533 | 3.696415628 | 127.7858929 |
| 106 | LH5 | 2016 | 4 | 10 | 2 | Hereward | wheat | 5.05 | 185.2034761 | 2.847429565 | 139.3105846 |
| 107 | LH5 | 2016 | 4 | 11 | 2 | Duxford | wheat | 10.8 | 503.839243 | 65.69725997 | 388.4389646 |
| 108 | LH5 | 2016 | 4 | 12 | 2 | Barley | Barley | 11.8 | 553.1084884 | 7.781838322 | 828.5048123 |

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|---|---|---|---|---|---|---|---|---|---|---|---|
| 109 | LH5 | 2016 | 1 | 13 | 3 | Xi19 | wheat | 13.3 | 698.8183913 | 10.19590356 | 589.710582 |
| 110 | LH5 | 2016 | 1 | 14 | 3 | Hereward | wheat | 9.78 | 433.6645508 | 4.859835904 | 322.2200324 |
| 111 | LH5 | 2016 | 1 | 15 | 3 | Duxford | wheat | 13.9 | 639.3265303 | 9.869660765 | 499.6319818 |
| 112 | LH5 | 2016 | 2 | 16 | 3 | Barley | Barley | 11.2 | 534.3163399 | 11.08728964 | 884.4459543 |
| 113 | LH5 | 2016 | 2 | 17 | 3 | Cadenza | wheat | 10.9 | 568.2148627 | 8.830219307 | 536.4855239 |
| 114 | LH5 | 2016 | 2 | 18 | 3 | Hereford | wheat | 9.69 | 629.9946235 | 8.189805266 | 405.7816994 |
| 115 | LH5 | 2016 | 3 | 19 | 4 | Duxford | wheat | 7.54 | 394.3681414 | 6.858129845 | 282.7537642 |
| 116 | LH5 | 2016 | 3 | 20 | 4 | Barley | Barley | 3.93 | 155.6712685 | 5.967625864 | 126.429062 |
| 117 | LH5 | 2016 | 3 | 21 | 4 | Hereward | wheat | 12 | 552.9553964 | 7.535513368 | 491.211746 |
| 118 | LH5 | 2016 | 4 | 22 | 4 | Hereford | wheat | 9.89 | 432.3988941 | 5.220403857 | 232.2780412 |
| 119 | LH5 | 2016 | 4 | 23 | 4 | Xi19 | wheat | 12.2 | 597.2085132 | 7.265153622 | 490.879063 |
| 120 | LH5 | 2016 | 4 | 24 | 4 | Cadenza | wheat | 14.9 | 697.5316232 | 11.6011231 | 483.6452468 |
| 121 | LH5 | 2017 | 1 | 1 | 1 | Barley | Barley | 16.7 | 722.2829134 | 12.92118315 | 460.2553312 |
| 122 | LH5 | 2017 | 1 | 2 | 1 | Cadenza | wheat | 10.2 | 353.0754167 | 10.13784277 | 458.9874948 |
| 123 | LH5 | 2017 | 1 | 3 | 1 | Hereford | wheat | 12.7 | 540.0763153 | 6.682896864 | 581.1635474 |
| 124 | LH5 | 2017 | 2 | 4 | 1 | Xi19 | wheat | 14.1 | 586.5876471 | 15.11018278 | 631.2369615 |
| 125 | LH5 | 2017 | 2 | 5 | 1 | Hereward | wheat | 14.9 | 704.1524534 | 13.92994279 | 839.5558072 |
| 126 | LH5 | 2017 | 2 | 6 | 1 | Duxford | wheat | 14 | 600.7521242 | 13.73270375 | 776.0809087 |
| 127 | LH5 | 2017 | 3 | 7 | 2 | Cadenza | wheat | 14.5 | 746.5464788 | 8.896009492 | 928.8392135 |
| 128 | LH5 | 2017 | 3 | 8 | 2 | Hereford | wheat | 16 | 1307.66 | 11.61364186 | 534.5393181 |
| 129 | LH5 | 2017 | 3 | 9 | 2 | Xi19 | wheat | 12.7 | 594.6126511 | 10.66474664 | 534.4130481 |
| 130 | LH5 | 2017 | 4 | 10 | 2 | Hereward | wheat | 16.1 | 781.7652271 | 18.38540433 | 701.7225359 |
| 131 | LH5 | 2017 | 4 | 11 | 2 | Duxford | wheat | 10.5 | 431.4717647 | 6.975082877 | 249.6594533 |
| 132 | LH5 | 2017 | 4 | 12 | 2 | Barley | Barley | 13.5 | 649.2840537 | 9.288424764 | 560.2445771 |
| 133 | LH5 | 2017 | 1 | 13 | 3 | Xi19 | wheat | 15.7 | 593.2786291 | 6.158721939 | 753.3571944 |
| 134 | LH5 | 2017 | 1 | 14 | 3 | Hereward | wheat | 9.38 | 376.4109236 | 6.698560646 | 288.7368025 |
| 135 | LH5 | 2017 | 1 | 15 | 3 | Duxford | wheat | 14.4 | 550.0628235 | 3.739084802 | 384.8384749 |
| 136 | LH5 | 2017 | 2 | 16 | 3 | Barley | Barley | 14.9 | 837.1259506 | 19.83609781 | 799.9681195 |
| 137 | LH5 | 2017 | 2 | 17 | 3 | Cadenza | wheat | 14 | 596.9386111 | 3.818052158 | 1067.230338 |

| | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 138 | LH5 | 2017 | 2 | 18 | 3 | Hereford | wheat | 13.2 | 834.4122255 | 2.594535421 | 489.1743064 |
| 139 | LH5 | 2017 | 3 | 19 | 4 | Duxford | wheat | 14.6 | 738.6279559 | 3.855195653 | 622.6942588 |
| 140 | LH5 | 2017 | 3 | 20 | 4 | Barley | Barley | 14 | 743.2022336 | 5.620532353 | 500.1630501 |
| 141 | LH5 | 2017 | 3 | 21 | 4 | Hereward | wheat | 13.8 | 653.095598 | 9.975436103 | 499.8922036 |
| 142 | LH5 | 2017 | 4 | 22 | 4 | Hereford | wheat | 14.7 | 565.750491 | 6.045497287 | 497.212555 |
| 143 | LH5 | 2017 | 4 | 23 | 4 | Xi19 | wheat | 12.6 | 480.789468 | 5.25242159 | 605.4612256 |
| 144 | LH5 | 2017 | 4 | 24 | 4 | Cadenza | wheat | 11.4 | 596.9730026 | 5.902601112 | 460.7702086 |
| 145 | LH4 | 2016 | 1 | 1 | 1 | Xi19 | wheat | 8.92 | 383.5312661 | 1.722628905 | 181.6229022 |
| 146 | LH4 | 2016 | 1 | 2 | 1 | Cadenza | wheat | 7.64 | 368.464645 | 1.475845053 | 144.4818537 |
| 147 | LH4 | 2016 | 1 | 3 | 1 | Hereward | wheat | 12.2 | 510.8396613 | 2.361974861 | 218.1003901 |
| 148 | LH4 | 2016 | 2 | 4 | 1 | Hereward | wheat | 7.17 | 328.2530163 | 2.676909841 | 113.7666613 |
| 149 | LH4 | 2016 | 2 | 5 | 1 | Barley | Barley | 9.82 | 380.9484474 | 2.45240682 | 155.7586762 |
| 150 | LH4 | 2016 | 2 | 6 | 1 | Duxford | wheat | 11.6 | 529.7623604 | 3.728854734 | 322.7281367 |
| 151 | LH4 | 2016 | 3 | 7 | 2 | Duxford | wheat | 8.11 | 317.0303499 | 3.525850417 | 114.3488718 |
| 152 | LH4 | 2016 | 3 | 8 | 2 | Hereward | wheat | 11.2 | 476.5377255 | 2.93418656 | 192.6061255 |
| 153 | LH4 | 2016 | 3 | 9 | 2 | Barley | Barley | 8.98 | 384.2594824 | 3.286283877 | 152.7165562 |
| 154 | LH4 | 2016 | 4 | 10 | 2 | Duxford | wheat | 9.75 | 409.2062255 | 2.270219661 | 157.3622447 |
| 155 | LH4 | 2016 | 4 | 11 | 2 | Barley | Barley | 9.59 | 384.7335183 | 1.657572607 | 93.56386978 |
| 156 | LH4 | 2016 | 4 | 12 | 2 | Hereford | wheat | 8.67 | 460.2728143 | 2.172255022 | 124.0516008 |
| 157 | LH4 | 2016 | 1 | 13 | 3 | Hereford | wheat | 10.2 | 415.5562857 | 2.132771892 | 138.4535093 |
| 158 | LH4 | 2016 | 1 | 14 | 3 | Barley | Barley | 10.6 | 443.8520878 | 4.479854279 | 271.6713036 |
| 159 | LH4 | 2016 | 1 | 15 | 3 | Duxford | wheat | 8.14 | 508.2232181 | 1.984494908 | 172.0423176 |
| 160 | LH4 | 2016 | 2 | 16 | 3 | Xi19 | wheat | 7.13 | 329.2821071 | 1.405139538 | 129.5394498 |
| 161 | LH4 | 2016 | 2 | 17 | 3 | Cadenza | wheat | 7.54 | 358.6729423 | 1.508878244 | 136.790877 |
| 162 | LH4 | 2016 | 2 | 18 | 3 | Hereford | wheat | 10.4 | 477.9026256 | 2.254323415 | 162.0064569 |
| 163 | LH4 | 2016 | 3 | 19 | 4 | Cadenza | wheat | 10.4 | 507.2091653 | 2.538173009 | 229.9994185 |
| 164 | LH4 | 2016 | 3 | 20 | 4 | Xi19 | wheat | 11.8 | 506.2814294 | 6.896472035 | 564.9976472 |
| 165 | LH4 | 2016 | 3 | 21 | 4 | Hereford | wheat | 6.18 | 278.8124599 | 1.441431788 | 164.1079783 |
| 166 | LH4 | 2016 | 4 | 22 | 4 | Hereward | wheat | 10 | 395.4409897 | 1.111206902 | 103.1172262 |

| | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 167 | LH4 | 2016 | 4 | 23 | 4 | Cadenza | wheat | 11.4 | 513.9455294 | 2.917759196 | 145.5479782 |
| 168 | LH4 | 2016 | 4 | 24 | 4 | Xi19 | wheat | 9.43 | 357.650844 | 1.749962427 | 85.74758875 |
| 169 | LH4 | 2017 | 1 | 1 | 1 | Xi19 | wheat | 12.1 | 692.9901066 | 4.289395153 | 564.6130103 |
| 170 | LH4 | 2017 | 1 | 2 | 1 | Cadenza | wheat | 11 | 502.9370673 | 1.835553712 | 352.7480264 |
| 171 | LH4 | 2017 | 1 | 3 | 1 | Hereward | wheat | 11.6 | 589.2476624 | 4.156438454 | 569.8460837 |
| 172 | LH4 | 2017 | 2 | 4 | 1 | Hereward | wheat | 12 | 542.0584143 | 4.870589157 | 506.4539558 |
| 173 | LH4 | 2017 | 2 | 5 | 1 | Barley | Barley | 16.3 | 1692.632437 | 8.016724199 | 841.3151073 |
| 174 | LH4 | 2017 | 2 | 6 | 1 | Duxford | wheat | 15.5 | 775.5701833 | 5.279926857 | 732.6043109 |
| 175 | LH4 | 2017 | 3 | 7 | 2 | Duxford | wheat | 17.8 | 829.8103546 | 7.380025329 | 533.6126225 |
| 176 | LH4 | 2017 | 3 | 8 | 2 | Hereward | wheat | 14.7 | 618.0604348 | 5.175998563 | 652.5513265 |
| 177 | LH4 | 2017 | 3 | 9 | 2 | Barley | Barley | 17.9 | 767.1258779 | 10.88181415 | 527.3535663 |
| 178 | LH4 | 2017 | 4 | 10 | 2 | Duxford | wheat | 17.3 | 828.7392308 | 9.861652123 | 1537.626996 |
| 179 | LH4 | 2017 | 4 | 11 | 2 | Barley | Barley | 12.3 | 527.7008048 | 4.775280855 | 450.472208 |
| 180 | LH4 | 2017 | 4 | 12 | 2 | Hereford | wheat | 17.3 | 735.0695989 | 8.449182403 | 505.2845658 |
| 181 | LH4 | 2017 | 1 | 13 | 3 | Hereford | wheat | 14.6 | 826.6795865 | 9.728273995 | 601.2183415 |
| 182 | LH4 | 2017 | 1 | 14 | 3 | Barley | Barley | 15.9 | 817.8483624 | 7.262768635 | 909.4063752 |
| 183 | LH4 | 2017 | 1 | 15 | 3 | Duxford | wheat | 16.2 | 802.1401412 | 7.923182164 | 722.2636187 |
| 184 | LH4 | 2017 | 2 | 16 | 3 | Xi19 | wheat | 12.7 | 564.7360049 | 6.50737205 | 374.9659462 |
| 185 | LH4 | 2017 | 2 | 17 | 3 | Cadenza | wheat | 15.6 | 643.6927059 | 6.943737374 | 2743.530746 |
| 186 | LH4 | 2017 | 2 | 18 | 3 | Hereford | wheat | 13.3 | 577.2544453 | 2.089706753 | 650.8857953 |
| 187 | LH4 | 2017 | 3 | 19 | 4 | Cadenza | wheat | 20.5 | 1406.388599 | 7.077651145 | 804.1082879 |
| 188 | LH4 | 2017 | 3 | 20 | 4 | Xi19 | wheat | 28.3 | 656.9451944 | 2.617954606 | 228.7555963 |
| 189 | LH4 | 2017 | 3 | 21 | 4 | Hereford | wheat | 21.7 | 970.1626781 | 4.440151592 | 410.7372294 |
| 190 | LH4 | 2017 | 4 | 22 | 4 | Hereward | wheat | 24.3 | 916.3680882 | 4.134877882 | 474.0514898 |
| 191 | LH4 | 2017 | 4 | 23 | 4 | Cadenza | wheat | 26.9 | 1061.896878 | 8.480923814 | 1574.096986 |
| 192 | LH4 | 2017 | 4 | 24 | 4 | Xi19 | wheat | 20.1 | 851.7807673 | 5.309078427 | 634.941649 |

**9.4.3 Appendix III: Ggt qPCR metadata.** Where F: field, Y:year, SS: sampling season, T: time; B: block, P: plot, R:replicate, Q:qubit, Pre: present.

| no. | F | Y | SS | T | B | P | R | Cultivar | Crop | TAB | Q | soil.dry.weight | DNA.g.dry.soil | Ggt DNA.pg.g.soil | Pre_Absent | logten_Ggt |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | NZ | 2015 | 1 | 1 | 1 | 1 | 1 | Hereford | wheat | Low | 8.06 | 0.2223 | 3625.8716 | 0 | 0 | * |
| 2 | NZ | 2015 | 1 | 1 | 1 | 2 | 1 | Duxford | wheat | High | 11.3 | 0.2215 | 5100.8056 | 56.2966 | 1 | 1.7505 |
| 3 | NZ | 2015 | 1 | 1 | 1 | 3 | 1 | Barley | Barley | Unknown | 12.1 | 0.2214 | 5464.2996 | 51.0136 | 1 | 1.7077 |
| 4 | NZ | 2015 | 1 | 1 | 1 | 4 | 1 | Xi19 | wheat | Low | 12.5 | 0.2333 | 5357.2736 | 0 | 0 | * |
| 5 | NZ | 2015 | 1 | 1 | 1 | 5 | 1 | Hereward | wheat | High | 15.9 | 0.2170 | 7328.3130 | 0 | 0 | * |
| 6 | NZ | 2015 | 1 | 1 | 1 | 6 | 1 | Cadenza | wheat | Low | 16.8 | 0.2275 | 7383.0547 | 0 | 0 | * |
| 7 | NZ | 2015 | 1 | 1 | 2 | 7 | 2 | Xi19 | wheat | Low | 7.96 | 0.2222 | 3582.9036 | 0 | 0 | * |
| 8 | NZ | 2015 | 1 | 1 | 2 | 8 | 2 | Duxford | wheat | High | 6.69 | 0.2147 | 3115.3260 | 0 | 0 | * |
| 9 | NZ | 2015 | 1 | 1 | 2 | 9 | 2 | Barley | Barley | Unknown | 11.7 | 0.2157 | 5424.2571 | 0 | 0 | * |
| 10 | NZ | 2015 | 1 | 1 | 2 | 10 | 2 | Hereward | wheat | High | 10.4 | 0.2134 | 4873.4624 | 0 | 0 | * |
| 11 | NZ | 2015 | 1 | 1 | 2 | 11 | 2 | Cadenza | wheat | Low | 15.3 | 0.2164 | 7069.8029 | 0 | 0 | * |
| 12 | NZ | 2015 | 1 | 1 | 2 | 12 | 2 | Hereford | wheat | Low | 9.18 | 0.2200 | 4172.4037 | 0 | 0 | * |
| 13 | NZ | 2015 | 1 | 1 | 3 | 13 | 3 | Hereford | wheat | Low | 11 | 0.2211 | 4974.4087 | 0 | 0 | * |
| 14 | NZ | 2015 | 1 | 1 | 3 | 14 | 3 | Barley | Barley | Unknown | 14.8 | 0.2162 | 6846.1360 | 0 | 0 | * |
| 15 | NZ | 2015 | 1 | 1 | 3 | 15 | 3 | Cadenza | wheat | Low | 11.5 | 0.2190 | 5251.7682 | 0 | 0 | * |
| 16 | NZ | 2015 | 1 | 1 | 3 | 16 | 3 | Xi19 | wheat | Low | 12.5 | 0.2169 | 5763.7092 | 0 | 0 | * |
| 17 | NZ | 2015 | 1 | 1 | 3 | 17 | 3 | Hereward | wheat | High | 9.75 | 0.2148 | 4538.3246 | 0 | 0 | * |
| 18 | NZ | 2015 | 1 | 1 | 3 | 18 | 3 | Duxford | wheat | High | 16.2 | 0.2024 | 8004.2448 | 0 | 0 | * |
| 19 | NZ | 2015 | 1 | 1 | 4 | 19 | 4 | Hereford | wheat | Low | 11.9 | 0.2155 | 5521.0311 | 0 | 0 | * |
| 20 | NZ | 2015 | 1 | 1 | 4 | 20 | 4 | Cadenza | wheat | Low | 13.4 | 0.2181 | 6145.1966 | 95.274 | 1 | 1.9790 |
| 21 | NZ | 2015 | 1 | 1 | 4 | 21 | 4 | Duxford | wheat | High | 14.3 | 0.2181 | 6556.0229 | 0 | 0 | * |

| | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 22 | NZ | 2015 | 1 | 1 | 4 | 22 | 4 | Xi19 | wheat | Low | 11.1 | 0.2396 | 4631.7690 | 0 | 0 | * |
| 23 | NZ | 2015 | 1 | 1 | 4 | 23 | 4 | Barley | Barley | Unknown | 14.8 | 0.2131 | 6946.2474 | 0 | 0 | * |
| 24 | NZ | 2015 | 1 | 1 | 4 | 24 | 4 | Hereward | wheat | High | 10.2 | 0.2153 | 4736.7067 | 0 | 0 | * |
| 25 | NZ | 2016 | 2 | 2 | 1 | 1 | 1 | Hereford | wheat | Low | 7.45 | 0.2211 | 3369.8614 | 176.565 | 1 | 2.2469 |
| 26 | NZ | 2016 | 2 | 2 | 1 | 2 | 1 | Duxford | wheat | High | 22.1 | 0.2169 | 10187.5868 | 5381.35 | 1 | 3.7309 |
| 27 | NZ | 2016 | 2 | 2 | 1 | 3 | 1 | Barley | Barley | Unknown | 13.5 | 0.1987 | 6795.8145 | 2452.545 | 1 | 3.3896 |
| 28 | NZ | 2016 | 2 | 2 | 1 | 4 | 1 | Xi19 | wheat | Low | 14.2 | 0.1519 | 9350.5367 | 18593.48 | 1 | 4.2694 |
| 29 | NZ | 2016 | 2 | 2 | 1 | 5 | 1 | Hereward | wheat | High | 15.3 | 0.1958 | 7812.4351 | 4438.53 | 1 | 3.6472 |
| 30 | NZ | 2016 | 2 | 2 | 1 | 6 | 1 | Cadenza | wheat | Low | 15.5 | 0.2292 | 6764.0164 | 195.548 | 1 | 2.2913 |
| 31 | NZ | 2016 | 2 | 2 | 2 | 7 | 2 | Xi19 | wheat | Low | 19.2 | 0.2345 | 8189.3739 | 4318.08 | 1 | 3.6353 |
| 32 | NZ | 2016 | 2 | 2 | 2 | 8 | 2 | Duxford | wheat | High | 20.7 | 0.2119 | 9769.7882 | 1629.297 | 1 | 3.2120 |
| 33 | NZ | 2016 | 2 | 2 | 2 | 9 | 2 | Barley | Barley | Unknown | 11 | 0.2018 | 5449.7707 | 618.64 | 1 | 2.7914 |
| 34 | NZ | 2016 | 2 | 2 | 2 | 10 | 2 | Hereward | wheat | High | 17.2 | 0.2158 | 7968.7015 | 2495.376 | 1 | 3.3971 |
| 35 | NZ | 2016 | 2 | 2 | 2 | 11 | 2 | Cadenza | wheat | Low | 19 | 0.2117 | 8976.0743 | 3837.62 | 1 | 3.5841 |
| 36 | NZ | 2016 | 2 | 2 | 2 | 12 | 2 | Hereford | wheat | Low | 16.7 | 0.2161 | 7727.2353 | 629.089 | 1 | 2.7987 |
| 37 | NZ | 2016 | 2 | 2 | 3 | 13 | 3 | Hereford | wheat | Low | 17.1 | 0.2057 | 8313.7671 | 660.402 | 1 | 2.8198 |
| 38 | NZ | 2016 | 2 | 2 | 3 | 14 | 3 | Barley | Barley | Unknown | 16.8 | 0.2411 | 6968.8529 | 3300.36 | 1 | 3.5186 |
| 39 | NZ | 2016 | 2 | 2 | 3 | 15 | 3 | Cadenza | wheat | Low | 16.3 | 0.2151 | 7578.9479 | 355.992 | 1 | 2.5514 |
| 40 | NZ | 2016 | 2 | 2 | 3 | 16 | 3 | Xi19 | wheat | Low | 17.9 | 0.2156 | 8300.8723 | 1610.821 | 1 | 3.2070 |
| 41 | NZ | 2016 | 2 | 2 | 3 | 17 | 3 | Hereward | wheat | High | 6.86 | 0.2499 | 2744.6864 | 0 | 0 | * |
| 42 | NZ | 2016 | 2 | 2 | 3 | 18 | 3 | Duxford | wheat | High | 16.9 | 0.2154 | 7846.8979 | 14763.84 | 1 | 4.1692 |
| 43 | NZ | 2016 | 2 | 2 | 4 | 19 | 4 | Hereford | wheat | Low | 21.1 | 0.2021 | 10441.4318 | 1243.212 | 1 | 3.0945 |
| 44 | NZ | 2016 | 2 | 2 | 4 | 20 | 4 | Cadenza | wheat | Low | 23.1 | 0.2157 | 10711.7185 | 2453.451 | 1 | 3.3898 |
| 45 | NZ | 2016 | 2 | 2 | 4 | 21 | 4 | Duxford | wheat | High | 19.8 | 0.2072 | 9554.8560 | 3001.878 | 1 | 3.4774 |
| 46 | NZ | 2016 | 2 | 2 | 4 | 22 | 4 | Xi19 | wheat | Low | 16.3 | 0.2092 | 7792.6195 | 1277.594 | 1 | 3.1064 |
| 47 | NZ | 2016 | 2 | 2 | 4 | 23 | 4 | Barley | Barley | Unknown | 18 | 0.2014 | 8935.9959 | 383.652 | 1 | 2.5839 |
| 48 | NZ | 2016 | 2 | 2 | 4 | 24 | 4 | Hereward | wheat | High | 23 | 0.2126 | 10815.9756 | 7017.3 | 1 | 3.8462 |
| 49 | NZ | 2017 | 3 | 3 | 1 | 1 | 1 | Hereford | wheat | Low | 11.4 | 0.2320 | 4914.0999 | 4774.32 | 1 | 3.6789 |
| 50 | NZ | 2017 | 3 | 3 | 1 | 2 | 1 | Duxford | wheat | High | 10.7 | 0.2336 | 4579.7953 | 9892.15 | 1 | 3.9953 |

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|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 51 | NZ | 2017 | 3 | 3 | 1 | 3 | 1 | Barley | Barley | Unknown | 13.2 | 0.2362 | 5588.3894 | 297.6864 | 1 | 2.4738 |
| 52 | NZ | 2017 | 3 | 3 | 1 | 4 | 1 | Xi19 | wheat | Low | 15 | 0.2313 | 6484.0953 | 3670.2 | 1 | 3.5647 |
| 53 | NZ | 2017 | 3 | 3 | 1 | 5 | 1 | Hereward | wheat | High | 15.4 | 0.2255 | 6828.6888 | 1493.184 | 1 | 3.1741 |
| 54 | NZ | 2017 | 3 | 3 | 1 | 6 | 1 | Cadenza | wheat | Low | 10.7 | 0.2279 | 4695.4740 | 750.177 | 1 | 2.8752 |
| 55 | NZ | 2017 | 3 | 3 | 2 | 7 | 2 | Xi19 | wheat | Low | 16.4 | 0.2270 | 7225.4318 | 1837.128 | 1 | 3.2641 |
| 56 | NZ | 2017 | 3 | 3 | 2 | 8 | 2 | Duxford | wheat | High | 16.6 | 0.2227 | 7453.1270 | 3214.424 | 1 | 3.5071 |
| 57 | NZ | 2017 | 3 | 3 | 2 | 9 | 2 | Barley | Barley | Unknown | 13.1 | 0.2263 | 5789.0695 | 522.035 | 1 | 2.7177 |
| 58 | NZ | 2017 | 3 | 3 | 2 | 10 | 2 | Hereward | wheat | High | 10.9 | 0.2247 | 4850.1041 | 717.438 | 1 | 2.8558 |
| 59 | NZ | 2017 | 3 | 3 | 2 | 11 | 2 | Cadenza | wheat | Low | 11.1 | 0.2235 | 4965.3787 | 2278.941 | 1 | 3.3577 |
| 60 | NZ | 2017 | 3 | 3 | 2 | 12 | 2 | Hereford | wheat | Low | 15.4 | 0.2214 | 6955.2459 | 4840.22 | 1 | 3.6849 |
| 61 | NZ | 2017 | 3 | 3 | 3 | 13 | 3 | Hereford | wheat | Low | 17.4 | 0.2185 | 7964.1025 | 942.384 | 1 | 2.9742 |
| 62 | NZ | 2017 | 3 | 3 | 3 | 14 | 3 | Barley | Barley | Unknown | 14.4 | 0.2176 | 6616.8882 | 1293.552 | 1 | 3.1118 |
| 63 | NZ | 2017 | 3 | 3 | 3 | 15 | 3 | Cadenza | wheat | Low | 15.1 | 0.2294 | 6583.5909 | 351.5129 | 1 | 2.5459 |
| 64 | NZ | 2017 | 3 | 3 | 3 | 16 | 3 | Xi19 | wheat | Low | 9.92 | 0.2284 | 4343.1785 | 959.1648 | 1 | 2.9819 |
| 65 | NZ | 2017 | 3 | 3 | 3 | 17 | 3 | Hereward | wheat | High | 10.7 | 0.2337 | 4579.1766 | 481.5 | 1 | 2.6826 |
| 66 | NZ | 2017 | 3 | 3 | 3 | 18 | 3 | Duxford | wheat | High | 12.5 | 0.2387 | 5236.9729 | 769.5 | 1 | 2.8862 |
| 67 | NZ | 2017 | 3 | 3 | 4 | 19 | 4 | Hereford | wheat | Low | 15.5 | 0.2327 | 6661.3742 | 514.2435 | 1 | 2.7112 |
| 68 | NZ | 2017 | 3 | 3 | 4 | 20 | 4 | Cadenza | wheat | Low | 13.6 | 0.2328 | 5842.3875 | 18256.64 | 1 | 4.2614 |
| 69 | NZ | 2017 | 3 | 3 | 4 | 21 | 4 | Duxford | wheat | High | 10.2 | 0.2374 | 4295.9364 | 740.316 | 1 | 2.8694 |
| 70 | NZ | 2017 | 3 | 3 | 4 | 22 | 4 | Xi19 | wheat | Low | 19.1 | 0.2347 | 8138.8774 | 1242.646 | 1 | 3.0943 |
| 71 | NZ | 2017 | 3 | 3 | 4 | 23 | 4 | Barley | Barley | Unknown | 20.9 | 0.2302 | 9079.9631 | 128.7022 | 1 | 2.1096 |
| 72 | NZ | 2017 | 3 | 3 | 4 | 24 | 4 | Hereward | wheat | High | 13.8 | 0.2416 | 5711.8309 | 933.57 | 1 | 2.9701 |
| 73 | LH5 | 2015 | 0 | 0 | 1 | 1 | 1 | Barley | Barley | Unknown | 6.62 | 0.2120 | 3122.0159 | 0 | 0 | * |
| 74 | LH5 | 2015 | 0 | 0 | 1 | 2 | 1 | Cadenza | wheat | Low | 3.8 | 0.2100 | 1809.8931 | 0 | 0 | * |
| 75 | LH5 | 2015 | 0 | 0 | 1 | 3 | 1 | Hereford | wheat | Low | 6.5 | 0.2125 | 3059.3507 | 0 | 0 | * |
| 76 | LH5 | 2015 | 0 | 0 | 2 | 4 | 1 | Xi19 | wheat | Low | 2.3 | 0.2090 | 1100.2703 | 0 | 0 | * |
| 77 | LH5 | 2015 | 0 | 0 | 2 | 5 | 1 | Hereward | wheat | High | 4.9 | 0.2107 | 2325.3318 | 0 | 0 | * |
| 78 | LH5 | 2015 | 0 | 0 | 2 | 6 | 1 | Duxford | wheat | High | 4.09 | 0.1788 | 2287.1021 | 0 | 0 | * |
| 79 | LH5 | 2015 | 0 | 0 | 3 | 7 | 2 | Cadenza | wheat | Low | 3.9 | 0.2124 | 1836.4342 | 0 | 0 | * |

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|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 80 | LH5 | 2015 | 0 | 0 | 3 | 8 | 2 | Hereford | wheat | Low | 4.6 | 0.2061 | 2232.3111 | 0 | 0 | * |
| 81 | LH5 | 2015 | 0 | 0 | 3 | 9 | 2 | Xi19 | wheat | Low | 10.6 | 0.2140 | 4952.7565 | 0 | 0 | * |
| 82 | LH5 | 2015 | 0 | 0 | 4 | 10 | 2 | Hereward | wheat | High | 12.1 | 0.2132 | 5674.4047 | 0 | 0 | * |
| 83 | LH5 | 2015 | 0 | 0 | 4 | 11 | 2 | Duxford | wheat | High | 6.54 | 0.2146 | 3047.9759 | 0 | 0 | * |
| 84 | LH5 | 2015 | 0 | 0 | 4 | 12 | 2 | Barley | Barley | Unknown | 6.04 | 0.2159 | 2797.2414 | 0 | 0 | * |
| 85 | LH5 | 2015 | 0 | 0 | 1 | 13 | 3 | Xi19 | wheat | Low | 4.05 | 0.2077 | 1950.2997 | 0 | 0 | * |
| 86 | LH5 | 2015 | 0 | 0 | 1 | 14 | 3 | Hereward | wheat | High | 4.99 | 0.2140 | 2331.6914 | 0 | 0 | * |
| 87 | LH5 | 2015 | 0 | 0 | 1 | 15 | 3 | Duxford | wheat | High | 5.42 | 0.2073 | 2614.8375 | 0 | 0 | * |
| 88 | LH5 | 2015 | 0 | 0 | 2 | 16 | 3 | Barley | Barley | Unknown | 6.3 | 0.2097 | 3004.6154 | 0 | 0 | * |
| 89 | LH5 | 2015 | 0 | 0 | 2 | 17 | 3 | Cadenza | wheat | Low | 6.73 | 0.1951 | 3450.2496 | 0 | 0 | * |
| 90 | LH5 | 2015 | 0 | 0 | 2 | 18 | 3 | Hereford | wheat | Low | 6.5 | 0.2481 | 2619.9440 | 0 | 0 | * |
| 91 | LH5 | 2015 | 0 | 0 | 3 | 19 | 4 | Duxford | wheat | High | 7.75 | 0.2058 | 3766.5959 | 0 | 0 | * |
| 92 | LH5 | 2015 | 0 | 0 | 3 | 20 | 4 | Barley | Barley | Unknown | 5.1 | 0.2154 | 2367.9776 | 0 | 0 | * |
| 93 | LH5 | 2015 | 0 | 0 | 3 | 21 | 4 | Hereward | wheat | High | 3.37 | 0.1995 | 1688.8074 | 0 | 0 | * |
| 94 | LH5 | 2015 | 0 | 0 | 4 | 22 | 4 | Hereford | wheat | Low | 2.14 | 0.2129 | 1005.1715 | 0 | 0 | * |
| 95 | LH5 | 2015 | 0 | 0 | 4 | 23 | 4 | Xi19 | wheat | Low | 6.67 | 0.2089 | 3192.8899 | 0 | 0 | * |
| 96 | LH5 | 2015 | 0 | 0 | 4 | 24 | 4 | Cadenza | wheat | Low | 5.19 | 0.2318 | 2238.9259 | 0 | 0 | * |
| 97 | LH5 | 2016 | 1 | 1 | 1 | 1 | 1 | Barley | Barley | Unknown | 8.04 | 0.2260 | 3557.8061 | 0 | 0 | * |
| 98 | LH5 | 2016 | 1 | 1 | 1 | 2 | 1 | Cadenza | wheat | Low | 10.7 | 0.2303 | 4645.6234 | 0 | 0 | * |
| 99 | LH5 | 2016 | 1 | 1 | 1 | 3 | 1 | Hereford | wheat | Low | 10.7 | 0.2305 | 4641.5979 | 0 | 0 | * |
| 100 | LH5 | 2016 | 1 | 1 | 2 | 4 | 1 | Xi19 | wheat | Low | 7.4 | 0.2292 | 3229.1348 | 0 | 0 | * |
| 101 | LH5 | 2016 | 1 | 1 | 2 | 5 | 1 | Hereward | wheat | High | 10.7 | 0.2443 | 4379.9383 | 0 | 0 | * |
| 102 | LH5 | 2016 | 1 | 1 | 2 | 6 | 1 | Duxford | wheat | High | 10.3 | 0.2287 | 4502.7510 | 141.9031 | 1 | 2.1520 |
| 103 | LH5 | 2016 | 1 | 1 | 3 | 7 | 2 | Cadenza | wheat | Low | 11.7 | 0.2268 | 5158.8687 | 0 | 0 | * |
| 104 | LH5 | 2016 | 1 | 1 | 3 | 8 | 2 | Hereford | wheat | Low | 12.5 | 0.2304 | 5425.9186 | 87.4375 | 1 | 1.9417 |
| 105 | LH5 | 2016 | 1 | 1 | 3 | 9 | 2 | Xi19 | wheat | Low | 4.64 | 0.2271 | 2043.0222 | 73.81312 | 1 | 1.8681 |
| 106 | LH5 | 2016 | 1 | 1 | 4 | 10 | 2 | Hereward | wheat | High | 5.05 | 0.2282 | 2212.7085 | 177.3055 | 1 | 2.2487 |
| 107 | LH5 | 2016 | 1 | 1 | 4 | 11 | 2 | Duxford | wheat | High | 10.8 | 0.2298 | 4699.1889 | 0 | 0 | * |
| 108 | LH5 | 2016 | 1 | 1 | 4 | 12 | 2 | Barley | Barley | Unknown | 11.8 | 0.2172 | 5432.0246 | 0 | 0 | * |

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|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 109 | LH5 | 2016 | 1 | 1 | 1 | 13 | 3 | Xi19 | wheat | Low | 13.3 | 0.2220 | 5990.4453 | 0 | 0 | * |
| 110 | LH5 | 2016 | 1 | 1 | 1 | 14 | 3 | Hereward | wheat | High | 9.78 | 0.2201 | 4443.5828 | 88.66548 | 1 | 1.9478 |
| 111 | LH5 | 2016 | 1 | 1 | 1 | 15 | 3 | Duxford | wheat | High | 13.9 | 0.2277 | 6103.9646 | 58.4495 | 1 | 1.7668 |
| 112 | LH5 | 2016 | 1 | 1 | 2 | 16 | 3 | Barley | Barley | Unknown | 11.2 | 0.2085 | 5372.3306 | 0 | 0 | * |
| 113 | LH5 | 2016 | 1 | 1 | 2 | 17 | 3 | Cadenza | wheat | Low | 10.9 | 0.1865 | 5843.8592 | 44.363 | 1 | 1.6470 |
| 114 | LH5 | 2016 | 1 | 1 | 2 | 18 | 3 | Hereford | wheat | Low | 9.69 | 0.1718 | 5640.7914 | 0 | 0 | * |
| 115 | LH5 | 2016 | 1 | 1 | 3 | 19 | 4 | Duxford | wheat | High | 7.54 | 0.2241 | 3364.9905 | 30.6878 | 1 | 1.4870 |
| 116 | LH5 | 2016 | 1 | 1 | 3 | 20 | 4 | Barley | Barley | Unknown | 3.93 | 0.1988 | 1976.5614 | 0 | 0 | * |
| 117 | LH5 | 2016 | 1 | 1 | 3 | 21 | 4 | Hereward | wheat | High | 12 | 0.2318 | 5176.0879 | 625.44 | 1 | 2.7962 |
| 118 | LH5 | 2016 | 1 | 1 | 4 | 22 | 4 | Hereford | wheat | Low | 9.89 | 0.2322 | 4258.7265 | 0 | 0 | * |
| 119 | LH5 | 2016 | 1 | 1 | 4 | 23 | 4 | Xi19 | wheat | Low | 12.2 | 0.2283 | 5344.1977 | 0 | 0 | * |
| 120 | LH5 | 2016 | 1 | 1 | 4 | 24 | 4 | Cadenza | wheat | Low | 14.9 | 0.2265 | 6578.5596 | 509.9674 | 1 | 2.7075 |
| 121 | LH5 | 2017 | 2 | 2 | 1 | 1 | 1 | Barley | Barley | Unknown | 16.7 | 0.2374 | 7034.2915 | 0 | 0 | * |
| 122 | LH5 | 2017 | 2 | 2 | 1 | 2 | 1 | Cadenza | wheat | Low | 10.2 | 0.2419 | 4217.2303 | 90.7596 | 1 | 1.9579 |
| 123 | LH5 | 2017 | 2 | 2 | 1 | 3 | 1 | Hereford | wheat | Low | 12.7 | 0.2451 | 5180.6235 | 55.245 | 1 | 1.7423 |
| 124 | LH5 | 2017 | 2 | 2 | 2 | 4 | 1 | Xi19 | wheat | Low | 14.1 | 0.2428 | 5807.7897 | 4079.13 | 1 | 3.6106 |
| 125 | LH5 | 2017 | 2 | 2 | 2 | 5 | 1 | Hereward | wheat | High | 14.9 | 0.2392 | 6229.7978 | 10376.36 | 1 | 4.0160 |
| 126 | LH5 | 2017 | 2 | 2 | 2 | 6 | 1 | Duxford | wheat | High | 14 | 0.2422 | 5779.9873 | 950.32 | 1 | 2.9779 |
| 127 | LH5 | 2017 | 2 | 2 | 3 | 7 | 2 | Cadenza | wheat | Low | 14.5 | 0.2401 | 6038.9851 | 234.5375 | 1 | 2.3702 |
| 128 | LH5 | 2017 | 2 | 2 | 3 | 8 | 2 | Hereford | wheat | Low | 16 | 0.2388 | 6700.3264 | 121.488 | 1 | 2.0845 |
| 129 | LH5 | 2017 | 2 | 2 | 3 | 9 | 2 | Xi19 | wheat | Low | 12.7 | 0.2400 | 5290.6092 | 248.158 | 1 | 2.3947 |
| 130 | LH5 | 2017 | 2 | 2 | 4 | 10 | 2 | Hereward | wheat | High | 16.1 | 0.2404 | 6695.8010 | 2255.127 | 1 | 3.3532 |
| 131 | LH5 | 2017 | 2 | 2 | 4 | 11 | 2 | Duxford | wheat | High | 10.5 | 0.2418 | 4342.6354 | 667.8 | 1 | 2.8246 |
| 132 | LH5 | 2017 | 2 | 2 | 4 | 12 | 2 | Barley | Barley | Unknown | 13.5 | 0.2348 | 5749.8126 | 0 | 0 | * |
| 133 | LH5 | 2017 | 2 | 2 | 1 | 13 | 3 | Xi19 | wheat | Low | 15.7 | 0.2373 | 6616.4950 | 2983.942 | 1 | 3.4748 |
| 134 | LH5 | 2017 | 2 | 2 | 1 | 14 | 3 | Hereward | wheat | High | 9.38 | 0.2329 | 4026.6534 | 3985.562 | 1 | 3.6005 |
| 135 | LH5 | 2017 | 2 | 2 | 1 | 15 | 3 | Duxford | wheat | High | 14.4 | 0.2353 | 6118.8997 | 638.64 | 1 | 2.8053 |
| 136 | LH5 | 2017 | 2 | 2 | 2 | 16 | 3 | Barley | Barley | Unknown | 14.9 | 0.2321 | 6419.4013 | 0 | 0 | * |
| 137 | LH5 | 2017 | 2 | 2 | 2 | 17 | 3 | Cadenza | wheat | Low | 14 | 0.2398 | 5837.7329 | 132.552 | 1 | 2.1224 |

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|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 138 | LH5 | 2017 | 2 | 2 | 2 | 18 | 3 | Hereford | wheat | Low | 13.2 | 0.2384 | 5536.1219 | 131.7228 | 1 | 2.1197 |
| 139 | LH5 | 2017 | 2 | 2 | 3 | 19 | 4 | Duxford | wheat | High | 14.6 | 0.2384 | 6123.6359 | 225.789 | 1 | 2.3537 |
| 140 | LH5 | 2017 | 2 | 2 | 3 | 20 | 4 | Barley | Barley | Unknown | 14 | 0.2348 | 5961.6806 | 0 | 0 | 0.0000 |
| 141 | LH5 | 2017 | 2 | 2 | 3 | 21 | 4 | Hereward | wheat | High | 13.8 | 0.2355 | 5859.5090 | 2096.772 | 1 | 3.3216 |
| 142 | LH5 | 2017 | 2 | 2 | 4 | 22 | 4 | Hereford | wheat | Low | 14.7 | 0.2318 | 6341.1191 | 1000.923 | 1 | 3.0004 |
| 143 | LH5 | 2017 | 2 | 2 | 4 | 23 | 4 | Xi19 | wheat | Low | 12.6 | 0.2324 | 5421.8997 | 173.628 | 1 | 2.2396 |
| 144 | LH5 | 2017 | 2 | 2 | 4 | 24 | 4 | Cadenza | wheat | Low | 11.4 | 0.2285 | 4989.5580 | 0 | 0 | * |
| 145 | LH4 | 2016 | 0 | 0 | 1 | 1 | 1 | Xi19 | wheat | Low | 8.92 | 0.2067 | 4314.4506 | 0 | 0 | * |
| 146 | LH4 | 2016 | 0 | 0 | 1 | 2 | 1 | Cadenza | wheat | Low | 7.64 | 0.2116 | 3610.3146 | 0 | 0 | * |
| 147 | LH4 | 2016 | 0 | 0 | 1 | 3 | 1 | Hereward | wheat | High | 12.2 | 0.2203 | 5538.5805 | 0 | 0 | * |
| 148 | LH4 | 2016 | 0 | 0 | 2 | 4 | 1 | Hereward | wheat | High | 7.17 | 0.2191 | 3272.0451 | 0 | 0 | * |
| 149 | LH4 | 2016 | 0 | 0 | 2 | 5 | 1 | Barley | Barley | Unknown | 9.82 | 0.2376 | 4132.8152 | 0 | 0 | * |
| 150 | LH4 | 2016 | 0 | 0 | 2 | 6 | 1 | Duxford | wheat | High | 11.6 | 0.2094 | 5539.0151 | 0 | 0 | * |
| 151 | LH4 | 2016 | 0 | 0 | 3 | 7 | 2 | Duxford | wheat | High | 8.11 | 0.2107 | 3849.2010 | 15.056215 | 1 | 1.1777 |
| 152 | LH4 | 2016 | 0 | 0 | 3 | 8 | 2 | Hereward | wheat | High | 11.2 | 0.2081 | 5382.4632 | 0 | 0 | * |
| 153 | LH4 | 2016 | 0 | 0 | 3 | 9 | 2 | Barley | Barley | Unknown | 8.98 | 0.2095 | 4286.8738 | 62.12364 | 1 | 1.7933 |
| 154 | LH4 | 2016 | 0 | 0 | 4 | 10 | 2 | Duxford | wheat | High | 9.75 | 0.2090 | 4664.6877 | 14.937 | 1 | 1.1743 |
| 155 | LH4 | 2016 | 0 | 0 | 4 | 11 | 2 | Barley | Barley | Unknown | 9.59 | 0.2103 | 4560.6851 | 0 | 0 | * |
| 156 | LH4 | 2016 | 0 | 0 | 4 | 12 | 2 | Hereford | wheat | Low | 8.67 | 0.2067 | 4193.9529 | 0 | 0 | * |
| 157 | LH4 | 2016 | 0 | 0 | 1 | 13 | 3 | Hereford | wheat | Low | 10.2 | 0.2136 | 4775.8068 | 218.688 | 1 | 2.3398 |
| 158 | LH4 | 2016 | 0 | 0 | 1 | 14 | 3 | Barley | Barley | Unknown | 10.6 | 0.2079 | 5098.0050 | 0 | 0 | * |
| 159 | LH4 | 2016 | 0 | 0 | 1 | 15 | 3 | Duxford | wheat | High | 8.14 | 0.2083 | 3908.3829 | 0 | 0 | * |
| 160 | LH4 | 2016 | 0 | 0 | 2 | 16 | 3 | Xi19 | wheat | Low | 7.13 | 0.2080 | 3427.0926 | 277.9274 | 1 | 2.4439 |
| 161 | LH4 | 2016 | 0 | 0 | 2 | 17 | 3 | Cadenza | wheat | Low | 7.54 | 0.2089 | 3609.4979 | 0 | 0 | * |
| 162 | LH4 | 2016 | 0 | 0 | 2 | 18 | 3 | Hereford | wheat | Low | 10.4 | 0.2083 | 4993.2702 | 0 | 0 | * |
| 163 | LH4 | 2016 | 0 | 0 | 3 | 19 | 4 | Cadenza | wheat | Low | 10.4 | 0.2072 | 5018.9862 | 0 | 0 | * |
| 164 | LH4 | 2016 | 0 | 0 | 3 | 20 | 4 | Xi19 | wheat | Low | 11.8 | 0.2046 | 5767.3921 | 0 | 0 | * |
| 165 | LH4 | 2016 | 0 | 0 | 3 | 21 | 4 | Hereford | wheat | Low | 6.18 | 0.2069 | 2987.2023 | 470.1126 | 1 | 2.6722 |
| 166 | LH4 | 2016 | 0 | 0 | 4 | 22 | 4 | Hereward | wheat | High | 10 | 0.2058 | 4858.2244 | 76.93 | 1 | 1.8861 |

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|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 167 | LH4 | 2016 | 0 | 0 | 4 | 23 | 4 | Cadenza | wheat | Low | 11.4 | 0.2057 | 5541.6573 | 0 | 0 | * |
| 168 | LH4 | 2016 | 0 | 0 | 4 | 24 | 4 | Xi19 | wheat | Low | 9.43 | 0.2065 | 4565.7250 | 0 | 0 | * |
| 169 | LH4 | 2017 | 1 | 1 | 1 | 1 | 1 | Xi19 | wheat | Low | 12.1 | 0.2320 | 5216.1514 | 0 | 0 | * |
| 170 | LH4 | 2017 | 1 | 1 | 1 | 2 | 1 | Cadenza | wheat | Low | 11 | 0.2332 | 4717.7529 | 0 | 0 | * |
| 171 | LH4 | 2017 | 1 | 1 | 1 | 3 | 1 | Hereward | wheat | High | 11.6 | 0.2344 | 4948.9284 | 0 | 0 | * |
| 172 | LH4 | 2017 | 1 | 1 | 2 | 4 | 1 | Hereward | wheat | High | 12 | 0.2285 | 5251.0221 | 0 | 0 | * |
| 173 | LH4 | 2017 | 1 | 1 | 2 | 5 | 1 | Barley | Barley | Unknown | 16.3 | 0.2268 | 7186.2637 | 0 | 0 | * |
| 174 | LH4 | 2017 | 1 | 1 | 2 | 6 | 1 | Duxford | wheat | High | 15.5 | 0.2286 | 6780.5140 | 0 | 0 | * |
| 175 | LH4 | 2017 | 1 | 1 | 3 | 7 | 2 | Duxford | wheat | High | 17.8 | 0.2290 | 7771.9350 | 312.568 | 1 | 2.4949 |
| 176 | LH4 | 2017 | 1 | 1 | 3 | 8 | 2 | Hereward | wheat | High | 14.7 | 0.2254 | 6522.9218 | 1022.973 | 1 | 3.0099 |
| 177 | LH4 | 2017 | 1 | 1 | 3 | 9 | 2 | Barley | Barley | Unknown | 17.9 | 0.2196 | 8152.1425 | 0 | 0 | * |
| 178 | LH4 | 2017 | 1 | 1 | 4 | 10 | 2 | Duxford | wheat | High | 17.3 | 0.2236 | 7738.6009 | 193.6389 | 1 | 2.2870 |
| 179 | LH4 | 2017 | 1 | 1 | 4 | 11 | 2 | Barley | Barley | Unknown | 12.3 | 0.2190 | 5617.5663 | 0 | 0 | * |
| 180 | LH4 | 2017 | 1 | 1 | 4 | 12 | 2 | Hereford | wheat | Low | 17.3 | 0.2216 | 7807.0108 | 0 | 0 | * |
| 181 | LH4 | 2017 | 1 | 1 | 1 | 13 | 3 | Hereford | wheat | Low | 14.6 | 0.2249 | 6490.7505 | 3209.08 | 1 | 3.5064 |
| 182 | LH4 | 2017 | 1 | 1 | 1 | 14 | 3 | Barley | Barley | Unknown | 15.9 | 0.2481 | 6409.6501 | 101.2194 | 1 | 2.0053 |
| 183 | LH4 | 2017 | 1 | 1 | 1 | 15 | 3 | Duxford | wheat | High | 16.2 | 0.2451 | 6610.2292 | 0 | 0 | * |
| 184 | LH4 | 2017 | 1 | 1 | 2 | 16 | 3 | Xi19 | wheat | Low | 12.7 | 0.2446 | 5191.7340 | 5027.93 | 1 | 3.7014 |
| 185 | LH4 | 2017 | 1 | 1 | 2 | 17 | 3 | Cadenza | wheat | Low | 15.6 | 0.2450 | 6367.9917 | 96.2676 | 1 | 1.9835 |
| 186 | LH4 | 2017 | 1 | 1 | 2 | 18 | 3 | Hereford | wheat | Low | 13.3 | 0.2449 | 5429.7920 | 82.6063 | 1 | 1.9170 |
| 187 | LH4 | 2017 | 1 | 1 | 3 | 19 | 4 | Cadenza | wheat | Low | 20.5 | 0.2417 | 8482.0336 | 0 | 0 | * |
| 188 | LH4 | 2017 | 1 | 1 | 3 | 20 | 4 | Xi19 | wheat | Low | 28.3 | 0.2427 | 11662.7281 | 0 | 0 | * |
| 189 | LH4 | 2017 | 1 | 1 | 3 | 21 | 4 | Hereford | wheat | Low | 21.7 | 0.2402 | 9032.4818 | 15947.33 | 1 | 4.2027 |
| 190 | LH4 | 2017 | 1 | 1 | 4 | 22 | 4 | Hereward | wheat | High | 24.3 | 0.2402 | 10118.3903 | 1736.235 | 1 | 3.2396 |
| 191 | LH4 | 2017 | 1 | 1 | 4 | 23 | 4 | Cadenza | wheat | Low | 26.9 | 0.2344 | 11474.1770 | 0 | 0 | * |
| 192 | LH4 | 2017 | 1 | 1 | 4 | 24 | 4 | Xi19 | wheat | Low | 20.1 | 0.2332 | 8619.1174 | 0 | 0 | * |

**9.4.4 Appendix IV:** Percent infected roots and take-all index data for fields New Zealand, Long Hoos 5 and Long Hoos 4. The (*) data not available.

| Tube No | Field | Year | Plot | Cultivar | % roots infected post-harvest soil core bioassay | Plant Samples Take-all Index |
|---|---|---|---|---|---|---|
| 1 | NZ | 1 | 1 | Hereford | 24.08 | 0.67 |
| 2 | NZ | 1 | 2 | Duxford | 59.91 | 0.67 |
| 3 | NZ | 1 | 3 | Barley | 3.54 | 1.05 |
| 4 | NZ | 1 | 4 | Xi19 | 40.89 | 0.00 |
| 5 | NZ | 1 | 5 | Hereward | 39.91 | 4.44 |
| 6 | NZ | 1 | 6 | Cadenza | 15.35 | 3.03 |
| 7 | NZ | 1 | 7 | Xi19 | 34.71 | 0.00 |
| 8 | NZ | 1 | 8 | Duxford | 55.11 | 1.21 |
| 9 | NZ | 1 | 9 | Barley | 7.62 | 0.00 |
| 10 | NZ | 1 | 10 | Hereward | 49.77 | 1.08 |
| 11 | NZ | 1 | 11 | Cadenza | 9.84 | 8.57 |
| 12 | NZ | 1 | 12 | Hereford | 36.82 | 0.00 |
| 13 | NZ | 1 | 13 | Hereford | 22.40 | 0.00 |
| 14 | NZ | 1 | 14 | Barley | 22.46 | 0.00 |
| 15 | NZ | 1 | 15 | Cadenza | 16.00 | 0.00 |
| 16 | NZ | 1 | 16 | Xi19 | 33.91 | 0.00 |
| 17 | NZ | 1 | 17 | Hereward | 30.96 | 1.00 |
| 18 | NZ | 1 | 18 | Duxford | 46.22 | 1.62 |
| 19 | NZ | 1 | 19 | Hereford | 36.36 | 0.00 |
| 20 | NZ | 1 | 20 | Cadenza | 32.77 | 0.69 |
| 21 | NZ | 1 | 21 | Duxford | 39.62 | 4.62 |
| 22 | NZ | 1 | 22 | Xi19 | 45.02 | 1.60 |

| | | | | | | |
|---|---|---|---|---|---|---|
| 23 | NZ | 1 | 23 | Barley | 8.81 | 0.00 |
| 24 | NZ | 1 | 24 | Hereward | 51.21 | 0.71 |
| 25 | NZ | 2 | 1 | Hereford | 60.58 | * |
| 26 | NZ | 2 | 2 | Duxford | 67.13 | * |
| 27 | NZ | 2 | 3 | Barley | 38.14 | * |
| 28 | NZ | 2 | 4 | Xi19 | 45.61 | * |
| 29 | NZ | 2 | 5 | Hereward | 42.86 | * |
| 30 | NZ | 2 | 6 | Cadenza | 57.97 | * |
| 31 | NZ | 2 | 7 | Xi19 | 71.09 | * |
| 32 | NZ | 2 | 8 | Duxford | 70.37 | * |
| 33 | NZ | 2 | 9 | Barley | 52.01 | * |
| 34 | NZ | 2 | 10 | Hereward | 48.93 | * |
| 35 | NZ | 2 | 11 | Cadenza | 59.87 | * |
| 36 | NZ | 2 | 12 | Hereford | 63.84 | * |
| 37 | NZ | 2 | 13 | Hereford | 76.83 | * |
| 38 | NZ | 2 | 14 | Barley | 35.02 | * |
| 39 | NZ | 2 | 15 | Cadenza | 28.11 | * |
| 40 | NZ | 2 | 16 | Xi19 | 75.80 | * |
| 41 | NZ | 2 | 17 | Hereward | 73.38 | * |
| 42 | NZ | 2 | 18 | Duxford | 50.00 | * |
| 43 | NZ | 2 | 19 | Hereford | 38.16 | * |
| 44 | NZ | 2 | 20 | Cadenza | 75.00 | * |
| 45 | NZ | 2 | 21 | Duxford | 72.31 | * |
| 46 | NZ | 2 | 22 | Xi19 | 34.98 | * |
| 47 | NZ | 2 | 23 | Barley | 38.06 | * |
| 48 | NZ | 2 | 24 | Hereward | 38.71 | * |
| 49 | NZ | 3 | 1 | Hereford | * | 62.86 |

| | | | | | | |
|---|---|---|---|---|---|---|
| 50 | NZ | 3 | 2 | Duxford | * | 61.25 |
| 51 | NZ | 3 | 3 | Barley | * | 28.00 |
| 52 | NZ | 3 | 4 | Xi19 | * | 80.00 |
| 53 | NZ | 3 | 5 | Hereward | * | 66.96 |
| 54 | NZ | 3 | 6 | Cadenza | * | 60.71 |
| 55 | NZ | 3 | 7 | Xi19 | * | 80.00 |
| 56 | NZ | 3 | 8 | Duxford | * | 98.75 |
| 57 | NZ | 3 | 9 | Barley | * | 34.29 |
| 58 | NZ | 3 | 10 | Hereward | * | 89.03 |
| 59 | NZ | 3 | 11 | Cadenza | * | 67.14 |
| 60 | NZ | 3 | 12 | Hereford | * | 59.41 |
| 61 | NZ | 3 | 13 | Hereford | * | 68.89 |
| 62 | NZ | 3 | 14 | Barley | * | 21.33 |
| 63 | NZ | 3 | 15 | Cadenza | * | 60.00 |
| 64 | NZ | 3 | 16 | Xi19 | * | 90.77 |
| 65 | NZ | 3 | 17 | Hereward | * | 78.06 |
| 66 | NZ | 3 | 18 | Duxford | * | 78.71 |
| 67 | NZ | 3 | 19 | Hereford | * | 27.50 |
| 68 | NZ | 3 | 20 | Cadenza | * | 73.33 |
| 69 | NZ | 3 | 21 | Duxford | * | 70.00 |
| 70 | NZ | 3 | 22 | Xi19 | * | 45.56 |
| 71 | NZ | 3 | 23 | Barley | * | 14.29 |
| 72 | NZ | 3 | 24 | Hereward | * | 57.50 |
| 73 | LH5 | 0 | 1 | Barley | * | * |
| 74 | LH5 | 0 | 2 | Cadenza | * | * |
| 75 | LH5 | 0 | 3 | Hereford | * | * |
| 76 | LH5 | 0 | 4 | Xi19 | * | * |

| | | | | | | |
|---|---|---|---|---|---|---|
| 77 | LH5 | 0 | 5 | Hereward | * | * |
| 78 | LH5 | 0 | 6 | Duxford | * | * |
| 79 | LH5 | 0 | 7 | Cadenza | * | * |
| 80 | LH5 | 0 | 8 | Hereford | * | * |
| 81 | LH5 | 0 | 9 | Xi19 | * | * |
| 82 | LH5 | 0 | 10 | Hereward | * | * |
| 83 | LH5 | 0 | 11 | Duxford | * | * |
| 84 | LH5 | 0 | 12 | Barley | * | * |
| 85 | LH5 | 0 | 13 | Xi19 | * | * |
| 86 | LH5 | 0 | 14 | Hereward | * | * |
| 87 | LH5 | 0 | 15 | Duxford | * | * |
| 88 | LH5 | 0 | 16 | Barley | * | * |
| 89 | LH5 | 0 | 17 | Cadenza | * | * |
| 90 | LH5 | 0 | 18 | Hereford | * | * |
| 91 | LH5 | 0 | 19 | Duxford | * | * |
| 92 | LH5 | 0 | 20 | Barley | * | * |
| 93 | LH5 | 0 | 21 | Hereward | * | * |
| 94 | LH5 | 0 | 22 | Hereford | * | * |
| 95 | LH5 | 0 | 23 | Xi19 | * | * |
| 96 | LH5 | 0 | 24 | Cadenza | * | * |
| 97 | LH5 | 1 | 1 | Barley | 23.21 | * |
| 98 | LH5 | 1 | 2 | Cadenza | 29.35 | * |
| 99 | LH5 | 1 | 3 | Hereford | 20.06 | * |
| 100 | LH5 | 1 | 4 | Xi19 | 39.16 | * |
| 101 | LH5 | 1 | 5 | Hereward | 42.37 | * |
| 102 | LH5 | 1 | 6 | Duxford | 13.83 | * |
| 103 | LH5 | 1 | 7 | Cadenza | 21.32 | * |

| | | | | | | |
|---|---|---|---|---|---|---|
| 104 | LH5 | 1 | 8 | Hereford | 22.11 | * |
| 105 | LH5 | 1 | 9 | Xi19 | 26.49 | * |
| 106 | LH5 | 1 | 10 | Hereward | 34.69 | * |
| 107 | LH5 | 1 | 11 | Duxford | 25.57 | * |
| 108 | LH5 | 1 | 12 | Barley | 16.71 | * |
| 109 | LH5 | 1 | 13 | Xi19 | 25.43 | * |
| 110 | LH5 | 1 | 14 | Hereward | 15.64 | * |
| 111 | LH5 | 1 | 15 | Duxford | 19.08 | * |
| 112 | LH5 | 1 | 16 | Barley | 32.12 | * |
| 113 | LH5 | 1 | 17 | Cadenza | 46.01 | * |
| 114 | LH5 | 1 | 18 | Hereford | 11.65 | * |
| 115 | LH5 | 1 | 19 | Duxford | 11.97 | * |
| 116 | LH5 | 1 | 20 | Barley | 18.27 | * |
| 117 | LH5 | 1 | 21 | Hereward | 50.58 | * |
| 118 | LH5 | 1 | 22 | Hereford | 1.70 | * |
| 119 | LH5 | 1 | 23 | Xi19 | 6.23 | * |
| 120 | LH5 | 1 | 24 | Cadenza | 8.49 | * |
| 121 | LH5 | 2 | 1 | Barley | * | 18.89 |
| 122 | LH5 | 2 | 2 | Cadenza | * | 20.83 |
| 123 | LH5 | 2 | 3 | Hereford | * | 44.21 |
| 124 | LH5 | 2 | 4 | Xi19 | * | 52.26 |
| 125 | LH5 | 2 | 5 | Hereward | * | 99.33 |
| 126 | LH5 | 2 | 6 | Duxford | * | 44.62 |
| 127 | LH5 | 2 | 7 | Cadenza | * | 16.84 |
| 128 | LH5 | 2 | 8 | Hereford | * | 8.00 |
| 129 | LH5 | 2 | 9 | Xi19 | * | 22.61 |
| 130 | LH5 | 2 | 10 | Hereward | * | 53.33 |

| | | | | | | |
|---|---|---|---|---|---|---|
| 131 | LH5 | 2 | 11 | Duxford | * | 46.67 |
| 132 | LH5 | 2 | 12 | Barley | * | 3.75 |
| 133 | LH5 | 2 | 13 | Xi19 | * | 47.00 |
| 134 | LH5 | 2 | 14 | Hereward | * | 84.80 |
| 135 | LH5 | 2 | 15 | Duxford | * | 42.86 |
| 136 | LH5 | 2 | 16 | Barley | * | 7.10 |
| 137 | LH5 | 2 | 17 | Cadenza | * | 16.84 |
| 138 | LH5 | 2 | 18 | Hereford | * | 20.00 |
| 139 | LH5 | 2 | 19 | Duxford | * | 8.89 |
| 140 | LH5 | 2 | 20 | Barley | * | 0.54 |
| 141 | LH5 | 2 | 21 | Hereward | * | 40.83 |
| 142 | LH5 | 2 | 22 | Hereford | * | 41.90 |
| 143 | LH5 | 2 | 23 | Xi19 | * | 30.30 |
| 144 | LH5 | 2 | 24 | Cadenza | * | 2.50 |
| 145 | LH4 | 0 | 1 | Xi19 | * | * |
| 146 | LH4 | 0 | 2 | Cadenza | * | * |
| 147 | LH4 | 0 | 3 | Hereward | * | * |
| 148 | LH4 | 0 | 4 | Hereward | * | * |
| 149 | LH4 | 0 | 5 | Barley | * | * |
| 150 | LH4 | 0 | 6 | Duxford | * | * |
| 151 | LH4 | 0 | 7 | Duxford | * | * |
| 152 | LH4 | 0 | 8 | Hereward | * | * |
| 153 | LH4 | 0 | 9 | Barley | * | * |
| 154 | LH4 | 0 | 10 | Duxford | * | * |
| 155 | LH4 | 0 | 11 | Barley | * | * |
| 156 | LH4 | 0 | 12 | Hereford | * | * |
| 157 | LH4 | 0 | 13 | Hereford | * | * |

| | | | | | | |
|---|---|---|---|---|---|---|
| 158 | LH4 | 0 | 14 | Barley | * | * |
| 159 | LH4 | 0 | 15 | Duxford | * | * |
| 160 | LH4 | 0 | 16 | Xi19 | * | * |
| 161 | LH4 | 0 | 17 | Cadenza | * | * |
| 162 | LH4 | 0 | 18 | Hereford | * | * |
| 163 | LH4 | 0 | 19 | Cadenza | * | * |
| 164 | LH4 | 0 | 20 | Xi19 | * | * |
| 165 | LH4 | 0 | 21 | Hereford | * | * |
| 166 | LH4 | 0 | 22 | Hereward | * | * |
| 167 | LH4 | 0 | 23 | Cadenza | * | * |
| 168 | LH4 | 0 | 24 | Xi19 | * | * |
| 169 | LH4 | 1 | 1 | Xi19 | * | 0.85 |
| 170 | LH4 | 1 | 2 | Cadenza | * | 5.68 |
| 171 | LH4 | 1 | 3 | Hereward | * | 2.67 |
| 172 | LH4 | 1 | 4 | Hereward | * | 8.11 |
| 173 | LH4 | 1 | 5 | Barley | * | 0.65 |
| 174 | LH4 | 1 | 6 | Duxford | * | 0.80 |
| 175 | LH4 | 1 | 7 | Duxford | * | 14.65 |
| 176 | LH4 | 1 | 8 | Hereward | * | 16.34 |
| 177 | LH4 | 1 | 9 | Barley | * | 0.65 |
| 178 | LH4 | 1 | 10 | Duxford | * | 9.57 |
| 179 | LH4 | 1 | 11 | Barley | * | 11.04 |
| 180 | LH4 | 1 | 12 | Hereford | * | 7.33 |
| 181 | LH4 | 1 | 13 | Hereford | * | 13.45 |
| 182 | LH4 | 1 | 14 | Barley | * | 0.23 |
| 183 | LH4 | 1 | 15 | Duxford | * | 16.99 |
| 184 | LH4 | 1 | 16 | Xi19 | * | 20.11 |

| | | | | | | |
|---|---|---|---|---|---|---|
| 185 | LH4 | 1 | 17 | Cadenza | * | 4.64 |
| 186 | LH4 | 1 | 18 | Hereford | * | 6.67 |
| 187 | LH4 | 1 | 19 | Cadenza | * | 6.98 |
| 188 | LH4 | 1 | 20 | Xi19 | * | 15.67 |
| 189 | LH4 | 1 | 21 | Hereford | * | 35.88 |
| 190 | LH4 | 1 | 22 | Hereward | * | 19.10 |
| 191 | LH4 | 1 | 23 | Cadenza | * | 5.92 |
| 192 | LH4 | 1 | 24 | Xi19 | * | 0.00 |

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**9.4.5 Appendix V:** The OTU data file is very large, will be provided on request.