Evaluating populations derived from complex crosses involving both bread wheat and durum wheat parentage for partial resistance to crown rot

By

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A thesis submitted for the degree of

Master of Agricultural Science

at

The University of Adelaide

Faculty of Sciences

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Waite Campus

April 2014

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Abstract

Crown rot in durum, caused by *Fusarium pseudograminearum* and *Fusarium culmorum*, can reduce yields up to 90% in seasons characterised by limited spring rainfall. To decrease this potential loss, breeding of partially resistant cultivars could complement agronomic approaches. However, the limited variation in durum has meant that development of partially resistant lines is still a major objective to overcome. The aim of this study was to evaluate, through genotypic and phenotypic-based approaches, durum lines with partial resistance to crown rot. The germplasm under study consisted of 252 durum lines obtained by crossing durum parents with partially resistant bread wheat varieties. Phenotypic assessment of the symptoms, accomplished by visual assessment of the fungal necrosis of the stems, led to the identification of 120 partially resistant lines. Genotypic assessment, performed through a SNP array, identified associations between marker genotype and crown rot severity for the family originating from the parents EGA Bellaroi 38a and Sumai 3. Moreover, the frequency of QTL for crown rot partial resistance already published was investigated in the populations under study through the multiplex ready PCR technique. These findings confirm that bread wheat varieties can be exploited to reduce crown rot severity in durum.

Declaration

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Domenico Deserio

December 2013

Acknowledgements

CNR, Bari) for the opportunity and scientific support. Finally, a huge and warm thank you to Dr Federico Baruzzi, Dr Leonardo Caputo, Dr Maria Morea and (last but not least!) Dr Laura Quintieri, for their regular support despite the distance during my time in Australia, and since the very beginning of my scientific experience which started 10 years ago.

This achievement would not have been possible to reach without the support that my parents gave me from the other side of the world. I'm also grateful to my brother and his family, my grandmother, my uncles and cousins, and all of my friends back home to whom I have felt close to during these years. Thank you to all my new friends in Australia, all the people that lived with me during this deep experience, sharing the precious value of friendship far away from home.

Above all, this is for you, N.

Glossary of Abbreviations

Abbreviation	Full term
AGRF	Australian Genome Research Facility
ANOVA	Analysis of variance
ASOs	Allele-specific oligonucleotides
AUD	Australian Dollars
cDNA	Complementary deoxyribonucleic acid
CGIAR	Consultative Group for International Agricultural Research
CIMMYT	International Maize and Wheat Improvement Centre
CR	Crown rot
DArT	Diversity arrays technology
DNA	Deoxyribonucleic acid
EDTA	Ethylenediaminetetraacetic acid
EST	Expressed sequence tags
FHB	*Fusarium* Head Blight
GxE	Genotype by environment
I	Inoculated treatment
IARCs	International Agricultural Research Centre
ICARDA	International Centre for Agricultural Research in the Dry Areas
LOD	Logarithm of odds
LSD	Least significant difference
LSO	Locus specific oligonucleotide
MAS	Marker assisted selection
mRNA	messenger ribonucleic acid

Abbreviation	Full term
MRT	Multiplex Ready Technology
NI	Not inoculated treatment
PCNB	Pentachloronitrobenzene
PCR	Polymerase chain reaction
PDA	Potato Dextrose Agar
Ph1	Pairing homoeologous 1
QTL	Quantitative trait loci
R	Correlation coefficient
RCF	Rotation centrifugal force
RFLP	Restriction fragment length polymorphism
S	Susceptible line
SARDI	South Australian Research and Development Institute
SNP	Single nucleotide polymorphism
SSR	Simple sequence repeat

1 LITERATURE REVIEW

1.1 Durum wheat

1.1.1 Origin and domestication of durum wheat

Durum wheat evolved in the area of eastern Mediterranean and north-eastern Africa as a result of a cross between the diploid wheat *Triticum monococcum* (genome AA) and an unknown species (genome BB). Although this type of cross usually leads to sterile and/or diploid progeny, amphiploidy occurred and tetraploid wheat evolved (Luo *et al.* 2007; Dvorak *et al.* 2011). Hexaploidization also occurred, taking place spontaneously through an alloploid cross between the tetraploid durum wheat (*Triticum turgidum*, genome AABB) and the diploid *Aegilops tauschii* ssp. *strangulata* (genome DD) (Dvorak *et al.* 1998; Luo *et al.* 2007).

Prior to the domestication of tetraploid wheat, emmer (*T. turgidum* spp. *dicoccoides*) was the most widespread wheat, with records dating back to about 9,600 to 9,000 years ago in the Middle East (Nesbitt and Samuel, 1996; Luo *et al.* 2007). The precise localisation of the first domestication of the tetraploid wheat, then the evolution of durum wheat (*T. turgidum* spp. *durum*) from emmer wheat, has been indicated by Nesbitt and Samuel (1996) as the southern Levant and south-eastern Turkey. Molecular studies have been carried out to discover exactly which region of the Middle East has been involved in this evolution but the results have been found to be often discordant. In fact, although Mori *et al.* (2003) localised, through DNA analysis, this domestication in the Turkish region Kartal Dagi, Ozkan *et al.* (2005) and Luo *et al.* (2007) suggested that the area of interest is Karaca Dag.

Successful domestication of durum wheat is principally due to several characteristics acquired and selected throughout the years, which have made it

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more suitable than landraces and wild progenitors for harvesting as an agricultural crop of importance. One of these characteristics is yield improvement due to the reduction of spike shattering, which in emmer wheat is controlled by genes located on chromosomes 3A and 3B (*brittle rachis* loci) (Nalam *et al.* 2006; Dubcovsky and Dvorak, 2007). Spike shattering had previously been an important trait that allowed the easy release of the seed into the environment for the dissemination of wild wheat (Nalam *et al.* 2006; Dubcovsky and Dvorak, 2007). Another advantage acquired by durum wheat through domestication is the loss of glume hardness, which subsequently made the spike suitable for free-threshing (Jantasuriyarat *et al.* 2004; Dubcovsky and Dvorak, 2007). Other characteristics acquired by durum are the reduction in tiller number, seed dormancy, more erect plant form and increased seed size (Dubcovsky and Dvorak, 2007). Even though the domestication of the durum wheat has reduced the biodiversity of the cultivars (bottleneck effect), the loss of variability of the genomes, especially for quantitative trait, is compensated by the polyploidy with the abundance of repetitive elements (Dubcovsky and Dvorak, 2007).

1.1.2 The genetics of durum wheat

Genetically, wheat species are grouped into three categories: diploids with genome A (2n=2x=14; e.g. *T. monococcum*), tetraploids with genomes A and B (2n=4x=28; e.g. *T. turgidum*) and hexaploid (2n=6x=42; e.g. *Triticum aestivum*), constituted by the genomes A, B and D (Chantret *et al.* 2005). Several phenotypic characteristics are related to specific genome(s), consequently the different arrangement of genomes gives distinguishing features to the different species. For instance, in hexaploid wheat, variation in grain hardness is controlled by the *Ha* locus of chromosome 5D, a locus that is absent in diploid and tetraploid species

(Turner *et al.* 1999). These genomic differences occur despite the common ancestral origin of the genomes.

Polyploidisation of the wheat genome has resulted in the reorganisation of non-coding DNA, epigenetic changes, DNA methylation rearrangements and modified gene expression regulation (for example silencing or reducing the transcription of redundant genes) (Ozkan *et al.* 2001; Kashkush *et al.* 2002). Diploid-like behaviour during meiosis is a result of what is known as the pairing homoeologous 1 (*Ph1*) locus, which has been isolated to a region located on the long arm of chromosome 5B (Griffiths *et al.* 2006; Al-Kaff *et al.* 2008). The *Ph1* locus tightly regulates chromosome pairing behaviour, and in the absence of this locus, homoeologous chromosome associations have been shown (for example, chromosome 1A could potentially pair with either 1B or 1D) (Al-Kaff *et al.* 2008; Able *et al.* 2009). Moreover, Ozkan *et al.* (2001) declared that, in the preservation of the diploid-like behaviour, the *Ph1* effect has also been enhanced by the elimination of several genome specific sequences and chromosome specific sequences. As a consequence, and although the chromosomes in each of these genomes are similar with respect to gene content and gene order, during meiosis chromosomes will only pair with their homologue.

Another source of variability among the genomes A, B and D is provided by the broad presence of retrotransposons that, replicating themselves in other loci of DNA through mRNA intermediates, have a role in the stabilisation of mutations that may occur, and in the inactivation of the redundant genes among the genomes (Kumar and Bennetzen 1999; Gu *et al.* 2004). The majority of the retrotransposons occurred after the divergence of the three wheat genomes, as suggested by the low level of colinearity, and they have provided a useful source

for evolutionary study of the three wheat's genomes through comparison between orthologous and homologous DNA regions (Anderson *et al.* 2003).

With potentially two or three copies of each locus, duplication (in the case of durum wheat) and triplication (in hexaploid wheat) of loci has conferred significant evolutionary advantages (Kashkush *et al.* 2002). In fact, the added genomes made the polyploids more suitable than the diploids to adapt in different habitats, allowing for broad levels of genetic diversity and heterozygosity, hence partially compensating the loss of biodiversity of domesticated wheat (Matsuoka 2011; Soltis and Soltis 2000).

To sustain a high level of variability even in the modern cultivar, several international organisations play an important role in sustaining a high level of variability in domesticated wheats. A broad network called the Consultative Group for International Agricultural Research (CGIAR) has been established by different Agricultural Research Centres (IARCs), such as the International Maize and Wheat Improvement Centre (CIMMYT) of Mexico and the International Centre for Agricultural Research in the Dry Areas (ICARDA) of Syria (Reynolds and Borlaug 2006). These organisations aim to coordinate the agricultural efforts in the development and spread of new crop varieties (including durum wheat), and provide 'storage facilities' of landraces and wild progenitors of modern crops.

1.1.3 Physiology and agronomy of durum wheat

Production of durum is manly limited to areas which are typically characterised by a Mediterranean climate (such as Europe, North Africa, Australia), but it is also tilled in other areas such as North America (Connell *et al.* 2004). On the contrary, bread wheat has a greater ability to adapt to different environments, making this species much more widespread (Matsuoka, 2011). During the

Australian growing season, durum requires rainy and mild winters, warm days and cold nights, fertile soils and dry conditions around the grain filling stage. Within Australia, climates like these occur in some areas of Queensland, New South Wales, Victoria, South Australia and Western Australia. However durum is predominantly grown in New South Wales, Queensland and South Australia (Fig. 1; Connell *et al.* 2004).

Fig. 1. Production areas that are typically sown to durum. Areas shaded include South-East Queensland, Northern New South Wales, and South Australia (across to the border of Victoria) (Source: Australian Wheat Board Ltd).

The life cycle of durum wheat is constituted by a continuity of vegetative, reproductive and grain filling phases, with the timing of these processes being tightly related to a genotype by environment (GxE) interaction (Satorre and Slafer, 1999).

For durum breeding programs yield and yield stability are among the most important traits that need continual monitoring and improvement. Yield is a very complex trait and, in durum wheat, it is influenced by the interaction between environment and several quantitative trait loci (QTL). This has been proved by

Maccaferri *et al.* (2008) by finding 16 different QTL in a population of 249 recombinant inbred lines grown in 16 Mediterranean environments differing in thermal requirements and water availability conditions. Of the 16 QTL identified, 14 were discovered in the same environment, while the remainder were found in different combinations in three or more environments. This variability makes breeding for yield *per se* very time consuming and complex.

The durum grain is typically large, golden and hard, the hardest of all wheat species (Connell *et al.* 2004). This characteristic is due to the hardness of the endosperm that, during milling, allows for the production of the semolina, used to make spaghetti, pasta and couscous. In contrast the bread wheat endosperm breaks down during milling and results in ordinary flour (Connell *et al.* 2004). In durum wheat, grain quality attributes such as protein content, moisture, gluten strength and seed weight (typically measured as thousand grain weight) are correlated with the firmness, springiness and stability of the pasta and other products (Troccoli *et al.* 2000; Sharma *et al.* 2002; Gianibelli *et al.* 2005).

To enhance yield and quality of durum wheat, appropriate agronomic management is required, particularly with respect to soil nutrition and nitrogen availability for the crop (López-Bellido *et al.* 2006). The ability of durum wheat to take up nitrogen is related to the genotype and to the timing of nitrogen application (Limaux *et al.* 1999). This timing has been further emphasised by Lopez-Bellido *et al.* (2006), who reported that the period between tillering and the formation of the first node is the most suitable for nitrogen application.

1.1.4 Production

Durum holds a minor but important position in the global wheat market, and consequently is exported and processed among numerous countries. In the seasons from 2009-10 to 2012-13 (data for 2013 is a forecast), an average of 36.9 million tonnes per year of durum was produced, which was approximately 5.5% of the world's wheat production (Ponzielli 2013). During these seasons, the main producers of durum were the European Union (23% of world production) and North America (18.2%); while Australia produced approximately 1.4% (Ponzielli 2013). Although the amount of durum produced in Australia is comparatively low, it has been recognised as high quality and is exported to several countries including Italy (Troccoli *et al.* 2000; Connell *et al.* 2004).

1.2 *Fusarium* and wheat diseases

Of the many fungi that can cause plant disease, those belonging to the genus *Fusarium* have been reported to infect 81 of the 101 most economically important plant species, including wheat (Leslie and Summerell 2006). *Fusarium* diseases, such as *Fusarium* head blight (FHB) and crown rot (CR), may occur in different stages of plant growth, depending on the host involved and the fungal species. While FHB is principally due to *Fusarium graminearum*, CR is mostly caused by *Fusarium pseudograminearum* and *Fusarium culmorum* (Williams *et al.* 2002; Backhouse *et al.* 2004; Burgess and Bryden, 2012). The incidence of these diseases has risen in the last few years, partially due to farming practices such as stubble retention (MacLeod *et al.* 2008; Moretti and Susca, 2009).

1.2.1 *Fusarium*: an overview

Belonging to the phylum Ascomycota, class Ascomycetes and order Hypocreales, the genus *Fusarium* includes several toxigenic and phytopathogenic species that colonise a wide range of host plants (Burgess and Bryden, 2012). The biodiversity of the genus *Fusarium* allows it to colonise different environments, to act as a pathogen or opportunist agent on different crop types (Table 1; Moretti and Susca, 2009). Among *Fusarium* species, *F. pseudograminearum* and *F. culmorum* are highly aggressive pathogens and are the causal agent of CR in wheat (Burgess *et al.* 2001; Williams *et al.* 2002; Backhouse *et al.* 2004). *F. graminearum*, another species, results in mycotoxin production, including deoxynivalenol (DON) (Mudge *et al.* 2006; Covarelli *et al.* 2012). The sexual stage of *F. pseudograminearum*, *Gibberella coronicola,* can be important for its life cycle and pathogenicity. However, this sexual stage rarely occurs in the field, making clonal reproduction important for *F. pseudograminearum* (Aoki and O'Donnell 1999; Summerell *et al.* 2001; Bentley *et al.* 2008).

Table 1. Example of diseases caused by *Fusarium* species. The biodiversity of *Fusarium* species is highlighted by the broad range of diseases that they cause and the variability of the hosts affected. The symptoms of *Fusarium* diseases are usually related to colour anomalies and, in some cases, the production of mycotoxins (readapted from Burgess and Bryden 2012).

Species	Disease	Symptoms	Mycotoxins in grain
F. graminearum	Head blight of wheat and barley	Blighted spikelets, pink mouldy grain	Deoxynivalenol
F. graminearum	Stalk and cob rot of maize	Rotted stalks and pink mouldy kernels	Deoxynivalenol Zearalenone
F. verticilloides	Stalk and cob rot of maize	Rotted stalk and white mouldy kernels	Fumonisin group
F. pseudograminearum	Crown rot of wheat and barley	Browing of stem base, crown rot, whiteheads	Rarely affect heads
F. culmorum	Crown rot of wheat and barley	Browing of stem base, crown rot, whiteheads	Rarely affect heads
F. oxysporum f.sp. *cubense*	Vascular wilt of banana	Wilting, vascular browing, death	n.a.
F. oxysporum f.sp. *vasinfectum*	Vascular wilt of cotton	Wilting, vascular browing, death	n.a.
F. circinatum	Pitch canker complex of *Pinus* species	Cankers exuding resin, dieback, death	n.a.
F. fujikurol	Bakanae disease of rice	Stem elongation and crown rot	n.a.

Fusarium taxonomy has always been complex, with many unsolved issues. In this respect, *F. pseudograminearum* was previously considered *F. graminearum* group 1 (Aoki and O'Donnell 1999), due to morphological traits resembling those of *F. graminearum* which was denoted group 2. Indeed, *Fusarium* taxonomy has long been based on morphology: including the features of the macroconidia (septation and production in the aerial mycelium or in the sporodochia), microconidia (production in the aerial mycelium) and chlamydospores (intercalary and with thickened walls) (Leslie and Summerell 2006). Nucleic acid based methods have shown that *F. pseudograminearum* is a separate species: with studies on DNA sequences (Aoki and O'Donnell 1999), molecular markers such as restriction fragment length polymorphisms (RFLP) (Benyon *et al.* 2000) and isozymes (Láday *et al.* 2000) all confirming this. Further support in this evaluation is provided by the differentiation between the heterothallic nature of *F. pseudograminearum* (teleomorph: *G. coronicola*), characterised by two mating types (male and female), and the homothallic nature of *F. graminearum*, that is self-fertile (teleomorph: *Gibberella zeae*) (Aoki and O'Donnell 1999).

1.2.2 Life cycle

The life cycle of the *Fusarium* species is strictly connected to the mode of reproduction, since it may lead to different levels of genetic variability of the species, different aetiology of the CR, and therefore, have a different impact on infecting crops such as durum wheat. Although the sexual stage for *F. graminearum* and *F. pseudograminearum* have been characterised, no sexual stage is known in *Fusarium culmorum* (Miedaner *et al.* 2008).

G. Zeae frequently spreads through the air via its ascospores (sexually reproduced) that are essential for its pathogenicity, which is contrary to *G. coronicola* where production of perithecia (which contains ascospores) in the field is infrequent (Summerell *et al.* 2001), thus influencing the dissemination of *Fusarium* diseases. This is probably due to the fact that, to produce perithecia, the heterothallic nature of *G. coronicola* requires the presence of both the mating types in the same infected plant (Summerell *et al.* 2001). While the homothallic nature of *G. Zeae*, which contain the male and female structures on the same individual, enhances the likelihood that sexual reproduction occurs in the field (Aoki and O'Donnell, 1999). Moreover, the timing of the production of perithecia and ascospores may affect at what stage of plant growth the disease occurs (Summerell *et al.* 2001).

Although sexual reproduction of *Fusarium* species may improve their aggressiveness, even macroconidia (spores produced asexually) have an important role in the spreading of infection in durum wheat crops, especially during the growing season (Desjardins *et al.* 2004; Hayden *et al.* 2005). During the asexual stage of *Fusarium* species, the canoe-shaped macroconidia are produced by the sporodochia and splash-dispersed over a short distance (Burgess and Bryden, 2012). Although the sexual reproduction of *G. zeae* and *G. coronicola* is less frequent than asexual stage, several studies showed that macroconidia are less frequently detected from the field than ascospores. Trail *et al.* (2002) traced this back to the fact that, although ascospores are spread by wind once produced, macroconidia may be held in the sporodochia.

1.2.3 Crown rot

F. pseudograminearum and *F. culmorum* are the main casual agents responsible for CR in Australia. Other *Fusarium* species are involved as secondary pathogens, including *F. acuminatum*, *F. avenaceum*, *F. crookwellense* and *F. equiseti* (Burgess *et al.* 2001; Williams *et al.* 2002; Backhouse *et al.* 2004).

The common aetiology that is shared by FHB and CR has led researchers to conduct studies in parallel focussing on not just the pathology of the diseases but also the genetic mechanisms involved in plant resistance (Chakraborty *et al.* 2006; Li *et al.* 2010). Both FHB and CR can cause significant (>50%) yield loss in durum wheat. Although some of the drier environments in which durum wheat is usually sown will reduce the incidence of FHB, it has been detected in some areas of North Dakota, Canada and Australia (Clear and Patrick, 2000; Southwell *et al.* 2003; Vijayakumar *et al.* 2005). While FHB can cause substantial yield loss in durum, it is not widespread in Australia. It has been present, (season dependent) in limited areas of Queensland and northern New South Wales (Southwell *et al.* 2003).

Susceptibility to CR is of more concern as all durum varieties (at least those grown in Australia) are either susceptible or very susceptible to this disease and it is widespread in Australia (Wildermuth *et al.* 1997; Burgess *et al.* 2001; Backhouse *et al.* 2004; Wallwork *et al.* 2004; Miyan *et al.* 2013). The disease also occurs in Europe (Rossi *et al.* 1995; Pettitt and Parry 2001; Pettitt *et al.* 2003), North America (Fernandez and Zentner, 2005; Smiley *et al.* 2005), West Asia, North Africa (Nicol *et al.* 2004) and South Africa (Lamprecht *et al.* 2006). The amount of inoculum of the CR pathogens, *F. pseudograminearum* and *F. culmorum*, has a central role in the aetiology of the disease (Williams *et al.* 2002; Backhouse *et al.* 2004). CR is more likely to occur in durum wheat if it is sown

after other susceptible crops (for example, bread wheat), after long-term grass pastures, if the previous season's stubble is retained (CR affected residues can persist for at least two years), if dry conditions occur in the spring during grain fill and/or if humid weather persists while the disease develops (MacLeod *et al.* 2008).

MacLeod *et al.* (2008) described the phenotypic symptoms of CR as the colour aberration of the crown and lower stem that assume a honey-brown, dark brown or even pink discoloration (Fig. 2 c). The presence of whiteheads in the field has also been linked to CR, particularly when the remainder of the crop is comparatively green in colour during grain fill. The fungal colonisation of the stem reduces water and nutrients uptake resulting in poor grain fill (Fig. 2 a; MacLeod *et al.* 2008).

a)   b)   c)
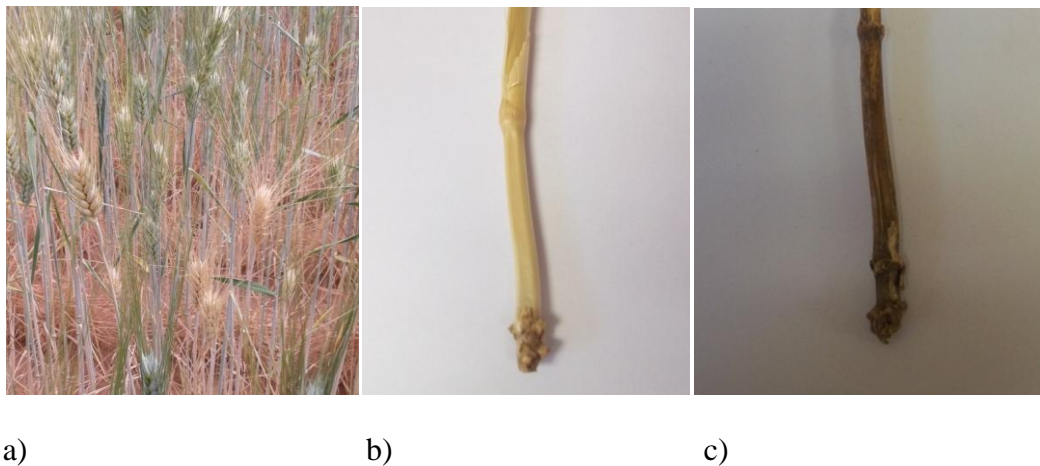
Fig. 2. Symptoms of CR on durum. *Fusarium* species, through the colonisation of root and stem, seem to reduce the uptake of water. This has been linked to the presence of whiteheads prior to crop maturity when the non-infected plants are still green (a). Non-infected (b) and infected (c) main stems are compared.

1.2.4 Environmental factors

As mentioned in section 1.2.3, severity, frequency and likelihood of CR are related to the environment, rainfall patterns, soil moisture, crop nutrition and the amount of stubble retention. A large number of studies have supported the theory that the rainfall and temperature influence the occurrence of *Fusarium* species. Backhouse and Burgess (2002) collected data related to the optimal climate conditions for growth of *F. pseudograminearum* and *F. culmorum*, the major CR agents, and *F. graminearum* (Table 2), which in some cases has been shown to be involved in CR aetiology (Akinsanmi *et al.* 2004). Their data demonstrated that environmental conditions may affect which pathogen will be involved in CR occurrence since, among the three *Fusarium* species cited, there are different optimum growth conditions (Table 2). In fact, Backhouse and Burgess (2002) reported that a pattern of high rainfall and low temperatures promotes the incidence of the fungus *F. culmorum*, whereas moderate temperatures and rainfall usually increases the incidence of *F. pseudograminearum* (Table 2). *F. graminearum* has its optimum growth conditions at rainfall patterns and temperatures higher than both *F. pseudograminearum* and *F. culmorum* (Table 2; Backhouse and Burgess 2002).

Table 2. Optimum climate parameters for *F. graminearum*, *F. pseudograminearum* and *F. culmorum* collected in Australia. The values indicate the ranges of temperature and rainfall patterns with the means in parentheses (Backhouse and Burgess 2002).

Climate parameter			
	F. graminearum	*F. pseudograminearum*	*F. culmorum*
Temperature			
Average annual (°C)	13.1-(18.2)-21.9	13.6-(17.0)-19.9	13.3-(16.3)-18.7
Min. of coldest month (°C)	0.6–(4.8)–10.9	0.1–(3.2)–6.2	1.3–(3.4)–6.2
Max. of warmest month (°C)	23.9–(29.2)–33.5	26.6–(31.4)–34.8	24.1–(29.8)–31.9
Rainfall			
Annual (mm)	343–(941)–1881	243–(510)–828	335–(564)–759
Wettest month (mm)	35–(147)–389	31–(65)–124	35–(76)–103
Driest month (mm)	9–(36)–66	8–(26)–42	12–(25)–35

Moisture is another important consideration that can effect CR severity in two ways: soil moisture and plant water potential. While wet soils will typically favour *Fusarium* growth and the colonisation of the plant by the fungus, dry soils will invariably reduce *Fusarium* inoculum (Liddell and Burgess 1988). Liddell and Burgess (1988) declared that moisture under the saturation level (within an optimal osmotic potential between -0.3 and -0.7 MPa) allows *Fusarium* species to grow in optimal osmotic condition, giving them a competitive advantage over other microorganisms in the up-take of nutrients. Nevertheless they confirmed that near the saturation level (within an osmotic potential range between -0.1 and 0.0 MPa), oxygen availability decreases which then promotes the growth of microaerobic and anaerobic bacteria. Contrary to soil moisture, the availability of water for the plant is inversely proportional to the likelihood of CR occurrence (Beddis and Burgess, 1992). This is due to the stress condition of the plant that plays a major role in the equilibrium between disease and resistance. In fact, as

Champeil *et al.* (2004) and Burgess and Bryden (2012) reported, the severity of infection of *F. culmorum* and *F. pseudograminearum* is higher in drought (or water-limiting) environments. For instance, if a rainy season occurs, the soil moisture would be high, promoting the growth of *Fusarium* species. On the other hand, if the wet soil provides optimal conditions for the plants, this would improve the plant vigour, making the symptoms of the disease less severe.

Another environmental factor that may influence incidence of CR is crop nutrient availability, especially zinc and nitrogen. Khoshgoftarmanesh *et al.* (2010) reported that adequate Zn availability can reduce *Fusarium* CR in bread wheat and durum wheat through enhancement of the cell membrane's integrity. Other nutrients that have been reported to also play a role include nitrogen and phosphorus. Too much nitrogen during the growing season may lead the plant to grow too fast vegetatively early in the season which could, in turn, increase its chances of being more susceptible to CR later in the season if there is a dry spring with limited rainfall (Kirkegaard *et al.* 2004).

The development and persistence of CR in the last two decades has been exacerbated through stubble retention. *Fusarium* species are able to survive in the stubble even beyond two years, which then becomes a source of infection across different seasons (MacLeod *et al.* 2008). Two important factors contribute to inoculum load: the biomass of stubble produced and the decomposition rate of the stubble (Backhouse, 2006). This cross-contamination, common in soilborne diseases hence defined as 'polyetic', results in the incidence of CR to rise across years until it reaches a plateau (Zadok, 1999; Backhouse, 2006). While the initial inoculum may be reduced by wet weather conditions through the acceleration of stubble breakdown (Zadok, 1999; MacLeod *et al.* 2008), the high influence which the initial inoculum has on the disease incidence leads CR to be defined as a

monocyclic disease (Burgess *et al.* 1993). In order to reduce the losses related to CR, the stubble management (for instance ploughing or burning) is considered important as it has been shown to reduce inoculum through enhanced stubble breakdown.

1.2.5 Economic aspects

Several reports confirm the direct connection between CR severity and yield loss. Smiley *et al.* (2005) estimated that in some areas of the USA, the yield reduction due to CR in commercial fields of winter and spring wheat has been up to 35%. They compared these data with those obtained in experiments inoculated with *F. pseudograminearum* and it led them to the conclusion that, even if in commercial fields the yield reduction was less severe than in the inoculated field trials, CR has a potential to reduce yield by up to 61%. In Australia the situation is analogous, with losses related to CR in wheat having been estimated to reach as high as AUD 83 million per year, 95% of these due to *F. pseudograminearum*, 5% to *F. culmorum* (Murray and Brennan, 2009). In fact studies carried out in New South Wales using the individual tiller method have determined yield reduction by up to 89% (Klein *et al.* 1991). In Australia, the states with the highest frequency of loss due to CR are Queensland, New South Wales and South Australia (Williams *et al.* 2002). Daniel and Simpfendorfer (2008) performed trials in New South Wales and Queensland, sowing durum wheat in soil infected by *F. pseudograminearum*. The yield reduction of durum wheat averaged 58%, and reached 90% for the most susceptible varieties.

The awareness of the economic damage caused by CR has led scientists to direct their studies from the examination of the aetiology towards working on understanding the mechanisms of resistance involved.

1.3 Control of crown rot

Compared with resistance to other plant diseases such as FHB, resistance to CR is still not well understood (Li *et al.* 2010). Li *et al.* (2010) investigated if FHB-resistant germplasm of hexaploid wheat may benefit the research based on CR resistance through understanding whether the same genes are involved. They assessed the resistance of the same genotypes to FHB and to CR and did not discover any relationship. In addition, they investigated potential loci that confer resistance to FHB and CR through QTL analysis and showed that they were located on different chromosomes.

1.3.1 Agronomic approaches

Agronomic approaches aimed to reduce CR incidence have included crops which are non-host to CR in rotations for at least two seasons before re-sowing with a susceptible crop in the same field (Burgess *et al.* 2001), weed control, reduction of moisture stress (sowing rates not too high, control of growth, addition of fertiliser in relation to water availability), improving soil nutrition (especially zinc), sowing in inter-rows, and stubble management (MacLeod *et al.* 2008). These practices have been shown to reduce the incidence of CR and the potential level of inoculum present, ensuring that the plant is less likely to be stressed.

As infected stubble is the primary source of inoculum (Backhouse, 2006; MacLeod *et al.* 2008), agricultural approaches to reduce CR should therefore be aimed at stubble management from season to season. One method, which involves ploughing the stubble, may reduce the soil infestation of *F. pseudograminearum* and *F. culmorum* respectively by 33% and 40% (Paulitz *et al.* 2010). However, Burgess *et al.* (1993) have clearly demonstrated that this method of stubble management does little to reduce the likelihood of CR infection. More effective

control measures, such as burning the stubble, have led to moderately successful results (Dodman and Wildermuth, 1989). Paulitz *et al.* (2010) reported that stubble burning reduces the soil infestation of *F. pseudograminearum* and *F. culmorum* by 47% and 30% respectively. However, this practice is not strongly recommended because it may favour the insurgence of other diseases and may reduce the quality of the soil (Simpfendorfer *et al.* 2005).

A more elegant and simple method which is aimed at reducing cross infection among subsequent crops is to sow the new seeds in the inter-rows, and this reduced CR severity by up to 61%, 51% and 53%, respectively, in the seasons 2004, 2005 and 2006 in field trials conducted in New South Wales (Rummery *et al.* 2007; MacLeod *et al.* 2008).

Comparing Zn-efficient cultivars with Zn-deficient cultivars, it is clear there is a reduced level of disease by *Fusarium* species in Zn-efficient lines (Grewal *et al.* 1996). Grewal *et al.* (1996) also demonstrated a correlation between Zn availability in soil which has been inoculated with *F. pseudograminearum* and the incidence of CR in bread wheat and durum wheat. They found that the more susceptible varieties expressed disease symptoms 12 days after sowing when the Zn concentration in the soil was less than 0.05 mg kg^{-1}, while the less susceptible cultivars showed less CR incidence in the same experimental conditions even 26 days after sowing. In all the varieties, CR symptoms decreased in the samples with a Zn concentration in the soil between 0.5 and 2 mg kg^{-1}. However the plant grown in soil with a Zn concentration of 2 mg kg^{-1} showed more vigour than the others. Grewal *et al.* (1996) have demonstrated that the Zn reduces CR incidence through a combination of its fungitoxicity and by having germplasm that displays early vigour. Other effects related to the application of Zn fertilisers which improve the plant vigour are the stabilisation of sulfhydryl groups in the

membrane proteins (Cakmak, 2000) and the role in the metabolism of carbohydrates, proteins and hormones (Alloway, 2008).

Beside the application of these chemical methodologies, some research has focused on the use of biological approaches to reduce CR incidence. These concern inoculation of the soil with competitive microorganisms which involve lower expenditures than fertiliser application. Some achievements have been obtained successfully in laboratories and glasshouses using *Burkholderia cepacia* and *Trichoderma* species as a containment measure of *Fusarium* soil infestation, and improved plant vigour (Huang and Wong, 1998; Wong *et al.* 2002; Vinale *et al.* 2008). There are limitations to the application of *Burkholderia cepacia*, in that it seems to be related to pulmonary hypertension and cystic fibrosis in humans (Fauroux *et al.* 2004), but the utilisation of *Trichoderma* species seems to be a valuable agronomic approach (Vinale *et al.* 2008).

1.3.2 The use of breeding and biotechnology

From a breeding perspective, a useful approach combines the crossing of very susceptible or susceptible materials with partially resistant lines that have previously been developed but have not necessarily been successful as varieties in their own right due to some other limiting factor(s) within their genetic make-up (for example, reduced yield or susceptibility to a different disease). This approach is also supported by the fact that, although the pathogenicity among *Fusarium* species seems to be potentially different, a partially resistant wheat line usually shows resistance against more than one species (Liu *et al.* 2004).

In this thesis the term 'partially resistant' is referring to less-susceptible varieties. Even though the former does not reflect the classical definition of resistance (a mechanism 'on-off'), it has been used extensively in several

published research articles in this area (Wallwork *et al.* 2004; Bovill *et al.* 2006; Collard *et al.* 2006; Bovill *et al.* 2010; Martin *et al.* 2013).

Many studies have been carried out in order to analyse the segregation of resistance in populations obtained by crossing two partially resistant parents, or a partially resistant and susceptible parent even if belonging to different, but still related, species. Specifically in bread wheat, Collard *et al.* (2005a) studied populations derived from the variety 2-49 (agronomically poor but partially resistant) and the susceptible variety Janz, while Bovill *et al.* (2010) used two doubled haploid populations obtained by crossing the parents 2-49 with W21MMT70 (partially resistant in seedlings), and 2-49 with Sunco, (partially resistant in adult plants). In the former study, the phenotypic assessment of the CR disease score in the segregating population was 50.8% while the parents showed a score of 39.3% (variety 2-49) and 68.1% (variety Janz), demonstrating a clear improvement in reducing CR susceptibility (based on the susceptible parent). Analogous achievements have been obtained by Bovill *et al.* (2010) that analysed two different double haploid populations across several years. Outcomes from this research resulted in lines with improved resistance to CR (compared with the parents W21MMT70, Janz and Sunco).

However, techniques based on phenotypic assessment are time consuming and not totally reliable due to being influenced by external factors such as the appearance of other diseases or the occurrence of favourable or unfavourable weather conditions for CR that lead to overestimating or underestimating its incidence (Wade *et al.* 2001; Wallwork *et al.* 2004). Consequently, pre-breeding research (Wallwork *et al.* 2004; Collard *et al.* 2005a; Bovill *et al.* 2010) has shifted towards combining both phenotypic approaches and molecular-based

(genomic) approaches to further our understanding of what contributes to reducing the susceptibility to CR in both bread wheat and durum wheat.

Studies carried out using molecular markers can assist breeders by providing them with tools to select the most favourable traits without the unreliability of the phenotypic-based selection (Fig. 3). These studies are aimed to identify, through the aid of molecular markers, the genetic traits, the quantitative trait loci (QTL), involved in the resistance and to evaluate their significance. For instance, Bovill *et al.* (2010), studying 2-49/W21MMT70 and 2-49/Sunco double haploid populations, detected 16 QTL: 6 of them were detected in at least one of the two trials performed in the population 2-49/W21MMT70, and 10 were detected in at least one of the three trials performed in the population 2-49/Sunco. Moreover they evaluated the significance regarding these QTL and the additive effect of their different combination in the segregating population. Such approaches have been built on the principle that molecular markers tightly linked to known QTL will segregate together. The importance of having QTL already mapped, is that breeders may select the most suitable phenotypes through marker analysis. As defined by Collard *et al.* (2005b), this methodology is called marker assisted selection (MAS) and is faster and more reliable than choosing the best lines using only phenotypic approaches (Fig. 3).
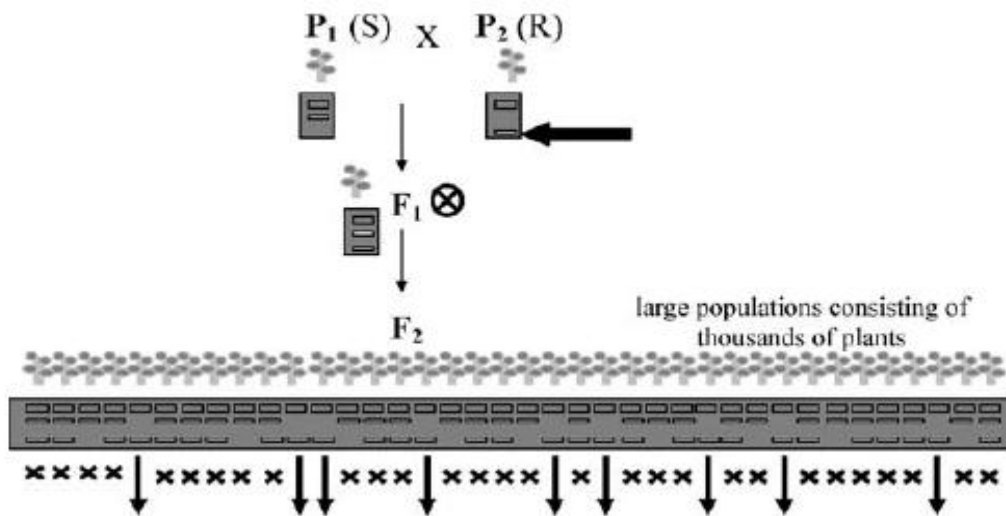
Fig. 3. Typical MAS of a breeding program for disease resistance. The susceptible parent (S) is crossed with the resistant (R). The F_1 plant is self-pollinated to produce an F_2 population. By using the marker, the most suitable phenotypes are selected (the arrows). This technique reduces the size of the population by quickly selecting the best phenotypes (Collard *et al*. 2005b).

Molecular approaches to understand CR and the related resistance mechanisms are therefore useful for the breeding programs to fast-track superior genotypes that can then be potentially deployed as new varieties. Such varieties could be used in combination with good agronomic practices to further reduce the incidence of CR.

1.4 Molecular approaches to understand crown rot

Plant responses to biotic and abiotic stresses can be controlled by a single gene or a complex 'net' of several genes. In plant breeding, if a phenotypic trait of interest is controlled by a single gene it is relatively simple to study its inheritance or to select for it in a new population with the aim to improve the crop's quality. On the

contrary, if the favourable traits are controlled by more than one gene, their expression and inheritance are usually more complex.

While some biotic diseases can be controlled by single gene inheritance, many diseases are polygenically controlled/regulated. Consequently, there has been considerable interest in extending the scientific community's knowledge on molecular markers and their potential to assist with understanding these polygenic controlled traits (Feuillet and Keller, 2004). Indeed, it has been suggested that DNA markers (through the process of molecular breeding) will contribute to the rise of food availability which will assist in sustaining world population growth (Ortiz, 1998; Kasha, 1999; Collard and Mackill, 2008).

1.4.1 Molecular markers and applications

With the advent of molecular markers, based on the detection of polymorphisms in the DNA sequence, plant breeding programs worldwide have embraced this technology (where available) so that they are not constrained by phenotyping and/or biochemical analysis alone (Winter and Kahl, 1995). In doing so, molecular marker systems have the capacity to 'fast-track' selection decisions in breeding programs.

Microsatellites markers, or SSRs, are based on short repeat units (up to 6 nucleotides) that explain the polymorphism in the variability of the number of copies present in the genome (they are not point mutations) and their utility comes from their abundance (Table 3; Ganal and Roder 2007). While microsatellites are still commonly used (particularly for plant species such as wheat), the high costs associated with their application may be not sustainable, especially if significant analysis needs to be undertaken. Consequently, other marker platforms have been developed, including Diversity Arrays Technology (DArT), which was first used

in rice by Jaccoud *et al.* (2001). Besides the low cost and high-throughput capacity of this technology, DArT markers do not need any prior sequence information and as such can be used for germplasm characterisation, genetic mapping, gene tagging, marker-assisted selection or the detection of genome methylation changes (Jaccoud *et al.* 2001). DArT marker technology has been used in wheat, through the analysis of the whole genome or for the identification of specific genes. For instance, Eberhard *et al.* (2010) based their research on the use of DArT markers in order to assess an F_2 population which was obtained by crossing durum and bread wheat parents. This study was based on the analysis of the introgression of the D genome and the segregation of genes within the A and B genomes. Besides broad analysis approaches, Mohler *et al.* (2012) conducted research that focused on identifying the transfer of a resistance gene for powdery mildew from a cultivated emmer parent.

In the past decade, single nucleotide polymorphisms (SNPs) have been the marker of choice, are considered third generation PCR-based markers (Rafalski 2002), and they are defined by a substitution of specific nucleotide(s) (Ganal and Roder 2007). This characteristic makes the SNP the smallest unit of genetic variation and a source of potentially unlimited polymorphisms (Ganal and Roder 2007). SNPs may be found using PCR techniques, thereby generating new sequence information, or exploiting databases' information through *in silico* analysis by studying genomic or cDNA sequences (Feuillet and Keller 2004). Although research on identifying SNPs is extensive in maize (Ching *et al.* 2002), barley and soybean (Zhu *et al.* 2003), Ganal and Roder (2007) reported that there was a lack of analogous studies in wheat, mostly due to the high expenditure involved and the difficulties of applying SNP technology to polyploid genomes. This made SNP analysis of wheat difficult and consequently delayed SSRs being

replaced by SNPs as the marker of choice for wheat. To improve this, an international project was started to develop SNP markers in wheat through *in silico* analysis (the Wheat SNP Development Project) (Khlestkina and Salina 2006). However, the success of this approach is limited by the availability of ESTs (expressed sequence tags). SNP array technology allows for whole genomes to be potentially analysed depending upon how many polymorphic markers are identified. In fact, even if each SNP is less informative than a SSR marker in terms of polymorphic content, the former markers are much more numerous than the microsatellites and this requires a high-throughput platform to exploit potential.

Among these, the Illumina GoldenGate assay, combined with the BeadArray Platform (Oliphant *et al.* 2002), utilises allele-specific oligonucleotides (ASOs) which can distinguish between two allelic variants of a SNP and it can also be applied for SNP genotyping of tetraploid and hexaploid wheat (Akhunov *et al.* 2009). While the ASO anneals the SNP locus keeping the discriminating nucleotide at the 3' end, just downstream a different annealing step involves another oligonucleotide (locus specific oligonucleotide, LSO) which contains address sequences called Illumicode (Akhunov *et al.* 2009). A PCR extension joins the two oligonucleotides creating a template which includes a sequence specific for the SNP allelic variant (ASO), and another specific for the locus (LSO) (Akhunov *et al.* 2009). During this PCR the ASOs are discriminately labeled with the fluorescents dyes Cy3 and Cy5. The amplified templates are ligated to beads through annealing specific oligonucleotides (on the beads), and the Illumicodes of the LSO. The analysis of the fluorescence emitted by the dyes gives information on the SNP allelic variant of the samples (Akhunov *et al.* 2009).

1.4.2 QTL and crown rot: what is known?

In order to extend knowledge on QTL and their use, several studies have focused on the discovery of new QTL, their mapping and their influence on phenotypic characteristics (Collard *et al.* 2005a; Bovill *et al.* 2006; Collard *et al.* 2006; Bovill *et al.* 2010; Martin *et al.* 2013). QTL mapping is constituted by linkage mapping and QTL analysis and is based on the principle that the closer the two genes are, the greater the likelihood they will segregate together during meiosis (Paterson 1996). Linkage mapping is aimed at identifying molecular markers which are tightly linked to QTL so they can be used as 'flags' (or 'tags') in a linkage map (which shows the chromosomal locations and genetic distances between markers) (Collard *et al.* 2005b). With a linkage map developed, it is possible to perform QTL analysis by studying the phenotype of individuals through the genotype of markers, using them as 'tags' for different genotypic groups (Collard *et al.* 2005b). This enables the position and significance (magnitude) of each QTL that may be classified as 'major' or 'minor' (Collard *et al.* 2005b) or as 'suggestive', 'significant' and 'highly significant' (Lander and Kruglyak, 1995) to be obtained. The position of a QTL is assumed to be where the likelihood, expressed by the logarithm of odds (LOD) score or another test statistic, has the maximum peak that has to be higher than a threshold evaluated by a permutation test (Collard *et al.* 2005b).

Bovill *et al.* (2010) carried out QTL analysis for CR resistance in 2-49/W21MMT70 and 2-49/Sunco double haploid populations, using the linkage map established by Collard *et al.* (2005a). In the segregating population 2-49/W21MMT70, 6 QTL were found. The QTL QCr.usq-3B.1 was the most significant, explaining up to 40.5% of the CR resistance. In 2-49/Sunco double haploid population, 10 QTL were found and the most significant had a magnitude

that explained 25% of the phenotypic variance. Among the 16 QTL for CR resistance found by Bovill *et al.* (2010) in the populations, 12 of them were located in the B genome. The high likelihood of the discovery of QTL for CR resistance in the B genome has also been confirmed by Collard *et al.* (2005a) who discovered 6 QTL, 3 of them located on chromosomes 2BS, 4BL and 7BS.

This has led research towards the utilisation and combination of these known QTL, by developing wheat genotypes that contain as many of these QTL as possible. Among many strategies applied through several years, the pyramiding of QTL has been evaluated as one of the most effective (Dekkers and Hospital 2002). For instance the populations studied by Bovill *et al.* (2010) have been obtained by setting-up a pyramiding of QTL for CR resistance in 2-49/W21MMT70 and 2-49/Sunco bread wheat populations. Although with different levels of resistance among the populations, some lines have shown a satisfactory resistance to CR. In 2006 the percentage of CR severity was on average 28% in the variety 2-49, 46.9% in the W21MMT170 and 43% in the segregating population (where the least susceptible individuals had a CR severity of 2.2%) (Bovill *et al.* 2010). Similar results have been collected with regard to the bread wheat population 2-49/Sunco: in 2004 the CR severity was on average 57.2% in Sunco, 40.3% in 2-49 and 52% in the segregating population (where the least susceptible individuals had a CR severity of 20.5%) (Bovill *et al.* 2010). In this experiment the segregating populations demonstrated an average CR susceptibility lower than the more susceptible parents W21MMT170 and Sunco, and some individuals even lower than the line 2-49.

In general, a successful approach to reduce the CR susceptibility in wheat concerns the use of partially resistant parents in crosses with varieties that provide good agronomic values. In bread wheat, this approach could be effective because

of the presence of several genetic sources of partial resistance to CR have already been discovered. Further, new QTL may be found by clarifying the susceptibility of some varieties such as Sumai 3, which is recognised as a source of resistance against FHB, and may have some partial resistance against CR (Liu and Anderson 2003; Collard *et al.* 2006; Li *et al.* 2010; Petrisko *et al.* 2010).

1.4.3 Towards improving crown rot partial resistance in durum

The large amount of QTL for partial resistance to CR already discovered in the bread wheat genome has allowed geneticists and breeders to develop novel hexaploid lines more resistant to CR (Collard *et al.* 2005a; Bovill *et al.* 2010). On the contrary, the absence of QTL for partial resistance to CR in durum wheat has been a limitation in developing less susceptible (partially resistant) durum lines.

Given the evolutionary relationship between bread wheat and durum wheat, QTL discovered in bread wheat may be utilised for potentially reducing CR severity in durum populations by performing crosses between the two species and selecting the durum progeny which have inherited the resistant traits from the bread wheat parents. For instance, Ma *et al.* (2012) evaluated phenotypically the CR severity in a segregating population obtained by crossing the hexaploid wheat CSCR6 with the durum variety EGA Bellaroi. The severity score was 10% for the resistant parent CSCR6 and 70% for the susceptible durum wheat Bellaroi. The durum progeny demonstrated on average a CR severity score of 56.7%, which is a moderate reduction when compared with the durum parent Bellaroi. A similar study has been conducted by Martin *et al.* (2013) using durum populations that were obtained by crossing with the partial resistant bread wheat 2-49. Results from within the populations reported a CR severity score which was reduced when compared to the durum parent, thus indicating that bread wheat QTL may

have segregated in the durum progeny (Martin *et al.* 2013). Preliminary molecular analysis carried out in this study led the authors to identify the introgression of QTL for partial resistance to CR in chromosomes 1A, 1B and 4B derived from 2-49.

However, the low number of studies aiming to reduce CR severity in durum has left the development of partial resistant durum lines still an unsolved issue. Therefore, the present study aimed to assess, phenotypically and genotypically, the CR susceptibility of six durum wheat populations obtained by crossing bread wheat varieties 2-49 and Sumai 3, with three different susceptible durum parents. The phenotypic analysis involved the assessment of CR severity symptoms of durum lines grown in the field infested by *F. pseudograminearum* and *F. culmorum*, while the genotypic approach utilised SNP array marker analysis. Moreover CR susceptibility for the bread wheat variety Sumai 3, where data has not been clearly defined in published literature, has also been investigated.

1.5 Rationale and significance

Durum is a tetraploid wheat required to produce pasta, spaghetti and couscous and for which the World mean annual production has been 36.9 million tonnes per year over the last 4 seasons (Ponzielli 2013). Australia, although averaging just 1.4% of world production, has been recognised as a producer of high-quality durum wheat (Connell et al., 2004; Troccoli et al., 2000).

However, a disease called CR (caused by the fungi *F. pseudograminearum* and *F. culmorum*) can reduce the yield of durum wheat by up to 90% (Daniel and Simpfendorfer, 2008). Agronomic and marker-based (biotechnology) approaches have been applied in order to help alleviate the damage that this disease can cause.

Nevertheless, the former does not provide resistance mechanisms and the latter, largely because of the unreliable phenotypic assessment, needs to be strengthened through adopting other marker-based approaches that may contribute in identifying new information to breeding for disease resistance.

The absence of QTL for CR resistance in durum wheat does not permit (at present) the development of partially resistant lines that completely satisfy the breeder's requirements. Moreover, while some studies have been carried out on segregating populations obtained by crossing the partially resistant variety 2-49 (or other bread wheat) with durum wheat varieties (Ma *et al.* 2012; Martin *et al.* 2013), further studies are required in order to screen different pedigrees with the aim to detect durum lines provided by good agronomic value and partial resistance to CR. This screening needs to be assisted with molecular analysis (SNP and SSR markers), and in durum there are not anywhere near as many published papers when compared to bread wheat.
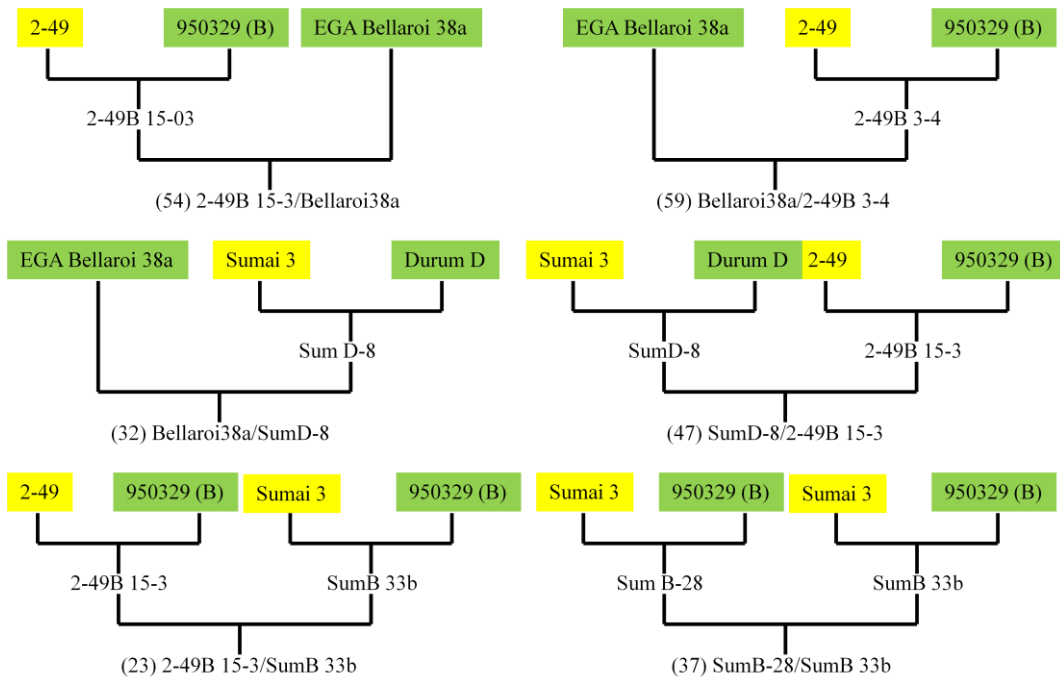
In the present study we therefore assessed the CR severity of six durum wheat populations obtained by crossing bread wheat varieties 2-49 and Sumai 3, with three different susceptible durum parents. These populations were supplied through the NSW Department of Primary Industries (NSW DPI). The lines from the field trial were also cross-analysed with SNP and SSR markers to assess the segregation of bread wheat loci potentially involved in reducing CR severity. Moreover CR susceptibility for the bread wheat variety Sumai 3, which from published literature is inconclusive and may/may not be as effective as 2-49, has also been defined.

2 MATERIALS AND METHODS

2 Materials and methods

2.1 Germplasm

Two hundred and fifty-two lines of tetraploid wheat were analysed. This germplasm was provided by the New South Wales Department of Primary Industries. It consisted of 6 populations, each derived from a different complex crossing program involving three durum parents (the cultivar EGA Bellaroi and durum breeding lines designated 950329 and Durum D) and two bread wheat parents (2-49 and Sumai 3) (Fig. 4). All materials used were F_4 single plant selections from the original cross which was made. These lines had been phenotypically selected in the field based on less crown rot (CR) severity and 'plant-type' (by Dr Steven Simpfendorfer and Dr Gururaj Kadkol, respectively). The bread wheat parents 2-49 and Sumai 3 (for which seed was provided by Associate Professor Hugh Wallwork of South Australian Research and Development Institute SARDI) were also analysed.

2-49   950329 (B)   EGA Bellaroi 38a   EGA Bellaroi 38a   2-49   950329 (B)

2-49B 15-03   2-49B 3-4

(54) 2-49B 15-3/Bellaroi38a   (59) Bellaroi38a/2-49B 3-4

EGA Bellaroi 38a   Sumai 3   Durum D   Sumai 3   Durum D   2-49   950329 (B)

Sum D-8   SumD-8   2-49B 15-3

(32) Bellaroi38a/SumD-8   (47) SumD-8/2-49B 15-3

2-49   950329 (B)   Sumai 3   950329 (B)   Sumai 3   950329 (B)   Sumai 3   950329 (B)

2-49B 15-3   SumB 33b   Sum B-28   SumB 33b

(23) 2-49B 15-3/SumB 33b   (37) SumB-28/SumB 33b

Fig. 4. Pedigree relationship for durum population investigated. Each population was generated from a complex cross involving both durum wheat (highlighted in green) and bread wheat (highlighted in yellow) parents. Progeny were obtained by self-pollinating up to the F_4 generation. For each population the number of lines under study is indicated in parentheses. Numbers appearing after 950329 (B) represent different selections from the original crosses.

2.2 Seed multiplication, glasshouse conditions

To obtain enough seed of each individual line for the phenotypic assessment study, a bulk-up of all seed material was conducted. Four seeds for each line were sown in a glasshouse located in 'The Plant Accelerator' at the Waite Campus (Adelaide, South Australia), where the temperature ranged from 16°C during the night to 22°C during the day, with a 16/8h photoperiod. Plants were watered as required to ensure suitable soil moisture was available.

2.3 DNA extraction and SNP assay

DNA was extracted from one plant of each of the 252 lines (F_5 generation) using a phenol/chloroform method readapted from Rogowsky *et al.* (1991) (Pallotta *et al.* 2000). The same extraction method was carried out on the parents 2-49, Sumai 3 and EGA Bellaroi 38a (however, the parents 950329 and Durum D were not provided through our collaborators at NSW DPI). A small piece of fresh leaf tissue was sampled from each plant three weeks after sowing in the glasshouse, then ground and diluted in 700 µL of DNA extraction buffer (1% sarkosyl, 100 mM Tris-HCl, 100 mM NaCl, 10 mM EDTA, 2% PolyVinyl-PolyPyrrolidone) and successively in 700 µL of phenol/chloroform/iso-amyalcohol (25:24:1). The solution was then transferred to an 8 mL silica matrix tube (Vacuette) and spun twice at 3220 RCF for 10 min (centrifuge 5810R, Eppendorf); adding 700 µL of phenol/chloroform/iso-amyalcohol between the 2 x 10 min centrifugations. A total of 60 µL of sodium acetate 3M (pH 4.8) and 600 µL of isopropanol was then added to the aqueous phase and the DNA was precipitated by centrifugation at 15871 RCF for 5 minutes (centrifuge 5424, Eppendorf). After decanting the liquid, 1 mL of 70% ethanol was added to the DNA pellet and the samples were centrifuged again at 15871 RCF for 5 min. After removing the ethanol the pellet was resuspended in 60 µL of R40 solution (RNase A (5 mg/mL, SIGMA R6513) and RNase buffer (5 mM Tris/HCl, 7.5 mM NaCl, pH 7.5), diluted 1:250 in 1X TE (10 mM Tris-HCl, 1 mM EDTA, pH 8.0)) and stored at 4°C. After adjusting the DNA concentrations for all the samples to approximately 50 and 60 ng µL^{-1} (using tris EDTA 10mM for the dilutions), they were sent to Dr Matthew Hayden of the Victorian Department of Primary Industries for genotyping with a 9K SNP array (Cavanagh *et al.* 2013). The genotyping, performed according to the Illumina protocol (Infinium iSelect SNP genotyping), exploited the instruments

BeadStatio, iScan and the 9k Infinum SNP chip PrivKSU_WheatCons_9k_11497518_A (Cavanagh *et al.* 2013). The genotype of the SNPs was called using the software GenomeStudio v2011.1 software (Illumina®, V2011.1).

2.4 Bird-proof enclosure layout and environmental conditions

Phenotypic assessment of all 252 lines and bread wheat parents was carried out in a bird-proof enclosure located at the Waite Campus. Soil of this enclosure has been naturally infested by *Fusarium culmorum* and *Fusarium pseudograminearum* over many years of continuous production of durum on durum stubble. The level of infestation had been detected in late 2011 by performing Predicta B tests. This DNA-based test (Ophel-Keller *et al.* 2008) provided the base level of *F. pseudograminearum* and *F. culmorum* (expressed as pg of fungal DNA/g of sample).

The experiment in the bird-proof enclosure was arranged in three blocks, each consisting of five pairs of two adjacent sub-blocks (Fig. 5). Each sub-block consisted of nine ranges of six 1-m-long rows. Within each pair of sub-blocks, the first sub-block was inoculated (I) and the second sub-block was not inoculated (NI) (Fig. 5). The additional inoculum of the I treatment was applied by sowing, in each row and together with the durum wheat seeds, 2 g of millet colonised by *F. pseudograminearum*. This contaminated millet was provided by Dr Cassandra Malligan from the Leslie Research Centre of Toowoomba (Queensland). Within each block, each of the 255 entries (252 lines and 3 parents) was assigned at random to a position within an inoculated sub-block and systematically to the corresponding position within the adjacent non-inoculated sub-block. The same principle, but with different randomised layouts, was applied to the other blocks.

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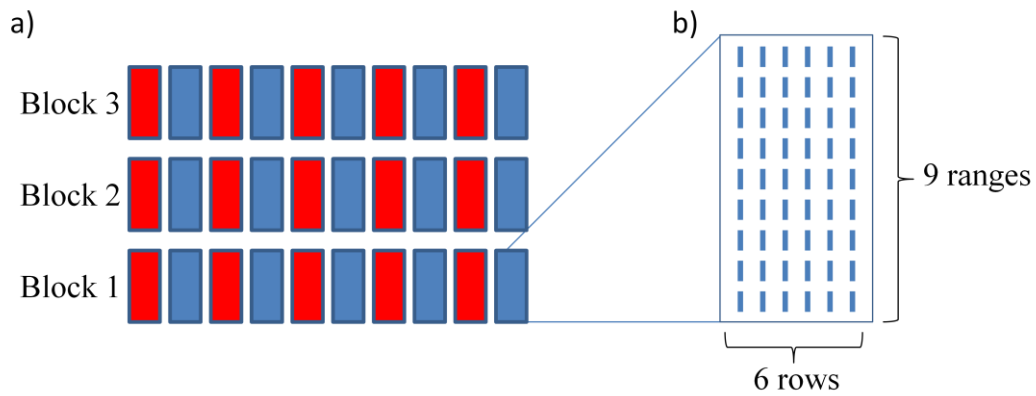
Fig. 5. Bird-proof enclosure layout. (a) The randomised complete block design of the experiment was composed of three replicated blocks, with each block having 10 sub-blocks. Two treatments were applied among the sub-blocks: the inoculated (I, red boxes) and the not inoculated (NI, blue boxes). (b) Each sub-block consisted of 6 rows (1 metre in length) and 9 ranges. Within the replicates, each entry was sown in one I sub-blocks and in the adjacent NI sub-blocks.

The experiment in the bird-proof enclosure was conducted during summer (December 2012 to March 2013). Based on weather reports (Bureau of Meteorology, 2012; 2013) in this four-month period the total rain pattern was approximately 54.6 mm (11.6 mm in December, 11.6 mm in January, 11.8 mm in February and 19.6 mm in March).

Irrigation was also applied to provide sufficient soil moisture for plant development. However, just prior to head emergence (9 weeks after sowing), moisture stress was imposed by withdrawing the irrigation in order to allow the infection to take place more readily than if adequate water was supplied. This was intended to simulate the conditions that would occur during a dry spring.

2.5 Inoculum viability and soil infestation

Fusarium infestation of the soil in the bird-proof enclosure was checked using a viability assessment of the inoculum and molecular analysis. Three and seven weeks after sowing, two viability assessments were performed according to the dilution plate technique methodology developed by Leslie and Summerell (2006). For each block, 162 soil cores were extracted from inter-rows and pooled in 18 samples (9 from I and 9 from NI sub-blocks). The samples were air-dried in a laminar flow cabinet for 48 hours and, after being ground to obtain small particles, they were diluted up to 1:400 in water agar (which has an agar concentration of 0.05%). They were then inoculated onto pentachloronitrobenzene (PCNB) agar medium which is selective for *Fusarium* species (Leslie and Summerell 2006). Cultures were incubated for 5 days at 25°C and colony-forming units (CFU) were counted. In addition, molecular analysis was performed 11 weeks after sowing by using the Predicta B test (Ophel-Keller *et al.* 2008). For each block 270 soil cores were collected from inter-rows and pooled into 6 samples, 3 from I and 3 from NI sub-blocks. These samples were processed by the SARDI Root Disease Testing Service to estimate the level of *Fusarium* infestation.

2.6 Phenotypic assessment

Crown rot severity was assessed at plant maturity by assigning to each individual a score related to the main stem browning (Fig. 6; Wallwork *et al.* 2004). To obtain main stems for assessment, all plants were pulled from the soil, all tillers from each plant were removed and each main stem was cut at approximately 7 cm above the crown. Each main stem was then assessed for the extent and severity of browning using a scale from 0 (no symptoms) to 5 (severe symptoms).
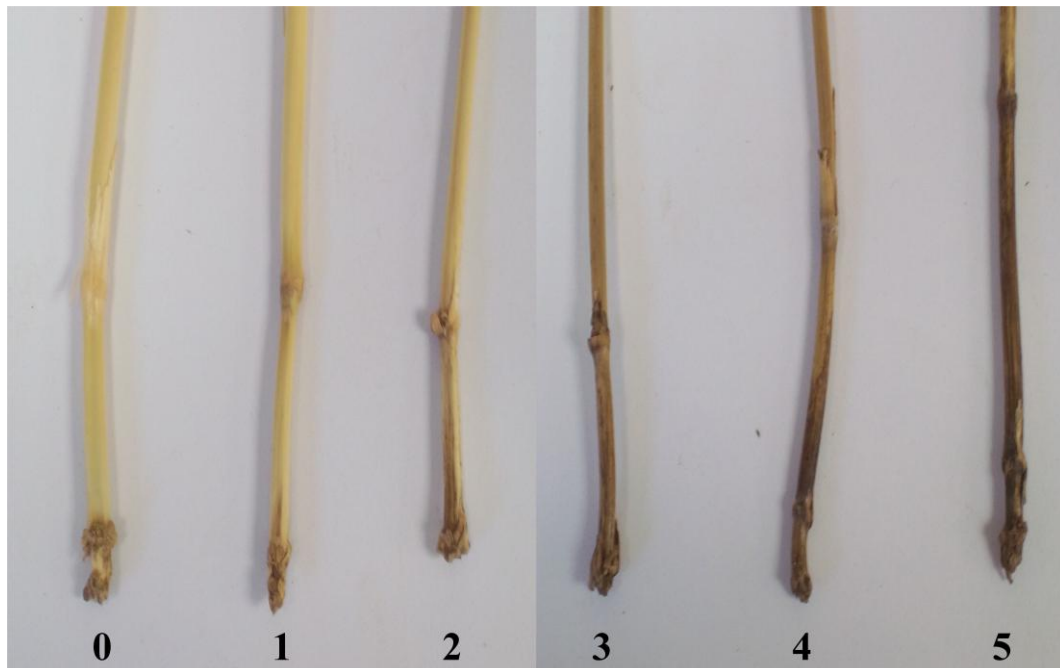
Fig. 6. Crown rot assessment scale. The severity of browning was assessed on the main stem of each mature plant on a scale of 0 to 5 (Wallwork *et al.* 2004). This figure illustrates the rating scale by showing one stem from each point on the scale.

2.7 Infection and viability of *Fusarium* in the main stem of the plants

Molecular analysis and viability assessment of the *Fusarium* infecting the plants were performed on the main stems of 8 samples per block. Within each block, the lines chosen were the bread wheat parents (2-49 and Sumai 3) one durum wheat line with a high and one durum wheat line with a low CR score. For each of these, samples were taken from both the I and NI sub-blocks. The molecular analysis was performed with the Predicta B test (Ophel-Keller *et al.* 2008) in 2 g of main stems for each sample. The viability of *Fusarium* in the stems was assessed using the moist incubation method (Burgess *et al.* 1988). Briefly, after cutting the first cm of the selected main stems and sterilising their surface, the samples were plated onto Potato Dextrose Agar (PDA) and incubated at room temperature, firstly in the dark (24 hours) and then in light conditions (48 hours). Afterwards,

the viability of the *Fusarium* was determined by visual naked-eye observation of mycelium and microscopic observation of canoe-shaped conidia.

2.8 Grain yield

The plant material harvested from each row was threshed, and the grain was weighed. Grain yield was calculated as g/m^2.

2.9 Statistical analysis of phenotypic data

The data collected were analysed using unbalanced ANOVA and correlation analysis tests (Genstat 15th edition). The analysis of variance was performed to determine the least significant difference (LSD) among the CR scores so that partially resistant lines (with a CR score significantly lower than the susceptible parent EGA Bellaroi 38a) could be identified. Moreover, a planned single-degree-of-freedom ANOVA enabled the comparison between the CR score of the parent EGA Bellaroi 38a, and the mean of each single family. The correlation analysis was carried out between CR scores, yield and 'Fusarium-inoculated' stems.

2.10 Association analysis

Results from the SNP array analysis were obtained from Dr Matthew Hayden as a GenomeStudio (Illumina®, V2011.1) file and a set of genotype calls (255 entries $\times$ 8632 markers) that had been automatically generated using a cluster file (TetraploidClusterFile_v.12) and SNP annotation file (TdDIVers SNP annotation_2.0) developed specifically for tetraploid wheat. For markers with ambiguous results, GenomeStudio was used to visually examine the distributions of R and Theta values and determine the final genotype calls for each entry. For each polymorphic marker, t-tests (Genstat, 15th edition) were conducted to

examine the association of the marker with CR severity scores across the full set of 252 lines, and for each of the families of lines in which that marker was polymorphic.

2.11 SSR marker analysis

The populations under study were also screened with SSR markers that had previously been reported to be linked with QTL for CR resistance in bread wheat populations derived from 2-49 (Martin *et al.* 2013). The analysis was conducted using the multiplex ready PCR technique (MRT) (Hayden *et al.* 2008) with separation of amplicons performed by AGRF (Australian Genome Research Facility). To prepare the samples, a preliminary PCR was performed. The PCR mastermix (final volume of 6 µL) contained 1.2 µL of 5x Mpx-Rdy Buffer, 0.03 µL of Immolase DNA polymerase (Bioline), 0.045 µL of dye labelled *tagF* (VIC for the markers *cfd39*, *gwm285* and *wmc216*; FAM for *gwm164*, *gwm11* and *wmc1*; NED for *wmc120* and *gwm376*; PET for *gwm513* and *barc148*), 0.045 µL of dye unlabelled *tagR*, 3 µL of template DNA and different amounts of selected SSR primer pairs (0.54 µL of *cfd39*, 0.075 µL of *gwm285*, 0.09 µL of *wmc216*, 0.21 µL of *gwm164*, 0.21 µL of *gwm11*, 0.24 µL of *wmc1*, 0.075 µL of *wmc120*, 0.48 µL of *gwm376*, 0.12 µL of *gwm513*, 0.21 µL of *barc148*). The Mpx-Rdy buffer contained 7.5 mM of $MgCl_2$, 1mM of each dNTP and 2.5x of BSA. The stock solution of 100x of BSA contained 10 mg/mL of bovine serium albumin factor V, 50 mM NaCl, 5% of glycerol and 0.1 mM of EDTA (pH 8).

The PCR protocol (thermocycler DNA engine tetra 2, MJ Research) consisted of an initial step of 10 min at 95°C followed by 65 cycles organised in the following steps: 5 cycles of 30 sec of denaturation at 92°C, 90 sec of annealing at 50°C and 60 sec of elongation at 72°C; 20 cycles at 92°C for 30 sec,

63°C for 90 sec, 72°C for 60 sec; 40 cycles at 92°C for 15 sec, 54°C for 30 sec, 72°C for 60 sec; then a final extension of 10 min at 72°C.

The next step consisted of pooling the PCR products belonging to the same DNA samples. The samples were then desalted with ultra-filtration filter plates (AcroPrep 384 UF filter plate, PALL Life Science) and then resuspended with water. Three µL of each desalted sample was aliquoted into a well in a 96-well PCR plate, then 8 µL of Hi-Di formamide and 0.04 µL of GeneScan500-LIZ size standard were added for each sample. The solutions were heated at 90°C for 5 min then submitted to AGRF for separation of amplicons in an ABI3730 (Applied Biosystems). The results were analysed with the software GeneMapper v3.7 (Applied Biosystems).

3 RESULTS

3 Results

3.1 Soil infestation and inoculum viability

In soil samples taken three weeks after sowing, viable *Fusarium* were detected (Fig. 7), with a significant difference in the number of CFU/g of soil between the I and the NI treatments detected only in block 1 (Fig. 7a). Seven weeks after sowing the level of infestation was consistently higher in the I treatment than in the NI treatment (Fig. 7b).
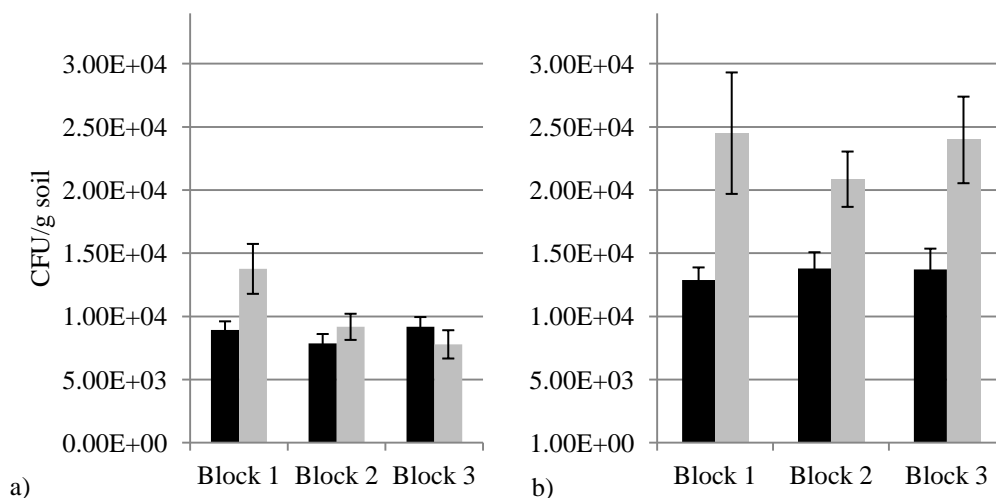

Fig. 7. Viability of *Fusarium* in the soil. Results of the CFU counts performed in according to the dilution plate technique (Leslie and Summerell 2006) are shown. Two identical analyses have been accomplished at 3 (a) and 7 weeks (b) after sowing. Means and standard errors are shown for sets of 18 samples taken from each of the treatments (NI: black bars; I: grey bars) within each of three blocks of the experiment.

However, the dilution plate technique does not discriminate among the *Fusarium* species counted. To determine which *Fusarium* species involved in CR infection, the Predicta B tests was also conducted on 6 soil samples taken from inter-rows. This analysis detected both *F. pseudograminearum* and *F. culmorum* in both the NI and I treatments (Fig. 8). For *F. culmorum*, the level of inoculum

was similar between the two treatments. For *F. Pseudograminearum*, only trace amounts were detected in the NI treatment, but high amounts were present in the soil treated with the infected millet (I treatment).
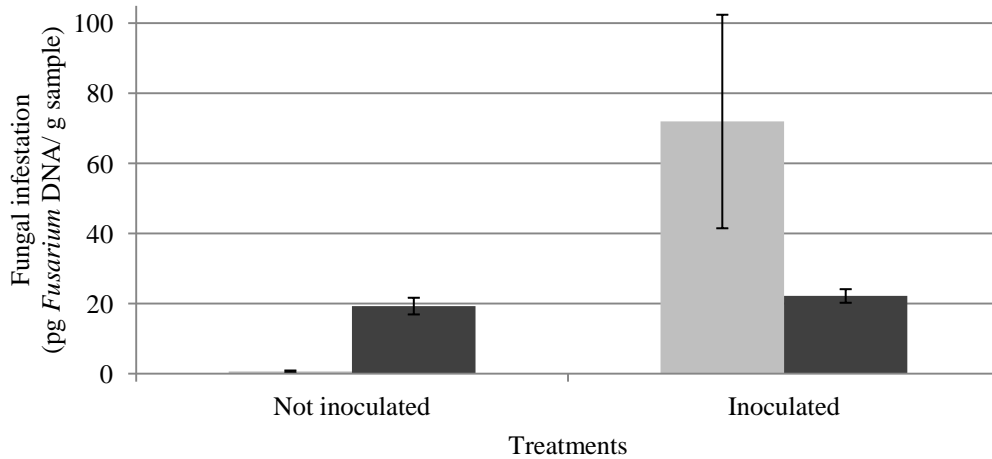

Fig. 8. Molecular analysis of *Fusarium* in the soil. This DNA-based analysis (Ophel-Keller *et al.* 2008) provided the base level of *F. pseudograminearum* and *F. culmorum* expressed as pg of fungal DNA/g per sample. Each column expresses the mean and SE (for 3 replications) of *F. pseudograminearum* (grey) or *F. culmorum* (black) infesting the soil. Both treatments are represented.

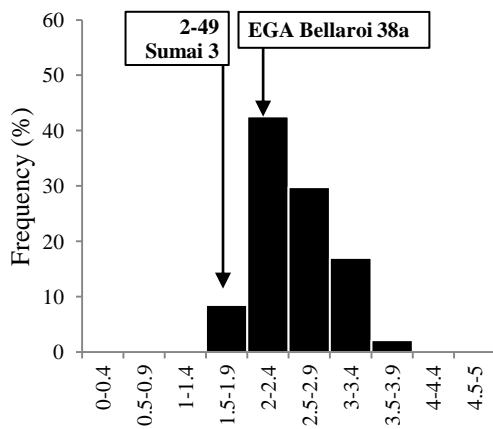**3.2 Phenotypic assessment of crown rot severity**

In both the NI and I treatments, the partially resistant bread wheat parent 2-49 had a mean CR severity score of 1.9. The results for the other bread wheat parent, Sumai 3, were not significantly different from this, with means of 1.7 and 2.1 for the NI and I treatments, respectively (LSD = 0.5, based on the ANOVA across all the entries). In the NI treatment, the durum parent EGA Bellaroi 38a had a mean of 2.3, also not significantly different from 2-49. In the I treatment, however, EGA Bellaroi 38a had significantly more severe symptoms (mean 3.1) than either bread wheat. Thus, in this experiment, only the I treatment effectively

discriminated between the well-known partial resistance of 2-49 and the susceptibility of EGA Bellaroi.
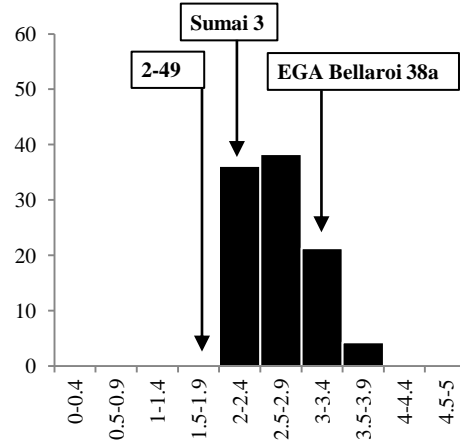
For five families out of six, a planned single-degree-of-freedom contrast indicated that the mean CR severity score of the progeny for the I treatment was significantly lower ($p < 0.001$) than that of EGA Bellaroi 38a (Fig. 9). The exception was the family Bellaroi38a/SumD-8 ($p = 0.058$).
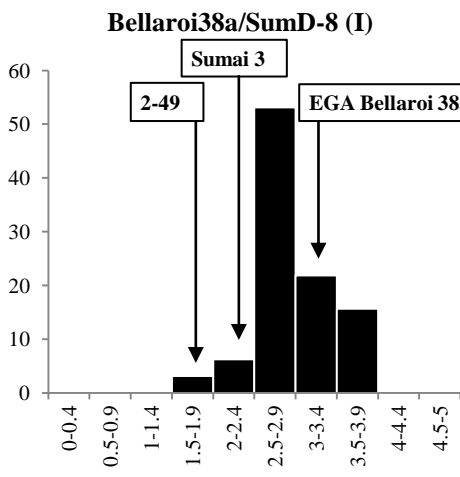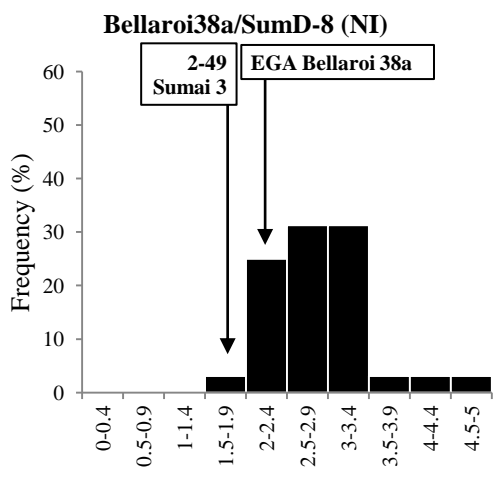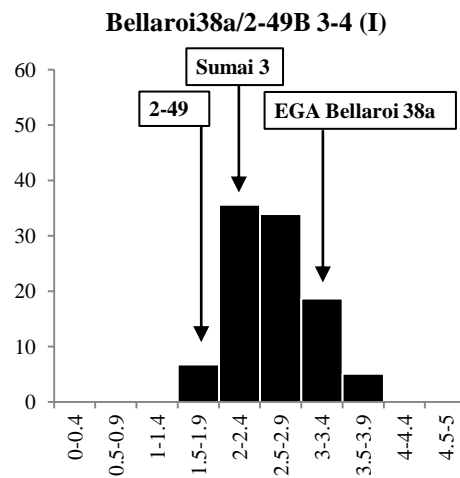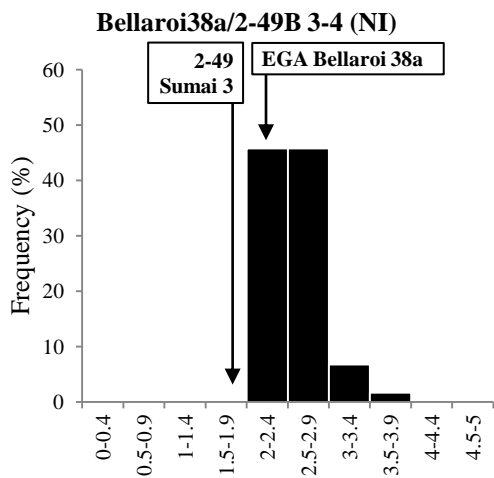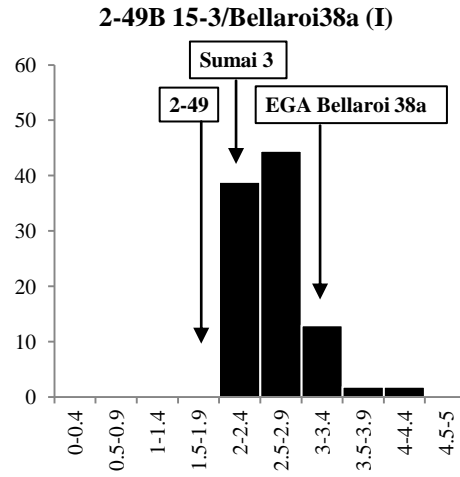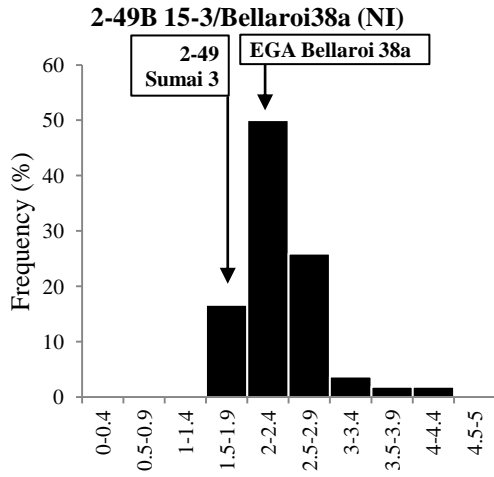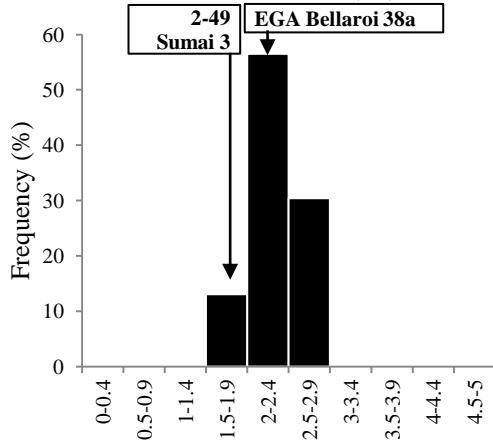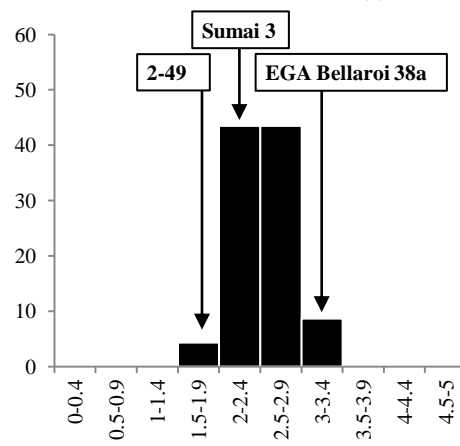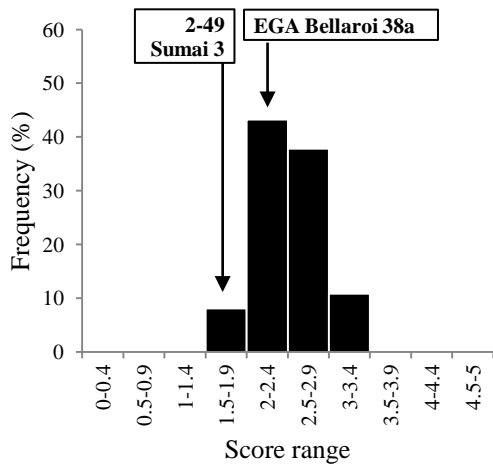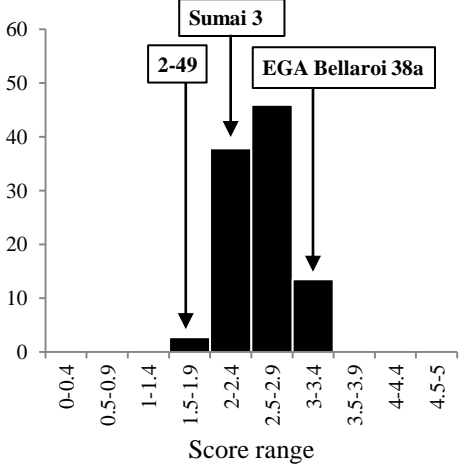
Fig. 9. Phenotypic assessment of CR severity. The methodology involved assigning a score from 0 (no symptoms) to 5 (severe symptoms) related to the intensity and extension of the main stem browning (Wallwork *et al.* 2004) for each line of each population. The data are expressed in frequencies (percentage) of the lines' scores grouped in ranges. Each pair of charts represents the CR scores of one family for NI and I treatments. The text boxes indicate which range of CR scores the parents belong to.

In the I treatment, 120 lines (48%) had significantly lower symptoms than EGA Bellaroi 38a, therefore suggesting that these lines may be consider partially resistant to CR. Within families, the percentage of partially resistant lines ranged from 19% in the family Bellaroi38a/SumD-8 to 65% in the family 2-49B 15-3/SumB 33b (Table 3).

Table 3. Partially resistant lines identified across all the populations studied. For each family, the percentage of lines which were partially resistant to CR are shown. In parentheses, the number of lines this represents is expressed. Each line has been defined as partially resistant if its CR score was significantly lower different than the score of EGA Bellaroi 38a for the I treatment, based on the analysis of variance and the LSD (least significant difference).

		Percentage of partially resistant lines
Family	Size of family (lines)	Treatment I
2-49B 15-3/Bellaroi38a	54	47% (26)
Bellaroi38a/2-49B 3-4	59	54% (32)
Bellaroi38a/SumD-8	32	19% (6)
SumD-8/2-49B 15-3	47	47% (22)
2-49B 15-3/SumB 33b	23	65% (15)
SumB-28/Sum33b	37	51% (19)
All families	252	48% (120)

While EGA Bellaroi 38a and many (59 of 132) of the durum progeny lines not classified as partially resistant had significantly more severe CR symptoms in the I treatment than in the NI treatment, there were no significant differences between the two treatments for the bread wheats 2-49 and Sumai, nor for 112 of 120 (93%) of the durum progeny lines that had been classified as partially resistant.

3.3 Yields of the lines under investigation

Grain yield from the plants under investigation were assessed and in the I treatment, the highest yielding entry was the parent Sumai 3 with 532 g/m^2, compared to only 264 g/m^2 for 2-49, 118 g/m^2 for EGA Bellaroi 38a and 127 g/m^2 for the mean across all entries (LSD = 101 g/m^2) (Fig. 10). Compared to the NI treatment, the yield losses in the I treatment were 5% for Sumai 3, 14% for 2-49, 54% for EGA Bellaroi 38a and 17% considering the mean across all the entries (Table 4).

Table 4. Yield losses between NI and I treatments. The table shows the yield for NI and I treatments, expressed in g/m^2, referred to the durum parents and the mean across all the entries. The yield losses between the two treatments shown in the last column are expressed in percentage.

	Treatment NI	Treatment I	Yield loss (%)
Sumai 3	560	532	5
2-49	308	264	14
EGA Bellaroi38a	256	118	54
Mean entries	153	127	17

The correlation coefficient (*r*) between mean CR score and mean yield was -0.44 across all durum lines. Analysing separately the susceptible (S) than the partially resistant lines, the *r* highlighted different correlations with values of -0.45 and -0.24 respectively. Among the families, 2-49B 15-3/Bellaroi38a, Bellaroi38a/SumD-8 and Bellaroi38a/2-49B 3-4 had the higher negative correlations.
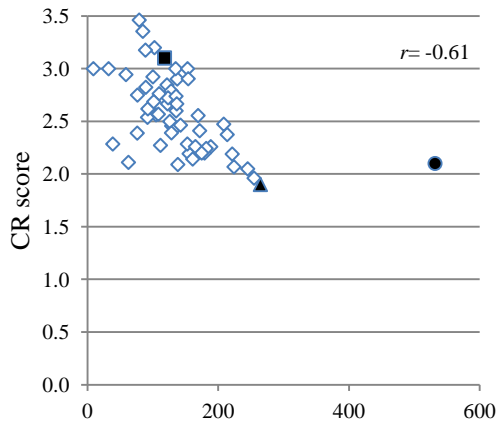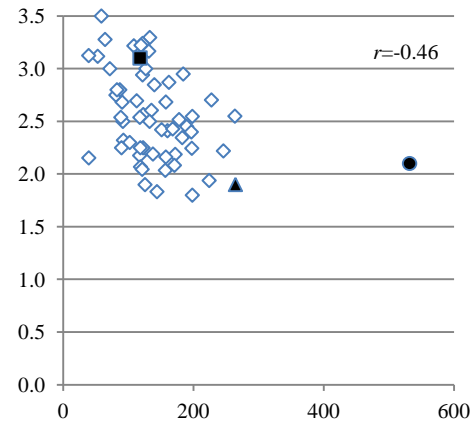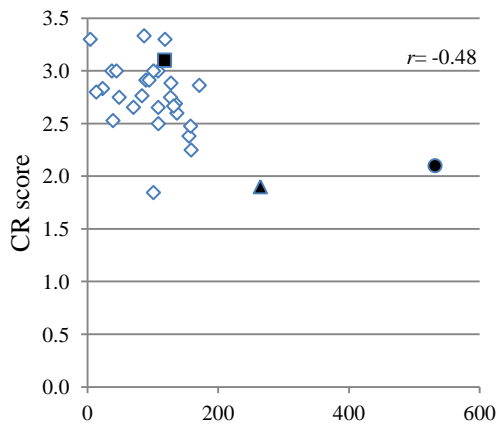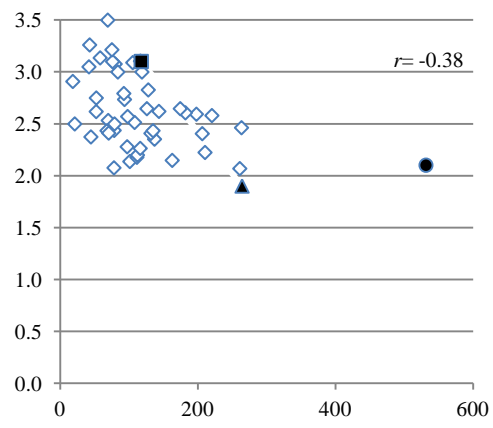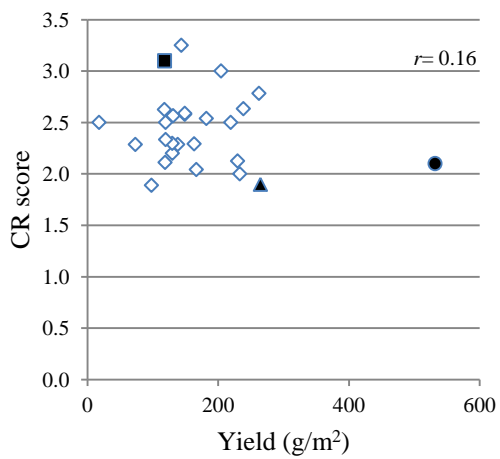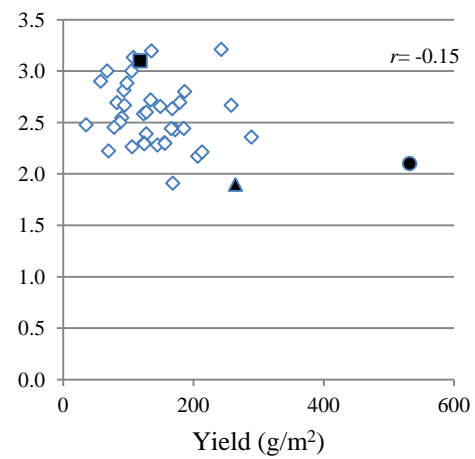
Fig. 10. 'Yield X CR score' scatter plots. The charts, one for each family, are a visual representation of CR score and yield for all lines under investigation in the I treatment. The parents displayed are EGA Bellaroi 38a (■), 2-49 (▲) and Sumai 3 (●). The correlation coefficients (*r*), calculated for each family excluding the parents' data, are also shown.

Comparing the yield of the treatments NI (mean of 151 g/m^2) and I (mean of 131 g/m^2), only 15 lines showed a significant reduction of yield in the samples subjected to the I treatments.

3.4 Infestation and viability of *Fusarium* infecting the main stem of the plants

The analysis of the *Fusarium* isolated from the main stems led to identify viability, taxonomy, and concentration of the pathogens responsible for the infection (Fig. 11; Fig. 12). Incubation onto PDA allowed the growth of pink mycelium (Fig. 11a), as expected for *F. pseudograminearum*, while the microscopic observation allowed the identification of canoe-shaped conidia, typical of *F. pseudograminearum* and *F. culmorum* (Fig. 11b). Both of the observations suggested that viable *Fusarium* were infecting the main stems of plant in this study.
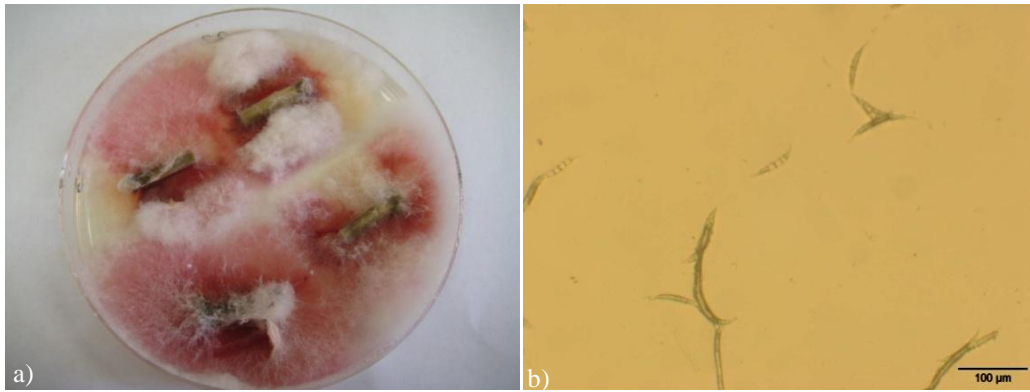
Fig. 11. Viability of *Fusarium* infecting the main stem. The viability of *Fusarium* infecting the main stems was assessed by using the 'moist incubation method' protocol (Burgess *et al.* 1988). Eight samples per replicate were chosen from each of the following: 2-49, Sumai 3, 1 durum line with a low CR score, and 1 durum with a high CR score. All of these samples were taken from NI and I sub-blocks. Visual (naked-eye) observations led to the identification of a pink mycelium in PDA culture (a), while the microscopic observation of the same mycelium led to observing canoe-shaped conidia (b). The pictures show, as demonstrative example, the susceptible line 174.

After the viability of the isolates was confirmed, molecular analysis was performed to identify the *Fusarium* species infecting the main stem (Fig. 12). In the samples from the NI sub-blocks, the bread wheat parents (2-49 and Sumai 3) and partially resistant lines showed only low levels of *F. culmorum* DNA and little or no *F. pseudograminearum* DNA. In contrast, high amounts of *F. culmorum* DNA were detected in the susceptible lines (Figure 12a). In the I treatment higher levels of *F. pseudograminearum* DNA were detected, especially for the durum lines, with no significant difference between those classified as partially resistant or susceptible based on symptom severity (Fig. 12b). Moreover, while the level of *F. culmorum* remained stable between the two treatments in 2-49, Sumai 3 and the susceptible samples, it increased in the I treatment of the partially resistant sample. However a high standard error has been calculated.
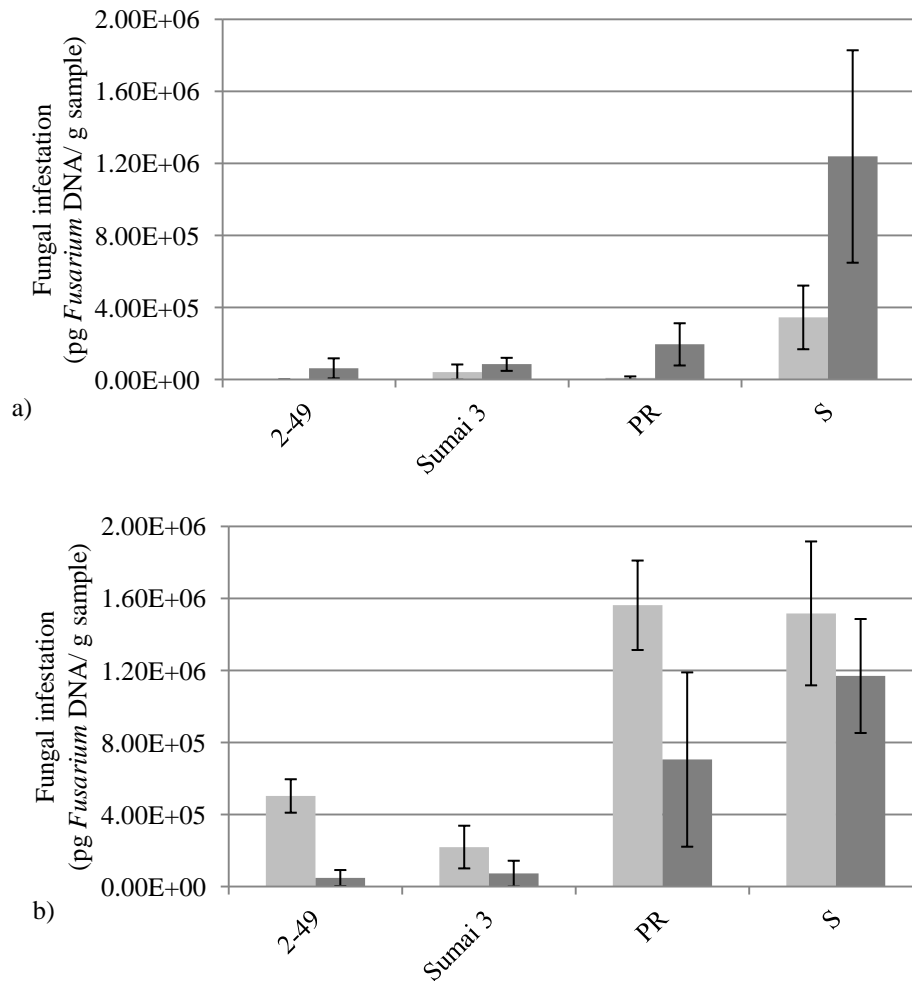
Fig. 12. Molecular analysis of *Fusarium* isolates infecting the main stem. Graphs show *F. pseudograminearum* (light grey bars) and *F. culmorum* (dark grey bars) infection of the main stems (expressed in pg of *Fusarium* DNA/ g of sample) of plants sown in NI (a) and I (b) sub-blocks. The data are expressed as means (with SE) of the 3 values for 2-49, Sumai 3, partial resistant lines (PR) and susceptible lines (S). The partial resistant entries chosen were lines 330 (with CR score of 1.8 for NI treatment and 2 for I, family 2-49B 15-3/Bellaroi38a), 471 (CR score 2 for NI and 1.9 for I, family Bellaroi38a/2-49B 3-4) and 486 (CR score 2.3 for NI and 2.1 for I, family Bellaroi38a/2-49B 3-4). The susceptible lines chosen were lines 174 (with CR score 2.6 for NI and 3.2 for I, family SumD-8/2-49B 15-3), 359 (CR score 2.4 for NI and 2.7 for I, family Bellaroi38a/SumD-8) and 508 (CR score of 3 for NI and 3.2 for I, family SumB-28/Sum33b).

3.5 Association analysis

The genotypes of the markers analysed with the 9K SNP array were recorded in the GenomeStudio file received by Dr Matthew Hayden. For those markers whose distributions of R and Theta values were ambiguous, the GenomeStudio charts were visually assessed and the genotypes re-called (Fig. 13).
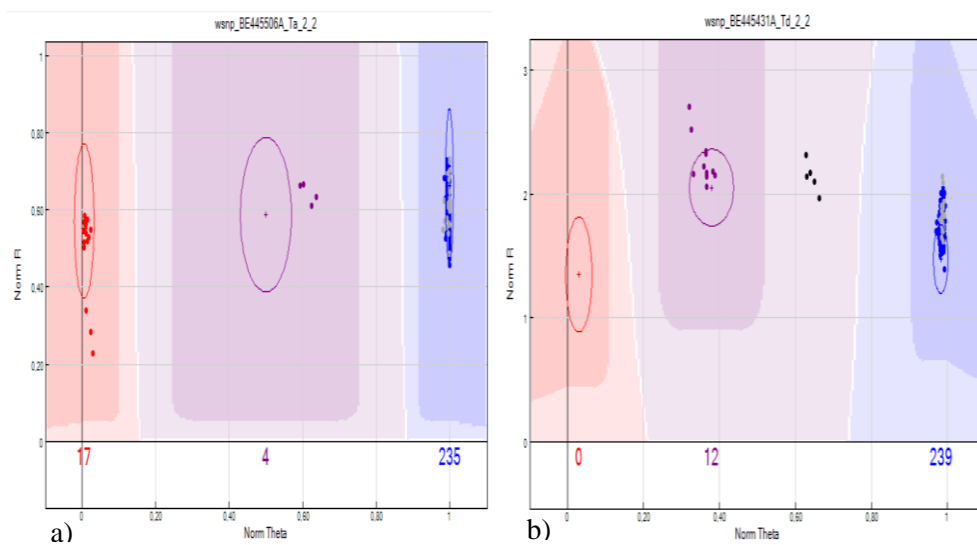
Fig. 13. GenomeStudio charts. The figure represents two examples of GenomeStudio charts. The axes represent Norm R and Norm Theta signals. Each chart is divided in three clusters, each of those represented by a different colour and referred to the three possible genotypes of a SNP: AA, AB and BB. The numbers below each cluster indicate the frequency of the entries that are automatically called with that genotype by the software. In the first example (a) the distribution of the fluorescent signals was clearly indicating the genotype of each marker, which had been then correctly recorded in the GenomeStudio file received. The other chart (b) shows an ambiguous result in which the Norm Theta signals are shifted and GenomeStudio was used to visually examine the genotypes and re-call them. In this example the entries represented as purple dots, previously identified as AB genotypes, have been visually re-called AA, and the entries not called (black dots) have been called AB.

Among 8634 SNPs on the array, 3122 were scored and 2453 exhibited polymorphism among the 252 durum progeny lines. The family with the highest number of polymorphic SNPs (1429) was Bellaroi38a/SumD-8, while 2-49B 15-3/Bellaroi38a and Bellaroi38a/2-49B 3-4 had only 160 and 301 polymorphic SNPs respectively. Only two SNPs (wsnp_Ku_c44600_51841068 and wsnp_JD_rep_c65181_41511534) exhibited significant association with CR symptom severity (Table 5) across all 252 lines and none exhibited significant association with CR symptom severity when analysed within individual families. The SNPs wsnp_Ku_c44600_51841068 and wsnp_JD_rep_c65181_41511534 were polymorphic only in the family Bellaroi38a/SumD-8 (Table 5) and the rare alleles have been detected in 12 and 10 individuals respectively.

Table 5. Association analysis across all the families. The table shows the association between SNPs genotypes and CR severity found analysing all the entries together. The rare alleles have been detected only in lines belonging to the family Bellaroi38a/SumD-8. No association has been detected when the analysis was performed on each family separately.

| SNP marker | Map position[1] | | Association with crown rot symptom severity | | | Mean crown rot severity of genotypic classes | | | |
| | | | | | | All lines | | Bellaroi38a/SumD-8 lines | |
	Chromosome	cM	p	Additive effect	% of phenotypic variation explained	Common allele	Rare allele	Common allele	Rare allele
wsnp_Ku_c44600_51841068	4A	144.1	<0.001	0.13	9.9	2.6	2.9	2.9	2.9
wsnp_JD_rep_c65181_41511534	7A	84.8	0.006	0.19	21.4	2.6	3.2	2.7	3.2

[1] As reported by Cavanagh *et al.* (2013) on a consensus genetic map for bread wheat

3.6 SSR marker analysis

Among the SSR markers that had been reported by Martin *et al.* (2013) as linked with QTL for CR resistance (*F. pseudograminearum*), only those that had been assessed as high-quality markers for MRT analysis (Hayden *et al.* 2008) were used here. Of 10 such markers, six exhibited polymorphism among the 183 lines from the four families with 2-49 in their pedigrees, and the same six SSRs exhibited the '2-49' allele in at least some of the lines under investigation with frequencies being in the range 0.5% and 21.8% (Table 6). There were no cases in which the mean CR symptom severity of the lines with the same genotype as '2-49' was significantly different from that of lines with other genotypes.

Table 6. Published QTL for partial resistance to CR. SSR markers associated to already published QTL found in the lines under study are shown. The table also shows the chromosomes in which the SSRs have been mapped, the frequency (and in parenthesis the number) of the lines carrying the '2-49' SSR allele. Two different means are expressed in the table: for the lines which had the '2-49' genotype and the lines with genotypes not associated to 2-49 (other genotype).

| | | | CR mean | |
SSR marker	Chromosome	Lines with the '2-49' genotype	'2-49' genotype	Other genotype(s)
wmc120	1A	6.5%(12)	2.7	2.6
barc148	1A	0.5% (1)	2.5	2.6
wmc216	1B	0.5% (1)	2.3	2.6
wmc1	3B	1% (2)	2.7	2.6
gwm376	3B	8.2%(15)	2.4	2.6
gwm513	4B	21.8%(40)	2.6	2.6

4 DISCUSSION AND CONCLUSION

4 Discussion and conclusion

This study investigated through phenotypic and genotypic approaches the susceptibility to CR of 252 lines of durum wheat obtained by crossing bread wheat varieties with durum parents. Crown rot symptoms were assessed in plants grown in a field infested by *F. culmorum* and inoculated with *F. pseudograminearum*, and genotypic analysis was performed using SNP and SSR markers to investigate genotype-phenotype associations for partial resistance to CR.

Despite some contrary results previously published (Li *et al.* 2010; Petrisko *et al.* 2010), this study demonstrates for the first time that the variety Sumai 3 has partial resistance to CR and it may be useful as a parent to improve the partial resistance to CR in durum progenies. This is consistent with what was reported by Collard *et al.* (2006), who cited unpublished data of Wildermuth and colleagues. The different behaviour of Sumai 3 in the experiments of Li *et al.* (2010) and Petrisko *et al.* (2010) may be traced back to the fact that, while the experiments reported here were conducted in the field, the previous published papers assessed CR symptoms of plants grown in pots only. Under their experimental conditions, Sumai 3 had much more severe symptoms than 2-49, but was not as highly susceptible as some other bread wheat and durum entries. In the field experiment conducted here, Sumai 3 displayed early vigour, and this may have contributed it to its partial resistance.

In order to assess which of the entries were partially resistant, just the data of the I treatment have been considered because, for the NI treatment, there was no significant difference between the CR scores of the partially resistant 2-49 and the susceptible EGA Bellaroi 38a. This indicates that the low level of infestation of the soil for this treatment did not allow to discriminate, for the parents in this

instance, between partial resistance and susceptibility. The results of the phenotypic assessment highlight the enhancement of partial resistance to CR in the progeny, compared to the durum parent. This improvement is likely to be due to the use of partially resistant bread wheat as parents, as found in other reports with different pedigrees being examined (Bovill *et al.* 2010; Ma *et al.* 2012; Martin *et al.* 2013). In fact, the level of partial resistance detected in the populations investigated in this study is (on average) greater than in populations originating from just durum parents and analysed in breeding programs (Jason Able, pers. comm.).

The ANOVA analysis performed for each family against the score of EGA Bellaroi 38a, pointed out that the means of the CR score were significantly reduced in five families out of six. Even if there was the family Bellaroi38a/SumD-8 for which the mean did not differ from the susceptible durum parent EGA Bellaroi 38a, six partially resistant lines were detected here. The high number of partially resistant lines found for the I treatment (48% of the total number of entries), is probably partly due to the selection against CR that was imposed during the development of the germplasm by the seed provider. Comparing the CR severity observed in the NI and I treatment, no significant difference arose in the majority of the entries, as detected for the parents 2-49 and Sumai 3. This similarity of scores across the treatments was due to the high number of partially resistant lines, of which only 7% exhibited a significant response to the inoculation.

The molecular analysis of the *Fusarium* species infecting soil led to consider that asymptomatic infections took part in this experiment; as similarly reported by Hollaway *et al.* (2013) who found, in the 20% of their entries, a lack of association between stem browning and *Fusarium* infestation of the soil.

Likewise, the molecular analysis of the stems, detected in partially resistant lines levels of *F. pseudograminearum* similar to those found in plants assessed as highly susceptible. This indicated that the partial resistance of some entries was due to mechanisms of tolerance to mycotoxins once the pathogen already infected the stem, instead of the ability of the plants to resist to the infection of the pathogen infesting the soil.

The analysis of the yield highlights how CR negatively affects good yielding varieties like EGA Bellaroi 38a which had the lower yield across the parents and reduced it from 256 g/m^2 (NI treatment) to 118 g/m^2 (I treatment). Interesting is the outcome for Sumai 3 with a CR severity comparable to 2-49, but a yield which was significantly higher than all the entries. Comparing the mean of the yield of all the lines for the I treatment with the mean for the NI treatment, no significant difference arose (Table 4). This result does not agree with that of Hollaway *et al.* (2013) which identified *F. pseudograminearum* as the pathogen responsible for more severe yield reduction than *F. culmorum*. This difference may be due to the fact that, in this study, even though the number of susceptible lines was not really high, the CR was severe which resulted in low or null yield. Consequently, the high variability of yield across the samples resulted in the average yield for the 2 treatments not being significantly different.

Given that *F. culmorum* and *F. pseudograminearum* were both present in this experiment, the latter especially higher in the I treatment, it is worth considering which kind of interactions took part in the experiment. Based on yield loss data, it is not clear whether the two species colonise the stems through a competitive or mutualistic mechanism. Considering the molecular analysis of *Fusarium* colonisation on the stem, the results suggest that the interaction between the two different species may be different among the different entries. For

example, the analysis of *Fusarium* DNA in the stems of partial resistant lines highlighted that, even if the infestation of *F. culmorum* in the soil was the same for both the treatments, a higher amount of DNA in the stems was detected for the I treatment than the NI (Fig. 12). This suggests that the presence of *F. pseudograminearum* in the I treatment favoured the colonisation of *F. culmorum*; acting then synergistically. However these results should be taken carefully because of the high standard error and the low number of samples analysed, the latter due to the high cost of the molecular analysis used. The fact that the same uncertainties arose in the study of Hollaway *et al.* (2013), suggests that further studies are required to quantify the different contributions of the two species to the yield reduction. New experiments could be designed in the field by applying three different treatments, one for each different block, to the same lines: soil infested with *F. pseudograminearum*, soil infested with *F. culmorum* and soil infested with both together.

While it was expected that a higher correlation between CR symptoms and yield would be found for partially resistant lines, surprisingly these had *r* of -0.24, while *r* of -0.45 was obtained for the susceptible lines. These differences may be due to the different families that contributed to the overall population of lines that were under study, as yield between these could be expected to vary. Consequently, when CR was not severe (for partially resistant lines) the differences in yield between the different families was more evident, leading to high variability and subsequently a low correlation between CR and yield. On the contrary, when CR was severe (susceptible lines) the yield reduction made the correlation between CR score and yield more evident. However, that said, when analysing the *r* separately for each family, for some populations there is still no clear trend between CR severity and yield due to the small size of these.

Considering those families with a larger number of individuals (for example the families 2-49B 15-3/Bellario38a and Bellaroi38a/2-49B 3-4, with 54 and 59 individuals respectively), higher *r* values were obtained. This may be traced back to that by analysing plants belonging to the same families, the variability in yield among the entries is reduced, inverting then the tendency found when analysing all the families together. Nevertheless, the results concerning the yield should be analysed carefully because the experiment was designed specifically for CR symptom analysis with the use of the rows; thus giving a slightly lower reliability for data concerning the yield.

With analysis conducted within families, no significant associations were detected between any individual SNP markers and the crown rot severity. With analysis conducted across all 252 progeny lines, significant associations were detected for two SNP markers. In both cases, the rarer allele (present in just 10 or 12 lines) was associated with greater symptom severity. In both cases, that allele was observed only in the Bellaroi38a/SumD-8 family, the family with the highest mean symptom severity. Accordingly, the significant associations detected for these two SNP markers simply reflect the overall difference in crown rot severity between the Bellaroi38a/SumD-8 progeny and the other lines. They are artefacts of population structure and cannot be considered to reflect true QTL effects.

Considering the high number of partially resistant lines found in this study and the large number of SNP markers evaluated, it is surprising that differences in resistance were not found to be associated with any markers. This may be partly due to the fact that the SNPs included on the array were chosen based on their ability to distinguish among bread wheat genotypes, whereas here the main interest was in distinguishing alleles from bread wheat from those present in durum wheat. A large number of the markers on the array failed to detect any

polymorphism among the materials used here. Further, since SNP markers are bi-allelic they would not have differentiated among all of the parents of the complex crosses used here. Also the small size of the families may play an important role in reducing the possibility to find an association between SNP genotype and partial resistance to CR.

Considering the markers associated with QTL previously identified in 2-49 by Martin *et al.* (2013), the SSR *gwm513* is the most frequently detected in this study. While this allele clearly differed from the ones detected for EGA Bellaroi 38a and Sumai 3, it is not possible to be certain that it was derived from 2-49 and not from the parents Durum D and 950329, which were not made available for marker analysis because of the non-supply of this parental germplasm. Although no overall difference in CR symptom severity was detected between the 40 lines with the same allele as 2-49 and the remaining 143 lines which had 2-49 as a parent, it is worth noting that there was a range in CR score from 2 to 4 among the 40 lines. It could be that the more resistant lines inherited their *gwm513* allele (and the associated resistance allele) from 2-49, while the more susceptible lines inherited their *gwm513* allele from one of the durum parents that was not genotyped. The same uncertainty was found for the other SSRs analysed, for which an allele similar to that of 2-49 was observed at low frequencies (ranging from 0.5% to 8.2%).

In general, the variability of results from crop species when placed under different environmental conditions and exposed to pathogens, suggests that much more research is still needed. As the scientific community moves further into exploring the area of molecular plant breeding, genotypic approaches have the potential to assist in reducing inconsistent results that can often be found between phenotypic-based reports. Coupled together, phenotypic and genotypic approaches

are crucial in order to minimise the impact of yield loss due to diseases such as CR. This is particularly important in crops like durum, which is highly susceptible and less studied than its bread wheat counterpart. Ironically, though, the traits (such as CR resistance) for which genotypic approaches could be the most valuable are also those in which genotype-phenotype associations are the most difficult to discover.

In conclusion, this research provided a phenotypic and a genotypic evaluation of 252 durum lines that had been developed with the objective of reducing CR susceptibility by crossing durum wheat with partially resistant bread wheat. A surprisingly high proportion of the lines were found to have some partial resistance to CR. The best of these could be further investigated in durum pre-breeding and breeding programs due to the yield that was obtained. Unfortunately, none of the molecular markers evaluated here were significantly associated with differences in CR resistance. While this may be partially due to the limitations of the materials and markers used here, it is also consistent with the widely held view that the inheritance of CR resistance is complex, and may involve small-effect alleles at numerous loci (Bovill *et al.* 2010). Moreover, this is the first report of results in the field that demonstrate the bread wheat variety Sumai 3 has partial resistance to CR.

5 References

Able, JA, Crismani, W & Boden, SA 2009, 'Understanding meiosis and the implications for crop improvement', *Functional Plant Biology*, vol. 36, pp. 575–588.

Akhunov, E, Nicolet, C & Dvorak, J 2009, 'Single nucleotide polymorphism genotyping in polyploid wheat with the Illumina GoldenGate assay', *Theoretical and Applied Genetics*, vol. 119, no. 3, pp. 507-517.

Akinsanmi, OA, Mitter, V, Simpfendorfer, S, Backhouse, D & Chakraborty, S 2004, 'Identity and pathogenicity of *Fusarium* spp. isolated from wheat fields in Queensland and northern New South Wales', *Australian Journal of Agricultural Research*, vol. 55, no. 1, pp. 97-107.

Al-Kaff, N, Knight, E, Bertin, I, Foote, T, Hart, N, Griffiths, S & Moore, G 2008, 'Detailed dissection of the chromosomal region containing the *Ph1* locus in wheat *Triticum aestivum*: with deletion mutants and expression profiling', *Annals of Botany*, vol. 101, no. 6, pp. 863-872.

Alloway, BJ 2008, *Zinc in Soils and Crop Nutrition*, International Fertilizer Industry Association and International Zinc Association, second edition, Brussels, Belgium and Paris, France.

Anderson, OD, Rausch, C, Moullet, O & Lagudah, ES 2003, 'The wheat D-genome HMW-glutenin locus: BAC sequencing, gene distribution, and retrotransposon clusters', *Functional & Integrative Genomics*, vol. 3, no. 1-2, pp. 56-68.

Aoki, T & O'Donnell, K 1999, 'Morphological characterization of *Gibberella coronicola* spp. obtained through mating experiments of *Fusarium pseudograminearum*', *Mycoscience*, vol. 40, pp. 443-453.

Australian Wheat Board Ltd, viewed 1 May 2012, www.awb.com.au/AWBL/Launch/Site/Customers/Content/GrainProducts/Wheat.

Backhouse, D 2006, 'Forecasting the risk of crown rot between successive wheat crops', *Australian Journal of Experimental Agriculture*, vol. 46, pp. 1499-1506.

Backhouse, D, Abubakar, AA, Burgess, LW, Dennis, JI, Hollaway, GJ, Wildermuth, GB, Wallwork, H & Henry, FJ 2004, 'Survey of *Fusarium* species associated with crown rot of wheat and barley in eastern Australia', *Australasian Plant Pathology*, vol. 33, pp. 255-261.

Backhouse, D & Burgess, LW 2002, 'Climatic analysis of the distribution of *Fusarium graminearum*, *F. pseudograminearum* and *F. culmorum* on cereals in Australia', *Australasian Plant Pathology*, vol. 31, no. 4, pp. 321-327.

Beddis, A & Burgess, L 1992, 'The influence of plant water stress on infection and colonization of wheat seedlings by *Fusarium graminearum* Group 1', *Phytopathology*, vol. 82, no. 1, pp. 78-83.

Bentley, AR, Summerell, BA & Burgess, LW 2008, 'Sexual compatibility in *Fusarium pseudograminearum* (*Gibberella coronicola*)', *Mycological Research*, vol. 112, no. 9, pp. 1101-1106.

Benyon, FHL, Burgess, L & Sharp, P 2000, 'Molecular genetic investigations and reclassification of *Fusarium* species in sections *Fusarium* and *Roseum*', *Mycological Research*, vol. 104, no. 10, pp. 1164-1174.

Bovill, WD, Horne, M, Herde, D, Davis, M, Wildermuth, GB & Sutherland, MW 2010, 'Pyramiding QTL increases seedling resistance to crown rot (*Fusarium pseudograminearum*) of wheat (*Triticum aestivum*)', *Theoretical and Applied Genetics*, vol. 121, no. 1, pp. 127-136.

Bovill, WD, Ma, W, Ritter, K, Collard, BCY, Davis, M, Wildermuth, GB & Sutherland, MW 2006, 'Identification of novel QTL for resistance to crown rot in the doubled haploid wheat population 'W21MMT70' x 'Mendos'', *Plant Breeding*, vol. 125, pp. 538-543.

Bureau of Meteorology 2012, viewed 30 September 2013, Daily Weather Observations. Available at: www.bom.gov.au/climate.

Bureau of Meteorology 2013, viewed 30 September 2013, Daily Weather Observations. Available at: www.bom.gov.au/climate.

Burgess, LW, Backhouse, D, Summerell, BA, Pattison, AB, Klein, TA, Esdaile, RJ & Ticehurst, G 1993, 'Long-term effects of stubble management on the incidence of infection of wheat by *Fusarium graminearum* Schw. Group 1'. *Australian Journal of Experimental Agriculture*, vol. 33, pp 451-456.

Burgess, LW, Backhouse, D, Summerell, BA & Swan, L 2001, *Crown rot of wheat. In 'Fusarium: Paul E. Nelson memorial symposium'.(Eds BA Summerell, JF Leslie, D Backhouse, WL Bryden, LW Burgess) pp. 271–294*, APS Press: St Paul, MN.

Burgess, LW & Bryden, WL 2012, '*Fusarium*: a ubiquitous fungus of global significance', *Microbiology*, vol. 22, pp. 22-25.

Burgess, LW, Liddell, CM & Summerell, BA 1988, *Laboratory Manual for Fusarium Research: Incorporating a Key and Descriptions of Common Species Found in Australia*, Second Edn, University of Sydney, Sydney, Australia.

Cakmak, I 2000, 'Tansley Review No. 111. Possible roles of zinc in protecting plant cells from damage by reactive oxygen species', *New Phytologist*, vol. 146, no. 2, pp. 185-205.

Cavanagh, CR, Chao, S, Wangc, S, Huang, BE, Stephen, S, Kiani, S, Forrest, K, Saintenac, C, Brown-Guedira, GL, Akhunova, A, See, D, Bai, G, Pumphrey, M, Tomar, L, Wonge, D, Konge, S, Reynoldsk, M, da Silva, ML, Bockelman, H, Talbert, L, Anderson, JA, Dreisigacker, S, Baenziger, S, Carter, A, Korzun, V, Morrell, PL, Dubcovsky, J, Morell, MK, Sorrells, ME, Hayden, MJ & Akhunov, E. 2013, 'Genome-wide comparative diversity uncovers multiple targets of selection for improvement in hexaploid wheat landraces and cultivars', *PNAS*, vol. 110, no. 20, pp. 8057-8062.

Chakraborty, S, Liu, CJ, Mitter, V, Scott, JB, Akinsanmi, OA, Ali, S, Dill-Macky, R, Nicol, J, Backhouse, D & Simpfendorfer, S 2006, 'Pathogen population structure and epidemiology are keys to wheat crown rot and *Fusarium* head blight management', *Australasian Plant Pathology*, vol. 35, pp. 643–655.

Champeil, A, Doré, T & Fourbet, JF 2004, '*Fusarium* head blight: epidemiological origin of the effects of cultural practices on head blight attacks and the production of mycotoxins by *Fusarium* in wheat grains', *Plant Science*, vol. 166, no. 6, pp. 1389-1415.

Chantret, N, Salse, J, Sabot, F, Rahman, S, Bellec, A, Laubin, B, Dubois, I, Dossat, C, Sourdille, P, Joudrier, P, Gautier, MF, Cattolico, L, Beckert, M, Aubourg, S, Weissenbach, J, Caboche, M, Bernard, M, Leroy, P & Chalhoub, B 2005, 'Molecular basis of evolutionary events that shaped the hardness locus in diploid and polyploid wheat species (*Triticum* and *Aegilops*)', *Plant Cell*, vol. 17, no. 4, pp. 1033-1045.

Ching, A, Caldwell, K, Jung, M, Dolan, M, Smith, O, Tingey, S, Morgante, M & Rafalski, A 2002, 'SNP frequency, haplotype structure and linkage disequilibrium in elite maize inbred lines', *BMC genetics*, vol. 3, no. 1, pp. 19-32.

Clear, R & Patrick, S 2000, '*Fusarium* head blight pathogens isolated from *Fusarium*-damaged kernels of wheat in western Canada, 1993 to 1998', *Canadian Journal of Plant Pathology*, vol. 22, no. 1, pp. 51-60.

Collard, BCY, Grams, RA, Bovill, WD, Percy, CD, Jolley, R, Lehmensiek, A, Wildermuth, G & Sutherland, MW 2005a, 'Development of molecular markers for crown rot resistance in wheat: mapping of QTLs for seedling resistance in a '2-49' X 'Janz' population', *Plant Breeding*, vol. 124, pp. 532-537.

Collard, BCY & Mackill DJ 2008, 'Marker-assisted selection: an approach for precision plant breeding in the twenty-first century', *The Phiosophical Transactions of the Royal Society of Biological Siences,* vol. 363, pp. 557-572.

Collard, BCY, Jahufer, MZZ, Brouwer, JB & Pang, ECK 2005b, 'An introduction to markers, quantitative trait loci (QTL) mapping and marker-assisted selection for crop improvement: The basic concepts', *Euphytica*, vol. 142, no. 1-2, pp. 169-196.

Collard, BCY, Jolley, R, Bovill, WD, Grams, RA, Wildermuth, GB & Sutherland, MW 2006, 'Confirmation of QTL mapping and marker validation for partial seedling resistance to crown rot in wheat line '2-49", *Australian Journal of Agricultural Research*, vol. 57, no. 9, pp. 967-973.

Connell, P, Lawrance, L & Nelson, R 2004, 'Durum Wheat Australia's role in world markets', *Australian Commodities*, vol. 11, no. 2, pp. 319-324.

Covarelli, L, Beccari, G, Steed, A & Nicholson, P 2012, 'Colonization of soft wheat following infection of the stem base by *Fusarium culmorum* and translocation of deoxynivalenol to the head', *Plant Pathology*, Vol. 61, pp.1121-1129.

Daniel, R & Simpfendorfer, S 2008, *The impact of crown rot on winter cereal yields*, viewed 20 July 2012, www.grdc.com.au/uploads/documents/GRDC%20update%202008%20crown%20 rot%20impact%20on%20yield.doc.

Dekkers, JCM & Hospital, F 2002, 'The use of molecular genetics in the improvement of agricultural populations', *Nature Review Genetics*, vol. 3, p. 23.

Desjardins, AE, Brown, DW, Yun, SH, Proctor, RH, Lee, T, Plattner, RD, Lu, SW & Turgeon, BG 2004, 'Deletion and complementation of the mating type (MAT) locus of the wheat head blight pathogen *Gibberella zeae*', *Applied and Environmental Microbiology*, vol. 70, no. 4, pp. 2437-2444.

Dodman, R & Wildermuth, G 1989, 'The effect of stubble retention and tillage practices in wheat and barley on crown rot caused by *Fusarium graminearum* Group 1', *Plant Protection Quarterly*, vol. 4, no. 3, pp 98-99.

Dubcovsky, J & Dvorak, J 2007, 'Genome plasticity a key factor in the success of polyploid wheat under domestication', *Science*, vol. 316, no. 5833, pp. 1862-1866.

Dvorak, J, Luo, M-C & Akhunovl, ED 2011, 'N.I. Vavilov's theory of centres of diversity in the light of current understanding of wheat diversity, domestication and evolution', *Czech Journal of Genetics and Plant Breeding*, vol. 47, pp. S20-S27.

Dvorak, J, Luo, MC, Yang, ZL & Zhang, HB 1998, 'The structure of the *Aegilops tauschii* genepool and the evolution of hexaploid wheat', *Theoretical and Applied Genetics*, vol. 97, no. 4, pp. 657-670.

Eberhard, FS, Zhang, P, Lehmensiek, A, Hare, RA, Simpfendorfer, S & Sutherland, MW 2010, 'Chromosome composition of an F2 *Triticum aestivum* x *T. turgidum* spp. *durum* cross analysed by DArT markers and MCFISH' *Crop and Pasture Science,* vol. 61, pp. 619-624.

Fauroux, B, Hart, N, Belfar, S, Boulé, M, Tillous-Borde, I, Bonnet, D, Bingen, E & Clément, A 2004, '*Burkholderia cepacia* is associated with pulmonary hypertension and increased mortality among cystic fibrosis patients', *Journal of Clinical Microbiology*, vol. 42, no. 12, pp. 5537-5541.

Fernandez, MR & Zentner, RP 2005, 'The impact of crop rotation and N fertilizer on common root rot of spring wheat in the brown soil zone of western Canada', *Canadian Journal of Plant Science*, pp. 569-575.

Feuillet, C & Keller, B 2004, '*Molecular markers for disease resistance. The example wheat*, *biotechnology in agriculture and forestry*', vol. 55, Springer-Verlag, Berlin Heidelberg, vol. 85, no. 3, pp. 353-370.

Ganal, MW & Roder, MS 2007, 'Microsatellite and SNP markers in wheat breeding', *Genomics Assisted Crop Improvement*, vol. 2, pp. 1-24.

Gianibelli, MC, Sissons, MJ & Batey, IL 2005, 'Effect of Source and Proportion of Waxy Starches on Pasta Cooking Quality', *Cereal Chemistry*, vol. 82, no. 3, pp. 321-327.

Grewal, HS, Graham, RD & Rengel, Z 1996, 'Genotypic variation in zinc efficiency and resistance to crown rot disease (*Fusarium graminearum* Schw. Group 1) in wheat', *Plant and Soil*, vol. 186, pp. 219-226.

Griffiths, S, Sharp, R, Foote, TN, Bertin, I, Wanous, M, Reader, S, Colas, I & Moore, G 2006, 'Molecular characterization of *Ph1* as a major chromosome pairing locus in polyploid wheat', *Nature*, vol. 439, pp. 749-752.

Gu, YQ, Coleman-Derr, D, Kong, X & Anderson, OD 2004, 'Rapid genome evolution revealed by comparative sequence analysis of orthologous regions from four triticeae genomes', *Plant Physiology*, vol. 135, no. 1, pp. 459-470.

Hayden, H, Carlier, J & Aitken, E 2005, 'The genetic structure of Australian populations of *Mycosphaerella musicola* suggests restricted gene flow at the continental scale', *Phytopathology*, vol. 95, no. 5, pp. 489-498.

Hayden, MJ, Nguyen, TM, Waterman, A & Chalmers, KJ 2008 'Multiplex-Ready PCR: A new method for multiplexed SSR and SNP genotyping', *BMC Genomics*, vol. 9, no. 80, pp 1-12.

Hollaway, GJ, Evans, ML, Wallwork, H, Dyson CB & McKay AC 2013, 'Yield Loss in Cereals, Caused by *Fusarium culmorum* and *F. pseudograminearum,* Is Related to Fungal DNA in Soil Prior to Planting, Rainfall, and Cereal Type', *Plant Disease*, vol. 97, pp. 977-982.

Huang, Y & Wong, P 1998, 'Effect of *Burkholderia* (*Pseudomonas*) *cepacia* and soil type on the control of crown rot in wheat', *Plant and Soil*, vol. 203, no. 1, pp. 103-108.

Jaccoud, D, Peng, K, Feinstein, D & Kilian, A 2001, 'Diversity Arrays: a solid state technology for sequence information independent genotyping', *Nucleic Acids Reearch,* vol. 29, no. 4 e25, pp.1-7.

Jantasuriyarat, C, Vales, MI, Watson, CJ & Riera-Lizarazu, O 2004, 'Identification and mapping of genetic loci affecting the free-threshing habit and spike compactness in wheat (*Triticum aestivum* L.)', *Theoretical and Applied Genetics*, vol. 108, no. 2, pp. 261-273.

Kasha, KJ 1999, 'Biotechnology and world food supply', *Genome*, vol. 42, no. 4, pp. 642-645.

Kashkush, K, Feldman, M & Levy, AA 2002, 'Gene loss, silencing and activation in a newly synthesized wheat allotetraploid', *Genetics*, vol. 160, pp. 1651-1659.

Khlestkina, EK & Salina, EA 2006, 'SNP markers: Methods of analysis, ways of development, and comparison on an example of common wheat', *Russian Journal of Genetics*, vol. 42, no. 6, pp. 585-594.

Khoshgoftarmanesh, AH, Kabiri, S, Shariatmadari, H, Sharifnabi, B & Schulin, R 2010, 'Zinc nutrition effect on the tolerance of wheat genotypes to *Fusarium* root-rot disease in a solution culture experiment', *Soil Science and Plant Nutrition*, vol. 56, no. 2, pp. 234-243.

Kirkegaard, J, Simpfendorfer, S, Holland, J, Bambach, R, Moore, K & Rebetzke, G 2004, 'Effect of previous crops on crown rot and yield of durum and bread wheat in northern NSW', *Australian Journal of Agricultural Research*, vol. 55, no. 3, pp. 321-334.

Klein, TA, Ellison, FW & Burgess, LW 1991, 'The incidence and spatial patterns of wheat plants infected by *Fusarium graminearum* Group 1 (soil borne fungus) and the effects of crown rot on yield', *Australian Journal of Agricultural Research*, vol. 42, pp. 399-407.

Kumar, A & Bennetzen, JL 1999, 'Plant retrotransposons', *Annual Review of Genetics*, vol. 33, no. 1, pp. 479-532.

Láday, M, Bagi, F, Mesterhazy, A & Szecsi, A 2000, 'Isozyme evidence for two groups of *Fusarium graminearum*', *Mycological Research*, vol. 104, no. 7, pp. 788-793.

Lamprecht, SC, Marasas, WFO, Hardy, MB & Calitz, FJ 2006, 'Effect of crop rotation on crown rot and the incidence of *Fusarium pseudograminearum* in wheat in the Western Cape, South Africa', *Australasian Plant Pathology*, vol. 35, pp. 419-426.

Lander, E & Kruglyak, L 1995, 'Genetic dissection of complex traits: guidelines for interpreting and reporting linkage results', *Nature genetics*, vol. 11, p. 241.

Leslie, JF & Summerell, BA 2006, *The Fusarium Laboratory Manual*, First edn, Blackwell Publishing Professional, Iowa, USA.

Li, HB, Xie, GQ, Ma, J, Liu, GR, Wen, SM, Ban, T, Chakraborty, S & Liu, CJ 2010, 'Genetic relationships between resistances to *Fusarium* head blight and crown rot in bread wheat (*Triticum aestivum* L.)', *Theoretical and Applied Genetics*, vol. 121, no. 5, pp. 941-950.

Liddell, CM & Burgess, LW 1988, 'Wax partionated soil columns to study the influence of soil moisture potential on the infection of wheat by *Fusarium graminearum* group 1', *The American Phytopathological Society*, vol. 2, pp. 185-189.

Limaux, F, Recous, S, Meynard, J-M & Guckert, A 1999, 'Relationship between rate of crop growth at date of fertiliser N application and fate of fertiliser N applied to winter wheat', *Plant and Soil*, vol. 214, pp. 49-59.

Liu, C, Magner, T, Mitter, V, Ban, T & Chakraborty, S 2004, 'Relationship between resistance to *Fusarium* head blight and crown rot in hexaploid wheat'. In *'4th International Crop Science Congress'*, Brisbane, Queensland, Australia, 26 Sep - 1 Oct 2004.

Liu, S & Anderson, JA 2003, 'Marker assisted evaluation of *Fusarium* head blight resistant wheat germplasm', *Crop Science*, vol. 43, no. 3, pp. 760-766.

López-Bellido, L, López-Bellido, RJ & López-Bellido, FJ 2006, 'Fertilizer nitrogen efficiency in durum wheat under rainfed mediterranean conditions: effect of split application', *Agronomy Journal*, vol. 98, no. 1, p. 55.

Luo, MC, Yang, ZL, You, FM, Kawahara, T, Waines, JG & Dvorak, J 2007, 'The structure of wild and domesticated emmer wheat populations, gene flow between them, and the site of emmer domestication', *Theoretical and Applied Genetics*, vol. 114, no. 6, pp. 947-959.

Ma, J, Zhang, CY, Liu, YX, Yan, GJ & Liu CJ 2012. 'Enhancing *Fusarium* crown rot resistance of durum wheat by introgressing chromosome segments from hexaploid wheat', *Euphytica*, vol. 186, pp. 67-73.

Maccaferri, M, Sanguineti, MC, Corneti, S, Ortega, JL, Salem, MB, Bort, J, DeAmbrogio, E, del Moral, LF, Demontis, A, El-Ahmed, A, Maalouf, F, Machlab, H, Martos, V, Moragues, M, Motawaj, J, Nachit, M, Nserallah, N, Ouabbou, H, Royo, C, Slama, A & Tuberosa, R 2008, 'Quantitative trait loci for grain yield and adaptation of durum wheat (*Triticum durum* Desf.) across a wide range of water availability', *Genetics*, vol. 178, no. 1, pp. 489-511.

MacLeod, B, Vanstone, V, Khangura, R, Perth, S & Beard, C 2008, *Root disease under intensive cereal production systems*, Bulletin of May 2008, Department of Agriculture and Food, State of Western Australia.

Martin, A, Simpfendorfer, S, Hare, RA & Sutherland, MW 2013, 'Introgression of hexaploid sources of crown rot resistance into durum wheat' *Euphytica*, vol. 192, no. 3, pp. 463-470.

Matsuoka, Y 2011, 'Evolution of polyploid triticum wheats under cultivation: the role of domestication, natural hybridization and allopolyploid speciation in their diversification', *Plant & Cell Physiology*, vol. 52, no. 5, pp. 750-764.

Miedaner, T, Cumagun, CJR & Chakraborty, S 2008, 'Population genetics of three important head blight pathogens *Fusarium graminearum*, *F. pseudograminearum* and *F. culmorum*', *Journal of Phytopathology*, vol. 156, pp. 129-139.

Miyan, S, Hüberli, D, Connor, M & MacLeod, B 2013 Management options for crown rot control in Western Australia, viewed 13 August 2013 researchrepository.murdoch.edu.au/14044/1/Management_options_for_Crown_ro t_in_Western_Australia.pdf.

Mohler, V, Bauer, C, Schweizer, G, Kempf, H & Hartl, L 2013, '*Pm50*: a new powdery mildew resistance gene in common wheat derived from cultivated emmer', *Journal of Applied Genetics*, vol. 54, pp. 259-263.

Moretti, A, & Susca, A 2009, '*Fusarium*' in Dongiou Liu edn, *Molecular Detection of Foodborne Pathogens*, pp. 577-592.

Mori, N, Ishi, T, Ishido, T, Hirosawa, S, Watatani, H, Kawahara, T, Nesbitt, M, Belay, G, Takumi, S, Ogihara, Y & Nakamura, C 2003, 'Origins of domesticated emmer and common wheat inferred from chloroplast DNA fingerprinting', Instituto Sperimentale per la Cerealicoltura, Rome, Italy Pogna NE, Romano M, Pogna EA, Galterio G edn, *Proceedings of the 10th International Wheat Genetics Symposium*, pp. 25-28.

Mudge, AM, Dill-Macky, R, Dong, Y, Gardiner, DM, White, RG & Manners JM 2006, 'A role for the mycotoxin deoxynivalenol in stem colonisation during crown rot disease of wheat caused by *Fusarium graminearum* and *Fusarium pseudograminearum*', *Physiological and Molecular Plant Pathology*, vol. 69, pp. 73-85.

Murray, GM & Brennan JP 2009, 'Estimating disease losses to the Australian wheat industry' *Australasian Plant Pathology*, vol. 38, pp. 558-570.

Nalam, VJ, Vales, MI, Watson, CJ, Kianian, SF & Riera-Lizarazu, O 2006, 'Map-based analysis of genes affecting the brittle rachis character in tetraploid wheat (*Triticum turgidum* L.)', *Theoretical and Applied Genetics*, vol. 112, no. 2, pp. 373-381.

Nesbitt, M, & Samuel, D 1996, 'From stable crop to extinction? The archaeology and history of the hulled wheats', In Hulled wheats, Rome (Italy), Padulosi S, Hammer K, Heller J, edn, *International Plant Genetic Resources Institute*, pp. 41-100.

Nicol, J, Bagci, A, Hekimhan, H, Bolat, N, Braun, H & Trethowan, R 2004, 'Strategy for the identification and breeding of resistance to dryland root rot complex for international spring and winter wheat breeding programs', in *4th International Crop Science Congress*, Brisbane, Queensland, Australia, 26 Sep - 1 Oct 2004 www.cropscience.org.au/icsc2004/poster/3/7/2/1322_nicol.htm.

Oliphant, A, Barker, DL, Stuelpnagel, JR & Chee, MS 2002, 'BeadArray Technology: Enabling an Accurate, Cost-Effective Approach to High-Throughput Genotyping', *BioTechniques*, vol. 32, pp. S56-S61.

Ophel-Keller, K, McKay, A, Hartley, D, Herdina, A, & Curran, J 2008, 'Development of a routine DNA-based testing service for soilborne diseases in Australia', *Australasian Plant Pathology*, vol. 37, pp. 243-253.

Ortiz, R 1998, 'Critical role of plant biotechnology for the genetic improvement of food crops: perspectives for the next millennium', *Electronic Journal of Biotechnology*, vol. 1, no. 3, pp. 16-17.

Ozkan, H, Brandolini, A, Pozzi, C, Effgen, S, Wunder, J & Salamini, F 2005, 'A reconsideration of the domestication geography of tetraploid wheats', *Theoretical and Applied Genetics*, vol. 110, no. 6, pp. 1052-1060.

Ozkan, H, Levy, AA & Feldman, M 2001, 'Allopolyploidy-Induced Rapid Genome Evolution in the Wheat (*Aegilops–Triticum*) Group', *The Plant Cell*, vol. 13, pp. 1735-1747.

Paterson, AH 1996, 'Making genetic maps', pp. 23-39 , in *Genome mapping in plants (Biotechnology intelligence unit)*, A.H. Paterson edn, Landes Company, San Diego, California; Academic Press, Austin, Texas.

Pallotta, MA, Graham, RD, Langridge, P, Sparrow, DHB & Barke, SJ 2000, 'RFLP mapping of manganese efficiency in barley', *Theoretical and Applied Genetics*, vol. 101, no. 7, pp. 1110-1108.

Paulitz, TC, Schroeder, KL & Schillinger, WF 2010, 'Soilborne pathogens of cereals in an irrigated cropping system: effects of tillage, residue management, and crop rotation', *Plant Disease*, vol. 94, no. 1, pp. 61-68.

Petrisko, JE, Knight, N & Sutherland, MW 2010, 'Progress in comparing *Fusarium pseudograminearum* infection levels and crown rot symptoms in stem internodes of cereals', in University of Southern Queensland Centre for Systems Biology, Toowoomba, 4350 Queensland (ed.), *6th Australasian Soilborne Diseases Symposium,* p. 74.

Pettitt, T, Xu, X & Parry, D 2003, 'Association of *Fusarium* species in the wheat stem rot complex', *European Journal of Plant Pathology*, vol. 109, pp. 769-774.

Pettitt, TR & Parry, DW 2001, 'Effect of temperature on *Fusarium* foot rot of wheat', in *Fusarium: Paul E Nelson Memorial Symposium*, eds BA Summerell, JF Leslie, D Backhouse, WL Bryden, LW Burgess pp. 145-160.

Ponzielli, P 2013 *24 maggio 2013 - 4th Incontro Internazionale della Filiera Cereagricola*, viewed 1 May 2012, www.romacereali.com/wp-content/uploads/2013/06/2013-ROMACEREALI_13-06-2013_DOC.pdf.

Rafalski, A 2002, 'Applications of single nucleotide polymorphisms in crop genetics', *Current Opinion in Plant Biology*, vol. 5, no. 2, pp. 94-100.

Reynolds, MP & Borlaug, NE 2006, 'Impacts of breeding on international collaborative wheat improvement', *The Journal of Agricultural Science*, vol. 144, no. 1, p. 3.

Rogowsky, PM, Guider, ELY, Langridge, P, Shepherd, KW & Koebner, RMD 1991, 'Isolation and characterization of wheat-rye recombinants involving chromosome arm 1DS of wheat', *Theoretical and Applied Genetics*, vol. 82, pp. 537-544.

Rossi, V, Cervi, C, Chiusa, G & Languasco, L 1995, 'Fungi associated with foot rots on winter wheat in northwest Italy', *Journal of Phytopathology*, vol. 143, no. 2, pp. 115-119.

Rummery, G, Daniel, R & Simpfendorfer, S 2007, 'Inter-row crown rot management – the results are in', *Southern Australia Focus*.

Satorre, EH & Slafer, GA 1999, *Wheat: Ecology and physiology of yield determination*, EH Satorre and GA Slafer edn, Binghamton, NY.

Sharma, R, Sissons, MJ, Rathjen, AJ & Jenner, CF 2002, 'The Null-4A Allele at the *Waxy* Locus in Durum Wheat affects Pasta Cooking Quality', *Journal of Cereal Science*, vol. 35, no. 3, pp. 287-297.

Simpfendorfer, S, Verrell, A, Nash, P & Moore, KJ 2005, 'Crown rot – the burning issue', In *The 15th Biennial Australasian Plant Pathology Society Conference*, Geelong 26-29 September 2005.

Smiley, RW, Gourlie, JA, Easley, SA, Patterson, LM & Whittaker, RG 2005, 'Crop Damage Estimates for Crown Rot of Wheat and Barley in the Pacific Northwest', *Plant Disease*, vol. 89, no. 6, pp. 595-604.

Soltis, PS & Soltis, DE 2000, 'The role of genetic and genomic attributes in the success of polyploids', *PNAS*, vol. 97, pp. 7051-7057.

Southwell, RJ, Moore, KJ, Manning, W & Hayman, PT 2003, 'An outbreak of *Fusarium* head blight of durum wheat on the Liverpool Plains in northern New South Wales in 1999', *Australasian Plant Pathology*, vol. 32, no. 4, pp. 465-471.

Summerell, BA, Burgess, LW, Backhouse, D, Bullock, S & Swan, LJ 2001, 'Natural occurrence of perithecia of *Gibberella coronicola* on wheat plants with crown rot in Australia', *Australasian Plant Pathology*, vol. 30, pp. 353-356.

Trail, F, Haixin, X, Rachel, L & David, G 2002, 'Physiological and environmental aspects of ascospore discharge in *Gibberella zeae* (anamorph *Fusarium graminearum*)', *Mycologia*, vol. 94, no. 2, pp. 181-189.

Troccoli, A, Borrelli, GM, De Vita, P, Fares, C & Di Fonzo, N 2000, 'Mini Review: Durum Wheat Quality: A Multidisciplinary Concept', *Journal of Cereal Science*, vol. 32, no. 2, pp. 99-113.

Turner, M, Mukai, Y, Leroy, P, Charef, B, Appels, R & Rahman, S 1999, 'The *Ha* locus of wheat: Identification of a polymorphic region for tracing grain hardness in crosses', *Genome*, vol. 42, pp. 1242-1250.

Vijayakumar, C, Wolf-Hall, C & Manthey, F 2005, '*Fusarium* Infection, DON Content and Microbial Loads in Durum Wheat from the Northern Plains: 2001-2004', p. 181 in SM Canty, T Boring, J Wardwell, L Siler & RW Ward eds, *Proceedings of the 2005 National Fusarium Head Blight Forum,* Hilton Milwaukee City Center Milwaukee, Wisconsin, 11-13 December 2005.

Vinale, F, Sivasithamparam, K, Ghisalberti, EL, Marra, R, Woo, SL & Lorito, M 2008, '*Trichoderma*–plant–pathogen interactions', *Soil Biology and Biochemistry*, vol. 40, no. 1, pp. 1-10.

Wade, MJ, Winther, RG, Agrawal, AF & Goodnight, CJ 2001, 'Alternative definitions of epistasis: dependence and interaction', *Trends in Ecology & Evolution*, vol. 16, no. 9, pp. 498-504.

Wallwork, H, Butt, M, Cheong, JPE & Williams, KJ 2004, 'Resistance to crown rot in wheat identified through an improved method for screening adult plants', *Australasian Plant Pathology*, vol. 33, pp. 1-7.

Wildermuth, GB, Thomas, GA, Radford, BJ, McNamara, RB & Kelly, A 1997, 'Crown rot and common root rot in wheat grown under different tillage and stubble treatments in southern Queensland, Australia', *Soil & Tillage Research*, vol. 44, pp. 211-224.

Williams, KJ, Dennis, JI, Smyl, C & Wallwork, H 2002, 'The application of species-specific assays based on the polymerase chain reaction to analyse *Fusarium* crown rot of durum wheat', *Australasian Plant Pathology*, vol. 31, pp. 119-127.

Winter, P & Kahl, G 1995, 'Molecular marker technologies for plant improvement', *World Journal of Microbiology and Biotechnology*, vol. 11, no. 4, pp. 438-448.

Wong, PTW, Mead, J & Croff, M 2002, 'Effect of temperature, moisture, soil type and *Trichoderma* species on the survival of *Fusarium pseudograminearum* in wheat straw', *Australasian Plant Pathology*, vol. 31, no. 3, pp. 253-257.

Zadok, JC 1999, 'Reflections on space, time and diversity', *Annual Review of Phytopathology*, vol. 37, pp. 1-17.

Zhu, Y, Song, Q, Hyten, D, Van Tassell, C, Matukumalli, L, Grimm, D, Hyatt, S, Fickus, E, Young, N & Cregan, P 2003, 'Single-nucleotide polymorphisms in soybean', *Genetics*, vol. 163, no. 3, pp. 1123-1134.