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Association mapping of stem rust resistance in durum wheat at the seedling and adult stages

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Association mapping of stem rust resistance in durum wheat at the seedling and adult plant stages

A dissertation

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Table of Contents

ACRONYMS

AFLP Amplified Fragment Length Polymorphism

AM Association Mapping

APR Adult Plant Resistance

CI Coefficient of Infection

CIMMYT International Maize and Wheat Improvement Center

cM centiMorgan

DArT Diversity Array Technology

DSS Disease Severity Score

DZARC Debre Zeit Agricultural Research Center

DZm Debre Zeit main season

DZo Debre Zeit off season

FDR False Discovery Rate

GWA Genome Wide Association

GLM General Linear Model

HR Hypersensitivity Reaction

ICARDA International Center for Agricultural Research in the Dry Areas

IT Infection Type

MAS Marker Assisted Selection

LD Linkage Disequilibrium

MLM Multiple Linear Model

NTSYS Numerical Taxonomy and Multivariate Analysis System

Pgt *Puccinia graminis* f. sp. *tritici*

QTL Quantitative Trait Loci

RIL Recombinant Inbred Line

SNP Single Nucleotide Polymorphism

SSR Simple Sequence Repeat

Sr Stem rust resistance

STS Sequence Tagged Site

TASSEL Trait Analysis by aSSociation, Evolution and Linkage

UPGMA Unweighted pair group method with arithmetic average

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SUMMARY

Diseases are the main factors that cause yield loss in wheat and are best controlled through the development of resistant cultivars. *Puccinia graminis* f. sp. *tritici*, the causative agent of stem rust in wheat, is known to rapidly evolve new virulence to resistance genes. While more than 50 stem rust resistance (*Sr*) loci have been identified in wheat, only a few remain effective, particularly against the highly virulent Ug99 (TTKSK) race and a mixture of durum-specific races. An association mapping (AM) approach based on 183 diverse durum wheat accessions was utilized to identify resistance loci for stem rust response in Ethiopia over four evaluation seasons by artificial inoculating with Ug99 and a mixture of durum-specific races under field conditions as well as in the greenhouse test at the seedling growth stage under controlled conditions for resistance to four highly virulent stem rust races: TRTTF, TTTTF, TTKSK (Ug99) and JRCQC. The panel was profiled with simple sequence repeat, diversity array technology and sequence tagged site markers (1253 markers). Five subpopulations, mostly comprised of lines of individual breeding programs, were identified. LD decayed across a range between 5 to 10 cM as determined by the pairwise r^2 (squared value of the correlation coefficient between two loci) method. AM using the MLM method was then conducted to reveal the genetic architecture of stem rust resistance in durum wheat breeding germplasm. The results under field conditions showed the oligogenic basis of resistance, with 12 QTL-tagging markers that were significant ($P < 0.05$) across three to four seasons and with R^2 values from 1.1 to 11.3%. Twenty-four additional single marker/QTL regions were found to be significant over two seasons. The AM results confirmed the role of *Sr13*, previously described in bi-parental mapping studies and the role of chromosome regions putatively harboring *Sr9*, *Sr14, Sr17* and *Sr28.* Additionally, 13 single marker/QTL regions were located in chromosome regions where no *Sr* genes/QTLs have been previously reported.

The results under controlled conditions showed that 15, 20, 19 and 19 chromosome regions harbored markers that showed significant effects for races TRTTF, TTTTF, TTKSK and JRCQC, respectively. These genomic regions showed marker R^2 values ranging from 1.13 to 8.34, 1.92 to 17.64, 1.75 to 23.12 and 1.51 to 15.33% for races TRTTF, TTTTF, TTKSK and JRCQC, respectively. The study demonstrates that stem

rust resistance in durum wheat is governed in part by shared loci and in part by race-specific ones. The QTLs identified in this study through AM will be useful in the marker-assisted development of durum wheat cultivars with durable stem rust resistance.

CHAPTER 1. INTRODUCTION

1.1 DURUM WHEAT: IMPORTANCE AND FUTURE PERSPECTIVES

Durum wheat (*Triticum turgidum* L. var. *durum*, 2n = 4x = 28; AABB genomes) is an important crop, mainly used for human consumption. Recently, this cereal has been the object of renewed interest, because of its valuable production and adaptation to low rainfall and semi-arid environments. More than half of the durum acreage lies in the Mediterranean Basin, mainly Italy, Spain, France, Greece and the West Asian and North African (WANA) countries, where through history this cereal has received special attention as an important commodity (Royo et al. 2000). In Ethiopia, about 60% of the wheat area is currently covered by bread wheat and 40% by durum wheat (Badebo et al. 2009). However, the estimate for 1967 indicated that about 15% of the wheat area was covered by bread wheat and 85% by durum wheat (Hailu, 1991). In Ethiopia, durum wheat is traditionally grown by smallholder farmers on heavy black clay soils (vertisol) of the highlands at altitude ranging from 1800-2800 m above sea level exclusively under rain-fed agriculture (Tesfaye and Getachew, 1991). Owing to its long history of cultivation by farmers in varied agrosystems of the country, Ethiopia has an amazing wealth of genetic variability for different desirable economic traits including disease resistance that can be utilized for improving the quality and productivity of durum wheat. Importantly, Ethiopia is also recognized as one of the Vavilovian centres of genetic diversity for this crop (Vavilov, 1951).

In Ethiopia, durum wheat is mainly utilized for the preparation of local traditional recipes such as dabo (Ethiopian bread), injera (thin, flattened bread), kolo (roasted grain), nifro (boiled whole grain, sometimes mixed with pulses) and kinche (crushed kernels, cooked with milk or water and mixed with spiced butter) and to some extent, for manufacturing of different pasta products (Solomon et al. 2006). However, in view of the current rapidly growing rate of urbanization, coupled with the increased expansion of existing as well as newly emerging food processing industries, durum wheat products such as macaroni and spaghetti are highly demanded in the local markets and have become an important part of the daily diet in the urban and peri-urban areas of Ethiopia. Importantly, almost all local pasta manufacturers depend on imported durum wheat. At present, the demand for imported durum is showing a

rising trend and annually the local pasta factories import thousands of tons of durum wheat from abroad, thus requiring tens of millions of dollars in foreign exchange. The rapidly increasing demand for more durum wheat both in global and domestic markets, combined with the availability of proven technologies and practices in the country offer an excellent opportunity for commercialization of the crop so that the Ethiopian smallholder farmers can significantly participate in the production of high quality durum wheat to improve their income and livelihood (Newai, 2006).

1.2 WHEAT STEM RUST: HISTORICAL AND CONTEMPORARY SIGNIFICANCE

Wheat stem rust caused by *Puccinia graminis*. f. sp. *tritici* is the most destructive of all the wheat rust pathogens due to its ability to decimate a healthy wheat crop late in the season and is capable of causing up to 100% yield loss from lodging and by damaging the stem of the plant as shown in **Figure 1** and disrupting water and photosynthate movement (Roelfs et al. 1992). For as long as agrarian societies have cultivated wheat, stem rust has been a threat to food security. The Bible refers to rust epidemics as punishments on the Israelites from God for their sins (Chester, 1946). Around 700 A.D, the Roman festival of Robigalia was celebrated annually to pacify the rust god Rubigus to ensure a healthy crop (Chester, 1946; Peterson, 2001).

During the last century, stem rust has caused major epidemics in all wheat-producing countries. In the 1940's and 1950's, China experienced major stem rust epidemics due to higher than average temperatures and rainfall leading to ideal conditions for the pathogen that flourishes under high temperature and moisture (Roelfs and Martens, 1987). Severe epidemics took place in North America in the first half of the 20th century (Roelfs and Bushnell, 1985; Hodson, 2011). In North America, the spring wheat-growing regions are most affected by stem rust epidemics causing yield losses up to 50% (Leonard, 2001).

Stem rust is potentially the most destructive of the three wheat rusts, which include stem (black), stripe (yellow) and leaf (brown) rusts. Stripe rust epidemics have been more frequent and widespread in recent years and under severe epidemics, yield losses are generally higher than usually recorded in large-scale production statistics

because the cool and wet conditions favorable for disease development are also favorable for wheat productivity. Stem rust, in contrast, is a biological firestorm with the potential to completely devastate an otherwise healthy crop just three weeks before harvest (Singh et al. 2006; Herrera-Foessel et al. 2011) at warmer temperatures less conducive for wheat productivity. It is arguably the most feared disease of wheat on all continents where wheat is grown.

In Ethiopia, where over 30 fungal diseases of wheat have been identified, stem rust is a major production constraint in most wheat-growing areas of the country and causes up to 100% yield loss in epidemic outbreaks (**Figure 1**; Admassu et al. 2004). The country also considered as one of the hot spot areas for the development of the present wheat stem rust complex (Leppik, 1970). The disease has become a major threat of wheat production after the epidemics of 1974 and 1993 that drove out of production the two bread wheat (*Triticum aestivum* L.) varieties, 'Lacketch' and 'Enkoy' (Badebo, 2002; Beteselassie et al. 2007). The discovery of the Ug99 race with virulence to *Sr31* in Uganda in 1999 (Pretorius et al. 2000) represented a real threat to wheat production in the world, including Ethiopia, where stem rust epidemics had not occurred since the resistant cultivar Enkoy lost its resistance in 1993 (Badebo, 2002). Since the first report in Uganda, race Ug99 has been detected in other parts of East Africa (Singh et al. 2006) and beyond in Yemen (Global Rust Initiative, 2007) and Iran (SeedQuest, 2008). In Ethiopia Ug99 was first detected in 2003 at six dispersed sites (Singh et al. 2006). It was more dominant in the Southeast and Central parts of the country than in Northwest Ethiopia. Therefore, Ug99 is a real threat to wheat growers of Ethiopia, requiring very close attention.

1.3 MOLECULAR MARKERS AND THEIR USE IN BREEDING FOR DISEASE RESISTANCE IN WHEAT

DNA-based molecular markers have several advantages over the traditional phenotypic selection and their potential benefits as marker-assisted selection (MAS) have been widely discussed (Melchinger, 1990; Paterson et al. 1991; Young, 1996; Mohan et al. 1997; Anderson, 2003; Varshney and Tuberosa, 2007), especially to provide solutions to overcome some of the problems faced by classical phenotypic screening approaches in plant breeding programs. Molecular markers can be used to

tag rust resistance genes and further their use can serve for the improvement of the efficiency of selection in plant breeding by MAS. Marker-assisted selection can be used at an early stage of plant development when multiple DNA markers are used to screen a segregating population for one or more genes simultaneously.

Even though single-gene resistance may be overcome by rapidly evolving races, the use of resistant cultivars is still the most effective and economical method of reducing yield losses due to stem rust (McIntosh, 1988). One way to increase the durability of stem rust resistance genes is to pyramid several *Sr* genes to increase broad-spectrum resistance to several races (Pederson and Leath, 1988; Admasu et al. 2011). With conventional methods in wheat breeding programs, the continuous pyramiding of genes in a single genotype will become difficult or even impossible when one or more genes in the background are effective against many races of the pathogen and also when different resistance genes produce similar infection types. In this case, identification of molecular markers linked to disease resistance genes facilitates MAS for pyramiding resistant alleles (Sharp et al. 2001; Babu et al. 2004; Haile et al. 2012; Miedaner and Korzun, 2012).

To date, more than 50 stem rust resistance (*Sr*) genes have been identified against different races of stem rust fungus (McIntosh et al. 2003, 2008) and molecular markers are available for some of them such as *Sr2* (Mago et al. 2011), *Sr13* (Admassu et al. 2011; Simons et al. 2011), *Sr22* (Olson et al. 2010; Periyannan et al. 2011), *Sr25* (Liu et al. 2010), *Sr26* (Liu et al. 2010), *Sr32* (Bariana et al. 2001), *Sr33* (Sambasivam et al. 2008), *Sr35* (Zhang et al. 2010), *Sr39* (Mago et al. 2009; Niu et al. 2011), *Sr40* (Wu et al. 2009), *Sr44* (Liu et al. 2012), *Sr45* (Sambasivam et al. 2008), *Sr47* (Faris et al. 2008), *Sr50* (Anugrahwati et al. 2008), *Sr51* (Liu et al. 2011a), *Sr52* (Qi et al. 2011), *Sr53* (Liu et al. 2011b), *SrCad* (Hiebert et al. 2011), *Sr57*(synonym *Lr34*/*Yr18*) (Pumphrey et al. 2012) and *SrWeb* (Hiebert et al. 2010). Although some of the markers have been used in MAS, some of them are not diagnostic and require improvement.

1.4 OVERVIEW OF ASSOCIATION MAPPING IN PLANTS

Elucidating the genetic basis of beneficial traits is the fundamental aim of many studies involving crop plants. Surprisingly, relatively little is known about the genetic architecture of several key traits (Mackay et al. 2009), particularly those quantitatively inherited. This notwithstanding, the introduction of genomics approaches now allows for an accurate dissection of the genetic basis of quantitative traits (Tuberosa et al. 2002; Semagn et al. 2010; Liu et al. 2012). There are two quantitative genetic approaches that are mainly used to identify associations between a genotype and phenotype. One of those is linkage mapping or linkage analysis. This method is based on the co-segregation of marker alleles with phenotypic observations on families of a segregating population to identify quantitative trait loci (QTLs) that contain causal variants. In a linkage or bi-parental mapping population, the studied progeny are usually just a few generations away from the two common ancestors, which results in high linkage disequilibrium (LD), i.e. the non-random association of alleles between two loci (Rafalski 2002). Thus, even rather distant markers are found to co-segregate with the causal variant. Although linkage mapping has proven successful in identifying QTL for hundreds of traits in many plant species (Mauricio, 2001; Doerge, 2002; Maccaferri et al. 2008, 2011; Mackay et al. 2009; Pasam et al. 2012), the identified QTL region can extend over several centiMorgans (cM) or more and contains hundreds of genes, making the identification of suitable candidates a very difficult task. Therefore, beneficial QTL alleles introgressed via marker-assisted selection are likely to suffer from linkage drag, i.e. the hitchhiking of deleterious loci with selected target loci. In addition, the construction of mapping populations of recombinant inbred lines (RILs) through controlled crosses followed by several generations of selfing is rather time-consuming, which further limits the use of linkage mapping.

An alternative method for identifying loci (genes and QTLs) is association mapping (AM) or LD mapping (Risch and Merikangas, 1996), which seeks to identify specific causal variants linked to phenotypic polymorphisms in populations of unrelated genotypes much more diverse than those derived from biparental crosses. Consequently, AM populations are usually many generations away from a common ancestor. Recombination events occurring throughout the evolutionary history of the

AM population contribute to the breakage of LD blocks within the genome (Nachman, 2002; Rafalski, 2002). Thus, LD decays much faster in AM populations than linkage mapping populations. AM can therefore achieve a higher resolution of causal trait polymorphism than linkage mapping. In addition to achieving higher resolution mapping, this method can also accommodate germplasm with broader genetic variation (i.e. from breeding lines to landraces and even wild progenitors) and allow for the mapping of many traits simultaneously. Thus, there is no need to develop expensive and time-consuming biparental populations for each target trait. However, because of the much reduced LD extent in AM populations compared to linkage mapping populations, a significantly greater number of genetic markers are needed to cover the whole genome and perform a genome-wide association scan (Nordborg and Weigel, 2008; Neuman et al. 2010). With the number of available robust genetic markers such as SSRs and Single Nucleotide Polymorphisms (SNPs) increasing and the cost of genotyping decreasing, AM has become a more attractive approach for revealing the genetic architecture of various traits in crop species (Rafalski, 2002; Oraguzie et al. 2007; Zhu et al. 2008; Ingvarsson and Street, 2010; Maccaferri et al. 2011), including also disease resistance in wheat (Maccaferri et al. 2011; Liu et al. 2012).

Generally, association mapping includes six steps as outlined in Figure 2: (1) a collection of diverse genotypes are selected that may include: landraces, elite cultivars, wild relatives and exotic accessions, (2) a comprehensive and precise phenotyping is performed over the traits such as yield, stress tolerance or quality-related traits of the selected genotypes in multiple replicates and years/environments, (3) the genotypes are then scanned with suitable molecular markers (AFLP, SSRs, SNPs), (4) population structure and kinships are determined to avoid false positives followed by (5) quantification of LD extent using different statistics like D, D' or r^2. Finally, (6) genotypic and phenotyping data are correlated using appropriate statistical software allowing tagging of molecular marker positioned in close proximity of gene(s) underlying a specific trait. Consequently, the tagged gene can be mobilized between different genotypes and/or cloned and annotated for a precise biological function.

1.4.1 Linkage disequilibrium

In AM studies, the hypothesis of interest is to test whether a marker is associated with the phenotype of interest. Despite a declared significant marker may or may not be within the functional gene; it is likely to be associated with the particular phenotype because it is in LD with the functional gene (Rafalski, 2002). LD is defined as the non-random association of alleles at different loci (Weir, 1979). In other words, alleles are co-inherited either more or less often than expected by chance. Three of the most common measures for describing LD are D, D', and r^2 (Weir, 1979; Jorde, 2000). Pairwise LD measured by the value of D according to Lewontin (1964) is as follows. Assume two diallelic loci are linked and let *Pij* be the proportion of chromosomes that have allele *i* at the first locus and allele *j* at the second locus. For example, p_{12} is the frequency of the haplotype with allele 1 at the first locus and allele 2 at the second locus. The disequilibrium coefficient D is the difference between the observed haplotype frequency p_{12} and the haplotype frequency expected under linkage equilibrium, the latter being the product of the two allele frequencies, i.e. p_1 and p_2. It may be written as follows:

$D = p_{12} - (p_1 x\ p_2)$.

A more commonly quoted measure of LD is D' (Lewontin, 1964). This is a normalized form, with a numerator equal to D and the denominator equal to the absolute maximum D that could be achieved given the allele frequencies at the two loci. D' can take values from -1 to +1 but, in general, its absolute value is presented and discussed. A value of 1 indicates the absence of recombination event, whereas values less than 1 indicate that two loci have been separated through recombination. Intermediate values of D' may be difficult to interpret, as the measure tends to be inflated when sample size is small or allele frequencies are low. The squared correlation coefficient, r^2 is sometimes preferred to quantify and compare the amount of LD between pairs of loci. In particular, r^2 is determined by dividing D' by the product of the four allele frequencies. When $r^2 = 1$, two markers provide identical information, not only having D' = 1 but also having equal allele frequencies. The main advantage of r^2 is its inverse relationship with the sample size required to detect genetic association between markers that are in complete LD (Pritchard and Przeworski, 2001). For instance, if cases and controls have only been genotyped for

markers in the vicinity of a functional variant, the sample size should be increased by a factor $1/r^2$ in order to achieve the same power as would have been achieved by generating data at the susceptible locus itself. However, r^2 is more sensitive to allele frequencies that /D'/ and can be difficult to interpret when the two loci in question differ in allele frequencies.

LD can be influenced by many factors. Mutation and recombination are the two main ones impacting LD. Mutation provides the raw material for generating LD between two loci. Recombination, however, is the main mechanism by which these ancestral haplotypes are broken down and LD is reduced. Because the recombination rate can vary across a chromosome, the extent of LD is also expected to vary in inverse relation to the local recombination rate (Nachman, 2002). Other factors that can influence the LD extent include: inbreeding, small population size, genetic isolation between lineages, population subdivision, founder effects, population admixture and selection (Flint-Garcia and Thornsberry, 2003; Oraguzie et al. 2007).

The average extent of LD in a species determines the density of molecular markers needed in AM. If LD extends over a longer distance, as for example in many self-pollinated species such as barley (Malysheva-Otto et al. 2006) and Arabidopsis (Nordborg et al. 2002), then fewer markers are needed to cover the entire genome. On the other hand, if LD extends over a very short distance, as in the out-crossing species of maize (Remington et al. 2001), many more markers are needed to cover the entire genome. In addition, because of the uneven distribution of recombination hotspots in both animal and plant systems (Lichten and Goldman, 1995; Mezard, 2006), LD will not only vary in different species, but also across different chromosomes in the same species and even in different regions on the same chromosome (Nachman, 2002; Rafalski and Morgante, 2004). If the marker density is not sufficiently high, the extent of LD across the entire genome cannot be rigorously assessed, and thus portions of the genome will remain poorly described (Rafalski, 2002). Thus, understanding the extent of LD both globally and locally in the studied population is necessary for the interpretation of AM results.

1.4.2 Population structure

Because of non-random mating, isolation or artificial selection, patterns exist more or less in any plant population. When population structure is present, an association may be found between a phenotype and one or more markers that have no physical linkage to the causal variants (Ewens and Spielman, 1995). Population structure results from selection and high levels of admixture (individual accession membership proportion found in multiple subpopulations) in a population and results in increased LD between unlinked markers (Farnir et al. 2000; Nordborg and Tavare, 2002; Cardon and Palmer, 2003; Rostoks et al. 2006; Zhu et al. 2008). The occurrence of such spurious associations is due to different phenotype frequencies across subpopulations. Population structure is often used in genetic studies to summarize relationships between individuals within and among populations and can provide insights into evolutionary relationships. The probability of a Type I error increases in AM studies if the population structure is not appropriately accounted for (Flint-Garcia and Thornsberry, 2003; Gupta et al. 2005; Maccaferri et al. 2011; Letta et al. 2013).

Several methods have been proposed for estimating population structure and modelling population structure in AM studies, including distance and model-based methods (Pritchard et al. 2000a; Ahmad, 2002; Lu et al. 2005; Maccaferri et al. 2005; Yu et al. 2006; Camus-Kulandaivelu et al. 2007; Peleg et al. 2008). Distance-based estimates of population structure are generally based on clustering of individuals based on pair-wise genetic distance estimates between individuals (Nei, 1972; Rogers, 1972; Nei, 1978; Maccaferri et al. 2005). Although visually appealing, distance-based methods are not suitable for statistical inference (Pritchard et al. 2000a). In contrast, model-based methods assign individuals probabilistically to one or more subpopulations (Pritchard et al. 2000a). The most common model-based approach is Bayesian modelling where allele frequencies are used to estimate the likelihood of an individual belonging to a particular subpopulation. This approach allows assignment of individuals to respective populations that can be integrated into statistical models to account for population structure in AM studies (Pritchard et al. 2000a). With Bayesian modelling, the number of subpopulations is usually estimated *a priori*. Often, known relationships (pedigree, origin of the individual) and/or genetic distance methods are used to estimate a realistic number of subpopulations for calculation of model-based

assignments (Liu et al. 2003, 2005; Agrama et al. 2007; Chao et al. 2007; Hai et al. 2007). Hai et al. (2007) found that when assigning population structure among 69 bread wheat accessions, both methods led to similar assignments of individuals to subpopulations. Maccaferri et al. (2005) reported similar findings in 183 durum accessions. In contrast, distance- and model-based methods were conflicting in a collection of US wheat cultivars and breeding lines, with model-based assignments detecting population structure missed by distance-based analysis (Chao et al. 2007). The software STRUCTURE (Pritchard et al. 2000a) has been developed to account for population structure (Pritchard et al. 2000a) and has been implemented in AM studies in a number of crop species including barley (Rostoks et al. 2006), wheat (Breseghello and Sorrells, 2006; Crossa et al. 2007; Tommasini et al. 2007), durum wheat (Maccaferri et al. 2010) and rice (Agrama et al. 2007). Structure utilizes a Bayesian modelling approach to assign individuals to a subpopulation to minimize the LD among unlinked markers among subpopulations. Yu et al. (2006) proposed a unified mixed-model method to determine relatedness of samples in populations, resulting in a reduction in both Type I and Type II errors, by combining population structure (Q) with relative kinship (K), accounting for multiple levels of relatedness. In an association study by Zhao et al. (2007), when used alone or when combined with estimates of population structure, inclusion of the kinship matrix resulted in a reduced false-positive rate. Pedigree information has been proposed as a means to estimate K, but factors such as missing/incorrect pedigree information; selection and genetic drift can make interpretation of pedigree information difficult (Liu et al. 2003).

Rare alleles (commonly defined as occurring at frequencies lower than 5-10%) (Tenaillon et al. 2001; Barnaud et al. 2006; Caldwell et al. 2006; Ravel et al. 2006a; Chao et al. 2007; Rhoné et al. 2007), inflate estimates of LD, reducing statistical power in AM studies (Wilson et al. 2004; Maccaferri et al. 2005; Crossa et al. 2007; Somers et al. 2007). The presence of rare alleles can also increase LD between unlinked markers and increase the Type I error rate in AM studies. Removal of rare alleles, or subsequently pooling rare alleles into their own class (Pritchard et al. 2000a; Pritchard et al. 2000b; Maccaferri et al. 2005; Somers et al. 2007) is a common practice prior to conducting AM studies. Tightly linked markers may result in

increased LD among unlinked markers and are best avoided when assessing structure (Falush et al. 2003).

Closely related individuals are more easily assigned to related populations, which may result in overestimating the number of subpopulations as a result of background LD (Pritchard et al. 2000a; Falush et al. 2003), which reduces the statistical power of AM studies (Yu et al. 2006). In addition, Camus-Kulandaivelu et al. (2007) noted that assignment of individuals to subpopulations was variable when closely related individuals were present in the AM population. However, removal of highly related individuals to estimate the number of subpopulations has been suggested as an approach to minimize overestimation of the number of subpopulations (Liu et al. 2003; Breseghello and Sorrells, 2006; Camus-Kulandaivelu et al. 2007).

1.4.3 Approaches for association mapping

Recently, several AM studies have been published on a variety of crops including common wheat (Breseghello and Sorrells, 2006; Ravel et al. 2006b; Roy et al. 2006; Crossa et al. 2007; Jing et al. 2007; Tommasini et al. 2007; Peng et al. 2008; Liu et al 2012), barley (Kraakman et al. 2004; Kraakman et al. 2006; Rostoks et al. 2006; Cockram et al. 2008), potato (*Solanum tuberosum* L.) (Malosetti et al. 2007), maize (Remington et al. 2001; Wilson et al. 2004; Weber et al. 2007), rice (Agrama et al. 2007), and durum wheat (Sanguineti et al. 2007; Maccaferri et al. 2010, 2011). Several studies provided support for the potential of AM with a number of the associations identified in their study in QTL regions previously identified through linkage analysis in barley (Kraakman et al. 2006) and wheat (Crossa et al. 2007; Jing et al. 2007; Tommasini et al. 2007; Yu et al. 2012). Two approaches are used for AM studies: whole-genome scans and candidate gene analysis (Thornsberry et al. 2001; Rafalski, 2002; Kraakman et al. 2004; Rostoks et al. 2006; Pasam et al. 2012).

1.4.3.1 Genome-wide association mapping

Genome-wide association (GWA) mapping also known as whole genome scanning approach is a comprehensive approach in which genotyping is done for all possible genetic markers across the genome to systematically search the genome for causal

genetic variation affecting the target trait(s) (Hirschhorn and Daly, 2005). GWA identifies genomic regions throughout the genome associated with the trait of interest. A large number of markers are tested for association with various complex traits and prior information regarding candidate genes is not required. For many traits, such prior knowledge may not exist or may be very vague; thus, for these cases, genome-wide AM is often used in association studies. The first association study to attempt a genome scan in plants was conducted in wild beet (*Beta vulgaris* ssp. *maritima*) for the requirement of vernalization prior to bolting, a feature determined by a single gene (Hansen et al. 2001).

The basis of genome-wide AM is to genotype a sufficient number of markers across the genome so the causal variants (i.e. the underlying gene(s) that control the trait) will likely be in LD with at least one marker. This approach is favoured in situations where LD extends for large distances, allowing for potential candidate regions associated with a trait of interest to be identified for further study (Remington et al. 2001). The extent of LD, therefore, is a critical factor in determining the number of markers needed to cover the genome and the mapping resolution that can be achieved. Association studies with high-density marker coverage; large sample size and minimum population structure offer great promise in complex trait dissection. To date, candidate-gene association studies have searched only a tiny fraction of the genome. The debate of candidate genes versus genome scans traces back to the original milestone paper of Risch and Merikangas (1996). As genomic technologies continue to evolve, more genome-wide association analyses conducted in different plant species are expected.

1.4.3.2 Candidate gene association mapping

A candidate gene is a coding sequence located in a chromosome region suspected of being involved in the expression of a trait whose protein product suggests that it could be the gene in question. Although the candidate gene approach has in some cases led to the identification of a few causal genes (Werner et al. 2005; Harjes et al. 2008; Zheng et al. 2008; Ramsay et al. 2011). It relies on some prior knowledge about the gene location and function. Candidate genes are selected based on prior knowledge of mutational analysis, biochemical pathway, or linkage and genome-wide association

analysis of the trait of interest. However, this low-cost, hypothesis-driven, and trait-specific approach will inevitably miss the role of other unknown non-coding loci that may actually be the primary cause of the observed phenotypic variability (Zhu et al. 2008). A remarkable example for this is provided by the work of Salvi et al. (2007) with *Vgt1*, a major QTL in maize that controls the transition from the vegetative to the reproductive stage, i.e. flowering time. The candidate gene approach directly tests the effects of genetic variants of a gene that may affect a particular trait. However, the candidate gene approach is limited by existing knowledge about the biology of the trait of interest and the genes underlying the QTL interval. This notwithstanding, the candidate gene approach is useful for quickly determining the association of a genetic variant with a trait and also for identifying genes of modest effect. Additionally, the increased availability of well-annotated genomes and the drastic reduction in sequencing costs are expected to facilitate the adoption of the candidate gene approach in future studies aimed to identify the loci governing the variability in traits of breeding interest.

Association analysis has the potential to identify the single polymorphism within a gene that is responsible for the differences in the phenotype. In addition, many plant species have a high level of diversity for which association approaches are well suited to evaluate the numerous alleles available (Flint-Garcia and Thornsberry, 2003). Several studies showed the use of this approach to identify causal molecular polymorphism responsible for several traits. As an example, SNPs in *dwarf8* were evaluated for association with flowering time and plant height in 92 maize inbred lines (Thornsberry et al. 2001), nine polymorphisms, including a miniature transposable element (MITE) insertion in the promoter were associated with flowering time (Thornsberry et al. 2001) and this gene has since been validated as a causal factor influencing flowering time in maize (Andersen et al. 2005). In maize, molecular differences at *Y1* were associated with phenotypic variation in grain carotenoid concentration (Palaisa et al. 2003) and this gene has since been identified as the causal factor for elevated carotenoids in maize. However, the association of SNPs with a trait still requires validation, as the SNP could be in disequilibrium with the causal factor, particularly if LD is high in the genomic region surrounding the gene. Thus, candidate gene approaches are generally utilized to eliminate putative candidates from more detailed functional studies.

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FIGURES

Figure 1. High disease epidemic caused by stem rust on wheat (Source http:// www.ars.usda.gov/ Main/docs.htm? docid-9910)

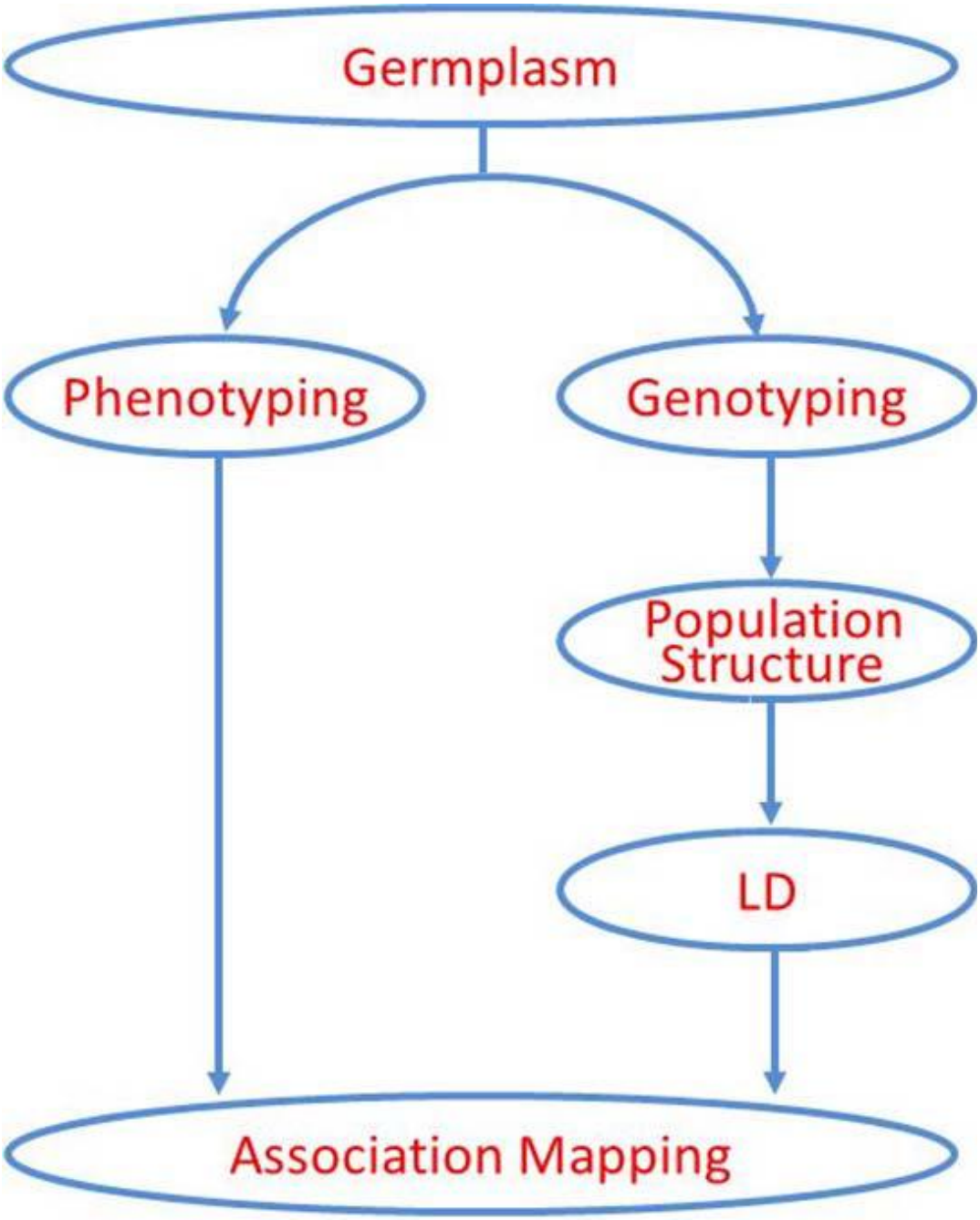

Figure 2. A simplified flow chart showing different stages of association mapping for tagging a gene of interest using germplasm accessions (Adapted from Al-Maskri et al. 2012).

CHAPTER 2. IMPROVING STEM RUST RESISTANCE IN WHEAT

2.1 INTRODUCTION

Resistance to stem rust was a top priority in the "Green Revolution" wheat varieties bred by the late Norman Borlaug and co-workers beginning in the 1950s and the combinations of resistance genes they created remained effective until the appearance of a new strain of stem rust identified from Africa that overcomes the major resistance genes used to combat stem rust (http:// 2blades.org/wheat-stem-rust.php). Improving stem rust resistance in wheat requires knowledge of the pathogen, including its life cycle, physiologic races and variation.

2.2 LIFE CYCLE OF WHEAT STEM RUST

The wheat stem rust fungus *Puccinia graminis* Pers. f. sp. *tritici* Eriks. E. Henn. (*Pgt*) has a complex life cycle consisting of both sexual and asexual reproductive cycles, different sexual and asexual hosts, multiple spore stages and nuclear conditions (**Figure 1**). *Pgt* is a heteroecious fungus requiring two hosts to complete the entire life cycle. Wheat stem rust is a biotrophic fungus and does not exist in nature apart from the primary host, wheat, or the secondary host, common barberry (*Berberis vulgaris* L.) (Leonard and Szabo, 2005).

Teliospores from wheat stem infected with *Pgt* are produced late in the growing season. These abiotic stress-tolerant spores are capable of overwintering on infected straw. Upon formation of the two cells of teliospores, two haploid nuclei (n+n) are present in each cell. These nuclei undergo karyogamy and form a single nucleus (2n). Meiosis takes place after karyogamy but is arrested until germination the following spring season (Boehm et al. 1992). Germination of teliospores is synchronized with new growth of the alternate host, barberry (*Berberis vulgaris*). Each teliospore produces a basidium from which four basidiospores are produced. Each basidiospore is haploid (n) and contains one of the four products of meiosis from the fusion of the haploid nuclei. The meiotic products are of different mating types, two basidiospores will be + and two will be -. A mitotic division in the basidiospores produces two haploid nuclei in each basidiospore. The basidiospores are products of recombination

and it is through meiosis in teliospores that novel variation in *Pgt* can be produced. The basidiospores ejected from the basidia infect the adaxial surface of barberry leaves (Roelfs, 1985).

The hyphae of basidiospores grow within the leaf mesophyll as haploid hyphae and produce a pycnium on the adaxial leaf surface. Haploid pycniospores (n) of + and – mating types are exuded from the top of the pycnium. Serving as male gametes, pycniospores are brought into contact with haploid female (n), flexuous hyphae of the opposite mating type that extrude from the top of the pycnium (Anikster et al. 1999). A dikaryon (n+n) consisting of two haploid nuclei is formed and the resulting hype grows throughout the leaf mesophyll to produce an aecium on the abaxial leaf surface. From the aecium, single celled, dikaryotic (n+n) aeciospores are produced which can then infect the wheat host.

The primary infection of wheat is by aeciospores that infect and produce hyphae within the host. These hyphae then produce uredinia that yield dikaryotic urediniospores (n+n) that represent the asexual stage of the life cycle. Urediniospores re-infect the host during the growing season and cause the principle damage to wheat plants resulting in yield losses. Upon maturity of the host, teliospores (n+n) are produced which will overwinter and begin the cycle the following growing season.

2.3 PHYSIOLOGIC RACES AND VARIATION OF STEM RUST

Puccinia graminis f. sp. *tritici* is divided into physiologic races based on virulence and avirulence specificities of isolates as related to a differential set of stem rust resistance genes (Roelfs and Martens, 1987). The differentials consist of five sets of genes, each comprised of four genes per set. To discriminate races, a letter code for each set is assigned. The specific pattern of high and the low infection type of genes within each set determines the letter code with a higher letter indicating virulence to more genes within the set.

Host genotype and spatial scale can dramatically affect the population structure of *Pgt.* The cultivation of a wheat variety carrying a single major gene for resistance to stem rust places an intense directional selection of existing avirulent *Pgt* genotypes

(Van der Plank, 1968). In the case of major genes encoding for NB-LRR type receptors that function in the detection of specific *Pgt* effector activity, the selective pressure on *Pgt* populations is for the loss of detection of effector activity which can simply be achieved through allelic changes by mutation or recombination during the sexual stage (McDonald and Linde, 2002). As only *Pgt* genotypes carrying the loss of avirulence are virulent on the host carrying the major resistance gene, these genotypes increases in frequency. The result of this virulence shift is a widespread disease on the newly-susceptible and widely cultivated variety. As the frequency of virulence increases in *Pgt* populations and epidemics worsen, the susceptible variety is planted on fewer acres, usually replaced by a new variety with a different source of major gene resistance, thereby perpetuating the boom-and-bust cycle (Sun and Yang, 1999).

Virulence shifts in *Pgt* populations take place during both sexual and asexual cycles. Sexual recombination allows the opportunity to bring virulence to multiple resistance genes together in a single genotype. The absence of the sexual cycle removes the possibility of sexual recombination and most common genotypes of *Pgt* have lost the ability to produce teliospores (Zambino et al. 2000). The barberry eradication program undertaken in the United States in the 20th century served to limit the diversity of *Pgt* populations by removing the impact of sexual reproduction and limiting evolutionary potential. The greatest diversity in aeciospore and urediniospore collections from Minnesota is from prior the large-scale barberry eradication efforts (Peterson et al. 2005).

2.4 MECHANISMS OF STEM RUST RESISTANCE

Stem rust resistance genes generally operate under two mechanisms. Of the designated *Sr* genes, 53 of them are single-locus major genes (McIntosh et al. 1995) conferring resistance at all stages of plant development, sometimes with varying effectiveness at the adult plant stage (Singh et al. 2011a). Resistance can also be quantitative, conferred by multiple minor genes that individually contribute small effects but together contribute significantly to the resistance phenotype (Poland et al. 2008). In particular, *Sr2* and *Sr55* confer quantitative adult plant resistance to stem rust and are pleiotropic (McIntosh et al. 1995; Krattinger et al. 2009), conferring resistance to other diseases such as leaf rust, stripe rust and powdery mildew.

The breeding methodology for developing wheat rust-resistant varieties varies greatly but usually includes mass screening of (i) seedlings for their reaction to specific rust pathotypes early in the breeding cycle and (ii) adult plants at a later date under field conditions.

2.4.1 Breeding for Hypersensitivity Response (HR)

Most of the resistance genes discovered and deployed in defence against the wheat rusts are classified as major resistance genes, also known as seedling resistance, vertical resistance, all-stage resistance or race-specific resistance genes. Major genes confer effective levels of resistance against specific physiologic races of the pathogen, generally throughout the life cycle of the host. In terms of disease phenotype, major resistance genes are often associated with a rapid programmed death of infected cells, a so-called ‘‘hypersensitive response’’ (HR) thought to play a role in limiting the expansion of biotrophic pathogens in the host tissue.

The race-specific resistance genes are also known as ‘‘R’’ genes and follow the gene-for-gene model (Flor, 1956). Host resistance requires the simultaneous presence of the resistance allele in the host and the corresponding avirulence allele in the pathogen. A race-specific “major” gene can be easily deployed due to their large phenotypic effects and hence are useful especially for short-term control options. Routine deployment of HR genes in combinations or pyramids has been less practical in the past due to the small number of broadly effective genes available at any given time and the lack of diagnostic markers needed to pyramid genes. A first step in achieving wheat lines with multiple sources of resistance is to have more genes at hand to work with. Stem rust resistance genes that are effective against Ug99 based on seedling and/or field testing (Singh et al. 2011b) and have been tagged by molecular markers include: *Sr2* (Mago et al. 2011), *Sr13* (Admassu et al. 2011; Simons et al. 2011), *Sr22* (Olson et al. 2010; Periyannan et al. 2011), *Sr25* (Liu et al. 2010), *Sr26* (Liu et al. 2010), *Sr32* (Bariana et al. 2001), *Sr33* (Sambasivam et al. 2008), *Sr35* (Zhang et al. 2010), *Sr39* (Mago et al. 2009; Niu et al. 2011), *Sr40* (Wu et al. 2009), *Sr44* (Liu et al. 2012), *Sr45* (Sambasivam et al. 2008), *Sr47* (Faris et al. 2008), *Sr50* (Anugrahwati et al. 2008), *Sr51* (Liu et al 2011a), *Sr52* (Qi et al. 2011), *Sr53* (Liu et al. 2011b),

SrCad (Hiebert et al. 2011), *Sr57* (synonym *Lr34*/*Yr18*) (Pumphrey et al. 2012) and *SrWeb* (Hiebert et al. 2010).

Seedling reactions are assessed under controlled conditions on a 0 to 4 scale (Stakman and Levine, 1922, as reported by McIntosh et al. 1995; **Figure 2**). Plants are considered to be immune to the race used if the seedling leaf showed no reaction to the rust (designated '0') or there is only a slight clearing of the chlorophyll to produce a fleck (designated as ';'). The seedling is classified as resistant when the reaction is a small lesion surrounded by a necrotic area or by a halo of chlorosis. These are classified as '1' and '2' type reactions, respectively. The plant is predicted to be susceptible if it produces a '3' (large healthy lesion surrounded by an area of chlorosis) or '4' where the lesion is large and surrounded by little or no chlorosis. A mixed or mesothetic reaction may also be produced and designated as 'X'. This methodology has been chosen as the heritability of rust resistance is quite high and it is much more cost effective for screening the very large populations associated with the early stages of the breeding cycle. Also, seedling screening for rust resistance can readily and effectively be undertaken in the off season.

The traditional emphasis on major gene resistance is understandable for many reasons:

1. Major genes are effective: such genes have, without question, provided significant economic benefits to wheat growers, as illustrated by the decades-long protection provided by the now defeated stem rust resistance gene *Sr31*;
2. They are relatively easy to identify and deploy: breeding for single-gene resistance is much simpler than breeding for oligo/polygenic resistance;
3. They are historically easier to combine: in the absence of clear additivity of the resistance conferred by different genes and in the absence of good molecular markers, race-specific disease phenotypes facilitate the efficient introgression and stacking of major genes using panels of different rust races;
4. They provide clear levels of protection: ''resistance'', rather than ''tolerance'', has long been held as an agronomic goal.

Despite these clear historic advantages, however, major resistance genes frequently lacked ''durability'', that is the ability of a widely deployed resistance gene to provide

an economic level of protection over an extended period of time (Johnson, 1984). Hence, recent epidemics have reinvigorated interest within the wheat breeding and research communities in partial resistance genes as sources of potentially more durable resistance.

2.4.2 Breeding for Adult Plant Resistance (APR)

Sources of resistance based on multiple genes, often termed quantitative resistance, which delay infection and also growth and reproduction of the pathogen in adult plants but not in seedlings, have been described as ‘‘adult plant resistance’’ (APR) (Gustafson and Shaner, 1982). The APR is usually more durable than resistance based on single *R* genes and can be identified in cultivars with defeated or no race-specific resistance genes. In most countries, especially developing countries that lack a competitive seed sector, variety turnover is slow, which makes APR and durable resistance particularly valuable for breeding purposes. These are compelling reasons for adopting an APR strategy in which wheat lines are, by necessity, bred for genetic complexity of resistance. The danger posed by inadequate monitoring of rapidly mutating and migrating stem rust races further supports the APR approach. Lastly, the use of resistance based on minor genes provides opportunities to other breeding programs to utilize race-specific resistance and further enhance the diversity for resistance in farmers’ fields.

Sr2 is one of the best characterized APR genes that confer resistance to stem rust. It is arguably the most important stem rust resistance gene and is closely associated with pseudo black chaff, which offers a morphological marker for breeders working with *Sr2*. *Sr2* was introduced from *Triticum turgidum* into hexaploid wheat in the 1920s and has remained durable since now (McIntosh et al. 1995). Less is known about other genes that contribute to adult plant resistance, but widely used APR genes *Lr34*/*Yr18* (recently designated *Sr57*) and *Lr46*/*Yr29* also contribute to stem rust APR (Bhavani et al. 2011) in bread wheat in combination with QTLs at other genomic locations identified through biparental and association mapping (Bhavani et al. 2011; Yu et al. 2011). However, early work by Knott and revisited by Singh indicates how the accumulation of four or five minor effect genes can provide an almost perfect immunity (Singh et al. 2000; **Figure 3**).

APR is assessed under field conditions based upon severity (percentage of rust infection on the plants; **Figure 4**) and field response (type of disease reaction; **Figure 5**) as described by Loegering (1966). Severity is recorded as a percentage, according to modified Cobb scale (Peterson et al. 1948). This recording process relies upon visual observations and it is common to use the following intervals: Trace, 5, 10, 20, 40, 60, 100% infection. Field response is recorded using the following letters:

- R = resistant; visible chlorosis or necrosis, no uredia are present
- MR = moderately resistant; small uredia are present and surrounded by either chlorotic or necrotic areas
- MS = moderately susceptible; medium size uredia are present and possibly surrounded by chlorotic areas
- S = susceptible; large uredia are present, generally with little or no chlorosis and no necrosis. Severity and field response readings are usually combined.

2.5 STEM RUST UG99 AND ITS IMPACT ON WHEAT PRODUCTION

Ug99 (Ug stands for Uganda and 99 for the year in which the race was named), more formally known in the scientific literature as TTKSK (Jin et al. 2007, 2008) was remarkable in that, at the time of its discovery, it was the only known race of *P. graminis* to overcome the race-specific stem rust resistant gene *Sr31* after more than 30 years of widespread deployment, leading to a wrong sense of "durability" associated with this resistance gene. Ug99 was uniquely virulent on *Sr31* as well as most of the resistance genes of wheat origin and other important genes found in CIMMYT, European, North American and Australian wheat germplasm (Reynolds and Borlaug, 2006; Jin et al. 2007) (**Table 1**). Today, the area under immediate threat along the projected migration pathway in North Africa, the Middle East and Asia (excluding China) amounts to 50 million hectares of wheat, i.e. approximately 25% of the world's wheat area responsible for 19% of global wheat production (Reynolds and Borlaug, 2006). An epidemic in this region would have a serious impact on the 1 billion people living within this zone. Subsequent estimates warned that over 90% of the world's wheat varieties are at risk imposed by Ug99 (Singh et al. 2006).

2.5.1 Distribution of Ug99 lineages

There is some evidence that Ug99 may have been present in Kenya as early as 1993, prior to its identification in Uganda (Davidson et al. 2012). By 2001, its presence was confirmed in Kenya, where it is now epidemic. In 2003, it was widely reported in Ethiopia and was poised to move across the Red Sea to the Arabian Peninsula and beyond to the world's breadbasket in South Asia. That jump happened in 2006 when virulence to *Sr31* was identified in Yemen. By 2007 it was carried into Iran, apparently by a tropical storm. To date, there are no reports that the pathogen has moved further into South Asia. However, the Ug99 family has also moved south from Kenya and is now present as far as South Africa. Other countries where Ug99 is present are Eritrea, Sudan, Tanzania, Mozambique and Zimbabwe, totalling 11 countries. The pathogen is not only moving, but it is evolving, overcoming additional stem rust resistance genes across the eastern areas of Africa. In total, the Ug99 family now has eight members (Hodson and Nazari, 2012). Surveillance data indicate that Ug99 variants with combined virulence to *Sr31* and *Sr24* are spreading rapidly. An update of the occurrence of the Ug99 family was compiled by Hodson and Nazari (2012) and is reproduced in **Figure 6**.

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FIGURES

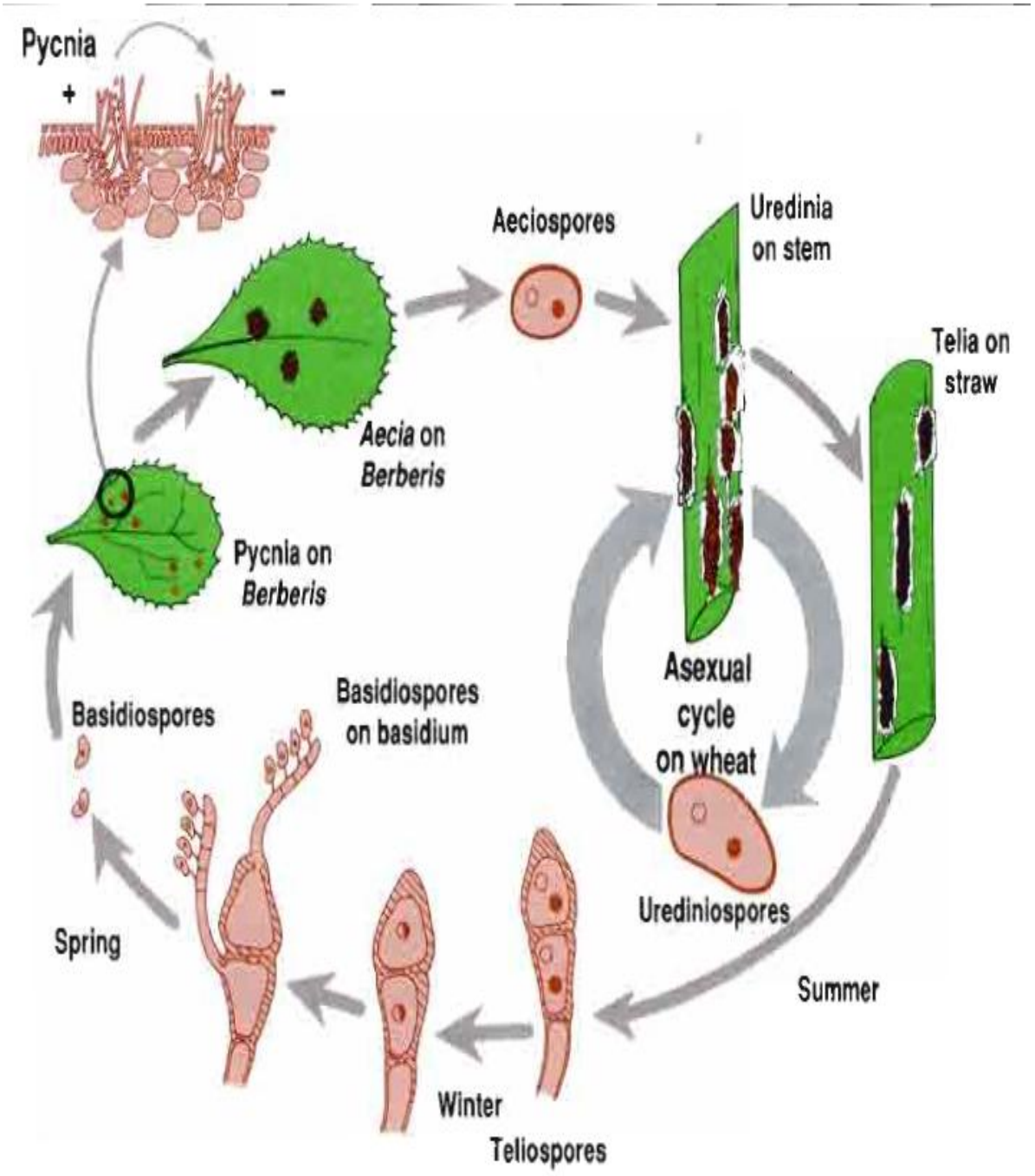
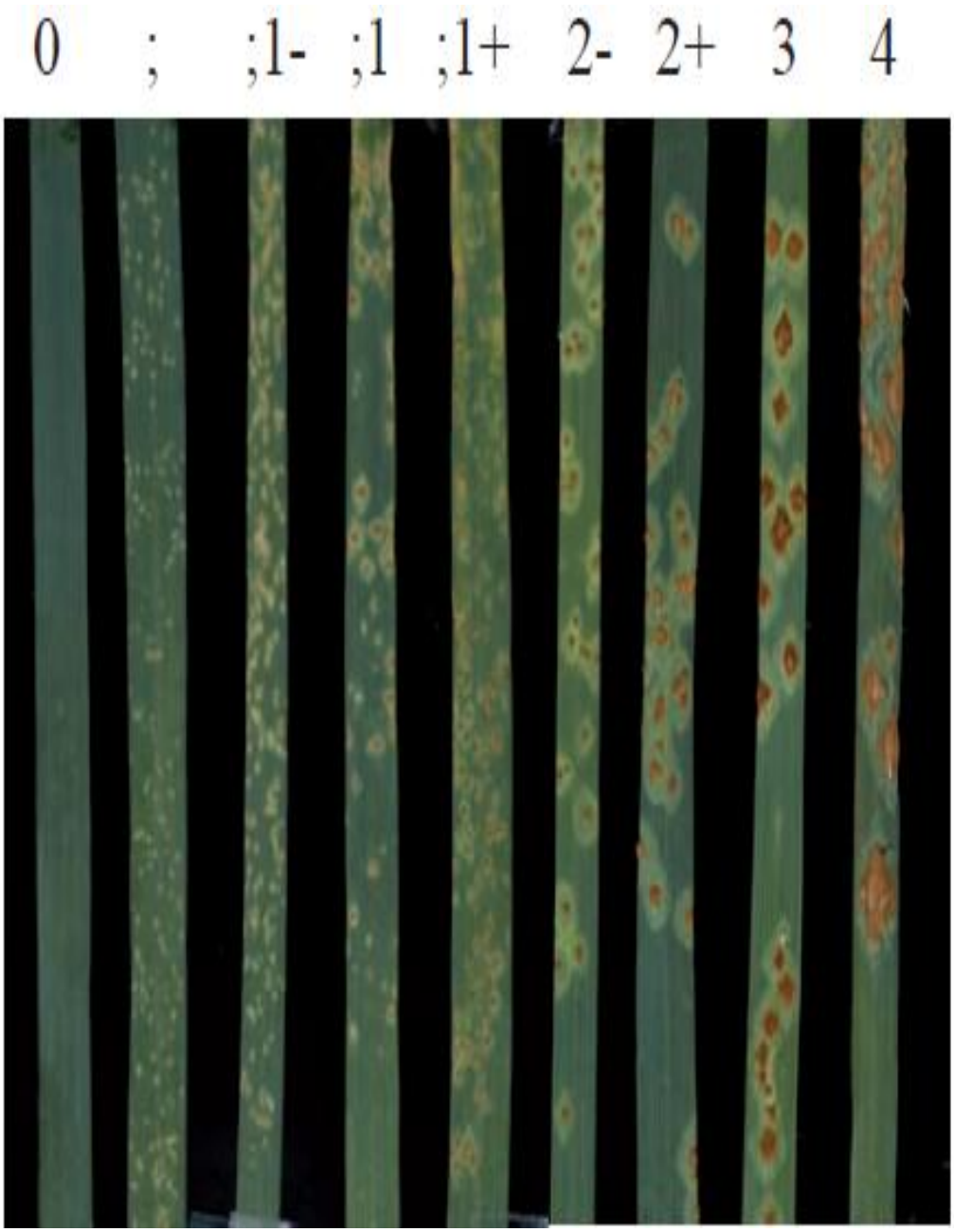


Figure 1. Life and disease cycles for *Puccinia graminis f.sp. tritici* (wheat stem rust) (Roelfs et al.1992).



----------Resistant----------{Susceptible =}

Figure 2. Seedling scoring of infection types on wheat differential using 0-4 evaluation scale. Photo credit: R. F. Park.

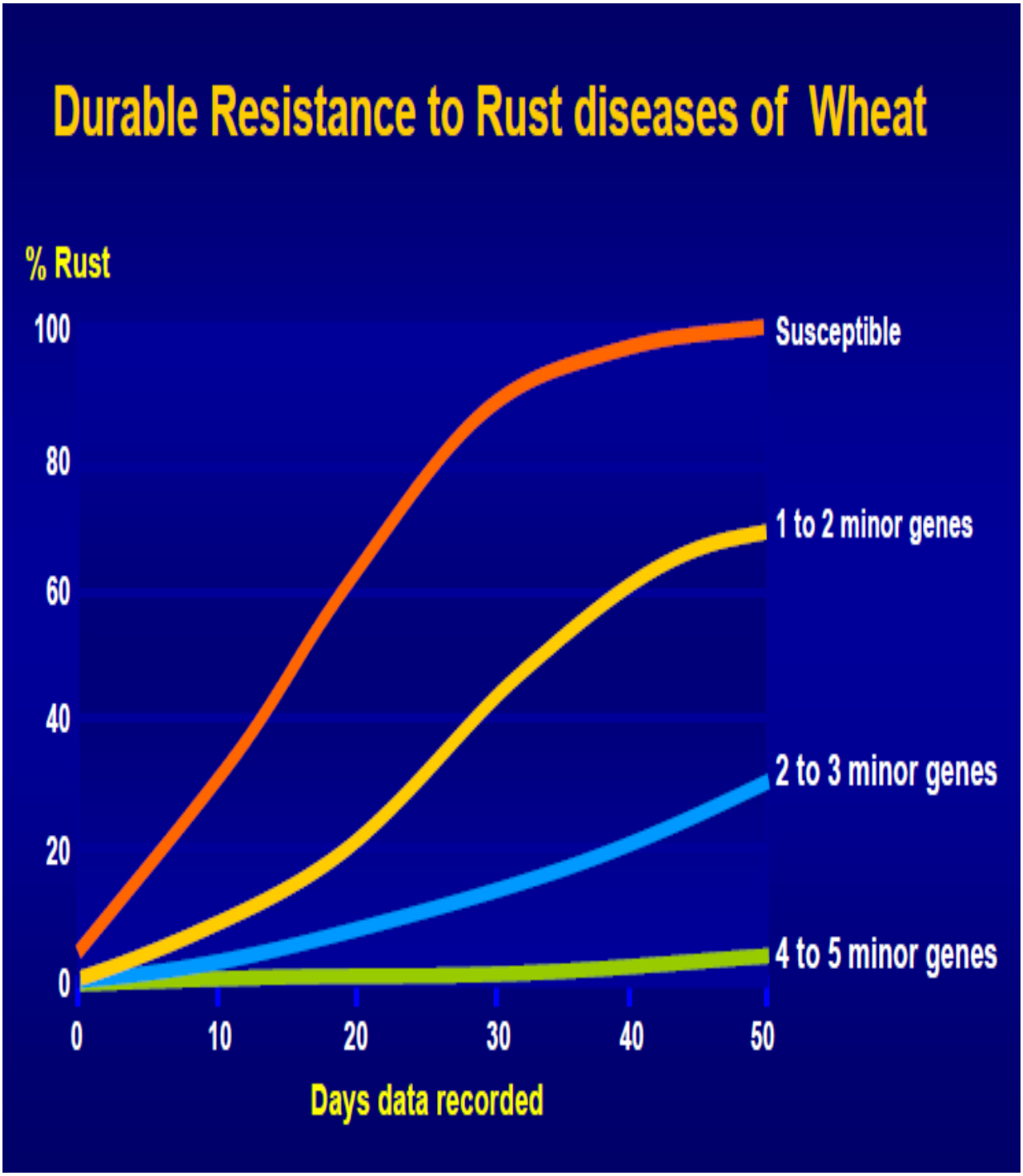


Figure 3. Effect of accumulating a different number of minor genes in wheat cultivar in reducing the level of disease infection (Adapted from Singh et al. 2010).

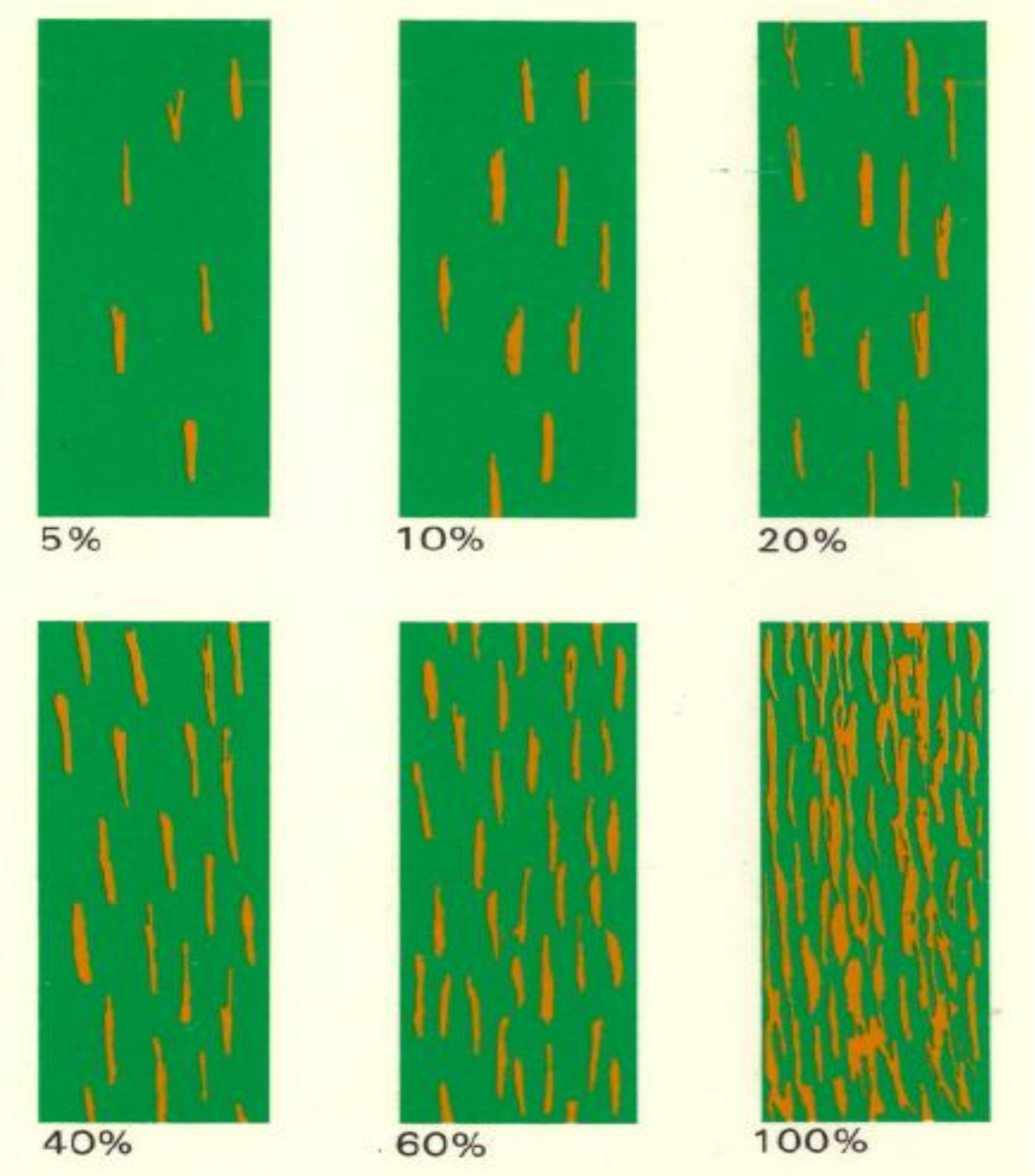


Figure 4. Stem rust severity scores (From Rust scoring guide booklet).

Figure 5. Adult plant field responses to stem rust disease (From Rust scoring guide booklet).

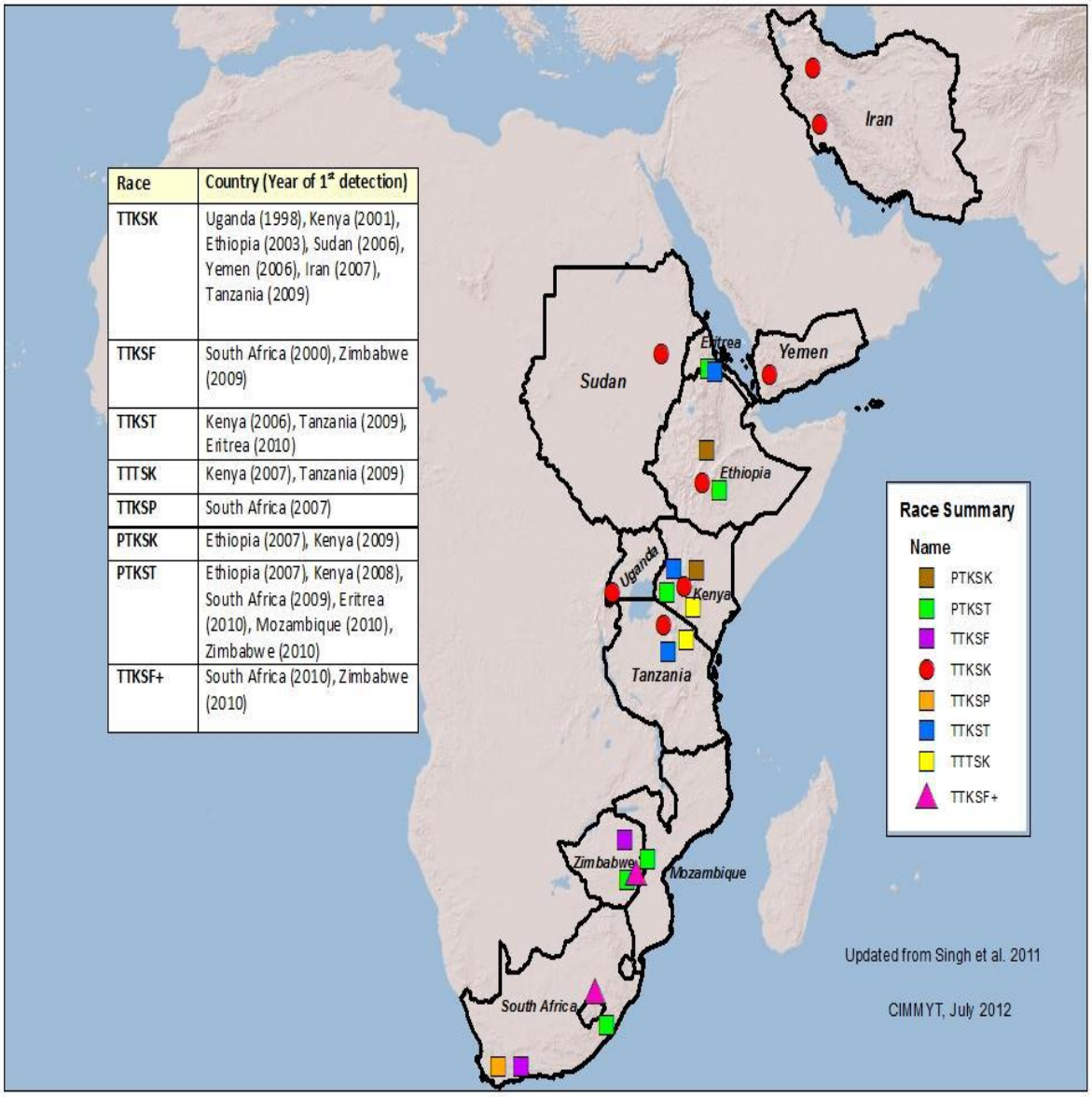


Figure 6. Distribution of Ug99 and its lineages (Hodson and Nazari, 2012).

TABLE

Table 1. Origin and *Sr* genes in conferring seedling and/or adult plant resistance to *Puccinia graminis* f. sp. *tritici* races belonging to the Ug99 lineage.

Origin of *Sr* genes	Stem rust resistance (*Sr*) genes	
	Ineffective	**Effective**
Triticum aestivum	*5, 6, 7a, 7b, 8a, 8b, 9a, 9b, 9f, 10, 15, 16, 18, 19, 20, 23, 30, 41, 42, 49, McN, Wid-1*	*28,*[a] *29,*[b,c] *48, Tmp,*[a] *AC-Cadillac, Sha7,*[b] *Huw234,*[b] *ND643*[b]
Triticum turgidum	*9d, 9e, 9g, 11, 12, 17*	*2,*[b] *13,*[a,b] *14*[a]
Triticum monococcum	*21*	*22, 35*
Triticum timopheevi	*36*	*37*[c]
Aegilops speltoides	-	*32*[c], *39*[c], *47*[d]
Aegilops tauschii	-	*33*[b], *45,*[ab] *46*[a,d]
Triticum comosum	*34*	-
Triticum ventricosum	*38*	-
Triticum urarticum	-	*40*[c]
Thinopyrum elongatum	*24*	*25,*[a] *26, 43*[c]
Thinopyrum intermedium	-	*44*[c]
Secale cereale	*31*	*27,*[a] *50, 1A.1R*[a,b]

[a] Virulence for the gene is known to occur in other races.

[b] Level of resistance conferred in the field usually inadequate under high disease pressure.

[c] Unsuitable for utilization due to linkage with undesirable traits in the translocation (adapted from Singh et al. 2011a).

CHAPTER 3. RESEARCH AIMS

Stem rust continues to cause huge losses worldwide in wheat production due to reliance on cultivars with narrow genetic basis for resistance and the high level of virulence variation in stem rust pathogens. Therefore, successful breeding relies on the identification of new resistance sources *via* gene mapping and incorporation of these resistance sources into breeding lines to release new resistant varieties. Furthermore, it is useful to identify stem rust resistance genes against highly virulent races of stem rust in durum wheat germplasm in order to increase the possibilities of broadening the genetic base of resistance by crossing elite varieties of durum wheat.

Hence, the objectives of this study were:

1. To survey virulence of stem rust races TRTTF, TTTTF, TTKSK (Ug99), and JRCQC in a set of durum wheat accessions suitable for an association mapping approach in order to identify genomic regions associated to seedling-based resistance to these virulent races of stem rust.
2. To evaluate the same set of durum wheat accessions under field conditions at an adult plant growth stage in order to identify genomic regions associated to field-based resistance to the combination of Ug99 with Ethiopian races of stem rust.

CHAPTER 4. SEARCHING FOR NOVEL SOURCES OF FIELD RESISTANCE TO UG99 AND ETHIOPIAN STEM RUST RACES IN DURUM WHEAT VIA ASSOCIATION MAPPING

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4.1 ABSTRACT

Puccinia graminis f. sp. *tritici*, the causative agent of stem rust in wheat, is a devastating disease of durum wheat. While more than 50 stem rust resistance (*Sr*) loci have been identified in wheat, only a few of them remained effective against Ug99 and other durum-specific Ethiopian races. An association mapping (AM) approach based on 183 diverse durum wheat accessions was utilized to identify resistance loci for stem rust response in Ethiopia over four field-evaluation seasons and artificial inoculation with Ug99 (TTKSK race) and a mixture of durum-specific races. The panel was profiled with simple sequence repeat, diversity array technology and sequence tagged site markers (1253 markers). The resistance turned out to be oligogenic, with twelve QTL-tagging markers that were significant ($P < 0.05$) across three to four seasons; R^2 values ranged from 1.1 to 11.3%. Twenty-four additional single marker/QTL regions were found to be significant over two seasons. The AM results confirmed the role of *Sr13*, previously described in bi-parental mapping studies, and the role of chromosome regions putatively harboring *Sr9*, *Sr14*, *Sr17* and *Sr28*. Three minor QTLs were coincident with those reported in hexaploid wheat and five overlapped with those recently reported in the Sebatel × Kristal durum mapping population. Thirteen single marker/QTL regions were located in chromosome regions where no *Sr* genes/QTLs have been previously reported. The allelic variation identified in this study is readily available and can be exploited for marker-assisted selection, thus providing additional opportunities for a more durable stem rust resistance under field conditions.

4.2 INTRODUCTION

Durum wheat (*Triticum durum* Desf.) is an important crop in the Mediterranean Basin, a region accounting for approximately 75% of global worldwide production (Belaid, 2000; Habash et al. 2009). In Sub-Saharan Africa, Ethiopia is the largest wheat-growing country and is considered one of the centers of diversity for tetraploid wheat (Vavilov, 1929, 1951). Durum wheat is grown on approximately 40% of the total wheat area in Ethiopia, with a tendency to increase due to the growing internal demand for pasta products (Badebo et al. 2009). Among the factors that negatively affect durum production and kernel quality, rust diseases play an important role (Singh et al. 2005). Historically, stem rust infections due to *Puccinia graminis* Pers. f. sp. *tritici* have caused severe losses to wheat production (Zwer et al. 1992; McIntosh and Brown, 1997; Eversmeyer and Kramer, 2000; Singh et al. 2011). Until the appearance of Ug99, stem rust control through the use of genetic resistance was considered a remarkable success story worldwide. Although more than 50 stem rust resistance (*Sr*) loci have been identified in wheat (Singh et al. 2006), including those introgressed from its wild relatives, only a few remain effective against Ug99 or its variants and even fewer are useful against the durum-specific Ethiopian races (Admassu et al. 2009). Susceptibility in some CIMMYT-derived germplasm was first noted in Uganda (Pretorius et al. 2000) and soon after was observed in all germplasm groups. This new race, designated as Ug99 or TTKS (Wanyera et al. 2006), spread to Kenya in 2001 and to Ethiopia in 2003 (Singh et al. 2006). By 2006, TTKS was identified in Sudan and Yemen (http://www.fao.org), and in 2008 its presence was confirmed in Iran (Nazari et al. 2009). Ug99 is projected to spread further into the major wheat growing regions of Asia (Singh et al. 2008). In Ethiopia, Ug99 and its variants were added to previously existing races, the latter specifically virulent on durum wheat. Two such races have been characterized as TRTTF and JRCQC with a combined virulence to *Sr9e* and *Sr13*, two genes present at high frequency in the durum wheat germplasm (Olivera et al. 2012). These races are predominant in durum-growing areas of Ethiopia and effective resistance to them (5.2% of the lines tested) was found in a collection of 996 tetraploid genotypes evaluated for field resistance at the Debre-Zeit Research Station in Ethiopia in 2009 (Olivera et al. 2010). Therefore, the combination of Ug99 + *Sr13*-virulent Ethiopian races currently poses a major threat to durum wheat production in Ethiopia and represents a tangible potential

danger elsewhere, should these virulent races reach distant durum-growing areas such as central India where conditions are known to be conducive to the epidemic development of this pathogen. Three different races from the TTKS or Ug99 lineage were identified in Kenya, which led to the re-designation of the original race as TTKSK, and the other two races as TTKST (with additional virulence on *Sr24*) (Jin et al. 2008) and TTTSK (with additional virulence on *Sr36*) (Jin et al. 2009). The effectiveness and durability of the genetic resistance approach to control the disease require the availability of many sources of resistance, preferably involving genes that act on adult plant field resistance, to counter the continuing evolution of new virulence in pathogen populations.

Selecting for the resistant phenotypes conferred by major, race-specific loci are relatively straightforward and initially rewarding though eventually becomes ineffective due to the fast evolution and selection of virulent strains of the pathogen, as seen with Ug99. Although a number of resistance genes have been introgressed into cultivated wheat from wild relatives (Ceoloni et al. 2005; Feuillet et al. 2008), the successful utilization of such materials has often been hampered by the inherent difficulties of operating with alien genomes.

Marker-based approaches allow us to identify genes/quantitative trait loci (QTL) governing plant response to diseases. The effective deployment of stem rust resistance alleles from different sources requires a thorough genetic characterization of the available germplasm. The standard approach is to use bi-parental mapping populations to relate phenotypic information to genotypic data obtained from molecular markers in order to determine the number and the chromosomal location of resistance loci (Gupta et al. 1999; Maccaferri et al. 2008; Simons et al. 2011). An alternative to the use of bi-parental mapping is association mapping (AM) or linkage disequilibrium (LD -based mapping in which genotype-phenotype relationships are explored in germplasm collections or natural populations (Rafalski, 2002, 2011; Flint-Garcia et al. 2003). The underlying principle of this approach is that LD tends to be maintained over many generations between loci that are genetically linked. With AM, statistical assessments are made for associations between genotypes based on molecular markers and phenotypes for various traits in reference germplasm sets (Buntjer et al. 2005). Since its first use in plants a decade ago (Thornsberry et al.

2001), AM has been used in many important crops thanks to advances in high-throughput genotyping technologies, increased interest in identifying useful and/or novel alleles, and improvements in statistical methods (Gupta et al. 2005; Yu et al. 2006; Zhu et al. 2008). In both tetraploid and hexaploid wheat, AM has already proven to be an effective strategy to identify marker-trait associations for agronomically valuable traits (Breseghello and Sorrells, 2006; Crossa et al. 2007; Maccaferri et al. 2010, 2011), including resistance to stem rust (Yu et al. 2011), *Stagonospora nodorum* Blotch (Tommasini et al. 2007), *Fusarium* head blight (Miedaner et al. 2011) in bread wheat and leaf rust (Maccaferri et al. 2010) and SBMCV (Maccaferri et al. 2011) in durum wheat.

The objective of this study was to evaluate a panel of durum wheat accessions well-suited for AM studies (Maccaferri et al. 2006, 2010, 2011) in order to identify genomic regions associated to field-based resistance to the combination of Ug99 with Ethiopian races of stem rust.

4.3 MATERIALS AND METHODS

4.3.1 Plant materials

A collection of 183 elite durum genotypes including cultivars released or breeding lines developed in Italy, Morocco, Spain, Syria, Tunisia, Southwestern USA and Mexico was assembled to represent different spring durum germplasm groups (**Appendix 1**). The genotypes included in the AM panel were chosen from a larger pool of 330 accessions obtained from various sources and evaluated in a field trial in 2003 in Cadriano, near Bologna, Italy (Maccaferri et al. 2006). The accessions of this panel were chosen based on their pedigrees and morpho-physiological traits critical to adaptation, such as plant height and heading date. Highly related accessions (e.g. sibs from the same cross, backcross lines, etc.) and/or with excessively large differences in heading date, a feature that could have biased the phenotypic evaluation of traits influenced by flowering time, were excluded. Most of the accessions were semi-dwarf, short- to medium-cycle elite cultivars and breeding lines released from the early ‘70s up to the late ‘90s. The collection comprises also ‘founder genotypes’ widely used as parents in breeding programs throughout the Mediterranean Basin and

at International CGIAR Centers (CIMMYT and ICARDA). The accessions were assembled for conducting AM studies and are hitherto collectively referred to as the 'AM durum panel'. A detailed phenotypic and molecular characterization of the panel was previously reported in Maccaferri et al. (2006 and 2010). Briefly, the panel included accessions belonging to one of five main population subgroups: accessions from ICARDA bred for the dryland areas (subgroup 1), from ICARDA bred for temperate areas (subgroup 2), from the Italian and early '70 CIMMYT breeding programs (subgroup 3), from CIMMYT in the late '70s-early '80s (subgroup 4) and from CIMMYT in the late '80s-early '90s (subgroup 5). As compared to the panel of accessions described in Maccaferri et al. (2010), 25 accessions were dropped due to their relatively high relatedness while 19 additional accessions from the CIMMYT breeding programs, mainly classified as belonging to subgroup 5, were added to the panel. Based on their molecular profiles, the accessions clustered into the five subgroups with balanced frequencies.

4.3.2 Stem rust response evaluation under field conditions

Field experiments were conducted in Ethiopia at the Debre-Zeit Agricultural Research Center (DZARC), located at an altitude of approximately 1,900 m above sea level, with latitude of 8^0 44' N and longitude of 38^0 85' E. DZARC is a hot spot for wheat stem rust during the main cropping season (July to November) as well as during the off-season (mid-January to May), if irrigation is provided to ensure proper plant development. DZARC has been identified as an international durum wheat screening site as part of the Borlaug Global Rust Initiative.

The AM durum panel was evaluated during four consecutive growing seasons in 2009 and 2010. In both years, the evaluation was carried out both in the off-season under supplementary irrigation and in the main season under rain-fed conditions. The off-season is warmer than the main season and as a result stem rust disease pressure is often higher than in the main season, depending on the moisture availability for disease development. The accessions were evaluated in non-replicated field trials, using an augmented design, with plots consisting of 1-m-long twin rows flanked by spreader rows that were sown with a seed mixture of PBW343, Morocco (bread wheat susceptible to Ug99) and Local Red or Arendeto (susceptible durum) accessions in

2:1:1 proportion, respectively. Spreader rows were artificially inoculated with Ug99 (TTKSK race) and a mixture of durum-specific races prevalent in Ethiopia. The Ug99 (TTKSK) stem rust race was isolated and maintained on the variety PBW343 under greenhouse conditions. Race purity was regularly checked on the North American stem rust differential lines. Additionally, bulk spores were collected directly from the durum wheat nurseries in the field and temporarily stored at 4 ^{o}C after drying. Field inoculation was carried out following the methodology described in Roelfs et al. (1992). Inoculation was carried out on spreader rows starting at stem elongation growth stage and was repeated two-three times at weekly intervals. The cultural practices including fertilizer, weeds and insect control were applied according to the local site recommendations.

Stem rust disease severity was recorded two to three times during the epidemics development using a modified Cobb's scale (Peterson et al. 1948). Disease severity score (DSS) was calculated as the percentage of the infected stem area covered with pustules (visually estimated over the whole canopy); at the same time, the major infection type was also recorded (Roelfs et al. 1992). Infection types were categorized into four discrete classes: resistant (R), moderately resistant (MR), moderately susceptible (MS) and susceptible (S). The DSS and the corresponding infection types were used to compute the values of the Coefficients of Infection (Stubbs et al. 1986). For each evaluation season, the terminal disease severity at the soft-dough stage (Zadoks scale, 85; Zadoks et al. 1974), in coincidence with the peak of disease severity, was considered as the most informative disease score and was therefore used to carry out the molecular-phenotype association tests.

4.3.3 Molecular profiling

A bulk of 25 seeds from the original pure stock of each accession was germinated and grown in a growth chamber at 20 ^{o}C. After 2 weeks, seedling leaves were collected, freeze-dried, ground and used for genomic DNA extraction as previously described in Maccaferri et al. (2010). The accessions were profiled with 350 simple sequence repeat loci (SSR), 900 Diversity Array Technology (DArT) markers and three additional sequence tagged site (STS) markers including those previously reported as markers associated to major stem rust resistance genes (Yu et al. 2010).

4.3.4 SSR and STS markers

The SSR primers were chosen among the publicly available sets catalogued in the GrainGenes database (http://wheat.pw.usda.gov) as BARC (*barc* marker loci), CFA, CFD and GPW from INRA (*cfa*, *cfd* and *gpw*, *respectively*), KSUM (*ksum*), WMC (*wmc*) and WMS (*gwm*); an additional subset of private genomic WMS primers from TraitGenetics (supplied by M. Ganal, TraitGenetics, Gatersleben, Germany) were also considered. The SSR loci used to genotype the accessions were preselected for (i) clarity and repeatability of the amplicons profile, (ii) polymorphism level and (iii) even distribution on all the A- and B-genome chromosomes (chrs.). The choice was carried out based on the results of a survey of SSR primer pairs conducted on a small subset of eight founder accessions and lines used as parents of mapping populations.

As described in Maccaferri et al. (2008), a unique thermo-cycling protocol was used for all primer sets and SSR profiles of the accessions were obtained using the automated LI-COR 4200 IR2 System (LiCor, Lincoln, NE, USA). Genotyping was performed for most SSR markers using the M13-labeled primers and amplification protocol (Schuelke, 2000). Alleles were scored using founder genotypes as an allele reference set. Most markers produced only one band assigned to a unique wheat chromosome in previous mapping studies. For SSR primer pairs amplifying two or more loci, each locus was independently scored and assigned to the respective linkage group based on either the score of the parental lines or the LD with adjacent markers.

4.3.5 DArT markers

In addition to SSR and STS markers, the panel was profiled with DArT markers. DArT markers were generated by Triticarte Pty. Ltd. (Canberra, Australia; http:// www.triticarte.com.au), a whole-genome profiling service company, as described by Akbari et al. (2006). The Durum wheat *Pst*I / *Taq*I array v 2.0, containing 7600 single DArT clones obtained as described in Mantovani et al. (2008) was used for genotyping the panel. The locus designation used by Triticarte Pty. Ltd. was adopted (‘wPt’, ‘rPt’ and ‘tPt’ loci corresponding to wheat, rye and triticale clones,

respectively), and alleles at polymorphic loci were scored as hybridization positive (1) or negative (0).

4.3.6 Construction of the consensus map

The majority of the SSR markers considered herein were previously mapped in five intra-specific durum recombinant inbred line (RIL)-based linkage maps whose genotypic data were used to obtain a consensus durum wheat specific-linkage map. Four mapping populations, i.e. Kofa × Svevo (KS RIL population, Maccaferri et al. 2008), Colosseo × Lloyd (CL RIL, Mantovani et al. 2008), Meridiano × Claudio (MC RIL, Maccaferri et al. 2011) and Simeto × Levante (SL RIL, Maccaferri et al. unpublished), were developed by DiSTA in collaboration with Produttori Sementi Bologna SpA (Argelato, BO, Italy); a fifth linkage map obtained from the cross Kofa × UC1113 (KU RIL population, Zhang et al. 2008) was considered for consensus mapping and the genotypic data were downloaded from the GrainGenes web database.

The consensus linkage map was obtained from the five datasets using the Carthagene v.4.0 software (de Givry et al. 2005). Merging was performed with the *dsmergen* command, after checking for marker order consistency across maps, so that for each marker pair a single recombination rate was estimated based on all available meioses. A framework-mapping method was applied. Non-framework markers were incorporated in the framework map by building a complete map using the framework map as a fixed order. The marker order and inter-marker genetic distances from the consensus map were used to report the LD and association results. The consensus map included a total of 2,031 markers (mostly SSR and DArT markers). Of those, 861 had genotypic scores available in the durum accessions (320 SSRs, 3 STSs and 538 DArT markers).

4.3.7 Association mapping

For association mapping analysis, only markers with non-rare alleles (allelic frequencies greater than 0.10) were considered for the LD and marker-trait association analyses, thus reducing the false-positive rate and the LD inflation effects that have

frequently been associated with the consideration of rare alleles (Myles et al. 2009). Similarly to rare alleles, data-points showing residual allelic heterogeneity within accession were considered as missing data. In total, 1,211 markers were used for marker-phenotype association tests. Among these, 320 SSRs, 3 STSs and 538 DArT markers were projected onto the consensus linkage map. The remaining 332 polymorphic and informative DArT markers and 18 SSRs that could not be integrated into the final map were not considered further.

4.3.8 Genetic structure and linkage disequilibrium analysis

Prior knowledge suggested the presence of significant population structure in the panel. To decrease the false-positive rate, this structure was accounted for in the association test models. The genetic structure of the panel has been investigated with a combination of model- and distance-based analyses. Model-based population structure using a selection of 96 loosely linked, highly informative and evenly spread SSRs was assessed using the program STRUCTURE v. 2 (Pritchard et al. 2000). Structure parameter settings were: linkage model, allele frequencies correlated, burn-in length 10,000 and 10,000 MCMC repetitions. An optimum number of five hypothetical subgroups were chosen to obtain the Q matrix of membership coefficients of each accession to all subgroups (for details see Maccaferri et al. 2011). In the distance-based analysis, pairwise genetic similarity values (GSij) were calculated for all possible pairs of accessions using the simple matching coefficient for multi-state markers: a co-ancestry K (kinship) matrix was thus obtained for SSRs (for details see Maccaferri et al. 2010). Similarly, the kinship matrix was also calculated for DArT markers separately.

Estimating LD between markers measures whether markers segregate independently or not. The program TASSEL, ver. 2.1 (www. maizegenetics.net, Yu et al. 2006) was used to estimate the LD parameters D' and r^2 values as a function of the corresponding inter-marker distances and the comparison-wise significance was computed with 10,000 permutations. The r^2 parameter was estimated for loci on the same chromosome and compared based on genetic distances measured in cM. If, within a chr. region, all pairs of adjacent loci were in LD, this region was referred to as an LD block (Stich et al. 2005).

4.3.9 Marker-phenotype association analysis

Genome-wide scans for AM of loci governing stem rust resistance were conducted using the coefficient of infection (CI) as reference phenotypic data. AM analysis was conducted using the TASSEL program, ver. 2.1. The 320 SSRs, 3 STSs and the 538 DArT markers were tested for significance of marker-trait associations under: (1) the fixed general linear model (GLM) including the Q population structure results as covariates (Q GLM), (2) the mixed linear model (MLM) including the Q population structure results plus the K kinship matrix (Q + K MLM). For GLM analysis, besides the marker-wise association probability values, the experiment-wise association significance probability was obtained based on a permutation test implemented in TASSEL (10,000 permutations in total). The experiment-wise test provides a much more severe threshold for significance as compared to the marker-wise test (Bradbury et al. 2007, 2011). In the MLM analysis, experiment-wise significance was inspected using the false discovery rate (FDR) approach according to Storey and Tibshirani (2003) and implemented in *Qvalue* program. Multiple adjacent co-segregating significant markers were assigned to a unique QTL region upon satisfaction of the following conditions: less than 20 cM of inter-marker genetic distance, the presence of significant and strong LD among markers (possibly with r^2 values $\geq$ 0.6), consistency of the marker allelic effects in sign (Massman et al. 2011).

Prior to analyzing the phenotype data, the homogeneity of experimental variance across experiments was verified through the Bartlett's test. Chr. regions with markers that were repeatedly associated to stem rust response over two or more seasons as well as using the mean data averaged across seasons were considered as putative QTLs, even though the experiment-wise significance threshold was not reached. For each putative QTL, the most significant marker associated to stem rust response was considered as the main QTL-tagging marker; further results on the allelic distribution and effects have been reported for the QTL-tagging markers only. Linear regression was used to investigate the fit of the accessions' haplotypes at the main QTLs (significant over three to four seasons) to the corresponding phenotypic responses (CIs averaged across seasons). Based on the results of the GLM and MLM tests, the non-rare alleles at the QTL-tagging markers significant over three to four seasons

were qualitatively classified as beneficial, intermediate or deleterious and the cumulative number of beneficial and deleterious alleles was counted for each accession. The accessions' disease response averaged across the four seasons was regressed on the cumulative numbers of both beneficial and deleterious alleles. The significance of the regression was estimated with an *F* test.

4.4 RESULTS

4.4.1 Response to stem rust

Stem rust infection was high in all four testing seasons, allowing for clear and unambiguous scoring of field reaction. The mean CI values of the panel accessions ranged from 33.6 for DZm-2010 to 49.3 for DZo-2010. In both years, the off-season experiment showed a disease pressure significantly ($P \leq 0.01$) higher than that recorded in the main-season (**Table 1**). In all seasons, a broad and continuous variation within the panel was noted, from close-to-immune, highly resistant reactions to highly susceptible ones, as indicated by the observed disease response ranges reported in **Table 1** and by the CI frequency distribution in each season and across seasons (reported in **Figures 1-5**).

The analysis of variance for stem rust reaction showed highly significant differences ($P \leq 0.0001$) among accessions and seasons (results not reported); the accession × season interaction was also significant ($P \leq 0.01$). The heritability coefficient of stem rust response, calculated across seasons using the data from the non-replicated experiments, was equal to 0.80 while the coefficient of variation reached 26.1%. The Pearson correlation coefficients between the stem rust responses recorded in the four seasons (data not reported) were always highly significant ($P \leq 0.001$), with values ranging from 0.40 (DZo-2010 *vs*. DZm-2010) to 0.58 (DZm-2009 *vs*. DZo-2010).

Based on the distribution of the stem rust responses averaged over the four seasons (**Table 2**), about 5% of the accessions (nine in total) were highly resistant (mean DSS < 10%) and 19% (36 accessions) were categorized as moderately resistant (mean DSS comprised between 10 and 30%). Additionally, 11 accessions (i.e. 6%) were classified as susceptible or highly susceptible (DSS equal to or higher than 70%); their number

increased to 51 (i.e. 30% of accessions) when considering the single DZo-2010 season that was characterized by an infection level significantly (higher than that reached in the other three seasons.

4.4.2 Relationship between population structure and response to stem rust

The genetic relationships among the accessions were investigated using both a genetic-similarity and a model-based Bayesian clustering method and the results have been reported in **Figure 6**. Both methods pointed out that the minimum and optimum number of hypothetical well-distinct subgroups present in the panel were equal to five. It was shown that the five subgroups corresponded to clearly distinct breeding lineages: 1) the ICARDA germplasm bred for the dryland areas (subgroup S1); 2) the ICARDA germplasm bred for the temperate areas (subgroup S2); 3) the Italian and early '70s CIMMYT germplasm (subgroup S3); 4) the late '70s CIMMYT germplasm, widely adapted to Mediterranean conditions (subgroup S4); 5) the late '80s, to early '90s CIMMYT germplasm, with increased yield potential (subgroup S5). Based on the molecular assignment of each accession to the subgroup with the highest Bayesian probability, the five subgroups included 11, 55, 26, 56 and 35 accessions, respectively. The membership coefficient for each of the five subgroups, averaged over all the accessions was equal to 0.09, 0.29, 0.14, 0.29 and 0.19 from S1 to S5, respectively. The differences for stem rust response among the five subgroups were highly significant ($P \leq 0.0001$, results not reported), with the differences among subgroups explaining 15.5% of the total variance. Although differences among subgroups were found significant, the within-group component of variance prevailed, accounting for 53.2% of the total variation. The effect of population structure on the stem rust response was also investigated by means of regression analysis. Using data of each season separately, a modest population structure effect was detected for the DZo-2009 and DZm-2010 seasons, with R^2 values of 8.9 and 7.7%, respectively, while a greater influence was detected for DZm-2009 and DZo-2010, with R^2 values of 14.7 and 20.8%, respectively. The mean and range of stem rust response values (CIs) of each of the five subgroups are reported in **Table 3**. These values clearly show that all five subgroups included accessions with a wide range of responses, from highly resistant to highly susceptible, thus indicating that all subgroups are equally informative and well-suited for AM purposes. Considering the mean subgroup values

across seasons and based on the least significant difference among subgroups, S4 and S5, which mainly included CIMMYT elite germplasm, showed significantly higher stem rust susceptibility than S1, S2 and S3. The complete data set of phenotypic response and population structure membership coefficients for each of the 183 accessions included in the association panel is reported as **Appendix 2**.

4.4.3 Association mapping for stem rust response

In view of the strong genotype by season interaction, marker-phenotype association tests were conducted separately for each season as well as for the responses averaged over the four seasons. The association mapping (AM) analysis was conducted by performing single-marker *F* tests using both the General Linear Model with Q covariate matrix (population structure correction: Q GLM) and the mixed linear model with Q + K matrices (population structure and familial relatedness correction: Q + K MLM). The genome-wide scan revealed chromosome regions harboring putative QTLs for stem rust response on all chromosomes except for 3B. Overall, 45 chr. regions harbored markers that were significant ($P \leq 0.05$) in at least two seasons under the Q GLM model as well as across the averaged data of the four seasons; 36 of these 45 chr. regions showed significant effects also using the Q + K MLM model.

Introducing the experiment-wise correction, eight chr. regions showed significant ($P \leq 0.05$) effects in the Q GLM model while in the Q + K MLM model the significance was limited to one region on chr. 6A which showed the strongest association with stem rust response. Based on these findings, we decided to present detailed results of the 36 chr. regions which were detected in the marker-wise analysis and considered as putative QTLs.

Figure 7 summarizes the results of the Q + K MLM genome scan for the disease response averaged across the four seasons. In several cases, the presence of a QTL was evidenced by multiple significant associations at linked SSR and DArT markers with inter-marker distances always comprised within 10 cM, as estimated from the durum consensus map, and, in most cases, LD r^2 values higher than 0.6. For each of the QTLs that were identified as linkage-blocks of adjacent markers, all the markers significantly associated to the phenotype were checked for consistency of their effects

and the marker with the most significant association to the trait was considered as the QTL-tagging marker**.**

For 12 of the 36 chr. regions considered as putatively harboring QTLs, the significance of the effects on stem rust response was confirmed across three to four seasons (QTL features reported in **Table 4;** see also **Figure 7**) while the 24 additional regions showed significant, consistent effects in two seasons (**Table 5** and **Figure 7**). The QTLs with consistent effects across three to four seasons (**Table 4**) were also those with the highest overall R^2 values based on the season-mean data (in most cases comprised between 4.0 and 7.0%) as well as for single seasons (values ranging from 1.0 to 11.3%). In particular, regions on chromosomes 1BS (QTL-tagging marker *barc8*), 2AS (*gwm1045*), 3AS (*wPt-7972*), 6AL (*gwm427* and *CD926040*) and 7AS (*wPt-2799*) showed the highest R^2 values and all these QTLs were tagged by a series of adjacent markers that supported the primary QTL effect. Regions on chromosomes 2BL, 3AL and 5AL had consistently high R^2 values but were identified by single markers.

The QTL tagged by *barc8* on chr. 1BS at 32.0 cM showed strong LD (r^2 range of 0.60-0.67) along a 9.0 cM interval that included nine DArT markers (following the mapping order of the consensus map: wPt-2999, wPt-4605, wPt-3582, tPt-8831, wPt-9864, wPt-4133, wPt-1876, wPt-5899 and wPt-4729) and one SSR marker (*gwm1100*). In the distal region of chr. 6AL, highly significant effects were detected at three closely adjacent chr. regions/linkage that overall spanned 15.8 cM on the durum consensus linkage map, but showed low LD as to each others.. Each of these three chr. regions were identified respectively by: i) the marker pair *gwm427-wmc580* (at chro. position 139.5 cM, r^2 LD value between the two markers = 0.98), ii) the EST-derived marker *CD926040* (chr. position 144.0 cM), associated to wPt-9474, wPt-4229, wPt-5654, wPt-3247 and wPt-4663 (spanning a 9.3 cM interval with moderate LD among markers and r^2 values ranging from 0.12 to 0.58) and iii) *barc104* (chr. position 155.3 cM).The marker pair *gwm427-wmc580* showed low LD values with all the other markers in the region (r^2 values from 0.01 to 0.20) while LD was detected between the linkage block of markers associated to *CD926040* and *barc104* **(**r^2 from 0.26 to 0.55).

As compared to the QTLs identified across three to four seasons, those (24 in total) with significant effects in only two seasons (**Table 5**) showed in general lower effects and R^2 values both on a mean- (values from 1.0 to 3.8%) and single-season basis. Nonetheless, some of these QTLs (e.g. those on chrs. 1AS, 1BL, 2B, 3AL, 6A and 7B) showed relatively high R^2 values in specific seasons (from 3.6 to 8.0%).

The least square phenotypic means (based on CIs) of non-rare alleles at the QTL-tagging markers with significant effects in three to four seasons are reported in **Table 6.** The SSR marker *gwm427* (chr. 6AL) showed two common alleles (molecular weight equal to 212 and 188 bp) that differed significantly ($P \leq 0.05$) for the associated stem rust response, with the 188 bp allele being associated with lower CI values. The EST-derived marker *CD926040* (chr. 6AL) carried three common alleles with phenotypic effects that were estimated to be beneficial for one allele (855 bp) over all seasons and detrimental (i.e. associated to increased susceptibility) for the other two alleles (851 and 845 bp). At *barc104* (chr. 6AL) the 202 and 206 bp alleles were both considered as beneficial as compared to the 172 bp allele (detrimental).

Table 7 reports the frequencies in the five main germplasm subgroups of the non-rare alleles at the QTL-tagging markers that were significant in three to four seasons. Inspection of allele frequencies as reported in **Table 7** indicates that allele fixation within subgroups was rare and further suggests that, in most cases, the frequency of the resistant alleles and of the other common alleles can be considered as balanced (> 0.20), hence informative. In general, common alleles were present with balanced frequencies - the best condition to maximize the reliability of the association assay - in two to three subgroups; while *barc104* (chr. 6AL), wPt-2799 (chr. 7AS) and wPt-7785 (chr. 7AS) showed balanced allele frequencies across four or five subgroups. For each QTL-tagging marker, the frequency of the beneficial allele/s across the five germplasm subgroups was highly variable. As an example, in five cases beneficial alleles were observed at relatively high frequencies (> 0.50) in more than one subgroup, i.e. in all the five subgroups (wPt-7992, chr. 3AS), in four subgroups (*barc8* and *gwm1045*, chr. 1BS and 2AS, respectively), in three subgroups (*barc104*, chr. 6AL) and in two subgroups (*wmc388,* chr. 3AL).

Overall, subgroup 1 (ICARDA accessions bred for drylands) had higher frequencies of resistance allele at the QTLs on chr. 5A; subgroup 5 (CIMMYT accessions released in the late ’80s - early ’90s), though characterized by mean phenotypic responses higher than those showed by the other groups, had higher frequencies of resistance allele at QTLs on chr. 6A compared with the other subgroups.

For each locus consistently associated to stem rust resistance over seasons, in addition to reporting the allelic effects estimated as phenotypic least squared means over the whole association panel and the consistency of their significant differences (**Table 6**) were further inspected within panel subgroups. Markers associated to the main stem rust resistance on chrs. 1B (*barc8*), 6A (*CD926040* and *barc104*) and 7A (wPt-2799) were considered for the comparison of the allelic phenotypic values in the entire panel and its subpopulations as these markers accounted for the largest proportion of phenotypic variation. Accessions carrying the 255 bp allele at *barc8*, the 855 bp allele at *CD926040*, the 202 or 206 bp allele at *barc104* as well as the presence of the band at wPt-2799 had significantly ($P \leq 0.05$) lower stem rust infection than the other accessions across three or more of the five subgroups that composed the panel.

The relevance of the QTL-tagging markers significant over three or four seasons in predicting the accessions’ stem rust response was further investigated by regressing CI values on the cumulated number of beneficial alleles of the accessions. The scatter plot thus obtained is reported in **Figure 8**. Although the significance of the linear regression was high ($P \leq 0.001$), the R^2 value of the regression was very low (5.6%). As expected, the regression coefficient was negative (b = -1.75). The increase in resistance associated to the cumulative effects of the beneficial alleles is also revealed by the comparison between the response values predicted for zero beneficial alleles (CI = 48.3) and the maximum number (9) of cumulated beneficial alleles (CI = 32.5). The significance of the regression was also tested for the pool of QTL-tagging markers when considering only the accessions with the susceptible allele at *CD926040*, the maker most associated to the *Sr13* region; also in this case the regression on the number of beneficial alleles was highly significant ($P \leq 0.001$), with the b coefficient and the R^2 value equal to -3.52 and 16.1%, respectively.

4.5 DISCUSSION

A better understanding of the genetic basis underlying the durum wheat response to Ug99 and durum-specific Ethiopian races of stem rust will help enhance disease resistance of this crop globally while shedding light on the evolution of durum wheat-stem rust relationships in East Africa. To this end, association mapping (AM) is a useful approach as indicated by the growing interest in its application to identify disease-resistance genes/QTLs in a wide range of crops (Ersoz et al. 2009; Hall et al. 2010; Maccaferri et al. 2010; Haile et al. 2012).

The AM durum panel evaluated in the present study encompasses a large portion of the genetic variation present in the elite germplasm pools commonly used by durum breeders. Only very few landraces/pre-Green Revolution genotypes were kept because of their "founders" role and significant contribution to the development of some of the modern germplasm groups. The predominance of elite germplasm in this panel was justified for several reasons. First, the presence in the elite germplasm of LD which extends over rather long distances, as shown in Maccaferri et al. (2005, 2006 and 2011) enabled us to conduct a genome-wide scan with an average marker density matching the genotyping capacity allowed by the marker systems currently available for durum wheat, mainly SSR and DArT markers (Maccaferri et al. 2003; Mantovani et al. 2008). Second, very little information about useful loci for quantitative stem rust field resistance is available in durum wheat and thus the modern germplasm pool was considered as the primary target for such investigation. Finally, the high homogeneity in phenology of the elite materials herein considered (Maccaferri et al. 2006) as compared to the higher heterogeneity in phenology observed in other AM collections, particularly those including landraces (Wang et al. 2012), allowed for a more meaningful assessments of the disease responses.

4.5.1 Response of the elite durum wheat germplasm to stem rust under field conditions

Highly significant genotype × season interactions were detected within the AM panel used in this study. These interactions were due not only to magnitude effects, since the stem rust response of some accessions varied from resistant in one season to

clearly susceptible in another season. This finding was confirmed by the values of correlation coefficients between accession responses in different seasons that even if highly significant were quite low ($r < 0.58$). These interactions could be explained in part by the different growing conditions prevailing in different seasons, which are known to affect disease incidence and intensity. Such inter-season effect on disease intensity is clearly seen in the increase in average intensity in the warmer off-seasons compared to the more temperate conditions during the main-seasons. Most importantly perhaps, genotype × season interactions may have been due to the use of a mixture of races with different virulence spectra rather than a single race. The different races, especially the least characterized durum-specific ones, may have impacted differently on final reaction in different seasons, due to different starting relative quantities, fitness or interactions with season-specific environmental and/or inoculation conditions. However, the use of such mixture rather than single race inoculum, while predictably complicating the interpretation of the results, was essential for this study to address comprehensively stem rust threats that are relevant to durum wheat breeding under field conditions.

The use of Ug99 or its more recent variants alone, all avirulent on *Sr13*, would have had limited relevance to global durum wheat breeding as resistance to them is present in the most germplasm groups worldwide. On the other hand, the exclusive use of the Ethiopian races, as single isolates or mixtures, because of their unclear virulence spectrum, would have likely provided incomplete information as to the global usefulness of sources of resistance or genomic regions involved in controlling such resistance. Also, the presence of Ug99 in the mixture was important, since this is the only race that so far has migrated out of Africa into Asia and could therefore become the first threat to the South Asian durum-growing areas. Whatever the reason for the highly significant genotype × season interaction, its effects were mitigated and the robustness of our conclusions was supported by the analysis of single season data in addition to the results averaged over the seasons. Genotypes were considered resistant or susceptible only when they performed as such consistently across seasons and phenotype-marker associations, as discussed below, were considered relevant only when they were significant in at least three of the four seasons. Nevertheless, clear trends in the distribution of genetic resistance present in this AM panel were observed and reliable conclusions could be drawn. First is the very low frequency (5% of all

accessions) of high-level resistance, expressed as reactions that are consistently close-to-immune or always below 10% DSS, supporting the conclusions from previous studies that elite durum wheat germplasm is relatively poor in genes with major effects providing complete field resistance to stem rust (Singh et al. 1992; Bonman et al. 2007). This is also in agreement with results from evaluations conducted in Ethiopia at the onset of the Borlaug Global Rust Initiative in 2007-08 which showed only 3% of resistant lines within the CIMMYT elite germplasm tested in that year (Ammar and Badebo, unpublished). This trend seems to extend to wider germplasm groups as shown by Olivera et al. (2010) who reported 5.2% of field resistance in a worldwide collection of 996 durum wheat accessions and other tetraploid relatives under conditions and with races similar to those used in the present study.

Another interesting reaction group includes genotypes showing DSS between 10 and 20%, mostly with R-MR to MS type pustules, with a reaction type very similar to that of local Ethiopian cultivars such as Boohai or Ude, considered adequately resistant to be competitive in most areas of Ethiopia. In the present study, 9% of the genotypes were consistently classified in this group and therefore could be considered as resistance sources for breeding programs, possibly providing usable resistance genes.

In contrast to the low frequency of accessions with high levels of resistance, a sizeable portion (at least 28%) showed a DSS consistently between 30 and 40%. Such intermediate, albeit susceptible, values can indicate, when accompanied by seedling susceptibility to the races investigated in this study, the presence in the durum germplasm, in relatively high frequency, of minor genes conferring quantitative and partial field resistance to both Ug99 and the Ethiopian durum races of stem rust. The accumulation of such genes in a single genotype, provided their effects are additive and seedling susceptibility confirmed, should result in an improved resistance comparable to that conferred by major gene-based resistance but otherwise race non-specific and thus more likely to be durable (Skovmand et al. 1978; Lagudah 2011; Singh et al. 2011). Alternatively, the low rust response observed in some accessions included in the present study may be due to the presence and possible accumulation of race-specific seedling genes, which exhibit moderate resistance to moderate susceptibility at the adult plant growth stage. Genotypes useful as sources of minor gene-based resistance to leaf rust have been identified in durum wheat (Herrera-

Foessel et al. 2007) and the improvement of resistance through the accumulation of such genes has been demonstrated (Herrera-Foessel et al. 2009).

4.5.2 Genetic basis of the resistance to stem rust in durum wheat and relevance to breeding

Based on the observation that complete immunity to the Ethiopian races was seldom observed in the field under heavy infection conditions, it has been suggested that resistance in durum wheat was likely to be based on additivity, i.e. resulting from the cumulative effect of additive beneficial alleles from multiple loci (major and minor) of variable effect (Osman Abdallah, personal communication; Ayele Badebo, personal communication). This finding is clearly supported in the present study by the fact that resistance was always associated with several genomic regions, each contributing a small fraction of the variability associated with field reaction while none of them was individually capable of providing a high level of resistance. When estimated in single seasons, each QTL identified in this study explained not more than 13% of the phenotypic variation for stem rust resistance, unlike previous QTL studies based on wide crosses where QTL with R^2 values as high as 37% have been reported (Bansal et al. 2008). Even though QTL effects estimated via AM are usually lower than those estimated through biparental mapping, the fact that even the most resistant, close-to-immune genotypes did not owe their resistance to a single major QTL, indicates the marginal role of classical major genes, as often seen in bread wheat. Furthermore, it is also known that most of the seedling major genes described for stem rust resistance in wheat, including *Sr13* native of tetraploid wheat, at the adult plant stage confer medium-resistance to medium-susceptiblity rather than complete resistance/immunity. The oligogenic nature of the resistance is also supported by the negative, albeit low, relationship between the number of accumulated favorable alleles and field reaction, whether or not the genomic region corresponding to *Sr13*, a major gene known to provide complete resistance to Ug99, was included in the analysis. Recently, this hypothesis has been strengthened by the results obtained from the genetic mapping of the factors responsible for the resistant response of the ICARDA elite cultivar 'Sebatel' (Haile et al. 2012), also included in this study. The genetic basis of Sebatel' resistance turned out to be oligogenic, with nine major and minor QTLs identified in the RIL population and R^2 values ranging from 5.0 to

34.0%. Another aspect that can contribute to explain the different results found between durum and bread wheat is that the elite breeding germplasm of durum wheat has not been improved in the past decades by means of an extensive use of wide-crosses to introgress alleles with strong phenotypic effects (Maccaferri et al. 2005), as has been the case with hexaploid wheat.

In the absence of single-race analysis at the seedling and adult stages with a wide collection of races, conclusive evidence cannot be drawn as to the nature of the resistance observed in the present study. Nevertheless, this report on the oligogenic nature and likely minor-gene basis of stem rust resistance in durum wheat has important implications for breeding activities. It suggests that deploying the sources identified in this study in a resistance-breeding program would result in an increase in resistance that would likely be more durable as compared to a monogenic, major-gene resistance. However, unlike the large-effect QTLs that are easily identified and maintained in breeding populations through phenotypic selection and can be easily managed via marker-assisted selection (MAS), the simultaneous handling of small-effect QTLs is much more complex. In fact, an effective phenotypic selection for small-effect loci requires well-planned populations and intense, uniform epidemics at every cycle of visual selection in order to readily detect and accurately score transgressive segregants. Under these conditions, the availability of useful markers reliably tagging the minor QTLs and the ready access to MAS facility becomes critical. In the near future, the availability of high-density single nucleotide polymorphism (SNP) platforms including thousands of highly multiplexed assays will allow for a nearly complete genome coverage and the possibility to switch from single-marker to haplotype-based analyses, thus enabling a full exploitation of the potential of AM (Akhunov et al. 2009; Kaur et al. 2011; Trebbi et al. 2011; You et al. 2011; van Poecke et al. 2013). The use of the same SNP assays in applied breeding programs will also facilitate the simultaneous selection of multiple beneficial alleles for partial resistance. Thus, MAS strategies that can effectively deal with a relatively high number of markers and haplotypes are required to accumulate and maintain the beneficial alleles at these small-effect QTLs in order to achieve an acceptable and durable level of resistance for stem rust within durum breeding populations (Kuchel et al. 2007). With this aim, recent advances in the implementation of genomic selection in crop species, in particular to improve stem rust resistance in hexaploid wheat

(Rutkoski et al. 2010), indicate that this could be the most efficient approach to exploit the potential of high-density molecular marker screening tools.

4.5.3 QTLs identified through association mapping and relationship with previously described QTLs and Sr loci

The joint Q GLM and Q + K MLM association analyses highlighted several chr. regions putatively harboring QTLs with main effects of varying magnitudes on field stem rust response. As expected, multiple-test correction drastically reduced the number of significant regions, a condition not well-suited for an exploratory analysis like the present one. In addition, our goal was to keep a reasonable power to identify loci conferring partial resistance with alleles characterized by relatively small effects. Therefore, also the most significant chr. regions based on the less stringent marker-wise significance test has been considered, provided that the associations were significant on the season average data and in at least two of the four seasons.

Several QTLs identified in this study co-located with previously reported major *Sr* loci as well as with a number of QTLs recently identified through AM in hexaploid wheat (Yu et al. 2011) and in tetraploid wheat (Haile et al. 2012). Others, namely those discovered on chrs. 1A, 1B, 3A, 4A, 4B, 5A5B, 7A and 7B were not reported elsewhere. These results highlight the effectiveness of AM to dissect the genetic basis of moderately complex traits while showing its potential to unveil the presence of previously unknown QTLs, provided that an appropriately balanced and phenologically suitable set of accessions are evaluated.

In chr. 1A, significant effects were identified in the distal end and near the centromere of chr. 1AS. In both cases, significant effects were reported in hexaploid and tetraploid wheat, respectively, within 10 cM distance from the significant markers, with associated R^2 values of ca. 5.0%. Highly significant effects were detected near the centromere of chr. 1B. This region is known to harbor *Sr14* as well as the 1B.1R translocation-based *Sr31* (Zeller 1973). However, due to the absence of the 1B.1R translocation in the present panel, the effect herein detected is likely due to *Sr14*. A recent AM study in hexaploid wheat (Yu et al. 2011) showed the presence of a QTL associated to stem rust response in this same region, precisely on the chr. arm 1BS.

Moreover, three DArT markers significantly associated to stem rust response were reported in the same region by Crossa et al. (2007). Significant markers on the proximal region of chr. arm 1BL, such as *cfd65*, wPt-8168 and *gwm947* are located in the same region as *Sr14*, which is effective against Ug99 races (Singh et al. 2006). The presence of the *Sr14* resistance allele in the durum wheat germplasm can be traced back to *Triticum dicoccum* Schrank accessions such as Khapli emmer, which is known to carry *Sr14* and is also considered as one of the few founders of the modern durum wheat germplasm (Autrique et al. 1996). *Sr14* has been considered as one of the causes of stem rust resistance in some synthetic wheat-derived lines (Njau et al. 2010).

Overlap of the QTL location in our study and a minor QTL described by Haile et al. (2012) in the durum wheat RIL population was found on chr. 2AS. On chr. arm 2BL, *gwm1300* and *wmc356* (50.9 cM apart) were significantly associated with stem rust resistance for two and three seasons, respectively. These markers mapped to regions corresponding to the putative locations of *Sr9/SrWeb* and *Sr28/Sr16*, respectively. At the *Sr9* region, two alleles are known: *Sr9e* which was reported to be ineffective against Ug99 at the seedling stage while showing MR to MS infection types in the field nurseries (Jin et al. 2007) and *Sr9g*, which provides field resistance to Ug99 and to the Ethiopian races. *Sr9e* is present in many durum wheat genotypes, including the CIMMYT landmark Yavaros C79 and its sister line Karim 80, which were classified as moderately resistant to moderately susceptible under the present study's conditions. *Sr9g* is one of the resistance alleles reported to be present in the durum cultivar Iumillo (McIntosh et al. 1995).

Several regions with significant associations to field reaction to stem rust were detected on chr. 3A where *Sr27* and *Sr35*, both effective against Ug99, have been reported (McIntosh et al. 1995; Singh et al. 2006). However, as *Sr27* originated from a wheat-rye translocation engineered exclusively in bread wheat and *Sr35* from *Triticum monococcum*, transferred to some tetraploids of Canadian origin, none of which was present in this study or in the pedigree of the accessions of the AM panel, the chr. 3A related associations detected herein are likely to involve novel loci or alleles. The distal region of chr. 3BS is known to harbor *Sr2*, a gene that confers partial resistance to Ug99 at the adult plant stage. In hexaploid wheat, the beneficial

Sr2 allele selected by MAS originates from the tetraploid wheat germplasm (Yaroslav emmer). Although the *Sr2* locus has been reported as the major component of resistance in the durum wheat RIL population Sebatel × Kristal (R^2 value equal to 34.0%, Haile et al. 2012), in the durum elite germplasm considered in this study it was irrelevant. The SSR markers used to provide information on the *Sr2*-associated haplotype (*gwm533* and *barc133*) showed that this haplotype was very rare in the sample of elite durum wheat accessions herein considered, under the allele frequency cutoff threshold used for the AM test (10.0%).

On chr. 6A, the AM analysis highlighted six QTLs with significant effects on field stem rust reaction. One of these regions (approximately 8 cM wide) tagged by wPt-7330, in the distal portion of chr. arm 6AS co-locates with the region known to harbor *Sr8*, a gene known to be ineffective against Ug99 (Singh et al. 2006). Interestingly, this region completely overlapped with a QTL for stem rust resistance recently reported in an AM study in hexaploid wheat (Yu et al. 2011). A wide region on chr. arm 6AL, about 40 cM wide, plays a major role in controlling stem rust response in the durum wheat germplasm tested herein. This region includes two distinct sub-regions harboring effective but most probably distinct genes.

The first, proximal sub-region, tagged by tPt-4209, *gwm1150* and *gwm169* and associated with stem rust resistance in this study, co-locates with *Sr26*, a gene effective against Ug99 (Singh et al. 2006) and the Ethiopian races (Ayele and Ammar, unpublished results). However, the presence of the known *Sr26* allele in the AM panel or in the durum wheat germplasm at large is unlikely, the *Sr26*-resistant allele having been introgressed from the wild relative *Thinopirum ponticum* exclusively into bread wheat. A novel gene/allelic variant other than *Sr26* should be located in this sub-region. Remarkably, this QTL region has been independently confirmed in the Sebatel × Kristal durum population and reported as *QSr.IPK-6A* (Haile et al. 2012), a QTL with R^2 value equal to 9.3% and tagged by the SSR markers *gwm494-gwm1150*. The second, distal sub-region of chr. 6AL includes three further sub-regions (tagged by *gwm427*, CD926040 and *barc104*) strongly associated to stem rust response. These sub-regions co-locate with *Sr13*, mapped in tetraploid wheat to chr. 6AL within a 1.2 to 2.8 cM interval flanked by the EST-derived markers *CD926040* and *BE471213* (Simons et al. 2011). In our study, *CD926040* showed the maximum R^2 value and was

consistently significant across all four seasons. *Sr13* is effective against the TTKS complex of *Puccinia graminis* ssp. *tritici*, namely TTKSK (Ug99), TTKST and TTTSK. However, virulence for *Sr13* within Ethiopian stem rust populations has been suspected for some time, and recently confirmed by the characterization of the TRTTF and JRCQC isolates collected from the Ethiopian site in Debre-Zeit (Olivera et al. 2010 and 2012). Therefore, while very effective against the TTKSK or Ug99 lineage (the only ones so far to have migrated out of Africa), its presence alone is not sufficient for adequate protection in Ethiopia. This is clearly seen when comparing the field reaction and the long-range haplotype in the extended *Sr13* chr. region of two US desert-durum cultivars, namely Kronos and Kofa, which were considered in the present study. While both cultivars exhibited the haplotype of Khapli Emmer, known to carry *Sr13* (Knott, 1962), Kronos had one of the most consistently resistant reactions over seasons while Kofa was regularly susceptible. Taking into account all of the above information, the presence of the resistant allele(s) in the *Sr13* region is valuable for breeding activities and should be pursued for pyramiding multiple useful alleles. Specifically for the *Sr13* locus, Simons et al. (2011) found different linked marker alleles among the *Sr13* donors, suggesting that breeding programs used different sources of *Sr13* or that independent recombination event occurred between loci. In our study, the durum wheat accessions Khapli, Kofa and Kronos were the donors of resistant *Sr13* alleles (Simons et al. 2011). The LD decay among the three main linkage blocks (tagged by *gwm427-wmc580, CD926040* and *barc104*) near *Sr13* and the variation in band sizes of the marker alleles indicate that the current markers are not fully diagnostic in a wide range of backgrounds and, therefore, cannot be used to predict with high confidence the presence of *Sr13* in unknown sets of germplasm. This notwithstanding, these markers can be used to follow the *Sr13* resistant alleles in segregating populations involving parental lines (e.g. Khapli, Kofa and Kronos) related to any of the known *Sr13* sources. The future availability of high-density, SNP platforms (Trebbi et al. 2011; van Poecke et al. 2012) will likely provide much better haplotype resolution.

The significant effects identified in chrs. 6BS, 7AS and 7BS are specific of the durum germplasm herein considered. Accordingly, these regions have not been reported as QTL locations neither in t bread wheat germplams nor in the Sebatel × Kristal population. On the distal portion of chr. 7AL, DArT markers with significant effects

on stem rust resistance in our study overlapped with the locations of *Sr22* and *QSr.ipk-7AL* (Haile et al. 2012). This is additional independent evidence for the relevance of this QTL region for stem rust response. Finally, AM detected QTLs at the distal end of chr. arm 7BL (QTL-tagging marker wPt-8615), with several DArT markers associated with stem rust resistance. This region co-locates with that known to harbor *Sr17*, a gene linked to *Lr14a* and *Pm5* in bread wheat (Crossa et al. 2007). It also is consistent with a region reported to include a stem rust QTL in the Arina x Forno RIL population (Bansal et al. 2008). *Sr17*-related resistance to stem rust has been reported in tetraploid wheat as well as synthetic bread wheat (Bariana et al 2009). In the present study, there was no relationship, either in coupling or repulsion, between stem rust resistance and the presence of *Lr14a* (known from previous studies on the same panel) present in the same region. This may indicate that both genes are widely spaced so no linkage could be detected. Further research is underway to confirm the presence of known resistance/susceptibility alleles for the aforementioned designated stem rust genes at the seedling stage.

Based on the results herein presented, it is clear that quantitative, additive variation is present in the elite germplasm at chromosome regions known to carry well-characterized resistance genes (*Sr14*, *Sr28-Sr16*, *Sr8* and particularly *Sr13*) whose alleles are tagged by known molecular markers and are known to be frequently defeated by specific races or non-effective at the seedling stage. Nevertheless, our results show that the same genes may have residual appreciable effects (though of quantitative and additive nature) at the adult, open field stage, particularly when present in multiple combinations.

It is also known that most of the seedling stem rust resistance genes produce medium-susceptible to medium-resistant responses, thus more quantitative in nature as compared to classical resistant responses and more subjected to genotype × environment interaction at the adult plant stage (including *Sr13*, and with the exception of *Sr6*, *Sr9e*, *Sr17*, *Sr19*, *Sr21* and *Sr31*). The low rust response phenotypes observed in the present study can therefore be ascribed to the presence of combinations of resistance genes including major designated genes and additional novel genes/QTLs.

4.6 CONCLUSIONS

Association mapping in elite germplasm has the potential to accelerate the translation of basic genetic information towards applications in crop improvement and cultivar release. Our study shows that AM effectively complement bi-parental mapping studies by providing independent validation of previously detected QTLs and discovering new QTLs. Additionally, our study highlighted the presence of valuable genetic variation that could be exploited to sustainably enhance stem rust resistance in durum wheat. The oligogenic nature or minor-gene basis of resistance to Ug99 and the Ethiopian races of stem rust in durum wheat have been clearly documented. Several chr. regions harboring putative QTLs involved in the stem rust response in the field under high infection were consistently detected across seasons; the allelic variation at these QTLs can be exploited for further validation studies and utilization in MAS programs. The AM results reported herein confirm the important role of the *Sr13* region but also its limitation in individually addressing the presence of the Ethiopian races. Our analysis also highlighted the role of chr. regions putatively harboring *Sr14* and *Sr9, Sr17,* to be further dissected as providing alleles with beneficial effects on final resistance, but again not sufficiently strong individually. Additionally, the AM analysis strengthen the role of five QTLs recently described in the Sebatel × Kristal durum mapping population and located in chromosome regions where no designated resistance genes were mapped. New regions, so far not reported to be associated with stem rust resistance either in durum or bread wheat, have been detected. These regions contribute minor genes that can be accumulated through MAS towards a more durable resistance to stem rust in durum wheat.

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FIGURES

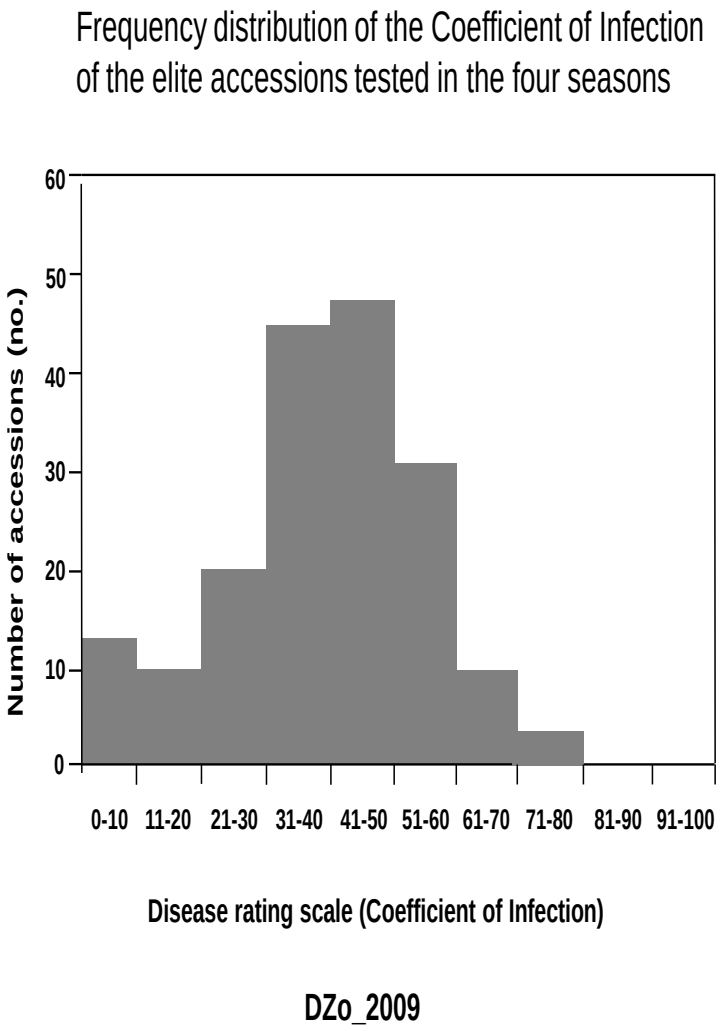

Figure 1. Frequency distribution of the Coefficient of Infection of the elite accessions tested at Debrezeit, 2009 off cropping season.

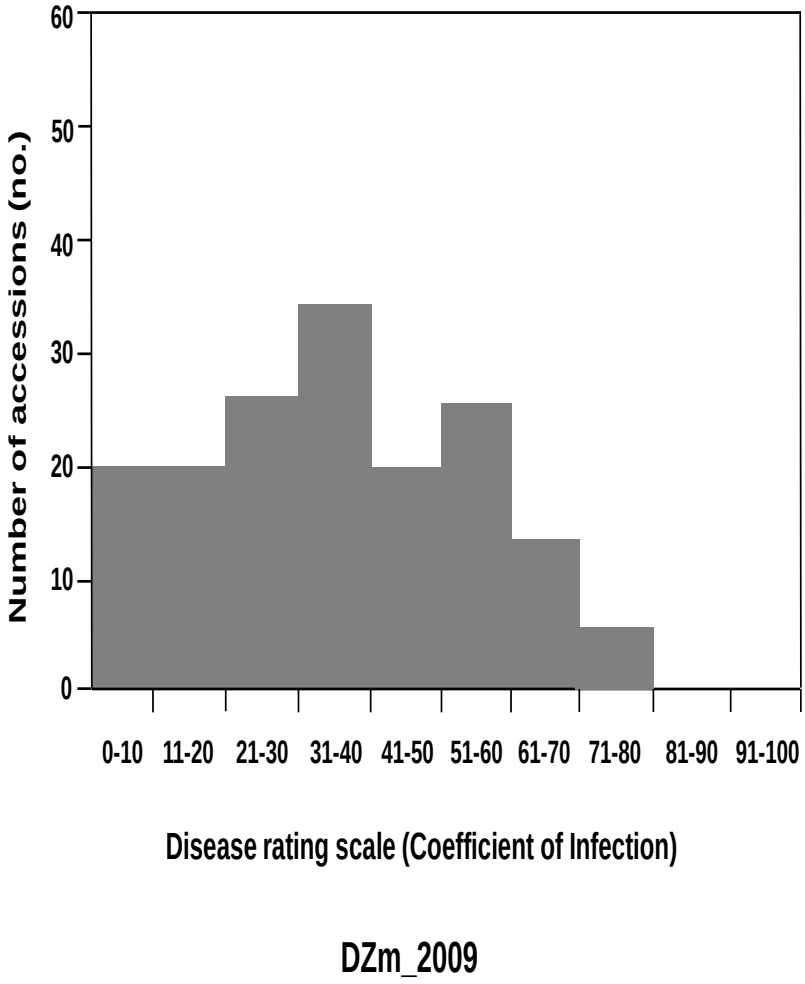

Figure 2. Frequency distribution of the Coefficient of Infection of the elite accessions tested at Debrezeit, 2009 main cropping season.

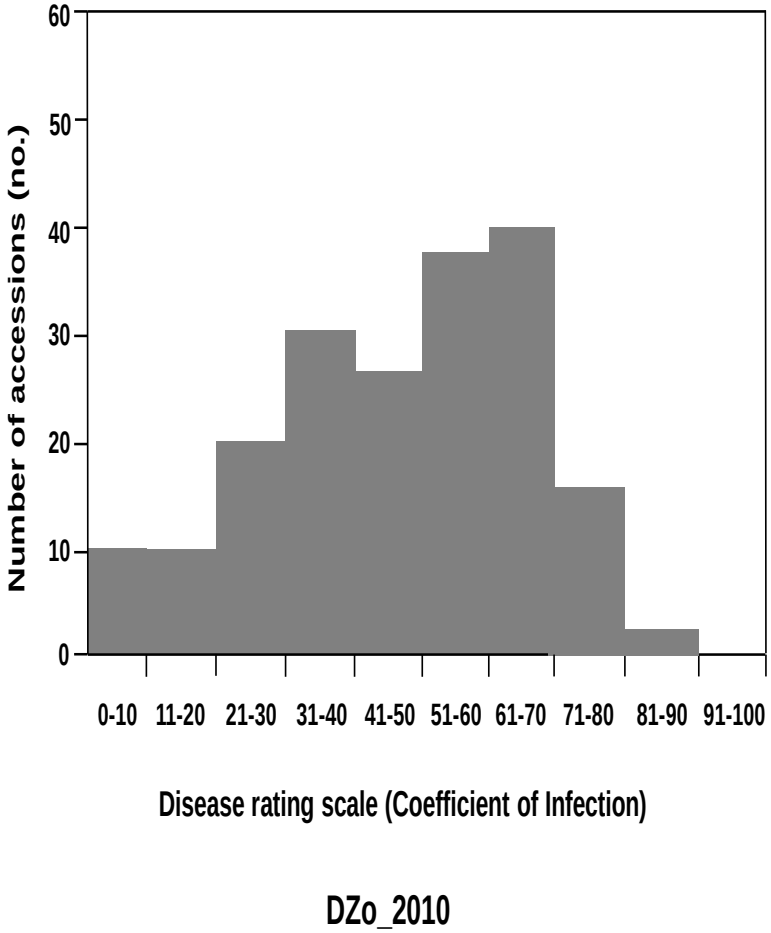

Figure 3. Frequency distribution of the Coefficient of Infection of the elite accessions tested at Debrezeit, 2010 off-cropping season.

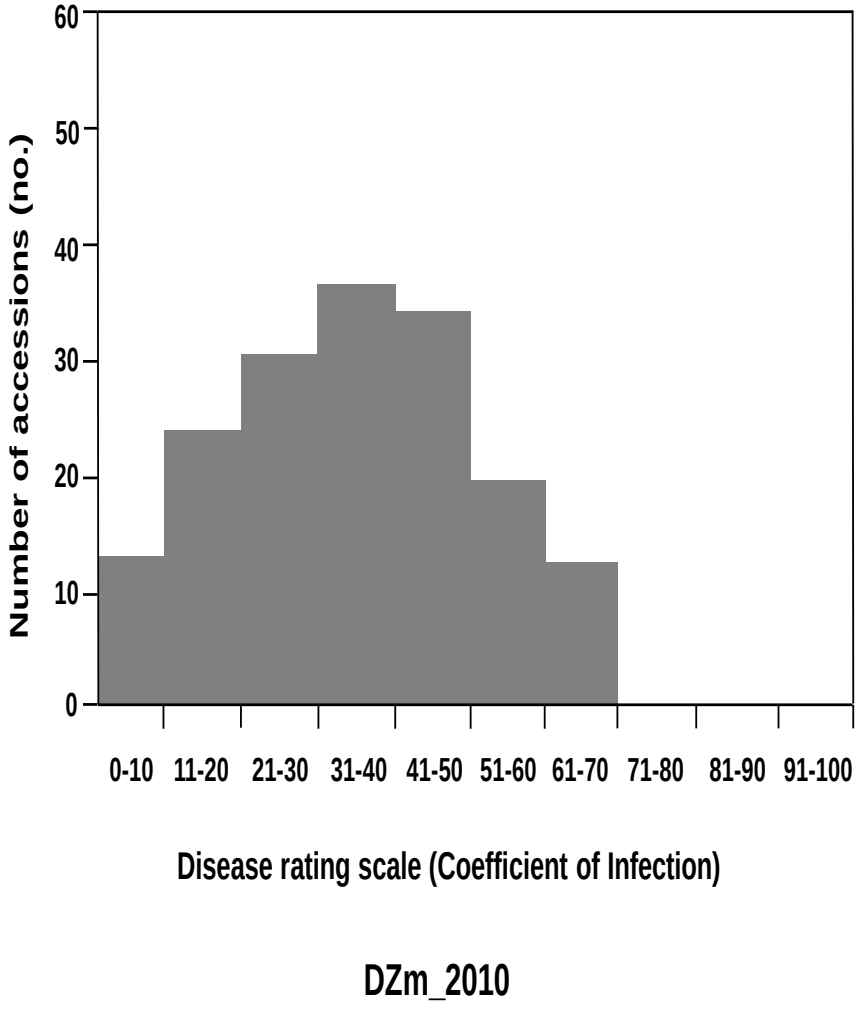

Figure 4. Frequency distribution of Coefficient of Infection of the elite accessions tested at Debrezeit, 2010 main cropping season.

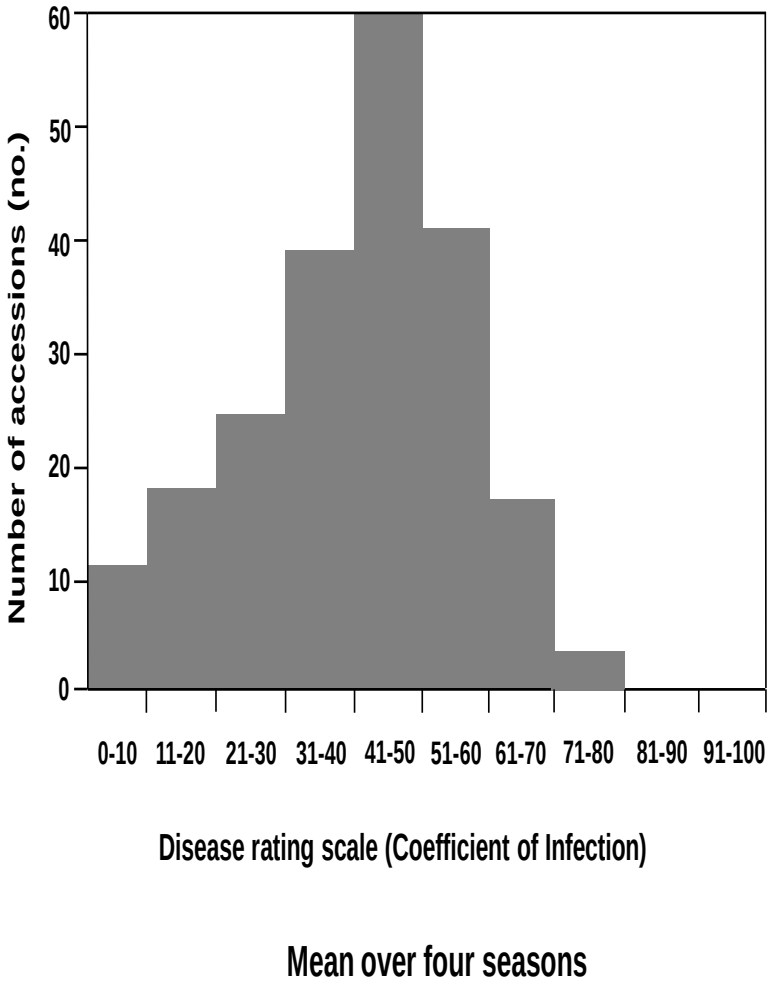

Figure 5. Frequency distribution of Coefficient of Infection of the elite accessions tested at Debrezeit, mean over four seasons cropping season.

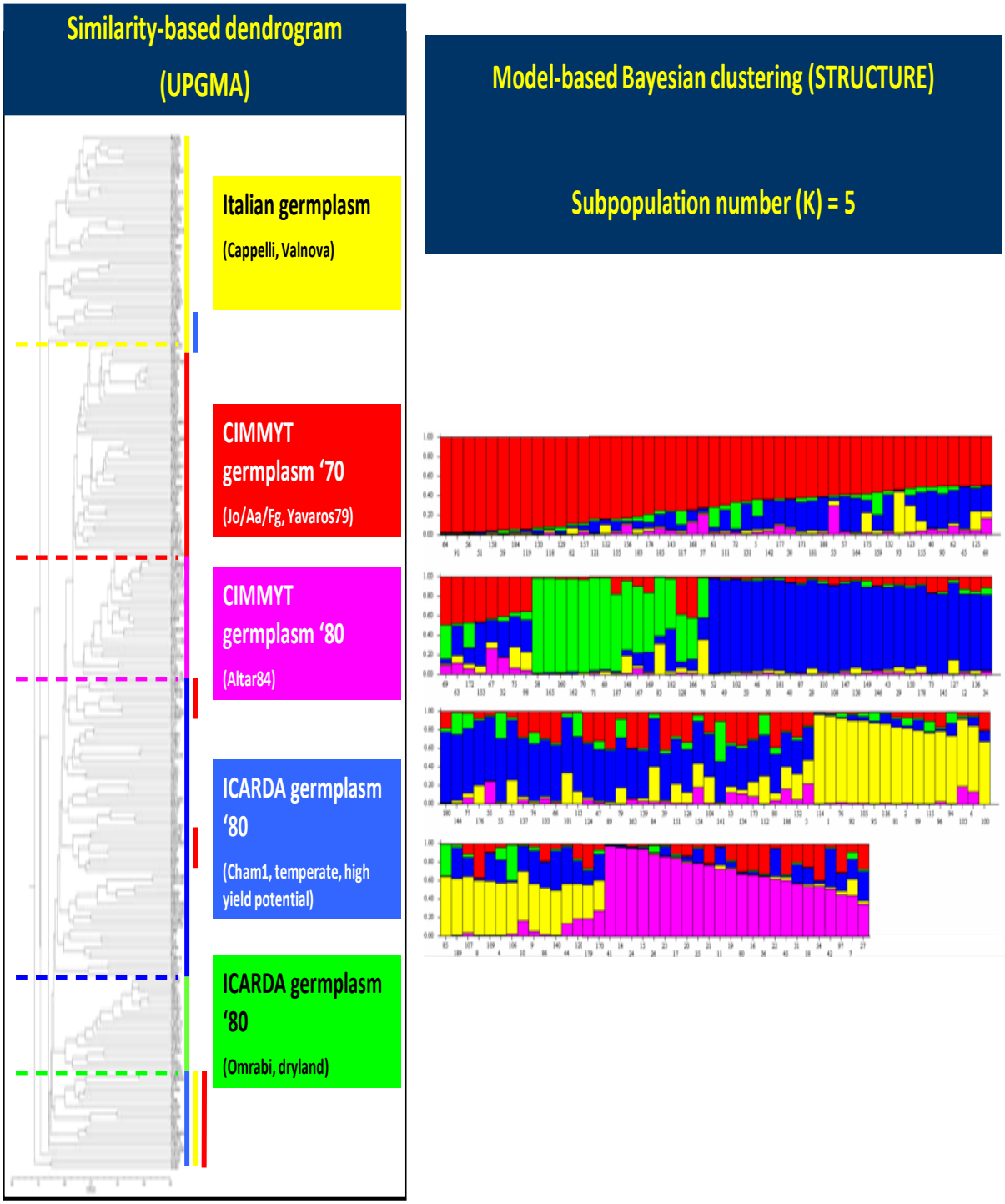

Figure 6. Population structure investigated by both distance and model-based Bayesian analysis.

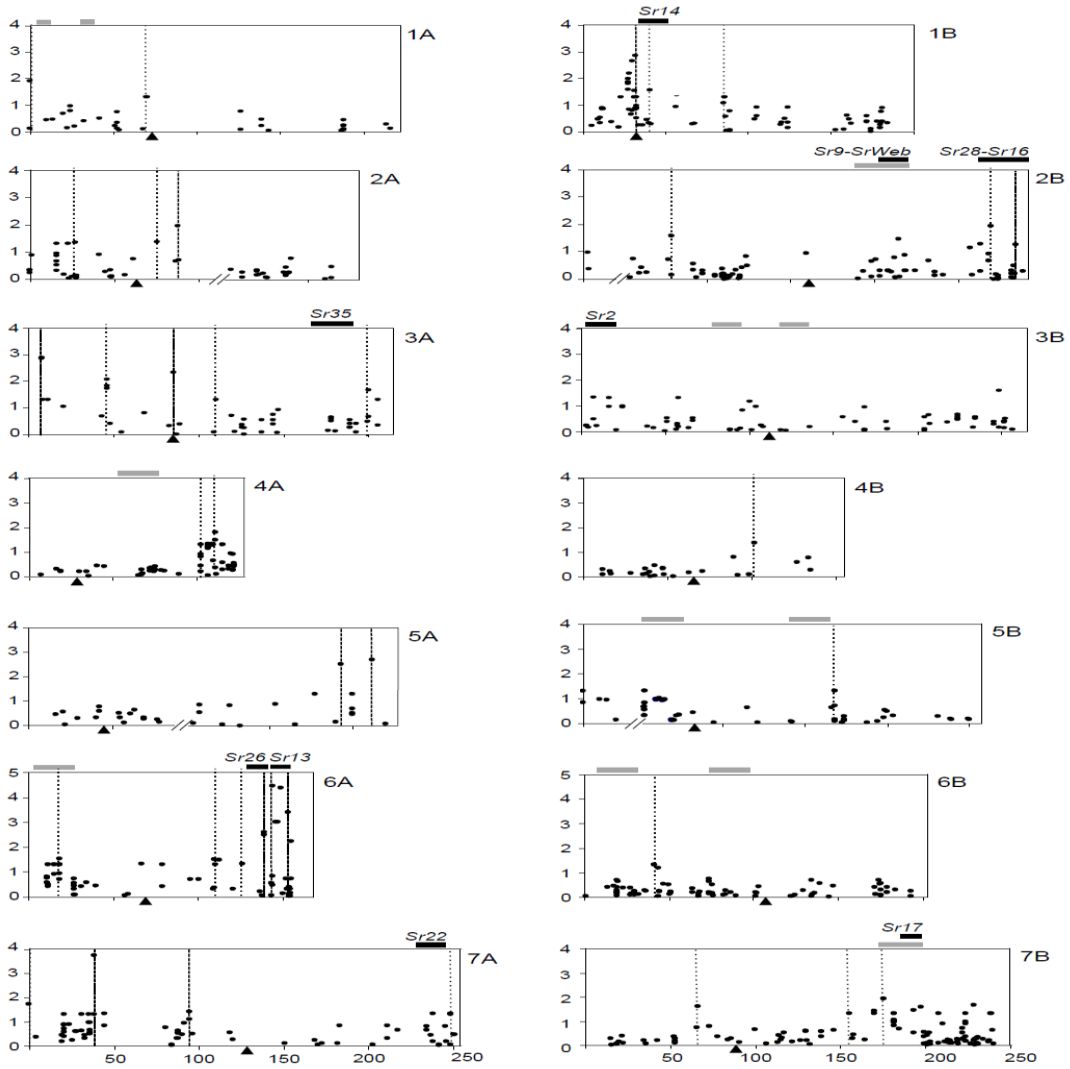

Figure 7. Association mapping probabilities, reported as -log (*p*), of the mapped markers tested for association to stem rust response of 183 elite accessions of durum wheat. Results are shown for the stem rust response averaged over four evaluation seasons, reported on a chromosome-by-chromosome basis. The -log 0.05 significance threshold value is equal to 1.35. Centromeres have been indicated as solid filled triangles. Vertical, dashed lines indicate the 12 markers with significant effects ($P < 0.05$) in three or four seasons; vertical, dotted lines indicate the 24 markers tagging QTL regions with significant effects ($P < 0.05$) in two seasons only. Chromosome intervals corresponding to the locations of stem rust (*Sr*) resistance loci reported by previous studies in hexaploid and tetraploid wheat have been reported as black bars above the graph of each chromosome. Chromosome linkage blocks associated to stem rust response in hexaploid wheat (Crossa et al. 2007; Yu et al. 2011) and in tetraploid wheat (Haile et al. 2012) have been reported as gray and crossed-bars, respectively.

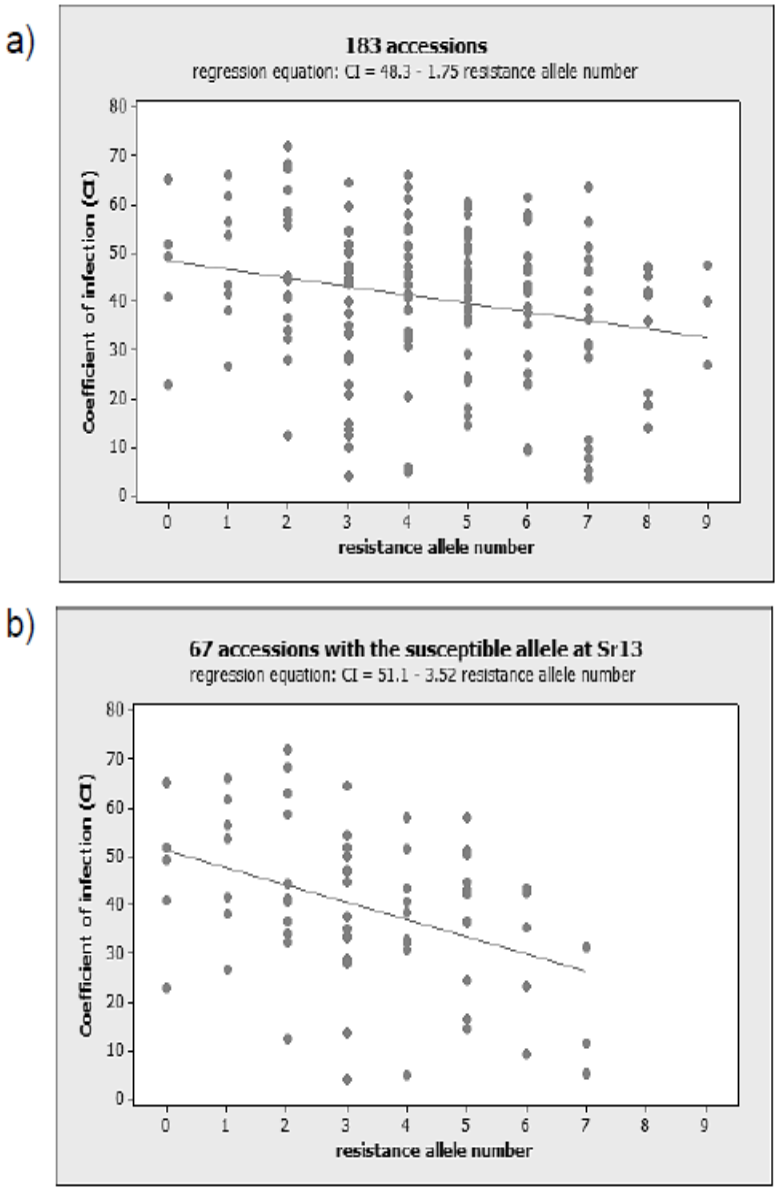

Figure 8. Scatter plot of the coefficient of infection values of the elite accessions of durum wheat on the cumulated number of beneficial alleles at the QTL-tagging markers significant ($P < 0.05$) in three or four seasons. Results are shown for the stem rust response averaged over four evaluation seasons of the 183 accessions (**a**) and of the 67 accessions (**b**) with the susceptible allele at *CD926040*, the marker most associated to the *Sr13* region.

TABLES

Table 1. Descriptive statistics for field stem rust response (reported as Coefficient of Infection) of the 183 elite durum wheat accessions evaluated in four growing seasons in Ethiopia.

Season [a]	CI		
	Mean	Min	Max
DZo-2009	42.2	0.2	80.0
DZm-2009	36.9	0.0	80.0
DZo-2010	49.3	0.0	90.0
DZm-2010	33.6	7.9	68.2
Mean	40.5	3.5	72.0

[a]: stem rust response evaluation carried out in Debre Zeit (DZ) Agricultural Research Center; DZo-2009: off season field trial evaluation carried out in 2009 (January to May); DZm-2009: main season evaluation in 2009 (July to November); DZo-2010: off season evaluation in 2010; DZm-2010: main season evaluation in 2010.

Table 2. Frequency distribution of stem rust responses averaged over four growing seasons in Ethiopia for the 183 elite durum wheat accessions included in the association mapping durum panel.

Season	Stem rust response [a]					
	(DSS < 10%)	(DSS 10-20%)	(DSS 30%)	(DSS 40%)	(DSS 50-60%)	(DSS 70-100%)
DZo-2009	0.06 (10) [b]	0.06 (10)	0.12 (20)	0.26 (43)	0.45 (75)	0.05 (8)
DZm-2009	0.11 (16)	0.12 (17)	0.19 (27)	0.18 (26)	0.28 (40)	0.13 (19)
DZo-2010	0.05 (9)	0.06 (10)	0.10 (17)	0.16 (27)	0.34 (58)	0.30 (51)
DZm-2010	0.15 (22)	0.10 (14)	0.17 (25)	0.22 (31)	0.30 (43)	0.06 (8)
Mean	0.05 (9)	0.09 (17)	0.10 (19)	0.18 (33)	0.51 (94)	0.06 (11)

[a]: classification of response based on the Disease Severity Score (DSS) as reported in Singh et al. (2009)
[b]: frequencies values; values within brackets report the actual accession numbers

Table 3. Mean and range of stem rust response (reported as Coefficient of Infection) in the five main germplasm subgroups of the association mapping durum wheat panel.

Environment	Subgroup 1 (S1) ICARDA drylands (11)[a]			Subgroup 2 (S2) ICARDA temperate (55)			Subgroup 3 (S3) Italian and early '70s CIMMYT (26)			Subgroup 4 (S4) late '70s CIMMYT (56)			Subgroup 5 (S5) late '80s CIMMYT (35)		
	Mean	Min	Max	Mean	Min	Max	Mean	Min	Max	Mean	Min	Max	Mean	Min	Max
DZo-2009	40.6	9	70	41.6	0.2	70	32.3	0.2	60	44.9	6	80	45.4	4	70
DZm-2009	27.4	6	54	34.5	2	80	22.7	0	63	42.7	3	80	44	3	80
DZo-2010	45.7	27	70	44.5	3	80	38.3	0	80	49	9	90	66.2	12	80
DZm-2010	43.5	9.4	60.	32.2	7.9	60.5	27.4	7.9	60.5	33.7	11	52.7	36.2	7.9	68.2
Mean	39.6	14.6	58	38.6	3.5	67.5	29.4	3.8	58.7	42.7	7.5	66.2	47.5	9.5	72

Least significant difference (LSD) among subgroups = 4.99 (*P* 0.05)

[a]: number of accessions belonging to each subgroup

Table 4. Quantitative trait loci (QTLs) for stem rust response identified through association mapping in a panel of 183 elite durum wheat accessions evaluated in Ethiopia, with significant effects observed over three to four evaluation seasons. For each QTL, the chromosome position, the associated markers and the QTL features are reported.

Chr.	Most associated marker	Position (cM)[a]	Seasons with significant marker-trait associations	R^2 range (%)[b]	R^2 (%)[c]	Associated markers in the QTL region	Interval width (cM)[a]
1BS	*barc8*	32	DZm-2009, DZo-2010, DZm-2010	3.2 - 5.6	4.6	*gwm1100*, wPt-2999, wPt-4605, tPt-8831, wPt-9864, wPt -3582, wPt-4133, wPt-1876, wPt-5899, wPt-4729	9
2AS	*gwm1045*	87.7	DZm-2009, DZo-2010, DZm-2010	3.3 - 5.8	3.9	*gwm425*, *cfa2263*	12.5
2BL	*wmc356*	220	DZo-2009, DZm-2009, DZo-2010	3.2 - 6.6	4.1	-	0
3AS	wPt-7992	8	DZo-2009, DZm-2009, DZo-2010, DZm-2010	1.7 - 4.7	3.3	wPt-6854, *barc12*, wPt-1111	3.5
3AL	*wmc388*	85.6	DZo-2009, DZo-2010, DZm-2010	1.9 -4.1	4	-	0
5AL	*gwm126*	93.3	DZo-2009, DZo-2010, DZm-2010	1.8 -4.8	4.1	-	0
5AL	*gwm291*	111.7	DZo-2009, DZm-2009, DZo-2010,	2.7 -5.7	4.4	-	0
6AL	*gwm427*	139.5	DZo-2009, DZm-2009, DZm-2010	1.7 -6.8	3.5	*wmc580*	0.1
6AL	*CD926040*	144	DZo-2009, DZm-2009, DZo-2010, DZm-2010	3.5 - 11.3	7.1	wPt-9474, wPt-4229, wPt-5654, wPt-3247, wPt-4663	9.3
6AL	*barc104*	155.3	DZo-2009, DZm-2009, DZm-2010	6.1 - 9.7	4.5	-	0
7AS	wPt-2799	38.2	DZo-2009, DZm-2009, DZo-2010, DZm-2010	1.7 - 4.9	5.2	*barc70*, *gwm1187*, *wmc479*	6.3
7AS	wPt-7785	94.8	DZo-2009, DZo-2010, DZm-2010	1.1 - 2.3	1.5	-	0

[a]: position of the QTL most associated marker as from the durum consensus map used as reference
[b]: range of R^2 value across the three to four evaluation seasons with significant marker-trait association
[c]: R^2 value for the marker most associated to the QTL (averaged over the four evaluation seasons)

Table 5. Quantitative trait loci (QTLs) for stem rust response identified through association mapping in a panel of 183 elite durum wheat accessions evaluated during four seasons in Ethiopia, with significant effects observed in two out of four evaluation seasons. For each QTL, the chromosome position, the associated markers and QTL features are reported.

Chr.	Most associated marker	Position (cM)[a]	Seasons with significant marker-trait associations	R^2 range (%)[b]	R^2 (%)[c]	Associated markers in the QTL region	Interval width (cM)[a]
1AS	*gpw2246*	0	DZo-2009, DZm-2009	2.3 - 4.7	3.1	-	0
1AS	wPt-5411	69.6	DZm-2009, DZo-2010	1.4 - 1.6	1.3	*gwm164*	1
1BL	*cfd65*	40.8	DZm-2009, DZm-2010	3.5 - 3.8	2.4	wPt-8168, *gwm947*	11
1BL	wPt-0202	85.7	DZo-2009, DZm-2009	1.4 - 2.0	1	wPt-0506, wPt-3227	0.6
2AS	wPt-7049	26.9	DZo-2010, DZm-2010	1.7 - 3.2	1.6	*barc212*	4
2BS	wPt-8404	75.7	DZm-2009, DZm-2010	2.2 - 6.1	1.6	*wmc257*, *wmc243*, *wmc25*	2
2BL	*wmc361*	29	DZm-2009, DZo-2010	2.5 - 4.3	2	-	0
2BL	*gwm1300*	169.1	DZo-2009, DZm-2010	1.6 - 8.0	1.7	wPt-5242	1
3AL	wPt-1923	46.4	DZm-2009, DZm-2010	2.2 - 4.5	2.2	wPt-3348, wPt-1652	0
3AL	*wmc428*	110.5	DZo-2009, DZm-2009	4.4 - 6.8	3.8	-	0
3AL	wPt-8203	200.3	DZo-2009, DZm-2009	1.9 - 2.5	1.8	*barc1177*	5.9
4AL	wPt-9196	102.4	DZo-2010, DZm-2010	1.0 - 1.5	1	wPt-2985, wPt-8886 wPt-8271 wPt-8167 wPt-3108 wPt-3796 wPt-6502 wPt-7821	6.9

[a]: position of the QTL most associated marker as from the durum consensus map used as reference
[b]: range of R^2 value across the evaluation seasons with significant marker-trait association

[c]: R^2 value for the marker most associated to the QTL (average over the four evaluation seasons)

Table 5. Continued….

Chr.	Most associated marker	Position (cM)[a]	Seasons with significant marker-trait associations	R^2 range (%)[b]	R^2 (%)[c]	Associated markers in the QTL region	Interval width (cM)[a]
4AL	wPt-0798	111.0	DZo-2009, DZm-2010	2.8 - 2.9	1.9	wPt-5055	0.0
4BL	wPt-8543	101.9	DZo-2009, DZo-2010	1.2 - 2.9	1.4	-	0.0
5BL	wPt-9300	118.9	DZm-2009, DZo-2010	1.2 - 1.9	1.1	wPt-2453, wPt-1733	0.0
6AS	wPt-7330	18.6	DZm-2009, DZo-2010	1.2 - 3.6	1.6	wPt-1742, wPt-5395, wPt-5633, tPt-6710, wPt-1377, wPt-9075, wPt-6520, wPt-7754, wPt-4016,wPt-4017, wPt-3468	7.5
6AL	tpt-4209	109.6	DZm-2009, DZo-2010	2.3 - 2.6	1.5	*gwm1150*	8.4
6AL	*gwm169*	126.6	DZo-2009, DZm-2010	2.0 - 3.0	1.5		0.0
6BS	wPt-1437	41.9	DZo-2009, DZo-2010	2.2 - 2.3	1.3	wPt-2095, wPt-7935	2.4
7AS	wPt-5489	0.0	DZo-2009, DZo-2010	1.5 - 1.8	2.0		0.0
7AL	wPt-0745	248.4	DZo-2009, DZm-2010	1.7 - 2.2	1.3	wPt-7763	0.0
7BS	*gwm573*	66.6	DZo-2009, DZm-2009	2.9 - 5.7	3.4	*gwm1184*,*wmc182*	6.2
7BL	*wmc517*	155.6	DZm-2009, DZm-2010	3.5 - 3.6	2.3	-	0.0
7BL	wPt-8615	175.9	DZo-2010, DZm-2010	2.3 - 2.7	2.1	wPt-5343, wPt-1715, wPt-4298, wPt-4869, wPt-7362, wPt-4010, wPt-7191, wPt-7351, Pt-8417, wPt-4045, *gwm611*	21.0

[a]: position of the QTL most associated marker as from the durum consensus map used as reference

[b]: range of R^2 value across the evaluation seasons with significant marker-trait association

[c]: R^2 value for the marker most associated to the QTL (average over the four evaluation seasons)

Table 6. Allele frequencies and phenotypic coefficients of infection (CI) least square means for the markers most associated to the QTLs consistently observed over three to four evaluation seasons. Data are reported for the common allelic variants only (frequency ≥ 0.10).

				CI least square means				
Chromosome	Marker	Allele [a, b]	Allele frequency	DZo-2009	DZm-2009	DZo-2010	DZm-2010	Mean over four seasons
1BS	*barc8*	257	0.23	52.7	**70**	**87.2**	**48.3**	**63.6 b**
		255*	0.77	46.9	**46.4**	**67.9**	**36.3**	**49.4 a**
2AS	*gwm1045*	Null	0.12	52.3	**60.9**	**84.8**	**51.9**	**62.1 b**
		180*	0.76	50.5	**47.5**	**74.4**	**39.1**	**52.9 a**
		172	0.12	56.5	**64.1**	**95.2**	**48.1**	**65.9 c**
2BL	*wmc356*	180*	0.12	**22.4**	**20**	**61.1**	23.3	**32.4 a**
		178	0.69	**40.2**	**34.2**	**74.3**	33.7	**45.5 b**
		176	0.19	**47.6**	**37.9**	**89.9**	27.5	**49.9 c**
3AS	wPt-7992	1	0.21	**59.9**	**64.1**	**80.9**	**47.8**	**62.3 b**
		0*	0.79	**50.2**	**49.9**	**73.3**	**40.4**	**53.3 a**
3AL	*wmc388*	250	0.29	**61.4**	53.3	**75.7**	**43.4**	**57.4 b**
		258	0.38	**60.2**	55.9	**81.6**	**44.6**	**60.8 c**
		275*	0.33	**47.9**	45.9	**70.7**	**35.5**	**49.6 a**
5AL	*gwm126*	nu11	0.46	**51**	48.8	**74.8**	**41.3**	**53.7 b**
		214*	0.42	**41.8**	41	**67.2**	**32.9**	**44.8 a**
		208	0.12	**49.8**	44.9	**77.2**	**42.3**	**52.5 b**
5AL	*gwm291*	166	0.45	**49.9**	**48.3**	**71.8**	39.1	**51.9 b**
		160	0.4	**54.6**	**59.2**	**81.9**	43.2	**59.6 c**
		139*	0.15	**42.9**	**44.9**	**60.7**	39.7	**47.3 a**
6AL	*gwm427*	212	0.72	**55.1**	**53.6**	**76.4**	45.1	**57.5 b**
		188*	0.28	**45.8**	**43.6**	**69.9**	33.4	**48.5 a**
6AL	*CD926040*	855*	0.32	**50.1**	**49.8**	**73.1**	**39.8**	**53.4 a**
		851	0.4	**68.9**	**61.3**	**83.8**	**55.7**	**66.9 b**
		845	0.28	**61.8**	**67.8**	**89.6**	**48.6**	**67.3 b**
6AL	*barc104*	206*	0.21	**50.6**	**68.6**	76.6	**32.9**	**56.2 b**
		202*	0.3	**50**	**48.9**	73.7	**37.9**	**52.4 a**
		172	0.49	**62.9**	**68.4**	81.9	**49.1**	**63.8 c**
7AS	wPt-2799	1*	0.42	**48.2**	**44.7**	**70.5**	**36.6**	**49.9 a**
		0	0.58	**55.6**	**59.3**	**78.2**	**45.3**	**59.6 b**
7AS	wPt-7785	1	0.78	**48.6**	46	**71.8**	**38.7**	**50.9 b**
		0*	0.12	**40.9**	41.9	**64.9**	**31.3**	**45.1 a**

[a]: molecular weight (bp) of the alleles at SSR markers; presence (1) or absence (0) of the band at DArT markers (wPt-).
[b]: "*" indicates the most resistant allele
Least square means reported with bold font refer to the marker-environment pairs showing significant associations. For each locus, the least significant difference between the allele means over four seasons was calculated: means followed by different letters are significantly different ($P \leq 0.05$).

Table 7. Allele frequency within each of the five germplasm subgroups (S1 to S5) for the markers most associated to the QTLs consistently observed over three to four evaluation seasons.

			Frequency with in subgroups				
Chromosome	Marker	Allele [a, b]	Subgroup 1 (S1), ICARDA drylands ((11) a)	Subgroup 2 (S2), ICARDA temperate, (55)	Subgroup 3 (S3) Italian and early'70s CIMMYT (26)	Subgroup 4 (S4) Late '70s CIMMYT (56)	Subgroup 5 (S5) late '80s CIMMYT (35)
1BS	*barc8*	257	0	**0.2**	**0.73**	0.12	0.15
		255*	1	**0.8**	**0.27**	0.89	0.85
2AS	*gwm1045*	Null	**0.2**	**0.18**	**0.13**	0.1	0.07
		180*	**0.8**	**0.79**	**0.25**	0.88	0.93
		172	**0**	**0.03**	**0.69**	0.02	0
2BL	*wmc356*	180*	0	**0.3**	0	0.05	0
		178	1	**0.62**	0.17	0.84	1
		176	0	**0.08**	0.83	0.11	0
3AS	wPt-7992	1	0.09	**0.33**	**0.29**	0.17	0.06
		0*	0.91	**0.67**	**0.71**	0.83	0.94
3AL	*wmc388*	250	0	**0.37**	0.65	**0.28**	**0.06**
		258	0	**0.43**	0.25	**0.52**	**0.25**
		275*	1	**0.2**	0.1	**0.2**	**0.69**
5AL	*gwm126*	nu11	0.18	**0.43**	**0.2**	**0.43**	0.85
		214*	0.82	**0.41**	**0.48**	**0.48**	0.15
		208	0.1	**0.16**	**0.32**	**0.09**	0
5AL	*gwm291*	166	0.1	0.47	**0.13**	0.42	0.77
		160	0	0.39	**0.63**	0.54	0.2
		139*	0.9	0.14	**0.24**	0.04	0.02
6AL	*gwm427*	212	0	**0.63**	0.87	**0.8**	**0.58**
		188*	1	**0.37**	0.13	**0.2**	**0.42**
6AL	*CD926040*	855*	0.18	**0.24**	0.08	**0.22**	**0.79**
		851	0.72	**0.44**	0.65	**0.36**	**0.12**
		845	0.1	**0.32**	0.27	**0.42**	**0.09**
6AL	*barc104*	206*	**0.33**	**0.28**	**0.24**	**0.2**	0.06
		202*	**0.33**	**0.24**	**0.05**	**0.12**	0.84
		172	**0.33**	**0.48**	**0.71**	**0.67**	0.1
7AS	wPt-2799	1*	**0.3**	**0.37**	**0.45**	**0.25**	**0.73**
		0	**0.7**	**0.63**	**0.55**	**0.75**	**0.27**
7AS	wPt-7785	1	**0.5**	**0.78**	**0.73**	**0.8**	1
		0*	**0.5**	**0.22**	**0.27**	**0.2**	0

[a]: molecular weight (bp) of the alleles at SSR markers; presence (1) or absence (0) of the band at DArT markers (wPt-).
[b]: "*" indicates the most resistant allele.
Least square means reported with bold font refer to the marker-environment pairs showing significant associations.

CHAPTER 5. GENOME-WIDE SEARCH OF STEM RUST RESISTANCE LOCI AT THE SEEDLING STAGE IN DURUM WHEAT

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5.1 ABSTRACT

Stem rust, caused by *Puccinia graminis* f. sp. *tritici* (*Pgt*), is one of the most destructive diseases of both durum and bread wheat. Recently emerged races in East African such as TTKSK (or Ug99), possess broad virulence to durum cultivars and only a limited number of known genes were effective. Association mapping (AM) was used to identify quantitative trait loci (QTLs) for resistance to stem rust races in a panel of 183 elite durum wheat accessions tested under controlled conditions at seedling stage. The panel was genotyped with 1,250 SSR and DArT® markers. Overall 15, 20, 19 and 19 chromosome regions were significantly associated to the response to TRTTF, TTTTF, TTKSK (Ug99) and JRCQC races, respectively. QTL R^2 values ranged from 1.13 to 23.12%. One QTL on chromosome 5A and two on chromosome 6A provided resistance to all tested races whilst the majority showed significant effects to one or two races. Some QTLs were co-located with known *Sr* genes (e.g. *Sr9*, *Sr13* and *Sr14*) while others mapped in chromosome regions where no *Sr* genes have previously been reported. Two major QTLs on chromosome arm 6AL mapped in the region known to harbour *Sr13* and showed major effects on TTTTF, TTKSK and JRCQC (R^2 up to 23.12%) and a minor effect on TRTTF. The combined analysis of the results of this study with those on adult plant resistance in Ethiopia where such races are present provide valuable indications to identify suitable parental lines for further improving stem rust resistance of durum wheat.

5.2 INTRODUCTION

Durum wheat (*Triticum turgidum* ssp. *durum* Desf.) is an important crop for tens of millions in the Mediterranean Basin, particularly in West Asia and North Africa (WANA) where durum wheat is grown annually over more than 13 million hectares. Mediterranean countries account for approximately 75% of global worldwide durum wheat production (Belaid, 2000; Habash et al. 2009). In Sub-Saharan Africa, Ethiopia is the largest wheat-growing country and is one of the centers of diversity for tetraploid wheat. Durum wheat represents approximately 40% of the total wheat area in Ethiopia, with a tendency to increase in response to the growing internal demand for pasta (Badebo et al. 2009).

Durum production and kernel quality can be negatively affected by rust diseases (Singh et al. 2005). Historically, stem rust infections of *Puccinia graminis* Pers. f. sp. *tritici*, (*Pgt*) have caused severe losses to wheat production (McIntosh and Brown, 1997; Eversmeyer and Kramer, 2000; Singh et al. 2011). While more than 50 stem rust resistance (*Sr*) loci have been identified in cultivated wheat and wild relatives, only a few of them remained effective against the new emerging races in East Africa, including TTKSK = Ug99 (Pretorious et al. 2000) and its variants, and even fewer are effective against the durum-specific Ethiopian races (Admassu et al. 2009; Rouse et al. 2012). In Ethiopia, Ug99 was in fact added to previously existing races, several of them specifically virulent on durum wheat (Admassu and Fekedu. 2005; Haile et al. 2012). Two such races have been characterised as TRTTF and JRCQC with a combined virulence on stem rust resistance genes *Sr9e* and *Sr13* (Olivera et al. 2012), and virulence on *Sr13* appears to be widespread in Ethiopia (Admassu et al. 2009). Very limited effective resistance (5.2%) to races TRTTF and JRCQC was found in a highly diverse collection of 996 tetraploid genotypes evaluated for field reaction at the Debre Zeit Research Station in Ethiopia (Olivera et al. 2012). Therefore, the combination of Ug99 and *Sr13*- and *Sr9e*-virulent Ethiopian races represents a major threat to the viability of the Ethiopian durum wheat production. Achieving higher and more durable stem rust resistance requires the characterisation of the genetic basis underlying the resistance present in improved germplasm or in exotic sources used for introgression in breeding. Only then can breeding programmes develop strategies to pre-emptively counter the emerging new virulence types in the pathogen populations. Whereas field resistance is the ultimate goal

sought in breeding programmes, seedling tests are a good complement for resistance characterisation as they allow large number of lines to be screened for reaction to multiple races, one race at a time, in a short period and with modest space requirements (Sun and Steffenson, 2005). Seedling screening provides information allowing postulating the presence of certain genes, based on the series of races available, without confounding effects of having several races acting at the same time, as it is often the case in field experiments.

Marker-based approaches can be used to identify genes/quantitative trait loci (QTLs) governing plant response to diseases. The standard approach is to use biparental mapping populations to relate phenotypic information to genotypic data obtained from molecular markers in order to determine the number and chromosomal location of resistance loci (Maccaferri et al. 2008; Simons et al. 2011; Singh et al. 2013). An alternative to the use of biparental mapping is association mapping (AM) or linkage disequilibrium (LD)-based mapping in which genotype-phenotype relationships are explored in germplasm collections or natural populations (Rafalski, 2002; Flint-Garcia et al. 2003). Since its first use in plants a decade ago (Thornsberry et al. 2001), AM has been used in many crops due to advances in high-throughput genotyping technologies, increased interest in identifying useful and/or novel alleles, and improvements in statistical methods (Gupta et al. 2005; Yu et al. 2006; Zhu et al. 2008). In both tetraploid and hexaploid wheat, AM has already proven to be an effective strategy to identify marker-trait associations for agronomically valuable traits (Breseghello and Sorrells, 2006; Crossa et al. 2007; Maccaferri et al. 2010, 2011), including resistance to stem rust (Yu et al. 2011) and leaf rust (Maccaferri et al. 2010) in durum wheat. Linkage disequilibrium (LD) in the cultivated durum wheat germplasm ranges from 5 to 10 cM (Maccaferri et al. 2005), thus enabling a whole-genome scan analysis for marker-trait associations with a relatively modest number of markers as compared to species with lower LD.

The objectives of this study were a genome-wide search in durum wheat for resistance loci to races TRTTF, TTTTF, TTKSK and JRCQC of *P. graminis* f. sp. *tritici* at the seedling stage and the identification of genomic regions suitable for marker-assisted selection and further genetic dissection.

5.3 MATERIALS AND METHODS

5.3.1 Plant materials

One hundred eighty-three accessions from different durum wheat-growing regions of Mediterranean countries (Italy, Morocco, Spain, Syria and Tunisia), Southwestern USA and Mexico already used in previous AM analysis for stem rust resistance under field conditions (Letta et al. 2013) were analysed in this study (**Appendix 1**). A detailed description of the accessions at the molecular and phenotypic level is reported in Maccaferri et al. (2006 and 2010).

5.3.2 Stem rust evaluation at seedling stage

Pathogen races

The AM panel was evaluated for reaction to four *Pgt* races: TRTTF, TTTTF, TTKSK, and JRCQC. The race designation is based on the letter code nomenclature system (Roelfs and Martens, 1988; Roelfs et al. 1993), modified to further delineate races in the TTKS lineage (Jin et al. 2008). These races were selected based on their differential virulence pattern and/or importance for durum wheat. Race TTKSK (Ug99) has a wide virulence spectrum and is rapidly evolving in East Africa. Race TTTTF is the most widely virulent race known in the United States, producing high infection types on the majority of stem rust differential lines (Jin et al. 2007). Races TRTTF and JRCQC, both present in Ethiopia, possess a virulence combination that overcomes both the resistance genes *Sr13* and *Sr9e,* two genes present at high frequency in durum wheat (Klindworth et al. 2007). Information about the stem rust isolates used in the disease phenotyping tests is summarised in **Table 1**.

5.3.3 Inoculation, incubation and disease assessment

The AM panel was evaluated under controlled conditions using a completely randomised design with two replications (over time) for each of the four races. Five to six seedlings per line were inoculated on the fully expanded primary leaves 8 to 9 days after planting. This work was conducted at the Cereal Disease Laboratory, St. Paul, MN, USA and the

experimental procedures in inoculation and disease assessment were done as described by Jin et al. (2007). Wheat cultivar McNair 701 (CItr 15288) was used as susceptible control. Plants were evaluated for their infection types (ITs) 14 days post-inoculation using the 0-4 scale according to Stakman et al. (1962), where ITs of 0 - ; - 1 - 2 or X are considered as low IT and ITs of 3 or 4 are considered as high ITs. Lines giving variable reactions between experiments were repeated again to confirm the most likely reactions.

5.3.4 Statistical analysis

Stakman's ITs were converted to a linear scale using a conversion algorithm proposed by Zhang et al. (2011). Briefly, infection types are converted as follows: 0, 1^-, 1, 1^+, 2^-, 2, 2^+, 3^-, 3 and 3^+ are coded as 0, 1, 2, 3, 4, 5, 6, 7, 8 and 9, respectively. The symbol for hypersensitive flecks (;) is converted to 0 and IT 4 is converted to 9. Special annotation codes C and N are ignored. Double minus and double plus annotations are converted to single minus and single plus, respectively. Complex ranges such as; 12^+ are first collapsed to $;2^+$. Then the first and last ITs of the ranges are converted and averaged; with the first IT double-weighted because the most prevalent IT is listed first. Infection types X^-, X, and X^+ are converted to linearised scores of 4, 5, and 6, respectively. These linearised 0-9 scale values were used for subsequent statistical analysis.

The heritability of linearised infection type responses was calculated for each of the four races on a mean basis across two replications according to the following: $h^2 = \sigma^2_G / (\sigma^2_G + \sigma^2_E/r)$ where r = number of replicates, σ^2_G = genotypic component of the MS among accessions and $\sigma^2_E = MS_{error}$, with MS indicating the mean square values as from the ANOVA results.

The dendrogram analysis was carried out using NTSYS-pc software version 2.0 (Rohlf 1997) and was based on the virulence phenotypes (Infection Types estimated with a 0-4 scale) of the races; the distances among races have been computed using the standardised Manhattan distances ('city-block' method).

5.3.5 Molecular profiling

Genomic DNA extraction and other molecular procedures were carried out as described in Maccaferri et al. (2010). The accessions were profiled with 350 simple sequence repeat loci (SSR), 900 Diversity Array Technology (DArT) markers and three additional sequence tagged site (STS) markers including those previously reported to be associated to major stem rust resistance genes (Yu et al. 2010).

5.3.6 SSR markers

The SSR primers were chosen among the publicly available sets catalogued in the GrainGenes database (http://wheat.pw.usda.gov) as BARC (*barc* marker loci), CFA-CFD-GPW from INRA (*cfa*, *cfd* and *gpw,* respectively), KSUM (*ksum*), WMC (*wmc*) and WMS (*gwm*); an additional subset of private genomic WMS primers from Trait Genetics (supplied by M. Ganal, TraitGenetics, Gatersleben, Germany) were also used. The SSR loci used to genotype the accessions were preselected for (i) clarity and repeatability of the amplicons, (ii) polymorphism level and (iii) even distribution on the A- and B-genome linkage groups. The choice was carried out based on the results of a survey of SSR primer pairs conducted on a small subset of eight germplasm founder accessions and lines used as parents of mapping populations. A unique thermo-cycling protocol (Maccaferri et al. 2008) was implemented for all primer sets and SSR profiles using an automated LI-COR 4200 IR2 System (LiCor, Lincoln, NE, USA). Genotyping was performed for most SSR markers using the M13-labeled primers and amplification protocol (Schuelke, 2000). Alleles were scored using founder genotypes as an allele reference set.

5.3.7 DArT markers

DArT markers were generated by Triticarte Pty. Ltd. (Canberra, Australia; http:// www.triticarte. com. au), as described by Akbari et al. (2006). The Durum wheat *Pst*I / *Taq*I array v 2.0, containing 7600 single DArT clones (Mantovani et al. 2008) was used for genotyping the panel. The locus designation used by Triticarte Pty. Ltd. was adopted (‘wPt’,

'rPt' and 'tPt' loci corresponding to the respective clones of the genomic representation), and alleles at polymorphic loci were scored as hybridisation positive (1) or negative (0).

5.3.8 Association mapping

For AM analysis, only markers with non-rare alleles (frequency > 0.10) in the AM Durum Panel were considered for the LD and marker-trait association analysis, thus reducing the false positives and the LD inflation effects which have frequently been attributed to the use of rare alleles (Myles et al. 2009). Rare alleles were considered as missing data. Data-points showing residual allelic heterogeneity within accession were also considered as missing data. In total, 1,211 markers were used for marker-phenotype association tests. Among these, 323 SSRs and STSs and 538 DArT markers were projected onto a consensus linkage map obtained from the genotypic data of five recombinant inbred line (RIL) mapping populations, using the Carthagene v. 4.0 software (de Givry et al. 2005). The RIL mapping populations included four populations from the crosses Kofa × Svevo (KS RIL, Maccaferri et al. 2008), Colosseo × Lloyd (CL RIL, Mantovani et al. 2008), Meridiano × Claudio (MC RIL, Maccaferri et al. 2011) and Simeto × Levante (SL RIL, Maccaferri et al. unpublished), developed by a joint effort of Produttori Sementi Bologna SpA (Argelato, BO, Italy) and DipSA, Bologna, Italy, and one population obtained from the cross Kofa × UC1113 (KU RIL population, Zhang et al. 2008) whose genotypic data were downloaded from the GrainGenes web database. The consensus linkage map was obtained from the five data-sets using the Carthagene v.4.0 software (de Givry et al. 2005). Merging was performed with the *dsmergen* command, after checking for marker order consistency across maps, so that for each marker pair a single recombination rate was estimated based on all available meioses. A framework-mapping method was applied. Non-framework markers were incorporated in the framework map by building a complete map using the framework map as a fixed order. The marker order and inter-marker genetic distances from the consensus map were used to report the LD and association results. The remaining 332 polymorphic and informative DArT markers and 17 SSRs that could not be integrated into the final map were not further considered. Details on the consensus map are reported in Letta et al. (2013).

5.3.9 Genetic structure and linkage disequilibrium analysis

The genetic structure of the panel has been investigated with a combination of model- and distance-based analyses. Model-based population structure was assessed deploying a selection of 96 loosely linked, highly informative and evenly spaced SSRs and using the program STRUCTURE v. 2 (Pritchard et al. 2000). Details for structure and kinship analysis were reported in Maccaferri et al. (2010, 2011). The program TASSEL (http://www.maizegenetics. net) was used to estimate the LD parameters *D'* and r^2 values as a function of the corresponding inter-marker distances and the comparison-wise significance was computed with 10,000 permutations. The r^2 parameter was estimated for all loci on the same chromosome and compared based on the genetic distances measured in cM. If all pairs of adjacent loci within a given chromosomal region were in LD, this region was referred to as an LD block (Stich et al. 2005).

5.3.10 Marker-phenotype association analysis

Genome-wide scan for loci governing stem rust resistance at the seedling stage was conducted using phenotypic data converted to a linear scale. AM analysis was carried out with TASSEL, ver. 2.1 (www. maizegenetics.net; Yu et al. 2006). The 323 SSRs and STSs and 538 DArT markers were tested for significance of marker-trait associations under: (i) the fixed general linear model (GLM) including the Q population structure results as covariates (Q GLM), (ii) the mixed linear model (MLM) including the Q population structure results plus the K kinship matrix (Q + K MLM).

For GLM analysis, besides the marker-wise association probability values, the experiment-wise association significance probability was obtained based on a permutation test implemented in TASSEL (10,000 permutations were used). The experiment-wise *P*-value provides a much more severe test of significance (adjusted *P*-value) that corresponds to the experiment-wise error and was used to make decisions about the significance of marker effects (Bradbury et al. 2007). In the MLM analysis, experiment-wise significance was

inspected using the False Discovery Rate (FDR) approach according to Storey and Tibshirani (2003) and implemented in *Qvalue* program.

Multiple adjacent co-segregating significant markers were assigned to a unique QTL region upon meeting the following conditions: less than 20 cM of inter-marker genetic distance, presence of significant and strong LD among the markers (with r^2 values ≥ 0.4) within the QTL region and consistency of allelic effects across significant markers (Massman et al. 2011).

5.4 RESULTS

5.4.1 Seedling evaluations

Seedling ITs for each of the 183 durum accessions are presented in the **Appendix 3**. The ITs frequency distribution presented in **Figure 1** depicts a continuous variation for all four races, with that for JRCQC being skewed toward susceptibility scores (3 and 4). The analysis of variance for stem rust seedling response showed highly significant differences ($P \leq 0.0001$) among races and accessions with highly significant effects of subgroups of accessions and subgroup × race interaction (results not reported). The highly variable classification and ranking of the accessions (**Appendix 3**) based on their responses to the different races supports the significance of the race × accession interaction. Heritability of the linearised IT values was high for all four races, ranging from h^2 = 93.0% for race TTTTF to h^2 = 98.9% for TTKSK.

The frequencies of accessions categorised as resistant, susceptible and heterogeneous in their reaction to the four races varied markedly depending on the race (**Table 2**). Seedling resistance to TRTTF, TTTTF, TTKSK and JRCQC was observed in 149 (81.4%), 117 (63.9%), 106 (57.9%) and 87 (47.5%) accessions, respectively (**Table 2**), with the race TRTTF from Yemen showing the lowest degree of virulence and the Ethiopian race JRCQC showing the highest degree of virulence among the four races. Sixty-six (36.1%) accessions were resistant (IT = 0 to 23) to all four races. **Figure 2** reports the pattern of diversity among the four stem rust races based on the UPGMA-cluster analysis of the avirulence/virulence patterns on the 183 durum accessions. The dendrogram clearly shows that the races grouped

into three well-distinct groups with TTTTF and TTKSK that clearly clustered together while TRTTF and JRCQC showed independent virulence patterns. Highly significant correlations of ITs among genotypes were observed for all the four races. In particular, relatively high *r*-values were observed for the pair-correlations of TTKSK *vs*. TTTTF (0.72), TTTTF *vs.* JRCQC (0.57), TRTTF *vs.* TTTTF (0.51), TTKSK *vs*. TRTTF (0.46). The correlation of ITs between JRCQC and TTKSK or JRCQC and TRTTF was rather weak (0.36 and 0.15, respectively).

5.4.2 Relationship between population structure and seedling response to stem rust

The genetic relationships among the accessions were investigated using both a genetic-similarity and a model-based Bayesian clustering method and the results have been reported elsewhere (Maccaferri et al. 2006, 2011; Letta et al. 2013). Both methods pointed out that the minimum and optimum number of hypothetical well-distinct subgroups present in the panel was equal to five, corresponding to clearly distinct breeding lineages (from S1 to S5). Each subgroup contains 11, 55, 26, 56 and 35 accessions, respectively. The differences for seedling stem rust response among the five subgroups were highly significant ($P \leq 0.001$, data not shown). The coefficients of membership to the five main subgroups as estimated with STRUCTURE were used to assess the effect of population structure to single race responses by means of multiple regressions. The percentage of phenotypic variation accounted for by population structure ranged from a minimum of 9.09% for race TTKSK to a maximum of 12.15% for race JRCQC.

The percentages of resistant and susceptible accessions for each of the five main subgroups are reported in **Table 3**. This clearly shows that all five subgroups included accessions with different responses thus indicating that all subgroups are equally informative for AM purposes. The complete dataset of seedling phenotypic response and population structure membership coefficients for each of the 183 accessions included in the association panel is reported as **Appendix 3**.

The ranking values of the four races based on their frequencies of avirulence/ virulence interactions considering the germplasm collection as a whole (with TRTTF showing the

highest degree of avirulent interactions, followed by TTTTF, TTKSK and finally JRCQC, which showed the highest frequency of virulent interactions) was roughly confirmed when considering each of the five different subgroups of germplasm accessions separately (**Table 3**). One exception was observed for the race virulence spectrum to accessions of subgroup 3 (including the Italian and early '70s CIMMYT germplasm) where race TTTTF showed the highest frequency of avirulence and race TTKSK resulted the most virulent. Differences among subgroups for frequency of resistance were observed in the proportion of accessions resistant to a given race. For all four races, subgroup 5 (CIMMYT germplasm of late 80s, early 90s) had the highest frequency of seedling resistant accessions, mostly scored as IT = 2 (**Table 3**). On the other hand, subgroup 1 (ICARDA accessions for rainfed environments), which is also the least represented within the panel, had the highest frequency of susceptible accessions, except when considering TRTTF, for which only subgroup 3 showed a higher frequency of susceptible accessions. Overall, more accessions in subgroups 4 and 5 showed resistance to all the four races than in the other subgroups.

5.4.3 Association mapping for seedling response to stem rust

The results of AM are reported in **Table 4**. AM revealed several putative QTLs for stem rust resistance at the seedling stage using the four races. In total, 41 distinct QTLs represented by either single markers or sets of closely linked markers, were found to be significantly associated to the seedling response to the four tested races under the Q + K MLM models, with 15, 20, 19, and 19 QTLs for the response to TRTTF (marker R^2 from 1.13 to 8.34%), TTTTF (R^2 from 1.92 to 17.64%), TTKSK (R^2 from 1.75 to 23.12%), and JRCQC (R^2 from 1.51 to 15.33%), respectively (**Table 4**). All these regions identified with the Q + K MLM showed significant effects also with the Q GLM model. In some cases, the presence of a QTL was evidenced by multiple significant associations at linked SSR and DArT markers within 10 cM, as estimated from the durum consensus map and LD r^2 values higher than 0.4 in most cases (result not reported). Using a more stringent model including the False Discovery Rate (FDR) multiple testing correction and Q + K MLM model, the number of chromosomal regions (QTLs) that showed significant ($P \leq 0.05$) associations were 4, 3, 4 and 4 for races TRTTF, TTTTF, TTKSK and JRCQC, respectively (**Table 4**), while the Q GLM model detected a higher number of significant markers.

The most important region in terms of significance and R^2 effects was observed on chromosome arm 6AL, a 28.7 cM interval harboring four distinct QTLs with R^2 values ranging from 1.51 to 23.12%. Within this large interval, noticeable associations across the four races were found at the two sites tagged by *CD926040* (143.9 cM on the consensus linkage group) and *barc104* (155.3 cM). These two sites (*CD926040* and *barc104*) showed consistently high R^2 values (from 10.47 to 23.12%) for races TTTTF, TTKSK and JRCQC. Conversely, *CD926040* and *barc104* showed only a limited effect, though still significant, for race TRTTF (R^2 values equal to 3.52 and 2.84% respectively).

In terms of significance across all four races, apart from the two sites on chromosome arm 6AL, only one QTL on chromosome 5A (*gwm410*) showed significant effects in response to all the four races considered in this study. Two genomic regions were identified on chromosomes 1B (*barc61*) and 2A (*wPt-5839*) that were putatively effective across three races (TRTTF, TTTTF and TTKSK but not for race JRCQC at both regions). The R^2 values of marker on chromosome 1B ranged from 2.27 to 2.45% while marker on chromosome 2A explained from 1.60 to 2.44% of the phenotypic variation. On chromosome 3A, marker *wPt-1923* tagged a region significant for TTTTF, TTKSK and JRCQC with R^2 values from 2.09 to 3.98%. Race-specific effects ($P < 0.001$) were observed for each race as following: for race TRTTF, putative genomic regions significantly affecting the response were found on chromosomes 2A, 2B and two regions on chromosome 7A. The region with the largest effect (R^2 = 8.34%) was tagged by *gwm47* on chromosome 2BL. The second and third regions with a sizeable effect to the response to race TRTTF were tagged by markers *wPt-6668* and *gwm344* on chromosome 7A with R^2 values of 2.70 and 5.79%, respectively. Marker *wPt-2293* on chromosome 2A tagged an additional region with a sizeable effect (R^2 = 4.20%) on this race. For race TTTTF, marker *wmc517* on chromosome 7B showed a significant effect (R^2 = 8.00%) that was shared with race TTKSK (R^2 = 4.96%). A QTL (R^2 = 4.87% and 2.75%) specific for TTKSK and TTTTF respectively was observed on chromosome 3B (*wmc43*). Remarkably, up to ten QTLs showed specificity for race JRCQC. These ten QTLs were tagged by *wPt-1876*, *wPt-9049*, *barc78*, *gwm1570*, *barc165*, *gwm234*, *wPt-2991*, *gwm816*, *gwm573* and *gwm333*. Among those QTLs, the most important in terms of significance and R^2 value was located on chromosome 4A, tagged by *barc78* (R^2 = 9.36%).

5.5 DISCUSSION

There is a growing interest in applying association mapping (AM) to a wide range of crops to identify genes/QTLs responsible for quantitatively inherited variation (Ersoz et al. 2009; Hall et al. 2010; Stich and Melchinger 2010; Kollers et al. 2013; Li et al. 2013;). Accordingly, a better understanding of the genetic basis underlying the naturally occurring genetic diversity for stem rust response in durum wheat could help to accelerate the progress for enhancing stem rust resistance of this crop while shedding light on the evolution of the host-pathogen relationships. Along this line, the panel of accessions herein evaluated surveys the genetic variation present in elite germplasm pool commonly used by durum breeders, a feature that makes our results more readily transferable to and more valuable for pre-breeding activities.

5.5.1 Relationship between QTLs for resistance at seedling and field evaluations

A previous study has examined the present collection for resistance to stem rust under field conditions (Letta et al. 2013) in Ethiopia, using an inoculum, which included three of the four races (TTKSK, TRTTF and JRCQC) tested herein. Such study highlighted the presence of 24 QTLs with significant effects in two out of four seasons and 12 QTLs in three out of four seasons (Letta et al. 2013). QTLs detected at seedling stage associated with resistance to the four races, namely those tagged by *CD926040* and *barc104*, were significantly and consistently (over seasons) associated with resistance under field conditions as well. *CD926040* and *barc104*, located on the long arm of chromosome 6A are linked to *Sr13* (Simons et al. 2011). Marker *gwm410* on chromosome 5AL also showed a significant effect for three seasons under field conditions and for all four races at the seedling stage. QTLs tagged by *gwm1300*, *gwm169* and *wmc517* showed significant effect to race TTKSK as well as under field conditions for two seasons. Among the markers associated with resistance to this race, *gwm1300* and *gwm169* were in the same regions harbouring *SrWeb* and *Sr26*, respectively, which are known to be effective against race TTKSK (Ug99) (Yu et al. 2011). The seedling QTL on chromosome 2B tagged by *wmc356*, which showed a significant effect with races TTTTF and JRCQC also showed significant effects in the field condition for two seasons. Seedling QTL specific to race JRCQC tagged by marker *gwm573* showed significant

effect under field conditions for two seasons. Other specific QTLs detected for the same race at loci *gwm1620* and *barc78* on chromosomes 3A and 4A, respectively, were located in regions very close to markers which showed significant effects under field conditions for four and two seasons, respectively.

5.5.2 significant markers linked to previously identified Sr genes

The analysis of the results obtained with both the Q GLM and Q + K MLM models highlighted several chromosome regions putatively harboring QTLs with main effects for stem rust response at the seedling stage. To determine whether any known resistance gene coincides with the putative genomic regions identified in this study, the current results were compared with previous findings for stem rust resistance in wheat. Several QTLs identified in this study co-located at previously reported major *Sr* loci as well as to a number of QTLs recently identified through linkage mapping in tetraploid wheat (Haile et al. 2012) and AM in hexaploid wheat (Yu et al. 2011, 2012). One QTL tagged by wPt-1876 (chromosome 1B) for response to race JRCQC corresponds to a region previously shown to influence stem rust resistance in two independent studies (Crossa et al. 2007; Yu et al. 2011). This region harbours *Sr14,* which appears effective against several stem rust races (Singh et al. 2006). However, this region did not show significant effects for race TTKSK, in accordance to the *Sr14* seedling IT reported by Jin et al. (2007). The genomic region on the distal part of chromosome 1B, tagged by *wmc44,* and associated with seedling resistance to TTTTF and JRCQC has been shown to harbour genes for multiple diseases: *Lr46/Yr29/Pm39* and a yet to be named gene for APR to stem rust (Bhavani et al. 2011; Ravi Singh, personal communication). On chromosome 2A, *cfa2201* and wPt-5839 co-located with the region known to host *Sr38* and *Sr34,* respectively. However, both genes are ineffective against races of the Ug99 lineage (Jin et al. 2007; Singh et al. 2011) and originate from *Triticum comosum* and *Triticum ventricosum*, which makes their presence in durum wheat highly unlikely. Consequently, *cfa2201* and wPt-5839 could be linked to putatively new loci. Several *Sr* genes are located on chromosome arm 2BL, including *Sr9, Sr16, Sr28* (McIntosh et al. 1995) and *SrWeb* (Hiebert et al. 2010). *SrWeb* confers resistance to Ug99 while none of the several alleles of *Sr9* confers resistance to the same race (Jin et al. 2007). Hence, the significant effects detected by *gwm1300* for race TTKSK might be tagging the presence of *SrWeb*

(Hiebert et al. 2010) while *gwm47*, detected for race TRTTF, might tag potential new alleles near or at the *Sr9* locus. Additionally, at the end of chromosome 2B, significant effects of *wmc356* to races TTTTF and JRCQC were detected, but it is unlikely that *Sr16* or *Sr28* plays any role since both genes are ineffective to these races. At chromosome arm 3BS, several *Sr* genes including *Sr2, Sr51, Sr12* and *SrB* (Yu et al. 2009) are known. Among more than 50 stem rust resistance loci identified so far, *Sr2* is one of the most widely deployed (McIntosh et al. 1995) and has provided durable adult plant rust resistance for more than 50 years. A previous study suggested that the *Sr2* APR allele is rare in the AM panel (Letta et al. 2013). Similarly, in the present study, none of the markers on chromosome arm 3BS showed significant effects near the position of *Sr2* (Mago et al. 2010). The chances of detecting *Sr2* at the seedling stage based on the IT responses were minimal, being this gene an APR locus. Nevertheless, *Sr2* has been reported to be tightly linked to a specific leaf chlorosis (mosaic) phenotype (Brown 1997), which was not observed in the entire panel (Olivera, personal communication). However, the genomic region at *wmc43* on chromosome arm 3BS conferring resistance to races TTKSK and TTTTF is in the same region of a previously reported QTL region for field stem rust resistance (Yu et al. 2011).

Four markers mapped in a 28.70 cM-wide region on chromosome arm 6AL with low LD as to each other (*gwm169*, *gwm427*, *CD926040* and *barc104*) showed significant effects in the same region previously reported to harbour genes for stem rust resistance. For instance, *gwm427*, *CD926040* and *barc104* correspond to the region reported by Simons et al. (2011) and Letta et al. (2013) while *gwm169* co-locates with *Sr26*, a gene effective against Ug99 (Singh et al. 2006) and the Ethiopian races (Badebo and Ammar, unpublished results). However, *Sr26* has been reported to be present exclusively in bread wheat following an introgression from the wild relative *Thynopirum elongatum*, thus its presence within the elite durum wheat germplasm included in this study is mostly unlikely.

A wide chromosome region including three subregions (marked by *gwm427*, *CD926040* and *barc104*) at the end of chromosome arm 6AL was strongly associated with resistance to all stem rust races. This region co-locates with *Sr13*, mapped in tetraploid wheat to the long arm of chromosome 6A within a 1.2 to 2.8 cM interval, flanked by the EST-derived markers *CD926040* and *BE471213* (Simons et al. 2011; Admassu et al. 2011; Dubcovsky et al. 2011).

In our study, *CD926040* showed the largest R^2 value and significance effects for resistance to all four races. *Sr13* is effective against the TTKS complex of *Pgt* namely TTKSK (Ug99), TTKST and TTTSK. However, virulence for *Sr13* within Ethiopian stem rust populations has been reported (Admassu et al. 2009), and recently confirmed by the characterisation of two races (TRTTF and JRCQC) collected at the site near Debre-Zeit (Olivera et al. 2012). The strong association between markers at this location and resistance to stem rust, both in seedlings inoculated with the four races and in field-grown adult plants inoculated with a mixture including *Sr13*-virulent inoculum, suggests the presence on chromosome arm 6AL of an additional gene linked to *Sr13*. Fine mapping and more precise characterisation of allelic variation present in the germplasm, on adequate genetic stocks, are needed to sort-out the precise genetic basis underlying the chromosome 6AL-related resistance to stem rust in durum wheat. Other Sr genes were mapped to chromosome 6A, such as *Sr5* and *Sr8a* which showed to be a highly effective gene for races TRTTF and JRCQC (Yue Jin, personal communication). However their mapping locations did not coincide with the 6AL-distal region.

Two significant QTLs for resistance to stem rust were found on chromosome arm 7AL, where *Sr22* and *Sr15* are located. *Sr15* is distally located on chromosome arm 7AL near *gwm344* while wPt-7299 appears to be linked to *Sr22*. Finally, AM detected a QTL at the distal end of chromosome arm 7BL near *wmc517* a region known to harbour *Sr17*, a gene linked to *Lr14a* and *Pm5* in bread wheat (Crossa et al. 2007). It is also consistent with a region reported to include a stem rust QTL in the Arina x Forno RIL population (Bansal et al. 2008).

5.5.3 Significant markers for putatively novel stem rust resistance at seedling stage

Several significant markers tagged regions where no stem rust genes had previously been reported. These regions with significant associations (one for TTKSK, one for TRTTF, two for TTTTF and three for race JRCQC) were detected on chromosome 3A where *Sr27* and *Sr35*, both effective against Ug99, have been reported (McIntosh et al. 1995; Singh et al. 2006; Jin et al. 2007). However, as *Sr27* originated from a wheat-rye translocation present mostly in triticales and *Sr35* from *Triticum monococcum* and then transferred to some

tetraploids of Canadian origin, none of which were present in this study or in the pedigree of the accessions of the AM panel, the chromosome 3A-related associations detected herein are likely to involve alternative and unknown loci. Additionally, no *Sr* gene was reported on chromosome arm 3BL and thus the significant effects associated with *wmc418* and wPt-9049 could be due to putatively novel loci. Similarly, the QTL on chromosome arm 4AL with a major effect for race JRCQC and the QTL on chromosome 4BL for race TTTTF could represent new race-specific loci for stem rust resistance. Although the significant markers identified on chromosomes 5A and 5B did not overlap with any reported major *Sr* gene, resistance QTLs for response to Ug99 have been mapped in similar locations on chromosome arm 5BS (Yu et al. 2011). The markers on chromosome 5A were located where no *Sr* gene or QTL has been previously reported and may represent one or more new QTL for stem rust resistance. All significant markers identified on chromosome arm 6BS did not coincide with any of the reported major *Sr* genes and may thus represent new loci. Similarly, two genomic regions were detected on chromosome 7AS (wPt-6668 and wPt-7188) and were not significantly associated with stem rust resistance in previous reports. Although no *Sr* genes have been reported for chromosome arm 7BS, two distinct QTLs were detected for race JRCQC near *gwm573* and *gwm333*, which could also be considered as novel *Sr* loci. Following further characterisation and validation, diagnostic markers for these resistance loci would be useful for enhancing APR to stem rust, provided they are also associated with broad range, adult plant resistance.

5.5.4 Reaction of race-specific resistance genes

Although more than 50 different stem rust resistance loci have been cataloged, and multiple alleles are known for three genes (Singh et al. 2011), additional resistance loci that are likely different from designated genes have recently been identified. All designated genes, except *Sr2* and the recently characterised *Sr55* and *Sr57* are race specific. The majority of stem rust resistance genes confer varying levels of intermediate resistance both at seedling and adult stages, and some genes did not confer adequate protection in the field if present alone (Singh et al. 2006, 2011). In our study, several race-specific QTLs associated to resistance to three or four of the races were detected that, upon pyramiding, may reduce susceptibility and enhance durability. Most studies in which several races were used to detect QTLs for resistance have

reported either race-specific QTLs (Niks et al. 2000; Zhu et al. 2003) or a combination of broad-spectrum QTLs and race-specific QTLs with various effects on resistance (Qi et al. 1999; Arru et al. 2003; Chen et al. 2003). Our results are more complex, since (i) some of the race-specific QTLs had strong effects on resistance (for instance the QTL on chromosome 2B tagged by *gwm47* for race TRTTF and the QTL on chromosome 4A tagged by *barc78* for race JRCQC), (ii) some QTLs had strong albeit quantitative effect (QTL on chromosome 7B tagged by *wmc517* for races TTKSK and TTTTF), (iii) some minor QTLs were effective across all races (QTL on chromosome 5A tagged by *gwm410*) and (iv) the QTL cluster on chromosome 6A tagged by *CD926040* and *barc104* showed broad spectrum resistance with major effect for races TTKSK, TTTTF and JRCQC and relatively minor effects for race TRTTF. The greater complexity observed in our study could partly derive from the evaluation of a large number of accessions with different genetic background and races with distinct virulence.

5.5.5 QTLs with minor effects

Except for the QTLs tagged by *CD926040* and *barc104*, each significant, association identified in this study explained less than 10% of the phenotypic variation for stem rust resistance at the seedling stage. Previous AM studies have also identified QTLs with minor phenotypic effects (Roy et al. 2010; Maccaferri et al. 2011; Massman et al. 2011). QTL effects estimated via AM are usually lower in R^2 and effect values than those estimated in biparental populations because of the greater complexity at the effector loci explored in the AM panels as compared to biparental mapping populations, including the presence of more complex epistatic interactions among loci. More importantly, the elite breeding germplasm of durum wheat, in the past decades, has not been improved by means of an extensive use of wide-crosses to introgress alleles with strong phenotypic effects (Maccaferri et al. 2005). Lastly, the effect of the marker is a function of the QTL effect and of the LD between the marker and the QTL and insufficient marker density could lead to markers that are in low LD with the QTL (Massman et al. 2011).

5.5.6 Breeding perspectives

This study shows that the level of seedling resistance to stem rust in elite durum is largely governed by minor QTLs and one major QTL on chromosome 6A. Selection for markers closely linked to these loci have thus the potential to improve stem rust resistance in populations generated from this set of accessions. Other promising sources of seedling resistance have been identified and demonstrated their effectiveness under highly conducive environments for stem rust epidemics. Markers are already available for *Sr9* (Tsilo et al. 2007), *Sr13* (Simons et al 2011), *Sr26* (Mago et al. 2005; Liu et al. 2010) and *Sr25* (Yu et al. 2010). If further confirmed, the QTLs reported here for seedling resistance and the corresponding closely linked molecular markers will contribute to broadening the genetic basis of seedling and potentially also field resistance to stem rust, an important goal of durum wheat breeding. We have identified several loci for resistance to highly virulent stem rust races that can be used in breeding programmes. Notably, some of the durum wheat lines that were tested herein carry resistance to all four races of *Pgt*.

Our results indicate the suitability of AM to provide valuable information to accelerate durum wheat improvement and cultivar release. In particular, the results confirm the role of of *Sr9*, *Sr13* and *Sr14* previously described in biparental mapping studies while unveiling the presence of putatively novel loci that could be exploited to enhance stem rust resistance in durum wheat *via* marker-assisted selection. With proper planning and an accurate deployment of *Sr* genes, long-term, broad-spectrum APR can thus be attained more effectively. Attaining APR is essential for the release of novel cultivars of durum wheat because stem rust susceptibility is low until heading. Therefore, stem rust resistance at the seedling stage, while easy and fast to assay, may not be indicative of the reaction at the adult plant stage. Combining the results of this study with those on APR in the field where races such as TTKSK, TRTTF and JRCQC prevail (Letta et al. 2013) will facilitate the selection of suitable parental lines for further improving stem rust resistance of durum wheat. In summary, this study provides novel information that can be exploited for pre-emptive breeding efforts to reduce the vulnerability of durum wheat to stem rust. The first step would be to identify a small subset of accessions that carry resistance alleles at different QTLs. These accessions would then be used as donor parents in a marker-assisted backcrossing scheme aimed to select lines with resistance alleles at different loci in an elite genetic background. Further

characterisation of these sets of near-isogenic lines would confirm the QTL effects while providing more accurate estimates of allelic effects and their epistatic interactions. In the near future, the availability of high-density SNP platforms including thousands of markers will allow for studies with almost complete genome coverage and a much more refined resolution at the haplotype level (Akhunov et al. 2009; Kaur et al. 2011; Trebbi et al. 2011; You et al. 2011; van Poeke et al. 2013). The use of the same SNP assays in applied breeding programmes will also facilitate the simultaneous selection of multiple, beneficial alleles for partial resistance. Finally, the relatively large number and small effects of the QTLs herein described suggest that a more comprehensive selection strategy, such as genomic selection (Heffner et al. 2009; Rutkoski et al. 2011), may prove more cost-effective than traditional MAS strategies at accumulating beneficial alleles in breeding populations.

5.6 CONCLUSIONS

Association mapping has the promising potential to dissect quantitative traits including disease resistance to generated information that facilitate crop improvement and variety development. As shown in this and other studies, AM complements bi-parental mapping for an independent QTL validation, identification of novel QTLs and potentially more precise QTL location. The present study highlighted the presence of novel genetic variation that could be exploited to enhance stem rust resistance in durum wheat via marker-assisted selection. Our results confirm the role of of *Sr9*, *Sr13* and *Sr14* previously described in biparental mapping studies while unveiling the presence of putatively novel loci, thus supporting the validity of AM.

Identifying new sources of resistance to stem rust including Ug99 and other virulent races provides wheat breeders with an increased diversity of *Sr* genes to be combined in new cultivars. With proper planning and an accurate selection of *Sr* genes, long-term, broad-spectrum resistance can thus be attained. Attaining adult plant resistance is essential for the release of novel cultivars of durum wheat because stem rust susceptibility is not high until heading. Nonetheless, stem rust resistance at the seedling stage, while easy and fast to assay, may not be indicative of the reaction at the adult plant stage because some genes are effective only at specific growth stages. Combining the results of this study with those in adult plant

resistance in the field at Debrezeit where races such as TTKSK, TRTTF and JRCQC are prevalent will provide valuable indications to select suitable parental lines for further improving stem rust resistance of durum wheat. Additionally, accessions carrying putative novel alleles should be tested against a collection of different stem rust isolates in the greenhouse to determine whether they possess a broad-based resistance.

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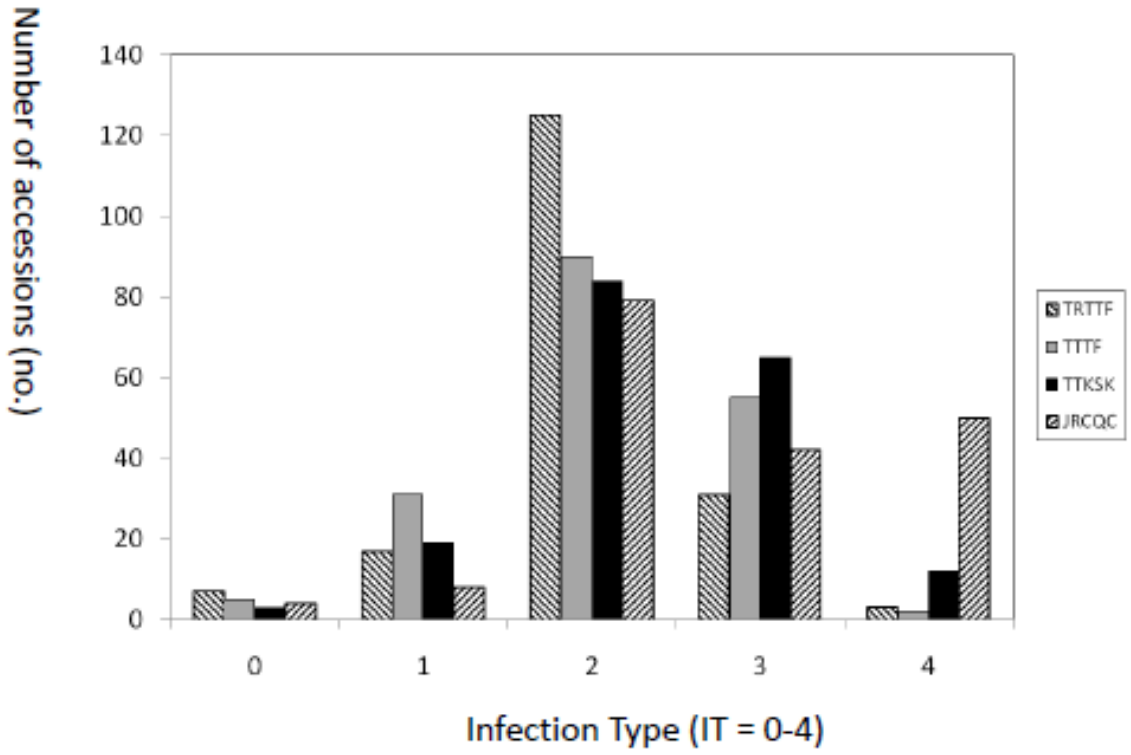
FIGURES

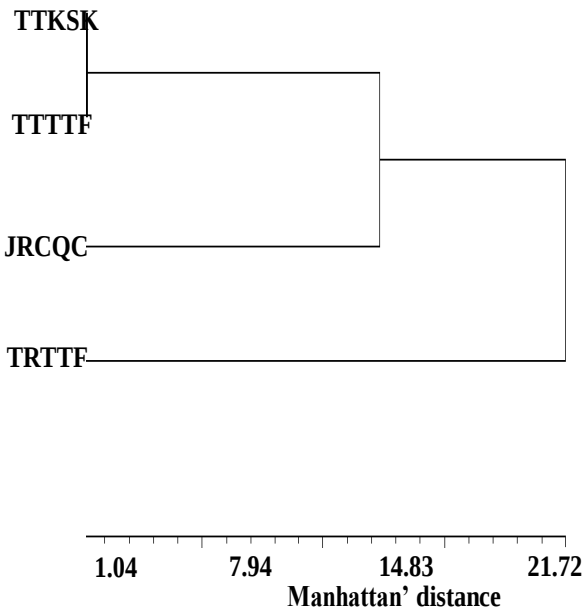
Figure 1. Frequency distribution of Infection Types (ITs) within an AM panel of 183 durum genotypes, challenged at seedling stage with 4 stem rust races.



ITs were assigned according to Long and Kolmer (1989) with resistant reactions indicated by ITs between 0-2 and susceptible reactions indicated by ITs of 3 or 4.

Figure 2. UPGMA-dendrogram of the 4 stem rust (*Puccinia graminis* Pers. F. sp. *tritici*) races used to characterize the durum germplasm collection.

The dendrogram is based on the races' virulence phenotypes (Infection types estimated with a 0-4 scale). Distances were computed using the standardized Manhattan distances ('city-block' method).



TABLES

Table 1. Isolate designation, origin, virulence phenotype and infection type (IT) on *Sr13* of *Puccinia graminis* f. sp. *tritici* races used to evaluate the durum panel.

Race	Isolate	Origin	Avirulence	Virulence	IT on *Sr13*[1]
TRTTF	06YEM34-1	Yemen	*Sr8a 24 31*	*Sr5 6 7b 9a 9b 9d 9e 9g 10 11 17 Sr21 30 36 38 McN*	3+
TTTTF	01MN84A-1-2	United States	*Sr24 31*	*Sr5 6 7b 8a 9a 9b 9d 9e 9g 10 11 Sr17 21 30 36 38 McN*	2
TTKSK (Ug99)	04KEN156/04	Kenya	*Sr24 36 Tmp*	*Sr5 6 7b 8a 9a 9b 9d 9e 9g 10 11 Sr17 21 30 31 38 McN*	22-
JRCQC	09ETH08-3	Ethiopia	*Sr5 7b 8a 36 9b 30 Tmp 24 31 38*	*Sr21 9e 11 6 9g 17 9a 9d McN*	3

[1]Infection types observed on seedlings at 14 days post-inoculation using a 0 to 4 scale according to Stakman et al. (1962), where infection types of ; 1, 2, or X are considered as a low IT and ITs of 3 or higher are considered as a high IT

Table 2. Numbers and frequencies of infection types (IT) and resistant, susceptible and heterogeneous reactions of the 183 durum genotypes included in the AM panel to four races of *Puccinia graminis* f. sp. *tritici* and the combined reaction to all races.

IT[1] / Reaction	TRTTF		TTTTF		TTKSK (Ug99)		JRCQC		All races	
	Lines	%	Lines	%	Lines	%	Lines	%	Lines	%
"0" or ";"	10	5.5	3	1.6	2	1.1	4	2.2	0	0
"1"	1	0.5	10	5.5	1	0.5	5	2.7	0	0
"2" or "23" or "X"	138	75.4	104	56.8	103	56.3	78	42.6	66	36.1
Resistant Reaction	149	81.4	117	63.9	106	57.9	87	47.5	66	36.1
"3"	22	12.0	47	25.7	53	29.0	39	21.3	10	5.5
"4"	4	2.2	2	1.1	13	7.1	50	27.3	2	1.1
Susceptible Reaction	26	14.2	49	26.8	66	36.1	89	48.6	12	6.6
Heterogeneous[2]	8	4.4	17	9.3	11	6.0	7	3.8	1	0.5

[1]Infection types observed on seedlings at 14 days post-inoculation using a 0 to 4 scale according to Stakman et al. (1962), where infection types of ; 1, 2, or X are considered as a low IT and ITs of 3 or higher are considered as a high IT

[2]Accessions that contained both resistant and susceptible plants

Table 3: Numbers and frequencies of reactions of 183 durum genotypes included in the AM panel to 4 races of *Puccinia graminis* f. sp. *tritici*, classified by origin subgroup

Durum Panel subgroup[1]		TRTTF			TTTTF			TTKSK			JRCQC			Across races		
		Res.[2]	Sus.[3]	% Res.[4]	Res.	Sus.	% Res.	Res.	Sus.	% Res.	Res.	Sus.	% Res.	Res.	Sus.	% Res.
		no.	no.	Rank	no.	no.	rank	no.	no.	rank	no.	no.	rank	no.	no.	rank
S1 (11 accessions)	Lines	9	2	4	5	5	5	3	7	5	2	9	5	2	1	4
	%	81.8	18.2		50	50		30	70		18.2	81.8		66.7	33.3	
S2 (55 accessions)	Lines	42	9	3	41	11	2	30	21	2	23	28	3	22	6	3
	%	82.4	17.6		78.8	21.2		58.8	41.2		45.1	54.9		78.6	21.4	
S2 (55 accessions)	Lines	14	8	5	17	5	3	12	11	4	16	10	2	8	4	5
	%	63.6	36.4		77.3	22.7		52.2	47.8		61.5	38.5		66.7	33.3	
S2 (55 accessions)	Lines	53	3	2	32	16	4	31	23	3	21	33	4	19	2	2
	%	94.6	5.4		66.7	33.3		57.4	42.6		38.9	61.1		90.5	9.5	
S2 (55 accessions)	Lines	35	0	1	29	5	1	29	5	1	25	9	1	26	0	1
	%	100	0		85.3	14.7		85.3	14.7		73.5	26.5		100	0	

[1] Durum Panel subgroups: S1: ICARDA germplasm for dryland areas; S2: ICARDA germplasm for temperate areas; S3: Italian and early ’70 CIMMYT germplasm; S4: CIMMYT germplasm (late ’70-early ’80); S5: CIMMYT germplasm (late ’80-early ’90)

[2] Resistant accessions: infection types of ; 1, 2, or X

[3] Susceptible accessions: ITs of 3 or higher

[4] Subgroup rank values based on their % of resistant accessions

Table 4. Most significant markers for each QTL associated with stem rust races TRTTF, TTTTF, TTKSK and JRCQC resistance in 183 durum association panel at seedling stage

			P value (Q+K model)[1]				R^2 (%)				Number of significant tests over four races	
Marker	Chr.	cM	TRTTF	TTTTF	TTKSK	JRCQC	TRTTF	TTTTF	TTKSK	JRCQC	Marker-wise tests	Genome-wise tests
wPt-1876	1B	31.3	-	-	-	0.0033	-	-	-	3.13	1	0
barc61	1B	87.2	0.0306	0.0393	0.0328	-	2.45	2.27	2.41	-	3	0
barc81	1B	119.9	-	0.0144	0.0131	-	-	2.42	2.37	-	2	0
wmc44	1B	158.1	-	0.0283	-	0.0093	-	3.88	-	4.86	2	0
wPt-5839	2A	0	0.0165	0.0098	0.0155	-	1.60	2.44	2.17	-	3	0
cfa2201	2A	47.8	0.0054	-	0.0137	-	4.25	-	3.50	-	2	0
wPt-2293	2A	63.7	**1.26E^{-4}**	-	-	-	**4.20**	-	-	-	1	1
gwm410	2B	64.1	0.009	-	-	-	3.70	-	-	-	1	0
gwm47	2B	158.9	**1.81E^{-5}**	-	-	-	**8.34**	-	-	-	1	1
gwm1300	2B	169.1	-	0.0215	0.0041	-	-	3.36	4.66	-	2	0
wmc356	2B	220	-	0.0421	-	0.008	-	2.44	-	4.22	2	0
wPt-1923	3A	46.4	-	0.0029	0.017	**6.94E^{-4}**	-	3.17	2.09	**3.98**	3	1
gwm1620	3A	83	-	0.0375	-	0.0152	-	2.47	-	3.09	2	0
wmc264	3A	119.6	0.0217	-	-	0.0322	3.7	-	-	3.49	2	0
wmc43	3B	57.9	-	0.0127	**0.0017**	-	-	2.75	**4.87**	-	2	1
wmc418	3B	122	0.0116	0.0143	-	-	3.66	3.56	-	-	2	0
wPt-9049	3B	182.5	-	-	-	0.0043	-	-	-	2.84	1	0
barc78	4A	110.6	-	-	-	**6.87E^{-7}**	-	-	-	**9.36**	1	1
gwm1084	4B	91.9	-	0.0081	-	-	-	3.67	-	-	1	0
gwm617	5A	4.4	-	-	0.0024	-	-	-	4.80	-	1	0
gwm1570	5A	32.6	-	-	-	0.004	-	-	-	2.94	1	0
barc165	5A	57.1	-	-	-	0.0248	-	-	-	3.50	1	0
gwm410	5A	120	0.0155	0.0191	0.009	0.0032	2.04	1.92	2.56	3.56	4	0
wPt-5514	5B	0	-	0.011	0.0067	-	-	2.48	2.89	-	2	0
gwm234	5B	27.2	-	-	-	0.007	-	-	-	3.51	1	0
wPt-0566	5B	189.1	0.0415	0.0021	-	-	1.13	3.56	-	-	2	0

[1] *P* values of markers that showed significant ($P \leq 0.05$) associations at the genome-wise level are reported in bold. (continued)

Table 4. Continued….

			P value (Q+K model) [1]				R^2 (%)				Number of significant tests	
Marker	Chr.	cM	TRTTF	TTTTF	TTKSK	JRCQC	TRTTF	TTTTF	TTKSK	JRCQC	Marker-wise tests	Genome-wise tests
gwm169	6°	126.6	-	0.002	0.0345	-	-	3.68	1.75	-	2	0
gwm427	6°	139.5	-	-	0.0153	0.0323	-	-	2.49	1.51	2	0
CD926040	6°	143.9	0.0087	**$3.43E^{-12}$**	**$4.17E^{-16}$**	**$1.56E^{-10}$**	3.52	**17.64**	**23.12**	**15.33**	4	3
barc104	6°	155.3	0.037	**$7.52E^{-7}$**	**$3.35E^{-10}$**	**$4.02E^{-6}$**	2.84	**11.95**	**17.82**	**10.47**	4	3
wPt-2991	6B	19.4	-	-	-	0.0095	-	-	-	2.4	1	0
wPt-0470	6B	51.6	-	0.0088	0.0086	-	-	2.45	2.50	-	2	0
gwm518	6B	84.1	-	-	0.0032	-	-	-	4.31	-	1	0
gwm816	6B	102.1	-	-	-	0.0307	-	-	-	2.60	1	0
wPt-6668	7°	32.7	**$8.35E^{-4}$**	-	-	-	**2.70**	-	-	-	1	1
wPt-7188	7°	80.4	0.0056	-	0.0341	-	2.11	-	1.77	-	2	0
wPt-7299	7°	150.7	-	-	0.0037	-	-	-	3.14	-	1	0
gwm344	7°	241.9	**$5.25E^{-4}$**	0.0088	-	-	**5.79**	3.79	-	-	2	1
gwm573	7B	66.6	-	-	-	0.0347	-	-	-	3.18	1	0
gwm333	7B	99.7	-	-	-	0.0182	-	-	-	3.90	1	0
wmc517	7B	155.6	-	**$1.73E^{-5}$**	**0.0016**	-	-	**8.0**	**4.96**	-	**2**	**2**
Total significant regions Marker-wise test			**15**	**20**	**19**	**19**						
Total Regions after FDR Genome-wise test			**4**	**3**	**4**	**4**						

[1] *P* values of markers that showed significant ($P \leq 0.05$) associations at the genome-wise level are reported in bold.

GENERAL CONCLUSIONS AND PERSPECTIVES

Ethiopia is the second largest wheat producer in the sub-Saharan Africa. The country is rich in genetic resources of tetraploid wheat and has suitable environments for wheat production. However, the country is a net importer of wheat particularly durum wheat (hard wheat). The demand for durum wheat is continuously increasing because of the new emerging food processing industries. But the productivity of wheat in Ethiopia is low due to the contrains caused by biotic and abiotic stresses. Among the biotic factors, stem rust plays a role of paramount importance in yield reduction. As a result of a recent emergence and spread of Ug99, a new and highly virulent race of leaf rust, and evolution of new durum-specific races which overcame widely deployed stem rust resistance genes (e.g. *Sr31*) that had been effective for many years, stem rust is becoming a serious threat to wheat production in Ethiopia as well as in other East African and wheat producing countries across the globe. Therefore, it is important to identify new resistance sources conferring resistance to stem rust races including Ug99 and its lineages and to develop codominant molecular markers suitable for marker-assisted selection towards a more sustainable control of stem rust.

To identify loci conferring resistance to stem rust races both at seedling and adult stages in durum wheat, 183 elite accessions representing the broad geographic origin were employed. The accessions were genotyped with 350 SSRs and 900 Diversity Array Technology (DArT) markers. Phenotypic data were collected on these accessions for stem rust resistance at the adult plant stage in Ethiopia in four evaluation seasons. Additionally, seedling phenotypic data on races TRTTF, TTTTF, TTKSK and JRCQC were collected at the Cereal Disease Laboratory, USDA and University of Minnesota, St. Paul, on the same set of durum panel.

Based on field data, twelve QTL-tagging markers significant ($P < 0.05$) across three to four seasons were detected. The role of *Sr13, Sr9, Sr14, Sr17* and *Sr28* was confirmed. Thirteen markers with significant effects on leaf rust resistance were located in regions with no *Sr* genes/QTLs and thus provide novel opportunities for marker-assisted selection. At the seedling stage and under controlled conditions, the number of chr. regions with significant effects on resistance to the TRTTF, TTTTF, TTKSK and JRCQC races was 15, 20, 19 and

19, respectively. Additionally, the results of this study show that stem rust resistance in durum is governed by shared loci and by race-specific ones. The QTLs identified in this study through AM will be useful in the marker-assisted development of durum wheat cultivars with durable stem rust resistance and for selecting suitable parental lines for further improving stem rust resistance of durum wheat. Additionally, accessions carrying putative novel alleles should be tested against a collection of different stem rust isolates in the greenhouse to determine whether they possess a broad-based resistance.

In conclusion, based on the results obtained in this study, a short list of elite accessions of durum wheat carrying beneficial alleles at different QTLs for stem rust resistance has been identified and can be used as donors to initiate a MAS program to improve stem rust resistance in the adapted Ethiopian durum wheat germplasm.

APPENDICES

Appendix 1. Name and origin of accessons of durum wheat used in this study.

Accession code	Accession name	ORIGIN
IDUWUE-002	CANNIZZO	ITALY
IDUWUE-003	CLAUDIO	ITALY
IDUWUE-004	LESINA	ITALY
IDUWUE-005	MERIDIANO	ITALY
IDUWUE-006	MONGIBELLO	ITALY
IDUWUE-007	NORBA	ITALY
IDUWUE-008	PIETRAFITTA	ITALY
IDUWUE-010	TORREBIANCA	ITALY
IDUWUE-011	BISU_1/PATKA_3	CIMMYT
IDUWUE-012	CMH82A.1062/3/GGOVZ394//SBA81/PLC/4/AAZ_1/C REX/5/HUI//CIT71/CII	CIMMYT
IDUWUE-013	DUKEM/3/RUFF/FGO//YAV79	CIMMYT
IDUWUE-015	KULRENGI-BALIKCIL_8	CIMMYT
IDUWUE-016	PLATA_16	CIMMYT
IDUWUE-017	PORTO_5	CIMMYT
IDUWUE-018	ROK/FGO//STIL/3/BISU_1	CIMMYT
IDUWUE-020	ACUATICO/YAZI_1	CIMMYT
IDUWUE-021	FOCHA_1/5*ALAS	CIMMYT
IDUWUE-023	BUSHEN_4/TARRO_2//BUSHEN_4	CIMMYT
IDUWUE-024	GS/CRA//SBA81/3/HO/4/MEXI_1/5/MEMO/6/2*ALT AR 84	CIMMYT
IDUWUE-025	RASCON_37/2*TARRO_2	CIMMYT
IDUWUE-027	SRN_3/AJAIA_15//DUKEM_1/3/DION_2	CIMMYT
IDUWUE-028	ALDEANO	IRTA-SPAIN
IDUWUE-029	ARIESOL	IRTA-SPAIN
IDUWUE-030	ARTENA	IRTA-SPAIN
IDUWUE-031	ASTIGI	IRTA-SPAIN
IDUWUE-032	BOABDIL	IRTA-SPAIN
IDUWUE-033	BOLENGA	IRTA-SPAIN
IDUWUE-034	BOLIDO	IRTA-SPAIN
IDUWUE-035	BOLO	IRTA-SPAIN
IDUWUE-036	BOMBASI	IRTA-SPAIN
IDUWUE-037	BORLI	IRTA-SPAIN
IDUWUE-038	CANYON	IRTA-SPAIN
IDUWUE-039	DURCAL	IRTA-SPAIN
IDUWUE-040	DUROI	IRTA-SPAIN
IDUWUE-041	GALLARETA	IRTA-SPAIN
IDUWUE-042	ILLORA	IRTA-SPAIN
IDUWUE-044	SENADUR	IRTA-SPAIN
IDUWUE-045	SULA	IRTA-SPAIN
IDUWUE-047	NASSIRA (MOROCCO_1805)	INRA-MOROCCO
IDUWUE-048	CHAOUI (MOROCCO_1807)	INRA-MOROCCO
IDUWUE-049	AMRIA (MOROCCO_1808)	INRA-MOROCCO
IDUWUE-050	MAROUANE (MOROCCO_1809)	INRA-MOROCCO
IDUWUE-053	JAWHAR	INRA-MOROCCO
IDUWUE-054	MARJANA	INRA-MOROCCO
IDUWUE-055	MARZAK	INRA-MOROCCO

Appendix 1. Continued…

Accession code	Accession name	ORIGIN
IDUWUE-056	OURGH	INRA-MOROCCO
IDUWUE-057	TAREK	INRA-MOROCCO
IDUWUE-060	AWALBIT	ICARDA
IDUWUE-061	BCR/3/CHAM_1//GTA/STR	ICARDA
IDUWUE-062	CHHB88/DERAA	ICARDA
IDUWUE-063	CHACAN	ICARDA
IDUWUE-064	KARIM	ICARDA
IDUWUE-065	HML/CHHB88	ICARDA
IDUWUE-066	KRS/HCN	ICARDA
IDUWUE-067	MURLAGOST-3	ICARDA
IDUWUE-068	MOULSABIL_2	ICARDA
IDUWUE-069	OMBAR	ICARDA
IDUWUE-071	MRB589_5	ICARDA
IDUWUE-072	QUADALETE//ERP/MAL/3/UNKNOWN(VSGI,ODESSA)	ICARDA
IDUWUE-073	SEBAH	ICARDA
IDUWUE-074	STOJOCRI_3	ICARDA
IDUWUE-075	ZEINA_1	ICARDA
IDUWUE-076	ANTON	ICARDA
IDUWUE-077	APPIO	ITALY
IDUWUE-079	ARCANGELO	ITALY
IDUWUE-080	ARCOBALENO	ITALY
IDUWUE-081	BRAVADUR	DESERT
IDUWUE-082	BRONTE	ITALY
IDUWUE-083	CAPEITI 8	ITALY
IDUWUE-084	CAPPELLI	ITALY
IDUWUE-085	CICCIO	ITALY
IDUWUE-086	COLORADO-DW	DESERT
IDUWUE-087	COLOSSEO	ITALY
IDUWUE-088	CORTEZ	DESERT
IDUWUE-089	CRESO	ITALY
IDUWUE-090	DON PEDRO	ITALY
IDUWUE-091	DUILIO	ITALY
IDUWUE-093	FLAMINIO	ITALY
IDUWUE-094	FORTORE	ITALY
IDUWUE-095	GARGANO	ITALY
IDUWUE-096	GRAZIA	ITALY
IDUWUE-097	IRIDE	ITALY
IDUWUE-098	ITALO	ITALY
IDUWUE-099	IXOS	ITALY
IDUWUE-100	KRONOS	DESERT
IDUWUE-102	MESSAPIA	ITALY
IDUWUE-103	MEXICALI 75	ITALY
IDUWUE-104	MOHAWK	ITALY
IDUWUE-105	OFANTO	ITALY
IDUWUE-106	PLATANI	ITALY
IDUWUE-107	PLINIO	ITALY
IDUWUE-108	PRODURA	ITALY
IDUWUE-109	REVA	ITALY
IDUWUE-110	ROQUENO	ITALY

Appendix 1. Continued…

Accession code	Accession name	ORIGIN
IDUWUE-111	SVEVO	ITALY
IDUWUE-112	TRINAKRIA	ITALY
IDUWUE-113	VALBELICE	ITALY
IDUWUE-114	VALNOVA	ITALY
IDUWUE-116	WESTBRED 881	DESERT
IDUWUE-117	WESTBRED TURBO	DESERT
IDUWUE-118	AGHRASS_1	ICARDA
IDUWUE-119	AINZEN_1	ICARDA
IDUWUE-120	ANGRE	ICARDA
IDUWUE-121	AMEDAKUL-1	ICARDA
IDUWUE-122	AMMAR-1	ICARDA
IDUWUE-123	ARISLAHN-5	ICARDA
IDUWUE-124	ATLAST-1	ICARDA
IDUWUE-125	AUS1	ICARDA
IDUWUE-126	AWALI_1	ICARDA
IDUWUE-127	RADIOSO	ITALY
IDUWUE-128	AZEGHAR_2	ICARDA
IDUWUE-130	BICRE	ICARDA
IDUWUE-131	BICREDERAA_1	ICARDA
IDUWUE-132	BIGOST-1	ICARDA
IDUWUE-133	BELIKH 2	ICARDA
IDUWUE-134	BRACHOUA	ICARDA
IDUWUE-135	CHAHBA88	ICARDA
IDUWUE-136	CHAM_1	ICARDA
IDUWUE-137	DERAA	ICARDA
IDUWUE-139	GEROMTEL-1	ICARDA
IDUWUE-140	GEZIRA-17	ICARDA
IDUWUE-141	GIDARA_2	ICARDA
IDUWUE-142	GUEROU_1	ICARDA
IDUWUE-144	HAURANI	ICARDA
IDUWUE-145	HEIDER	ICARDA
IDUWUE-146	OSL_1/4/BUC/CHRC//PRL/3/PVN/5/HEL/3/YAV/CORM//SHWA	ICARDA
IDUWUE-147	SEBOU	ICARDA
IDUWUE-148	BLK2//134XS-69-186/368-1/3/MRB589_5/4/ALBT_3	ICARDA
IDUWUE-149	ARIC31708.70/3/BO-DW//CDECH/BR-DW/4/CIT71/GTA	ICARDA
IDUWUE-150	JORDAN	ICARDA
IDUWUE-151	KABIR 1	ICARDA
IDUWUE-153	KHABUR_1	ICARDA
IDUWUE-154	KORIFLA	ICARDA
IDUWUE-155	LAGONIL-2	ICARDA
IDUWUE-156	LAHN	ICARDA
IDUWUE-157	LOUKOS_1	ICARDA
IDUWUE-158	MAAMOURI-1	ICARDA
IDUWUE-159	MARSYR-1	ICARDA
IDUWUE-160	MASSARA_1	ICARDA
IDUWUE-161	MIKI-1	ICARDA
IDUWUE-163	MURLAGOST-1	ICARDA
IDUWUE-164	NILE	ICARDA
IDUWUE-166	OMGENIL_3	ICARDA
IDUWUE-167	OMLAHN-3	ICARDA
IDUWUE-168	OMRUF-2	ICARDA
IDUWUE-169	OMSNIMA-1	ICARDA

Appendix 1. Continued….

Accession code	Accession name	ORIGIN
IDUWUE-170	ORONTE 1	ICARDA
IDUWUE-171	OTB-6	ICARDA
IDUWUE-172	OUASERL_1	ICARDA
IDUWUE-173	OUASLAHN-1	ICARDA
IDUWUE-175	QUABRACH-1	ICARDA
IDUWUE-176	QUADALETE	ICARDA
IDUWUE-177	RAZZAK	INRAT
IDUWUE-178	SAADA3/DDS//MTL-1	ICARDA
IDUWUE-179	SAJUR	ICARDA
IDUWUE-181	SHABHA	ICARDA
IDUWUE-182	TELSET_5	ICARDA
IDUWUE-183	TENSIFT_1	ICARDA
IDUWUE-184	TERBOL 97_3	ICARDA
IDUWUE-185	TUNSYR-1	ICARDA
IDUWUE-186	WADALMEZ_1	ICARDA
IDUWUE-187	YOUNES-1	ICARDA
IDUWUE-188	YOUSEF_1	ICARDA
IDUWUE-189	KOFA	ITALY
CIMMYT-251	1A.1D 5+10-6/3*MOJO//RCOL	CIMMYT
CIMMYT-252	SOOTY_9/RASCON_37	CIMMYT
CIMMYT-253	STOT//ALTAR 84/ALD	CIMMYT
CIMMYT-254	SOMAT_4/INTER_8	CIMMYT
CIMMYT-255	CHEN_1/TEZ/3/GUIL//CIT71/CII/4/SORA/PLATA_12/5/STOT//ALTAR 84/ALD	CIMMYT
CIMMYT-256	MALMUK_1//LOTUS_5/F3LOCAL(SEL.ETHIO.135.85)	CIMMYT
CIMMYT-257	1A.1D 5+10-6/2*WB881//1A.1D 5+10-6/3*MOJO/3/BISU_1/PATKA_3	CIMMYT
CIMMYT-258	HESSIAN-F_2/3/STOT//ALTAR 84/ALD	CIMMYT
CIMMYT-259	AJAIA_12/F3LOCAL(SEL.ETHIO.135.85)//PLATA_13/3/SOMAT_3/4/SOOTY_9/RASCON_37	CIMMYT
CIMMYT-260	USDA595/3/D67.3/RABI//CRA/4/ALO/5/HUI/YAV_1/6/ARDENTE/7/HUI/YAV79/8/POD_9	CIMMYT
CIMMYT-261	CNDO/PRIMADUR//HAI-OU_17/3/SNITAN	CIMMYT
CIMMYT-262	GEDIZ/FGO//GTA/3/SRN_1/4/TOTUS/5/ENTE/MEXI_2//HUI/3/YAV_1/GEDIZ/6/SOMBRA_20/7/STOT//ALTAR 84/ALD	CIMMYT
CIMMYT-263	VANRRIKSE_6.2//1A-1D 2+12-5/3*WB881	CIMMYT
CIMMYT-264	RANCO//CIT71/CII/3/COMDK/4/TCHO//SHWA/MALD/3/CREX/5/SNITAN	CIMMYT
CIMMYT-265	PLATA_10/6/MQUE/4/USDA573//QFN/AA_7/3/ALBA-D/5/AVO/HUI/7/PLATA_13/8/THKNEE_11/9/CHEN/ALTAR 84/3/HUI/POC//BUB/RUFO/4/FNFOOT	CIMMYT
CIMMYT-266	EUDO//CHEN_1/TEZ/3/TANTLO_1/4/PLATA_6/GREEN_17	CIMMYT
CIMMYT-267	ROLA_5/3/AJAIA_12/F3LOCAL(SEL.ETHIO.135.85)//PLATA_13/4/MALMUK_1/SERRATOR_1	CIMMYT
CIMMYT-268	ARMENT//SRN_3/NIGRIS_4/3/CANELO_9.1	CIMMYT
CIMMYT-269	SOMAT_3/PHAX_1//TILO_1/LOTUS_4	CIMMYT

Appendix 2. Complete data set of field phenotypic response and population structure membership coefficients for each of the 183 accessions included in the association panel.

	Stem rust response					Membership's Coefficients					
Accession code	Dzo-2009	DZm-2009	Dzo-2010	DZm-2010	Mean	Subgroup1 (ICARDA germplasm for dry areas	Subgroup 2 (ICARDA germplasm for temperate areas	Subgroup 3 (Italian and early '70s CIMMYT germplasm	Subgroup 4 (CIMMYT germplasm of late '70s-early '80s)	Subgroup 5 (CIMMYT late '80s-early '90s)	Main subgroup
IDUWUE-002	20	.	45	37.3	32.9	0.09	0.03	0.8	0.06	0.02	3
IDUWUE-003	.	27	70	45	48	0.01	0.36	0.26	0.15	0.22	2
IDUWUE-004	40	50	80	45	53.7	0.14	0.25	0.57	0.04	0.01	3
IDUWUE-005	60	.	80	37.3	57.9	0.17	0.13	0.03	0.64	0.03	4
IDUWUE-006	6	6	16	37.3	16.3	0.01	0.09	0.72	0.04	0.14	3
IDUWUE-007	.	.	70	45	56.7	0.08	0.22	0.18	0.08	0.45	5
IDUWUE-008	40	18	20	37.3	28.8	0.01	0.02	0.6	0.37	0.01	3
IDUWUE-010	50	27	70	60.5	51.9	0.01	0.27	0.54	0.01	0.17	3
IDUWUE-011	40	40	70	45	48.7	0.01	0.19	0.04	0.03	0.73	5
IDUWUE-012	45	32	80	24.9	45.5	0.05	0.78	0.01	0.13	0.03	2
IDUWUE-013	.	.	70	52.7	60.6	0.03	0.45	0.06	0.33	0.13	2
IDUWUE-015	45	45	80	20.2	47.6	0.01	0.02	0.01	0.02	0.94	5
IDUWUE-016	40	36	80	29.5	46.4	0.01	0.03	0.01	0.28	0.67	5
IDUWUE-017	40	24	60	45	42.2	0.02	0.02	0.01	0.1	0.84	5
IDUWUE-018	36	45	54	27.2	40.6	0.01	0.18	0.02	0.25	0.54	5
IDUWUE-020	40	.	60	45	47.2	0.01	0.05	0.01	0.12	0.81	5
IDUWUE-021	30	16	40	37.3	30.8	0.01	0.01	0.01	0.21	0.78	5
IDUWUE-023	40	24	70	34.2	42	0.02	0.1	0.01	0.01	0.87	5
IDUWUE-024	50	40	70	24.9	46.2	0.01	0.01	0.01	0.03	0.95	5
IDUWUE-025	50	32	70	52.7	51.2	0.01	0.16	0.01	0.03	0.79	5
IDUWUE-027	36	27	60	21.8	36.2	0.02	0.32	0.03	0.27	0.36	5
IDUWUE-028	50	.	60	.	50.3	0.02	0.9	0.06	0.02	0.01	2
IDUWUE-029	50	40	.	.	45.8	0.01	0.83	0.05	0.08	0.04	2
IDUWUE-030	60	32	60	45	49.2	0.01	0.92	0.03	0.01	0.03	2

Appendix 2 Continued…

	Stem rust response					Membership's Coefficients					
Accession code	Dzo-2009	DZm-2009	Dzo-2010	DZm-2010	Mean	Subgroup1 (ICARDA germplasm for dry areas	Subgroup 2 (ICARDA germplasm for temperate areas	Subgroup 3 (Italian and early '70s CIMMYT germplasm	Subgroup 4 (CIMMYT germplasm of late '70s-early '80s)	Subgroup 5 (CIMMYT late '80s-early '90s)	Main subgroup
IDUWUE-031	60	54	80	45	59.7	0.02	0.2	0.01	0.2	0.57	5
IDUWUE-032	60	27	60	18.7	41.4	0.05	0.36	0.01	0.4	0.17	4
IDUWUE-033	6	12	.	11	12.2	0.03	0.65	0.26	0.06	0.01	2
IDUWUE-034	50	45	70	.	53	0.07	0.77	0.01	0.11	0.04	2
IDUWUE-035	40	.	60	.	45.3	0.01	0.69	0.01	0.04	0.25	2
IDUWUE-036	60	36	70	60.5	56.6	0.01	0.01	0.01	0.32	0.66	5
IDUWUE-037	9	18	36	45	27	0.01	0.04	0.01	0.72	0.21	4
IDUWUE-038	30	3	50	11	23.5	0.01	0.29	0.01	0.63	0.06	4
IDUWUE-039	.	70	70	60.5	67.5	0.03	0.51	0.02	0.41	0.03	2
IDUWUE-040	40	45	36	34.2	38.8	0.04	0.38	0.01	0.51	0.06	4
IDUWUE-041	45	72	60	11	47	0	0.01	0	0.01	0.98	5
IDUWUE-042	50	18	70	27.2	41.3	0.01	0.42	0.02	0.03	0.52	5
IDUWUE-044	60	63	70	34.2	56.8	0.01	0.4	0.43	0.02	0.14	3
IDUWUE-045	36	8	50	20.2	28.6	0.01	0.02	0.04	0.33	0.61	5
IDUWUE-047	60	18	60	37.3	43.8	0.1	0.56	0.01	0.31	0.03	2
IDUWUE-048	30	36	70	15.6	37.9	0.01	0.91	0.01	0.05	0.01	2
IDUWUE-049	0.2	.	6	7.9	3.5	0.02	0.94	0.02	0.02	0.01	2
IDUWUE-050	70	70	80	45	66.2	0.01	0.93	0.01	0.03	0.02	2
IDUWUE-053	70	70	70	45	63.7	0.02	0.03	0.05	0.6	0.3	4
IDUWUE-054	4	9	.	7.9	9.5	0.01	0.03	0.03	0.39	0.54	5
IDUWUE-055	30	60	50	.	44.7	0.28	0.68	0.02	0.01	0.01	2
IDUWUE-056	27	60	50	.	43.7	0.01	0.01	0	0.97	0.01	4
IDUWUE-057	50	54	36	45	46.2	0.02	0.35	0.01	0.59	0.03	4
IDUWUE-060	30	.	50	52.7	43.1	0.94	0.01	0.03	0.01	0.01	1

Appendix 2 Continued…..

	Stem rust response					Membership's Coefficients					
Accession code	Dzo-2009	DZm-2009	Dzo-2010	DZm-2010	Mean	Subgroup1 (ICARDA germplasm for dry areas	Subgroup 2 (ICARDA germplasm for temperate areas	Subgroup 3 (Italian and early '70s CIMMYT germplasm	Subgroup 4 (CIMMYT germplasm of late '70s-early '80s)	Subgroup 5 (CIMMYT late '80s-early '90s)	Main subgroup
IDUWUE-061	40.0	70.0	50.0	45.0	51.2	0.21	0.04	0.01	0.72	0.02	4
IDUWUE-062	40.0	40.0	36.0	37.3	38.3	0.04	0.34	0.03	0.51	0.09	4
IDUWUE-063	32.0	63.0	30.0	37.3	40.6	0.02	0.31	0.08	0.48	0.12	4
IDUWUE-064	.	.	60.0	45.0	51.7	0.01	0.01	0.00	0.98	0.01	4
IDUWUE-065	40.0	50.0	80.0	.	54.7	0.01	0.43	0.02	0.50	0.04	4
IDUWUE-066	10.0	24.0	4.0	34.2	18.0	0.02	0.61	0.01	0.34	0.02	2
IDUWUE-067	50.0	70.0	60.0	37.3	54.3	0.02	0.23	0.07	0.42	0.27	2
IDUWUE-068	50.0	80.0	80.0	37.3	61.8	0.01	0.26	0.07	0.50	0.17	4
IDUWUE-069	.	60.0	45.0	.	50.5	0.35	0.05	0.02	0.49	0.10	4
IDUWUE-071	.	54.0	70.0	45.0	57.0	0.94	0.01	0.01	0.01	0.02	1
IDUWUE-072	6.0	.	9.0	11.0	7.5	0.27	0.03	0.01	0.67	0.03	4
IDUWUE-073	30.0	70.0	60.0	.	51.4	0.01	0.81	0.01	0.16	0.01	2
IDUWUE-074	50.0	70.0	50.0	52.7	55.7	0.12	0.62	0.02	0.23	0.01	2
IDUWUE-075	30.0	54.0	27.0	52.7	40.9	0.04	0.31	0.22	0.36	0.07	4
IDUWUE-076	0.2	3.0	27.0	18.7	12.2	0.05	0.01	0.91	0.01	0.03	3
IDUWUE-077	60.0	80.0	60.0	60.5	65.1	0.16	0.71	0.05	0.02	0.07	2
IDUWUE-079	.	27.0	36.0	27.2	30.7	0.20	0.55	0.17	0.08	0.01	2
IDUWUE-080	24.0	60.0	70.0	34.2	47.0	0.01	0.01	0.01	0.30	0.67	5
IDUWUE-081	20.0	20.0	27.0	.	20.4	0.02	0.09	0.83	0.05	0.01	3
IDUWUE-082	.	60.0	40.0	37.3	46.4	0.02	0.02	0.07	0.88	0.01	4
IDUWUE-083	9.0	18.0	.	9.4	14.6	0.60	0.08	0.31	0.01	0.01	1
IDUWUE-084	50.0	70.0	60.0	52.7	58.2	0.05	0.52	0.38	0.02	0.03	2
IDUWUE-085	.	.	70.0	20.2	44.3	0.34	0.01	0.64	0.01	0.01	3
IDUWUE-086	.	13.5	60.0	29.5	35.0	0.01	0.28	0.51	0.19	0.02	3

Appendix 2 Continued……

	Stem rust response					Membership's Coefficients					
Accession code	Dzo-2009	DZm-2009	Dzo-2010	DZm-2010	Mean	Subgroup1 (ICARDA germplasm for dry areas	Subgroup 2 (ICARDA germplasm for temperate areas	Subgroup 3 (Italian and early '70s CIMMYT germplasm	Subgroup 4 (CIMMYT germplasm of late '70s-early '80s)	Subgroup 5 (CIMMYT late '80s-early '90s)	Main subgroup
IDUWUE-087	40.0	50.0	40.0	29.5	39.9	0.01	0.90	0.01	0.06	0.02	2
IDUWUE-088	30.0	.	70.0	37.3	44.6	0.07	0.42	0.08	0.38	0.05	2
IDUWUE-089	30.0	50.0	50.0	34.2	41.0	0.02	0.55	0.02	0.39	0.01	2
IDUWUE-090	30.0	70.0	70.0	.	54.7	0.08	0.35	0.03	0.51	0.04	4
IDUWUE-091	.	27.0	50.0	45.0	41.3	0.01	0.01	0.00	0.97	0.01	4
IDUWUE-093	50.0	.	36.0	27.2	36.6	0.01	0.01	0.43	0.54	0.01	4
IDUWUE-094	.	2.0	0.0	11.0	5.0	0.11	0.13	0.74	0.01	0.01	3
IDUWUE-095	40.0	8.0	36.0	7.9	23.0	0.09	0.02	0.87	0.01	0.01	3
IDUWUE-096	.	8.0	.	9.4	13.4	0.02	0.02	0.77	0.16	0.03	3
IDUWUE-097	12.0	3.0	.	11.0	11.2	0.01	0.19	0.04	0.30	0.46	5
IDUWUE-098	40.0	60.0	70.0	.	54.7	0.09	0.33	0.18	0.35	0.05	4
IDUWUE-099	16.0	.	3.0	12.5	9.4	0.02	0.12	0.79	0.06	0.01	3
IDUWUE-100	6.0	2.0	27.0	.	9.7	0.01	0.12	0.68	0.19	0.01	3
IDUWUE-102	9.0	36.0	.	11.0	21.2	0.01	0.94	0.02	0.02	0.01	2
IDUWUE-103	12.0	16.0	.	.	14.8	0.02	0.02	0.73	0.04	0.20	3
IDUWUE-104	60.0	.	60.0	52.7	56.4	0.02	0.46	0.29	0.22	0.02	2
IDUWUE-105	.	0.0	0.2	9.4	3.8	0.04	0.04	0.90	0.01	0.02	3
IDUWUE-106	45.0	.	30.0	52.7	41.4	0.39	0.02	0.56	0.01	0.02	3
IDUWUE-107	50.0	60.0	60.0	27.2	49.3	0.04	0.21	0.61	0.11	0.04	3
IDUWUE-108	50.0	.	60.0	27.2	44.6	0.01	0.89	0.02	0.08	0.01	2
IDUWUE-109	40.0	.	18.0	7.9	20.8	0.01	0.30	0.58	0.09	0.01	3
IDUWUE-110	60.0	36.0	30.0	52.7	44.7	0.03	0.90	0.01	0.04	0.02	2
IDUWUE-111	50.0	45.0	40.0	24.9	40.0	0.05	0.22	0.02	0.70	0.02	4
IDUWUE-112	.	2.0	4.0	7.9	5.3	0.22	0.44	0.30	0.03	0.01	2

Appendix 2 Continued…..

	Stem rust response					Membership's Coefficients					
Accession code	Dzo-2009	DZm-2009	Dzo-2010	DZm-2010	Mean	Subgroup1 (ICARDA germplasm for dry areas	Subgroup 2 (ICARDA germplasm for temperate areas	Subgroup 3 (Italian and early '70s CIMMYT germplasm	Subgroup 4 (CIMMYT germplasm of late '70s-early '80s)	Subgroup 5 (CIMMYT late '80s-early '90s)	Main subgroup
IDUWUE-113	40.0	12.0	27.0	13.3	23.1	0.26	0.59	0.13	0.01	0.01	2
IDUWUE-114	30.0	.	8.0	45.0	26.5	0.01	0.01	0.97	0.01	0.01	3
IDUWUE-116	4.0	4.0	6.0	9.4	5.9	0.01	0.11	0.86	0.01	0.02	3
IDUWUE-117	40.0	.	36.0	.	33.3	0.14	0.06	0.02	0.75	0.02	4
IDUWUE-118	32.0	36.0	60.0	.	40.7	0.02	0.01	0.03	0.92	0.03	4
IDUWUE-119	36.0	24.0	40.0	15.6	28.9	0.01	0.03	0.01	0.94	0.01	4
IDUWUE-120	60.0	60.0	70.0	45.0	58.7	0.02	0.25	0.38	0.16	0.19	3
IDUWUE-121	60.0	60.0	50.0	.	54.7	0.02	0.12	0.01	0.84	0.01	4
IDUWUE-122	60.0	50.0	50.0	.	51.4	0.01	0.04	0.09	0.84	0.01	4
IDUWUE-123	45.0	60.0	18.0	12.5	33.9	0.07	0.11	0.27	0.53	0.02	4
IDUWUE-124	50.0	54.0	27.0	37.3	42.1	0.01	0.58	0.02	0.33	0.06	2
IDUWUE-125	40.0	.	22.5	37.3	32.1	0.03	0.23	0.19	0.50	0.04	4
IDUWUE-126	36.0	50.0	50.0	.	43.4	0.08	0.47	0.12	0.32	0.02	2
IDUWUE-127	50.0	27.0	16.0	18.7	27.9	0.03	0.79	0.14	0.03	0.01	2
IDUWUE-128	40.0	16.0	.	45.0	36.2	0.48	0.06	0.05	0.38	0.03	1
IDUWUE-130	50.0	36.0	32.0	45.0	40.7	0.02	0.04	0.01	0.93	0.01	4
IDUWUE-131	60.0	18.0	27.0	45.0	37.5	0.02	0.27	0.04	0.66	0.01	4
IDUWUE-132	50.0	36.0	60.0	37.3	45.8	0.04	0.37	0.01	0.55	0.03	4
IDUWUE-133	20.0	.	24.0	15.6	18.7	0.02	0.62	0.02	0.29	0.06	2
IDUWUE-134	40.0	40.0	60.0	.	44.7	0.03	0.45	0.04	0.36	0.12	2
IDUWUE-135	40.0	50.0	70.0	45.0	51.2	0.02	0.10	0.01	0.84	0.03	4
IDUWUE-136	6.0	12.0	27.0	11.0	14.0	0.03	0.78	0.03	0.15	0.01	2
IDUWUE-137	.	2.0	3.0	27.2	11.4	0.02	0.63	0.03	0.27	0.05	2
IDUWUE-139	50.0	12.0	36.0	29.5	31.9	0.02	0.52	0.03	0.39	0.04	2

Appendix 2 Continued…..

	Stem rust response					Membership's Coefficients					
Accession code	Dzo-2009	DZm-2009	Dzo-2010	DZm-2010	Mean	Subgroup1 (ICARDA germplasm for dry areas	Subgroup 2 (ICARDA germplasm for temperate areas	Subgroup 3 (Italian and early '70s CIMMYT germplasm	Subgroup 4 (CIMMYT germplasm of late '70s-early '80s)	Subgroup 5 (CIMMYT late '80s-early '90s)	Main subgroup
IDUWUE-140	40.0	.	.	.	38.1	0.02	0.43	0.49	0.06	0.01	2
IDUWUE-141	50.0	16.0	18.0	52.7	34.2	0.43	0.45	0.01	0.10	0.01	2
IDUWUE-142	40.0	.	70.0	.	50.3	0.01	0.29	0.02	0.64	0.04	4
IDUWUE-144	40.0	4.0	36.0	45.0	31.2	0.24	0.71	0.03	0.01	0.01	2
IDUWUE-145	50.0	27.0	70.0	7.9	38.7	0.02	0.80	0.01	0.16	0.01	2
IDUWUE-146	60.0	6.0	16.0	29.5	27.9	0.01	0.85	0.01	0.09	0.05	2
IDUWUE-147	50.0	8.0	9.0	24.9	23.0	0.02	0.88	0.02	0.05	0.03	2
IDUWUE-148	12.0	6.0	27.0	18.7	15.9	0.70	0.06	0.18	0.04	0.02	1
IDUWUE-149	30.0	9.0	27.0	7.9	18.5	0.06	0.86	0.02	0.04	0.02	2
IDUWUE-150	40.0	.	50.0	.	40.3	0.02	0.83	0.05	0.09	0.01	2
IDUWUE-151	30.0	.	70.0	.	45.3	0.03	0.49	0.20	0.26	0.02	2
IDUWUE-153	40.0	.	40.0	11.0	29.2	0.01	0.44	0.07	0.46	0.02	4
IDUWUE-154	60.0	.	60.0	52.7	56.4	0.06	0.46	0.26	0.03	0.19	2
IDUWUE-155	40.0	27.0	45.0	29.5	35.4	0.06	0.30	0.10	0.51	0.04	4
IDUWUE-156	40.0	.	60.0	.	45.3	0.04	0.02	0.01	0.83	0.11	4
IDUWUE-157	30.0	.	60.0	20.2	35.6	0.02	0.06	0.02	0.87	0.03	4
IDUWUE-158	60.0	40.0	60.0	24.9	46.2	0.01	0.02	0.01	0.95	0.01	4
IDUWUE-159	80.0	45.0	90.0	37.3	63.1	0.23	0.15	0.04	0.57	0.01	4
IDUWUE-160	70.0	40.0	70.0	.	58.0	0.96	0.01	0.01	0.02	0.01	1
IDUWUE-161	54.0	27.0	40.0	29.5	37.6	0.03	0.30	0.04	0.62	0.01	4
IDUWUE-163	50.0	.	36.0	37.3	39.9	0.01	0.53	0.01	0.39	0.06	2
IDUWUE-164	50.0	.	36.0	.	38.3	0.03	0.35	0.02	0.59	0.01	4
IDUWUE-166	60.0	27.0	50.0	37.3	43.6	0.42	0.12	0.03	0.42	0.02	1
IDUWUE-167	40.0	18.0	36.0	52.7	36.7	0.68	0.13	0.03	0.09	0.07	1

Appendix 2 Continued….

	Stem rust response					Membership's Coefficients					
Accession code	Dzo-2009	DZm-2009	Dzo-2010	DZm-2010	Mean	Subgroup1 (ICARDA germplasm for dry areas	Subgroup 2 (ICARDA germplasm for temperate areas	Subgroup 3 (Italian and early '70s CIMMYT germplasm	Subgroup 4 (CIMMYT germplasm of late '70s-early '80s)	Subgroup 5 (CIMMYT late '80s-early '90s)	Main subgroup
IDUWUE-168	50.0	16.0	40.0	37.3	35.8	0.01	0.10	0.02	0.75	0.12	4
IDUWUE-169	50.0	27.0	36.0	60.5	43.4	0.61	0.20	0.02	0.16	0.02	1
IDUWUE-170	40.0	.	70.0	18.7	41.7	0.04	0.28	0.33	0.06	0.28	3
IDUWUE-171	30.0	20.0	60.0	18.7	32.2	0.05	0.30	0.02	0.62	0.01	4
IDUWUE-172	50.0	50.0	50.0	37.3	46.8	0.26	0.16	0.05	0.47	0.06	4
IDUWUE-173	60.0	27.0	22.5	24.9	33.6	0.02	0.44	0.16	0.29	0.09	2
IDUWUE-175	36.0	24.0	15.0	.	23.0	0.07	0.13	0.21	0.58	0.02	4
IDUWUE-176	50.0	.	36.0	.	38.3	0.02	0.70	0.20	0.08	0.01	2
IDUWUE-177	50.0	.	50.0	.	45.3	0.02	0.23	0.01	0.64	0.10	4
IDUWUE-178	40.0	27.0	12.0	18.7	24.4	0.01	0.82	0.06	0.08	0.03	2
IDUWUE-179	40.0	30.0	45.0	.	36.4	0.02	0.16	0.37	0.26	0.20	3
IDUWUE-181	50.0	24.0	70.0	45.0	47.2	0.02	0.92	0.04	0.01	0.01	2
IDUWUE-182	45.0	32.0	32.0	60.5	42.4	0.51	0.44	0.02	0.02	0.01	1
IDUWUE-183	40.0	27.0	54.0	.	38.4	0.04	0.08	0.05	0.82	0.01	4
IDUWUE-184	40.0	30.0	50.0	.	38.0	0.02	0.01	0.01	0.94	0.01	4
IDUWUE-185	30.0	24.0	27.0	.	25.0	0.03	0.13	0.01	0.76	0.07	4
IDUWUE-186	27.0	32.0	16.0	24.9	25.0	0.01	0.37	0.25	0.21	0.17	2
IDUWUE-187	50.0	36.0	40.0	52.7	44.7	0.79	0.01	0.01	0.17	0.01	1
IDUWUE-188	50.0	36.0	70.0	45.0	50.2	0.01	0.35	0.02	0.61	0.02	4
IDUWUE-189	60.0	40.0	60.0	.	51.4	0.03	0.34	0.61	0.01	0.01	3
CIMMYT-251	60.0	36.0	60.0	.	50.0	0.05	0.02	0.03	0.29	0.62	5
CIMMYT-252	60.0	60.0	70.0	68.2	64.5	0.02	0.06	0.03	0.02	0.89	5
CIMMYT-253	70.0	60.0	80.0	34.2	61.0	0.00	0.02	0.01	0.01	0.96	5
CIMMYT-254	80.0	60.0	80.0	45.0	66.2	0.01	0.02	0.01	0.51	0.46	4

Appendix 2 Continued…..

	Stem rust response					Membership's Coefficients					
Accession code	**Dzo-2009**	**DZm-2009**	**Dzo-2010**	**DZm-2010**	**Mean**	**Subgroup1 (ICARDA germplasm for dry areas**	**Subgroup 2 (ICARDA germplasm for temperate areas**	**Subgroup 3 (Italian and early '70s CIMMYT germplasm**	**Subgroup 4 (CIMMYT germplasm of late '70s-early '80s)**	**Subgroup 5 (CIMMYT late '80s-early '90s)**	**Main subgroup**
CIMMYT-255	60.0	70.0	80.0	45.0	63.7	0.01	0.02	0.01	0.02	0.94	5
CIMMYT-256	50.0	60.0	60.0	45.0	53.7	0.02	0.03	0.02	0.18	0.75	5
CIMMYT-257	60.0	50.0	80.0	.	61.4	0.01	0.01	0.01	0.02	0.96	5
CIMMYT-258	40.0	60.0	60.0	37.3	49.3	0.00	0.01	0.00	0.01	0.98	5
CIMMYT-259	50.0	36.0	70.0	34.2	47.5	0.03	0.05	0.01	0.03	0.88	5
CIMMYT-260	70.0	32.0	45.0	.	47.0	0.01	0.01	0.01	0.49	0.48	4
CIMMYT-261	50.0	50.0	36.0	37.3	43.3	0.01	0.03	0.02	0.57	0.38	4
CIMMYT-262	60.0	36.0	60.0	29.5	46.4	0.01	0.03	0.02	0.05	0.91	5
CIMMYT-263	12.0	.	12.0	7.9	9.5	0.11	0.08	0.09	0.11	0.61	5
CIMMYT-264	70.0	70.0	80.0	68.2	72.0	0.06	0.08	0.02	0.15	0.69	5
CIMMYT-265	60.0	70.0	70.0	37.3	59.3	0.02	0.06	0.01	0.05	0.86	5
CIMMYT-266	60.0	.	60.0	.	55.3	0.00	0.01	0.00	0.75	0.24	4
CIMMYT-267	50.0	60.0	70.0	52.7	58.2	0.10	0.02	0.01	0.02	0.85	5
CIMMYT-268	45.0	70.0	70.0	.	59.7	0.04	0.23	0.11	0.04	0.58	5
CIMMYT-269	60.0	80.0	80.0	52.7	68.2	0.10	0.04	0.03	0.07	0.76	5
Mean						0.09	0.29	0.14	0.29	0.19	

Appendix 3 Complete data set of phenotypic response to four races (TRTTF, TTKSK, TTTTF and JRCQC) and population structure membership coefficients for each of the 183 accessions included in the association panel.

	Stem rust response				Membership's Coefficients					
Accession code	TRTTF	TTKSK	TTTTF	JRCQC	Subgroup1 (ICARDA germplasm for dry areas	Subgroup 2 (ICARDA germplasm for temperate areas	Subgroup 3 (Italian and early '70s CIMMYT germplasm	Subgroup 4 (CIMMYT germplasm of late '70s-early '80s)	Subgroup 5 (CIMMYT late '80s-early '90s)	Main subgroup
IDUWUE-002	3- / 2-	2 / 3	2-;	1;	0.086	0.027	0.803	0.064	0.02	3
IDUWUE-003	22-	2 / 3	2-	2	0.009	0.36	0.26	0.149	0.222	2
IDUWUE-004	2	33+	2-; / 33+	1; / 3+	0.136	0.248	0.568	0.038	0.011	3
IDUWUE-005	2-	4	4	4	0.173	0.131	0.031	0.64	0.025	4
IDUWUE-006	3	2+/2+3-	2+ / 3-	2-	0.013	0.094	0.715	0.041	0.136	3
IDUWUE-007	2-	;2-	2-	3+ / 2-;	0.08	0.218	0.176	0.079	0.448	5
IDUWUE-008	33+	3	2+	;N	0.009	0.019	0.598	0.365	0.01	3
IDUWUE-010	33+	33+	3+	4	0.012	0.27	0.538	0.013	0.168	3
IDUWUE-011	2-	2-	2-	2-	0.008	0.192	0.042	0.025	0.733	5
IDUWUE-012	2-	2	2-	4	0.047	0.784	0.014	0.127	0.029	2
IDUWUE-013	2	3+	3+	3+	0.027	0.448	0.064	0.331	0.13	2
IDUWUE-015	2-	2	2-	2-	0.012	0.018	0.006	0.02	0.944	5
IDUWUE-016	2-;	2	2-;	2	0.011	0.029	0.01	0.282	0.667	5
IDUWUE-017	2-	2	2-	2	0.021	0.018	0.014	0.103	0.844	5
IDUWUE-018	2-	2	2-	22+	0.012	0.176	0.023	0.245	0.544	5
IDUWUE-020	2-	2-;	2-	4	0.007	0.053	0.013	0.122	0.805	5
IDUWUE-021	2-	2	2-	2	0.005	0.008	0.006	0.206	0.775	5
IDUWUE-023	2-	22-	2-	2	0.015	0.099	0.007	0.013	0.865	5
IDUWUE-024	2-;	2	2-	2-	0.005	0.01	0.007	0.028	0.951	5
IDUWUE-025	2-	2-	2-	2-	0.013	0.155	0.007	0.031	0.793	5
IDUWUE-027	;N	2-;	;N	22+	0.019	0.323	0.034	0.267	0.358	5
IDUWUE-028	2-	2-	2-	0; / 3+	0.016	0.898	0.06	0.016	0.01	2
IDUWUE-029	;	2-;	2-	2-	0.01	0.827	0.05	0.078	0.035	2
IDUWUE-030	3+	3	3+ / 2	4	0.014	0.92	0.026	0.014	0.026	2

Appendix 3 Continued…..

	Stem rust response				Membership's Coefficients					
Accession code	TRTTF	TTKSK	TTTTF	JRCQC	Subgroup1 (ICARDA germplasm for dry areas	Subgroup 2 (ICARDA germplasm for temperate areas	Subgroup 3 (Italian and early '70s CIMMYT germplasm	Subgroup 4 (CIMMYT germplasm of late '70s-early '80s)	Subgroup 5 (CIMMYT late '80s-early '90s)	Main subgroup

IDUWUE-031	2-	3	33+	4	0.015	0.202	0.011	0.2	0.573	5
IDUWUE-032	2-	4	3+	0	0.054	0.362	0.008	0.403	0.172	4
IDUWUE-033	;N	;	;1-	2+	0.026	0.653	0.255	0.06	0.006	2
IDUWUE-034	2-	2-	2	2+	0.073	0.769	0.011	0.109	0.038	2
IDUWUE-035	2-	2	2-;	2-	0.013	0.69	0.01	0.041	0.246	2
IDUWUE-036	2-	2	2-	2-	0.01	0.006	0.006	0.321	0.657	5
IDUWUE-037	2-	2-;	2-	2-	0.009	0.044	0.012	0.723	0.212	4
IDUWUE-038	2-	2-	2-	3+1;N	0.01	0.286	0.01	0.632	0.061	4
IDUWUE-039	22-	3+	3+	3+	0.032	0.507	0.015	0.412	0.033	2
IDUWUE-040	2-	2-	22-	22+	0.042	0.378	0.009	0.51	0.062	4
IDUWUE-041	2-	;2-	2-;	2-	0.004	0.005	0.004	0.006	0.98	5
IDUWUE-042	2-;	2-	2-	2-	0.013	0.416	0.019	0.033	0.518	5
IDUWUE-044	;N	0;	;1	2+3-	0.014	0.397	0.43	0.02	0.138	3
IDUWUE-045	2-	2 / 2+	2-;	2-	0.01	0.018	0.035	0.327	0.609	5
IDUWUE-047	2	3++	3+	4	0.097	0.559	0.01	0.308	0.026	2
IDUWUE-048	2	2-	22-	3	0.011	0.914	0.014	0.051	0.01	2
IDUWUE-049	2-	;	2-	2-	0.015	0.942	0.015	0.017	0.011	2
IDUWUE-050	33+	3	3+	3+	0.012	0.934	0.011	0.025	0.018	2
IDUWUE-053	2- / 22+	2-	2-	2-	0.022	0.034	0.047	0.599	0.298	4
IDUWUE-054	2-	2-	2-	2-	0.011	0.026	0.033	0.386	0.544	5
IDUWUE-055	2+	2-	2-	2	0.276	0.683	0.016	0.014	0.011	2
IDUWUE-056	2	2+3- / 3	3+	3+	0.008	0.007	0.004	0.969	0.012	4
IDUWUE-057	;2-N	33+	22-	3+	0.019	0.35	0.009	0.591	0.032	4
IDUWUE-060	2	3-	3	3+	0.94	0.013	0.031	0.009	0.006	1

Appendix 3 Continued….

	Stem rust response				Membership's Coefficients					
Accession code	TRTTF	TTKSK	TTTTF	JRCQC	Subgroup1 (ICARDA germplasm for dry areas	Subgroup 2 (ICARDA germplasm for temperate areas	Subgroup 3 (Italian and early '70s CIMMYT germplasm	Subgroup 4 (CIMMYT germplasm of late '70s-early '80s)	Subgroup 5 (CIMMYT late '80s-early '90s)	Main subgroup
IDUWUE-061	2-	3+	2 / 3	3+	0.207	0.043	0.01	0.72	0.021	4
IDUWUE-062	2-	3	3	4	0.038	0.339	0.028	0.506	0.089	4
IDUWUE-063	22-	3-	2-	3+	0.02	0.307	0.075	0.477	0.121	4
IDUWUE-064	22-	3-	3+	4	0.006	0.008	0.004	0.975	0.007	4
IDUWUE-065	2 / 3+	2- / 3	2 / 3+	2+ / 4	0.009	0.428	0.018	0.501	0.044	4
IDUWUE-066	2-	2-	;1-	2-	0.018	0.608	0.013	0.34	0.021	2
IDUWUE-067	0; / ;1	2+3- / 22-	2+3-	3+	0.019	0.225	0.066	0.422	0.267	2
IDUWUE-068	4	3	33+	3+	0.007	0.258	0.068	0.499	0.168	4
IDUWUE-069	2 / 2-;	2- / 3+	;2- / 3	2	0.352	0.049	0.015	0.489	0.096	4
IDUWUE-071	22+	3+	3	4	0.944	0.012	0.011	0.012	0.02	1
IDUWUE-072	2-	2-	2-	2-	0.274	0.027	0.006	0.668	0.025	4
IDUWUE-073	22- / ;	2-	2-	2+	0.01	0.809	0.011	0.16	0.01	2
IDUWUE-074	22-	2-	2	22+	0.122	0.617	0.023	0.228	0.01	2
IDUWUE-075	3	3	2-	;N	0.039	0.314	0.216	0.363	0.069	4
IDUWUE-076	3	3	2-	11+;	0.046	0.013	0.907	0.009	0.025	3
IDUWUE-077	X	2+	2+	4	0.164	0.706	0.046	0.015	0.069	2
IDUWUE-079	3-	2+3-	3-	33+	0.196	0.548	0.168	0.077	0.011	2
IDUWUE-080	2-	22+	2-;	2-	0.005	0.012	0.011	0.299	0.673	5
IDUWUE-081	2-	2-;	2-	3+	0.021	0.086	0.829	0.052	0.012	3
IDUWUE-082	2	3	33+	4	0.023	0.02	0.069	0.88	0.008	4
IDUWUE-083	2+3	2+	22+	33+	0.598	0.076	0.314	0.005	0.007	1
IDUWUE-084	3- / 4	2+3	2+	4	0.053	0.517	0.384	0.016	0.03	2
IDUWUE-085	33+	3	33+	4	0.338	0.014	0.639	0.005	0.005	3
IDUWUE-086	3+ / 2;	4	2+	4	0.011	0.275	0.511	0.186	0.017	3

Appendix 3 Continued…..

Stem rust response					Membership's Coefficients					
Accession code	**TRTTF**	**TTKSK**	**TTTTF**	**JRCQC**	**Subgroup1 (ICARDA germplasm for dry areas**	**Subgroup 2 (ICARDA germplasm for temperate areas**	**Subgroup 3 (Italian and early '70s CIMMYT germplasm**	**Subgroup 4 (CIMMYT germplasm of late '70s-early**	**Subgroup 5 (CIMMYT late '80s-early '90s)**	**Main subgroup**

								'80s)		
IDUWUE-087	3+	3+	3	4	0.012	0.902	0.007	0.063	0.015	2
IDUWUE-088	33+	3	33+	4	0.071	0.419	0.079	0.383	0.048	2
IDUWUE-089	23	3	2-	33+	0.02	0.554	0.023	0.393	0.009	2
IDUWUE-090	2-	2-	2-;	4	0.078	0.353	0.025	0.507	0.036	4
IDUWUE-091	2	3	3	4	0.007	0.008	0.004	0.973	0.008	4
IDUWUE-093	2	33-	3	4	0.01	0.01	0.431	0.538	0.011	4
IDUWUE-094	3+	2+3 / 3	33+	4	0.111	0.134	0.735	0.006	0.013	3
IDUWUE-095	3+	3	33+	3+3	0.091	0.022	0.87	0.007	0.01	3
IDUWUE-096	2-; / ;	2-	;	2-	0.018	0.022	0.765	0.161	0.034	3
IDUWUE-097	2-	2	2-	2	0.01	0.193	0.038	0.302	0.456	5
IDUWUE-098	;N	2-N	;2-	2	0.09	0.329	0.182	0.352	0.047	4
IDUWUE-099	33+	3	2+	2-	0.018	0.117	0.79	0.063	0.012	3
IDUWUE-100	2-	2-	2- / 3+	2-	0.008	0.115	0.679	0.193	0.006	3
IDUWUE-102	2	2-	2-	2-	0.01	0.94	0.024	0.017	0.01	2
IDUWUE-103	2-	2-	2-	2	0.02	0.015	0.729	0.041	0.195	3
IDUWUE-104	2-	3	22+	1	0.018	0.457	0.291	0.218	0.015	2
IDUWUE-105	33+ / 2+	3+	2-	2-	0.038	0.04	0.895	0.008	0.019	3
IDUWUE-106	2-	2+2	2	22+	0.389	0.019	0.561	0.009	0.021	3
IDUWUE-107	3+3	3	33+	4	0.035	0.21	0.606	0.109	0.041	3
IDUWUE-108	22+	22+	22-	2+	0.007	0.89	0.015	0.082	0.006	2
IDUWUE-109	2-;	2- / 3	;2-	3+	0.01	0.304	0.584	0.09	0.011	3
IDUWUE-110	32+; / 2;	2+3	31+;	4	0.027	0.897	0.013	0.042	0.021	2
IDUWUE-111	2-	2-	2-	4	0.047	0.216	0.017	0.7	0.02	4
IDUWUE-112	2+3-	22+	2+	3	0.224	0.44	0.299	0.025	0.011	2

Appendix 3 Continued….

Stem rust response	Membership's Coefficients

Accession code	TRTTF	TTKSK	TTTTF	JRCQC	Subgroup1 (ICARDA germplasm for dry areas	Subgroup 2 (ICARDA germplasm for temperate areas	Subgroup 3 (Italian and early '70s CIMMYT germplasm	Subgroup 4 (CIMMYT germplasm of late '70s-early '80s)	Subgroup 5 (CIMMYT late '80s-early '90s)	Main subgroup
IDUWUE-113	2+3-	22+	2+	3	0.261	0.591	0.133	0.01	0.006	2
IDUWUE-114	3	2+3-	2	;1	0.006	0.01	0.97	0.006	0.008	3
IDUWUE-116	2-;	2-	22-	2+	0.011	0.105	0.857	0.007	0.02	3
IDUWUE-117	22-	3	3	3+	0.143	0.064	0.024	0.751	0.018	4
IDUWUE-118	2-	2-	2- / 3+	22+	0.021	0.008	0.026	0.919	0.025	4
IDUWUE-119	2-	2-	2-	2-	0.006	0.028	0.013	0.939	0.014	4
IDUWUE-120	2-	2-	2+	33+	0.022	0.25	0.383	0.158	0.187	3
IDUWUE-121	2-	2-;	2-;	4	0.022	0.115	0.011	0.842	0.011	4
IDUWUE-122	2	2	22+	22+	0.01	0.044	0.093	0.84	0.013	4
IDUWUE-123	2-;	2-;	2-;	33+	0.071	0.106	0.274	0.526	0.023	4
IDUWUE-124	2-	4/X	3	4	0.014	0.583	0.02	0.328	0.056	2
IDUWUE-125	3+	3+	3+	4	0.027	0.234	0.194	0.501	0.044	4
IDUWUE-126	2-	2-;	2-;	4	0.081	0.465	0.115	0.323	0.017	2
IDUWUE-127	2	2+	2-	2-;	0.029	0.787	0.14	0.032	0.012	2
IDUWUE-128	2	4	3+	4	0.483	0.056	0.05	0.384	0.026	1
IDUWUE-130	2	3	2+ / 3	4	0.018	0.038	0.009	0.927	0.009	4
IDUWUE-131	2-	3+	2-	3+	0.022	0.27	0.037	0.661	0.01	4
IDUWUE-132	2-	2-	2-	2+3-	0.041	0.372	0.01	0.551	0.026	4
IDUWUE-133	2-;	2-	2-;	2-	0.016	0.615	0.02	0.287	0.062	2
IDUWUE-134	2-;	2-;	2-;	33+	0.032	0.448	0.042	0.359	0.118	2
IDUWUE-135	2-	4	3+	3+	0.024	0.101	0.01	0.839	0.026	4
IDUWUE-136	22+	2-	2- / 3-	1; / 4	0.028	0.779	0.033	0.149	0.011	2
IDUWUE-137	3-	4	2-;	2	0.017	0.63	0.034	0.272	0.047	2
IDUWUE-139	2-	3	2-	2+	0.023	0.521	0.029	0.386	0.041	2

Appendix 3 Continued….

Stem rust response	Membership's Coefficients

Accession code	TRTTF	TTKSK	TTTTF	JRCQC	Subgroup1 (ICARDA germplasm for dry areas	Subgroup 2 (ICARDA germplasm for temperate areas	Subgroup 3 (Italian and early '70s CIMMYT germplasm	Subgroup 4 (CIMMYT germplasm of late '70s-early '80s)	Subgroup 5 (CIMMYT late '80s-early '90s)	Main subgroup
IDUWUE-140	2-2	3	22+	3	0.022	0.427	0.487	0.055	0.009	2
IDUWUE-141	2-	3+	3+	4	0.429	0.449	0.014	0.1	0.007	2
IDUWUE-142	2-	2-	2- / 3+	2-	0.008	0.292	0.023	0.64	0.037	4
IDUWUE-144	4	4	4	4	0.238	0.712	0.032	0.009	0.009	2
IDUWUE-145	2 / 2+	2-	2	2+	0.02	0.804	0.006	0.155	0.014	2
IDUWUE-146	2	3	2-	2-	0.007	0.847	0.011	0.09	0.045	2
IDUWUE-147	;N	2	2- / ;N1	2- / 3	0.022	0.884	0.016	0.048	0.03	2
IDUWUE-148	2-	;2-	2-;	2-;	0.703	0.059	0.176	0.042	0.02	1
IDUWUE-149	;N / 2-	2-;	;N	2-	0.059	0.855	0.022	0.042	0.022	2
IDUWUE-150	2	2	22-	22+	0.022	0.825	0.052	0.09	0.011	2
IDUWUE-151	2-	2-;	2-	4	0.028	0.489	0.204	0.261	0.018	2
IDUWUE-153	2-	2-	2-	33+	0.009	0.44	0.072	0.461	0.018	4
IDUWUE-154	33+	3+	3 / ;2-	3+	0.06	0.458	0.26	0.033	0.188	2
IDUWUE-155	;2- / ;N	2+ / 3	3 / 2;	3+	0.055	0.295	0.099	0.514	0.037	4
IDUWUE-156	2- / 3-	2-	2	4	0.035	0.016	0.013	0.829	0.108	4
IDUWUE-157	2-	4	3	4	0.022	0.055	0.018	0.872	0.033	4
IDUWUE-158	2-	2-	2-	2-	0.009	0.016	0.01	0.951	0.014	4
IDUWUE-159	2	3+	3+	4	0.234	0.152	0.038	0.566	0.01	4
IDUWUE-160	2-	3+	3	4	0.956	0.009	0.006	0.018	0.012	1
IDUWUE-161	2-	2	;2-	2-	0.034	0.301	0.036	0.62	0.008	4
IDUWUE-163	2-;	2-	2-;	2-	0.008	0.532	0.009	0.391	0.059	2
IDUWUE-164	2-	2-	2	22-	0.033	0.352	0.015	0.589	0.011	4
IDUWUE-166	2-;N	3+	2+3-	4	0.421	0.117	0.029	0.415	0.017	1
IDUWUE-167	2-	4	33+	4	0.679	0.132	0.025	0.093	0.072	1

Appendix 3 Continued……

Stem rust response					Membership's Coefficients					
Accession code	**TRTTF**	**TTKSK**	**TTTTF**	**JRCQC**	**Subgroup1 (ICARDA germplasm for dry areas**	**Subgroup 2 (ICARDA germplasm for temperate areas**	**Subgroup 3 (Italian and early '70s CIMMYT germplasm**	**Subgroup 4 (CIMMYT germplasm of late '70s-early '80s)**	**Subgroup 5 (CIMMYT late '80s-early '90s)**	**Main subgroup**

IDUWUE-168	2-	2-	2-;	2-	0.011	0.102	0.016	0.746	0.124	4
IDUWUE-169	4	3+	3+	4	0.606	0.204	0.016	0.159	0.016	1
IDUWUE-170	2-;	2-;	2-;	2-	0.044	0.281	0.33	0.061	0.284	3
IDUWUE-171	2 / 3-	3+	2-	2	0.045	0.302	0.016	0.624	0.013	4
IDUWUE-172	2-	2+	3+	3+	0.259	0.159	0.054	0.469	0.059	4
IDUWUE-173	2-	3 / 2	;2-	;1 / 4	0.015	0.444	0.158	0.292	0.09	2
IDUWUE-175	2-	2-	2-	2	0.065	0.128	0.213	0.579	0.015	4
IDUWUE-176	3+3	X-	3	4	0.023	0.698	0.197	0.076	0.007	2
IDUWUE-177	22+	;2-	2-	4	0.02	0.233	0.013	0.635	0.1	4
IDUWUE-178	2- / 3+	2-	2- / 3	3+	0.013	0.824	0.06	0.076	0.027	2
IDUWUE-179	2-	2-;	2-;	2-	0.022	0.158	0.365	0.259	0.196	3
IDUWUE-181	3+3	3	3	3+	0.016	0.92	0.038	0.014	0.012	2
IDUWUE-182	2-	22+	2- / 3	2-	0.514	0.435	0.022	0.015	0.013	1
IDUWUE-183	2-	2-;	2-	3+	0.042	0.078	0.054	0.817	0.009	4
IDUWUE-184	2-	2-	2- / 3-	2-	0.023	0.013	0.013	0.94	0.011	4
IDUWUE-185	2-	4 / 2-	2-	3+	0.03	0.131	0.013	0.757	0.069	4
IDUWUE-186	2-	2-	2-	;2-	0.01	0.37	0.245	0.207	0.169	2
IDUWUE-187	2-	33+	3+	4	0.789	0.014	0.014	0.173	0.01	1
IDUWUE-188	2-	3+	3+	4	0.006	0.346	0.018	0.61	0.019	4
IDUWUE-189	2-	2-	;2-	2-	0.026	0.339	0.614	0.013	0.008	3
CIMMYT-251	;N	33+	3	3+	0.053	0.015	0.025	0.286	0.621	5
CIMMYT-252	2-	4	3	3+	0.019	0.056	0.025	0.015	0.885	5
CIMMYT-253	2-	2-	2-;	2	0.004	0.016	0.007	0.014	0.959	5
CIMMYT-254	2	4	3+	4	0.008	0.015	0.01	0.505	0.462	4

Appendix 3 Continued…..

	Stem rust response				Membership's Coefficients					
Accession code	**TRTTF**	**TTKSK**	**TTTTF**	**JRCQC**	**Subgroup1 (ICARDA germplasm for dry areas**	**Subgroup 2 (ICARDA germplasm for temperate areas**	**Subgroup 3 (Italian and early '70s CIMMYT germplasm**	**Subgroup 4 (CIMMYT germplasm of late '70s-early '80s)**	**Subgroup 5 (CIMMYT late '80s-early '90s)**	**Main subgroup**
CIMMYT-255	2-	2	2	2+	0.008	0.017	0.008	0.024	0.944	5
CIMMYT-256	2-	2-	2-	22+	0.018	0.034	0.024	0.175	0.749	5
CIMMYT-257	2-	;2= / 3	;2-	;	0.008	0.009	0.005	0.021	0.956	5
CIMMYT-258	2-	2-	2-;	2-	0.004	0.006	0.004	0.007	0.98	5
CIMMYT-259	2-	2-	2-	4	0.026	0.048	0.013	0.032	0.88	5
CIMMYT-260	2-	2-	2	2	0.011	0.013	0.011	0.49	0.475	4
CIMMYT-261	2-	3	3+	4	0.011	0.029	0.018	0.565	0.377	4
CIMMYT-262	2-	2-	2-	3	0.006	0.028	0.015	0.045	0.906	5
CIMMYT-263	2-	2	2-	2	0.111	0.079	0.085	0.113	0.613	5
CIMMYT-264	2-	3	3+	4	0.058	0.084	0.018	0.153	0.686	5
CIMMYT-265	2-	2-	2-;	4	0.016	0.061	0.014	0.054	0.855	5
CIMMYT-266	2-	2-	2-	3+	0.004	0.006	0.004	0.747	0.238	4
CIMMYT-267	2-;	2	2- / 3	2	0.099	0.018	0.012	0.021	0.851	5
CIMMYT-268	2-	2	2+	2+	0.036	0.229	0.108	0.043	0.584	5
CIMMYT-269	2-	3	3+	4	0.099	0.036	0.028	0.073	0.764	5
Average membership coefficient					**0.09**	**0.29**	**0.14**	**0.29**	**0.19**	