ASSOCIATION MAPPING FOR DETECTING QTLS FOR FUSARIUM HEAD BLIGHT AND YELLOW RUST RESISTANCE IN BREAD WHEAT

By

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ABSTRACT

ASSOCIATION MAPPING FOR DETECTING QTLS FOR FUSARIUM HEAD BLIGHT AND YELLOW RUST RESISTANCE IN BREAD WHEAT

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Yellow rust (YR), caused by *Puccinia striiformis*, and Fusarium head blight (FHB), caused by *Fusarium graminearum*, are two of the most important wheat diseases in the world. Both pathogens cause severe losses in yield and in the case of FHB, there is an additional concern related with mycotoxin production, which induces serious toxicological problems in human and animals. Breeding for resistance for both diseases has been considered as the most practical strategy of control. To identify sources of resistance and detect regions responsible of resistance to these diseases in wheat germplasm, an association mapping panel (AMP) of 297 spring wheat lines developed by the International Maize and Wheat Improvement Center (CIMMYT) was assembled. The AMP was evaluated for resistance to *P. striiformis* and *F. graminearum* in Mexico and Ecuador over two years. The AMP was screened with 8,632 SNP markers included in the wheat 9K chip from Illumina® and 66 SSR markers from the wheat consensus map. A total of 3,701 SNP and 33 SSR markers were informative and were used to perform analyses in the wheat AMP. Genotypic data was used to estimate the population structure and determine the extent of linkage disequilibrium in the panel. Genotypic and phenotypic data was used to identify marker trait associations.

The structure analysis determined that the panel can be separated in three sub-populations. The extent of LD was different for each genome with major differences between linkage groups in the D-genome. Association analysis with GLM method

detected significant regions associated with yellow rust resistance on chromosomes 1A, 2A, 5A, 6A, 7A, 2B, 5B, 6B, 7B, and 3D, however, the analysis with the MLM method detected significant regions on chromosomes 1A and 2A. The association analysis conducted for Fusarium head blight resistance using the GLM detected regions significantly associated with resistance on chromosomes 4A, 7A, 2B, 5B, and 7B and using the MLM method the regions associated with resistance were located on chromosomes 2B and 7B. In the association analysis for DON concentration with GLM the regions associated with resistance were detected on chromosomes 4A, 5B, 7B, and 2D. However, no significant regions were detected with the MLM method.

This study allowed the identification of several sources of resistance for yellow rust and Fusarium head blight as well as the identification of several molecular markers linked to regions responsible for resistance to these two important diseases. Additionally, the wheat AMP panel showed to be a source of genetic diversity. The findings reported here can be applied to wheat breeding by different programs interested in spring wheat. Finally, the SNP chip utilized to conduct the genotypic analysis was found to be a very useful tool to conduct association analysis studies. However, more coverage on the D-genome might be necessary in spring wheat populations.

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CHAPTER 1

YELLOW RUST AND FUSARIUM HEAD BLIGHT IN BREAD WHEAT: IMPORTANCE, PATHOLOGY AND DISEASE RESISTANCE

Bread wheat: Origin and importance

The origin of bread wheat (*Triticum aestivum* L.) can be traced back to southwest Asia between 8,000 to 12,000 years ago (Giles and Brown, 2006; McFadden and Sears, 1946). Bread wheat is a hexaploid species with three genomes A, B, and D. Hexaploid wheat arose from the hybridization of cultivated tetraploid emmer wheat (*T. turgidum* ssp. *dicoccum* Schrank) with the wild diploid wheat species *Aegilops tauschii* Coss.(Caldwell et al., 2004; Matsuoka, 2011). Each of the three genomes has seven chromosomes and the total chromosome number is (2n = 6x = 42) (Gill and Friebe, 2009). *Triticum aestivum* and all polyploidy wheat species are disomic in inheritance due to genome-specific chromosome-pairing (Gustafson et al., 2009), controlled by pairing suppressor genes *Ph1*, *Ph2* and other minor genes (Ceoloni and Feldman, 1987; Sears, 1976; Sears, 1977). This characteristic has allowed full fertility in the species and, moreover, the action of favorable effect of an extra gene dosage or the build-up of positive inter-genomic interactions (Feldman et al., 2012).

The allelic diversity found in hexaploid wheat is reduced compared with its diploid ancestors (Haudry et al., 2007). This severe bottleneck originated by limited number of hybridizations during its formation (Talbert et al., 1998). Fortunately, diploid wheat species can naturally or artificially be crossed with other polyploid wheat species (Gill and Raupp, 1987). These interspecific crosses have helped to increase the diversity in hexaploid wheat (Chen and Li, 2007; Sharma and Gill, 1983). Furthermore, production

of interspecific crosses has resulted in the development of wheat lines with resistance to many biotic and abiotic constrains (Mujeeb-Kazi et al., 1996; van Ginkel and Ogbonnaya, 2007) and are being used in wheat breeding programs and in some cases have resulted in improved wheat varieties (Yang et al., 2009).

The wheat genome is one of the largest crop genomes with ~16 000 Mb (Gill et al., 2004) of which 80% are repetitive sequences (Smith and Flavell, 1975). Wheat has a complex and extremely large genome compared with other crops, therefore its genome has not yet been totally sequenced. Efforts to sequence the genome are being led by the International Wheat Genome Sequencing Consortium (IWGSC) which aims to establish a high quality reference sequence of the wheat genome using cv. 'Chinese Spring' (www.wheatgenome.org). Currently, only chromosome 3B is completely sequenced by a French group from INRA.

Wheat is one of the most important crops in the world and is grown on 20% of the cultivated land area of the world. It is grown on more than 216 million hectares with an approximate production of 675 million tons of grain annually (FAOSTAT, 2012). It is the staple food of nearly 35% of the world's population (Rajaram, 2010). Most of its production is for human consumption mostly as flour and a small portion as whole grain is used to feed animals (Harlan, 1981). Wheat provides 20% of the total caloric inputs and protein to the world population (Reynolds et al., 2008; Shiferaw et al., 2013). It is also the most widely adapted crop plant and wheat is produced between 30º - 60º north latitude and between 27º - 40º south latitude (Bockus et al., 2010). Likewise, wheat is produced at high altitudes in the tropics such as the Andean region or valleys in equatorial countries in Africa (Dubin and Rajaram, 1996; Lantican et al., 2005). The

diversity of environments where wheat is grown also allows the occurrence of vast number of diseases which affect seed quality and yield. A complete review of diseases affecting wheat can be found in (Bockus et al., 2010). Among this large group of wheat diseases, yellow rust (*Puccinia striiformis* Westend. f. sp. *tritici*) and fusarium head blight (*Fusarium* spp.) are considered two of the most severe.

Yellow Rust

Yellow Rust (YR), also known as stripe rust, is caused by *Puccinia striiformis* Westend. f. sp. *tritici* (McIntosh et al., 1995). Yellow rust is one of the major wheat diseases in temperate regions around the world (Roelfs et al., 1992). High losses can arise due to reduced number and size of flowering spikes, shriveled grain, and damaged tillers, especially when the infection occurs in early growth stages (Wellings, 2010). Losses from 20 to 75% have been recorded in the western states of the US during severe epidemics (Roelfs, 1978). *Puccinia striiformis* has been a constant threat to wheat production. Significant regional epidemics have been recorded since 1725 (Wellings, 2011). Such recurrent epidemics occur due to a combination of specific virulence in the pathogens population and wide-scale cultivation of genetically similar varieties (Danial et al., 1994).

The infection can occur throughout the life of a plant. Symptoms first appear as chlorotic patches on leaves. Tiny, yellow to orange uredia develop in these chlorotic areas (Chen, 2010). Narrow stripes are formed on the leaves due to the production of pustules containing orange-yellow urediospores. Yellow rust usually infects leaves; however, the disease can also infect the glumes of the spikelets in susceptible cultivars.

Biology of *Puccinia striiformis*

Puccinia striiformis is an obligate parasite that shows optimal development under high relative humidity conditions and low temperatures (8-15°C), particularly cool nights (< 10°C). The optimum temperature for urediospore germination is between 7 and 12°C, with limits near 0 and 21°C. Disease development is most rapid between 10 and 18°C with intermittent rain or dew (Chen, 2010).

Puccinia striiformis is considered a highly diverse pathogen since large number of different races have been reported worldwide (Kolmer et al., 2009). This pathogenic variability has been observed between and within geographical areas (Chen et al., 2009; Chen et al., 2002; Mboup et al., 2009). The main mechanism generating variability is thought to be the result of mutations and asexual recombination (Stubbs, 1988). An alternate host of *P. striiformis* was unknown, so it was though that the pathogen has a micro-cyclic life cycle (McIntosh et al., 1995). However, Jin et al. (2010) recently demonstrated that several *Berberis* spp. in China can be naturally infected by *P. striiformis* and act as alternate hosts. In consequence, *P. striiformis* is a macrocyclic rust with five different spore stages: uredinial, telial, basidia, pycnial, and aecial stages (Figure 1-1).

Yellow rust control

The use of resistance genes is considered the most effective strategy to control yellow rust. The incorporation of resistance genes for yellow rust along with other resistance

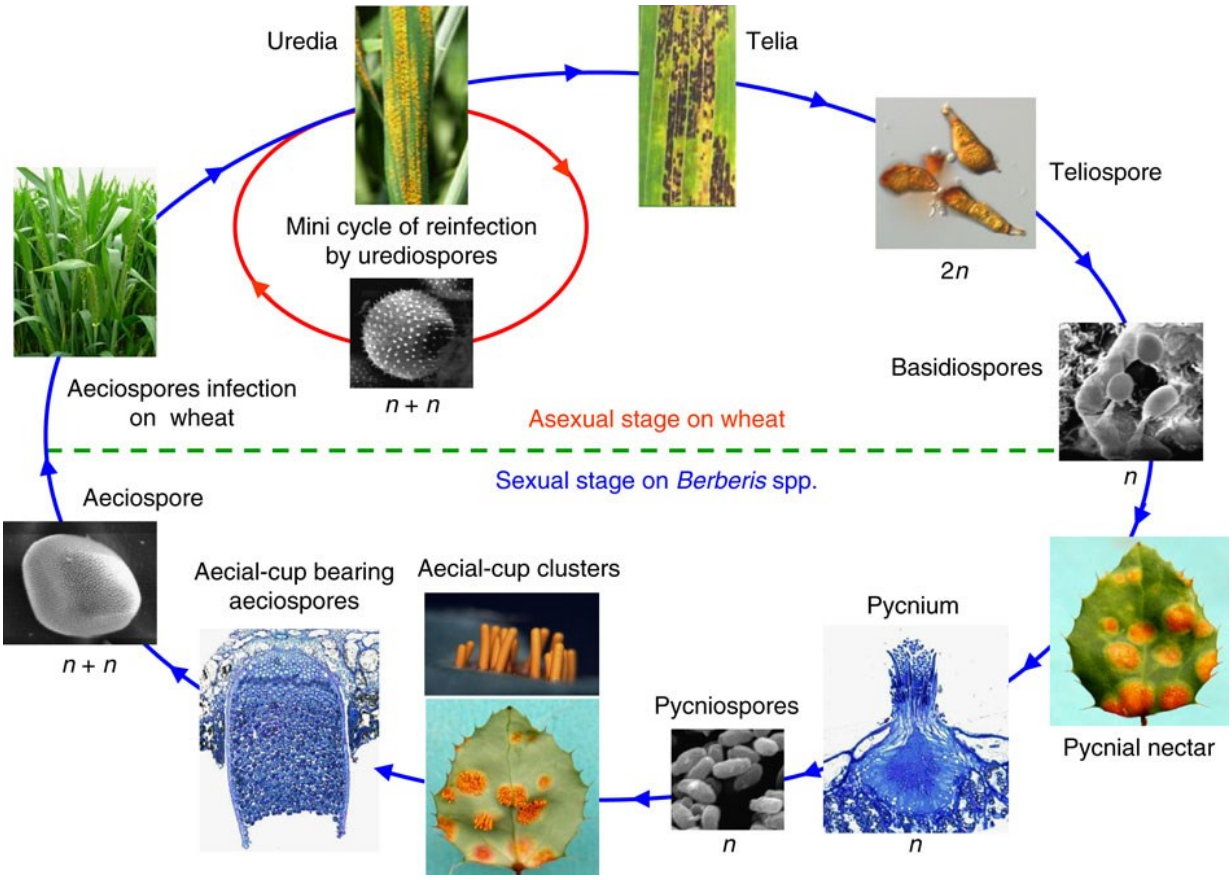

Figure 1-1. Life cycle of *Puccinia striiformis* Westend. Two types of disease symptoms may appear on a wheat primary host, the uredinial stage with urediniospores and the telial stage with teliospores. The two-celled teliospores may germinate with a basidium developing into four basidiospores. In the alternal host, the pathogen can produce pycniopores. Finally, aeciospores are produced and wheat can be infected completing the cycle (Zheng et al., 2013). For interpretation of the references to color in this and all other figures, the reader is referred to the electronic version of this dissertation.

genes has been the primary objective of most of the wheat breeding programs (Johnson, 1992).

Many sources of resistance carrying major or minor genes have been reported (Roelfs et al., 1992; Wellings, 2011). However, the large genetic variability and high mutation rate that it exhibits has allowed the yellow rust pathogen to overcome many major resistance genes. For example, the resistance conferred from *Yr27* resistance gene has broken down in some regions in Asia (Hodson, 2011). For this reason, it is necessary to

develop cultivars with high and durable resistance that combine effective genes. A promising long-term control strategy is to breed and deploy cultivars carrying durable resistance based on minor, slow rusting genes with additive effects (Singh et al., 2004) The use of multi-lines has been proposed to control cereal diseases (Wolfe, 1985); however, the success of this strategy depends on several factors such as the genetic background of the pathogen race, host, and interaction among pathogen races (Dileone and Mundt, 1994) resulting in a very complex approach.

Cultural practices, such as the removal of volunteer plants from previous seasons, are always part of integrated control to avoid early infections. Several fungicides are effective to control the disease. Seed treatment and timely application of fungicides can be used (Chen, 2010); however, the use of fungicides significantly increase the production cost (Wellings, 2007).

Resistance to yellow rust

Genetic resistance to yellow rust is conferred by race-specific and/or non-race-specific genes. The race-specific resistance is usually conferred by a single dominant gene, which results in a hypersensitive reaction that can be observed after the pathogen infection. Whereas non-race-specific resistance or horizontal resistance is controlled by QTLs that act additively (Lindhout, 2002). Race-specific genes have been extensively used; however, this type of resistance has been overcome by some rust pathogen biotypes (Johnson, 2000). The capability of the pathogen to develop new virulent races via mutations is relatively high (Chen et al., 2009; Sharma-Poudyal et al., 2013; Wellings et al., 2000). More than 50 yellow resistance genes have been identified and

catalogued and several more are under characterization (Boyd, 2005; McIntosh et al., 2012; Yamazaki et al., 1998). The majority of the genes that have been cataloged are expressed throughout the life of the plant; however, some genes are expressed at later growth stages and the resistance type that they confer has been designated as field or adult plant resistance (APR)(Johnson, 1992), and some particular APR genes are only expressed at high temperatures (> 10ºC) (Qayoum and Line, 1985; Uauy et al., 2005). Several QTLs conferring resistance to yellow rust have been reported and mapped (Table 1-1).

Table 1-1. QTLs for field or adult plant resistance to yellow rust in wheat. Adapted from Boyd (2005).

Chromosomal location of QTL	Source of QTL	gene name	
3BS	'Opata85'		Singh et al. 2000
3DS	'Opata85'		
5DS	'Opata85'		
7DS	'Opata85'; '*Yr18*/6*AvS'	*Yr18*	(Singh et al., 2000b)
2BS	'Opata85'		Borner et al. 2000
2AL	'Opata85'		
2BS	'Opata85'		Boukhatem et al. 2002
3DS	'Opata85'		
5AL	'Opata85'		
6DL	'Opata85'		
7DS	'Opata85'		
3BS	'Lgst79-74'	*Yrns-B1*	
1BL	'Pavon76'	*Yr29*	
3BS	'Pavon76'; 'Parula'	*Yr30*	
4B	'Pavon76'		
6[a]	'AvocetS'		
6B	'Pavon76'		
1BL	'Parula'	*Yr29*	
3BS	'Parula'	*Yr30*	
7DS	'Parula'	*Yr18*	
2BS	'Kariega'		
7DS	'Kariega'		
7B	'Kukri'	*QYr.sun-7B*	
2D	Quaiu #3	*Yr54*	(Basnet et al., 2013)
4DS		*Yr28*	(Singh et al., 2000b)

The most promising long-term control strategy is to breed and deploy cultivars carrying durable resistance based on minor, slow rusting genes with additive effects (Singh et al., 2004). Wheat breeding lines with high yield potential and resistance levels reaching near-immunity to yellow rust have been successfully developed by CIMMYT through combination of several QTLs (3 – 5) with small to intermediate effects (Singh et al., 2000a). In this context, CIMMYT has been successful with the development of hundreds

of wheat lines that have been released as new improved cultivars in many countries of the world, especially in developing countries (Reynolds and Borlaug, 2006).

Resistance genes widely used in developing wheat lines with resistance to yellow rust are many. Among them, *Yr18* is one of the most widely deployed (Reynolds and Borlaug, 2006). *Yr18* confers moderate levels of adult plant resistance (Singh and Rajaram, 1992). Additionally, this gene is completely linked to other genes that confer resistance to other diseases such as leaf rust, barley yellow dwarf (BYD) virus, and powdery mildew (Singh, 1993; Spielmeyer et al., 2005). These combined characteristics were the reason to develop molecular markers to conduct marker assisted selections for these specific region (Suenaga et al., 2003).

Yr25 is another gene frequently deployed in wheat cultivars (Boshoff and Pretorius, 1999) and it is also present in 'Strubes Dickkopf' used to differentiate *P. striiformis* races. This gene was located on chromosome 1D. Interestingly, it has been observed that genes located in other chromosomes might suppress or reduce the levels of resistance of this gene (Calonnec and Johnson, 1998).

Another example of a resistance gene frequently deployed is *Yr32* (Hovmøller, 2007). Gene *Yr32* is located in chromosome 2AL (Eriksen et al., 2004), and it is present in the differential cultivar 'Cartens V' (McIntosh et al., 1995).

Other genes have been widely deployed in wheat breeding for resistance such as *YrA*, *Yr1*, *Yr2*, *Yr9*, and *Yr17*; however, several reports have been published indicating that resistance have been overcome by new strains of *P. striiformis* (Bayles et al., 2000; Boyd, 2005; Hovmøller, 2001; Lupton and Johnson, 1970; Wellings, 2011). None of these major genes are recommended to be used alone.

Fusarium Head Blight

Fusarium head bight (FHB), also known as Fusarium ear blight or scab, is one of the most important diseases affecting wheat. The major causal organism of this disease worldwide is *Gibberella zeae* (Schwein) Petch (anamorph: *Fusarium graminearum* Schwabe) (Schmale III and Bergstrom, 2003). However, FHB several other species of *Fusarium* and one species of *Microdochium* can also cause FHB. *Fusarium graminearum* and *F. culmorum* are the most important species due to their wide distribution in wheat fields around the world (Bottalico and Perrone, 2002; Parry et al., 1995). The infection of *Fusarium* on wheat causes yield reduction and losses as high as 50% (Ireta and Gilchrist, 1994). FHB epidemics are cyclic and severe outbreaks of the disease have been reported in many regions where the crop is grown resulting in millions of dollars in crop losses (McMullen et al., 1997). The pathogen also produces mycotoxins, which are a major concern. These metabolites have toxic effects in humans and mono-gastric animals (Bottalico and Perrone, 2002). These toxins can induce a spectrum of effects in farm and laboratory animals including emesis immunotoxic effects, and suppression of appetite and growth (Voss, 2010). The most common mycotoxins are Deoxynivalenol (DON), Zearalenone, Moniliformin, 3-Acetyldeoxynivalenol (3-ADON), Nivalenol, and T-2 toxin (Bottalico and Perrone, 2002; Placinta et al., 1999). Mycotoxins are commonly present in wheat fields and the health risk associated with them has prompted several countries to create a policy regarding maximum allowable levels in food. For instance, the United States allows a maximum concentration of DON of 1000 µg/kg in wheat products finished for human consumption (Richard, 2007); whereas the European Nations do not allow flour with more than 750

µg/kg (van Egmond and Jonker, 2004). Unfortunately, several countries lack regulations for mycotoxins concentrations in food or allow relatively high concentrations in wheat products (Dohlman, 2004).

FHB was first described in 1884 in England and was considered a major threat to wheat and barley during the early years of the twentieth century (Stack, 2003). The first symptoms of FHB appear shortly after flowering. Diseased spikelets exhibit premature bleaching as the pathogen grows and spreads within the head (Ireta and Gilchrist, 1994). One or more spikelets located on the top, middle, or bottom of the head may be bleached. Over time, the premature bleaching of the spikelets may progress throughout the entire head (Schmale III and Bergstrom, 2003). Other symptoms include tan to brown discoloration at the base of the head, a pink or orange colored mold at the base of the florets under moist conditions, and kernels that are shriveled, white, and chalky in appearance (Buhariwalla et al., 2011). The pathogen can infect wheat spikes from flowering to late stages of kernel development (Del Ponte et al., 2007). Initial source of *Fusarium* inoculum comes from the soil, which survives either as saprophytic mycelium or as chlamydospores (Parry et al., 1995). Later in the season, macroconidia and ascospores carried by air currents to wheat heads are considered the primary inoculum (Dill-Macky, 2010). Warm temperatures and high relative humidity favor pathogen growth, and aggregations of light pink/salmon colored spores (sporodochia) may appear on the rachis and glumes of individual spikelets (Schmale III and Bergstrom, 2003). Later in the season, bluish- black perithecia bodies may appear on the surface of infected spikelets. These bodies are sexual structures of the fungus known as perithecia. As symptoms progress, the fungus colonizes the developing grain, causing it

to shrink and wrinkle inside the head (Dill-Macky, 2010). The cycle is completed when *Fusarium*-infected seeds or host residues remaining in the soil provide source of inoculum for the next cropping cycle (Parry et al., 1995) (Figure 1-2).

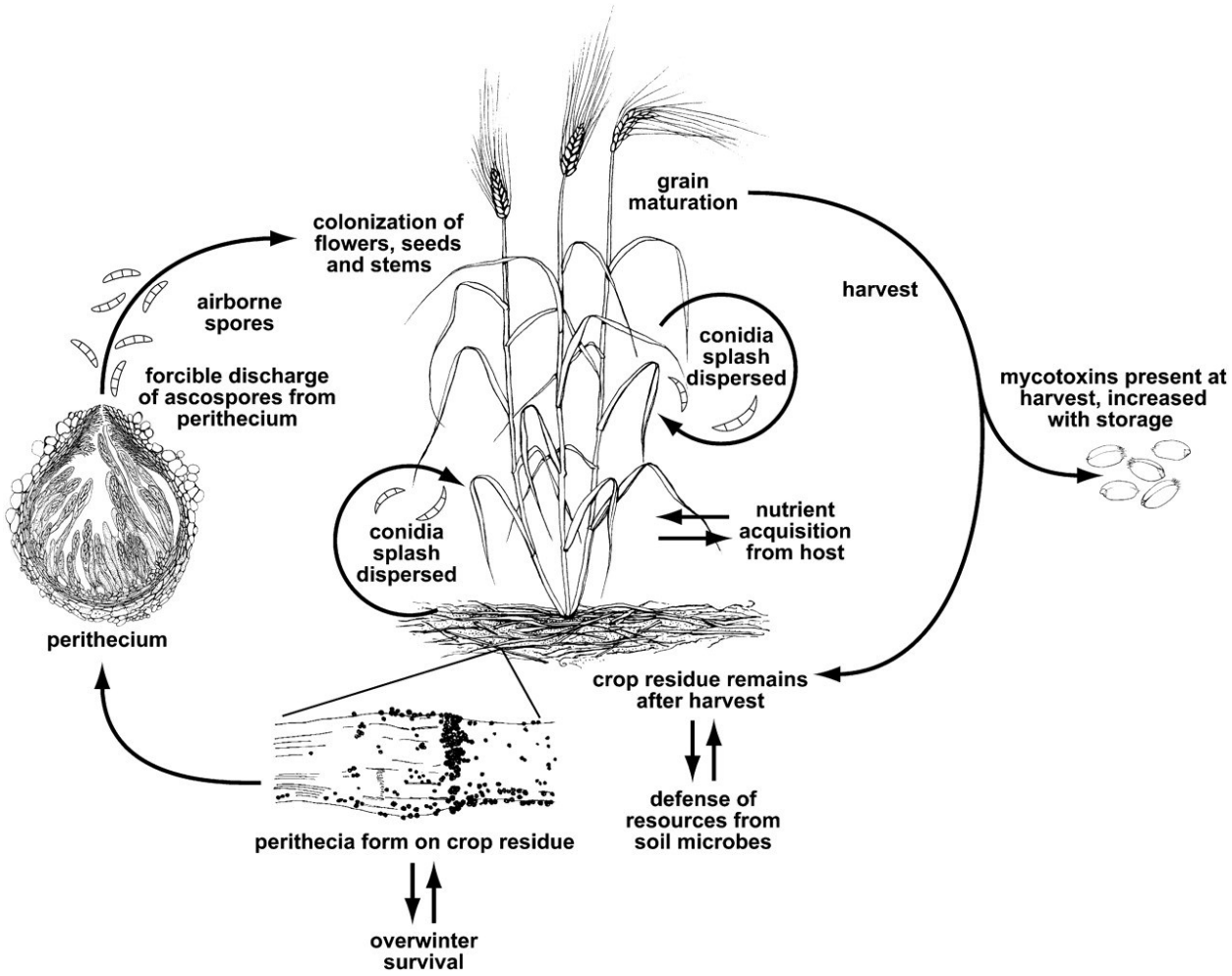

Figure 1-2. *Fusarium graminearum* life cycle in wheat. The pathogen overwinters on infested crop residues. Ascospores from perithecium are produced and infect wheat spikes. Infected seed or crop residues become the source of inoculum for the next season (Trail, 2009).

Control of FHB

There is agreement that no single strategy is 100% effective against FHB (Gilbert and Haber, 2013). Cultural and management practices, such as crop rotations with at least a

1-year break from the cultivation of a host crop (corn, wheat, barley, and other cereals), thorough tillage (McMullen et al., 2012; Parry et al., 1995; Pereyra and Dill-Macky, 2008) and the use disease-free or treated seeds (Gilbert and Tekauz, 2000), may reduce the damage caused by FHB in wheat cultivars. However, these practices do not completely control the disease (Dill-Macky, 2010; Dill-Macky and Jones, 2000). Fungicides partially control the disease under optimal application conditions (Jones, 2000). However, fungicide application is not always effective because not all fungicides used can control FHB (Mesterházy et al., 2011). Moreover, it has been reported that some fungicides such as azoxystrobin partially controlled the disease but resulted in an increase of DON toxin concentration (Mesterházy et al., 2003). It is also common to get incomplete crop coverage of spikes because differences in flowering or inadequate equipment use (Mesterházy, 2003). Incorrect timing of application can also be another reason for control failure. Some fungicides such as tebuconzole or carbendazim are reported as useful to control FHB (Dill-Macky, 2010); however these fungicides do not totally prevent the disease (Jones, 2000; Mesterházy et al., 2011). The increase in cost is also a constraint for some farmers who want to avoid additional production costs (Lewis, 2010, pers. com.). Additionally, chemical control may represent health risks to farmers who are exposed to pesticides and do not take enough care to protect themselves or simply ignore safety measures (Ecobichon, 2001; Jeyaratnam, 1990). Therefore, the development of new cultivars, with high levels of FHB resistance, is the most promising cost-effective strategy for FHB control.

Resistance to FHB

The resistance to FHB has been grouped based on mechanisms. The most studied types of FHB resistance are: type I, (resistance to initial infection) and type II, (resistance to fungal spread within the inoculated head). Other types are resistance to deoxynivalenol (DON) accumulation (also known as type III), and resistance to the development of Fusarium-damaged kernels (FDK) (Schroeder and Christensen, 1963). Presently, no cultivar has been reported as immune to FHB infection; however, large genetic variation for FHB resistance has been observed in wheat germplasm (Mesterhazy et al., 2005; Ruckenbauer et al., 2001). QTL mapping studies have shown that resistance genes for FHB are present on all wheat chromosomes except chromosome 7D (Buerstmayr et al., 2009). Several sources of resistance have been reported and widely used. One of these sources is the Chinese cultivar 'Sumai 3', that possesses two well-known and exploited loci (*Fhb1* and *Fhb2*) (Waldron et al., 1999). However, none of these genes confer complete resistance to the pathogen (Miller and Greenhalgh, 1988; Snijders, 1994). Other Chinese wheat cultivars used as sources of resistance include 'Ning7840', 'Wuhan 1' and 'Nyuubai' (McCartney et al., 2007), 'Chokwang' (Yang et al., 2005). Another popular source of resistance widely used for more than 50 years ago is the Brazilian cultivar 'Frontana' (Schroeder and Christensen, 1963). Sources from Europe have been also reported, and the Swiss cultivar 'Arina', are the most studied and used from that region (Snijders, 1990).

Table 1-2. Most common sources of FHB resistance, location of the QTLs and type of resistance. Adapted from Buerstmayr et al. (2009).

Source of resistance	**Country of origin**	**Chromosome**	**Type of resistance**
'Sumai 3'	China	3BS	FHB spread (II)

Table 1-2 (cont'd)

		6BS	FHB spread (II)
'Ning 7840'	China	3BS	FHB spread (II)
		2BL	FHB spread (II)
		2AS	FHB spread (II)
'Stoa'	USA	2AL	FHB spread (II)
		4BS	FHB spread (II)
'ND-2603'	USA	3BS	FHB spread (II)
		6AS	FHB spread (II)
		3AL	FHB spread (II)
'CM-82036'	Mexico	3BS	FHB spread (II)
		5[a]	FHB spread (II)
		1B	FHB spread (II)
'Alondra'	Mexico/Brasil	2DS	FHB spread (II)
		1B	FHB spread (II)
'Ning 894037'	China	3BS	FHB spread (II)
		6BS	FHB spread (II)
'Huapei 57-2'	China	3BS	FHB spread (II)
		3BL	FHB spread (II)
		3AS	FHB spread (II)
'Wuhan 1'	China	2DL	FHB spread (II)
'Patterson'	USA	5BL	FHB spread (II)
		3D	FHB spread (II)
'Nyu Bai'	China	3BS	FHB spread (II) and DON content
		3BS	FHB Severity
		5AS	DON content
		2D	DON content
'Wangshuibai'	China	3BS	FHB spread (II)
		6B	FHB spread (II)
		1B	FHB spread (II)
		7A	FHB spread (II)
		3BS	FHB spread (II) and DON content
		2D	FHB Severity
		4B	FHB Severity
		5B	FHB Severity
		2DL	FHB Severity
		5A	FHB Incidence
		3AS	FHB Incidence
		5DL	DON content and FHB Incidence
'Frontana'	Brasil	3A	FHB Severity (II) and FHB Incidence
		5[a]	FHB Severity
		2B	FHB incidence

Table 1-2 (cont'd)

		6B	FHB Severity and FHB Incidence
		7AS	FHB Severity
'Arina'	Switzerland	4AL	FHB Severity
		6DL	FHB Severity
		3BL	FHB Severity
		5AL	FHB Severity
		2AL	FHB Severity
		1BL	FHB Severity
		6BS	FHB Severity
		4DS	FHB Severity
		6BL	FHB Severity

The selection of wheat germplasm with resistance is conducted mainly in the field, but greenhouse inoculations can be performed to assess type II resistance (Buerstmayr et al., 2002). The screening techniques may differ and depend on factors such as project goals, precision needed, number of lines under evaluation and resources (Rudd et al., 2001). The environment plays an important role in the development of the disease, so the infection in the field might be improved with the use of sprinklers to provide adequate levels of humidity. Since resistance to *Fusarium* head blight is horizontal and non-race specific (Mesterhazy et al., 1999), selection of any aggressive strain of *F. graminearum* or *F. culmorum* for screening purposes should be satisfactory (Eeuwijk et al., 1995). To ensure infection in the trials, some researchers use a mixture of isolates to do not completely depend in only one isolate (Lu et al., 2013; Van Ginkel et al., 1996; Yoshida and Nakajima, 2010). The inoculum concentration is an important factor in screening for resistance. Stein et al. (2009) reported that disease incidence and severity increased sharply in relation to inoculum

concentration. In general, a recommendation will be to use inoculum with concentration of 50,000 spores/ml (Gilbert and Woods, 2006).

Numerous QTLs in wheat have been mapped onto chromosomes of resistance sources from many Asian, North American, South American, and European countries using traditional QTL analysis methods (Ma et al., 2006; Paillard et al., 2004). More than 100 QTLs conditioning FHB resistance in wheat have been reported (Buerstmayr et al., 2009; Liu et al., 2009; Loeffler et al., 2009). However, the discovery of such QTLs has been conducted in bi-parental populations (Buerstmayr et al., 2009) and most of the QTLs have minor effects. New methods to identify QTL for FHB and other wheat diseases are being employed which are described with more detail in the following two sections.

Association mapping

Association Mapping (AM), also known as Association Analysis or Linkage Disequilibrium Mapping, is a method used to detect QTLs controlling traits based on correlating genotype with phenotype (Neumann et al., 2011). Association mapping can also be employed as an approach to validate the presence and position of QTLs previously reported (Aranzana et al., 2005). The principle of AM methodology is based on linkage disequilibrium (LD) (Breseghello and Sorrells, 2006), which is the non-random association of alleles at different loci (Flint-Garcia et al., 2003). LD tends to be maintained over many generations between loci which are genetically linked to one another. The approach was developed originally in the field of human genetics (Lander

and Schork, 1994) and now, with the development of complex statistical methods, association mapping is being employed in plants (Thornsberry et al., 2001).

One of the advantages of association mapping is the use of existing populations, which could be obtained from gene banks or germplasm collections. Therefore, there is no need to develop specific crosses resulting in saving time (Oraguzie and Wilcox, 2007). The population can be assembled with breeding lines, cultivars, landaraces or mixtures of all of them. In order to successfully detect QTLs controlling traits of interest in such populations using AM approaches, a diverse population with a considerable allelic variation for the trait/s of interest must be assembled (Yu et al., 2006). If the population is rich in allele diversity for a specific trait, the likelihood to discover large number of significantly important and novel alleles will increase. Less frequent alleles significantly associated with a trait can exist, however, rare alleles are usually not considered for analysis (Adhikari et al., 2012; Reimer et al., 2008), since association analysis require rare alleles to be filtered to avoid errors that could lead to false positive associations (Brachi et al., 2010; Maccaferri et al., 2010).

Two methods are extensively used in association analysis: The general linear model (GLM) and the mixed linear model (MLM). With the GLM method, associations between markers and phenotype are detected using the population membership estimates of each individual as covariates to control for population structure (Pritchard and Rosenberg, 1999), since population structure can cause spurious associations (Kang et al., 2008). MLM, additionally to the population structure, incorporates kinship in the association analysis allowing an improved control of type I and type II error rates over GLM due to relatedness and population structure (Yu et al., 2006).

False discoveries are a common problem in association studies. A false discovery refers to the situation when one concludes erroneously that a genomic region harbors a gene contributing to a quantitative trait (Sabatti, 2007). False discoveries are common in association studies due to the multiple hypotheses testing (Sabatti, 2007; Storey, 2003). In order to control false discoveries in association studies, several methods have been proposed. Bonferroni multiple correction test is one of the most well known methods (Shaffer, 1995), which defines a cut-off value based on the proposed threshold divided by number of tests (aka markers employed in the analysis) as a new threshold. However, this method has been considered too conservative (Perneger, 1998). Some other methods such as Holm-Bonferroni have been cited in the literature of association mapping studies (Miedaner et al., 2011), which are described as more powerful test since is more likely to detect an effect it exists (Abdi, 2010). Finally, the Q value method proposed by Storey (2002) is also used in association studies, where q-values are calculated based on p-values.

Association mapping in wheat

Association mapping in wheat has become a popular method to detect QTLs, based on numerous studies published. For example, association mapping have been used to detect markers associated with agronomic traits (Yao et al., 2009), quality traits such as kernel size and milling quality (Breseghello and Sorrells, 2006; Reimer et al., 2008), and resistance to diseases such as yellow rust (Wang and Chen, 2013), leaf rust (Maccaferri et al., 2010), *Fusarium* head blight (Hao et al., 2012; Kollers et al., 2013), and *Septoria tritici* blotch (Goudemand et al., 2013).

The number and distribution of molecular markers in the genome are critical for association mapping studies. In this sense, microsatellites (SSRs), Diversity Array Technology (DArT) and single nucleotide polymorphisms (SNP) markers are considered the best choices (Crossa et al., 2007; Jing et al., 2009; Zhu et al., 2008). These markers are highly polymorphic in the wheat genome or any plant species and can be automated or semi-automated (Akbari et al., 2003; Zhu et al., 2008). Association mapping studies using SSRs have been published where important agronomic traits such as plant height, spike length, spikelets per spike, grains per spike, thousand kernel weight have been associated with SSR markers (Maccaferri et al., 2008; Maccaferri et al., 2010; Reimer et al., 2008; Yao et al., 2009). DArT markers have been successfully employed in association mapping to find associations between markers and resistance to stem rust, leaf rust, yellow rust, and powdery mildew, grain yield in wheat from CIMMYT (Crossa et al., 2007). Currently, there are around 7,000 DArT markers available for wheat (Goudemand et al., 2013). In the case of SNPs, there is a large list of SNPs markers available at databases such as Graingenes (http://wheat.pw.usda.gov), the Triticease tool box (http://triticeaetoolbox.org/wheat/) or CerealsDB (http://www.cerealsdb.uk.net/). The wheat community now has a valuable tool which will facilitate the screening of wheat populations with almost 9,000 SNP markers distributed in the wheat genome. This is the 9K SNP chip developed by a research consortium (Cavanagh et al., 2013) and commercialized by Illumina. The chip was developed from 27 wheat cultivars from the US and Australia (Akhunov et al., 2011) funded by USDA-AFRI and Grains Research and Development Corporation (GRDC, Australia) (http://www.triticeaecap.org/). The wheat SNP chip is now available to the wheat

community and results from its use are already being published. Wang and Chen (2013) have used the SNP chip to detect markers linked with regions conferring resistance to yellow rust, Zhao et al. (2013) detected frost tolerance locus on Central European winter wheat, and Würschum et al. (2013) used the SNP chip to conduct a study of genetic diversity in a population of winter wheat.

Linkage disequilibrium (LD) in plants

Linkage disequilibrium is the nonrandom association of alleles at different loci (Flint-Garcia et al., 2003). Alleles at two or more loci are said to be in LD if they are non-randomly co-inherited as determined by their individual and joint allele frequencies (Slatkin, 2008). Consequently, for two loci, the alleles at one locus are predictive of those present at the other. Given its dependence on allele frequencies, any measure of LD is population-specific (Waugh et al., 2009). The extent of LD differs for each crop species and LD can vary between different populations of he same crop species (Chao et al., 2010). Factors affecting LD can be domestication, mating system, inbreeding (Kim et al., 2007; Wright et al., 2005), selection of favorable alleles (Cavanagh et al., 2013; Kane and Rieseberg, 2007), and admixture (Flint-Garcia et al., 2003).
It has been observed in wheat that LD extends differently depending on population origin and genome (A, B, or D), however, LD commonly extends more than 10 cM (Chao et al., 2010). In corn, due the diversity and the mating system, LD decays relatively fast. The LD decay distance ranged from 1 to 10 kb (Yan et al., 2009). LD does not decay as fast in self-pollinated crops (Flint-Garcia et al., 2003). LD in soybean extended from 90 to 574 kb in three cultivated groups which presented highly variable

patterns of LD (Hyten et al., 2007). However, this is not always a constant. In wild barley, a self-pollinated species, LD may decay faster than expected (Morrell et al., 2005) or in the case of *Arabidopsis thaliana*, LD decays within 10 kb on average which is faster than previously estimated (Kim et al., 2007).

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CHAPTER 2

STUDY OF THE POPULATION STRUCTURE IN THE WHEAT ASSOCIATION MAPPING PANEL

Abstract

Wheat is the number one cereal grown in the world based on production area and direct human consumption. Fifty percent of this wheat is produced in developing countries where spring wheat type is the most abundant. CIMMYT, based in Mexico, and its branches located in many countries are the main source of spring wheat germplasm in the world. As a result, thousands of wheat varieties have been released in the world. CIMMYT continues the effort of producing wheat germplasm with high yield and enhanced disease resistance to distribute potential new varieties or sources of valuable alleles with the mission to end hunger in the world. One major concern of breeders at CIMMYT is the reduction of genetic diversity. Therefore, CIMMYT breeders focus on maintaining high levels of diversity in international nurseries. In the current study, population structure and extent of linkage disequilibrium (LD) were examined in a wheat association mapping panel (AMP) with 297 wheat accessions developed by CIMMYT with many elite accessions. To conduct this study, a SNP chip with 9K markers and 20 SSR markers were used. Analysis of the population structure determined that the wheat AMP can be separated in three sub-populations. Linkage disequilibrium extended between 13 – 15 cM on chromosomes in the A and B-genome. On the D-genome, LD decayed at different distances from 3 cM on chromosomes 2, 4, and 7D to 40 cM on chromosome 6D. The results of the population structure analysis showed that the AMP includes wheat accessions genetically distant which is important to conduct wheat

breeding. The LD analysis showed that LD extends considerably as is expected in a self-crossing species such as wheat. Based on the LD results, it was concluded that association studies can be accurately conducted with the 9K SNP chip; however, there is low marker coverage on the D-genome. Therefore, it is necessary to include more molecular markers on D-genome to increase the likelihood of finding favorable alleles and increase the confidence of the results in association studies.

Introduction

Wheat (*Triticum aestivum* L.) is one of the most ancient crops cultivated by humankind (McFadden and Sears, 1946) and, nowadays, wheat is the most widely cultivated cereal in the world with approximately 220 million ha planted annually (FAOSTAT, 2012). Fifty percent of the wheat is produced in developing countries (Shiferaw et al., 2013). Most of the wheat cultivated in this region of the world is spring wheat type and the spring wheat germplasm developed by the International Maize and Wheat International Improvement Center (CIMMYT) is predominant. According to Lantican et al. (2005), 86% of all spring bread varieties releases in developing countries (excluding Eastern Europe and Former Soviet Union) were originated by or had some form of CIMMYT ancestry. The genetic characteristics of CIMMYT's wheat germplasm are some of the reasons to find this type of wheat distributed in many regions of the world. CIMMYT germplasm have *Rht* genes, which stands for 'reduced height' (Ellis et al., 2005), and indirectly increase harvest index and reduce lodging by inhibition of gibberellin sensitivity in wheat cultivars (Flintham et al., 1997; Youssefian et al., 1992). Additionally, CIMMYT focuses its efforts

on the incorporation of genes to confer resistance to the major and most frequent biotic and abiotic constraints that occur around the world (Reynolds and Borlaug, 2006). Concern over the reduction of genetic diversity in crop species by widespread adoption of modern cultivars by farmers exist which results in replace of local cultivars and land races (Frankel, 1970). However, CIMMYT gives singular attention to maintain high levels of genetic diversity to minimize the risk of genetic vulnerability (Dreisigacker et al., 2012; Reeves, 1999). Evidence of this strategy can be observed in the pedigrees of wheat lines that are part of the international nurseries distributed by CIMMYT around the world, where exotic alleles from wild species and landraces are usually incorporated (Chen and Li, 2007; Mujeeb-Kazi et al., 2000; Mujeeb-Kazi et al., 1996; Reynolds et al., 2007).

Elite lines from CIMMYT germplasm contain valuable genes for numerous traits of interest. Assembly of populations from elite germplasm to discover and exploit these genes can be a useful tool in wheat breeding. However, association studies on existing populations used to map QTLs require clear estimation of the population structure to avoid spurious associations between molecular markers and regions in the genome that have no effect on phenotype (Pritchard and Rosenberg, 1999). Additionally, it is also important to estimate how linkage disequilibrium extends in this type of population to determine the proper number and distribution of molecular markers in the genome in this association studies (Ball, 2005; Ball, 2013).

Population structure occurs when there is a population subdivision caused by non-random mating between individuals and an unequal distribution of alleles exists within these subpopulations (Flint-Garcia et al., 2003). Genetic markers can be used to

estimate the genetic structure of germplasm by inferring individual identity or relatedness between individuals (Dreisigacker et al., 2012). Several methods have been proposed to estimate population structure. Among the most popular, it is the model-based clustering method performed by the software STRUCTURE which uses multi-locus genotype data to infer population structure and assign individuals to subpopulations (Porras-Hurtado et al., 2013; Pritchard et al., 2000). Another method proposed to estimate population structure is principal component analysis (Patterson et al., 2006), which models ancestry differences between samples of the population giving accurate estimation of population stratification (Price et al., 2006).

The wheat association mapping panel has been developed by Singh, Huerta-Espino, and Duveiller at CIMMYT to conduct association mapping studies of yellow rust and fusarium head blight. Wheat lines come from CIMMYT elite spring wheat yield trials (IBWSN44, IBWSN45, SAWYT27, HRWSN20), and other lines selected by the wheat Pathology Program based on response to *Fusarium* head blight.

This study aims to estimate the population structure and linkage disequilibrium decay in a 297 line wheat association mapping panel assayed with the 9K SNP chip.

Materials and Methods

Plant Material

A group of 297 spring wheat accessions was assembled to conduct the current study (Table 2-1). This collection of accessions will be referred to as the association mapping panel (AMP) from now on. The AMP was obtained from the International Center for Maize and Wheat Improvement (CIMMYT) and it included breeding lines, cultivars, and

landraces from different origins as well as control wheat lines used for Fusarium head blight (FHB) and yellow rust (YR) studies. The panel was selected because of its variability for FHB and YR response observed in previous evaluations in experimental stations at CIMMYT. The AMP represents a considerable number of the resistant alleles employed by CIMMYT's to develop improved wheat lines.

Table 2-1. Wheat accessions from the association mapping panel developed by CIMMYT listed with the germplasm identifier (GID), pedigree and origin from CIMMYT trials.

No	GID	Pedigree	Origin*
1	6175653	SAUAL/KRONSTAD F2004	C45IBWSN
2	6178206	TUKURU//BAV92/RAYON*2/3/PVN	C45IBWSN
3	6179223	PBW343*2/KUKUNA*2//FRTL/PIFED	C45IBWSN
4	6176225	FRET2/TUKURU//FRET2/3/MUNIA/CHTO//AMSEL/4/FRET2/TUKURU//FRET2	C45IBWSN
5	6176235	ROLF07*2/KACHU #1	C45IBWSN
6	6179254	WBLL1*2/4/BABAX/LR42//BABAX/3/BABAX/LR42//BABAX	C45IBWSN
7	6176332	BAV92//IRENA/KAUZ/3/HUITES*2/4/MURGA	C45IBWSN
8	6176335	WBLL1*2/CHAPIO*2//MURGA	C45IBWSN
9	6176395	KACHU/5/REH/HARE//2*BCN/3/CROC_1/AE.SQUARROSA (213)//PGO/4/HUITES/6/KACHU	C45IBWSN
10	6176409	ATTILA*2/PBW65*2//W485/HD29	C45IBWSN
11	6176428	BAV92//IRENA/KAUZ/3/HUITES*2/4/CROC_1/AE.SQUARROSA (224)//KULIN/3/WESTONIA	C45IBWSN
12	6176474	KACHU #1/4/CROC_1/AE.SQUARROSA (205)//KAUZ/3/SASIA/5/KACHU	C45IBWSN
13	6176600	BAV92//IRENA/KAUZ/3/HUITES*2/4/GONDO/TNMU	C45IBWSN
14	6176914	MUNAL #1/FRANCOLIN #1	C45IBWSN
15	6178972	PFAU/SERI.1B//AMAD/3/WAXWING/4/BABAX/LR42//BABAX*2/3/KURUKU	C45IBWSN
16	6174887	BECARD/KACHU	C45IBWSN
17	6177148	TRCH/HUIRIVIS #1	C45IBWSN
18	6177159	TRCH/KBIRD	C45IBWSN
19	6177324	ROLF07/MUU	C45IBWSN
20	6177667	PBW343*2/KUKUNA//TECUE #1	C45IBWSN
21	6175076	NAC/TH.AC//3*PVN/3/MIRLO/BUC/4/2*PASTOR/5/KACHU/6/KACHU	C45IBWSN
22	6175172	YAV_3/SCO//JO69/CRA/3/YAV79/4/AE.SQUARROSA (498)/5/LINE 1073/6/KAUZ*2/4/CAR//KAL/BB/3/NAC/5/KAUZ/7/KRONSTAD F2004/8/KAUZ/PASTOR//PBW343	C45IBWSN

Table 2-1 (cont'd)

23	6175216	WAXWING/4/BL 1496/MILAN/3/CROC_1/AE.SQUARROSA (205)//KAUZ/5/FRNCLN	C45IBWSN
24	6175409	WAXWING*2/HEILO	C45IBWSN
25	6178018	KIRITATI/4/2*BAV92//IRENA/KAUZ/3/HUITES	C45IBWSN
26	6178123	KZA//WH 542/2*PASTOR/3/BACEU #1	C45IBWSN
27	6179218	KFA/2*KACHU	C45IBWSN
28	6085788	QUAIU #1	C45IBWSN
29	5895861	PASTOR//HXL7573/2*BAU/3/WBLL1	C45IBWSN
30	5686762	KLDR/PEWIT1//MILAN/DUCULA	C45IBWSN
31	5893342	PUB94.15.1.12/FRTL	C45IBWSN
32	6178964	FRET2*2/4/SNI/TRAP#1/3/KAUZ*2/TRAP//KAUZ*2/5/BOW/URES//2*WEAVER/3/CROC_1/AE.SQUARROSA (213)//PGO	C45IBWSN
33	6175500	ATTILA*2/PBW65//WBLL1*2/VIVITSI	C45IBWSN
34	6175667	ALTAR 84/AE.SQUARROSA (221)//3*BORL95/3/URES/JUN//KAUZ/4/WBLL1/5/REH/HARE//2*BCN/3/CROC_1/AE.SQUARROSA (213)//PGO/4/HUITES	C45IBWSN
35	6175679	MURGA//WAXWING/KIRITATI	C45IBWSN
36	6175694	MURGA/KRONSTAD F2004	C45IBWSN
37	6175740	ATTILA*2/PBW65//MURGA	C45IBWSN
38	6175757	BAV92//IRENA/KAUZ/3/HUITES/6/ALD/CEP75630//CEP75234/PT7219/3/BUC/BJY/4/CBRD/5/TNMU/PF85487	C45IBWSN
39	6175897	WBLL1*2/CHAPIO//HEILO	C45IBWSN
40	6175902	WBLL1*2/CHAPIO//HEILO	C45IBWSN
41	6175989	WAXWING*2/4/BOW/NKT//CBRD/3/CBRD	C45IBWSN
42	6176021	ROLF07*2/3/PRINIA/PASTOR//HUITES	C45IBWSN
43	6176024	ROLF07*2/4/CROC_1/AE.SQUARROSA (205)//BORL95/3/2*MILAN	C45IBWSN
44	6176045	WBLL1*2/KUKUNA/5/PSN/BOW//SERI/3/MILAN/4/ATTILA/6/WBLL1*2/KKTS	C45IBWSN
45	6178273	WAXWING*2/DIAMONDBIRD	C45IBWSN
46	6178335	BAV92//IRENA/KAUZ/3/HUITES*2/4/MILAN/KAUZ//CHIL/CHUM18	C45IBWSN

Table 2-1 (cont'd)

47	6178362	BAV92//IRENA/KAUZ/3/HUITES*2/4/PVN	C45IBWSN
48	6176134	BAV92//IRENA/KAUZ/3/HUITES*2/4/TNMU	C45IBWSN
49	6178476	WBLL1/DIAMONDBIRD//WBLL1*2/VIVITSI	C45IBWSN
50	6178527	SAUAL/YANAC//SAUAL	C45IBWSN
51	6178539	SAUAL/KIRITATI//SAUAL	C45IBWSN
52	6178575	CS/TH.SC//3*PVN/3/MIRLO/BUC/4/URES/JUN//KAUZ/5/HUITES/6/YANAC/7/CS/TH.SC//3*PVN/3/MIRLO/BUC/4/MILAN/5/TILHI	C45IBWSN
53	6178591	FINSI/METSO//FH6-1-7/3/FINSI/METSO	C45IBWSN
54	6179244	INQALAB 91*2/KUKUNA*2//PVN	C45IBWSN
55	6176173	UP2338*2/4/SNI/TRAP#1/3/KAUZ*2/TRAP//KAUZ/5/MILAN/KAUZ//CHIL/CHUM18/6/UP2338*2/4/SNI/TRAP#1/3/KAUZ*2/TRAP//KAUZ	C45IBWSN
56	6178240	UP2338*2/KKTS*2//YANAC	C45IBWSN
57	6176189	WAXWING/2*ROLF07	C45IBWSN
58	6176903	WBLL1*2/5/CNO79//PF70354/MUS/3/PASTOR/4/BAV92	C45IBWSN
59	6176298	ATTILA*2/PBW65*2//MURGA	C45IBWSN
60	6176361	WBLL1/FRET2//PASTOR*2/3/MURGA	C45IBWSN
61	6176368	KACHU #1/4/CROC_1/AE.SQUARROSA (205)//BORL95/3/2*MILAN/5/KACHU	C45IBWSN
62	6176403	SAUAL/4/CROC_1/AE.SQUARROSA (205)//BORL95/3/2*MILAN/5/SAUAL	C45IBWSN
63	6176431	ROLF07*2/4/CROC_1/AE.SQUARROSA (224)//KULIN/3/WESTONIA	C45IBWSN
64	6176455	KACHU*2/3/CHUM18/BORL95//CBRD	C45IBWSN
65	6176480	KACHU #1/4/CROC_1/AE.SQUARROSA (205)//KAUZ/3/SASIA/5/KACHU	C45IBWSN
66	6176509	SAUAL*2/6/CNDO/R143//ENTE/MEXI_2/3/AEGILOPS SQUARROSA (TAUS)/4/WEAVER/5/2*PASTOR	C45IBWSN
67	6176556	ATTILA*2/PBW65*2/4/BOW/NKT//CBRD/3/CBRD	C45IBWSN
68	6176583	BAV92//IRENA/KAUZ/3/HUITES/4/FN/2*PASTOR/5/BAV92//IRENA/KAUZ/3/HUITES	C45IBWSN

Table 2-1 (cont'd)

69	6176584	BAV92//IRENA/KAUZ/3/HUITES/4/FN/2*PASTOR/5/BAV92//IRENA/KAUZ/3/HUITES	C45IBWSN
70	6176611	ROLF07*2/4/BOW/NKT//CBRD/3/CBRD	C45IBWSN
71	6178881	WBLL1/4/BOW/NKT//CBRD/3/CBRD/5/WBLL1*2/TUKURU	C45IBWSN
72	6176647	WBLL1*2/4/YACO/PBW65/3/KAUZ*2/TRAP//KAUZ*2/5/GONDO	C45IBWSN
73	6176696	PFAU/WEAVER*2//BRAMBLING/3/KAUZ//TRAP#1/BOW/4/PFAU/WEAVER*2//BRAMBLING	C45IBWSN
74	6178897	KACHU*2//CHIL/CHUM18	C45IBWSN
75	6178898	KACHU*2//CHIL/CHUM18	C45IBWSN
76	6176829	SAUAL/3/ACHTAR*3//KANZ/KS85-8-4/4/SAUAL	C45IBWSN
77	6176848	BAV92//IRENA/KAUZ/3/HUITES*2/4/YUNMAI 47	C45IBWSN
78	6178715	WAXWING/KIRITATI*2/3/C80.1/3*BATAVIA//2*WBLL1	C45IBWSN
79	6178734	BAV92//IRENA/KAUZ/3/HUITES*2/4/WHEAR	C45IBWSN
80	6178760	KACHU #1*2/WHEAR	C45IBWSN
81	6178768	KACHU #1/3/C80.1/3*BATAVIA//2*WBLL1/4/KACHU	C45IBWSN
82	6178790	SAUAL/WHEAR//SAUAL	C45IBWSN
83	6177845	FRNCLN/BECARD	C45IBWSN
84	6176924	PAURAQ/3/KIRITATI//PRL/2*PASTOR	C45IBWSN
85	6178999	QUAIU/5/FRET2*2/4/SNI/TRAP#1/3/KAUZ*2/TRAP//KAUZ	C45IBWSN
86	6179596	BECARD/KACHU	C45IBWSN
87	6177095	FRANCOLIN #1/HAWFINCH #1	C45IBWSN
88	6177127	FRNCLN/TECUE #1	C45IBWSN
89	6177147	TRCH/HUIRIVIS #1	C45IBWSN
90	6179044	QUAIU/TECUE #1	C45IBWSN
91	6177439	KBIRD//WBLL1*2/KURUKU	C45IBWSN
92	6177509	KINGBIRD #1/KACHU	C45IBWSN
93	6177552	WAXWING/4/SNI/TRAP#1/3/KAUZ*2/TRAP//KAUZ/5/AKURI	C45IBWSN
94	6177562	WAXWING/4/SNI/TRAP#1/3/KAUZ*2/TRAP//KAUZ/5/TECUE #1	C45IBWSN
95	6177652	PBW343*2/KUKUNA//TECUE #1	C45IBWSN
96	6174927	WBLL1*2/BRAMBLING//FN/2*PASTOR	C45IBWSN

Table 2-1 (cont'd)

97	6174952	QUAIU #3//MILAN/AMSEL	C45IBWSN
98	6177898	ATTILA*2/PBW65//MUU #1/3/FRANCOLIN #1	C45IBWSN
99	6174993	ATTILA*2/PBW65*2//TOBA97/PASTOR	C45IBWSN
100	6175057	WBLL1*2/VIVITSI//PRINIA/PASTOR/3/WBLL1*2/BRAMBLING	C45IBWSN
101	6175078	SAUAL/5/REH/HARE//2*BCN/3/CROC_1/AE.SQUARROSA (213)//PGO/4/HUITES/6/KACHU	C45IBWSN
102	6179159	MUU #1//PBW343*2/KUKUNA/3/MUU	C45IBWSN
103	6175232	WBLL1*2/KURUKU/6/CNDO/R143//ENTE/MEXI_2/3/AEGILOPS SQUARROSA (TAUS)/4/WEAVER/5/2*JANZ/7/WBLL1*2/KURUKU	C45IBWSN
104	6175312	TUKURU//BAV92/RAYON*2/7/YAV_3/SCO//JO69/CRA/3/YAV79/4/AE. SQUARROSA (498)/5/LINE 1073/6/KAUZ*2/4/CAR//KAL/BB/3/NAC/5/KAUZ	C45IBWSN
105	6175382	NG8675/CBRD//FN/2*PASTOR/4/THELIN/3/2*BABAX/LR42//BABAX	C45IBWSN
106	6175444	BAV92//IRENA/KAUZ/3/HUITES/4/GONDO/TNMU/5/BAV92//IRENA/KAUZ/3/HUITES	C45IBWSN
107	6177980	CONI#1/2*HUIRIVIS #1	C45IBWSN
108	6178005	TECUE #1/2*WAXWING	C45IBWSN
109	6178080	KBIRD//WH 542/2*PASTOR/3/WBLL1*2/BRAMBLING	C45IBWSN
110	6178083	MUU/5/TRAP#1/BOW/3/VEE/PJN//2*TUI/4/BAV92/RAYON/6/MILAN/S87230//BAV92	C45IBWSN
111	6179559	KFA/3/PFAU/WEAVER//BRAMBLING/4/PFAU/WEAVER*2//BRAMBLING	C45IBWSN
112	6179479	ATTILA*2/PBW65//KRONSTAD F2004	C45IBWSN
113	6179497	WBLL1*2/TUKURU//KRONSTAD F2004	C45IBWSN
114	6179417	CHIL/CHUM18//GONDO	C45IBWSN
115	6179293	WBLL1*2/KUKUNA//KIRITATI/3/WBLL1*2/KUKUNA	C45IBWSN
116	6176149	NORM/WBLL1//WBLL1/3/TNMU/4/WBLL1*2/TUKURU	C45IBWSN
117	6179562	PBW343*2/KHVAKI*2//YANAC	C45IBWSN
118	6179345	FRANCOLIN #1/4/BABAX/LR42//BABAX*2/3/KURUKU	C45IBWSN
119	6177057	PANDORA//WBLL1*2/BRAMBLING	C45IBWSN

Table 2-1 (cont'd)

120	6181746	WBLL1*2/BRAMBLING//JUCHI	C45IBWSN
121	6177408	WBLL1*2/KKTS//KINGBIRD #1	C45IBWSN
122	6179457	TACUPETO F2001//WBLL1*2/KKTS/3/WBLL1*2/BRAMBLING	C45IBWSN
123	6179471	WBLL1/KUKUNA//TACUPETO F2001/3/KRONSTAD F2004/4/ROLF07	C45IBWSN
124	6175213	ATTILA*2/PBW65*2/5/REH/HARE//2*BCN/3/CROC_1/AE.SQUARROSA (213)//PGO/4/HUITES	C45IBWSN
125	6181759	HEILO/7/IVAN/6/SABUF/5/BCN/4/RABI//GS/CRA/3/AE.SQUARROSA (190)/8/VORB/FISCAL	C45IBWSN
126	6178136	KSW/SAUAL//SAUAL	C45IBWSN
127	6179481	KAUZ/PASTOR//PBW343/3/KRONSTAD F2004	C45IBWSN
128	6175720	REH/HARE//2*BCN/3/CROC_1/AE.SQUARROSA (213)//PGO/4/HUITES/5/KRONSTAD F2004	C45IBWSN
129	6179510	PRL/2*PASTOR//VORB	C45IBWSN
130	6179534	TRCH*2/3/WUH1/VEE#5//CBRD	C45IBWSN
131	6179423	KACHU #1/3/SHA3/SERI//SHA4/LIRA/4/KACHU	C45IBWSN
132	6178918	PBW343/PASTOR*2/6/TURACO/5/CHIR3/4/SIREN//ALTAR 84/AE.SQUARROSA (205)/3/3*BUC	C45IBWSN
133	6179013	WBLL1*2/BRAMBLING/4/BABAX/LR42//BABAX*2/3/KURUKU	C45IBWSN
134	6177099	FRANCOLIN #1/KIRITATI	C45IBWSN
135	6177771	BABAX/LR42//BABAX*2/3/KUKUNA/4/TAM200/PASTOR//TOBA97	C45IBWSN
136	6179553	MURGA/KRONSTAD F2004//QUAIU #3	C45IBWSN
137	6181750	KENYA NYANGUMI/3/2*KAUZ/PASTOR//PBW343	C45IBWSN
138	5793255	PARUS/PASTOR//INQALAB 91*2/KUKUNA	ELITE2NDYEAR
139	5793394	CROC_1/AE.SQUARROSA (205)//KAUZ/3/SASIA/4/TROST	ELITE2NDYEAR
140	5793395	CROC_1/AE.SQUARROSA (205)//KAUZ/3/SASIA/4/TROST	ELITE2NDYEAR
141	5793605	PFAU/MILAN//SOVA/3/PBW65/2*SERI.1B	ELITE2NDYEAR
142	5793920	PASTOR/KAUZ/6/CNDO/R143//ENTE/MEXI_2/3/AEGILOPS SQUARROSA (TAUS)/4/WEAVER/5/2*KAUZ	ELITE2NDYEAR
143	5793926	PASTOR/3/VORONA/CNO79//KAUZ/4/MILAN/OTUS//ATTILA/3*BCN	ELITE2NDYEAR

Table 2-1 (cont'd)

144	5793927	PASTOR/3/VORONA/CNO79//KAUZ/4/MILAN/OTUS//ATTILA/3*BCN	ELITE2NDYEAR
145	5793974	CHIBIA/WEAVER//KACHU	ELITE2NDYEAR
146	5793975	CHIBIA/WEAVER//KACHU	ELITE2NDYEAR
147	5793991	PRINIA/PASTOR//HUITES/3/MILAN/OTUS//ATTILA/3*BCN	ELITE2NDYEAR
148	5794010	C80.1/3*BATAVIA//2*WBLL1/3/TOBA97/PASTOR	ELITE2NDYEAR
149	5794027	WHEAR/3/PBW343/PASTOR//ATTILA/3*BCN	ELITE2NDYEAR
150	5794033	PBW343/HUITES/3/MILAN/OTUS//ATTILA/3*BCN	ELITE2NDYEAR
151	5794348	WBLL1*2/KURUKU//KRONSTAD F2004	ELITE2NDYEAR
152	5794812	MONARCA F2007/KRONSTAD F2004	ELITE2NDYEAR
153	5794547	PBW343*2/KUKUNA//PBW343*2/KUKUNA/3/PBW343	ELITE2NDYEAR
154	5794843	WHEAR/2*KRONSTAD F2004	ELITE2NDYEAR
155	5794845	C80.1/3*BATAVIA//2*WBLL1/3/2*KRONSTAD F2004	ELITE2NDYEAR
156	5794846	C80.1/3*BATAVIA//2*WBLL1/3/2*KRONSTAD F2004	ELITE2NDYEAR
157	10004	SUMAI #3	ELITE2NDYEAR
158	5536	GAMENYA	ELITE2NDYEAR
159	4936163	FALCIN/AE.SQUARROSA (312)/3/THB/CEP7780//SHA4/LIRA	ELITE2NDYEAR
160	4877754	GONDO/CBRD	ELITE2NDYEAR
161	2589783	HEILO	ELITE2NDYEAR
162	6121919	PICUS/3/KAUZ*2/BOW//KAUZ/4/KKTS/5/HEILO	PCFUSWRYRG
163	6121935	HUIRIVIS #1/GONDO	PCFUSWRYRG
164	6121938	HUIRIVIS #1/GONDO	PCFUSWRYRG
165	6121967	KAUZ/PASTOR//PBW343/3/HEILO	PCFUSWRYRG
166	6121989	FRET2/WBLL1//TACUPETO F2001/3/HEILO	PCFUSWRYRG
167	6122002	WBLL1*2/4/YACO/PBW65/3/KAUZ*2/TRAP//KAUZ/5/GONDO	PCFUSWRYRG
168	6122022	WBLL1*2/CHAPIO//HEILO	PCFUSWRYRG
169	6122036	WBLL1*2/KURUKU//HEILO	PCFUSWRYRG
170	6122042	WBLL1*2/KURUKU//HEILO	PCFUSWRYRG
171	6122072	WBLL1*2/VIVITSI//GONDO	PCFUSWRYRG
172	6122079	ATTILA/2*PASTOR//FN/2*PASTOR	PCFUSWRYRG
173	6122123	KACHU #1/4/CROC_1/AE.SQUARROSA (205)//KAUZ/3/SASIA/5/KACHU	PCFUSWRYRG

Table 2-1 (cont'd)

174	6122128	KACHU #1/4/CROC_1/AE.SQUARROSA (205)//KAUZ/3/SASIA/5/KACHU	PCFUSWRYRG
175	6122172	SAUAL*2/6/CNDO/R143//ENTE/MEXI_2/3/AEGILOPS SQUARROSA (TAUS)/4/WEAVER/5/2*PASTOR	PCFUSWRYRG
176	6122349	BAV92//IRENA/KAUZ/3/HUITES*2/4/GONDO/TNMU	PCFUSWRYRG
177	6122353	BAV92//IRENA/KAUZ/3/HUITES*2/4/GONDO/TNMU	PCFUSWRYRG
178	6122408	FRET2*2/KUKUNA*2//SHA4/CHIL	PCFUSWRYRG
179	6122546	WBLL1*2/KURUKU*2//TNMU	PCFUSWRYRG
180	6122554	WBLL1*2/TUKURU//WUH1/BOW/3/WBLL1*2/TUKURU	PCFUSWRYRG
181	6122590	WBLL1/FRET2//PASTOR*2/3/GONDO	PCFUSWRYRG
182	6122654	PFAU/WEAVER*2//BRAMBLING/7/IVAN/6/SABUF/5/BCN/4/RABI//GS/CRA/3/AE.SQUARROSA (190)/8/PFAU/WEAVER//BRAMBLING	PCFUSWRYRG
183	6122710	TRCH*2/TNMU	PCFUSWRYRG
184	6122741	KACHU*2//CHIL/CHUM18	PCFUSWRYRG
185	6122745	KACHU*2//CHIL/CHUM18	PCFUSWRYRG
186	6122847	SAUAL #1/TNMU//SAUAL	PCFUSWRYRG
187	6123007	PRINIA/PASTOR//CHIL/CHUM18/3/PRINIA/PASTOR	PCFUSWRYRG
188	6123164	PBW343*2/KHVAKI*2//CHIL/CHUM18	PCFUSWRYRG
189	6123179	PBW343/PASTOR*2/6/TURACO/5/CHIR3/4/SIREN//ALTAR 84/AE.SQUARROSA (205)/3/3*BUC	PCFUSWRYRG
190	6123188	PBW343/PASTOR*2/3/WUH1/VEE#5//CBRD	PCFUSWRYRG
191	6123193	PBW343/PASTOR*2/3/WUH1/VEE#5//CBRD	PCFUSWRYRG
192	6123199	PBW343/PASTOR*2/3/WUH1/VEE#5//CBRD	PCFUSWRYRG
193	6123225	NG8675/CBRD/7/IVAN/6/SABUF/5/BCN/4/RABI//GS/CRA/3/AE.SQUARROSA (190)/8/WBLL1*2/CHAPIO	PCFUSWRYRG
194	6123229	NG8675/CBRD/7/IVAN/6/SABUF/5/BCN/4/RABI//GS/CRA/3/AE.SQUARROSA (190)/8/WBLL1*2/CHAPIO	PCFUSWRYRG
195	6123240	SHA3/CBRD//TNMU/3/KACHU	PCFUSWRYRG
196	6123281	FN/2*PASTOR//GONDO/TNMU/3/FRANCOLIN #1	PCFUSWRYRG
197	6123311	HEILO//GONDO/TNMU/3/WBLL1*2/BRAMBLING	PCFUSWRYRG
198	6123623	CBRD/FILIN	PCFUSWRYRG

Table 2-1 (cont'd)

199	6123625	CBRD/FILIN	PCFUSWRYRG
200	6123661	CHIL/CHUM18//GONDO	PCFUSWRYRG
201	6122202	SAUAL/4/CROC_1/AE.SQUARROSA (205)//KAUZ/3/ATTILA/5/SAUAL	PCFUSWRYRG
202	6122272	WAXWING/KIRITATI*2/3/SHA3/SERI//SHA4/LIRA	PCFUSWRYRG
203	6122389	CNO79//PF70354/MUS/3/PASTOR/4/BAV92*2/5/SHA3/SERI//SHA4/LIRA	PCFUSWRYRG
204	6122425	FRET2/TUKURU//FRET2/3/WUH1/VEE#5//CBRD/4/FRET2/TUKURU//FRET2	PCFUSWRYRG
205	6122610	WBLL1/FRET2//PASTOR/3/SHA3/SERI//SHA4/LIRA/4/WBLL1/TACUPETO F2001//PASTOR	PCFUSWRYRG
206	6122665	PFAU/WEAVER*2//BRAMBLING/7/IVAN/6/SABUF/5/BCN/4/RABI//GS/CRA/3/AE.SQUARROSA (190)/8/PFAU/WEAVER//BRAMBLING	PCFUSWRYRG
207	6122704	PFAU/WEAVER//BRAMBLING*2/3/SHA3/SERI//SHA4/LIRA	PCFUSWRYRG
208	6122756	KACHU #1/3/SHA3/SERI//SHA4/LIRA/4/KACHU	PCFUSWRYRG
209	6123015	PRINIA/PASTOR//CHIL/CHUM18/3/PRINIA/PASTOR	PCFUSWRYRG
210	6123133	KETUPA*2/PASTOR/6/TURACO/5/CHIR3/4/SIREN//ALTAR 84/AE.SQUARROSA (205)/3/3*BUC/7/KACHU	PCFUSWRYRG
211	6123209	CHIL/CHUM18//FN/2*PASTOR/3/PRL/2*PASTOR	PCFUSWRYRG
212	6123221	CHIL/CHUM18//GONDO/3/WBLL1*2/KURUKU	PCFUSWRYRG
213	6123242	SHA3/CBRD//TNMU/3/KACHU	PCFUSWRYRG
214	6123283	FN/2*PASTOR//GONDO/TNMU/3/FRANCOLIN #1	PCFUSWRYRG
215	6123299	NG8675/CBRD//FN/2*PASTOR/4/THELIN/3/2*BABAX/LR42//BABAX	PCFUSWRYRG
216	6123343	HEILO/7/IVAN/6/SABUF/5/BCN/4/RABI//GS/CRA/3/AE.SQUARROSA (190)/8/VORB/FISCAL	PCFUSWRYRG
217	5993501	BAV92//IRENA/KAUZ/3/HUITES/4/DOLL	SELC44IBWSN
218	5993950	TRCH/SRTU//KACHU	SELC44IBWSN
219	5994110	PRL/2*PASTOR//SRTU/3/PRINIA/PASTOR	SELC44IBWSN
220	5994207	WAXWING*2/3/PASTOR//HXL7573/2*BAU	SELC44IBWSN
221	5994481	ATTILA*2/PBW65*2//TNMU	SELC44IBWSN
222	5994020	SERI.1B//KAUZ/HEVO/3/AMAD*2/4/KIRITATI	SELC44IBWSN

Table 2-1 (cont'd)

223	5995334	WBLL1*2/4/SNI/TRAP#1/3/KAUZ*2/TRAP//KAUZ/5/KACHU	SELC44IBWSN
224	5995338	WBLL1*2/4/YACO/PBW65/3/KAUZ*2/TRAP//KAUZ/5/KACHU #1	SELC44IBWSN
225	5995481	FRET2*2/4/SNI/TRAP#1/3/KAUZ*2/TRAP//KAUZ/5/KACHU	SELC44IBWSN
226	5995483	FRET2*2/KUKUNA//PRINIA/PASTOR	SELC44IBWSN
227	5995487	FRET2/KIRITATI/5/NAC/TH.AC//3*PVN/3/MIRLO/BUC/4/2*PASTOR	SELC44IBWSN
228	5995488	FRET2/KIRITATI/5/NAC/TH.AC//3*PVN/3/MIRLO/BUC/4/2*PASTOR	SELC44IBWSN
229	5995598	KAUZ//ALTAR 84/AOS/3/MILAN/KAUZ/4/SAUAL	SELC44IBWSN
230	5995609	KAUZ//ALTAR 84/AOS/3/MILAN/KAUZ/4/OTUS/TOBA97	SELC44IBWSN
231	5995635	SAUAL/3/KAUZ/PASTOR//PBW343	SELC44IBWSN
232	5995800	NG8675/CBRD//MILAN/3/SAUAL/6/CNDO/R143//ENTE/MEXI_2/3/AEGILOPS SQUARROSA (TAUS)/4/WEAVER/5/2*PASTOR	SELC44IBWSN
233	5996086	ATTILA/3*BCN//BAV92/3/TILHI/4/SHA7/VEE#5//ARIV92	SELC44IBWSN
234	5996092	ATTILA/3*BCN//BAV92/3/TILHI/4/SHA7/VEE#5//ARIV92	SELC44IBWSN
235	5996469	BABAX/KS93U76//BABAX/3/ATTILA/3*BCN//TOBA97/4/WBLL1*2/KURUKU	SELC44IBWSN
236	5849348	ATTILA*2/PBW65//KRONSTAD F2004	SELC44IBWSN
237	5996709	KANZ*4/KS85-8-4//2*WBLL1*2/KURUKU	SELC44IBWSN
238	5993900	FRET2/KUKUNA//FRET2/3/PASTOR//HXL7573/2*BAU/5/FRET2*2/4/SNI/TRAP#1/3/KAUZ*2/TRAP//KAUZ	SELC44IBWSN
239	5994089	PRL/2*PASTOR//PARUS/5/NAC/TH.AC//3*PVN/3/MIRLO/BUC/4/2*PASTOR	SELC44IBWSN
240	5996840	WAXWING*2/JUCHI	SELC44IBWSN
241	3826276	FUNDACEP 30	20HRWSNFHB
242	9774	SHANGHAI #8	20HRWSNFHB
243	3855011	VOROBEY	20HRWSNFHB
244	5685927	CPI8/GEDIZ/3/GOO//ALB/CRA/4/AE.SQUARROSA (208)/5/HAHN/2*WEAVER/6/SKAUZ/BAV92	20HRWSNFHB
245	5685928	CPI8/GEDIZ/3/GOO//ALB/CRA/4/AE.SQUARROSA (208)/5/HAHN/2*WEAVER/6/SKAUZ/BAV92	20HRWSNFHB
246	5685929	CPI8/GEDIZ/3/GOO//ALB/CRA/4/AE.SQUARROSA	20HRWSNFHB
247	5685994	NING MAI 96035/FINSI//HEILO	20HRWSNFHB

Table 2-1 (cont'd)

248	5685998	NING MAI 96035/FINSI//HEILO	20HRWSNFHB
249	5686022	ATTILA/HEILO	20HRWSNFHB
250	5686023	ATTILA/HEILO	20HRWSNFHB
251	5551988	WAXWING//PFAU/WEAVER	20HRWSNFHB
252	5398611	BABAX/LR42//BABAX*2/3/KURUKU	20HRWSNFHB
253	5535312	ND643//2*PRL/2*PASTOR	20HRWSNFHB
254	3855011	VOROBEY	27SAWSNFHB
255	5423325	BABAX/LR42//BABAX/3/ER2000	27SAWSNFHB
256	5422808	OASIS//TC14/2*SPER/3/ATTILA/4/WBLL4	27SAWSNFHB
257	5427957	FILIN/3/CROC_1/AE.SQUARROSA (205)//KAUZ/4/FILIN/5/VEE/MJI//2*TUI/3/PASTOR	27SAWSNFHB
258	5428538	T.DICOCCON PI94625/AE.SQUARROSA (372)//3*PASTOR	27SAWSNFHB
259	5428200	PASTOR/4/WEAVER/TSC//WEAVER/3/WEAVER/5/URES/PRL//BAV92	27SAWSNFHB
260	5427842	SW94.2690/SUNCO	27SAWSNFHB
261	5427852	SW94.2690/SUNCO	27SAWSNFHB
262	5427940	VEE/MJI//2*TUI/3/PASTOR/4/BERKUT	27SAWSNFHB
263	5427955	BERKUT/3/ATTILA*2//CHIL/BUC	27SAWSNFHB
264	5423682	TAN//TEMPORALERA M 87/AGR/3/FRET2/4/URES/PRL//BAV92	27SAWSNFHB
265	5423717	A93324S.7197.29/4/KAUZ//ALTAR 84/AOS/3/KAUZ/5/PASTOR	27SAWSNFHB
266	5423751	OASIS//TC14/2*SPER/3/ATTILA/10/ATTILA*2/9/KT/BAGE//FN/U/3/BZA /4/TRM/5/ALDAN/6/SERI/7/VEE#10/8/OPATA	27SAWSNFHB
267	5436044	MEX94.27.1.20/3/SOKOLL//ATTILA/3*BCN	27SAWSNFHB
268	5686798	KS82W418/SPN//WBLL1/3/BERKUT	ELITE2NDYEAR
269	5686808	CNDO/R143//ENTE/MEXI75/3/AE.SQ/4/2*FCT/5/KAUZ*2/YACO//KAUZ /6/BERKUT	ELITE2NDYEAR
270	5687025	SOKOLL/EXCALIBUR	ELITE2NDYEAR
271	5687066	PASTOR/SLVS//FRAME	ELITE2NDYEAR
272	5687067	PASTOR/SLVS//FRAME	ELITE2NDYEAR
273	5687100	BAXTER*2/4/CHEN/AEGILOPS SQUARROSA (TAUS)//BCN/3/BAV92	ELITE2NDYEAR
274	5894425	BERKUT/3/ALTAR 84/AE.SQUARROSA (219)//SERI	ELITE2NDYEAR
275	5894548	MILAN/DUCULA//SUNCO/2*PASTOR	ELITE2NDYEAR

Table 2-1 (cont'd)

276	5894637	SW89-5124*2/FASAN//PARUS/PASTOR	ELITE2NDYEAR
277	5894655	SOKOLL//SUNCO/2*PASTOR	ELITE2NDYEAR
278	5894659	CROC_1/AE.SQUARROSA (224)//OPATA/3/ALTAR	ELITE2NDYEAR
279	5894787	SUNSTATE/SD 3195//SOKOLL	ELITE2NDYEAR
280	5894800	SOKOLL*2/GLE	ELITE2NDYEAR
281	5894832	TEMPORALERA M 87	ELITE2NDYEAR
282	5894851	FINSI/3/ATTILA/BAV92//PASTOR/4/PBW343*2/KUKUNA	ELITE2NDYEAR
283	5894933	CO99W329/2*BERKUT	ELITE2NDYEAR
284	5895167	PSN/BOW//MILAN/3/2*BERKUT	ELITE2NDYEAR
285	5895192	CROC_1/AE.SQUARROSA (224)//OPATA/3/RAC655/4/SLVS/PASTOR	ELITE2NDYEAR
286	5895200	SLVS/PASTOR/3/PASTOR//MUNIA/ALTAR 84	ELITE2NDYEAR
287	5895215	YAV79//DACK/RABI/3/SNIPE/4/AE.SQUARROSA (460)/5/2*EX	ELITE2NDYEAR
288	5895222	CNDO/R143//ENTE/MEXI_2/3/AEGILOPS SQUARROSA	ELITE2NDYEAR
289	5895241	CROC_1/AE.SQUARROSA (205)//BORL95/3/KENNEDY/6/	ELITE2NDYEAR
290	5895245	D67.2/PARANA 66.270//AE.SQUARROSA	ELITE2NDYEAR
291	5895256	CALINGIRI/SOKOLL	ELITE2NDYEAR
292	5837084	SOKOLL//SLVS/PASTOR/3/ATTILA*2//CHIL/BUC	ELITE2NDYEAR
293	5895311	BERKUT/HTG	ELITE2NDYEAR
294	5895333	SOKOLL/FRAME	ELITE2NDYEAR
295	5895337	SOKOLL/SLVS	ELITE2NDYEAR
296	5895423	ASTREB*2/NING MAI 9558	ELITE2NDYEAR
297	5895427	ASTREB*2/3/WUH1/VEE#5//CBRD	ELITE2NDYEAR

* C45IBWSN = Cycle 45 International Bread Wheat Screening Nursery; ELITE2NDYEAR, PCFUSWRYRG and PCFUSWRYRG = Selections from the Pathogy Program at CIMMYT; 27SAWSNFHB= Cycle 27 Semi-arid wheat screening nursery for Fusarium Head Blight; 20HRWSNFHB= Cycle 20 Haigh-reinfall wheat screening nursery for Fusarium Head Blight.

Genotyping

Ten seeds of each accession of the AMP were planted in a greenhouse at Michigan State University (MSU) in 2011. A leaf sample from one seedling, between 2 and 3 wk old, was harvested. The tissue was frozen in liquid nitrogen and stored at -80 ºC prior to DNA extraction. Genomic DNA was extracted with the Wizard® Genomic DNA purification (Promega®) according to the manufacturer's protocol to obtain 20 mL sample of DNA concentration of 50 ng/uL from each sample. The DNA was genotyped the by Illumina Infinium® genotyping facility at MSU for whole-genome profiling using 8,632 SNP markers integrated in the 9K SNP chip from Illumina (Cavanagh et al., 2013).

Three day assays using the 9K chip were carried out to genotype the wheat AMP samples with the 8,632 SNPs at MSU using iScan screener from Illumina®. Quality of SNP markers was determined by GenomeStudio® data analysis software from Illumina®. SNP markers with unexpected genotype AB (heterozygous) were recoded as either AA or BB based on the graphical interface visualization tool of the software. SNP markers that did not show clear clustering patterns were excluded. In addition, 66 simple sequence repeats (SSR) markers were screened in the AMP in the Biotechnology laboratory at INIAP using a 4300 DNA analyzer from LI-COR® to obtain a larger number of polymorphic markers in the D genome. To visualize and score the SSR markers, the forward primer of each marker was tagged with a M13 tail with the following primer tag sequence: "5'-CACGACGTTGTAAAACGAC-3'". The sequences of the SSR primer markers can be found in Table 2-2.

Table 2-2. Microsatellite markers (SSRs) employed to screen the wheat association mapping panel, sequences of the primers, and comments from the results of the amplifications.

Marker name	Forward sequence 5' - 3'	Reverse sequence 5' - 3'	Chromosome Location	Comments
Barc133	AGCGCTCGAAAAGTCAG	GGCAGGTCCAACTCCAG	3BS, 5D	Linked to *Fhb-1*
Gwm493	TTCCCATAACTAAAACCGCG	GGAACATCATTTCTGGACTTTG	3BS	Linked to *Fhb-1*
Barc19	GCGACCCGAGTAGCCTGAA	GGTGGACCATTAGACGCTTACTTG	3AS	Linked to a FHB QTL
Wmc44	GGTCTTCTGGGCTTTGATCCTG	TGTTGCTAGGGACCCGTAGTGG	1BL	*Yr29*
Gwm261	CTCCCTGTACGCCTAAGGC	CTCGCGCTACTAGCCATTG	2D	*Rht8*
Xgwm259	AGGGAAAAGACATCTTTTTTTTC	CGACCGACTTCGGGTTC	1B	*Yr29*
Xgwm146	CCAAAAAAACTGCCTGCATG	CTCTGGCATTGCTCCTTGG	7BL	Septoria
Xgwm493	TTCCCATAACTAAAACCGCG	GGAACATCATTTCTGGACTTTG	3BS	*Fhb-1*
Xgwm533	AAGGCGAATCAAACGGAATA	GTTGCTTTAGGGGAAAAGCC	3BS	
Xbarc124	TGC ACC CCT TCC AAA TCT	TGC GAG TCG TGT GGT TGT	2A, 2B, 2D	*Yr61*
Xgwm359	AGC CGC GAA ATC TAC TTT GA	TTA AAC GGA CAG AGC ACA CG	2A	*Yr61*
Cfa2149	CTT GGA GCT CGG GTA GTA GC	AAG GCA GCT CAA TCG GAG TA	4B, 5A	*Yr48*
Snf-A2	TCC GTC TCC ATC ATT CAA CA	GTG TTG CGC AAG TTT GTG AC	5AL	*Yr48*
Xgwm130	AGC TCT GCT TCA CGA GGA AG	CTC CTC TTT ATA TCG CGT CCC	7D	*Yr18*
Wmc720	CACCATGGTTGGCAAGAGA	CTGGTGATACTGCCGTGACA	4D	
Cfd23	TAGCAGTAGCAGCAGCAGGA	GCAAGGAAGAGTGTTCAGCC	4D	
Cfd84	GTTGCCTCGGTGTCGTTTAT	TCCTCGAGGTCCAAAACATC	4D	
Barc196	GGTGGGTTTTATCGAATAGATTTGCT	GCGTTTCGTCAAGATTAATGCAGGTTT	6D	
Wmc14	ACCCGTCACCGGTTTATGGATG	TCCACTTCAAGATGGAGGGCAG	7D	
Wmc606	CCGATGAACAGACTCGACAAGG	GGCTTCGGCCAGTAGTACAGGA	7B, 7D	Easy to score and saparate genomes. Few bands on 7B

Table 2-2 (cont'd)

Gwm297	ATCGTCACGTATTTTGCAATG	TGCGTAAGTCTAGCATTTTCTG	7B	
Wmc581	CATGTTGCCATCAAACTCGC	GCTATTGACATGCAACTATGGACCT	7B	
Xbarc71	GCGCTTGTTCCTCACCTGCTCATA	GCGTATATTCTCTCGTCTTCTTGTTGGTT	3DL	Linked to *Sr24*
Wmc331	CCTGTTGCATACTTGACCTTTTT	GGAGTTCAATCTTTCATCACCAT	4D	
Gwm624	TTGATATTAAATCTCTCTATGTG	AATTTTATTTGAGCTATGCG	4D	
Gdm153	TATAGGCAAATTAATTAAGACG	ATCTTTATGTGAGTACACTGC	5D	
Barc130	CGGCTAGTAGTTGGAGTGTTGG	ACCGCCTCTAGTTATTGCTCTC	5D	
Wmc111	ATTGATGTGTACGATGTGCCTG	CATGTCAATGTCATGATGAAGC	2D	
Cfd2	GGTTGCAGTTTCCACCTTGT	CATCTATTGCCAAAATCGCA	Multilocation	
Barc228	CCCTCCTCTCTTTAGCCATCC	GCACGTACTATTCGCCTTCACTTA	2D	
Gwm301	GAGGAGTAAGACACATGCCC	GTGGCTGGAGATTCAGGTTC	2D	
Cfd35	GGGATGACACATAACGGACA	ATCAGCGGCGCTATAGTACG	2DL	
Wmc11	TTGTGATCCTGGTTGTGTTGTGA	CACCCAGCCGTTATATATGTTGA	3A, 3B, 3D	
Gwm161	GATCGAGTGATGGCAGATGG	TGTGAATTACTTGGACGTGG	3D	
Gwm314	AGGAGCTCCTCTGTGCCAC	TTCGGGACTCTCTTCCCTG	3D	
Wmc492	AGGATCAGAATAGTGCTACCC	ATCCCGTGATCAGAATAGTGT	3D, 5A	
Gwm456	TCTGAACATTACACAACCCTGA	TGCTCTCTCTGAACCTGAAGC	3D	linked to *Sd-1* character
Wmc656	AAGTAGGCGAGCGTTGT	TTTCCCTGGCGAGATG	3D	
Wmc549	TTGTCACACACGCACTCCC	GTCCTTCCCTCGTTCATCCT	3D	
Barc71	GCGCTTGTTCCTCACCTGCTCATA	GCGTATATTCTCTCGTCTTCTTGTTGGTT	3D	
Wmc617	CCACTAGGAAGAAGGGGAAACT	ATCTGGATTACTGGCCAACTGT	4A, 4B, 4D	Not possible to distinguish between genomes B and D

Table 2-2 (cont'd)

Wmc89	ATGTCCACGTGCTAGGGAGGTA	TTGCCTCCCAAGACGAAATAAC	4A, 4B, 4D	Easy to detect bands from the D genome
Wmc622	CAGGAAGAAGAGCTCCGAGAAA	CTTGCTAACCCGCGCC	4D	Multilocus all from the D genome
Wmc74	AACGGCATTGAGCTCACCTTGG	TGCGTGAAGGCAGCTCAATCGG	4B, 4D, 5A	Easy to distinguish. 234 bp on 5A, 256 bp on 4D, and 310 bp on 4B
Wmc233	GACGTCAAGAATCTTCGTCGGA	ATCTGCTGAGCAGATCGTGGTT	5DS	
Gwm205	CGACCCGGTTCACTTCAG	AGTCGCCGTTGTATAGTGCC	5A, 5D	
Gdm136	CTCATCCGGTGAGTGCATC	CCCGCATGTCTACATGAGAA	1A, 1B, 3D, 5D	Not possible to distinguish between genomes
Gwm174	GGGTTCCTATCTGGTAAATCCC	GACACACATGTTCCTGCCAC	5D	
Cfd183	ACTTGCACTTGCTATACTTACGAA	GTGTGTCGGTGTGTGGAAAG	5D	
Gwm654	TGCTGATGTTGTAAGAAGGC	TGCGTCAGATATGCCTACCT	5D	
Cfd49	TGAGTTCTTCTGGTGAGGCA	GAATCGGTTCACAAGGGAAA	6D	
Gdm132	ACCGCTCGGAGAAAATCC	AGGGGGGCAGAGGTAGG	6D	
Gwm469	CAACTCAGTGCTCACACAACG	CGATAACCACTCATCCACACC	5D, 6D	
Gwm325	TTTCTTCTGTCGTTCTCTTCCC	TTTTTACGCGTCAACGACG	6B, 6D	
Cfd76	GCAATTTCACACGCGACTTA	CGCTCGACAACATGACACTT	6D	
Barc204	CGCAGAAGAAAAACCTCGCAGAAAAACC	CGCAGTGTATCCAAATGGGCAAGC	1A, 6A, 6D	
Wmc773	GAGGCTTGCATGTGCTTGA	GCCAACTGCAACCGGTACTCT	5B, 6D	

Table 2-2 (cont'd)

Gwm350	ACCTCATCCACATGTTCTACG	GCATGGATAGGACGCCC	4A, 7A, 7D	Not possible to distinguish between genomes
Cfd41	TAAAGTCTCAGGCGACCCAC	AGTGATAGACGGATGGCACC	7D	
Wmc629	TTTGTGTGTTGGATGCGTGC	AATAAAACGCGACCTCCCCC	7D	
Wmc405	GTGCGGAAAGAGACGAGGTT	TATGTCCACGTTGGCAGAGG	Multilocation	Not possible to distinguish between genomes
Wmc121	GGCTGTGGTCTCCCGATCATTC	ACTGGACTTGAGGAGGCTGGCA	7D	
Gwm437	GATCAAGACTTTTGTATCTCTC	GATGTCCAACAGTTAGCTTA	7D	
Gwm121	TCCTCTACAAACAAACACAC	CTCGCAACTAGAGGTGTATG	5D, 7D	Possible to distinguish
Gwm37	ACTTCATTGTTGATCTTGCATG	CGACGAATTCCCAGCTAAAC	Multilocation	Not possible to distinguish between genomes
Cfd175	TGTCGGGGACACTCTCTCTT	ACCAATGGGATGCTTCTTTG	2D, 7D	

Population structure

Population structure was estimated with STRUCTURE version 2.3.4 software, which implements a model-based clustering algorithm (Pritchard et al., 2000). One condition to perform the analysis is the use of unlinked markers, so this analysis was conducted with 315 SNP distributed in the 21 wheat chromosomes and 22 SSR markers located exclusively in the D genome (Table 2-3). The admixture model was selected due the nature of the wheat AMP. The parameters were set to 10,000 burnings and the number of MCMC iterations after burning were 100,000 with subpopulation number (k) from k=1 to k=10. The optimum k value was determined with Evanno method, which consists of identifying the true number of clusters (*K*) in a sample of individuals using an ad hoc statistic Delta *K* based on the rate of change in the log probability of data between successive *K* values (Evanno et al., 2005). To apply the Evanno method, the online software Structure Harvester version 0.6.93 was employed (Earl and Vonholdt, 2012). The online software can be found at: (http://taylor0.biology.ucla.edu/structureHarvester/).

EIGENSTRAT software, which infers principal components (Price et al., 2006), was used to detect and correct for population structure. Principal Component Analysis was conducted with 3,701 SNP markers distributed in the wheat AMP genome with MAF > 5%.

Table 2-3. List of SSR markers that amplified in the wheat AMP genome.

SSR marker name	Chromosome	SSR marker name	Chromosome	SSR marker name	Chromosome
Barc83	1A	*Wmc728*	1B	*Gwm147*	1D
Gwm636	2A	*Barc133*	3B	*Wmc216*	1D
Wmc658	2A	*Gwm493*	3B	*Gwm261*	2D
Barc19	3A	*Wmc89*	4B	*Wmc111*	2D
Cfa2149	5A	*Gwm297*	7B	*Barc71*	3D
				Gwm161	3D
				Gwm314	3D
				Gwm456	3D
				Wmc492	3D
				Cfd84	4D
				Wmc331	4D
				Wmc720	4D
				Barc130	5D
				Gdm153	5D
				Barc204	6D
				Cfd49	6D
				Gdm132	6D
				Gwm325	6D
				Gwm469	6D
				Wmc773	6D
				Gwm121	7D
				Gwm437	7D

Linkage disequilibrium

For estimating linkage disequilibrium (LD), SNP alleles with minor allele frequency (MAF) higher than 0.05 were used. Pair-wise linkage disequilibrium (LD) was measured using the squared allele-frequency correlations (r^2) (Flint-Garcia et al., 2003). TASSEL 4.0 (Bradbury et al., 2007) was employed to estimate inter and intra-chromosomal LD. To confirm the results from TASSEL, a set of SNP markers located in different regions of the wheat AMP genome were selected and r^2 was calculated using GGT 2.0: Graphical Genotypes (van Berloo, 2008). LD decay were assessed by calculating r^2 for pairs of SNP loci and plotting them against genetic distance (cM) and the cutoff was set as $r^2 > 0.2$ is in LD.

Results

Genotyping

The wheat AMP was screened at MSU with 8,632 SNP markers included in the wheat SNP chip from Illumina®. The total number of markers with missing data (no call) was 2,324 (27%) (Figure 2.1). The remaining SNP markers (6,308) ranged from 100 to 13% calls. The quality of the 6,308 SNP markers was determined by GenomeStudio® data analysis software from Illumina®. From the total number of good quality SNP markers, 681 were coded as heterozygous by Genome Studio's automated SNP calling in some individuals of the wheat AMP, but they were actually homozygous. The 681 markers were re-coded from AB to AA or BB allele based on GenomeStudio results. A total of

1,629 SNP markers were not considered for the analysis because of poor quality or because the position in the genome was unknown. Additionally, markers with more than 10% no-calls were also not considered in the analysis. The final number of markers considered for association analysis were 4,679 SNP (Figure 2.2), which were part of the 7,497 SNP markers with known positions in the wheat genome (Cavanagh et al., 2013) and additionally 32 SSR markers, out of 66 SSR markers screened, were selected based on clarity to score and genome specificity. Twenty-two SSR markers out of the 32 were located on the D-genome.

The distribution of the SNP markers in the wheat chromosomes are shown in Table 2-4. The A and B-genome have the best coverage in every chromosome compared with marker coverage on D-genome. The number of markers in A and B-genome ranged from 87 SNP markers on chromosome 4B to 404 SNP markers on chromosome 2B. The total number of SNP markers distributed on the entire D-genome was only 227. Chromosomes 3, 5, 6, and 7 from the D-genome were subdivided in three linkage groups each, according to the original report of SNP positions from the consensus map (Cavanagh et al., 2013). The number of SNP per linkage group ranged from 6 on chromosome 4D to 65 on chromosome 1D (Table 2-4).

Table 2-4. Size of the wheat linkage groups (cM) and number of SNP markers from the 9K SNP chip after filtering for MAF(> 5%) and missing data (< 10%).

Chr.[1]	Size of Chr. (cM)	No of SNPs for AM	Chr.	Size of Chr. (cM)	No of SNPs for AM	Chr.	Size of Chr. (cM)	No of SNPs for AM
1A	183	254	1B	141	221	1D	145	65
2A	231	195	2B	272	404	2D	192	44
3A	172	241	3B	196	238	3D1	2	3
4A	211	210	4B	125	87	3D2	2	0
5A	196	279	5B	227	369	3D3	85	14
6A	218	255	6B	154	281	4D	102	6
7A	194	276	7B	169	173	5D3	48	15
						5D2	16	2
						5D1	54	17
						6D1	8	17
						6D2	78	21
						6D3	8	2
						7D1	7	3
						7D2	55	11
						7D3	8	7

[1] Chr. = Chromosome

Linkage disequilibrium (LD)

Linkage disequilibrium analysis was conducted with 3,701 SNP markers after filtering the selected 4,679 SNP showing good quality against alleles with minimum frequency > 5%. Linkage disequilibrium decay was different for each genome. In the A-genome, LD decayed to the proposed cutoff of r^2= 0.2 at about 13 cM (Figure 2.9), while in the B-genome, LD decayed at 15 cM (Figures 2.10). In the D genome, the lack of good coverage of markers resulted in unreliable estimate of the LD decay for the entire genome (Figure 2.11). Therefore, the LD decay calculation was not performed for the entire genome, but it is reported for each individual chromosome. Thus, LD on chromosome 1D decayed at 15 cM. LD on chromosomes 2D, 4D and 7D, decayed at 3 cM. On chromosome 3D, LD decayed at 10 cM. On chromosome 5D, LD decayed at 5 cM, and LD on chromosome 6D decayed at 40 cM Figures 2.7 and 2.8).

In the LD analysis, 6,857,956 pair-wise comparisons were performed between SNP markers of the wheat AMP. The percentage of comparisons with an $r^2 \leq 0.2$ was 98.8% and only 1.2% of the pair-wise comparisons between molecular markers were higher than 0.2.

The intra-chromosomal analysis of the linked markers showed that 16.8 and 16.7% of the pair-wise comparisons of each chromosome in the A and B-genome respectively had an $r^2 > 0.2$. However, for the D-genome, 21.3% of the pair-wise comparisons were $r^2 > 0.2$ (Figures 2.3 – 2.8).

In the A-genome, LD decays at different rates in each chromosome. Analyzing the pair-wise comparison of r^2 values it was possible to note that Chromosome 3A showed the

largest percentage of pair-wise comparison of SNP markers with r^2 values > 0.2 (20.9%). On other chromosomes such as 5A showed lower percentage of pair-wise comparisons with r^2 > 0.2 (7.9%) (Figures 2.3 and 2.4).

In the B-genome, Chromosome 6B had the largest percentage of pair-wise comparison of SNP markers with r^2 values > 0.2 (21.3%), while chromosome 7B had the lower percentage of pair-wise comparisons with r^2 > 0.2 (11.7%) (Figures 2.5 and 2.6).

Population structure analysis

The population structure of the AMP was determined with: 1) STRUCTURE software based on 315 SNP markers separated by at least 4.0 cM in the whole wheat genome, and 22 SSR markers located on linkage groups of the D-genome exclusively (Table 2-3), and 2). EIGENSTRAT software, which was employed to perform a principal component analysis (PCA) with the 3,701 SNP markers from the wheat AMP distributed on the 21 wheat chromosomes. The output from STRUCTURE was analyzed with Structure Harvester to obtain Delta K values and determine the number of subpopulations in the wheat AMP. The results indicated that there were three subpopulations (k=3) (Figure 2.12). The first subpopulation with 96 accessions, a second subpopulation with 94 accessions, and the third subpopulation with 107 accessions (Figure 2.13). The principal component analysis also showed three clusters when PCA1 was plotted against PCA2 as shown in Figure 2.14. In this Figure, colors have been assigned to each wheat accession based on STRUCTURE results (Red= sub-population 1, Green= sub-population 2, and Blue= sub-population 3). It can be

observed in Figure 2.14 that clusters from the PCA, shows agreement with the STRUCTURE results. Similar results between the STRUCTURE analysis and the Neighbor Joining (NJ) tree (Fig 2.15) analysis can also be observed where three clusters are formed. However, each of the three clusters from the tree includes wheat accessions that were assigned to a different group according to STRUCTURE results. Seven accessions that STRUCTURE assigned to subpopulation 3 (blue color) and five accessions assigned to subpopulation 2 (green color) were clustered in the tree where most of the wheat accessions that STRUCTURE assigned as subpopulation 1 (red color). In the same way, twelve accessions from subpopulation 1 (red color) and one accession from subpopulation two (green color) were clustered in the cluster that STRUCTURE determined as subpopulation 3 (blue color). Finally, 11 accessions from supopulation 1 (red color) and 31 accessions from subpopulation 3 (blue color) were clustered in subpopulation 2 (green color).

Discussion

Genotyping

The final number of SNP markers used for association analysis was 4,679. These markers were selected for three reasons. First, these markers showed good quality, which means that presented good allele calls and clustered clearly to differentiate between one or another allele for each individual. Second, these markers have less than 10% missing data in the wheat AMP. Third, these markers were part of the 7,497 SNP markers with known positions in the wheat genome (Cavanagh et al., 2013). In total, 27% of SNP markers did not function (no-call) in the wheat AMP. The percentage

of markers with no-calls was similar to the number of markers that did not produce signals obtained by Würschum et al. (2013), where the number of markers with no-calls was 26.9%. The number of no-calls differs widely within markers. A SNP marker could not be detected because poor quality of the DNA sample or the marker did not hybridize, or simply, the SNP was not present (Illumina, 2008).

Single nucleotide polymorphisms markers are ideal to study genetic structure and diversity in wheat (Chao et al., 2010) due to abundance and distribution in the whole genome. Here, using the 9K SNP chip from Illumina (Cavanagh et al., 2013), we have confirmed that this SNP platform system works for spring wheat.

All the SSR markers used in this study amplified and detected polymorphisms, but, not all were useful. The main problem observed with the SSR screening was the difficulty to identify the genome origin of each allele when a marker amplified in more than one *locus*. In wheat, it is relatively easy to determine the number of loci expected in the progeny based on the number of *locus* observed in the parents and if the marker amplifies in paralogous loci when biparental populations are screened with molecular markers (Song et al., 2005). However, in this study, the marker screening of wheat breeding lines with different ancestry resulted frequently in multi-locus amplification. It has been observed in complex genomes as wheat (Somers et al., 2004). As a consequence, only 32 SSR markers were scored and able to be assigned to the proper genome. Five SSR markers were located on A-genome, five on B-genome, and twenty two on D-genome (Table 2-3).

The distribution of useful SNP markers for this study was ideal for the A and B-genome and poor for D-genome. It is common to observe reduced number of polymorphic

number of markers at the D-genome in wheat (Pestsova et al., 2000; Somers et al., 2004). The reason for the reduced polymorphism number is the result of few hybridizations in the formation of the modern wheat by the fusion of the tetraploid wheat genome with the *T. tauschii* genome (Talbert et al., 1998).

Linkage disequilibrium

As expected, the extend of LD for SNP pairs decays as map distance increases (Du et al., 2007; Sorkheh et al., 2008) . In this study, LD declined to $r^2 \leq 0.2$ more slowly than in other studies where LD decayed at about 6.3 cM in the A-genome and 7 cM in the B-genome (Chao et al., 2007) and at 5 cM in a US winter wheat and a durum wheat population (Breseghello and Sorrells, 2006; Maccaferri et al., 2005). LD values must be different for different wheat populations (Chao et al., 2010) since LD is affected by several factors such as recombination, population size, admixture, or genetic bottlenecks (Flint-Garcia et al., 2003). In other crops such as soybean, LD decay pattern differed among four distinct populations of diverse origin (Hyten et al., 2007). LD can decay faster or slower, depending primary on the mating system. LD usually decays faster in open pollinated crops. For example, LD in maize, often measured as physical distance, has been shown to decay to an $r^2 \leq 0.2$ within 500 – 2,000 bp (Remington et al., 2001). This is extremely fast compared with hexaploid wheat if we consider that 1 cM from the consensus map (Cavanagh et al., 2013) represents an average of 3.4 Mb based on the wheat genome size of 16 Gb (Arumuganathan and Earle, 1991).
LD decay distance was different in each chromosome. It has also been observed that LD decay distance may differ among chromosomes (Yan et al., 2009).

The distance over which LD persist defines the number of markers needed to conduct association mapping analysis (Sorkheh et al., 2008). In this study, LD extended to about 13cM and 15 cM for SNP markers at the A and B- genome, while the distance at the D-genome varied from 3 to 15 cM with exception ofr chromosome 6D, which decayed at 40cM. The SNP map developed by (Cavanagh et al., 2013) has 3,500 cM, so the number of SNP markers utilized in this study tell us that there is 1 SNP per cM. This situation would be ideal, since LD extends > 3cM in every linkage group. However, this situation is not true for the D-genome due to the reduced presence of markers in this genome.

Population structure analysis

A population is structured if individuals of the population do not mate at random and alleles deviate from the Hardy Weinberg equilibrium which results in unequal distribution of alleles within these subpopulations (Flint-Garcia et al., 2003). In this study, three subpopulations were identified using three different methods to group individuals based on genotypic information. The computer software STRUCTURE was able to allocate each accession of the wheat AMP in one of the three subpopulations based on multiple locus genotype data using computationally intensive methods (Pritchard et al., 2000). The results from STRUCTURE differed slightly from the other two methods utilized to estimate population structure (principal component analysis or NJ three clustering method). However, it is clear that individuals can be separated in three subpopulations (Figures 2.13; 2.14; 2.15).

The three methods show to be efficient in this study. They separated the wheat accessions in three subpopulations. Some studies have mentioned that STRUCTURE software might have some limitations to accurately identify genetic clusters within species (Kalinowski, 2011; Price et al., 2006); however, for this specific study the results were similar.

Some wheat accessions assigned to one subpopulation were not genetically distant from other accessions assigned to other subpopulations as can be observed in the NJ tree (Fig 2.15). These lines could have same ancestors in common. The analysis with STRUCTURE using the Admixture model can show how these lines share loci from different subpopulations. Individual observations of the membership coefficients on each line from STRUCTURE (Appendix A) show how these lines have close values that could be used to assign these wheat lines in one or other sub-population. For instance, accession 135 (BABAX/LR42//BABAX*2/3/KUKUNA/4/TAM200/PASTOR//TOBA97), line from subpopulation 2 (Green color) according to STRUCTURE analysis, was clustered with lines from subpopulation three (blue color) in the NJ tree analysis. The membership coefficients were: Q1=0.38, Q2= 0.44, and Q3= 0.18. So, values for Q1 and Q2 were relatively close. Anthor example is accession 7 (BAV92//IRENA/KAUZ/3/HUITES*2/4/MURGA), assigned to subpopulation 2 (Green color) by STRUCTURE, was clustered with lines from population 1 (Red color). The membership coeficients of this accession were Q1= 0.44, Q2= 0.52, and Q3=0.03. Based on these values, it is not surprising that one analysis produced a different result.

Conclusions

Accessions in the wheat association mapping panel can be assigned to three different sub-populations. Three different methods based on genotypic data coincided to the allocation of most of the wheat accessions into these three clusters. In the same manner, these wheat accessions showed rich allele diversity based on SNP and SSR markers.

Linkage disequilibrium in the wheat AMP extends considerably as expected in self-crossing species; however, LD decay was different in each chromosome. These results indicated that 1-3 molecular markers per cM can be enough for association mapping studies. In other words, 3,000 to 4,000 molecular markers would be needed to accurately conduct an association study in wheat. The wheat SNP chip with 9K SNP markers is a great tool to study the genetic diversity of wheat and perform association mapping studies; however, low coverage and polymorphism was observed in most of the chromosomes of the D-genome. It will be advisable to include more molecular markers on the D-genome to provide more complete marker coverage and increase the chances to discover marker-trait associations.

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Daniel Zarka from MSU genotyped the wheat AMP with 9K SNP chip from Illumina.

Zixang Wen from MSU with software analysis.

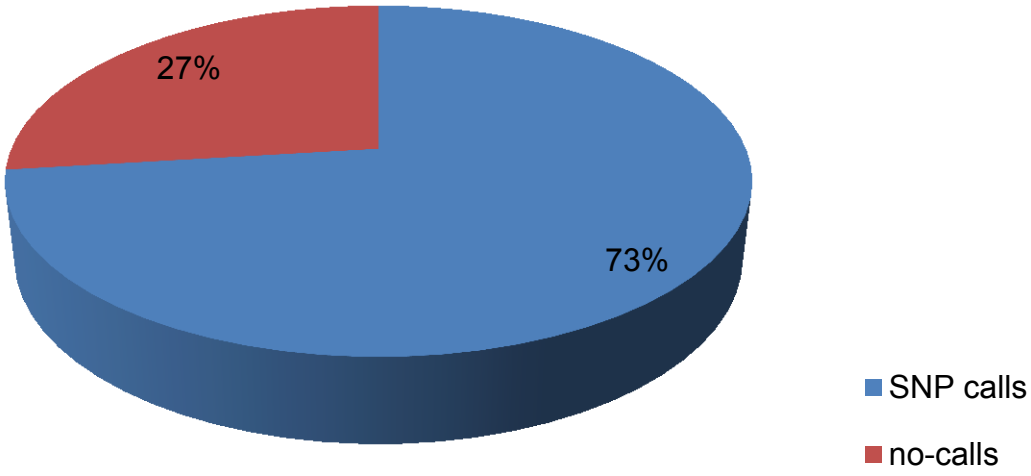

Figure 2-1. Results from the Illumina® iSelect scan: blue color corresponds to the percentage of SNP markers from the 9K SNP chip that were detected and red color corresponds to the percentage of SNP markers placed in the 9K SNP Chip from Illumina that were not detected.

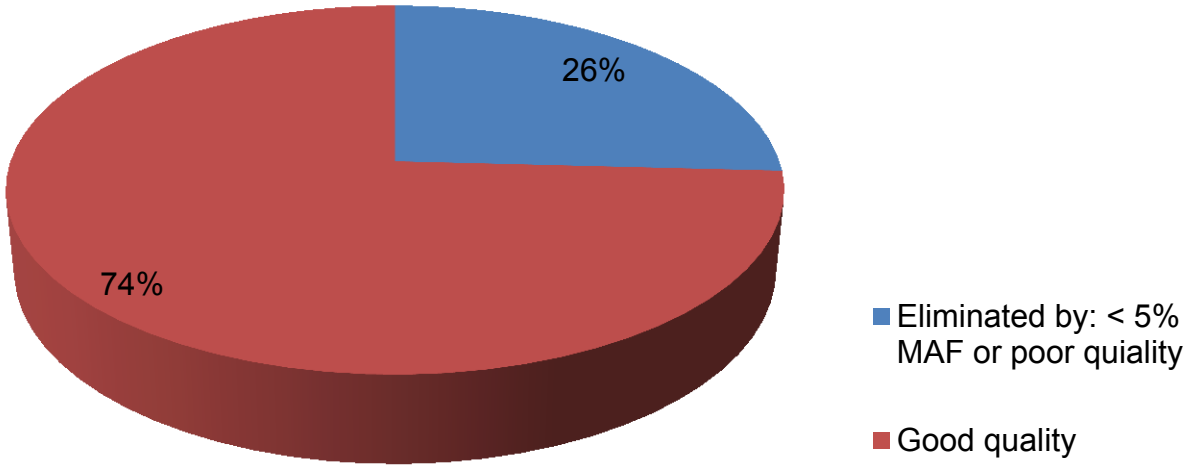

Figure 2-2. Percentage of SNP markers eliminated after filtering for poor quality or minimum frequency alleles (<5%) and SNP markers showing good quality and considered for analysis.

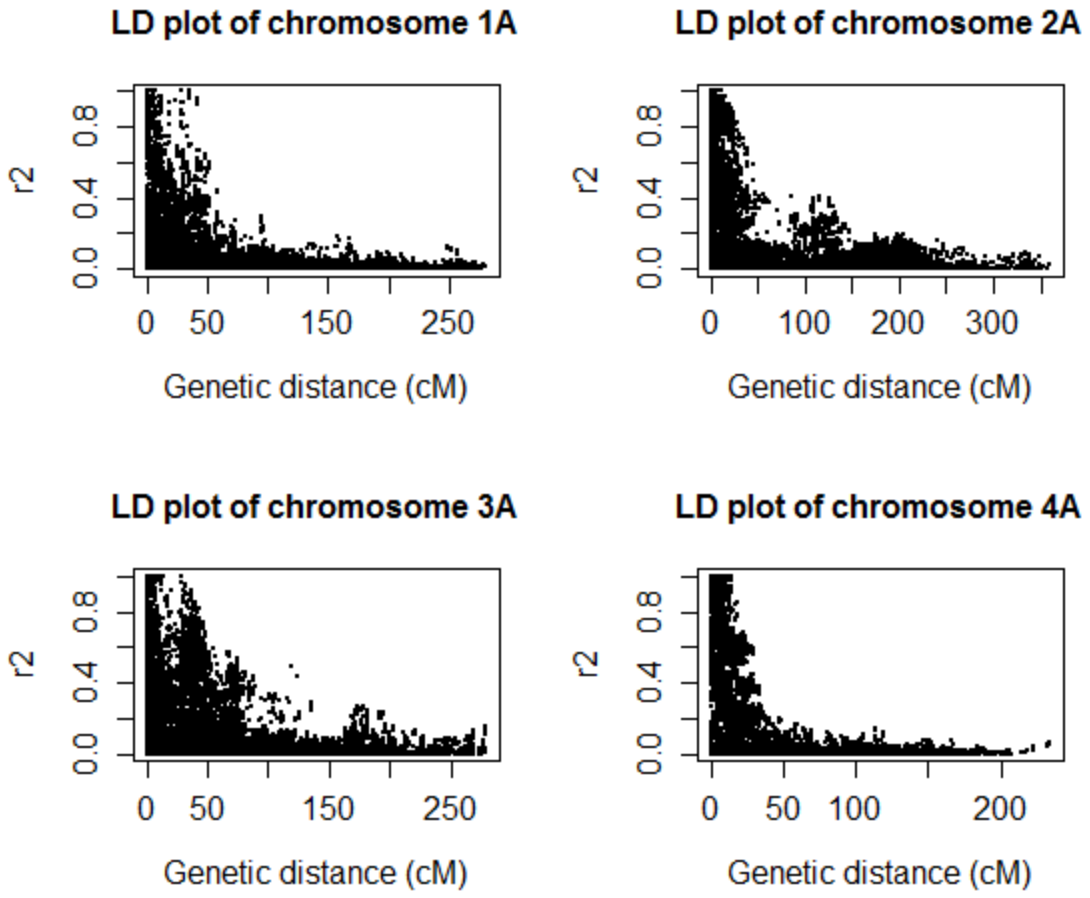

Figure 2-3. Scatter plot of LD values (r^2) against genetic distance (cM) of chromosomes 1A – 4A.

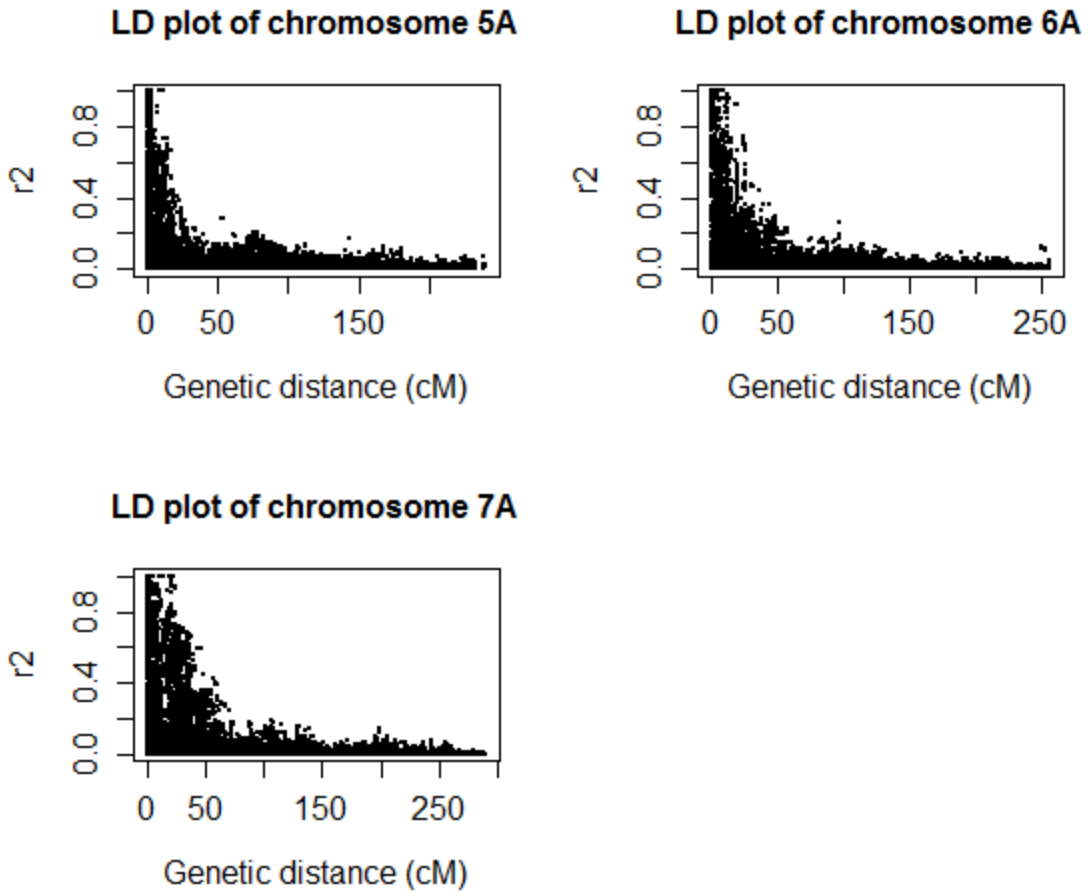

Figure 2-4. Scatter plot of LD values (r^2) against genetic distance (cM) of chromosomes 5A – 7A.

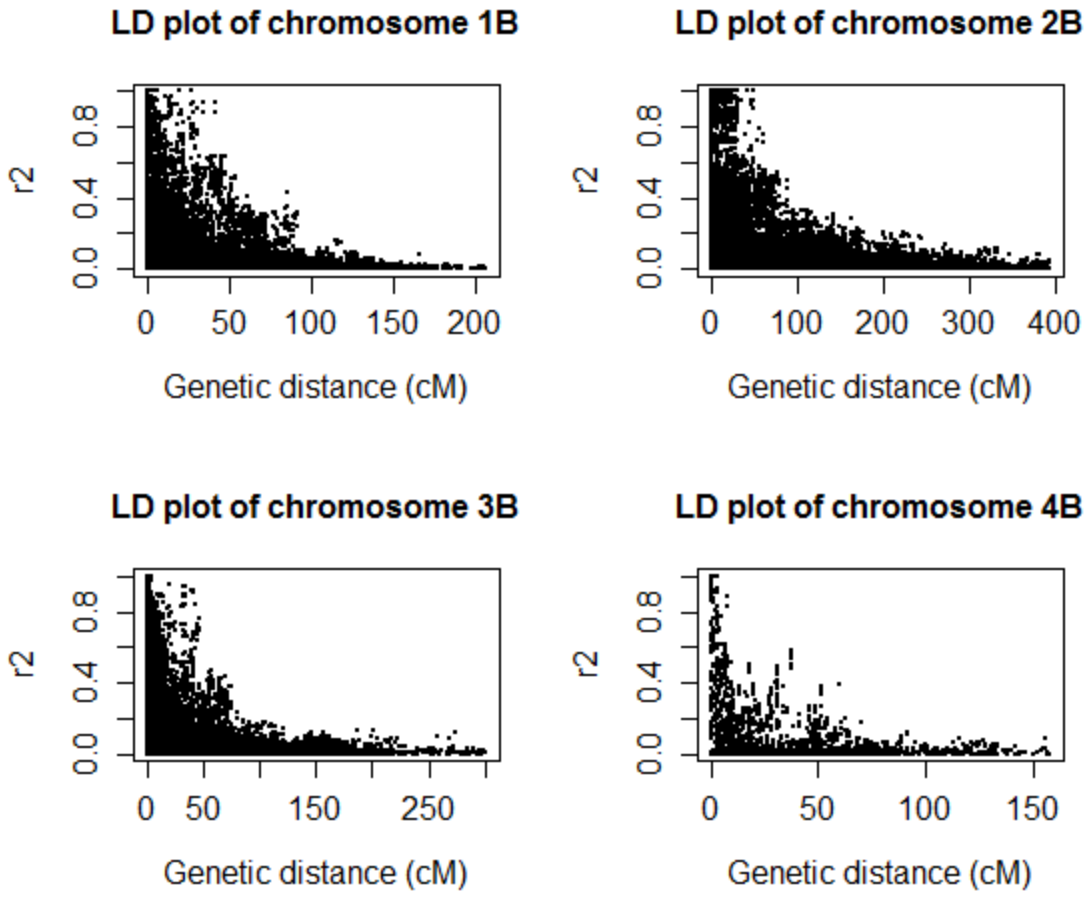

Figure 2-5. Scatter plot of LD values (r^2) against genetic distance (cM) of chromosomes 1B – 4B.

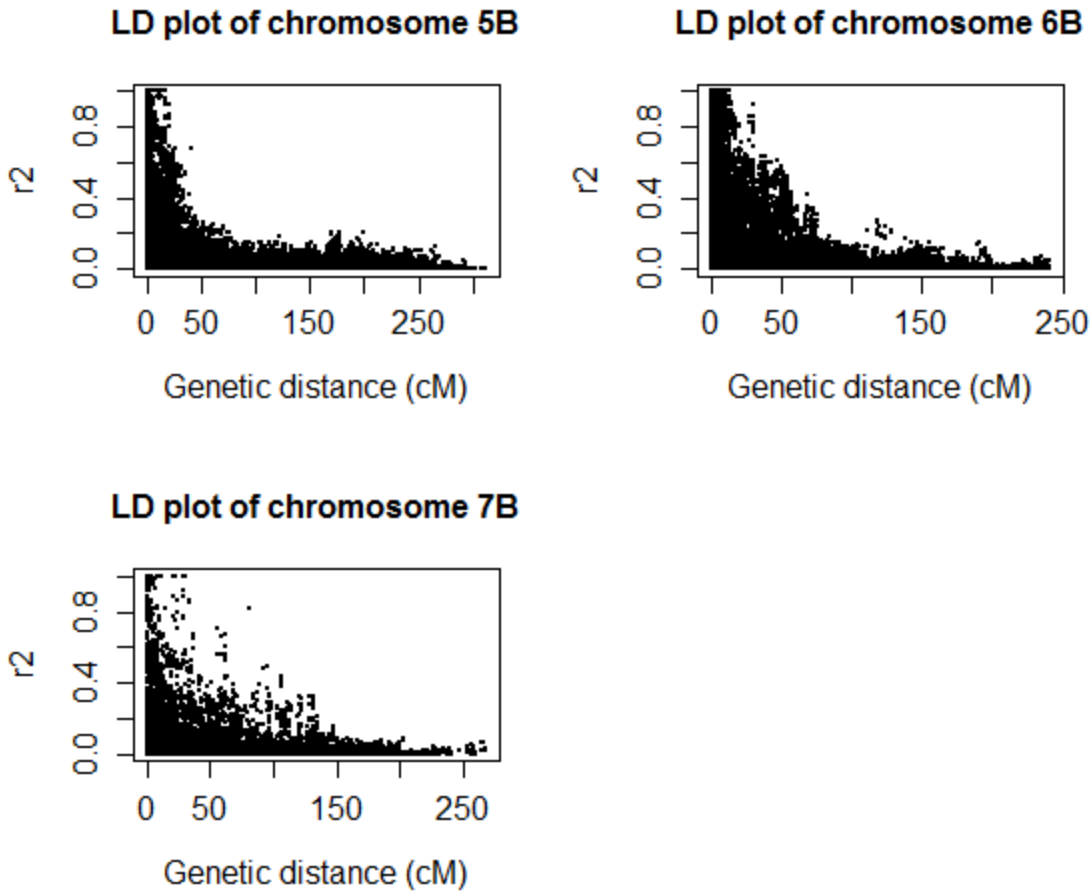

Figure 2-6. Scatter plot of LD values (r^2) against genetic distance (cM) of chromosomes 5B – 7B.

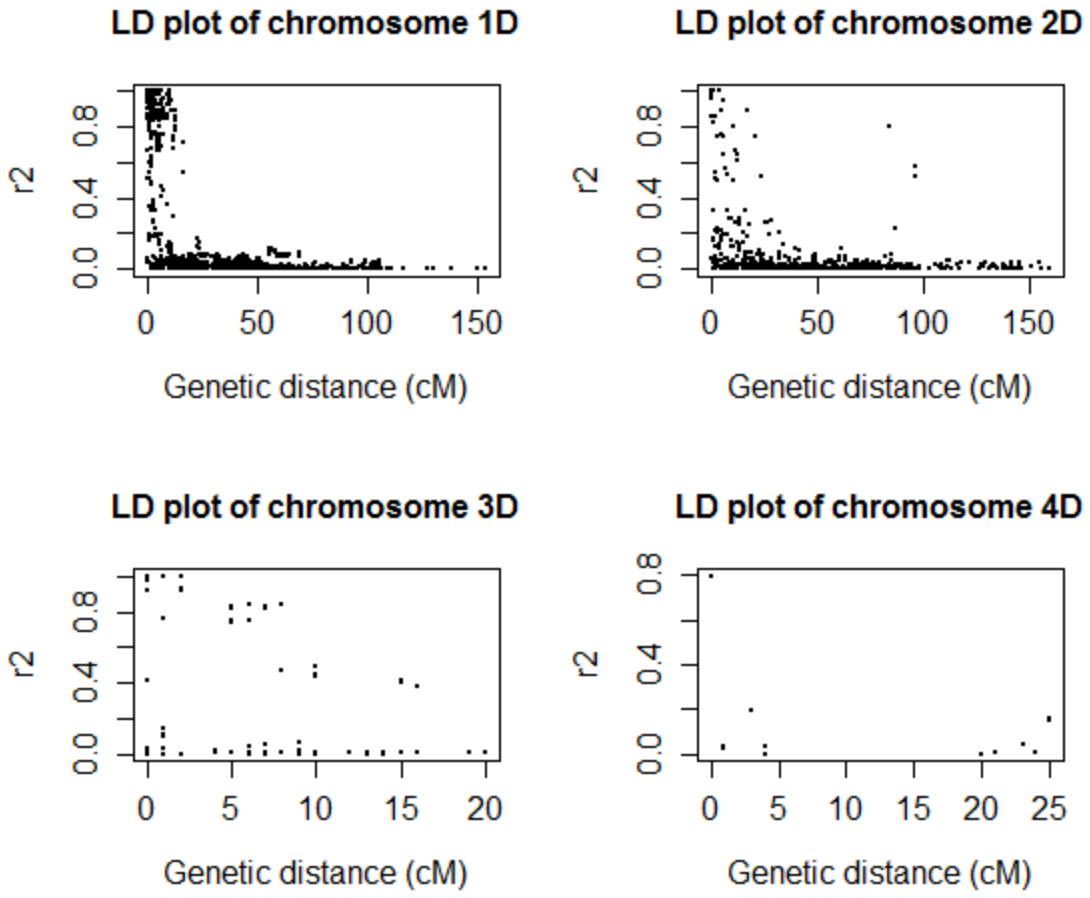

Figure 2-7. Scatter plot of LD values (r^2) against genetic distance (cM) of chromosomes 1D – 4D.

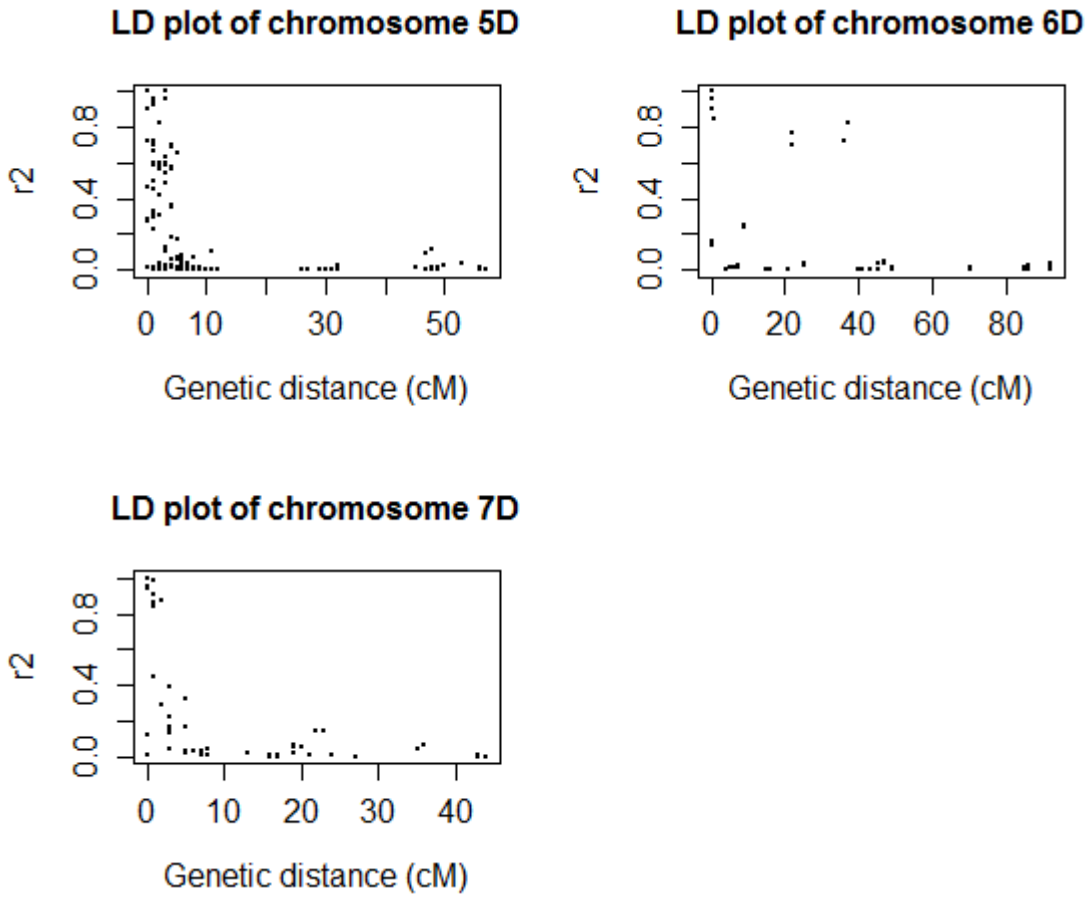

Figure 2-8. Scatter plot of LD values (r^2) against genetic distance (cM) of chromosomes 5D – 7D.

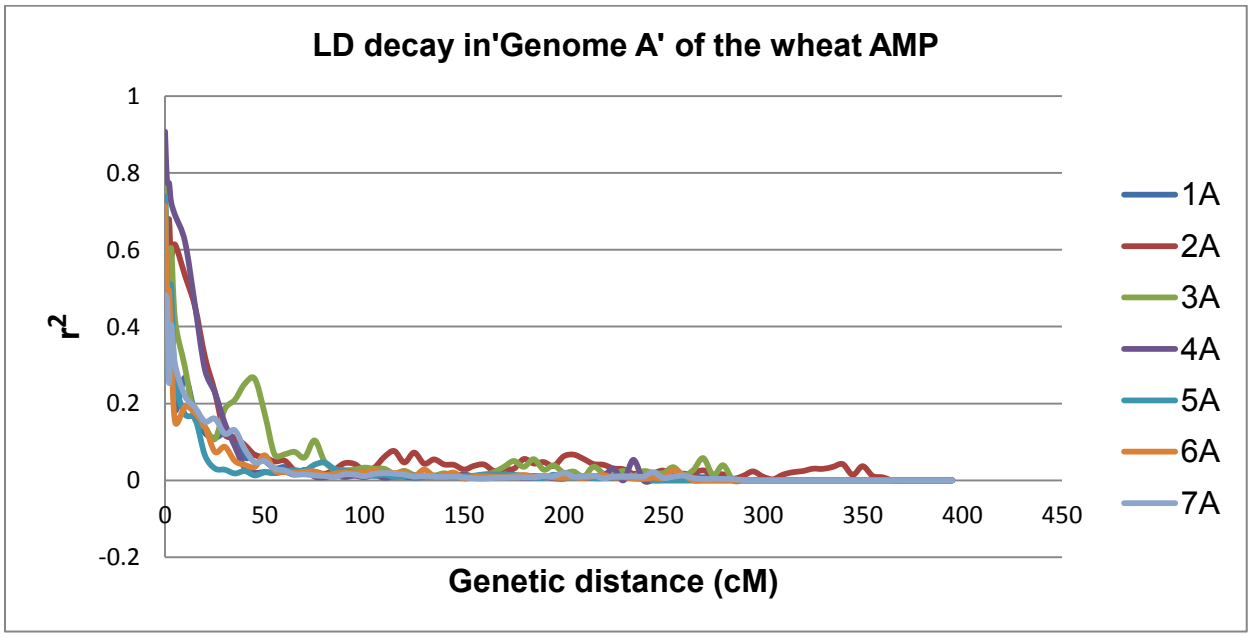

Figure 2-9. Intrachromosomal comparison of LD decay on chromosomes from the A genome of the wheat AMP.

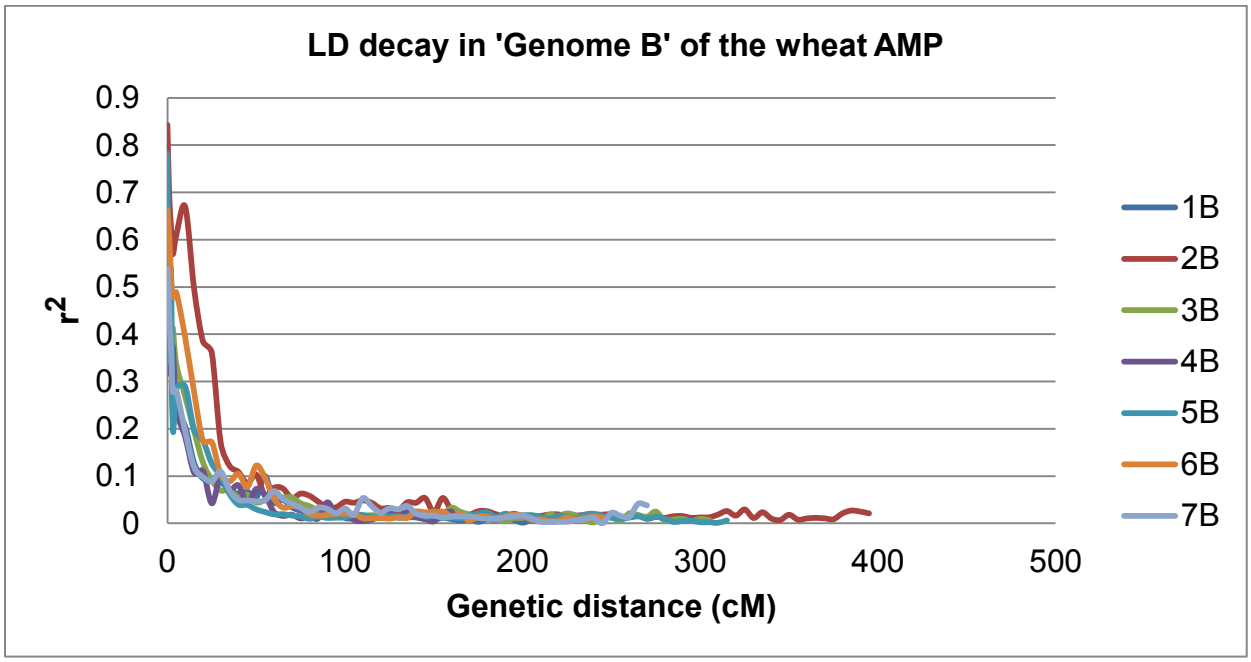

Figure 2-10. Intrachromosomal comparison of LD decay on chromosomes from the B genome of the wheat AMP.

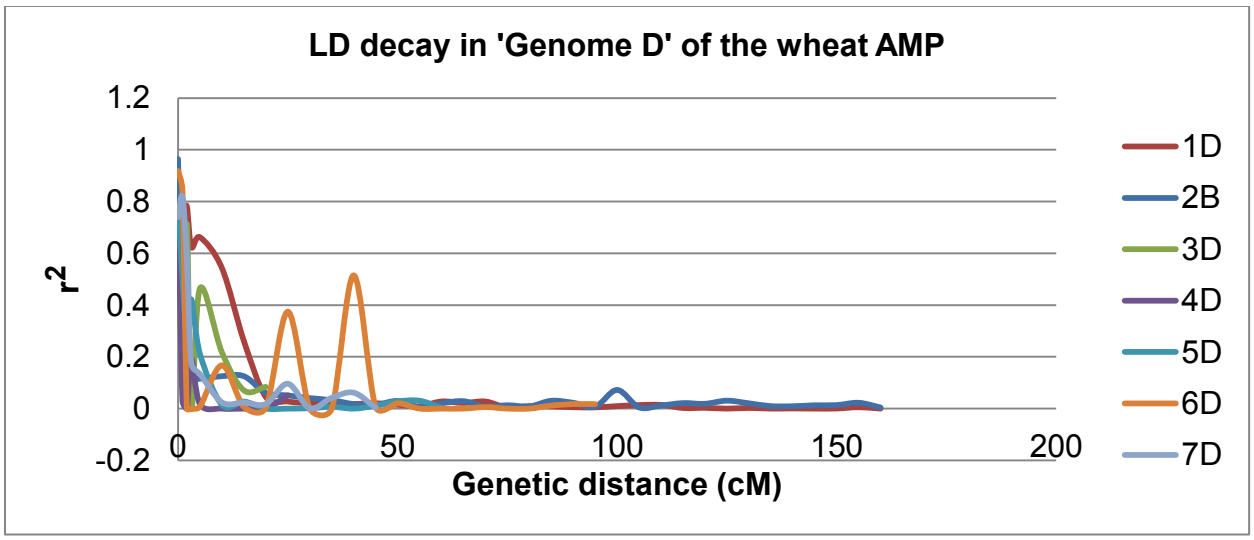

Figure 2-11. Intrachromosomal comparison of LD decay on chromosomes from the D genome of the wheat AMP.

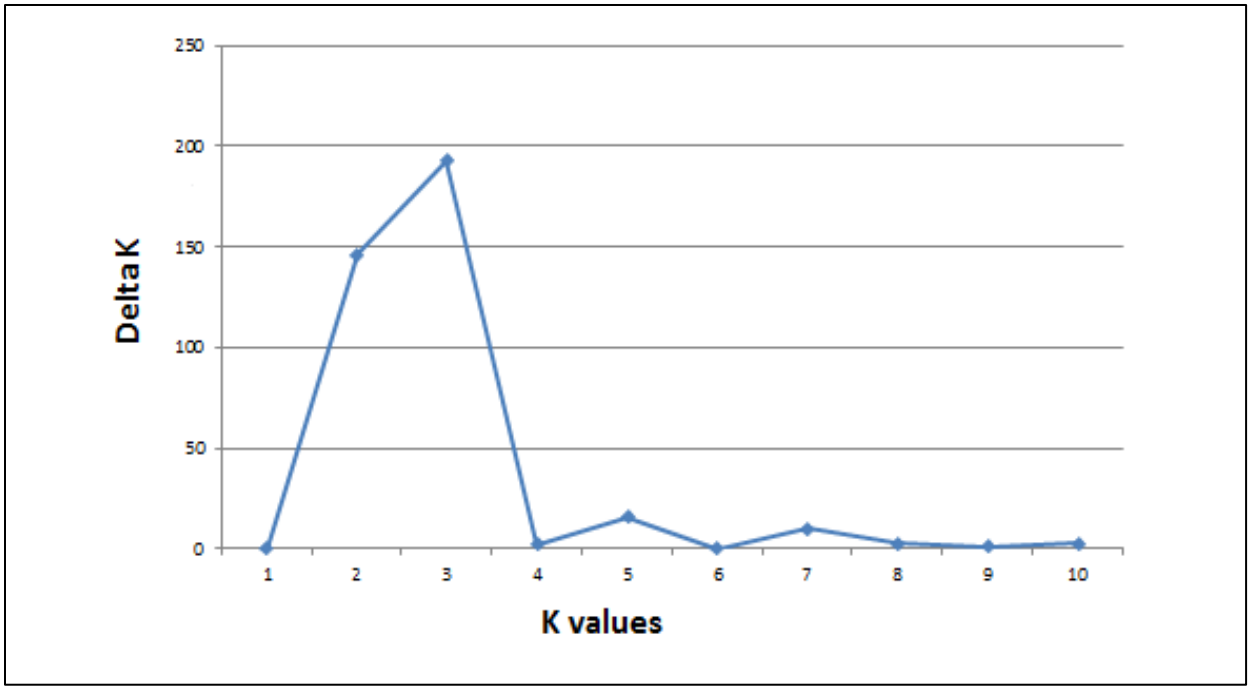

Figure 2-12. Distribution of Delta K values in wheat the association mapping panel based on STRUCTURE analysis. East Lansing. 2013.

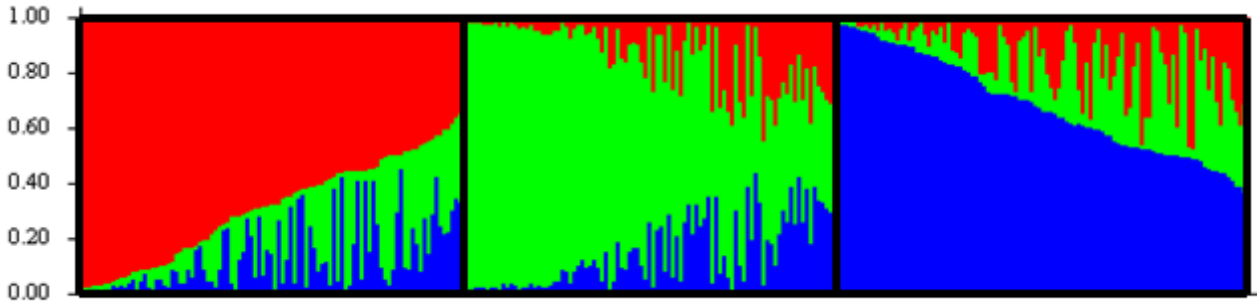

Figure 2-13. Population structure based on STRUCTURE software of the wheat association mapping panel. East Lansing. 2013.

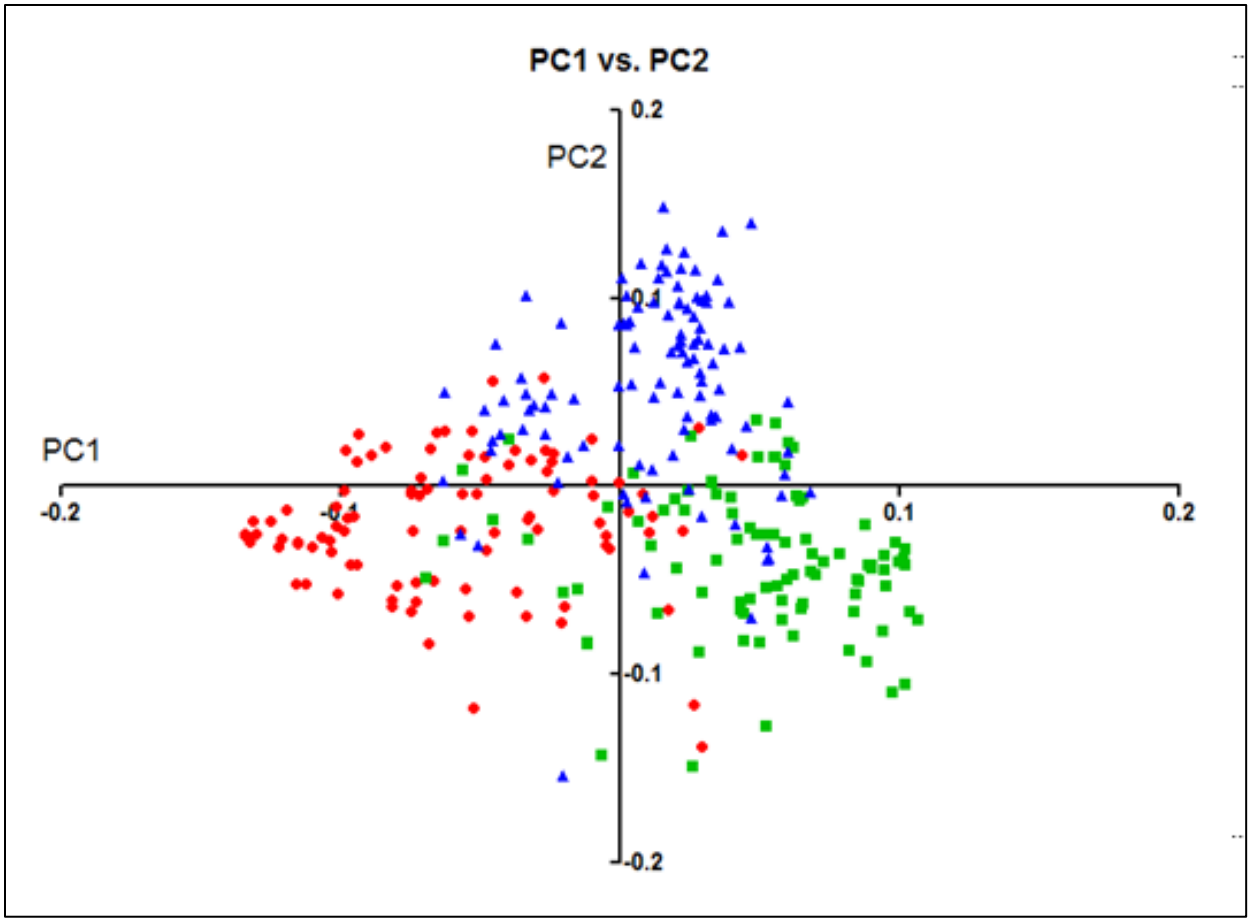

Figure 2-14. Principal component analysis of the wheat association mapping panel (red= sub-population one, green= sub-population two, blue= sub-population three) based on SNP markers. East Lansing. 2013.

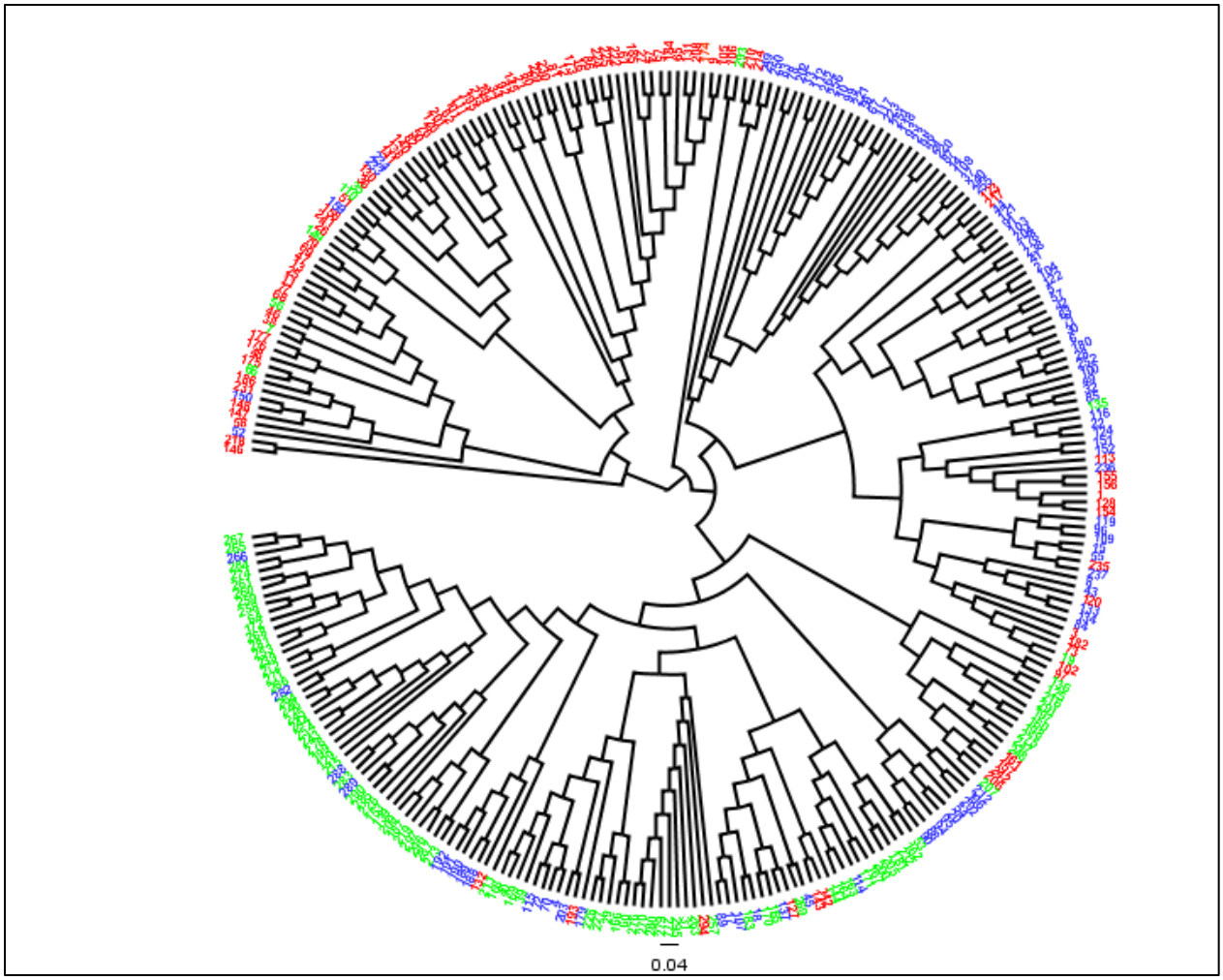

Figure 2-15. Neighbor joining tree of the wheat Association Mapping Panel. Accessions have been assigned colores based on STRUCTURE analysis. Red= sub-population 1, Green= sub-population 2, and Blue= subpopulation 3.

APPENDIX

Appendix: wheat association mapping panel and membership coefficients.

Table 2-5. Wheat AMP accessions and membership coefficients for each sub-population (Q) determined by STRUCTURE software.

	Accession pedigree	Q1	Q2	Q3
1	SAUAL/KRONSTAD F2004	0.97	0.02	0.01
2	TUKURU//BAV92/RAYON*2/3/PVN	0.26	0.16	0.57
3	PBW343*2/KUKUNA*2//FRTL/PIFED	0.70	0.09	0.21
4	FRET2/TUKURU//FRET2/3/MUNIA/CHTO//AMSEL/4/FRET2/TUKURU//FRET2	0.06	0.45	0.50
5	ROLF07*2/KACHU #1	0.03	0.09	0.88
6	WBLL1*2/4/BABAX/LR42//BABAX/3/BABAX/LR42//BABAX	0.04	0.15	0.81
7	BAV92//IRENA/KAUZ/3/HUITES*2/4/MURGA	0.44	0.52	0.03
8	WBLL1*2/CHAPIO*2//MURGA	0.22	0.19	0.59
9	KACHU/5/REH/HARE//2*BCN/3/CROC_1/AE.SQUARROSA (213)//PGO/4/HUITES/6/KACHU	0.97	0.01	0.01
10	ATTILA*2/PBW65*2//W485/HD29	0.05	0.06	0.90
11	BAV92//IRENA/KAUZ/3/HUITES*2/4/CROC_1/AE.SQUARROSA	0.69	0.25	0.06
12	KACHU #1/4/CROC_1/AE.SQUARROSA (205)//KAUZ/3/SASIA/5/KACHU	0.97	0.01	0.01
13	BAV92//IRENA/KAUZ/3/HUITES*2/4/GONDO/TNMU	0.55	0.39	0.06
14	MUNAL #1/FRANCOLIN #1	0.01	0.01	0.97
15	PFAU/SERI.1B//AMAD/3/WAXWING/4/BABAX/LR42//BABAX*2/3/ KURUKU	0.08	0.02	0.90
16	BECARD/KACHU	0.75	0.02	0.23
17	TRCH/HUIRIVIS #1	0.40	0.10	0.50
18	TRCH/KBIRD	0.20	0.07	0.73
19	ROLF07/MUU	0.13	0.44	0.42
20	PBW343*2/KUKUNA//TECUE #1	0.17	0.33	0.51
21	NAC/TH.AC//3*PVN/3/MIRLO/BUC/4/2*PASTOR	0.83	0.13	0.04
22	YAV_3/SCO//JO69/CRA/3/YAV79/4/AE.SQUARROSA (498)/5/LINE	0.35	0.04	0.61
23	WAXWING/4/BL 1496/MILAN/3/CROC_1/AE.SQUARROSA (205)//KAUZ/5/ FRNCLN	0.04	0.04	0.92
24	WAXWING*2/HEILO	0.02	0.15	0.84

Table 2-5 (cont'd)

25	KIRITATI/4/2*BAV92//IRENA/KAUZ/3/HUITES	0.60	0.29	0.11
26	KZA//WH 542/2*PASTOR/3/BACEU #1	0.50	0.44	0.05
27	KFA/2*KACHU	0.80	0.11	0.09
28	QUAIU #1	0.02	0.11	0.87
29	PASTOR//HXL7573/2*BAU/3/WBLL1	0.03	0.61	0.36
30	KLDR/PEWIT1//MILAN/DUCULA	0.05	0.41	0.54
31	PUB94.15.1.12/FRTL	0.02	0.94	0.04
32	FRET2*2/4/SNI/TRAP#1/3/KAUZ*2/TRAP//KAUZ*2/5/BOW/URES//2*WEAVER/3/CROC_1/AE.SQUARROSA (213)//PGO	0.20	0.04	0.76
33	ATTILA*2/PBW65//WBLL1*2/VIVITSI	0.04	0.06	0.90
34	ALTAR 84/AE.SQUARROSA (221)//3*BORL95/3/URES/JUN//KAUZ/4/WBLL1/5/REH/HARE//2*BCN/3/CROC_1/AE.SQUARROSA (213)//PGO/4/HUITES	0.78	0.18	0.04
35	MURGA//WAXWING/KIRITATI	0.12	0.64	0.25
36	MURGA/KRONSTAD F2004	0.66	0.30	0.04
37	ATTILA*2/PBW65//MURGA	0.01	0.01	0.98
38	BAV92//IRENA/KAUZ/3/HUITES/6/ALD/CEP75630//CEP75234/PT7219/3/BUC/BJY/4/CBRD/5/TNMU/PF85487	0.55	0.42	0.03
39	WBLL1*2/CHAPIO//HEILO	0.34	0.62	0.04
40	WBLL1*2/CHAPIO//HEILO	0.28	0.53	0.19
41	WAXWING*2/4/BOW/NKT//CBRD/3/CBRD	0.06	0.08	0.86
42	ROLF07*2/3/PRINIA/PASTOR//HUITES	0.04	0.44	0.51
43	ROLF07*2/4/CROC_1/AE.SQUARROSA (205)//BORL95/3/2*MILAN	0.11	0.23	0.66
44	WBLL1*2/KUKUNA/5/PSN/BOW//SERI/3/MILAN/4/ATTILA/6/WBLL1*2/KKTS	0.09	0.33	0.58
45	WAXWING*2/DIAMONDBIRD	0.07	0.21	0.72
46	BAV92//IRENA/KAUZ/3/HUITES*2/4/MILAN/KAUZ//CHIL/CHUM18	0.54	0.04	0.41
47	BAV92//IRENA/KAUZ/3/HUITES*2/4/PVN	0.71	0.16	0.13
48	BAV92//IRENA/KAUZ/3/HUITES*2/4/TNMU	0.56	0.39	0.05
49	WBLL1/DIAMONDBIRD//WBLL1*2/VIVITSI	0.33	0.14	0.53
50	SAUAL/YANAC//SAUAL	0.89	0.02	0.09
51	SAUAL/KIRITATI//SAUAL	0.90	0.05	0.05

Table 2-5 (cont'd)

52	CS/TH.SC//3*PVN/3/MIRLO/BUC/4/URES/JUN//KAUZ/5/HUITES/6/YANAC/7/CS/TH.SC//3*PVN/3/MIRLO/BUC/4/MILAN/5/TILHI	0.31	0.32	0.37
53	FINSI/METSO//FH6-1-7/3/FINSI/METSO	0.08	0.66	0.26
54	INQALAB 91*2/KUKUNA*2//PVN	0.39	0.18	0.44
55	UP2338*2/4/SNI/TRAP#1/3/KAUZ*2/TRAP//KAUZ/5/MILAN/KAUZ//CHIL/CHUM18/6/UP2338*2/4/SNI/TRAP#1/3/KAUZ*2/TRAP//KAUZ	0.15	0.21	0.64
56	UP2338*2/KKTS*2//YANAC	0.34	0.60	0.06
57	WAXWING/2*ROLF07	0.01	0.02	0.97
58	WBLL1*2/5/CNO79//PF70354/MUS/3/PASTOR/4/BAV92	0.76	0.15	0.09
59	ATTILA*2/PBW65*2//MURGA	0.12	0.05	0.83
60	WBLL1/FRET2//PASTOR*2/3/MURGA	0.03	0.94	0.03
61	KACHU #1/4/CROC_1/AE.SQUARROSA (205)//BORL95/3/2*MILAN/5/KACHU	0.89	0.08	0.02
62	SAUAL/4/CROC_1/AE.SQUARROSA (205)//BORL95/3/2*MILAN/5/SAUAL	0.92	0.07	0.01
63	ROLF07*2/4/CROC_1/AE.SQUARROSA (224)//KULIN/3/WESTONIA	0.14	0.41	0.45
64	KACHU*2/3/CHUM18/BORL95//CBRD	0.05	0.91	0.04
65	KACHU #1/4/CROC_1/AE.SQUARROSA (205)//KAUZ/3/SASIA/5/KACHU	0.97	0.01	0.02
66	SAUAL*2/6/CNDO/R143//ENTE/MEXI_2/3/AEGILOPS SQUARROSA (TAUS)/4/WEAVER/5/2*PASTOR	0.39	0.60	0.01
67	ATTILA*2/PBW65*2/4/BOW/NKT//CBRD/3/CBRD	0.01	0.02	0.97
68	BAV92//IRENA/KAUZ/3/HUITES/4/FN/2*PASTOR/5/BAV92//IRENA/KAUZ/3/HUITES	0.46	0.46	0.08
69	BAV92//IRENA/KAUZ/3/HUITES/4/FN/2*PASTOR/5/BAV92//IRENA/KAUZ/3/HUITES	0.59	0.29	0.12
70	ROLF07*2/4/BOW/NKT//CBRD/3/CBRD	0.21	0.24	0.55
71	WBLL1/4/BOW/NKT//CBRD/3/CBRD/5/WBLL1*2/TUKURU	0.07	0.23	0.70
72	WBLL1*2/4/YACO/PBW65/3/KAUZ*2/TRAP//KAUZ*2/5/GONDO	0.04	0.47	0.49
73	PFAU/WEAVER*2//BRAMBLING/3/KAUZ//TRAP#1/BOW/4/PFAU/WEAVER*2//BRAMBLING	0.42	0.33	0.24
74	KACHU*2//CHIL/CHUM18	0.94	0.03	0.03
75	KACHU*2//CHIL/CHUM18	0.91	0.07	0.02
76	SAUAL/3/ACHTAR*3//KANZ/KS85-8-4/4/SAUAL	0.80	0.16	0.04
77	BAV92//IRENA/KAUZ/3/HUITES*2/4/YUNMAI 47	0.55	0.27	0.18
78	WAXWING/KIRITATI*2/3/C80.1/3*BATAVIA//2*WBLL1	0.14	0.19	0.68

Table 2-5 (cont'd)

79	BAV92//IRENA/KAUZ/3/HUITES*2/4/WHEAR	0.51	0.39	0.10
80	KACHU #1*2/WHEAR	0.86	0.06	0.08
81	KACHU #1/3/C80.1/3*BATAVIA//2*WBLL1/4/KACHU	0.72	0.24	0.04
82	SAUAL/WHEAR//SAUAL	0.77	0.21	0.03
83	FRNCLN/BECARD	0.05	0.04	0.91
84	PAURAQ/3/KIRITATI//PRL/2*PASTOR	0.06	0.08	0.87
85	QUAIU/5/FRET2*2/4/SNI/TRAP#1/3/KAUZ*2/TRAP//KAUZ	0.05	0.09	0.86
86	BECARD/KACHU	0.81	0.02	0.17
87	FRANCOLIN #1/HAWFINCH #1	0.08	0.01	0.91
88	FRNCLN/TECUE #1	0.09	0.29	0.61
89	TRCH/HUIRIVIS #1	0.25	0.09	0.66
90	QUAIU/TECUE #1	0.16	0.23	0.60
91	KBIRD//WBLL1*2/KURUKU	0.27	0.04	0.69
92	KINGBIRD #1/KACHU	0.74	0.02	0.24
93	WAXWING/4/SNI/TRAP#1/3/KAUZ*2/TRAP//KAUZ/5/AKURI	0.03	0.02	0.95
94	WAXWING/4/SNI/TRAP#1/3/KAUZ*2/TRAP//KAUZ/5/TECUE #1	0.10	0.18	0.72
95	PBW343*2/KUKUNA//TECUE #1	0.09	0.38	0.53
96	WBLL1*2/BRAMBLING//FN/2*PASTOR	0.26	0.12	0.61
97	QUAIU #3//MILAN/AMSEL	0.55	0.30	0.15
98	ATTILA*2/PBW65//MUU #1/3/FRANCOLIN #1	0.02	0.03	0.94
99	ATTILA*2/PBW65*2//TOBA97/PASTOR	0.03	0.12	0.85
100	WBLL1*2/VIVITSI//PRINIA/PASTOR/3/WBLL1*2/BRAMBLING	0.08	0.21	0.71
101	SAUAL/5/REH/HARE//2*BCN/3/CROC_1/AE.SQUARROSA (213)//PGO/4/HUITES/6/KACHU	0.93	0.03	0.04
102	MUU #1//PBW343*2/KUKUNA/3/MUU	0.44	0.27	0.29
103	WBLL1*2/KURUKU/6/CNDO/R143//ENTE/MEXI_2/3/AEGILOPS SQUARROSA (TAUS)/4/WEAVER/5/2*JANZ/7/WBLL1*2/KURUKU	0.21	0.13	0.66
104	TUKURU//BAV92/RAYON*2/7/YAV_3/SCO//JO69/CRA/3/YAV79/4/AE.SQUARROSA (498)/5/LINE 1073/6/KAUZ*2/4/CAR//KAL/BB/3/NAC/5/KAUZ	0.05	0.25	0.70
105	NG8675/CBRD//FN/2*PASTOR/4/THELIN/3/2*BABAX/LR42//BABAX	0.83	0.01	0.16
106	BAV92//IRENA/KAUZ/3/HUITES/4/GONDO/TNMU/5/BAV92//IRENA/KAUZ/3/HUITES	0.48	0.28	0.24

Table 2-5 (cont'd)

107	CONI#1/2*HUIRIVIS #1	0.36	0.11	0.53
108	TECUE #1/2*WAXWING	0.03	0.01	0.96
109	KBIRD//WH 542/2*PASTOR/3/WBLL1*2/BRAMBLING	0.30	0.29	0.41
110	MUU/5/TRAP#1/BOW/3/VEE/PJN//2*TUI/4/BAV92/RAYON/6/MILAN/S87230//BAV92	0.30	0.44	0.25
111	KFA/3/PFAU/WEAVER//BRAMBLING/4/PFAU/WEAVER*2//BRAMBLING	0.94	0.04	0.03
112	ATTILA*2/PBW65//KRONSTAD F2004	0.21	0.03	0.77
113	WBLL1*2/TUKURU//KRONSTAD F2004	0.56	0.01	0.43
114	CHIL/CHUM18//GONDO	0.02	0.48	0.50
115	WBLL1*2/KUKUNA//KIRITATI/3/WBLL1*2/KUKUNA	0.04	0.28	0.68
116	NORM/WBLL1//WBLL1/3/TNMU/4/WBLL1*2/TUKURU	0.15	0.03	0.83
117	PBW343*2/KHVAKI*2//YANAC	0.01	0.02	0.97
118	FRANCOLIN #1/4/BABAX/LR42//BABAX*2/3/KURUKU	0.03	0.01	0.96
119	PANDORA//WBLL1*2/BRAMBLING	0.36	0.12	0.52
120	WBLL1*2/BRAMBLING//JUCHI	0.68	0.17	0.15
121	WBLL1*2/KKTS//KINGBIRD #1	0.12	0.05	0.83
122	TACUPETO F2001//WBLL1*2/KKTS/3/WBLL1*2/BRAMBLING	0.02	0.36	0.62
123	WBLL1/KUKUNA//TACUPETO F2001/3/KRONSTAD F2004/4/ROLF07	0.50	0.05	0.45
124	ATTILA*2/PBW65*2/5/REH/HARE//2*BCN/3/CROC_1/AE.SQUARROSA (213)//PGO/4/HUITES	0.30	0.05	0.65
125	HEILO/7/IVAN/6/SABUF/5/BCN/4/RABI//GS/CRA/3/AE.SQUARROSA (190)/8/VORB/FISCAL	0.06	0.92	0.03
126	KSW/SAUAL//SAUAL	0.83	0.10	0.06
127	KAUZ/PASTOR//PBW343/3/KRONSTAD F2004	0.67	0.05	0.27
128	REH/HARE//2*BCN/3/CROC_1/AE.SQUARROSA (213)//PGO/4/HUITES/5/KRONSTAD F2004	0.97	0.01	0.02
129	PRL/2*PASTOR//VORB	0.03	0.71	0.26
130	TRCH*2/3/WUH1/VEE#5//CBRD	0.36	0.29	0.35
131	KACHU #1/3/SHA3/SERI//SHA4/LIRA/4/KACHU	0.96	0.02	0.02
132	PBW343/PASTOR*2/6/TURACO/5/CHIR3/4/SIREN//ALTAR 84/AE.SQUARROSA (205)/3/3*BUC	0.49	0.42	0.09
133	WBLL1*2/BRAMBLING/4/BABAX/LR42//BABAX*2/3/KURUKU	0.17	0.29	0.53

Table 2-5 (cont'd)

134	FRANCOLIN #1/KIRITATI	0.36	0.11	0.54
135	BABAX/LR42//BABAX*2/3/KUKUNA/4/TAM200/PASTOR//TOBA97	0.38	0.44	0.18
136	MURGA/KRONSTAD F2004//QUAIU #3	0.30	0.52	0.18
137	KENYA NYANGUMI/3/2*KAUZ/PASTOR//PBW343	0.39	0.22	0.39
138	PARUS/PASTOR//INQALAB 91*2/KUKUNA	0.55	0.04	0.41
139	CROC_1/AE.SQUARROSA (205)//KAUZ/3/SASIA/4/TROST	0.02	0.66	0.33
140	CROC_1/AE.SQUARROSA (205)//KAUZ/3/SASIA/4/TROST	0.55	0.04	0.41
141	PFAU/MILAN//SOVA/3/PBW65/2*SERI.1B	0.69	0.03	0.28
142	PASTOR/KAUZ/6/CNDO/R143//ENTE/MEXI_2/3/AEGILOPS SQUARROSA (TAUS)/4/WEAVER/5/2*KAUZ	0.48	0.43	0.09
143	PASTOR/3/VORONA/CNO79//KAUZ/4/MILAN/OTUS//ATTILA/3*BCN	0.47	0.35	0.18
144	PASTOR/3/VORONA/CNO79//KAUZ/4/MILAN/OTUS//ATTILA/3*BCN	0.26	0.60	0.14
145	CHIBIA/WEAVER//KACHU	0.72	0.26	0.02
146	CHIBIA/WEAVER//KACHU	0.62	0.36	0.02
147	PRINIA/PASTOR//HUITES/3/MILAN/OTUS//ATTILA/3*BCN	0.68	0.25	0.07
148	C80.1/3*BATAVIA//2*WBLL1/3/TOBA97/PASTOR	0.64	0.04	0.32
149	WHEAR/3/PBW343/PASTOR//ATTILA/3*BCN	0.18	0.44	0.38
150	PBW343/HUITES/3/MILAN/OTUS//ATTILA/3*BCN	0.30	0.25	0.45
151	WBLL1*2/KURUKU//KRONSTAD F2004	0.43	0.15	0.43
152	MONARCA F2007/KRONSTAD F2004	0.23	0.05	0.73
153	PBW343*2/KUKUNA//PBW343*2/KUKUNA/3/PBW343	0.13	0.65	0.22
154	WHEAR/2*KRONSTAD F2004	0.70	0.02	0.28
155	C80.1/3*BATAVIA//2*WBLL1/3/2*KRONSTAD F2004	0.71	0.14	0.15
156	C80.1/3*BATAVIA//2*WBLL1/3/2*KRONSTAD F2004	0.63	0.03	0.34
157	SUMAI #3	0.38	0.31	0.30
158	GAMENYA	0.50	0.41	0.09
159	FALCIN/AE.SQUARROSA (312)/3/THB/CEP7780//SHA4/LIRA	0.32	0.32	0.36
160	GONDO/CBRD	0.29	0.49	0.22
161	HEILO	0.54	0.21	0.25
162	PICUS/3/KAUZ*2/BOW//KAUZ/4/KKTS/5/HEILO	0.25	0.41	0.34
163	HUIRIVIS #1/GONDO	0.17	0.43	0.39

Table 2-5 (cont'd)

164	HUIRIVIS #1/GONDO	0.12	0.67	0.21
165	KAUZ/PASTOR//PBW343/3/HEILO	0.17	0.45	0.39
166	FRET2/WBLL1//TACUPETO F2001/3/HEILO	0.27	0.71	0.03
167	WBLL1*2/4/YACO/PBW65/3/KAUZ*2/TRAP//KAUZ/5/GONDO	0.03	0.64	0.33
168	WBLL1*2/CHAPIO//HEILO	0.28	0.52	0.20
169	WBLL1*2/KURUKU//HEILO	0.22	0.73	0.05
170	WBLL1*2/KURUKU//HEILO	0.23	0.47	0.30
171	WBLL1*2/VIVITSI//GONDO	0.02	0.62	0.36
172	ATTILA/2*PASTOR//FN/2*PASTOR	0.03	0.81	0.16
173	KACHU #1/4/CROC_1/AE.SQUARROSA (205)//KAUZ/3/SASIA/5/KACHU	0.95	0.01	0.03
174	KACHU #1/4/CROC_1/AE.SQUARROSA (205)//KAUZ/3/SASIA/5/KACHU	0.98	0.01	0.01
175	SAUAL*2/6/CNDO/R143//ENTE/MEXI_2/3/AEGILOPS SQUARROSA (TAUS)/4/WEAVER/5/2*PASTOR	0.90	0.05	0.05
176	BAV92//IRENA/KAUZ/3/HUITES*2/4/GONDO/TNMU	0.50	0.46	0.03
177	BAV92//IRENA/KAUZ/3/HUITES*2/4/GONDO/TNMU	0.56	0.42	0.02
178	FRET2*2/KUKUNA*2//SHA4/CHIL	0.05	0.16	0.79
179	WBLL1*2/KURUKU*2//TNMU	0.16	0.37	0.48
180	WBLL1*2/TUKURU//WUH1/BOW/3/WBLL1*2/TUKURU	0.23	0.05	0.72
181	WBLL1/FRET2//PASTOR*2/3/GONDO	0.02	0.96	0.03
182	PFAU/WEAVER*2//BRAMBLING/7/IVAN/6/SABUF/5/BCN/4/RABI//GS/CRA/3/AE.SQUARROSA (190)/8/PFAU/WEAVER//BRAMBLING	0.45	0.27	0.28
183	TRCH*2/TNMU	0.30	0.39	0.31
184	KACHU*2//CHIL/CHUM18	0.90	0.08	0.02
185	KACHU*2//CHIL/CHUM18	0.61	0.14	0.25
186	SAUAL #1/TNMU//SAUAL	0.68	0.30	0.02
187	PRINIA/PASTOR//CHIL/CHUM18/3/PRINIA/PASTOR	0.06	0.70	0.24
188	PBW343*2/KHVAKI*2//CHIL/CHUM18	0.31	0.18	0.51
189	PBW343/PASTOR*2/6/TURACO/5/CHIR3/4/SIREN//ALTAR 84/AE.SQUARROSA (205)/3/3*BUC	0.19	0.39	0.42
190	PBW343/PASTOR*2/3/WUH1/VEE#5//CBRD	0.09	0.32	0.60
191	PBW343/PASTOR*2/3/WUH1/VEE#5//CBRD	0.14	0.32	0.54

Table 2-5 (cont'd)

192	PBW343/PASTOR*2/3/WUH1/VEE#5//CBRD	0.13	0.36	0.51
193	NG8675/CBRD/7/IVAN/6/SABUF/5/BCN/4/RABI//GS/CRA/3/AE.SQUARROSA (190)/8/WBLL1*2/CHAPIO	0.40	0.38	0.22
194	NG8675/CBRD/7/IVAN/6/SABUF/5/BCN/4/RABI//GS/CRA/3/AE.SQUARROSA (190)/8/WBLL1*2/CHAPIO	0.65	0.23	0.12
195	SHA3/CBRD//TNMU/3/KACHU	0.61	0.32	0.08
196	FN/2*PASTOR//GONDO/TNMU/3/FRANCOLIN #1	0.04	0.37	0.60
197	HEILO//GONDO/TNMU/3/WBLL1*2/BRAMBLING	0.10	0.60	0.31
198	CBRD/FILIN	0.10	0.62	0.28
199	CBRD/FILIN	0.28	0.67	0.05
200	CHIL/CHUM18//GONDO	0.02	0.87	0.11
201	SAUAL/4/CROC_1/AE.SQUARROSA (205)//KAUZ/3/ATTILA/5/SAUAL	0.94	0.03	0.02
202	WAXWING/KIRITATI*2/3/SHA3/SERI//SHA4/LIRA	0.02	0.08	0.90
203	CNO79//PF70354/MUS/3/PASTOR/4/BAV92*2/5/SHA3/SERI//SHA4/LIRA	0.04	0.33	0.63
204	FRET2/TUKURU//FRET2/3/WUH1/VEE#5//CBRD/4/FRET2/TUKURU//FRET2	0.19	0.08	0.73
205	WBLL1/FRET2//PASTOR/3/SHA3/SERI//SHA4/LIRA/4/WBLL1/TACUPETO F2001//PASTOR	0.03	0.85	0.12
206	PFAU/WEAVER*2//BRAMBLING/7/IVAN/6/SABUF/5/BCN/4/RABI//GS/CRA/3/AE.SQUARROSA (190)/8/PFAU/WEAVER//BRAMBLING	0.58	0.38	0.03
207	PFAU/WEAVER//BRAMBLING*2/3/SHA3/SERI//SHA4/LIRA	0.27	0.41	0.33
208	KACHU #1/3/SHA3/SERI//SHA4/LIRA/4/KACHU	0.96	0.02	0.02
209	PRINIA/PASTOR//CHIL/CHUM18/3/PRINIA/PASTOR	0.09	0.75	0.16
210	KETUPA*2/PASTOR/6/TURACO/5/CHIR3/4/SIREN//ALTAR 84/AE.SQUARROSA (205)/3/3*BUC/7/KACHU	0.68	0.16	0.16
211	CHIL/CHUM18//FN/2*PASTOR/3/PRL/2*PASTOR	0.04	0.77	0.19
212	CHIL/CHUM18//GONDO/3/WBLL1*2/KURUKU	0.26	0.11	0.64
213	SHA3/CBRD//TNMU/3/KACHU	0.63	0.33	0.04
214	FN/2*PASTOR//GONDO/TNMU/3/FRANCOLIN #1	0.40	0.37	0.23
215	NG8675/CBRD//FN/2*PASTOR/4/THELIN/3/2*BABAX/LR42//BABAX	0.02	0.59	0.39
216	HEILO/7/IVAN/6/SABUF/5/BCN/4/RABI//GS/CRA/3/AE.SQUARROSA (190)/8/VORB/FISCAL	0.06	0.91	0.03

Table 2-5 (cont'd)

217	BAV92//IRENA/KAUZ/3/HUITES/4/DOLL	0.85	0.11	0.04
218	TRCH/SRTU//KACHU	0.62	0.02	0.36
219	PRL/2*PASTOR//SRTU/3/PRINIA/PASTOR	0.02	0.69	0.29
220	WAXWING*2/3/PASTOR//HXL7573/2*BAU	0.02	0.07	0.92
221	ATTILA*2/PBW65*2//TNMU	0.09	0.07	0.84
222	SERI.1B//KAUZ/HEVO/3/AMAD*2/4/KIRITATI	0.83	0.08	0.09
223	WBLL1*2/4/SNI/TRAP#1/3/KAUZ*2/TRAP//KAUZ/5/KACHU	0.37	0.03	0.60
224	WBLL1*2/4/YACO/PBW65/3/KAUZ*2/TRAP//KAUZ/5/KACHU #1	0.91	0.04	0.05
225	FRET2*2/4/SNI/TRAP#1/3/KAUZ*2/TRAP//KAUZ/5/KACHU	0.91	0.02	0.07
226	FRET2*2/KUKUNA//PRINIA/PASTOR	0.03	0.84	0.13
227	FRET2/KIRITATI/5/NAC/TH.AC//3*PVN/3/MIRLO/BUC/4/2*PASTOR	0.15	0.76	0.09
228	FRET2/KIRITATI/5/NAC/TH.AC//3*PVN/3/MIRLO/BUC/4/2*PASTOR	0.16	0.73	0.11
229	KAUZ//ALTAR 84/AOS/3/MILAN/KAUZ/4/SAUAL	0.92	0.02	0.05
230	KAUZ//ALTAR 84/AOS/3/MILAN/KAUZ/4/OTUS/TOBA97	0.90	0.07	0.03
231	SAUAL/3/KAUZ/PASTOR//PBW343	0.61	0.22	0.17
232	NG8675/CBRD//MILAN/3/SAUAL/6/CNDO/R143//ENTE/MEXI_2/3/AEGILOPS SQUARROSA (TAUS)/4/WEAVER/5/2*PASTOR	0.03	0.94	0.03
233	ATTILA/3*BCN//BAV92/3/TILHI/4/SHA7/VEE#5//ARIV92	0.47	0.04	0.49
234	ATTILA/3*BCN//BAV92/3/TILHI/4/SHA7/VEE#5//ARIV92	0.47	0.03	0.50
235	BABAX/KS93U76//BABAX/3/ATTILA/3*BCN//TOBA97/4/WBLL1*2/KURUKU	0.57	0.05	0.38
236	ATTILA*2/PBW65//KRONSTAD F2004	0.46	0.02	0.53
237	KANZ*4/KS85-8-4//2*WBLL1*2/KURUKU	0.13	0.37	0.51
238	FRET2/KUKUNA//FRET2/3/PASTOR//HXL7573/2*BAU/5/FRET2*2/4/SNI/TRAP#1/3/KAUZ*2/TRAP//KAUZ	0.16	0.40	0.44
239	PRL/2*PASTOR//PARUS/5/NAC/TH.AC//3*PVN/3/MIRLO/BUC/4/2*PASTOR	0.01	0.95	0.04
240	WAXWING*2/JUCHI	0.01	0.01	0.98
241	FUNDACEP 30	0.45	0.41	0.14
242	SHANGHAI #8	0.35	0.32	0.33
243	VOROBEY	0.04	0.88	0.08
244	CPI8/GEDIZ/3/GOO//ALB/CRA/4/AE.SQUARROSA (208)/5/HAHN/2*WEAVER/6/SKAUZ/BAV92	0.05	0.90	0.05

Table 2-5 (cont'd)

245	CPI8/GEDIZ/3/GOO//ALB/CRA/4/AE.SQUARROSA (208)/5/HAHN/2*WEAVER/6/SKAUZ/BAV92	0.02	0.95	0.03
246	CPI8/GEDIZ/3/GOO//ALB/CRA/4/AE.SQUARROSA (208)/5/HAHN/2*WEAVER/6/SKAUZ/BAV92	0.02	0.96	0.02
247	NING MAI 96035/FINSI//HEILO	0.27	0.47	0.26
248	NING MAI 96035/FINSI//HEILO	0.31	0.39	0.29
249	ATTILA/HEILO	0.04	0.01	0.94
250	ATTILA/HEILO	0.05	0.02	0.93
251	WAXWING//PFAU/WEAVER	0.27	0.02	0.71
252	BABAX/LR42//BABAX*2/3/KURUKU	0.05	0.14	0.81
253	ND643//2*PRL/2*PASTOR	0.08	0.83	0.10
254	VOROBEY	0.06	0.85	0.09
255	BABAX/LR42//BABAX/3/ER2000	0.11	0.03	0.87
256	OASIS//TC14/2*SPER/3/ATTILA/4/WBLL4	0.04	0.05	0.91
257	FILIN/3/CROC_1/AE.SQUARROSA (205)//KAUZ/4/FILIN/5/VEE/MJI//2*TUI/3/PASTOR	0.06	0.71	0.23
258	T.DICOCCON PI94625/AE.SQUARROSA (372)//3*PASTOR	0.01	0.98	0.01
259	PASTOR/4/WEAVER/TSC//WEAVER/3/WEAVER/5/URES/PRL//BAV92	0.02	0.97	0.02
260	SW94.2690/SUNCO	0.04	0.94	0.03
261	SW94.2690/SUNCO	0.03	0.95	0.02
262	VEE/MJI//2*TUI/3/PASTOR/4/BERKUT	0.07	0.14	0.79
263	BERKUT/3/ATTILA*2//CHIL/BUC	0.10	0.74	0.17
264	TAN//TEMPORALERA M 87/AGR/3/FRET2/4/URES/PRL//BAV92	0.23	0.69	0.08
265	A93324S.7197.29/4/KAUZ//ALTAR 84/AOS/3/KAUZ/5/PASTOR	0.02	0.90	0.09
266	OASIS//TC14/2*SPER/3/ATTILA/10/ATTILA*2/9/KT/BAGE//FN/U/3/BZA/4/TRM/5/ALDAN/6/SERI/7/VEE#10/8/OPATA	0.03	0.45	0.52
267	MEX94.27.1.20/3/SOKOLL//ATTILA/3*BCN	0.05	0.84	0.10
268	KS82W418/SPN//WBLL1/3/BERKUT	0.07	0.88	0.04
269	CNDO/R143//ENTE/MEXI75/3/AE.SQ/4/2*FCT/5/KAUZ*2/YACO//KAUZ/6/BERKUT	0.36	0.59	0.04
270	SOKOLL/EXCALIBUR	0.03	0.95	0.02
271	PASTOR/SLVS//FRAME	0.04	0.94	0.02
272	PASTOR/SLVS//FRAME	0.06	0.92	0.02

Table 2-5 (cont'd)

273	BAXTER*2/4/CHEN/AEGILOPS SQUARROSA (TAUS)//BCN/3/BAV92	0.18	0.80	0.02
274	BERKUT/3/ALTAR 84/AE.SQUARROSA (219)//SERI	0.03	0.94	0.04
275	MILAN/DUCULA//SUNCO/2*PASTOR	0.31	0.59	0.10
276	SW89-5124*2/FASAN//PARUS/PASTOR	0.04	0.93	0.03
277	SOKOLL//SUNCO/2*PASTOR	0.05	0.92	0.03
278	CROC_1/AE.SQUARROSA (224)//OPATA/3/ALTAR 84/AE.SQ//2*OPATA	0.09	0.75	0.17
279	SUNSTATE/SD 3195//SOKOLL	0.13	0.83	0.05
280	SOKOLL*2/GLE	0.03	0.94	0.03
281	TEMPORALERA M 87/ROMO96/3/ATTILA/BAV92//PASTOR/4/PRL/2*PASTOR	0.02	0.96	0.02
282	FINSI/3/ATTILA/BAV92//PASTOR/4/PBW343*2/KUKUNA	0.11	0.43	0.46
283	CO99W329/2*BERKUT	0.30	0.44	0.26
284	PSN/BOW//MILAN/3/2*BERKUT	0.02	0.96	0.02
285	CROC_1/AE.SQUARROSA (224)//OPATA/3/RAC655/4/SLVS/PASTOR	0.32	0.60	0.08
286	SLVS/PASTOR/3/PASTOR//MUNIA/ALTAR 84	0.02	0.97	0.02
287	YAV79//DACK/RABI/3/SNIPE/4/AE.SQUARROSA (460)/5/2*EXCALIBUR/6/VEE/LIRA//BOW/3/BCN/4/KAUZ	0.17	0.78	0.05
288	CNDO/R143//ENTE/MEXI_2/3/AEGILOPS SQUARROSA (TAUS)/4/WEAVER/5/2*JANZ/6/D67.2/PARANA 66.270//AE.SQUARROSA (320)/3/CUNNINGHAM	0.26	0.29	0.46
289	CROC_1/AE.SQUARROSA (205)//BORL95/3/KENNEDY/6/CNDO/R143//ENTE/MEXI_2/3/AEGILOPS SQUARROSA (TAUS)/4/WEAVER/5/2*JANZ	0.34	0.27	0.39
290	D67.2/PARANA 66.270//AE.SQUARROSA (320)/3/CUNNINGHAM/4/PASTOR/SLVS	0.14	0.52	0.34
291	CALINGIRI/SOKOLL	0.03	0.53	0.44
292	SOKOLL//SLVS/PASTOR/3/ATTILA*2//CHIL/BUC	0.03	0.25	0.72
293	BERKUT/HTG	0.39	0.51	0.11
294	SOKOLL/FRAME	0.50	0.21	0.29
295	SOKOLL/SLVS	0.26	0.68	0.06
296	ASTREB*2/NING MAI 9558	0.16	0.76	0.09
297	ASTREB*2/3/WUH1/VEE#5//CBRD	0.03	0.89	0.08

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CHAPTER 3

ASSOCIATION MAPPING FOR DETECTING QTLs FOR YELLOW RUST IN BREAD WHEAT

Abstract

Yellow rust (*Puccinia striiformis*) is one of the most aggressive diseases of bread wheat (*Triticum aestivum* L.), which drastically can reduce the yield. Currently, yellow rust is a global concern, since the pathogen is now present in areas where it was not previously reported before. Adult plant resistance genes (APR) are considered the better approach to generate new wheat varieties with high levels of non-race specific resistance. In the current study, a wheat association mapping panel (AMP) with 297 spring wheat accessions developed by CIMMYT was evaluated in Mexico and Ecuador during two years to identify markers linked to regions in the wheat genome responsible for yellow rust resistance. SNP markers significantly associated with the resistance to *P. striiformis* were detected on chromosomes 1A, 2A, 5A, 6A, 7A, 2B, 5B, 6B, 7B, and 3D using the GLM method; whereas, the association analysis detected SNP markers significantly associated with the trait on chromosomes 1A and 2A using the MLM method.

Introduction

Yellow rust or stripe rust, caused by *Puccinia striiformis*, is considered one the most severe diseases of wheat (Roelfs et al., 1992) and also one of most frequent diseases to occur along with stem and leaf rust (McIntosh et al., 1995). Yield losses arise due to leaf tissue damaged by the infection, reduced number and size of flowering spikes,

shriveled grain, and damaged tillers, especially when the infection occurs in early growth stages (Wellings, 2010). It is possible to have yield losses over 70% when susceptible cultivars are planted and the weather favors pathogen development (Sharma-Poudyal and Chen, 2010). In the past, yellow rust was considered a disease common only in areas where cool and moist weather conditions prevail (Stubbs, 1988). Severe epidemics are now often reported in warmer areas, where yellow rust was absent before or not considered important (Hovmøller et al., 2010).

Complete resistance to the pathogen conferred by major resistance genes, which are race specific, have has been extensively used by wheat breeders in the past (Zadoks, 1961); however, it has been demonstrated that this mechanism of resistance is commonly overcome by the pathogen (Johnson, 1992). Some cases of these failures have been reported in the literature. For example, *Yr6* was released in the UK cultivar Rothwell Perdix in 1964, but isolates virulent to this cultivar were detected only two years later (Boyd, 2005). *Yr17* was introduced into northern European wheat cultivars in the mid-70s and after 20 years of extensive use of this gene in new wheat cultivars, the gene was no longer effective in some countries of this region (Bayles et al., 2000).

Partial resistance conferred by genes with minor effects in the control of yellow rust is currently the most popular mechanism of resistance employed in wheat breeding since it has been more durable over time (Morgounov et al., 2012; Qayoum and Line, 1985). Partial resistance is also non-race specific (Singh et al., 2004), and genes involved in the disease resistance possess additive effects, therefore these genes can be pyramided to provide high levels of resistance near immunity. Singh et al. (2011) reported that CIMMYT lines with combinations of 4 – 5 minor, slow rusting genes were

able to acquire high levels of resistance near-immunity to yellow rust (1 – 5% of disease severity) in environments which favors the development of the pathogen located in hotspots in Ecuador, Mexico and Kenya.

Many major and minor resistance genes for yellow rust resistance have been identified. From those, more than 50 genes have been catalogued and some more potential novel genes remains temporally catalogued (Boyd, 2005; McIntosh et al., 2012). Breeders have been taking advantage of QTL analysis studies to discover, map, and quantify the effects of these genes in plant germplasm with the purpose of using this knowledge to develop new improved varieties in more efficient ways. In wheat, many yellow rust resistance genes have been identified, but many still remain undiscovered. Additionally, it is important to validate already reported new QTL effects in different genetic backgrounds. Association mapping is a technique to map QTLs using existing populations. Using this approach in wheat elite, exotic, or landraces germplasm will allow the discovery of novel alleles and quantify these effects in different genetic backgrounds at the same time. Moreover, association mapping uses large numbers of molecular markers distributed in plants genome, therefore, new SNP markers closely linked to resistance genes are likely to be discovered and contribute to wheat breeding efforts.

The current research aims to evaluate the resistance against *Puccinia striiformis* in the wheat AMP and detect QTLs for yellow rust resistance using association mapping approach in this collection of germplasm.

Materials and methods

Plant material

A group of 297 spring wheat accessions was assembled to conduct the current study (Table 2-1, Chapter II). This collection of accessions will be referred to as the association mapping panel (AMP). The AMP was obtained from the International Center for Maize and Wheat Improvement (CIMMYT) located in Mexico. The wheat AMP contains breeding lines, cultivars, and landraces from different origins as well as control wheat lines used for yellow rust (YR) studies. The panel was selected because most of the wheat lines in the panel have shown variability for YR response observed in previous evaluations at CIMMYT. The AMP represents a considerable number of the resistant alleles employed by CIMMYT's to develop improved wheat lines.

Locations

The field research was conducted in Toluca - Mexico and Santa Catalina – Ecuador during 2011 and 2012. Phenotypic and genotypic data analyses were conducted at MSU as well as CIMMYT (Table 3-1).

Table 3-1. Locations and years of the wheat association mapping study on Yellow Rust.

Location	Years	Altitude (masl)	Type of study
East Lansing-MSU-USA	2011	262	Genotyping
Santa Catalina-INIAP-Ecuador	2011 - 2012	3,050	Field evaluation
Toluca-CIMMYT-Mexico	2011 - 2012	2,640	Field evaluation

Field management, inoculation, and phenotyping

The wheat AMP nurseries for YR studies were arranged in an alpha lattice design. Each plot was 1.0 m long with two rows separated by 0.25 m. Two replications of the wheat AMP were planted in Ecuador in 2011 and 2012 while one replication was sown in Mexico during 2011 and 2012.

For YR evaluations in Ecuador, the AMP was surrounded by a mixture of the susceptible cultivars 'Morocco', 'Tungurahua' and 'Cotopaxi'. The susceptible cultivars planted around the experiments were selected because these lines reach the highest level of susceptibility at different periods of time so a continuous supply of inoculum is produced. In 2011, the wheat AMP and the susceptible cultivars were inoculated three times every five days staring 45 DAP. In 2012, only the susceptible cultivars were inoculated three times every five days starting 45 DAP. The inoculum concentration was 80,000 spores per mL. The wheat plants were inoculated using a Micron Ulva-8 sprayer (Distributed by Micron Sprayers, Bromyard, UK.).

In the YR field experiments in Mexico 2011 and 2012, the AMP was surrounded by a mixture of six susceptible wheat cultivars derived from the cross 'Avocet /Attila' known to carry the *Yr27* stripe rust resistance gene. The cultivars were inoculated with the *P. striiformis* isolates Mex96.11 and Mex08, which are virulent to genes *Yr27* and *Yr31.*

The variables recorded from the field studies were:

Yellow Rust severity (%).- Percentage of surface area of the plant showing yellow rust infection according to the modified Cobb's scale recorded as: 0, 1, 5, 10, 20, 30, 40, 50, 60, 70, 80, 90 or 100% (Roelfs et al., 1992) (See Appendix B for visual representation).

Yellow Rust reaction.- Also known as the field response, recorded by using the codes listed in Table 3-2 (CIMMYT, 1986) (See also Appendix C for visual representation). Yield data was collected to keep record of the seed produced in the experiments, however, yield was not part of any statistical analysis since plots size were too small for yield evaluation.

Table 3-2. Codes for recording wheat reaction to Yellow Rust infection as used by CIMMYT (1986).

Reaction	Code	Description
No symptoms	0	No visible infection on plants.
Resistant	R	Visible chlorosis or necrosis without presence of uredia.
Moderately Resistant	MR	Small uredia are present and surrounded by chlorotic or necrotic areas.
Intermediate	M	Variable sized uredia are present, some with chlorosis, necrosis, or both
Moderately Susceptible	MS	Medium sized uredia are present and possibly surrounded by chlorotic areas
Susceptible	S	*Large uredia* are present, generally with little or no chlorosis and no necrosis

A Seedling test to confirm adult plant resistance (APR) was conducted in the greenhouse at INIAP-Ecuador with two isolates of *P. striiformis* collected from Santa Catalina Research Station on 2013. Plastic trays (60 x 40 x 10 cm) were used to plant the wheat AMP. Each tray was divided in 15 cells of 20 x 8 cm where 15 seeds were planted from each accession. Twenty days after planting, the wheat AMP was inoculated with each isolate separatedly. The test was replicated two times with each isolate. After 10 – 15 days, seedlings were evaluated and the infection type was recorded following the protocol described by Roelfs et al. (1992). Seedling with scores 0 – 3 were considered resistant reactions and scores from 4 – 9 were considered susceptible reaction according to Uauy et al. (2005).

Genotyping

A total of 1,666 SNP markers (selected by MAF > 5%) generated from the screening of 297 accessions from the wheat AMP with the 9K SNP chip and 32 microsatellites markers (SSR) were employed to conduct the association analysis (See chapter II).

Statistical analysis

The data were tested for normality using the Shapiro-Wilk normality test (Shapiro and Wilk, 1965) with R (Ihaka and Gentleman, 1996) version 2.15.3. Data sets that were not normal were transformed with square root function. Analysis of variance (ANOVA) for every trait was conducted in R with packages Agricolae version 1.1-4 and PBIB.test using REML (de Mendiburu, 2013).

The number of subpopulations in the wheat AMP was estimated with the software STRUCTURE v.2.3.4 (http://pritchardlab.stanford.edu/structure.html). Default setting of admixture model for the ancestry of individuals and correlated allele frequencies were used. Population structure was modelled with a burning of 10,000 cycles followed by 100,000 Markov Chain Monte Carlo (MCMC) repeats for assumed subpopulation number, k= 1,…10 according to Pritchard et al. (2010). The optimum k value was determined with Evanno method described in Chapter II (Evanno et al., 2005). Principal component analysis (PCA) was used to validate the number of subpopulations estimated by STRUCTURE. The software used to perform the PCA was EIGENSTRAT. The Principal Component Analysis was conducted with 3,701 SNP markers distributed in the wheat AMP genome with MAF > 5%.

Association analyses between markers and traits were conducted with TASSEL v 4.0 (http://www.maizegenetics.net/) using the general linear model (GLM) and the mixed linear model (MLM). The GLM includes population structure as covariable, whereas the MLM method includes, in addition to the population structure, a matrix of relatedness (Kinship matrix), which was estimated with TASSEL. The Kinship Matrix and the association analysis were performed with a set of 3,701 SNP markers, which were selected from the original set of 9 K SNP markers included in the SNP chip. The selection criteria to select these SNP markers were less than 10% of calls and MAF > 5%. Once the association analyses were done and p-values were obtained with both methods (GLM and MLM), significant markers linked to the traits were selected using false discovery rate (FDR) method, which controls the rate of false positives when testing several hypotheses simultaneously (Storey, 2002). FDR analysis was conducted with R using Q-value package version 1.0 (Dabney et al., 2004).

Results

The field evaluations of the wheat association mapping panel composed of 297 wheat accessions were conducted in Mexico and Ecuador for two years (2011 and 2012). Agronomic data and disease response to Yellow Rust were collected form the two locations as shown in Table 3-1. Yellow rust response (percentage of severity) from Mexico and Ecuador was used to conduct statistical analysis. The two agronomic variables analyzed from the experiments were flowering (Days after planting- DAP) and plant height (cm). The analyses of variance of these traits detected significant differences among locations, so the traits were analyzed independently as follows:

Germplasm evaluation

In the two years of evaluations in Ecuador and Mexico, more than 50% of the accessions from the wheat AMP showed high levels of resistance (0 – 5% of disease severity) (Figures 3-6 and 3-7). The resistance of most of the cultivars was conferred by APR genes since less than 25% of the accessions showed hypersensitivity to *P. striiformis* infection in the field evaluations in Mexico and Ecuador. Additionally, the seedling test conducted in Ecuador with two *P. striiformis* isolates showed that only 19% of the 297 wheat accessions showed resistance reaction according to Uauy et al. (2005) protocol.

The wheat accession 'KAUZ' was one of the common parents observed in the wheat AMP pedigrees. It was present in 58 accessions. Most of these wheat accessions were susceptible at seedling stage and resistant as adult plants in Mexico and Ecuador. Another wheat accession which is common in the pedigrees of the wheat accessions in the AMP with adult plant resistance in the two locations was 'ATTILA'. This wheat accession was present in 32 wheat accessions in the wheat AMP according their pedigrees. Most of the lines with 'ATTILA' in its pedigree showed high levels of adult plant resistance. Finally, another wheat line present 17 times in the pedigrees of the accessions was 'HEILO'. The lines with 'HEILO' in the pedigrees also showed susceptibility as seedlings and resistance in the field.

Another wheat line present in the pedigree of the accessions of the wheat AMP was 'QUAIU'. This line was present in the pedigree of five lines and all of them but one (MURGA/KRONSTADF2004//QUAIU) showed high levels of yellow rust resistance in the field.

Table 3-3. Yellow rust severity registered in the wheat AMP in Ecuador and Mexico. 2011-2012.

Acc. No.	Pedigree	Ecu. 2011	Ecu. 2012	Mex. 2011	Mex. 2012
		% of disease severity			
7	BAV92//IRENA/KAUZ/3/HUITES*2/4/MURGA	0	0	0	0
65	KACHU #1/4/CROC_1/AE.SQUARROSA (205)//KAUZ/3/SASIA/5/KACHU	0	0	0	0
86	BECARD/KACHU	0	0	0	0
88	FRNCLN/TECUE #1	0	0	0	0
90	QUAIU/TECUE #1	0	0	0	0
92	KINGBIRD #1/KACHU	0	0	0	0
116	NORM/WBLL1//WBLL1/3/TNMU/4/WBLL1*2/TUKURU	0	0	0	0
162	PICUS/3/KAUZ*2/BOW//KAUZ/4/KKTS/5/HEILO	0	0	0	0
190	PBW343/PASTOR*2/3/WUH1/VEE#5//CBRD	0	0	0	0
223	WBLL1*2/4/SNI/TRAP#1/3/KAUZ*2/TRAP//KAUZ/5/KACHU	0	0	0	0
226	FRET2*2/KUKUNA//PRINIA/PASTOR	0	0	0	0
9	KACHU/5/REH/HARE//2*BCN/3/CROC_1/AE.SQUARROSA (213)//PGO/4/HUITES/6/KACHU	0	0	0	1
17	TRCH/HUIRIVIS #1	0	0	0	1
27	KFA/2*KACHU	0	0	0	1
35	MURGA//WAXWING/KIRITATI	0	0	0	1
36	MURGA/KRONSTAD F2004	0	0	0	1
43	ROLF07*2/4/CROC_1/AE.SQUARROSA (205)//BORL95/3/2*MILAN	0	0	0	1
44	WBLL1*2/KUKUNA/5/PSN/BOW//SERI/3/MILAN/4/ATTILA/6/WBLL1*2/KKTS	0	0	1	0
59	ATTILA*2/PBW65*2//MURGA	0	0	0	1
61	KACHU #1/4/CROC_1/AE.SQUARROSA (205)//BORL95/3/2*MILAN/5/KACHU	0	0	0	1
74	KACHU*2//CHIL/CHUM18	0	0	0	1
76	SAUAL/3/ACHTAR*3//KANZ/KS85-8-4/4/SAUAL	0	0	1	0
89	TRCH/HUIRIVIS #1	0	0	0	1
105	NG8675/CBRD//FN/2*PASTOR/4/THELIN/3/2*BABAX/LR42//BABAX	0	0	0	1

Table 3-3 (cont'd)

125	HEILO/7/IVAN/6/SABUF/5/BCN/4/RABI//GS/CRA/3/AE.SQUARROSA (190)/8/VORB/FISCAL	0	0	0	1
130	TRCH*2/3/WUH1/VEE#5//CBRD	0	0	0	1
132	PBW343/PASTOR*2/6/TURACO/5/CHIR3/4/SIREN//ALTAR 84/AE.SQUARROSA (205)/3/3*BUC	0	0	0	1
173	KACHU #1/4/CROC_1/AE.SQUARROSA (205)//KAUZ/3/SASIA/5/KACHU	0	0	0	1
185	KACHU*2//CHIL/CHUM18	0	0	0	1
191	PBW343/PASTOR*2/3/WUH1/VEE#5//CBRD	0	0	0	1
209	PRINIA/PASTOR//CHIL/CHUM18/3/PRINIA/PASTOR	0	0	0	1
229	KAUZ//ALTAR 84/AOS/3/MILAN/KAUZ/4/SAUAL	0	0	0	1
282	FINSI/3/ATTILA/BAV92//PASTOR/4/PBW343*2/KUKUNA	0	0	0	1
15	PFAU/SERI.1B//AMAD/3/WAXWING/4/BABAX/LR42//BABAX*2/3/KU	0	0	1	1
32	FRET2*2/4/SNI/TRAP#1/3/KAUZ*2/TRAP//KAUZ*2/5/BOW/URES//2*WEAVER/3/CROC_1/AE.SQUARROSA (213)//PGO	0	0	1	1
54	INQALAB 91*2/KUKUNA*2//PVN	0	0	1	1
147	PRINIA/PASTOR//HUITES/3/MILAN/OTUS//ATTILA/3*BCN	0	0	1	1
208	KACHU #1/3/SHA3/SERI//SHA4/LIRA/4/KACHU	0	0	1	1
5	ROLF07*2/KACHU #1	2.5	0	0	0
62	SAUAL/4/CROC_1/AE.SQUARROSA (205)//BORL95/3/2*MILAN/5/SAUAL	2.5	0	0	0
67	ATTILA*2/PBW65*2/4/BOW/NKT//CBRD/3/CBRD	2.5	0	0	0
131	KACHU #1/3/SHA3/SERI//SHA4/LIRA/4/KACHU	2.5	0	0	0
169	WBLL1*2/KURUKU//HEILO	2.5	0	0	0
192	PBW343/PASTOR*2/3/WUH1/VEE#5//CBRD	2.5	0	0	0
249	ATTILA/HEILO	2.5	0	0	0
3	PBW343*2/KUKUNA*2//FRTL/PIFED	2.5	0	0	1
16	BECARD/KACHU	2.5	0	0	1
21	NAC/TH.AC//3*PVN/3/MIRLO/BUC/4/2*PASTOR/5/KACHU/6/KACHU	0	2.5	0	1

Table 3-3 (cont'd)

34	ALTAR 84/AE.SQUARROSA (221)//3*BORL95/3/URES/JUN//KAUZ/4/WBLL1/5/REH/HARE//2*BCN/3/CROC_1/AE.SQUARROSA (213)//PGO/4/HUITES	2.5	0	0	1
38	BAV92//IRENA/KAUZ/3/HUITES/6/ALD/CEP75630//CEP75234/PT7219/3/BUC/BJY/4/CBRD/5/TNMU/PF85487	2.5	0	0	1
60	WBLL1/FRET2//PASTOR*2/3/MURGA	0	2.5	1	0
97	QUAIU #3//MILAN/AMSEL	0	2.5	0	1
107	CONI#1/2*HUIRIVIS #1	2.5	0	0	1
164	HUIRIVIS #1/GONDO	2.5	0	0	1
170	WBLL1*2/KURUKU//HEILO	2.5	0	1	0
184	KACHU*2//CHIL/CHUM18	2.5	0	0	1
194	NG8675/CBRD/7/IVAN/6/SABUF/5/BCN/4/RABI//GS/CRA/3/AE.SQUARROSA (190)/8/WBLL1*2/CHAPIO	2.5	0	0	1
216	HEILO/7/IVAN/6/SABUF/5/BCN/4/RABI//GS/CRA/3/AE.SQUARROSA (190)/8/VORB/FISCAL	0	2.5	0	1
222	SERI.1B//KAUZ/HEVO/3/AMAD*2/4/KIRITATI	2.5	0	0	1
233	ATTILA/3*BCN//BAV92/3/TILHI/4/SHA7/VEE#5//ARIV92	2.5	0	0	1
235	BABAX/KS93U76//BABAX/3/ATTILA/3*BCN//TOBA97/4/WBLL1*2/KURUKU	2.5	0	1	0
247	NING MAI 96035/FINSI//HEILO	2.5	0	0	1
255	BABAX/LR42//BABAX/3/ER2000	2.5	0	1	0
291	CALINGIRI/SOKOLL	2.5	0	0	1
58	WBLL1*2/5/CNO79//PF70354/MUS/3/PASTOR/4/BAV92	0	2.5	1	1
174	KACHU #1/4/CROC_1/AE.SQUARROSA (205)//KAUZ/3/SASIA/5/KACHU	0	2.5	1	1
182	PFAU/WEAVER*2//BRAMBLING/7/IVAN/6/SABUF/5/BCN/4/RABI//GS/CRA/3/AE.SQUARROSA (190)/8/PFAU/WEAVER//BRAMBLING	2.5	0	1	1
199	CBRD/FILIN	2.5	0	1	1
225	FRET2*2/4/SNI/TRAP#1/3/KAUZ*2/TRAP//KAUZ/5/KACHU	2.5	0	1	1
286	SLVS/PASTOR/3/PASTOR//MUNIA/ALTAR 84	2.5	0	1	1

Table 3-3 (cont'd)

95	PBW343*2/KUKUNA//TECUE #1	5	0	0	0
108	TECUE #1/2*WAXWING	5	0	0	0
133	WBLL1*2/BRAMBLING/4/BABAX/LR42//BABAX*2/3/KURUKU	5	0	0	0
165	KAUZ/PASTOR//PBW343/3/HEILO	0	0	5	0
45	WAXWING*2/DIAMONDBIRD	5	0	0	1
80	KACHU #1*2/WHEAR	2.5	2.5	0	1
109	KBIRD//WH 542/2*PASTOR/3/WBLL1*2/BRAMBLING	5	0	1	0
114	CHIL/CHUM18//GONDO	0	5	0	1
160	GONDO/CBRD	2.5	2.5	0	1
163	HUIRIVIS #1/GONDO	5	0	0	1
176	BAV92//IRENA/KAUZ/3/HUITES*2/4/GONDO/TNMU	5	0	1	0
186	SAUAL #1/TNMU//SAUAL	5	0	0	1
187	PRINIA/PASTOR//CHIL/CHUM18/3/PRINIA/PASTOR	5	0	1	0
248	NING MAI 96035/FINSI//HEILO	5	0	0	1
287	YAV79//DACK/RABI/3/SNIPE/4/AE.SQUARROSA (460)/5/2*EXCALIBUR/6/VEE/LIRA//BOW/3/BCN/4/KAUZ	5	0	1	0
6	WBLL1*2/4/BABAX/LR42//BABAX/3/BABAX/LR42//BABAX	5	0	1	1
20	PBW343*2/KUKUNA//TECUE #1	0	5	1	1
40	WBLL1*2/CHAPIO//HEILO	5	0	1	1
142	PASTOR/KAUZ/6/CNDO/R143//ENTE/MEXI_2/3/AEGILOPS SQUARROSA (TAUS)/4/WEAVER/5/2*KAUZ	2.5	2.5	1	1
210	KETUPA*2/PASTOR/6/TURACO/5/CHIR3/4/SIREN//ALTAR 84/AE.SQUARROSA (205)/3/3*BUC/7/KACHU	2.5	2.5	1	1
284	PSN/BOW//MILAN/3/2*BERKUT	5	0	1	1
24	WAXWING*2/HEILO	7.5	0	0	1
29	PASTOR//HXL7573/2*BAU/3/WBLL1	2.5	0	5	1
46	BAV92//IRENA/KAUZ/3/HUITES*2/4/MILAN/KAUZ//CHIL/CHUM18	2.5	0	5	1
55	UP2338*2/4/SNI/TRAP#1/3/KAUZ*2/TRAP//KAUZ/5/MILAN/KAUZ//CHIL/CHUM18/6/UP2338*2/4/SNI/TRAP#1/3/KAUZ*2/TRAP//KAUZ	7.5	0	0	1
94	WAXWING/4/SNI/TRAP#1/3/KAUZ*2/TRAP//KAUZ/5/TECUE #1	7.5	0	0	1

Table 3-3 (cont'd)

166	FRET2/WBLL1//TACUPETO F2001/3/HEILO	7.5	0	0	1
195	SHA3/CBRD//TNMU/3/KACHU	2.5	0	1	5
212	CHIL/CHUM18//GONDO/3/WBLL1*2/KURUKU	7.5	0	0	1
215	NG8675/CBRD//FN/2*PASTOR/4/THELIN/3/2*BABAX/LR42//BABAX	2.5	0	5	1
250	ATTILA/HEILO	5	2.5	0	1
268	KS82W418/SPN//WBLL1/3/BERKUT	7.5	0	1	0
64	KACHU*2/3/CHUM18/BORL95//CBRD	7.5	0	1	1
135	BABAX/LR42//BABAX*2/3/KUKUNA/4/TAM200/PASTOR//TOBA97	5	2.5	1	1
143	PASTOR/3/VORONA/CNO79//KAUZ/4/MILAN/OTUS//ATTILA/3*BCN	7.5	0	1	1
31	PUB94.15.1.12/FRTL	7.5	2.5	0	0
66	SAUAL*2/6/CNDO/R143//ENTE/MEXI_2/3/AEGILOPS SQUARROSA (TAUS)/4/WEAVER/5/2*PASTOR	5	0	5	0
81	KACHU #1/3/C80.1/3*BATAVIA//2*WBLL1/4/KACHU	0	10	0	0
145	CHIBIA/WEAVER//KACHU	5	0	0	5
153	PBW343*2/KUKUNA//PBW343*2/KUKUNA/3/PBW343	0	10	0	0
218	TRCH/SRTU//KACHU	5	0	0	5
22	YAV_3/SCO//JO69/CRA/3/YAV79/4/AE.SQUARROSA (498)/5/LINE 1073/6/KAUZ*2/4/CAR//KAL/BB/3/NAC/5/KAUZ/7/KRONSTAD F2004/8/KAUZ/PASTOR//PBW343	5	0	5	1
85	QUAIU/5/FRET2*2/4/SNI/TRAP#1/3/KAUZ*2/TRAP//KAUZ	5	0	1	5
234	ATTILA/3*BCN//BAV92/3/TILHI/4/SHA7/VEE#5//ARIV92	2.5	2.5	1	5
264	TAN//TEMPORALERA M 87/AGR/3/FRET2/4/URES/PRL//BAV92	5	0	5	1
270	SOKOLL/EXCALIBUR	7.5	2.5	1	1
39	WBLL1*2/CHAPIO//HEILO	7.5	0	1	5
52	CS/TH.SC//3*PVN/3/MIRLO/BUC/4/URES/JUN//KAUZ/5/HUITES/6/YANAC/7/CS/TH.SC//3*PVN/3/MIRLO/BUC/4/MILAN/5/TILHI	5	7.5	0	1
87	FRANCOLIN #1/HAWFINCH #1	5	2.5	5	1
150	PBW343/HUITES/3/MILAN/OTUS//ATTILA/3*BCN	2.5	10	0	1
252	BABAX/LR42//BABAX*2/3/KURUKU	5	2.5	5	1
262	VEE/MJI//2*TUI/3/PASTOR/4/BERKUT	7.5	0	5	1

Table 3-3 (cont'd)

51	SAUAL/KIRITATI//SAUAL	2.5	10	1	1
297	ASTREB*2/3/WUH1/VEE#5//CBRD	12.5	0	1	1
137	KENYA NYANGUMI/3/2*KAUZ/PASTOR//PBW343	0	0.5	10	5
28	QUAIU #1	10	0	5	1
75	KACHU*2//CHIL/CHUM18	0	10	1	5
77	BAV92//IRENA/KAUZ/3/HUITES*2/4/YUNMAI	10	5	1	0
141	PFAU/MILAN//SOVA/3/PBW65/2*SERI.1B	5	10	0	1
177	BAV92//IRENA/KAUZ/3/HUITES*2/4/GONDO/TNMU	10	0	1	5
214	FN/2*PASTOR//GONDO/TNMU/3/FRANCOLIN #1	5	0	10	1
259	PASTOR/4/WEAVER/TSC//WEAVER/3/WEAVER/5/URES/PRL//BAV92	5	0	10	1
280	SOKOLL*2/GLE	5	5	5	1
148	C80.1/3*BATAVIA//2*WBLL1/3/TOBA97/PASTOR	5	10	1	1
50	SAUAL/YANAC//SAUAL	12	5	1	0
168	WBLL1*2/CHAPIO//HEILO	2.5	15	1	0
196	FN/2*PASTOR//GONDO/TNMU/3/FRANCOLIN #1	2.5	10	5	1
261	SW94.2690/SUNCO	7.5	0	10	1
119	PANDORA//WBLL1*2/BRAMBLING	12.5	5	1	1
219	PRL/2*PASTOR//SRTU/3/PRINIA/PASTOR	2.5	15	1	1
70	ROLF07*2/4/BOW/NKT//CBRD/3/CBRD	5	0	10	5
118	FRANCOLIN #1/4/BABAX/LR42//BABAX*2/3/KURUKU	20	0	0	0
138	PARUS/PASTOR//INQALAB 91*2/KUKUNA	5	0	10	5
140	CROC_1/AE.SQUARROSA (205)//KAUZ/3/SASIA/4/TROST	0	0	15	5
204	FRET2/TUKURU//FRET2/3/WUH1/VEE#5//CBRD/4/FRET2/TUKURU//FRET2	5	15	0	0
8	WBLL1*2/CHAPIO*2//MURGA	0	20	0	1
53	FINSI/METSO//FH6-1-7/3/FINSI/METSO	2.5	17.5	0	1
266	OASIS//TC14/2*SPER/3/ATTILA/10/ATTILA*2/9/KT/BAGE//FN/U/3/BZA/4/TRM/5/ALDAN/6/SERI/7/VEE#10/8/OPATA	10	0	10	1

Table 3-3 (cont'd)

213	SHA3/CBRD//TNMU/3/KACHU	15	2.5	0	5
244	CPI8/GEDIZ/3/GOO//ALB/CRA/4/AE.SQUARROSA (208)/5/HAHN/2*WEAVER/6/SKAUZ/BAV92	7.5	0	10	5
257	FILIN/3/CROC_1/AE.SQUARROSA (205)//KAUZ/4/FILIN/5/VEE/MJI//2*TUI/3/PASTOR	2.5	10	10	1
267	MEX94.27.1.20/3/SOKOLL//ATTILA/3*BCN	5	2.5	15	1
285	CROC_1/AE.SQUARROSA (224)//OPATA/3/RAC655/4/SLVS/PASTOR	7.5	0	15	1
12	KACHU #1/4/CROC_1/AE.SQUARROSA (205)//KAUZ/3/SASIA/5/KACHU	0	25	0	0
258	T.DICOCCON PI94625/AE.SQUARROSA (372)//3*PASTOR	5	0	10	10
260	SW94.2690/SUNCO	5	0	15	5
296	ASTREB*2/NING MAI 9558	15	0	5	5
271	PASTOR/SLVS//FRAME	5	0.5	15	5
100	WBLL1*2/VIVITSI//PRINIA/PASTOR/3/WBLL1*2/BRAMBLING	7.5	2.5	15	1
279	SUNSTATE/SD 3195//SOKOLL	5	15.5	5	1
33	ATTILA*2/PBW65//WBLL1*2/VIVITSI	7.5	0	15	5
82	SAUAL/WHEAR//SAUAL	2.5	25	0	1
121	WBLL1*2/KKTS//KINGBIRD #1	7.5	5	15	1
112	ATTILA*2/PBW65//KRONSTAD F2004	10	17.5	1	1
211	CHIL/CHUM18//FN/2*PASTOR/3/PRL/2*PASTOR	5	0	10	15
265	A93324S.7197.29/4/KAUZ//ALTAR 84/AOS/3/KAUZ/5/PASTOR	7.5	7.5	10	5
272	PASTOR/SLVS//FRAME	15	0	10	5
295	SOKOLL/SLVS	5	0	20	5
4	FRET2/TUKURU//FRET2/3/MUNIA/CHTO//AMSEL/4/FRET2/TUKURU	20	5	5	1
220	WAXWING*2/3/PASTOR//HXL7573/2*BAU	5	2.5	20	5
13	BAV92//IRENA/KAUZ/3/HUITES*2/4/GONDO/TNMU	30	2.5	0	1
106	BAV92//IRENA/KAUZ/3/HUITES/4/GONDO/TNMU/5/BAV92//IRENA/KAUZ/3/HUITES	20	7.5	1	5
63	ROLF07*2/4/CROC_1/AE.SQUARROSA (224)//KULIN/3/WESTONIA	20	5	5	5

Table 3-3 (cont'd)

181	WBLL1/FRET2//PASTOR*2/3/GONDO	5	5	20	5
203	CNO79//PF70354/MUS/3/PASTOR/4/BAV92*2/5/SHA3/SERI//SHA4/LIRA	5	0	15	15
274	BERKUT/3/ALTAR 84/AE.SQUARROSA (219)//SERI	5	0	20	10
263	BERKUT/3/ATTILA*2//CHIL/BUC	25	2.5	5	5
104	TUKURU//BAV92/RAYON*2/7/YAV_3/SCO//JO69/CRA/3/YAV79/4/AE.SQUARROSA (498)/5/LINE 1073/6/KAUZ*2/4/CAR//KAL/BB/3/NAC/5/KAUZ	12.5	2.5	15	10
228	FRET2/KIRITATI/5/NAC/TH.AC//3*PVN/3/MIRLO/BUC/4/2*PASTOR	10	0	20	10
30	KLDR/PEWIT1//MILAN/DUCULA	30	5	1	5
117	PBW343*2/KHVAKI*2//YANAC	15	15	10	1
139	CROC_1/AE.SQUARROSA (205)//KAUZ/3/SASIA/4/TROST	5	20	15	1
149	WHEAR/3/PBW343/PASTOR//ATTILA/3*BCN	10	30	0	1
197	HEILO//GONDO/TNMU/3/WBLL1*2/BRAMBLING	30	5	5	1
224	WBLL1*2/4/YACO/PBW65/3/KAUZ*2/TRAP//KAUZ/5/KACHU #1	5	30	1	5
47	BAV92//IRENA/KAUZ/3/HUITES*2/4/PVN	20	20	1	1
122	TACUPETO F2001//WBLL1*2/KKTS/3/WBLL1*2/BRAMBLING	22.5	0	15	5
277	SOKOLL//SUNCO/2*PASTOR	12.5	0	20	10
101	SAUAL/5/REH/HARE//2*BCN/3/CROC_1/AE.SQUARROSA (213)//PGO/4/HUITES/6/KACHU	40	2.5	1	0
11	BAV92//IRENA/KAUZ/3/HUITES*2/4/CROC_1/AE.SQUARROSA (224)//KULIN/3/WESTONIA	40	2.5	1	1
124	ATTILA*2/PBW65*2/5/REH/HARE//2*BCN/3/CROC_1/AE.SQUARROSA (213)//PGO/4/HUITES	22.5	20	1	1
273	BAXTER*2/4/CHEN/AEGILOPS SQUARROSA (TAUS)//BCN/3/BAV92	25	17.5	1	1
72	WBLL1*2/4/YACO/PBW65/3/KAUZ*2/TRAP//KAUZ*2/5/GONDO	10	0	30	5
198	CBRD/FILIN	0	0	30	15
245	CPI8/GEDIZ/3/GOO//ALB/CRA/4/AE.SQUARROSA (208)/5/HAHN/2*WEAVER/6/SKAUZ/BAV92	5	0	20	20

Table 3-3 (cont'd)

256	OASIS//TC14/2*SPER/3/ATTILA/4/WBLL4	22.5	2.5	15	5
269	CNDO/R143//ENTE/MEXI75/3/AE.SQ/4/2*FCT/5/KAUZ*2/YACO//KAUZ /6/BERKUT	5	0	30	10
278	CROC_1/AE.SQUARROSA (224)//OPATA/3/ALTAR 84/AE.SQ//2*OPATA	5	0	20	20
293	BERKUT/HTG	5	0	30	10
71	WBLL1/4/BOW/NKT//CBRD/3/CBRD/5/WBLL1*2/TUKURU	30	15	0	1
146	CHIBIA/WEAVER//KACHU	20	5	20	1
179	WBLL1*2/KURUKU*2//TNMU	15	0	30	1
48	BAV92//IRENA/KAUZ/3/HUITES*2/4/TNMU	30	15	1	1
69	BAV92//IRENA/KAUZ/3/HUITES/4/FN/2*PASTOR/5/BAV92//IRENA/KA UZ/3/HUITES	30	15	1	1
2	TUKURU//BAV92/RAYON*2/3/PVN	15	7.5	20	5
98	ATTILA*2/PBW65//MUU #1/3/FRANCOLIN #1	30	2.5	10	5
231	SAUAL/3/KAUZ/PASTOR//PBW343	40	2.5	5	1
73	PFAU/WEAVER*2//BRAMBLING/3/KAUZ//TRAP#1/BOW/4/PFAU/WEA VER*2//BRAMBLING	45	5	1	1
152	MONARCA F2007/KRONSTAD F2004	12.5	0	20	20
232	NG8675/CBRD//MILAN/3/SAUAL/6/CNDO/R143//ENTE/MEXI_2/3/AEGI LOPS SQUARROSA (TAUS)/4/WEAVER/5/2*PASTOR	12.5	0	30	10
239	PRL/2*PASTOR//PARUS/5/NAC/TH.AC//3*PVN/3/MIRLO/BUC/4/2*PAS TOR	42.5	0	5	5
243	VOROBEY	7.5	0	30	15
83	FRNCLN/BECARD	40	2.5	10	1
127	KAUZ/PASTOR//PBW343/3/KRONSTAD F2004	40	2.5	10	1
276	SW89-5124*2/FASAN//PARUS/PASTOR	40	2.5	10	1
205	WBLL1/FRET2//PASTOR/3/SHA3/SERI//SHA4/LIRA/4/WBLL1/TACUPE TO F2001//PASTOR	5	0	30	20
251	WAXWING//PFAU/WEAVER	30	5	15	5

Table 3-3 (cont'd)

23	WAXWING/4/BL 1496/MILAN/3/CROC_1/AE.SQUARROSA (205)//KAUZ/5/FRNCLN	50	5	1	1
115	WBLL1*2/KUKUNA//KIRITATI/3/WBLL1*2/KUKUNA	7.5	15	20	15
238	FRET2/KUKUNA//FRET2/3/PASTOR//HXL7573/2*BAU/5/FRET2*2/4/SNI/TRAP#1/3/KAUZ*2/TRAP//KAUZ	15	2.5	30	10
126	KSW/SAUAL//SAUAL	40	20	0	0
56	UP2338*2/KKTS*2//YANAC	30	30	0	1
236	ATTILA*2/PBW65//KRONSTAD F2004	35	10	15	1
57	WAXWING/2*ROLF07	22.5	20	15	5
110	MUU/5/TRAP#1/BOW/3/VEE/PJN//2*TUI/4/BAV92/RAYON/6/MILAN/S87230//BAV92	40	7.5	10	5
41	WAXWING*2/4/BOW/NKT//CBRD/3/CBRD	20	0	30	15
129	PRL/2*PASTOR//VORB	20	20	20	5
144	PASTOR/3/VORONA/CNO79//KAUZ/4/MILAN/OTUS//ATTILA/3*BCN	10	0	40	15
200	CHIL/CHUM18//GONDO	5	0	40	20
237	KANZ*4/KS85-8-4//2*WBLL1*2/KURUKU	40	5	15	5
283	CO99W329/2*BERKUT	50	10	5	0
202	WAXWING/KIRITATI*2/3/SHA3/SERI//SHA4/LIRA	50	7.5	10	1
221	ATTILA*2/PBW65*2//TNMU	30	15	15	10
254	VOROBEY	5	5	40	20
188	PBW343*2/KHVAKI*2//CHIL/CHUM18	50	15	5	1
183	TRCH*2/TNMU	5	2.5	50	15
151	WBLL1*2/KURUKU//KRONSTAD F2004	40	10	15	10
253	ND643//2*PRL/2*PASTOR	50	0	15	10
281	TEMPORALERA M 87/ROMO96/3/ATTILA/BAV92//PASTOR/4/PRL/2*PASTOR	20	5	40	10
113	WBLL1*2/TUKURU//KRONSTAD F2004	50	10	15	1
49	WBLL1/DIAMONDBIRD//WBLL1*2/VIVITSI	15	12.5	30	20

Table 3-3 (cont'd)

189	PBW343/PASTOR*2/6/TURACO/5/CHIR3/4/SIREN//ALTAR 84/AE.SQUARROSA (205)/3/3*BUC	10	7.5	30	30
25	KIRITATI/4/2*BAV92//IRENA/KAUZ/3/HUITES	60	20	1	0
275	MILAN/DUCULA//SUNCO/2*PASTOR	50	30	1	1
175	SAUAL*2/6/CNDO/R143//ENTE/MEXI_2/3/AEGILOPS SQUARROSA (TAUS)/4/WEAVER/5/2*PASTOR	52.5	15	10	5
18	TRCH/KBIRD	50	15	15	5
42	ROLF07*2/3/PRINIA/PASTOR//HUITES	40	30	10	5
227	FRET2/KIRITATI/5/NAC/TH.AC//3*PVN/3/MIRLO/BUC/4/2*PASTOR	22.5	2.5	40	20
68	BAV92//IRENA/KAUZ/3/HUITES/4/FN/2*PASTOR/5/BAV92//IRENA/KAUZ/3/HUITES	50	35	1	0
294	SOKOLL/FRAME	50	25	10	1
161	HEILO	32.5	30	10	15
103	WBLL1*2/KURUKU/6/CNDO/R143//ENTE/MEXI_2/3/AEGILOPS SQUARROSA (TAUS)/4/WEAVER/5/2*JANZ/7/WBLL1*2/KURUKU	60	15	10	5
120	WBLL1*2/BRAMBLING//JUCHI	40	25	20	5
159	FALCIN/AE.SQUARROSA (312)/3/THB/CEP7780//SHA4/LIRA	50	25	10	5
1	SAUAL/KRONSTAD F2004	70	20	1	1
246	CPI8/GEDIZ/3/GOO//ALB/CRA/4/AE.SQUARROSA (208)/5/HAHN/2*WEAVER/6/SKAUZ/BAV92	12.5	30	30	20
167	WBLL1*2/4/YACO/PBW65/3/KAUZ*2/TRAP//KAUZ/5/GONDO	7.5	0	50	40
292	SOKOLL//SLVS/PASTOR/3/ATTILA*2//CHIL/BUC	60	22.5	10	5
111	KFA/3/PFAU/WEAVER//BRAMBLING/4/PFAU/WEAVER*2//BRAMBLING	55	30	10	5
123	WBLL1/KUKUNA//TACUPETO F2001/3/KRONSTAD F2004/4/ROLF07	35	35	20	10
96	WBLL1*2/BRAMBLING//FN/2*PASTOR	70	25	5	1
26	KZA//WH 542/2*PASTOR/3/BACEU #1	60	22.5	10	10
240	WAXWING*2/JUCHI	70	10	20	5
201	SAUAL/4/CROC_1/AE.SQUARROSA (205)//KAUZ/3/ATTILA/5/SAUAL	60	40	5	1
154	WHEAR/2*KRONSTAD F2004	80	25	1	1

Table 3-3 (cont'd)

10	ATTILA*2/PBW65*2//W485/HD29	70	25	10	5
102	MUU #1//PBW343*2/KUKUNA/3/MUU	60	40	5	5
79	BAV92//IRENA/KAUZ/3/HUITES*2/4/WHEAR	70	35	5	1
99	ATTILA*2/PBW65*2//TOBA97/PASTOR	60	45	5	1
217	BAV92//IRENA/KAUZ/3/HUITES/4/DOLL	70	27.5	10	5
78	WAXWING/KIRITATI*2/3/C80.1/3*BATAVIA//2*WBLL1	60	30	20	5
19	ROLF07/MUU	50	50	10	10
37	ATTILA*2/PBW65//MURGA	80	20	15	5
178	FRET2*2/KUKUNA*2//SHA4/CHIL	80	42.5	1	1
14	MUNAL #1/FRANCOLIN #1	80	35	5	5
134	FRANCOLIN #1/KIRITATI	70	20	30	5
91	KBIRD//WBLL1*2/KURUKU	80	12.5	20	15
84	PAURAQ/3/KIRITATI//PRL/2*PASTOR	50	45	20	15
93	WAXWING/4/SNI/TRAP#1/3/KAUZ*2/TRAP//KAUZ/5/AKURI	80	40	10	1
242	SHANGHAI #8	60	50	15	10
157	SUMAI #3	80	50	5	1
128	REH/HARE//2*BCN/3/CROC_1/AE.SQUARROSA (213)//PGO/4/HUITES/5/KRONSTAD F2004	70	65	1	1
171	WBLL1*2/VIVITSI//GONDO	15	12.5	70	40
172	ATTILA/2*PASTOR//FN/2*PASTOR	80	17.5	30	10
155	C80.1/3*BATAVIA//2*WBLL1/3/2*KRONSTAD F2004	60	30	30	20
206	PFAU/WEAVER*2//BRAMBLING/7/IVAN/6/SABUF/5/BCN/4/RABI//GS/CRA/3/AE.SQUARROSA (190)/8/PFAU/WEAVER//BRAMBLING	80	55	5	1
193	NG8675/CBRD/7/IVAN/6/SABUF/5/BCN/4/RABI//GS/CRA/3/AE.SQUARROSA (190)/8/WBLL1*2/CHAPIO	50	30	50	15
230	KAUZ//ALTAR 84/AOS/3/MILAN/KAUZ/4/OTUS/TOBA97	80	50	10	5
288	CNDO/R143//ENTE/MEXI_2/3/AEGILOPS SQUARROSA (TAUS)/4/WEAVER/5/2*JANZ/6/D67.2/PARANA 66.270//AE.SQUARROSA (320)/3/CUNNINGHAM	70	55	20	1

Table 3-3 (cont'd)

290	D67.2/PARANA 66.270//AE.SQUARROSA (320)/3/CUNNINGHAM/4/PASTOR/SLVS	80	50	15	5
156	C80.1/3*BATAVIA//2*WBLL1/3/2*KRONSTAD F2004	70	15	40	30
136	MURGA/KRONSTAD F2004//QUAIU #3	70	30	40	30
207	PFAU/WEAVER//BRAMBLING*2/3/SHA3/SERI//SHA4/LIRA	70	80	10	10
241	FUNDACEP 30	80	50	30	20
289	CROC_1/AE.SQUARROSA (205)//BORL95/3/KENNEDY/6/CNDO/R143//ENTE/MEXI_2/3/AEGILOPS SQUARROSA (TAUS)/4/WEAVER/5/2*JANZ	80	85	10	5
180	WBLL1*2/TUKURU//WUH1/BOW/3/WBLL1*2/TUKURU	80	65	60	30
158	GAMENYA	90	65	60	40

Analysis of variance for Yellow Rust Severity

The ANOVA of the Ecuador trials indicated significant differences for yellow rust severity (%) among accessions in each location and year (Table 3-3). The average severity scores were 19.01 and 9.17% in 2011 and 2012, respectively. In 2011, the severity ranged from 0 - 90%, whereas, in 2012 the severity ranged from 0 – 65% (Table 3-4; Figure 3-1; Figure 3-2). The combined analysis among the two years detected significant differences between treatments, but the coefficient of variance was high cv = 82.9% (Table 3-3).

The ANOVA of the experiments evaluated in Mexico 2011 and 2012 for YR severity detected significant differences between accessions and years (Table 3-3). The means were 8.37 and 4.25% in 2011 and 2012, respectively. The percentage of severity ranged from 0 – 70% in 2011 and from 0 – 40 % in 2012 (Figure 3-6; Figure 3-7). According to Shapiro-Wilk normality test, the data distribution for yellow rust severity were not normal and showed skewedness in each experiment for each year and location (Figure 3-6)

A high positive correlation was observed between locations in the two years (Table 3-5). In Ecuador, the correlation $r^2 = 0.77$ ($P< 0.001$) between years. In Mexico the correlation was $r^2 = 0.85$ ($P< 0.001$) between years. However, the correlation between the two locations was relatively low ($r^2 = 0.30$; $P< 0.001$) (Figure 3-8; Table 3-5). Some wheat accessions were susceptible in Ecuador but resistant in Mexico. These differences of disease response in each location lowered the correlation between

locations and may be caused by a race specific effect of some major genes against different local races.

Table 3-4. Analysis of variance of yellow rust severity in the association mapping panel. Ecuador and Mexico. 2011-12.

Sources of variation	Df	Mean squares	F-value	P-value
Yellow rust (Ecuador 2011-12)				
Year	1	14384	105.47	< 0.001
Accession	296	693.2	5.0827	< 0.001
Block/Group	196	125.5	0.9199	0.69
Error	100	136.4		
CV(%)=	82.9			
Mean (%)=	14.1			
Yellow rust (Ecuador 2011)				
Accession	296	808.79	28.943	<0.001
Error	100	27.94		
CV(%) =	27.8			
Mean(%) =	19.0			
Yellow rust (Ecuador 2012)				
Accession	296	455.83	7.6414	<0.0001
Error	100	59.65		
CV(%) =	84.2			
Mean(%) =	9.17			
Yellow rust (Mexico 2011-12)				
Accession	296	14.9	17.2	<0.001
Error	280	0.9		
CV(%) =	28.9			
Mean(%) =	6.31			

Table 3-5. Disease severity in the association mapping panel planted in Ecuador and Mexico. 2011-12.

Location	Year	Range (%)	Average (%)
Santa Catalina – Ecuador	2011	0 - 90	19.01
	2012	0 - 65	9.17
El Batan – Mexico	2011	0 - 70	8.37
	2012	0 - 40	4.25

Table 3-6. Pearson correlation and p-values of correlations for yellow rust severity in the association mapping panel experiments in two locations and two years. Ecuador and Mexico. 2011 -12. All values were highly significant (P< 0.001).

	Ecuador 2011-12	Mexico 2011-12	Ecuador 2011	Ecuador 2012	Mexico 2011	Mexico 2012
Ecuador 2011-12	1					
Mexico 2011-12	0.30	1				
Ecuador 2011	0.97	0.30	1			
Ecuador 2012	0.91	0.25	0.77	1		
Mexico 2011	0.30	0.98	0.31	0.24	1	
Mexico 2012	0.27	0.94	0.26	0.24	0.85	1

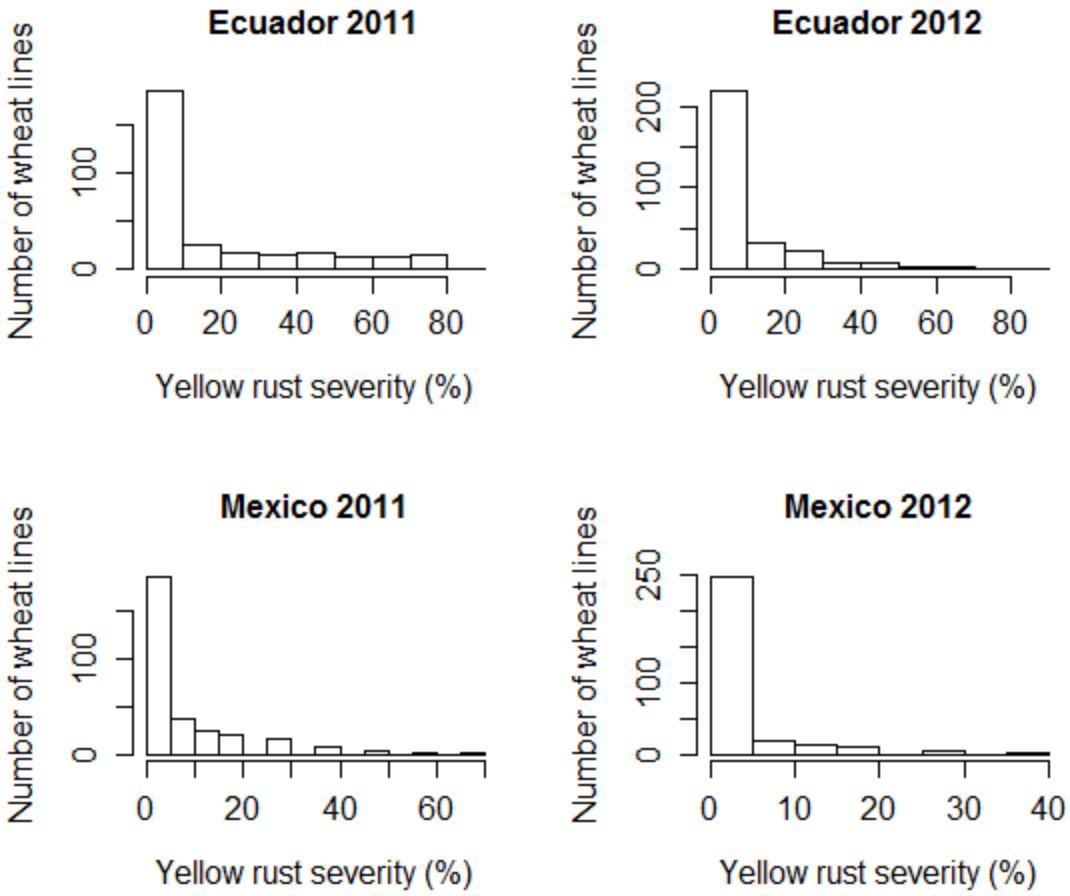

Figure 3-1. Histograms of yellow rust severity (%) in the wheat AMP evaluated in Ecuador and Mexico, 2011-12.

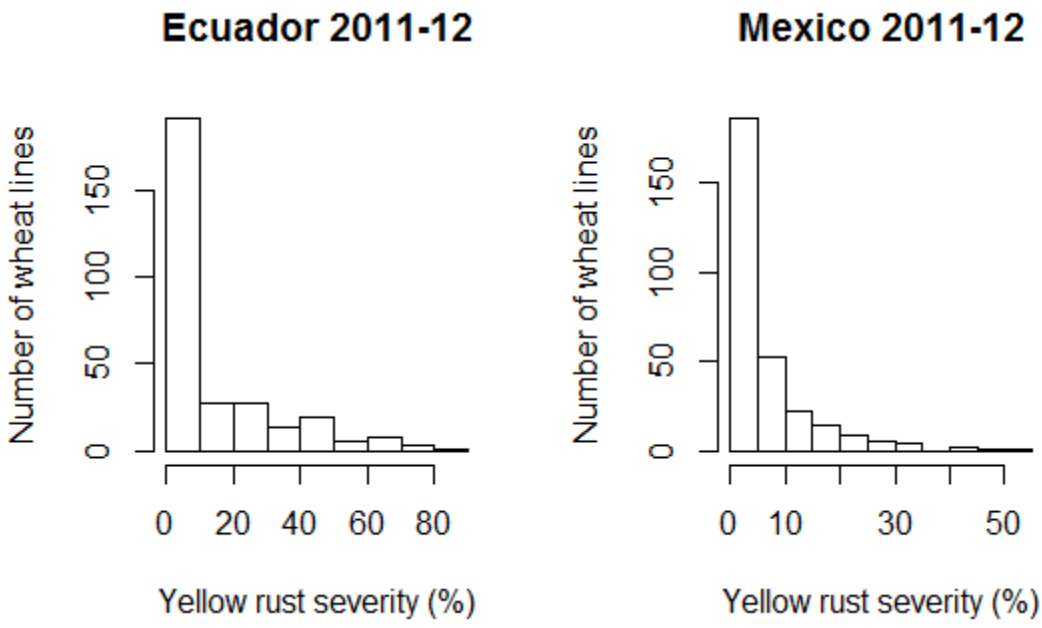

Figure 3-2. Histograms of two year averages of yellow rust severity (%) in the wheat AMP evaluated in Ecuador and Mexico, 2011-12.

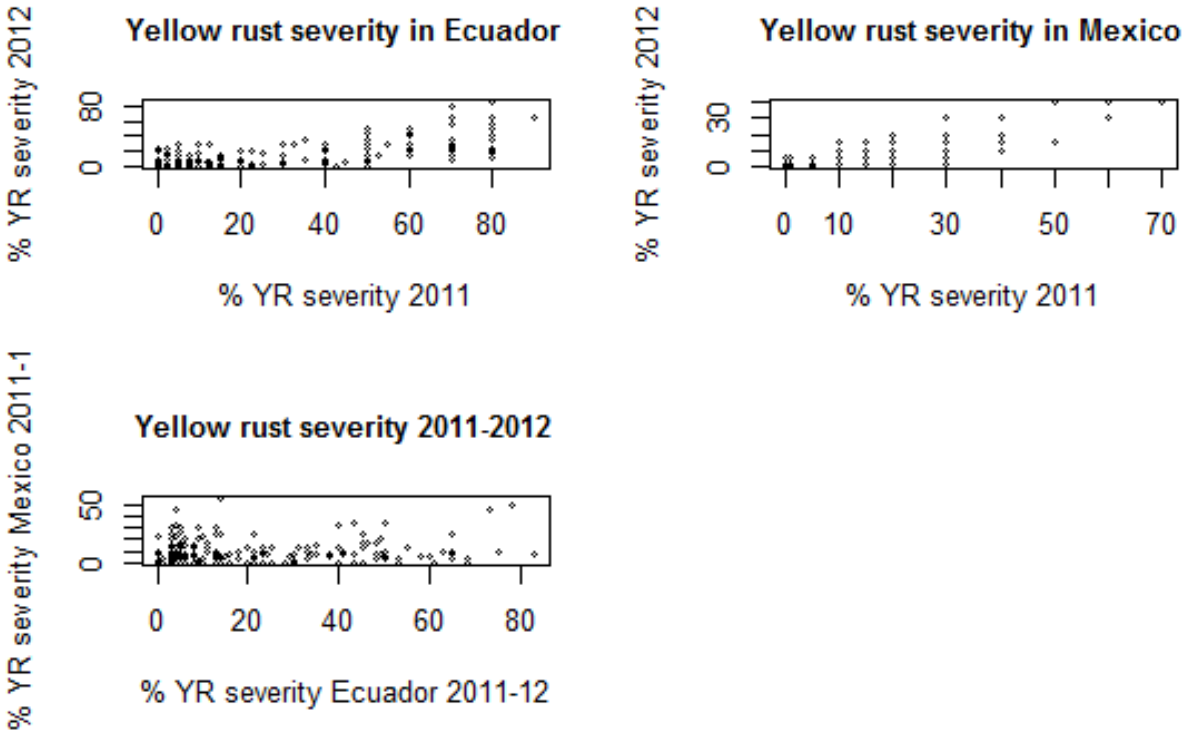

Figure 3-3. Scatter plots of yellow rust severity data from the wheat AMP evaluated in Ecuador and Mexico. 2011-12.

Association analysis for yellow rust severity

A total of 4,679 SNPs and 33 SSR markers showing good quality were considered for the association analysis with the traits collected in Mexico and Ecuador during 2011 and 2012. The markers were filtered to retain polymorphic markers with minor allele frequencies over 5% and one marker per locus avoiding markers in clusters with the same polymorphic pattern. The final number of molecular markers employed to perform the association analysis was 1,666.

The association analysis conducted in Mexico using the GLM method detected 17 and 9 significant SNP markers during 2011 and 2012, respectively. (Table 3-6; Figure 3-4; Figure 3-5). These SNP markers were located on chromosomes 2A, 5A, 6A, 7A, 2B, 5B, 6B, 3D, and 5D. On chromosome 2A, the markers were distributed between 5 and 53 cM. Two SNP markers (wsnp_Ku_c33374_42877546 and wsnp_RFL_Contig1951_1127302) showed the most significant p-valuel in both years (p-values < 3.7×10^{-8}). On chromosome 5A, two significant markers were detected only in the association analysis conducted in 2011. These two SNP markers were located at 121 and 172 cM. On chromosome 7A the region associated with the YR resistance was located at 41 and 51 cM. On chromosome 2B the region associated with the YR resistance was located at 5, 15, 112, and 220 cM. On chromosome 5B, only one significant SNP marker was detected at 100 cM. In the same way, only one marker was detected on chromosome 6B at 22 cM. On chromosome 7B there were two SNP markers located at 45 and 160 cM. On the D-genome, chromosomes 3D and 5D showed markers associated with yellow rust resistance at 15 and 13 cM, respectively.

In Ecuador, the association analysis conducted in the AMP with the combined data set collected in Ecuador 2011-12 detected regions associated with YR resistance on chromosome 1A, 2A, 5A, 6A, 7A, 1B, 2B, 3B, 4B, 5B, 6B, 7B, 1D, 2D, 3D, 5D, 6D, and 7D (Table 3-6; Figure 3-6; Figure 3-7).

The association analysis from the combined experiments in Ecuador 2011-12 resulted in more significant markers linked to YR resistance compared with those found in Mexico. In total, 72 and 56 SNP markers were associated with YR resistance in 2011 and 2012, respectively. However, some markers located on chromosome 2A (wsnp_Ku_c33374_42877546 and wsnp_RFL_Contig1951_1127302) were common for both locations (Table 3-6). Additionally, the analysis detected markers located on chromosomes 1A, 6A, 6B, and 7B. SNP marker on chromosome 1A was detected at 171 cm. SNP markers on chromosome 2A were distributed from 5 to 88 cM. SNP marker on chromosome 6A was located at 106 cM. SNP markers on chromosome 6B were found at 191 – 192 cM. Finally, SNP markers on chromosome 7B were found at 66 -67 cM.

The individual analysis in Ecuador 2011 using the GLM method detected significant markers on chromosomes 1A, 2A, 5A, 6A, 7A, 1B, 2B, 3B, 4B, 5B, 6B, 7B, 1D, 2D, 3D, 5D, 6D, and 7D (Table 3-6; Figure 3-6). On chromosome 2A, the significant SNP markers were distributed from 5 to 76 cM. On chromosome 5B the significant markers were located at 8, 23, 69, 71, 100, and 181 cM. On chromosome 7B, two significant SNP markers were detected at 31and 100 cM. Finally, chromosome 7D contained significant markers at 0-8 cM.

The association analysis conducted with the data collected in Ecuador 2012 for yellow rust severity with the GLM method detected SNP markers significantly associated with resistance to YR located on chromosomes 1A, 2A, 3A, 4A, 5A, 6A, 7A, 1B, 2B, 3B, 5B, 7B, 2D, 4D, and 7D (Figure 3-6; Figure 3-7). On chromosome 2A, eight SNP markers significantly associated with YR located at 5, 7, 10, 72, and 76 cM were detected. On chromosome 6A, eight SNP markers were detected at 21, 90. 99. 106, 117, 139, 189, and 206 cM. On chromosome 7A, three SNP markers were detected located at 104, 105, and 107 cM. On chromosome 1B, the significant markers were located at 46, 86, 91 cM. On chromosome 5B, three SNP markers were detected at 23, 39, and 134 cM. On chromosome 6B, there was one SNP marker detected at 192 cM. Finally, on chromosome 7B, three SNP markers were detected at 41, 47, 51, and 99 cM.

The association analysis conducted with the combined data set and individually data set per location in Ecuador and Mexico in the wheat AMP using the MLM method detected SNP markers significantly linked to YR resistance on chromosomes 1A, 2A, 3A, 6A, 1B, 2B, 7B, 6D, 7D . On chromosome 2A, the SNP markers were located at 7 and 10 cM (Table 3-7). The same results were found in the association analysis conducted with data collected from Ecuador using MLM method. In addition to the SNPs located on chromosome 2A, there two SNP markers located on chromosome 1A position 104 and 111cM.

Table 3-7. Association analysis for yellow rust severity of the wheat association mapping panel using GLM model. Mexico and Ecuador. 2011-12.

Marker	Chr	Position (cM)	P-value	r2	Alleles	Allele 1 Sev(%)	Allele 2 Sev(%)	Effect Sev(%)
Ecuador 2011								
wsnp_Ex_c48087_53105842	1A	36	0.00119	0.04	A/G	17.2	30.3	13.1
wsnp_BE403956A_Ta_2_3	1A	71	4.07E-04	0.05	T/C	17	21.7	4.7
wsnp_Ex_c6817_11761300	1A	71	6.58E-04	0.05	T/C	30	17	13
wsnp_BE517729A_Ta_2_1	1A	100	0.00445	0.04	A/G	13.5	19.4	5.9
wsnp_Ex_rep_c68085_66839109	1A	104	9.24E-05	0.07	A/G	20.3	15.1	5.2
wsnp_Ex_c43228_49605281	1A	104	0.00182	0.04	A/G	20.1	14.5	5.6
wsnp_Ex_c5550_9779698	1A	176	0.00262	0.04	T/C	17.1	27.3	10.2
wsnp_Ku_c23598_33524490	2A	5	7.61E-05	0.06	A/C	23.1	16.1	7
wsnp_Ku_c33374_42877546	2A	7	1.11E-24	0.31	A/G	26.8	3.4	23.4
wsnp_RFL_Contig1951_1127302	2A	10	1.41E-29	0.37	A/G	3.4	27.6	24.2
wsnp_Ex_rep_c68113_66877517	2A	72	6.25E-04	0.05	T/C	22.6	10	12.6
wsnp_CAP11_rep_c8768_3788007	2A	76	0.00229	0.04	T/G	22.5	10.2	12.3
wsnp_JG_c2509_1153697	3A	57	0.00315	0.04	A/G	18.5	16.8	1.7
wsnp_Ex_c5047_8963671	3A	100	4.18E-04	0.05	T/C	18.6	15.5	3.1
wsnp_Ex_c5623_9891584	3A	123	0.00149	0.04	A/G	21.2	13.3	7.9
wsnp_Ex_c361_708712	3A	162	6.47E-04	0.05	A/G	27.3	16.1	11.2
wsnp_Ex_c5072_9006666	4A	4	4.30E-05	0.07	A/G	16.8	31.5	14.7
wsnp_Ku_c9746_16265584	4A	5	1.02E-05	0.08	A/G	32.7	16.6	16.1
wsnp_Ex_c1246_2393978	4A	47	2.17E-05	0.07	T/G	16.1	26.1	10
wsnp_JD_c27162_22206547	4A	63	5.76E-04	0.05	T/C	21.3	14.6	6.7
wsnp_RFL_Contig4086_4599222	5A	114	9.91E-05	0.06	A/G	17.4	28.1	10.7
wsnp_Ex_c10231_16783750	5A	153	0.00424	0.04	T/C	20.4	17.3	3.1
wsnp_Ku_c9559_16000086	5A	185	9.30E-04	0.05	T/C	22.1	15	7.1
wsnp_Ex_c13230_20872924	6A	21	9.72E-04	0.05	T/C	17.5	26.7	9.2
wsnp_Ku_c17618_26749729	6A	43	0.00161	0.04	A/G	14.4	22.4	8
wsnp_Ex_c18965_27868480	6A	90	5.17E-04	0.05	A/G	17.3	21.1	3.8
wsnp_Ex_c34641_42914170	6A	139	0.00113	0.05	T/C	19.3	18.3	1

Table 3-7 (cont'd)

wsnp_JD_c5872_7032077	6A	187	0.00633	0.03	A/G	20.1	17.1	3
wsnp_Ex_rep_c66939_65371026	7A	6	0.00624	0.04	A/G	15.3	27.7	12.4
wsnp_BG313770A_Ta_2_1	7A	20	0.00474	0.04	T/C	21.4	16	5.4
wsnp_Ex_c20062_29096408	7A	51	4.55E-04	0.05	T/C	30.9	16.6	14.3
wsnp_Ra_c26491_36054023	7A	105	0.00119	0.04	A/G	37.2	17.9	19.3
wsnp_Ku_rep_c103889_90513365	7A	134	0.00319	0.04	A/G	22.5	14.9	7.6
wsnp_Ex_c6142_10746442	7A	173	9.03E-04	0.05	A/G	16.9	25.2	8.3
wsnp_Ex_c52474_56060204	1B	46	0.00753	0.03	A/G	19.8	14.8	5
wsnp_Ku_rep_c69901_69397257	1B	48	0.003	0.04	T/C	18.6	17.3	1.3
wsnp_BG606986B_Ta_2_1	1B	86	0.00186	0.04	T/C	16.6	20.3	3.7
wsnp_Ex_c194_381656	1B	91	0.00172	0.04	T/C	20.1	16.3	3.8
wsnp_Ex_c1597_3045682	1B	141	9.95E-04	0.05	A/G	22.2	15.4	6.8
wsnp_Ex_c7776_13247654	2B	5	2.58E-05	0.07	T/C	28.9	17.2	11.7
wsnp_JD_c12687_12877994	2B	76	0.00269	0.04	T/C	20.5	11.9	8.6
wsnp_Ex_c30447_39360584	2B	91	0.00721	0.03	A/G	13.4	19.7	6.3
wsnp_Ex_c17845_26604587	2B	170	0.00521	0.04	T/C	25.2	14.4	10.8
wsnp_Ku_c48694_54811376	2B	220	0.00304	0.03	T/C	17.4	32	14.6
wsnp_CAP11_c3742_1796552	3B	12	1.33E-04	0.06	T/C	5.3	20.5	15.2
wsnp_Ku_c33335_42844594	3B	61	2.34E-04	0.06	A/G	17.4	32.6	15.2
wsnp_Ex_c48922_53681502	4B	80	0.00333	0.03	T/C	20.1	4	16.1
wsnp_Ex_c26285_35531493	4B	86	0.00556	0.04	T/C	20.5	17.2	3.3
wsnp_JD_c12221_12509932	5B	8	0.00157	0.04	A/C	21.4	9.8	11.6
wsnp_JD_c8978_9893945	5B	23	2.38E-05	0.07	T/C	15.8	31.2	15.4
wsnp_Ex_rep_c68600_67448893	5B	69	0.00337	0.04	T/G	19.8	16.7	3.1
wsnp_Ra_c20970_30293078	5B	71	0.00411	0.04	A/C	22.4	14.3	8.1
wsnp_Ex_c2264_4243233	5B	100	2.39E-05	0.07	A/C	5.4	21	15.6
wsnp_Ex_rep_c103024_88075347	5B	181	0.00722	0.03	T/C	15	23.8	8.8
wsnp_JD_c15167_14703349	6B	12	0.00295	0.04	T/C	8.3	21.5	13.2
wsnp_Ku_c4910_8793327	6B	140	0.00305	0.04	A/G	15.3	23.2	7.9
wsnp_Ex_c6731_11634168	6B	153	0.00851	0.04	T/G	19.8	14.6	5.2

Table 3-7 (cont'd)

wsnp_Ku_c665_1371448	7B	31	0.00759	0.03	A/G	15.2	23.3	8.1
wsnp_Ex_c8963_14948293	7B	100	0.00323	0.04	T/C	16.7	30.5	13.8
wsnp_Ex_c278_538285	1D	0	7.78E-04	0.05	T/C	26.3	16.9	9.4
wsnp_Ex_c15396_23659859	1D	91	0.00772	0.02	A/G	21.6	18.2	3.4
wsnp_Ex_c14779_22892053	2D	0	1.39E-05	0.07	T/C	17.6	31.5	13.9
wsnp_Ex_c6400_11123059	2D	89	0.00395	0.04	A/G	19.7	13.1	6.6
wsnp_BE444144D_Ta_1_1	2D	101	0.00382	0.04	A/G	11.9	20.2	8.3
wsnp_Ex_rep_c70527_69450183	3D	2	0.00626	0.03	T/G	20.5	16.1	4.4
wsnp_BE497160D_Ta_2_1	3D	53	0.00379	0.04	T/C	21.7	12.7	9
wsnp_JD_c825_1223454	5D	13	1.30E-04	0.06	T/C	20.7	14.6	6.1
wsnp_Ex_c6942_11966469	6D	0	6.98E-04	0.05	T/C	17.4	19.4	2
wsnp_Ex_c1690_3206784	6D	44	0.00445	0.04	A/G	18	31.9	13.9
wsnp_Ex_c43083_49499652	7D	34	0.00389	0.04	A/G	16.5	20.8	4.3
wsnp_CAP11_c2839_1425826	7D	0	1.37E-06	0.09	A/G	15.8	31.8	16
wsnp_CAP11_c176_177381	7D	8	0.00203	0.04	T/C	19.2	12.4	6.8
Ecuador 2012								
wsnp_BE403956A_Ta_2_3	1A	71	2.36E-04	0.06	T/C	7.6	20.6	13
wsnp_BE495786A_Ta_2_1	1A	81	5.18E-04	0.05	T/G	10.9	4.6	6.3
wsnp_Ex_c1255_2411550	1A	178	1.83E-04	0.06	A/C	10.1	8.1	2
wsnp_Ku_c23598_33524490	2A	5	0.00375	0.04	A/C	10.9	7.7	3.2
wsnp_Ku_c33374_42877546	2A	7	1.99E-11	0.15	A/G	12.1	3.4	8.7
wsnp_RFL_Contig1951_1127302	2A	10	7.19E-12	0.16	A/G	3.7	12	8.3
wsnp_Ex_c5412_9565733	2A	53	9.89E-07	0.09	T/C	4.9	13.3	8.4
wsnp_BQ168780B_Ta_2_1	2A	67	2.79E-05	0.07	A/G	6.6	14.3	7.7
wsnp_Ex_rep_c68113_66877517	2A	72	0.00137	0.04	T/C	11.2	3.4	7.8
wsnp_JD_c13086_13174510	2A	72	0.00343	0.04	A/G	4.1	11.2	7.1
wsnp_CAP11_rep_c8768_3788007	2A	76	0.00337	0.04	T/G	11.2	3.9	7.3
wsnp_JG_c2509_1153697	3A	57	0.00157	0.04	A/G	8.6	9.4	0.8
wsnp_Ku_rep_c68484_67499824	3A	100	0.00352	0.04	T/C	2.1	8.3	6.2

Table 3-7 (cont'd)

wsnp_Ra_c4858_8709000	3A	105	2.40E-04	0.06	A/C	7.2	9.2	2
wsnp_Ex_c5623_9891584	3A	123	0.00302	0.04	A/G	10.7	5.2	5.5
wsnp_Ex_c361_708712	3A	162	0.00446	0.04	A/G	13.8	7.7	6.1
wsnp_Ex_c5072_9006666	4A	4	0.00308	0.04	A/G	7.9	15.7	7.8
wsnp_Ex_c1246_2393978	4A	47	3.42E-04	0.05	T/G	7.4	13.3	5.9
wsnp_Ku_c46057_52907637	4A	152	0.00114	0.05	A/C	8.7	8.4	0.3
wsnp_Ra_c25624_35192195	5A	12	0.00476	0.04	T/C	17.6	8.2	9.4
wsnp_JD_c21776_19013462	5A	25	0.00219	0.04	A/G	10.8	8	2.8
wsnp_RFL_Contig4086_4599222	5A	114	0.0048	0.04	A/G	8.2	16.2	8
wsnp_Ra_c11420_18529863	5A	153	0.00107	0.05	T/C	7.2	10.8	3.6
wsnp_Ex_c13230_20872924	6A	21	0.00198	0.04	T/C	8.3	14.2	5.9
wsnp_BF200644A_Ta_1_1	6A	90	1.14E-04	0.05	T/G	0	8.2	8.2
wsnp_Ex_c35545_43677576	6A	99	0.00141	0.04	A/G	13	7.7	5.3
wsnp_Ex_c2350_4403690	6A	106	0.00167	0.04	A/G	13.7	7.7	6
wsnp_Ku_c22358_32187765	6A	117	0.00394	0.04	A/G	13.6	7.5	6.1
wsnp_Ex_c34641_42914170	6A	139	0.0023	0.04	T/C	12	8.4	3.6
wsnp_Ex_c10718_17457870	6A	189	1.23E-04	0.06	A/G	8.3	19.8	11.5
wsnp_Ex_c1153_2213588	6A	206	0.00459	0.04	A/G	9.1	7.5	1.6
wsnp_Ku_c34643_43968242	7A	104	0.00171	0.04	T/C	8.4	14.2	5.8
wsnp_Ra_c26491_36054023	7A	105	0.0011	0.05	A/G	18.5	8.4	10.1
wsnp_BM134363A_Ta_2_4	7A	107	0.00499	0.03	A/G	20.5	9.6	10.9
wsnp_Ex_c52474_56060204	1B	46	1.78E-04	0.06	A/G	10.1	5.9	4.2
wsnp_BG606986B_Ta_2_1	1B	86	0.00171	0.04	T/C	6.6	11.4	4.8
wsnp_Ex_c194_381656	1B	91	0.00237	0.04	T/C	10	7.3	2.7
wsnp_Ex_c7776_13247654	2B	5	0.00248	0.04	T/C	12.3	8.8	3.5
wsnp_BE499478B_Ta_2_1	2B	94	0.0032	0.04	T/C	14.3	7	7.3
wsnp_Ex_rep_c101906_87187119	2B	112	4.70E-04	0.05	A/C	5.2	12.3	7.1
wsnp_Ku_c3000_5638635	2B	160	0.00168	0.04	A/G	11.1	3.3	7.8
wsnp_Ex_c10796_17575074	2B	185	0.00189	0.04	T/C	12.1	7.1	5
wsnp_Ku_c48694_54811376	2B	220	2.54E-04	0.04	T/C	19.9	8	11.9

Table 3-7 (cont'd)

wsnp_CAP11_c3742_1796552	3B	12	0.0013	0.04	T/C	3.5	9.8	6.3
wsnp_Ex_c29623_38630871	3B	102	0.00221	0.04	A/G	15.4	8.4	7
wsnp_JD_c8978_9893945	5B	23	0.00181	0.04	T/C	7.7	16	8.3
wsnp_Ku_c8953_15094606	5B	39	0.00418	0.03	A/G	17.7	8.2	9.5
wsnp_Ex_rep_c66375_64566565	5B	134	0.00348	0.04	T/C	13.1	7.3	5.8
wsnp_CAP7_c90_52035	7B	41	9.38E-05	0.06	T/C	7.9	23.8	15.9
wsnp_CAP11_rep_c6622_3044459	7B	47	0.00131	0.05	A/G	7.2	14.1	6.9
wsnp_Ex_c2539_4733110	7B	51	0.00345	0.04	A/G	7.4	12.5	5.1
wsnp_Ex_c10550_17231294	7B	99	0.00207	0.03	T/C	8.3	23	14.7
wsnp_Ex_c14779_22892053	2D	0	0.00156	0.04	T/C	8.4	14.9	6.5
wsnp_Ku_c13442_21433358	4D	46	0.00475	0.04	A/G	5.3	9.2	3.9
wsnp_Ex_c43083_49499652	7D	34	0.00224	0.04	A/G	8.1	9.4	1.3
wsnp_CAP11_c176_177381	7D	8	0.00274	0.04	T/C	9.4	4	5.4
Mexico 2011								
wsnp_Ku_c33374_42877546	2A	7	3.95E-18	0.21	A/G	12.1	1	11.1
wsnp_RFL_Contig1951_1127302	2A	10	1.14E-18	0.22	A/G	1.7	12.4	10.7
wsnp_Ex_c5412_9565733	2A	53	1.40E-05	0.07	T/C	6	11.5	5.5
wsnp_Ku_rep_c68259_67171095	5A	121	4.13E-04	0.05	T/C	11.7	4.5	7.2
wsnp_Ku_c29319_39227528	5A	172	4.56E-04	0.05	A/G	5.5	11.1	5.6
wsnp_Ku_c139_279238	7A	41	3.75E-05	0.06	T/C	13.2	5.9	7.3
wsnp_Ex_c20062_29096408	7A	51	2.64E-04	0.05	T/C	11.5	7.7	3.8
wsnp_Ex_c7776_13247654	2B	5	2.27E-07	0.09	T/C	15	7.4	7.6
wsnp_Ex_c25688_34949297	2B	15	5.85E-04	0.04	T/C	7.6	14.2	6.6
wsnp_Ex_rep_c101906_87187119	2B	112	2.75E-04	0.05	A/C	11.9	4.6	7.3
wsnp_Ku_c48694_54811376	2B	220	1.87E-04	0.04	T/C	15	7.7	7.3
wsnp_Ex_c2264_4243233	5B	100	2.49E-07	0.09	A/C	2	9.3	7.3
wsnp_Ex_c4815_8597139	6B	22	1.88E-04	0.05	T/C	5.1	9	3.9
wsnp_Ex_c27914_37074773	7B	45	8.97E-05	0.05	T/C	9.7	0.5	9.2
wsnp_BE445506B_Ta_2_2	7B	160	3.19E-04	0.05	T/C	7.1	9.6	2.5

Table 3-7 (cont'd)

wsnp_Ku_c7264_12545135	3D	15	2.31E-05	0.06	T/C	7	14.2	7.2
wsnp_JD_c825_1223454	5D	13	3.87E-04	0.05	T/C	10.4	3.6	6.8
Mexico 2012								
wsnp_Ku_c33374_42877546	2A	7	3.71E-08	0.10	A/G	5.8	1.3	4.5
wsnp_RFL_Contig1951_1127302	2A	10	8.64E-08	0.10	A/G	1.6	5.9	4.3
wsnp_Ex_c5412_9565733	2A	53	9.52E-05	0.06	T/C	2.8	6	3.2
wsnp_Ex_c7546_12900094	6A	217	1.11E-04	0.06	T/C	4	4.3	0.3
wsnp_Ex_c7776_13247654	2B	5	1.09E-04	0.06	T/C	7.2	3.8	3.4
wsnp_Ku_c48694_54811376	2B	220	7.99E-05	0.05	T/C	8.7	3.8	4.9
wsnp_Ra_c13424_21239985	5B	182	5.34E-05	0.06	A/C	3.1	7.3	4.2
wsnp_Ex_c1498_2868339	5B	182	8.87E-05	0.06	T/C	3.1	7.3	4.2
wsnp_Ex_c4815_8597139	6B	22	1.90E-04	0.06	T/C	2.1	4.6	2.5

Table 3-8. Association analysis for yellow rust severity of the wheat association mapping panel using MLM model. Mexico and Ecuador. 2011-12.

Marker	Chr.	Pos. (cM)	P-value	r2	Alleles	Allele 1 (% Sev.)	Allele 2 (% Sev.)	Effect (% Sev.)
Ecuador 2011								
wsnp_RFL_Contig1951_1127302	2A	10	2.56E-12	0.212	A/G	24.2	3.4	27.6
wsnp_Ku_c33374_42877546	2A	7	8.12E-12	0.192	A/G	23.4	26.8	3.4
wsnp_Ex_c34641_42914170	6A	139	1.01E-04	0.066	T/C	1	19.3	18.3
wsnp_Ex_rep_c68085_66839109	1A	104	1.02E-04	0.071	A/G	5.2	20.3	15.1
wsnp_Ex_c6942_11966469	6D	0	1.14E-04	0.064	T/C	2	17.4	19.4
wsnp_Ex_c43083_49499652	7D	34	1.24E-04	0.065	A/G	4.3	16.5	20.8
wsnp_Ex_c1597_3045682	2B	141	1.24E-04	0.068	A/G	6.8	22.2	15.4
wsnp_BG606986A_Ta_2_1	1A	111	1.31E-04	0.065	A/C	15.1	20.5	5.4
wsnp_Ex_c43228_49605281	1A	104	2.06E-04	0.063	A/G	5.2	20.3	15.1
wsnp_Ex_c2350_4403690	6A	106	2.75E-04	0.060	A/G	24.3	16.9	7.4

Table 3-8 (cont'd)

wsnp_Ex_c5623_9891584	3A	123	3.33E-04	0.05805	A/G	7.9	21.2	13.3
wsnp_Ex_c17692_26437459	6A	90	4.02E-04	0.05482	A/G	3.8	17.3	21.1
wsnp_Ex_c24777_34031473	1B	141	4.76E-04	0.05651	A/G	6.8	22.2	15.4
wsnp_Ex_c908_1754208	7B	41	5.21E-04	0.05521	T/C	15.1	31.7	15.6
Ecuador 2012								
wsnp_Ku_c33374_42877546	2A	7	5.08E-08	0.12013	T/C	7.6	20.6	13
wsnp_RFL_Contig1951_1127302	2A	10	2.87E-07	0.11185	T/G	10.9	4.6	6.3
Mexico 2011								
wsnp_RFL_Contig1951_1127302	2A	10	2.30E-10	0.16857	A/G	12.1	1	11.1
wsnp_Ku_c33374_42877546	2A	7	1.03E-09	0.15126	A/G	1.7	12.4	10.7
Mexico 2012								
wsnp_Ku_c33374_42877546	2A	7	1.31E-05	0.07789	A/G	5.8	1.3	4.5
wsnp_RFL_Contig1951_1127302	2A	10	1.50E-05	0.07825	A/G	1.6	5.9	4.3

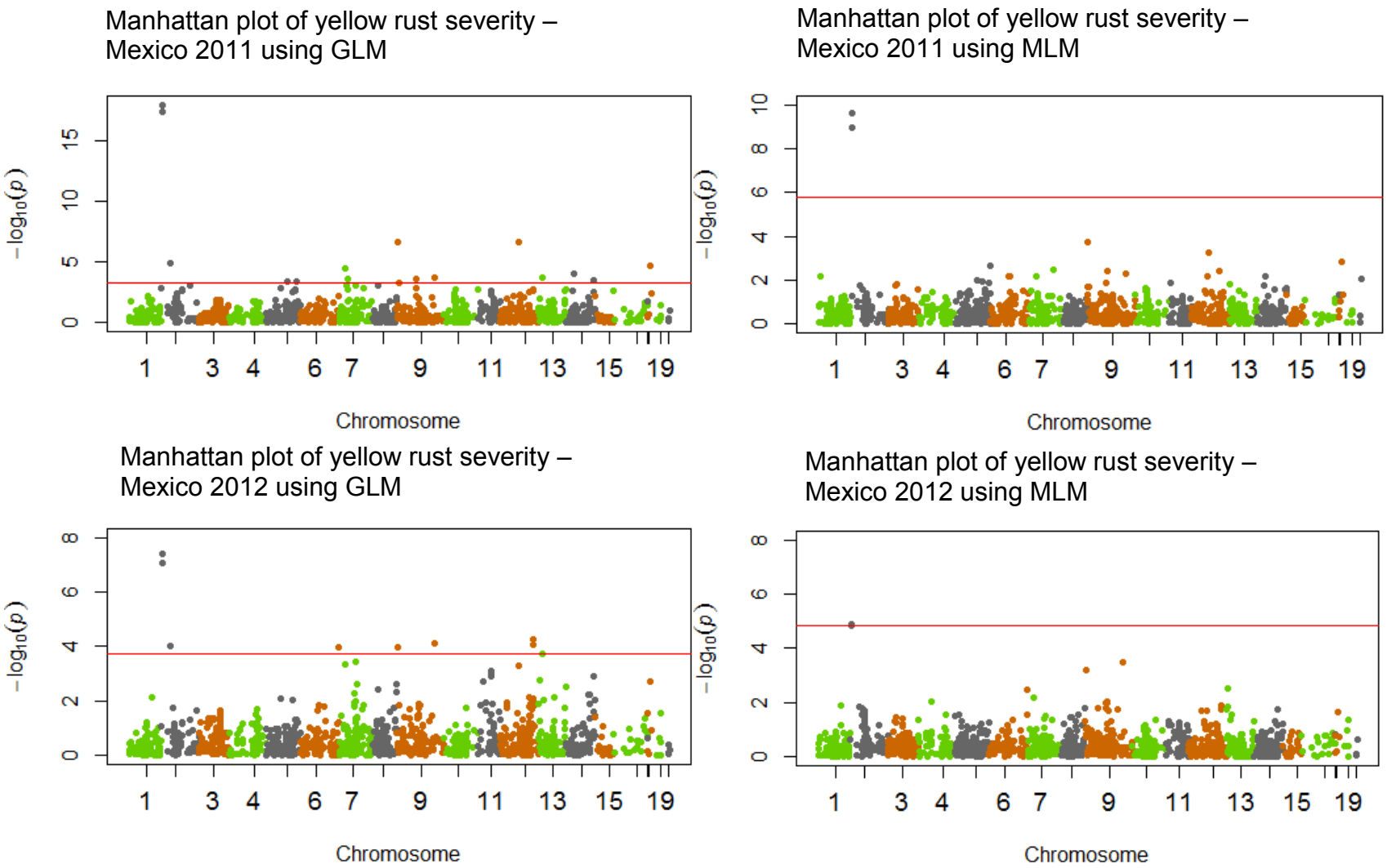

Figure 3-4. Manhattan plots of the association analysis for yellow rust severity in the wheat association mapping panel using GLM and MLM. Mexico 2011 and 2012.

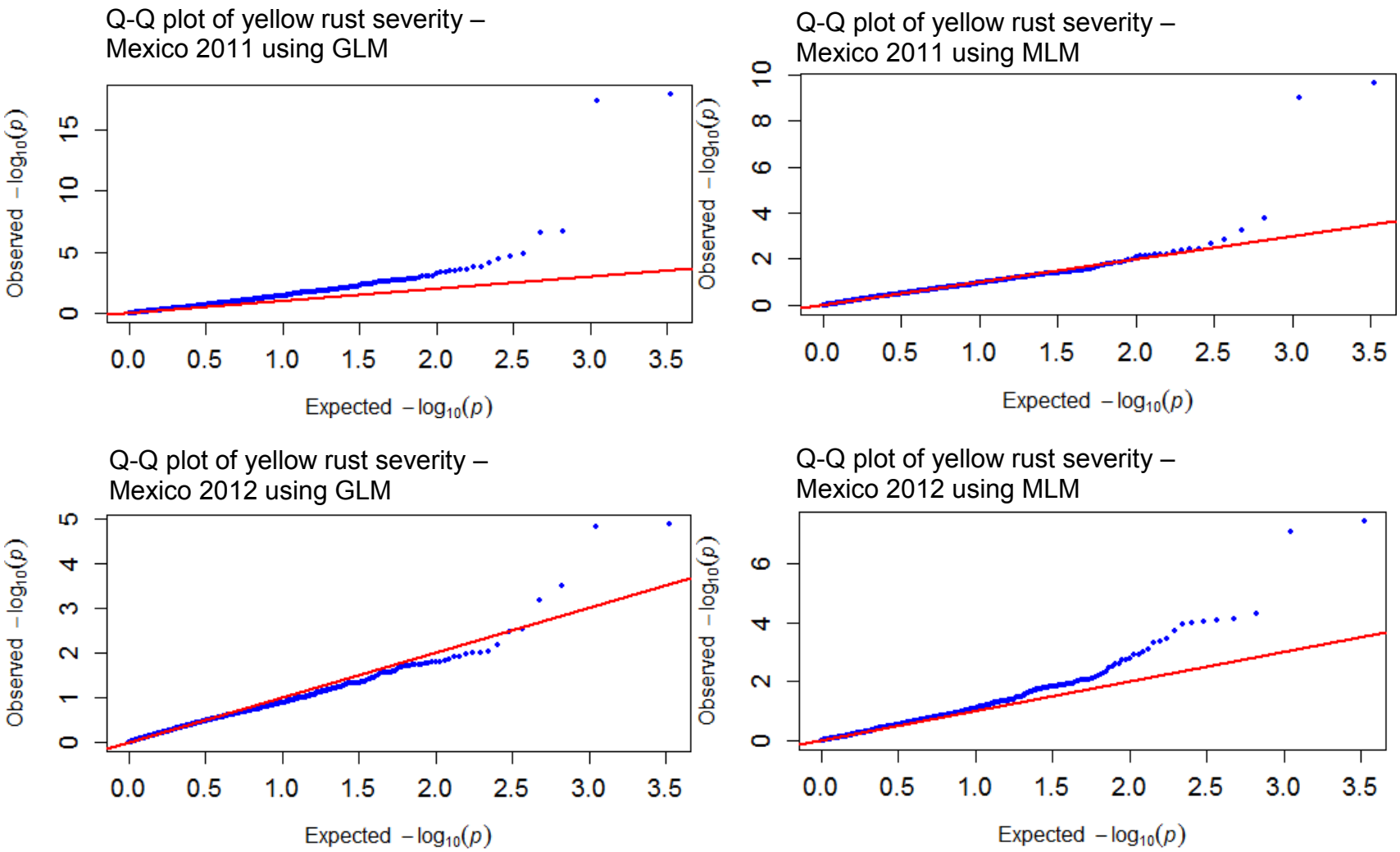

Figure 3-5. Q-Q plots of the of the association analysis for yellow rust severity in the wheat association mapping panel using GLM and MLM. Mexico 2011 and 2012.

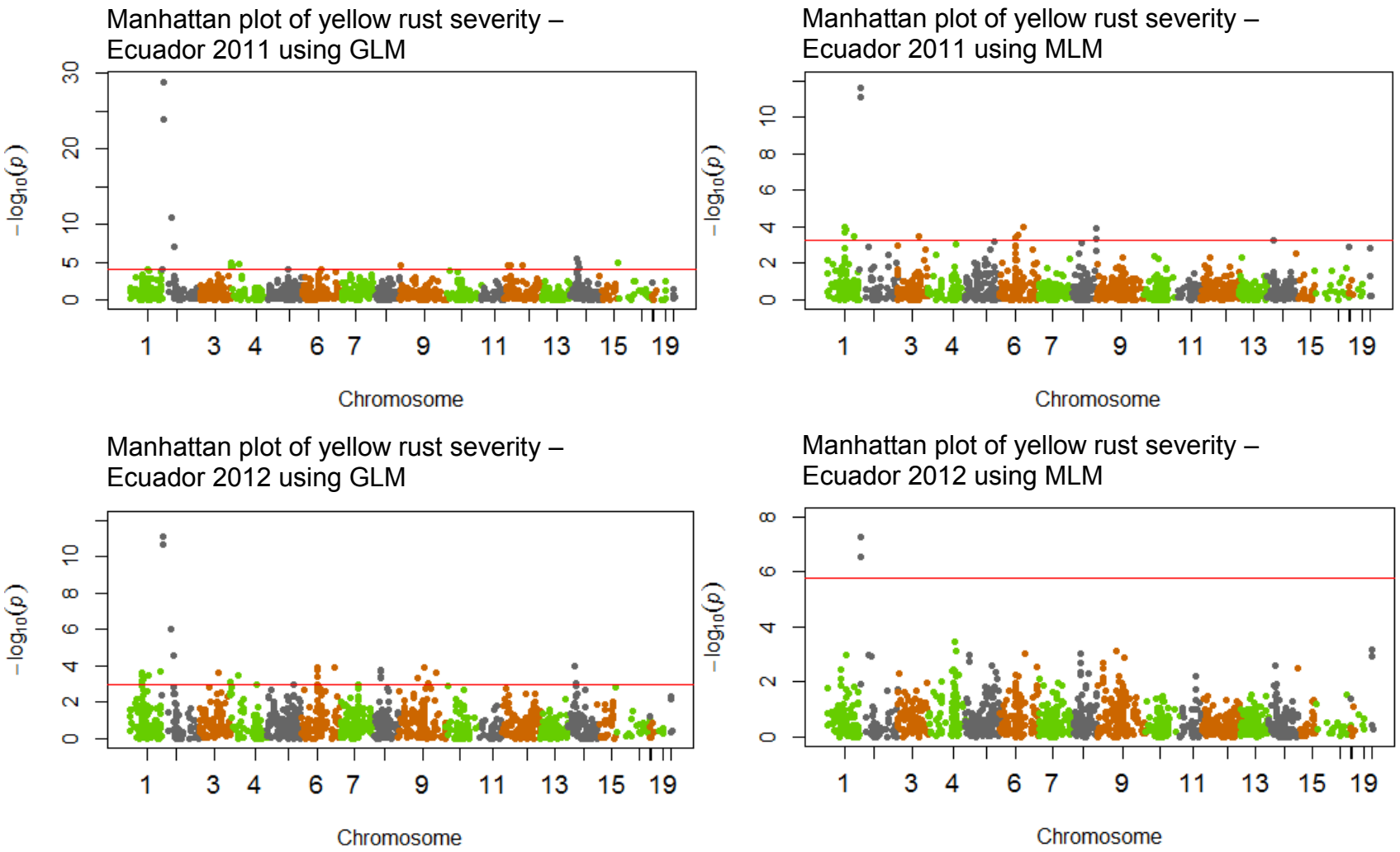

Figure 3-6. Manhattan plots of the association analysis for yellow rust severity in the wheat association mapping panel using GLM and MLM. Ecuador 2011 and 2012.

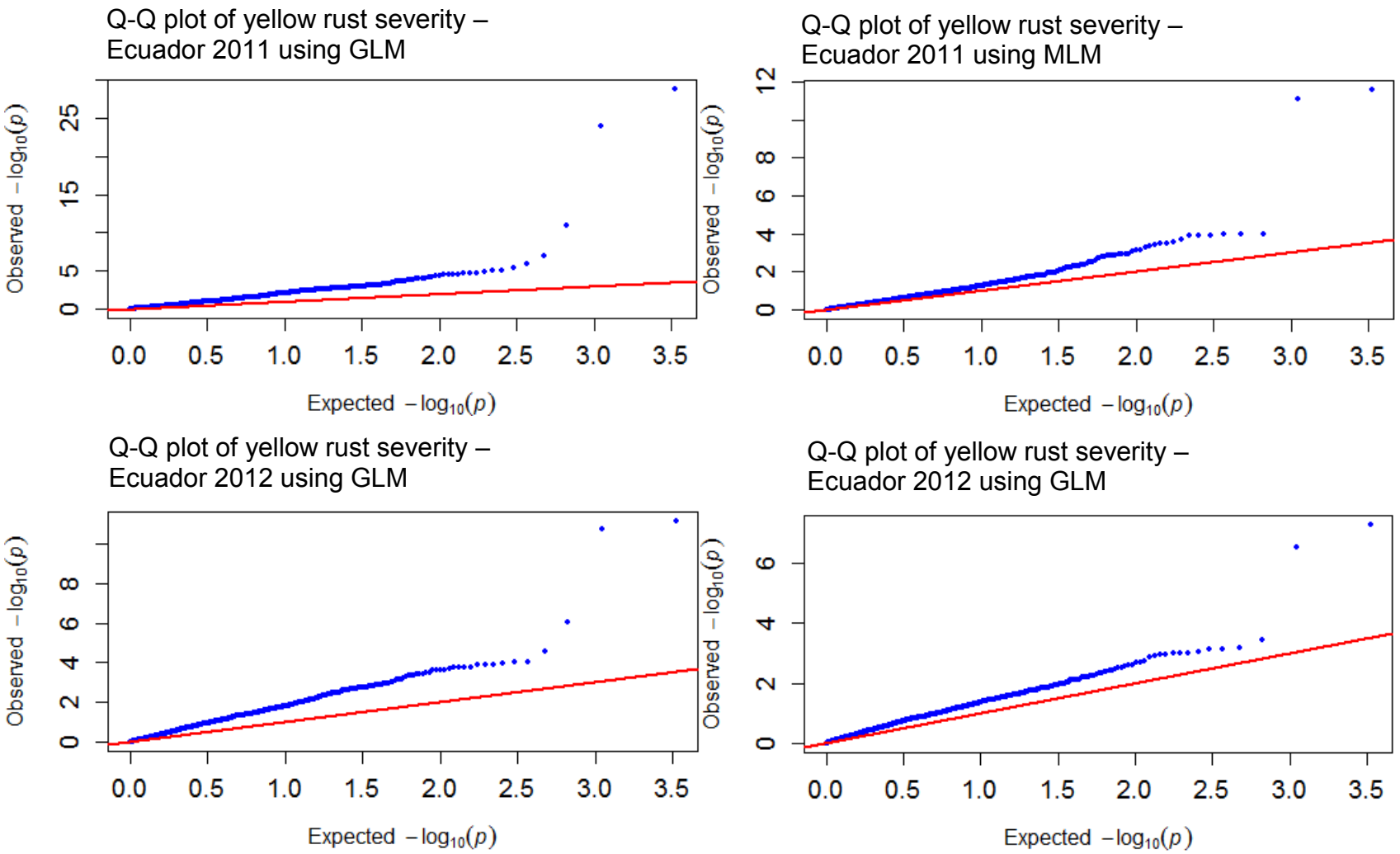

Figure 3-7. Q-Q plots of the association analysis for yellow rust severity in the wheat association mapping panel using GLM and MLM. Ecuador 2011 and 2012.

Analysis of variance of flowering time

The analysis of variance of the wheat AMP detected significant differences between accessions in Ecuador in 2011 and 2012 ($P < 0.05$) (Table 3-8). In 2011, the wheat accessions started flowering at 80 DAP and finished at 101 DAP with an average of 91.8 DAP and 21 days range. In 2012, the flowering started at 85 DAP and finished 103 DAP with an average of 94.7 DAP (Table 3-9; Figure 3-8).

The Shapiro-Wilk normality test determined that data distribution for flowering days in Ecuador was not normally distributed ($P = 0.04$).

In Mexico, the analysis of variance detected significant differences between treatments (Table 3-8). In 2011, the wheat accessions started flowering at 66 DAP and finished 85 DAP with an average of 76.7 DAP and a range of 19 days. In 2012, the wheat accessions started flowering 68 DAP and finished 93 DAP with an average of 77.5 DAP and 25 a range of 25 days (Table 3-9; Figure 3-8).

The Shapiro-Wilk normality test from the experiments carried out in combined data from Mexico 2011 and 2012 determined that data distribution was not normal ($P= 0.02$).

The analysis of correlation in the wheat AMP showed significant correlations between the two years of study in Ecuador and Mexico (Table 3-10).

Table 3-9. Analysis of variance of flowering days of the wheat association mapping panel. Ecuador 2011 – 2012.

Sources of variation	Df	Mean squares	F-value	P-value
Flowering days (Ecuador 2011-12)				
Year	1	1235	265.4	< 0.001***
Accession	296	18.95	4.1	< 0.001***
Block/Group	8	35.66	7.7	<0.001***
Error	288	4.65		
CV(%)=	2.3			
Mean (%)=	93.3			
Flowering days (Mexico 2011-12)				
Year	1	86.75	30.4	< 0.001***
Accession	296	22.5	7.9	< 0.001***
Block/Group	8	5.8	2	0.0422*
Error	288	2.9		
CV(%)=	2.2			
Mean (%)=	77.1			

Table 3-10. Flowering days of the wheat association mapping panel grown in Santa Catalina-Ecuador and El Batan-Mexico. 2011-2012.

Location	Year	Start (DAP)	End (DAP)	Average (days)	Range (days)
Santa Catalina – Ecuador	2011	80	101	91.8	21
	2012	87	103	94.7	16
El Batan – Mexico	2011	66	85	76.7	19
	2012	68	93	77.5	25

Table 3-11. Analysis of correlation (Pearson) for flowering days between the wheat association mapping panel planted in two locations and two years. Ecuador and Mexico. 2011-2012. All values were highly significant ($P < 0.001$).

	Mexico 2011	Mexico 2012	Ecuador 2011	Ecuador 2012
Mexico 2011	1			
Mexico 2012	0.77	1		
Ecuador 2011	0.46	0.47	1	
Ecuador 2012	0.3	0.35	0.57	1

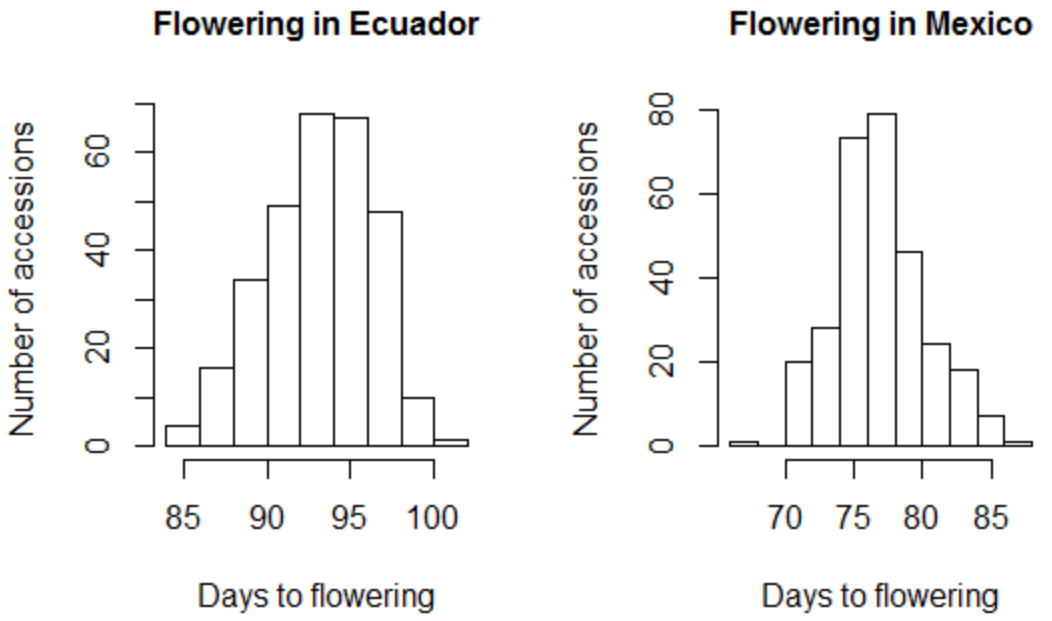

Figure 3-8. Histogram for flowering days in the Association Mapping Panel evaluated in Ecuador and Mexico. 2011 -2012.

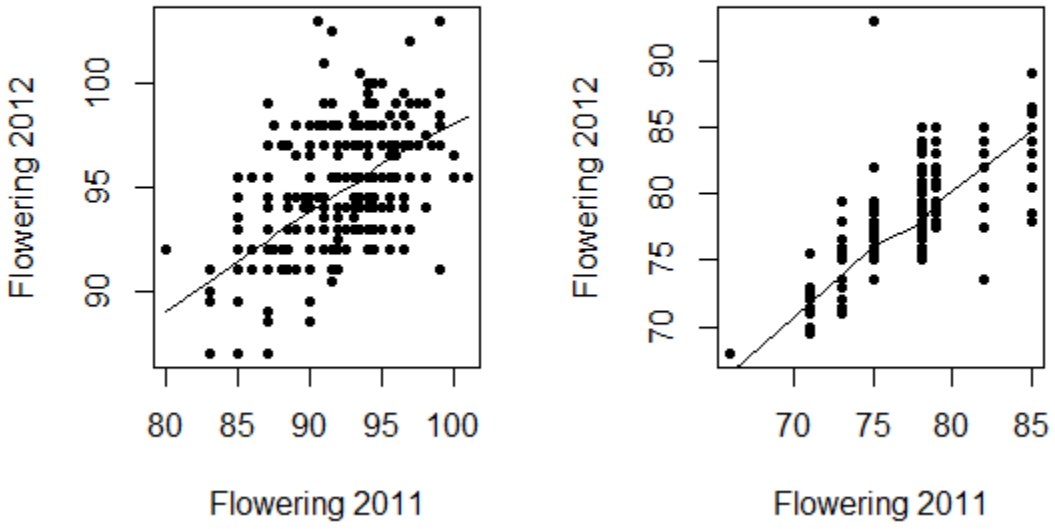

Figure 3-9. Scatter plot of flowering days of the wheat Association Mapping Panel. Ecuador and Mexico. 2011 – 2012.

Association Analysis for flowering time

The association analysis using the GLM method performed with data collected in Mexico during 2011 and 2012 in the wheat AMP detected SNP markers significantly associated with flowering time on chromosomes 3A, 5A, and 6D. On chromosome 3A, two markers located at 35 cM explained between 5.8 to 6.2% of the phenotypic variance of this trait. On chromosome 5A, there was only one SNP marker associated with flowering time. This marker was located at 146 cM and explained 6.1% of the phenotypic variance. Finally, on chromosome 6D, there were two SNP markers associated with flowering time. These markers were located at 58 cM and expalned between 6.2 and 6.5% of the phenotypic variance.

There were no SNP markers significantly associated with flowering time in Ecuador neither SNP markers significantly associated with the trait using the mixed model.

Analysis of variance of plant height

According to the analysis of variance, there were significant differences between accessions for plant height in Ecuador and Mexico (Table 3-13 and 3-8). The average plant height in Ecuador was 96.5 cm in 2011 and the average was 99.2 cm in 2012, with a overall average of 97.9 cm. The range for plant height was 75 – 125 cm in 2011 and from 80 – 125 cm in 2012 (Table 3-14; Figure 3-3; Figure 3-4).

The average plant height in Mexico 2011 was 94.3 cm with a range from 75 – 117 cm, whereas the AMP in 2012, the average in plant height was 102.7 with a range from 84 – 132 cm. The general average for plant height was 98.5 cm (Table 3-14; Figure 3-11).

Plant height in Mexico (p-value = 0.40) and Ecuador (p-value = 0.32) were normally distributed according to Shapiro-Wilk normality test.

The analysis of correlation for plant height in the wheat AMP detected significant correlation among data collected in 2011 and 2012. There were a high correlation between location and year for plant height (Table 3-15).

Association analysis for plant height

The association analysis using the general linear model (GLM) in the combined data set of Mexico 2011 and 2012 detected significant SNP markers related with plant height on chromosomes 2A, A, 7A, 2B, 6D (Table 3-16; Figure 3-12). SNP markers located on chromosome 2A were at 119 cM. This region explained from 5.5 to 5.9% of the phenotypic variation of the trait with an effect of 3.4 – 3.5 cm. The lagest effects were observed on SNP markers located on chromosome 7A at 8cM with 5 cm. No significant markers were detected in evaluations conducted in Ecuador using the GLM. Furthermore, no significant markers were located in any of the two locations using the MLM.

Discussion

Germplasm evaluation

In general, the wheat AMP has a large number of wheat accessions with high levels of disease resistance against yellow rust, especially adult plant resistance. Resistance was demonstrated with the yellow rust response in the greenhouse and the field. Adult

plant resistance genes can confer high levels of resistance near immunity (Singh et al., 2000). Analysis of the pedigrees showed that most of the wheat breeding lines in the wheat AMP have 'ATTILA', 'KAUZ', 'PASTOR' as one of its progenitor in complex crosses. These lines are not necessary the source of resistance for yellow rust in this study, but it is important to mention that these lines have been very popular in CIMMYT germplasm because they have wide range of adaptation and good agronomical and physiological traits (Rajaram et al., 2002). Some lines that may have more than one *Yr* gene are those that possess 'Quaiu' in their pedigrees, since it has been reported that this accession has *Yr54* gene (Basnet et al., 2013) and in this study almost all of these lines have high levels of disease resistance. The large number of wheat accessions in the panel with high levels of resistance to yellow rust demonstrates the value of the AMP as sources of resistance to any breeding program. This is expecially relevant because the two locations of the field evaluations are hot spots for *P. striiformis* where very aggressive races of this pathogen exist. CIMMYT has been evaluating germplasm in these two location for several years to enhance resistance (Singh et al., 2011).

Analysis of variance of yellow rust severity

Generally, the wheat breeding program relies on natural infection for wheat germplasm evaluations since environmental conditions of Santa Catalina favor YR infection and development annually (Bonjean and Angus, 2001; Dubin and Rajaram, 1996); however, in this study inoculations were carried out to ensure the infection.

The yellow rust severity data collected in the two locations where the evaluations were conducted did not follow a normal distribution. The reason for non normal distribution is

caused by the large number of wheat lines showing high resistance in the AMP. CIMMYT has been selecting for this characteristic in previous germplasm evaluation over years. The wheat accessions included in the AMP were chosen based on the diverse pedigree and segregation for disease response with the purpose to find a large number of novel alleles for disease resistance.

The high coefficient of variance (82.9%) observed in the analysis of variance of the experiments evaluated in Ecuador during 2011 and 2012 might be the result of the reduced disease pressure observed in the experiment in 2012. The overall disease severity mean in 2011 was 19.0%, whereas the overall mean in 2012 was 9.2%. The severity was higher in 2011 due to climatic conditions, since cooler temperatures and higher humidity allowed more rapid development of the disease in the susceptible wheat cultivars (McIntosh et al., 1995).

In Mexico, the overall mean in 2012 was also significantly lower than the mean in 2011. Similar to Ecuador, 2012 was a less humid year.

In general, around the 50% of the population showed resistance with a disease severity between 0 – 5%. In other to conduct further analysis, the data were transformed using root square transformation method to adjust to normality.

The correlation analysis between the two years in each location was high, however, low correlation was observed between the two locations. It was observed that some wheat accessions were susceptible in Ecuador but resistant in Mexico. These differences of disease response in each location reduced the correlation between locations and can be caused by a race specific effect of some major genes with local races. Broad sense

heritability (H^2) estimates were high in both locations. In Mexico 2011-12 the heritability for yellow rust severity (%) was 0.97 and heritability in Ecuador 2011-12 was 0.80.

Association analysis for yellow rust severity

The association analysis in the wheat AMP detected markers significantly associated with yellow rust resistance in each location and each year using GLM and MLM methods.

Genes for yellow rust resistance have been found in almost every chromosome of the wheat genome (Boyd, 2005). In this study, analyses conducted with data collected in Ecuador and Mexico detected significant SNP markers on chromosome 2A. The association analysis using the MLM method, which is a very conservative method of analysis, detected SNP markers located between 5 and 40 cM on chromosome 2A. One gene for yellow rust resistance on chromosome 2A is *Yr17* (Bariana and McIntosh, 1993). *Yr17* has been located in the short arm of chromosome 2A (Bariana and McIntosh, 1993; Jia et al., 2011), which is the region where the association analyses have detected significant markers. Interestingly, the same chromosome segment that contains *Yr17* also contains genes *Lr37* and *Sr38* which confers resistance to leaf rust and stem rust, respectively (Helguera et al., 2003). *Yr17* has been extensively used in CIMMYT's germplasm (Singh and Huerta-Espino, 2000) and it would not be surprising that these markers are linked to *Yr17*. Another well-known gene located on the long arm of chromosome 2A is *Yr1* (McIntosh and Arts, 1996). The probability that the gene associated with the SNP markers significantly associated with resistance to yellow rust detected in this study is lower since SNP markers identified in this study were located in

short arm of chromosome 2A. Additionally, races of *P. striiformis* occurring in Ecuador overcome *Yr1* (Ochoa et al., 2007) therefore phenotypic variation of resistance at this gene was unlikely in Ecuador.

Another gene that might be linked to the SNP markers detected on chromosome 2B (Mexico 2011) might be *Yr27*. The reason to make this assumption is that CIMMYT uses this gene frequently in the development of improved wheat lines. A known source of this gene is the accession 'Kauz'. This accession carries *Yr9* and *Yr27* and this accession was part of the pedigree of 58 lines in the wheat AMP. The isolates that were employed in Mexico to inoculate the susceptible cultivars overcome the resistance conferred by *Yr27* and the cultivars planted around the experiments carried *Yr27.* For this reason, the population of YR isolates was expected to be infective against *Yr27* so the QTL identified on chromosome 2B might be a different QTL or the population of YR contained isolates compatible and incompatible for *Yr27* (McDonald et al. 2004).

On chromosome 5A, the association analysis detected a significant region at 141 cM. Bariana et al. (2006) reported a gene on chromosome 5AL which confers APR. The origin of the source is the breeding line WAWHT-2046 from Australia (http://www.wheatpedigree.net/sort/show/82706). *Yr54* is another gene that has been reported on Chromosome 5AL. This gene comes from a synthetic derivative from CIMMYT's Wide Cross Program (Lowe et al., 2011). The wheat AMP includes 49 genotypes that have synthetic lines in the pedigrees, so it is not surprising that the significant region detected in this study contains *Yr54*.

Two genome regions associated with yellow rust resistance located on chromosome 7A were detected by the association analysis using both Mexio 2011 and Ecuador 2012

data using the GLM method. The region was located at 62 cM with Mexico 2011 data and at 159 – 161 cM with Ecuador 2012 data. This region is interesting since no QTLs have previously been reported on chromosome 7A (McIntosh et al., 2012).

Analysis of variance of flowering time

Accessions in the wheat AMP started flowering earlier in Mexico than Ecuador. The difference in days was expected since the wheat association mapping panel was planted at 2,640 masl at Toluca – Mexico whereas in Ecuador, the wheat panel was planted at 3,050 masl (Table 3-1). The temperatures were higher and days were warmer in Mexico as compared to Ecuador (Appendix D) which hastened the growth rate (Altenbach et al., 2003; Wiegand and Cuellar, 1980). There were statistical differences betwewen accessions for flowering days. The range observed in the two locations during the two years for flowering time (around 20 days) demonstrated that the wheat AMP includes accessions with a considerable diversity for this trait.

Association Analysis for flowering time

Three major groups of genes control flowering time in wheat. Those are photoperiod response genes (*Ppd* genes), vernalization response genes (*Vrn* genes), and developmental rate genes ('earliness per se', *Eps* genes) (Snape et al., 2001). From those, *Vrn-A1a* is located on chromosome 5A (Iwaki et al., 2002). *Vrn-A1a* gene is one of the major genes responsible for change in growth habit (spring vs. winter wheat). Itis highly conserved among spring wheat cultivars (Fu et al., 2005). It is known that *Vrn* genes contribute indirectly to yield by influencing flowering time, which makes this gene

important for plant breeders. A previous study conducted with spring wheat accessions from CIMMYT determined that *Vrn-D1* is the most frequent gene found in this specific germplasm (van Beem et al., 2005); however, no significant markers were associated with flowering time in any region on chromosome 5D.

Table 3-12. Association analysis for flowering time of the wheat association mapping panel using GLM model. Mexico and Ecuador. 2011-12.

Marker	Chr.	Pos. (cM)	p-value	r2	Allele	Allele 1	Allele 2	Effect
Mexico 2011-12								
wsnp_Ex_c33765_42199371	3A	35	7.34E-05	0.06276	A/G	77.6	75.5	2.1
wsnp_Ex_rep_c69816_68774932	3A	35	1.16E-04	0.05802	A/G	75.6	77.6	2
wsnp_BE444644A_Ta_2_2	5A	146	8.93E-05	0.06075	A/C	78.4	76.7	1.7
wsnp_Ex_c23383_32628864	6D	58	3.83E-05	0.06505	A/G	77.8	76.6	1.2
wsnp_Ex_c37749_45436366	6D	58	7.98E-05	0.06196	A/C	77.8	76.7	1.1
Ecuador 2011-12	NS							

Table 3-13. Association analysis for days to flowering of the wheat association mapping panel using MLM model. Mexico and Ecuador. 2011-12.

Marker	Chr.	Pos. (cM)	p-value	r2	Allele	Allele 1	Allele 2	Effect
Mexico 2011-12	NS							
Ecuador 2011-12	NS							

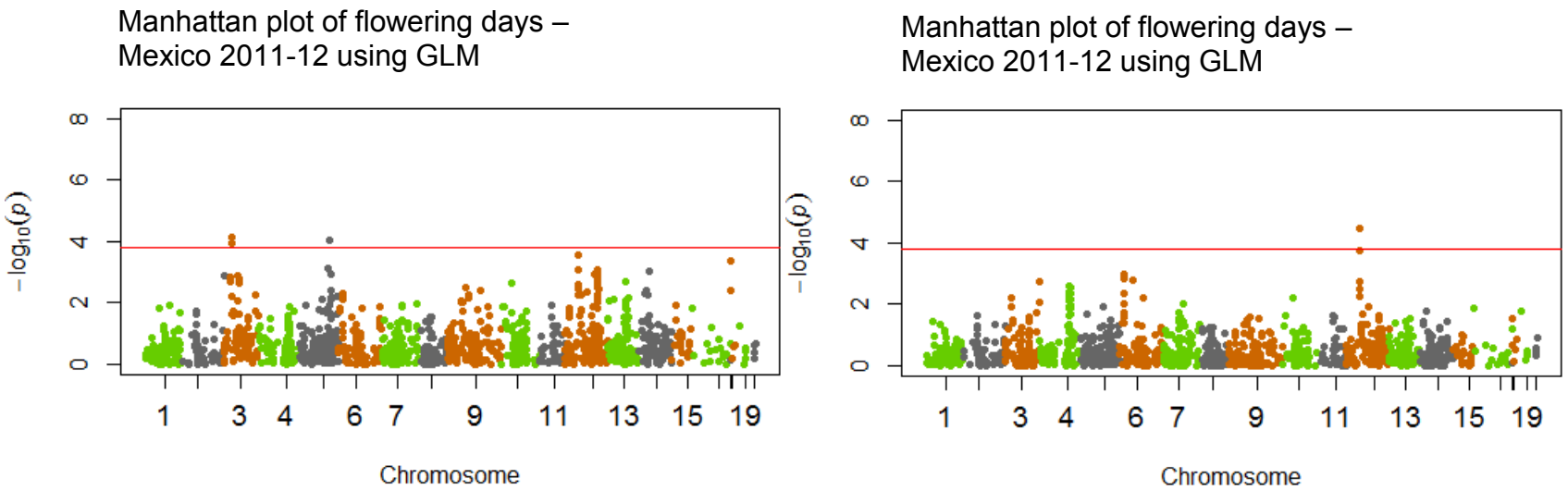

Figure 3-10. Manhattan plots of association analysis for flowering in the wheat association mapping panel using GLM (left) and MLM (right) method. Mexico 2011 and 2012.

Analysis of variance of plant height

The ANOVA detected statistical differences for plant height among accessions and also between years in the experiments evaluated in Mexico and Ecuador. Average plant height registered in Ecuador 2011 (96.5 cm) was slightly lower than the registered in 2012 (99.2 cm) and the difference (2.7 cm) was minor. However, the differences for plant height observed in Mexico 2011 (94.3 cm) versus Mexico 2012 (102.7 cm) were larger (8.4 cm). According to the literature, *Rht* genes can respond differently to different environments and plant height differences of more than 20 cm in the same genotype at different environment have been observed (Flintham et al., 1997). Irrigation and nitrogen fertilization can also have such effect on this trait (Cooper, 1980). However, the wheat plants carrying *Rht* genes tend to be always smaller than wheat genotypes without those genes, since *Rht* genes encode growth repressors that are normally suppressed by GA (Hedden, 2003). So, the differences observed for plant height in Mexico are considered normal.

Table 3-14. Analysis of variance of the wheat association mapping panel for plant height. Ecuador and Mexico 2011-12.

Sources of variation	Df	Mean squares	F-value	P-value
Plant height (Ecuador 2011-12)				
Year	1	269.4	42.3	<0.0001***
Accession	296	91.5	14.4	<0.0001***
Block/Group	16	17	2.7	0.0006**
Error	280	6.4		
CV(%)=	2.6			
Mean (cm) =	97.9			
Plant height (Mexico 2011-12)				
Year	1	9616.3	322.6	<0.0001***
Accession	296	76	2.6	<0.0001***
Block/Group	16	38.2	1.3	0.21^{ns}
Error	280	29.8		
CV(%) =	5.6			
Mean (cm) =	98.5			

Table 3-15. Mean and range for plant height of the wheat association mapping panel planted in Ecuador and Mexico. 2011-12.

Location	Year	Range (cm)	Average (cm)
Santa Catalina – Ecuador	2011	75 - 125	96.5
	2012	80 – 125	99.2
El Batan – Mexico	2011	75 – 117	94.3
	2012	84 - 132	102.7

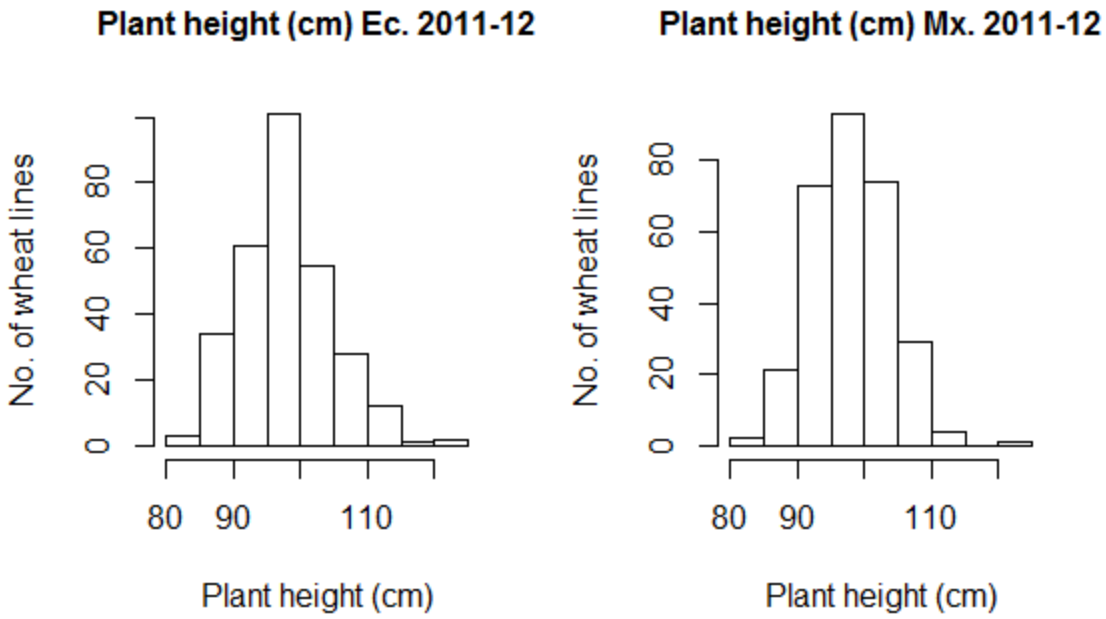

Figure 3-11. Histogram of plant heigh (cm) of the wheat AMP evaluated in Ecuador and Mexico 2011-12.

Table 3-16. Analysis of correlation (Pearson) for plant height in the wheat association mapping panel between wheat accessions in two locations and two years. Ecuador and Mexico. 2011-12. All values were highly significant (P< 0.001).

	Mex.2011	Mexico 2012	Ecuador 2011	Ecuador 2012	Ecuador 2011-12	Mexico 2011-12	Average 2011	Average 2012
Mexico 2011	1							
Mexico 2012	0.67	1						
Ecuador 2011	0.54	0.49	1					
Ecuador 2012	0.47	0.47	0.86	1				
Ecuador 2011-12	0.52	0.5	0.96	0.97	1			
Mexico 2011-12	0.92	0.91	0.57	0.52	0.56	1		
Average 2011	0.87	0.66	0.88	0.76	0.85	0.84	1	
Average 2012	0.65	0.83	0.81	0.89	0.88	0.81	0.83	1

Association analysis for plant height

In Mexico, the association analysis conducted in 2011 and 2012 using the GLM method detected one significant SNP markers related with plant height on chromosomes 2A, 4A, 7A, 2B, and 6D. A QTL has been reported on chromosome 2B (Talaat et al., 2000) with minor effects on plant height. Another QTL previously reported is located on chromosome 7A (Cadalen et al., 1998). Other plant height related genes expected to be present in the AMP population were *Rht-B1b* or *Rht-D1b*, which are known to be GA insensitive dwarfing genes and are present in the majority of the world semi-dwarf wheat lines (Flintham et al., 1997); however, the association analysis did not detect these since there was no segregation for these genes in the population.

Table 3-17. Association analysis for plant height of the wheat association mapping panel using GLM model. Mexico and Ecuador. 2011-12.

Marker	Chr.	Pos. (cM)	p-value	r2	Alleles	Allele 1 (cm)	Allele 2 (cm)	Effect (cm)
Mexico 2011-12								
wsnpCAP11_rep_c8469_3658252	2A	119	7.84E-05	0.059	A/G	95.6	99	3.4
wsnp_BF145580A_Ta_2_1	2A	119	1.48E-04	0.05518	A/G	95.3	99	3.7
wsnp_BF474615A_Ta_1_1	4A	133	2.13E-04	0.05419	A/G	98.9	96.4	2.5
wsnp_Ra_c5008_8947135	7A	8	9.15E-05	0.04774	A/G	93.8	98.8	5
wsnp_Ku_c5874_10384659	7A	8	1.37E-04	0.04558	A/C	93.8	98.8	5
wsnp_Ex_c22018_31193171	2B	112	5.96E-05	0.06218	T/C	96.5	99.7	3.2
wsnp_Ku_c7096_12264232	2B	112	2.22E-04	0.05412	T/C	100.3	97.2	3.1
wsnp_Ex_c37749_45436366	6D	58	1.46E-06	0.08451	A/G	100.7	96.8	3.9
wsnp_Ex_c23383_32628864	6D	58	9.77E-06	0.07182	A/C	100.7	96.8	3.9
Ecuador 2011-12	NS							

Table 3-18. Association analysis for plant height of the wheat association mapping panel using MLM model. Mexico and Ecuador. 2011-12.

Marker	Chr.	Pos. (cM)	p-value	r2	Alleles	Allele 1 (cm)	Allele 2 (cm)	Effect (cm)
Mexico 2011-12	NS							
Ecuador 2011-12	NS							

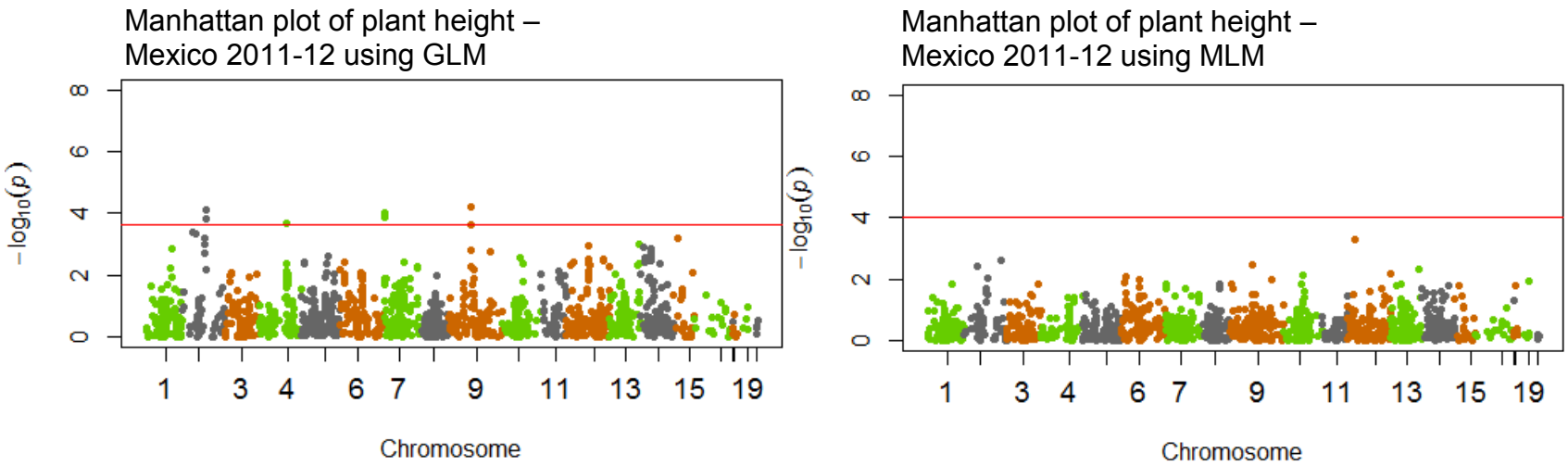

Figure 3-12. Manhattan plot of the association mapping analysis for plant height with the GLM method in the wheat association mapping population. Mexico 2011 -2012.

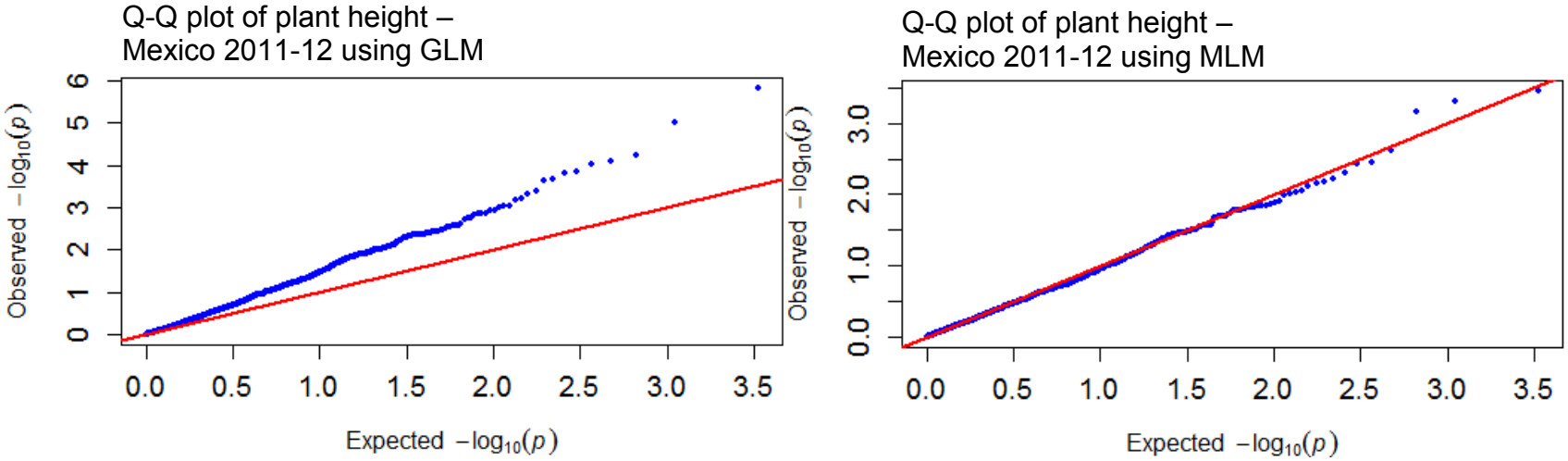

Figure 3-13. Q-Q plot for association analysis of the wheat association mapping panel for plant height. Mexico 2011 – 2012.

Conclusions

A large majority of the accessions in the wheat AMP have yellow rust resistance. The resistance was demonstrated during two years of evaluations in two locations with high disease pressure and favorable environmental conditions for disease progress. The two locations are considered as yellow rust hot spots where aggressive races of the pathogen occur. Based on the high level of resistance showed by most of the wheat accessions and the field and the greenhouse responses, the resistance of these wheat accessions appears to be conferred by several adult plant resistance genes combined in single accessions. For these reasons, we conclude that the germplasm evaluated in this study have great potential as sources of favorable alleles to develop future spring wheat populations with yellow rust resistance. Additionally, all the accessions in the wheat AMP were adapted to the two environments where the evaluations were conducted.

The association analyses detected markers significantly linked to regions responsible for yellow rust resistance. These regions could contain genes for yellow rust resistance that have been previously identified such as *Yr17*; however, these genes have been identified mostly using SSR markers. One interesting finding in this study is the discovery of new SNP markers linked to these genes. Other regions not reported previously are also valuable findins from this study. These regions have shown low effects, but it can be always useful to wheat breedeers to conduct indirect selection with molecular markers.

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APPENDICES

Appendix A: Modified Cobb's scale.

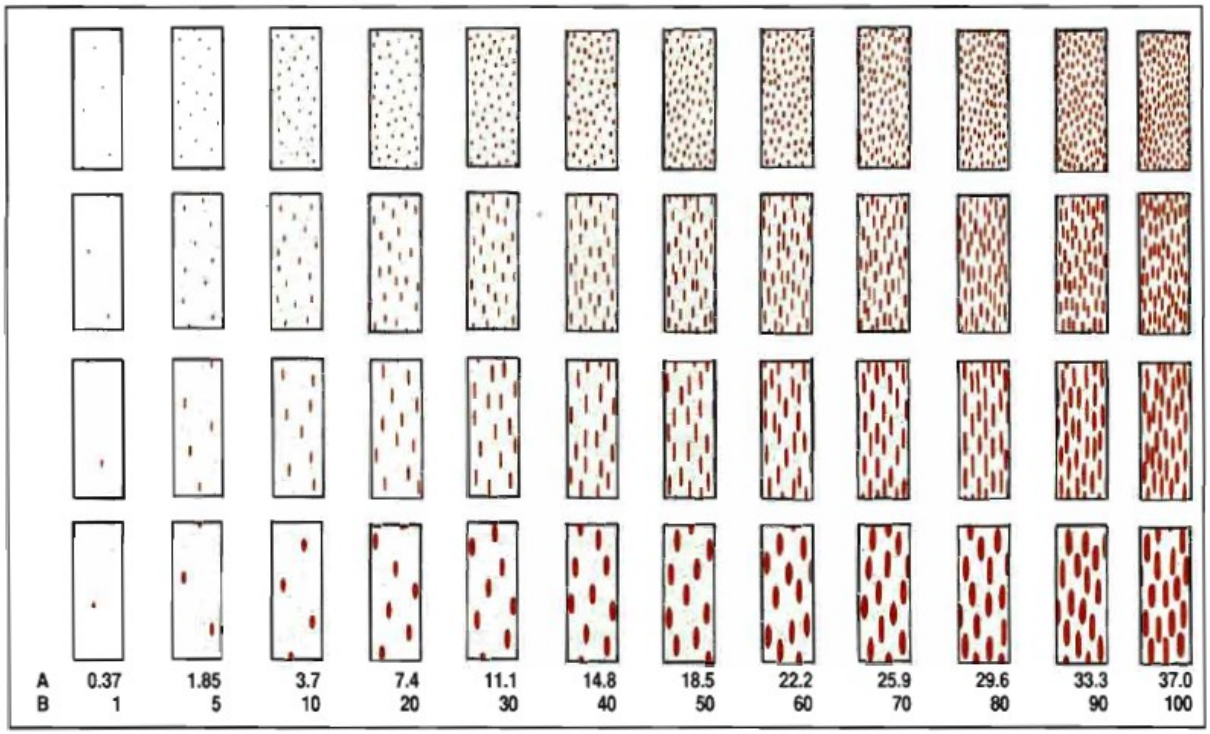

Figure 3-14. The modified Cobb's scale: A: Actual percentage occupied by rust uredinia; B: Rust severities of the modified Cobb's scale (Roelfs et al., 1992).

Appendix B: Yellow rust reaction

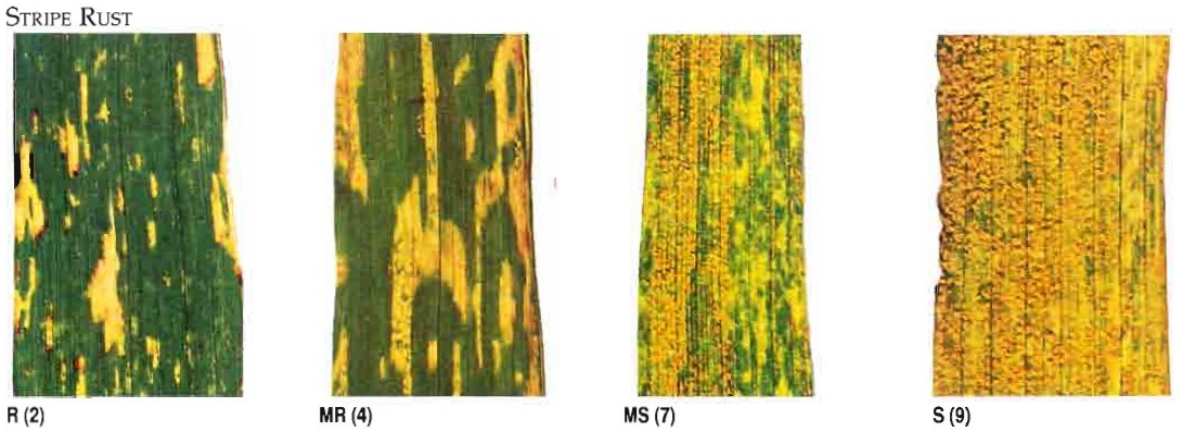

Figure 3-15. Adult plant responses to stripe rust (*P. striiformis*) (Roelfs et al., 1992).

Appendix C: Temperatures and precipitation in Ecuador and Mexico. 2011-12

Table 3-18. Temperature and precipitation data from Santa Catalina – Ecuador and Toluca Mexico during 2011-12.

Location	Year	Months	Average temp. (°C)	Temp. max. (°C)	Temp. min. (°C)	Precipitation (mm)
Santa Catalina*	2011	February	11.3	19.6	3.8	206
		March	11.2	20.5	2.6	143.7
		April	11.1	19.9	2.5	262.2
		May	12.1	21.6	2	91.7
		June	12	20.6	2.2	61.5
	2012	February	11.1	18.6	4.5	227.3
		March	12.2	20.6	5	197.4
		April	11.1	23.7	3.2	219.3
		May	11.8	19.8	4.2	62.9
		June	11.8	21.2	2.6	10.2
Toluca**	2011	Aug	15.2	21.1	9.9	113.3
		Sep	14.5	20.8	8.6	74.1
		Oct	12.2	20.5	4.6	51.6
	2012	Aug	14.8	19.9	9.9	177
		Sep	14.5	20.6	9.2	110.7
		Oct	13.2	21.6	5.4	118.3

* Data collected from the weather station of Santa Catalina Researc Station
** Data collected from the weather station located at the Lic. Adolfo López Mateos International Airport (Toluca, Mexico)
(http://weatherspark.com/history/32602/2012/Toluca-Mexico)

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CHAPTER 4

ASSOCIATION MAPPING FOR DETECTING QTLs FOR FUSARIUM HEAD BLIGHT IN BREAD WHEAT

Abstract

Fusarium head bight (FHB) caused by *Fusarium graminearum* Schwabe is one of the most important diseases in wheat due to the yield reduction, seed damage, and mycotoxins that results from the pathogen infection. Yield reduction and seed damage cause severe economic impacts, however, societal impacts caused by toxins produced by the pathogen, such as Deoxynivalenol (DON), deserve special attention. Cultivars with high levels of resistance are the most practical way to control the disease and CIMMYT has consider this disease as one of its priorities in the development of wheat germplasm with enhanced disease resistance. In the present study, a wheat association mapping panel from CIMMYT with 297 wheat accessions has been evaluated for Fusarium head blight resistance. The objectives of this study were to identify sources of resistance in the wheat AMP and conduct an association mapping study with 3,701 SNP markers incorporated in the 9K SNP wheat chip from Illumina and 32 SSR markers. The evaluations conducted in Mexico during 2011 and 2012 revealed that the wheat AMP has several wheat accessions with high FHB resistance that can be used in breeding programs focused on spring wheat. The wheat AMP showed allelic diversity for FHB resistance that come from different origins according to their pedigrees. Some of these accessions have synthetic wheat parents in its pedigrees. The association mapping studies for FHB resistance conducted with the GLM method detected SNP markers on chromosomes 4A, 7A, 2B, 5B, and 7B. When the MLM method was used,

significant markers were detected only on chromosome 2B and 7B. The association analysis also detected SNP markers associated with DON concentration on different chromosomes using the GLM method (4A, 5B, 7B, and 2D); however, no SNP markers were detected when the MLM method was used.

Introduction

Wheat (*Triticum aestivum* L.) is the most important cereal crop for human consumption as the global production of wheat almost reaches 700 million tons per year (FAOSTAT, 2012) and provides 20 % of the total dietary calories and proteins worldwide (Shiferaw et al., 2013). It has been estimated that global wheat production must increase 1.6% annually to meet the wheat demands from the growing population by 2020 (Dixon et al., 2009). However, the world production of wheat in the last two decades only increased 1.1% annually (Ortiz, 2011). It is evident that the increase in wheat production is not keeping pace with the future demand of the crop, so rapid action in the next years to increase yield potential is needed to avoid social and economic problems caused by food scarcity. One of the main causes for poor yields and increasing of the gap between potential and actual yield are wheat diseases (Bockus et al., 2010). One of the most important diseases affecting wheat production is Fusarium head bight (FHB), also known as Fusarium ear blight or scab (Dill-Macky, 2010). The major causal organism of this disease worldwide is *Gibberella zeae* (Schwein) Petch (anamorph: *Fusarium graminearum* Schwabe) (Schmale III and Bergstrom, 2003). However, there are 17 species in total associated with this disease (Parry et al., 1995). The infection of *Fusarium* on wheat causes yield reduction and

losses as high as 50% have been reported (Ireta and Gilchrist, 1994). The infection also affects wheat quality by reducing test weight, milling quality, and baking performance (Dexter et al., 1996; Dexter et al., 1997; Gilbert and Tekauz, 2000). However, the major concern with FHB is the fact that the pathogen produces secondary metabolites (mycotoxins), such as DON (Bottalico and Perrone, 2002; Placinta et al., 1999). This metabolite produces toxic effects in animals and humans (Pestka, 2010; Pestka, 2007), since it induces a spectrum of effects in farm and laboratory animals including emesis immunotoxic effects, and suppression of appetite and growth (Voss, 2010). As a consequence, strong regulations have been created in some countries, where limits for DON concentration have been established. This is the case of the United States where a maximum concentration 1000 µg/kg of DON is allowed (Richard, 2007) or no more than 750 µg/kg in the European Nations for wheat flour (van Egmond and Jonker, 2004).

Genetic resistance is considered the most practical way to control FHB disease (Bai and Shaner, 2004). The resistance to FHB has been grouped in four types based on the mechanisms used by the plant. Type I refers to resistance to initial infection, type II is used to describe resistance to fungal spread within the inoculated spike, type III refers to resistance to DON accumulation in the kernels, and type IV denotes resistance to the development of Fusarium-damaged kernels (FDK) (Schroeder and Christensen, 1963).

Quantitative trait loci (QTLs) studies to identify resistance for all four types of FHB resistance have been conducted, however, QTLs studies related to type II resistance are the most abundant in the literature (Buerstmayr et al., 2009). Discovery

of QTLs with medium to large effects or validate already reported QTLs in different genetic backgrounds can contribute to develop improved varieties with high levels of resistance to FHB. Association mapping is a novel approach which allows QTL mapping or validation in existing populations. Additionally, the QTLs detected through association mapping are associated with tightly linked SNP markers due to the dense coverage of SNP markers employed and the historical recombination exploited in breeding lines usually used to conduct such studies (Zhu et al., 2008).

The current research aims to detect QTLs for fusarium head blight in the wheat AMP using association mapping approach and evaluate the resistance against *Fusarium graminearum* in this collection of germplasm.

Materials and Methods

Plant material

A group of 297 spring wheat accessions was assembled to conduct the current study (Table 2-1). This collection of accessions will be referred to as the association mapping panel (AMP). The AMP was obtained from CIMMYT and it included breeding lines, cultivars, and landraces from different origins as well as control wheat lines used for Fusarium head blight (FHB) studies. Wheat accessions in the AMP panel were selected based on the variability for FHB response observed in previous evaluations at CIMMYT. Additionally, the AMP panel includes wheat accessions that are part of CIMMYT's elite germplasm and have showed wide adaptation, high yield, and resistance for several diseases.

Locations

The field research was conducted in El Batan – Mexico and Santa Catalina - Ecuador during 2011 and 2012. Genotyping was performed at Michigan State University (MSU), East Lansing, Michigan, USA in 2011 (Table 4-1). Phenotypic data for FHB were collected from El Batan and Santa Catalina during 2011 and 2012. At Santa Catalina Experimental Station of the National Institute for Agricultural Research (INIAP), the disease was evaluated at 3,050 masl. In Mexico, the AMP was evaluated for FHB in El Batan at 2,249 masl. Phenotypic and genotypic data analyses were conducted at MSU and CIMMYT.

Table 4-1. Locations and years of the wheat association mapping study on Yellow Rust.

Location	Years	Altitude (masl)	Type of study
East Lansing-MSU-USA	2011	262	Genotyping
Santa Catalina-INIAP-Ecuador	2011 - 2012	3,050	Field evaluation
El Batan-CIMMYT-Mexico	2011 - 2012	2,249	Field evaluation

Field management, inoculation, and phenotyping

The AMP nurseries for FHB studies were arranged in an alpha lattice design. Each plot was 1.0 m long with two rows separate with 0.25 m. Two replications of the wheat AMP for FHB were planted in Ecuador in 2011 and 2012 while one replication for FHB was sown in Mexico during 2011. In 2012, two replications for FHB evaluation were sown. The FHB nursery in Ecuador was inoculated with one *F. graminearum* isolate (SC01) collected from Santa Catalina Experimental Station. In 2011, the field was inoculated with corn seeds infected with the pathogen. The inoculum was broadcasted at rate of 50 g of infected seed/m^2. The inoculations with *F. graminearum* were performed twice, 3 and 2 weeks before the anticipated start of flowering. In 2012,

inoculum was broadcasted directly to the soil similar to 2011 and, additionally, the wheat spikes were sprayed with macroconidial suspension (50,000 spores/mL) at the rate of 50 mL per plot using 1-L hand sprayer. YR pressure in 2011 was high; therefore the FHB nursery was sprayed with Propiconazole (48.1%), which controls YR but does not control FHB (Paul et al., 2008), before flag leaf emergence to avoid or reduce rust infection.

In Mexico, plots were inoculated with five isolates of *F. graminearum* (CIMFU235, 702, 715, 720, and 770) at flowering (50% anthesis) by spraying a 30 mL macroconidial suspension of *F. graminearum* (50,000 spores/mL) using a CO_2-powered backpack sprayer (model T R&D Sprayers - Opelousas, LA) calibrated to 40 psi. A second inoculation was repeated after two days. Ten spikes from each inoculated plot were tagged to collect data. High relative humidity in the field site was maintained by a mist irrigation system which was activated for 10 min. every hour.

The FHB severity data were collected 20, 25, and 30 days after inoculation by counting spikelets showing FHB symptoms on tagged spikes. Data were transformed to percentage (FHB severity). Incidence (percentage of tagged spikes with symptoms) was also recorded at 30 days after inoculation.

At maturity, the plots were hand harvested. Spikes from each plot were air-dried in the greenhouse inside meshpolypropylene bags for 4 – 7 days. Each sample was threshed by a belt thresher Wintersteiger LD180 (Ecuador) and with a Large Vogel Plot Thresher (Mexico). In the two locations, Fusarium damaged kernels (FDK) from each plot was registered. The FDK refers to the percentage of visibly scabby kernels in a sample of seed.

From each plot, 50 – 100g sub-samples were collected. Sub-samples were ground to produce particles similar to whole wheat flour, with at least 60 % of the flour able to pass through a No. 20 sieve. A laboratory mill (Retsch ZM 200) was employed to grind the samples in Ecuador, and a coffee grinder was used at CIMMYT. Ground samples were analyzed for DON concentration at CIMMYT in the laboratory of wheat pathology with the Ridascreen® Fast DON™ (R-Biopharm) enzyme linked immuno-assay (ELISA) according to the manufacturer's instructions and at INIAP by the Laboratory of Nutrition and Quality with an Agilent 1100 series HPLC value system (Agilent Technologies) using the water extraction method in conjunction with DONPREP (R-Biopharm).

Genotyping

The genotypic data to conduct the association analysis included 3,701 SNP markers from the 9K SNP chip from Illumina®, which were selected based on good quality and MAF > 5%, and 32 microsatellites markers (SSR) distributed mostly in the D genome (20 SSRs) (See chapter II).

Statistical Analyses

Phenotypic data from 297 wheat accessions from the AMP were tested for normality using the Shapiro-Wilk normality test (Shapiro and Wilk, 1965) with the statistical package R ver.2.15.3 (Ihaka and Gentleman, 1996). Phenotypic data sets, which did not show normal distribution, were transformed using the square root method

of transformation (McDonald, 2009). Analysis of variance (ANOVA) for every trait was conducted in R with packages Agricolae version 1.1-4 and PBIB.test using REML (de Mendiburu, 2013).

A total of 3,701 SNP markers were utilized from the whole set of 8,632 SNP markers included in the 9K SNP wheat chip from Illumina®. The markers were selected based on minimum frequency of alleles ≥ 0.05 and missing data ≤10%.

Marker-trait association analyses were conducted with software TASSEL v.4.0 (http://www.maizegenetics.net/) using the general linear model (GLM), which includes population structure as co-variable, and the mixed linear model (MLM), which incorporates population structure (Q) and relative kinship (K) (Yu et al., 2006).

To estimate the population structure, a subset of 315 SNP and 22 SSR markers loosely linked and evenly distributed in the 21 wheat chromosomes were selected to be analyzed under the software STRUCTURE v. 2.3.4 (http://pritchardlab.stanford.edu/structure.html). STRUCTURE uses a Bayesian model-based clustering method which allows obtaining the optimum number of hypothetical sub-populationss and membership coefficients for each individual to create the Q matrix (Pritchard et al., 2000) that was included in the Association analysis.

The Kinship matrix, which estimates the relationships between individuals, was obtained with TASSEL using the genotypic data (Bradbury et al., 2007).

Significant markers linked to the traits were selected using false discovery rate (FDR) method described by Storey (2002). FDR analysis was conducted with R using Qvalue package version 1.0 (Dabney et al., 2004).

Marker effects were also calculated with TASSEL. It is important to note that the resulting marker effects calculated by TASSEL is not decomposed into additive and dominance effects but simply tested for overall significance (Bradbury et al., 2007).

Graphics of Q-Q plots were generated by TASSEL and Manhattan plots were generated by R using with all the p-values from each marker-trait association analysis and an R code developed by Turner (2011).

Results

Analysis of variance of Fusarium Head Blight Severity

The ANOVA for FHB severity in Mexico 2011 and 2012 detected significant differences between treatments and years (Table 4-2). In 2011, FHB severity ranged from 2.3 – 64.0% with a mean of 22.4%. In 2012 in this location, the FHB severity ranged from 1.0 – 80.0% with a mean of 9.6% (Table 4-3). Statistical analysis was not conducted for the wheat AMP in Ecuador 2011 and 2012. The reason to exclude this location from the analysis is due to the low disease pressure observed in the two years. The severity for FHB in Ecuador ranged from 0 -10% with a mean of 3.8%. Broad sense heritability of Fusarium head blight severity was H^2= 0.44.

The correlations were very low across years in Mexico (r^2 = 0.3, p-value=<0.001) (Table 4-4 and Figure 4-2).

Table 4-2. ANOVA for Fusarium Head Blight severity in the wheat association mapping panel from two years. Mexico 2011-12.

Sources of variation	Df	Mean Squares	F value	Pr(>F)
Year	1	24385.3	302.6	< 0.001***
Accession	296	143	1.8	< 0.001***
Block/Group	8	248.9	3.1	0.002**
Error	288	80.6		
CV(%)=	56.0			
Mean (%)=	16.0			
H^2= 0.44				

Table 4-3. Fusarium head blight severity in the wheat association mapping panel. Ecuador and Mexico. 2011 – 2012.

Location	Year	Range (%)	Average (%)
Santa Catalina – Ecuador	2011	0.0 – 10.0	3.8
	2012	NA	NA
El Batan – Mexico	2011	2.30 – 64.00	22.4
	2012	0.0– 80.0	9.6

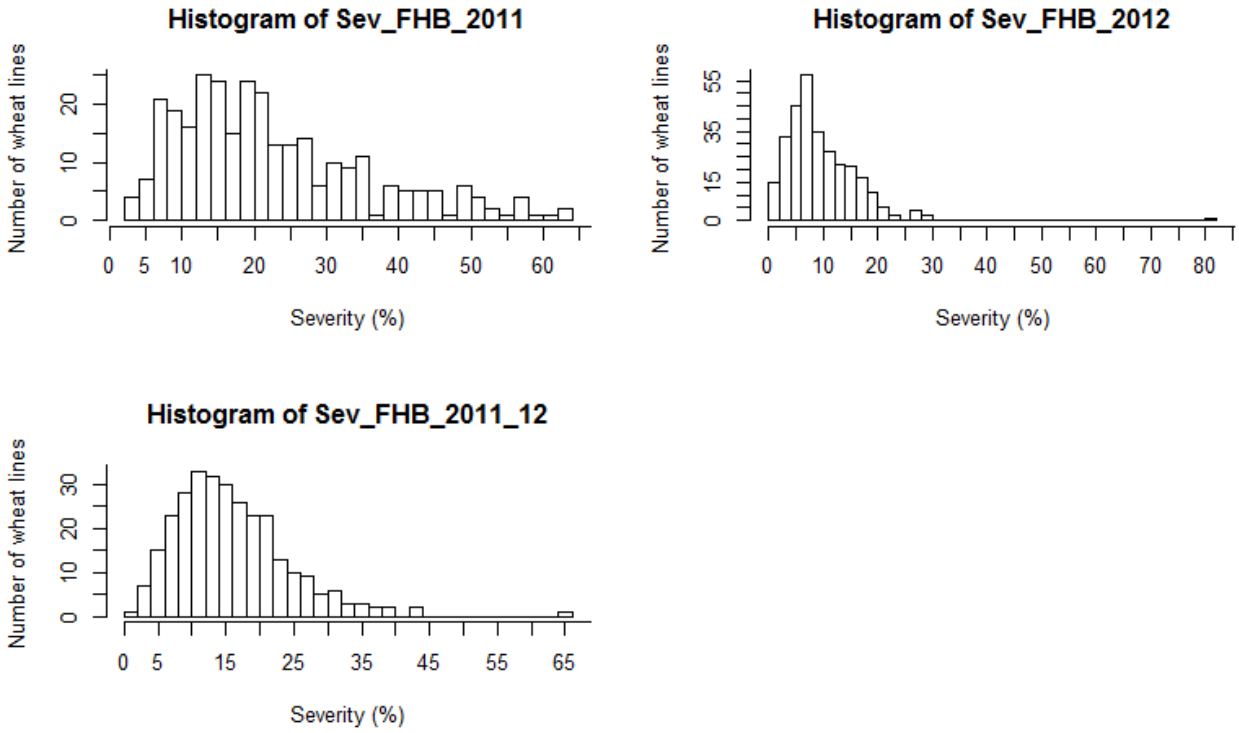

Figure 4-1. Distribution of percentage of FHB severity in the wheat AMP evaluated in Mexico 2011-12.

Table 4-4. Correlations and p-values in the Association Mapping panel between Mexico 2011 and 2012 for Fusarium Head Blight severity. Mexico 2011-12. All values were highly significant (*P*< 0.001).

	Mexico 2011	Mexico 2012	Mexico 2011-12
Mexico 2011	1		
Mexico 2012	0.3	1	
Mexico 2011-12	0.9	0.7	1

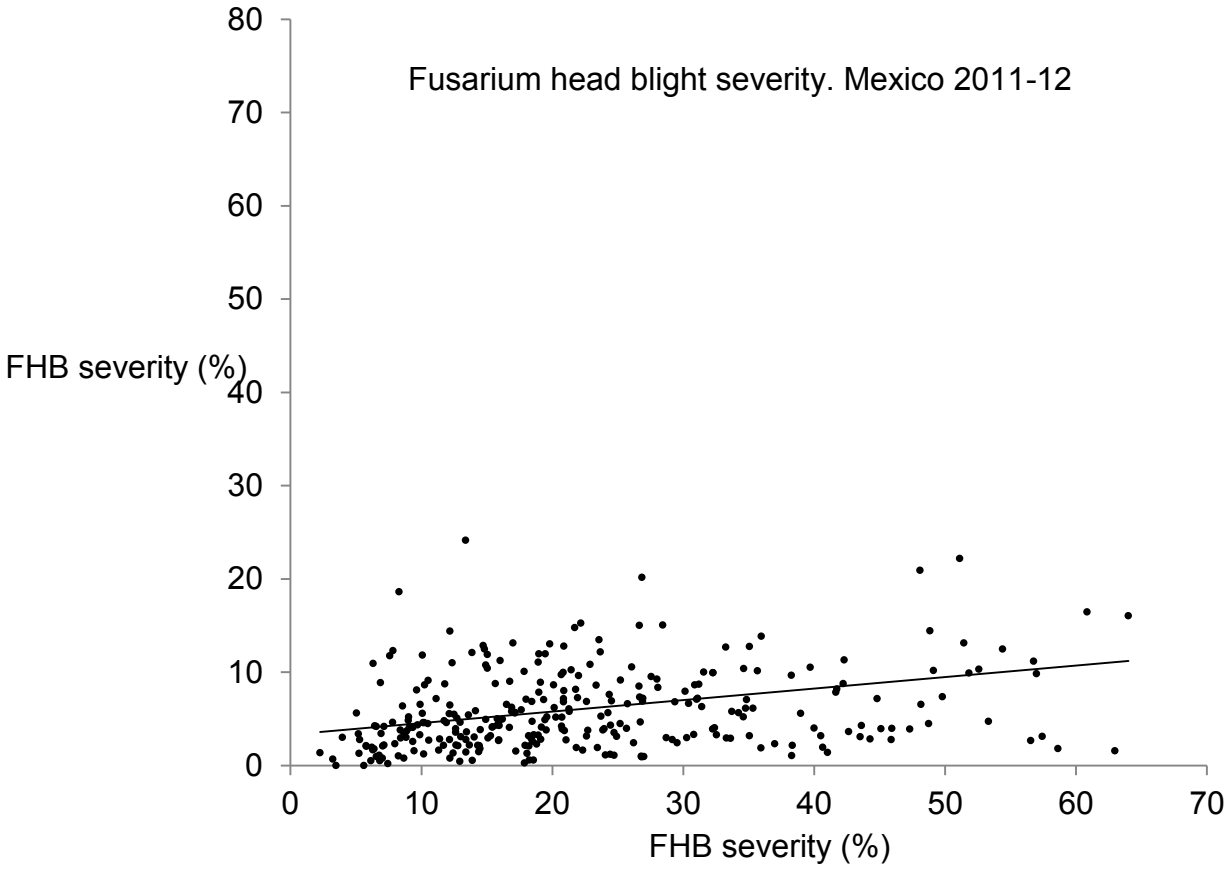

Figure 4-2. Scatter plot and regression line of FHB severity from the wheat AMP evaluated in Mexico, 2011-12.

Association analysis of Fusarium Head Blight Severity

The association analysis for FHB conducted in Mexico using the GLM method detected 59 SNP markers significantly associated with FHB resistance on chromosomes 7A, 2B, 5B, and 7B during 2011 and 31 SNP markers located on chromosomes 1A, 2A, 3A, 5A, 7A, 2B, 3B, 5B, 7B, and 2D during 2012 (Table 4-5; Figure 4-3). In 2011, the region showing the largest effect related with FHB resistance (9.5 and 12.3%) were located on chromosome 7A at 5-6 cM. At this region, SNP markers wsnp_ku_c14220_22456923 and wsnp_Ex_rep_c66939_65371026 were located. These markers explained 8.0 and 11.0% of the phenotypic variance observed

in the trait. Another region with a significant effect was located on chromosome 7B at 41-45 cM. Three markers located in this region also presented a relatively large effect for FHB resistance. The effects observed ranged from 11.1 – 12.9 % for FHB severity. These significant SNP markers were wsnp_CAP7_c90_52035, wsnp_be352570B_Ta_2_2, and wsnp_CAP8_c3593_1773371. The phenotypic variance (r2) observed for FHB severity explained in this region ranged from 5-7%.

Another region with moderate effect over FHB severity was located on chromosome 2B. Several SNP markers with significant effects were observed along this chromosome. Most SNP markers were located from 122 to 160 cM. SNP marker wsnp_BE445278B_Ta_2_1 showed the largest effect (5.9%). The phenotypic variance explained by this marker was 4%.

The largest number of SNP markers associated singnificantly with FHB severity were located on chromosome 5B with 46 SNP markers significantly associated with FHB resistance located in a region from 225 – 247 cM. The phenotypic variance explained by the regions where these SNP markers were located ranged from 6.3 – 9.8%.

The association analysis conducted with the data collected from Mexico 2012 from the wheat AMP for FHB resistance using the GLM method detected 31 SNP markers significantly associated with FHB resistance on chromosomes 1A, 2A, 3A, 5A, 7A, 2B, 3B, 5B, 7B, and 2D. All of them explained low percentages of the phenotypic variance for the trait with low effects. On chromosome 2B, four SNP markers were located at 122 – 126 cM. The phenotypic variance explained by the QTL ranged from 3.0 to 4 with effects between 5.4 to 11.1% for FHB severity. On chromosome 7B, SNP markers

associated with FHB resistance were detected at 32 - 45 cM with effects between 3.9 to 5.0% of disease severity.

The association analysis with the combined data from Mexico 2011-12 in the wheat AMP for FHB severity using the MLM method did not detected any SNP markers significantly associated with FHB resistance on any chromosome; however, the association analysis conducted with data collected from Mexico 2011 detected three SNP markers located at chromosome 7B at 41 – 51 cM. The QTL detected in this region explained from 5.0 – 8.4% of the phenotypic variance observed for FHB severity during this specific year. The association analysis conducted with data collected from Mexico 2012 detected one SNP marker associated with FHB resistance on chromosome 2B located at 126 cM. The QTL detected in this region explained 8.3% of the phenotypic variance observed for FHB severity.

Table 4-5. Association analysis for Fusarium head blight severity of the wheat association mapping panel using GLM model. Mexico. 2011-12.

Marker	Chr.	Pos.	P-value	r2	Alleles	Allele 1 (%Sev.)	Allele 2 (%Sev.)	Effect (%Sev.)
Mexico 2011								
wsnp_Ku_c14220_22456923	7A	5	2.04E-06	0.08	T/C	28,4	18,9	9.5
wsnp_Ex_rep_c66939_65371026	7A	6	7.15E-08	0.11	A/G	18,4	30,7	12.3
wsnp_Ku_c1809_3536072	7A	9	0.00266	0.04	A/G	20,9	24,9	4
wsnp_Ex_c14219_22169892	7A	11	5.57E-04	0.05	A/G	25,9	18,6	7.3
wsnp_Ex_rep_c66476_64726880	7A	13	2.58E-05	0.07	T/C	19,3	26,9	7.6
wsnp_JD_c6179_7344980	7A	16	8.12E-04	0.05	T/C	18,2	24,6	6.4
wsnp_BG313770A_Ta_2_1	7A	20	5.57E-06	0.07	T/C	26,4	19,1	7.3
wsnp_BG313770A_Ta_2_3	7A	20	9.19E-06	0.07	A/G	19,2	26,5	7.3
wsnp_Ku_rep_c105954_91953127	7A	57	9.48E-04	0.04	T/G	21	25,8	4.8
wsnp_Ex_c7776_13247654	2B	5	0.00424	0.03	T/C	19,1	23,5	4.4
wsnp_Ra_c16822_25566950	2B	73	1.21E-04	0.06	A/G	21	25,5	4.5
wsnp_Ku_c13905_22034406	2B	73	3.09E-04	0.05	A/C	20,1	25,7	5.6
wsnp_CAP8_c303_286918	2B	122	5.04E-04	0.05	T/G	22,1	24,5	2.4
wsnp_Ra_c2842_5399988	2B	126	6.79E-05	0.06	T/C	27,7	21,9	5.8
wsnp_BF291736B_Ta_1_1	2B	126	2.29E-04	0.05	T/C	21,9	26,9	5
wsnp_CAP11_c5474_2542512	2B	126	2.32E-04	0.04	A/G	22	25,3	3.3
wsnp_BE445278B_Ta_2_1	2B	126	3.29E-04	0.04	A/G	21,8	27,7	5.9
wsnp_BE445278B_Ta_2_3	2B	126	6.45E-04	0.04	A/G	22	24,9	2.9
wsnp_Ex_c38739_46195930	2B	126	0.00133	0.03	T/C	21,9	32,9	11
wsnp_Ku_c3000_5638635	2B	160	2.73E-04	0.05	A/G	23,9	18,1	5.8
wsnp_Ku_c3102_5811860	5B	97	3.49E-04	0.05	A/G	24	16,6	7.4
wsnp_Ku_c3102_5810751	5B	97	6.05E-04	0.05	T/C	16,3	24,1	7.8
wsnp_Ex_rep_c67690_66354931	5B	163	3.37E-06	0.08	A/G	21,1	24,7	3.6
wsnp_Ex_c48257_53217539	5B	163	1.92E-05	0.07	T/C	26,4	20,9	5.5
wsnp_Ex_c38105_45710671	5B	163	9.27E-04	0.04	A/G	26,3	20,7	5.6
wsnp_Ex_c4826_8610827	5B	164	7.80E-07	0.09	A/G	20,7	25,3	4.6
wsnp_Ku_c8270_14083963	5B	164	3.35E-06	0.08	A/G	20,7	26,3	5.6

Table 4-5 (cont'd)

wsnp_CAP8_c1594_914839	5B	164	1.20E-05	0.07	A/G	28,4	20,7	7.7
wsnp_BE606403B_Ta_2_1	5B	164	4.72E-05	0.06	T/C	21	23,7	2.7
wsnp_Ex_rep_c108314_91592072	5B	164	4.47E-04	0.05	A/G	20,7	28,2	7.5
wsnp_Ku_c28491_38419391	5B	166	3.54E-05	0.06	T/C	20,9	26,8	5.9
wsnp_Ex_c39535_46808105	5B	166	4.07E-05	0.06	A/C	21	25,5	4.5
wsnp_Ku_c15630_24304954	5B	167	4.52E-04	0.05	A/G	19,7	26	6.3
wsnp_Ex_c7469_12780118	5B	168	1.11E-04	0.06	T/C	29,4	20,1	9.3
wsnp_Ex_c1938_3656802	5B	168	1.21E-04	0.06	T/C	21	28,4	7.4
wsnp_Ku_c1661_3262505	5B	168	1.40E-04	0.05	T/C	21,2	28,4	7.2
wsnp_Ex_c49809_54305634	5B	168	1.45E-04	0.05	A/C	28,4	21,2	7.2
wsnp_Ex_c658_1293780	5B	168	1.50E-04	0.05	A/G	28,4	21,2	7.2
wsnp_Ku_c1661_3262637	5B	168	1.64E-04	0.05	A/C	28,4	21,1	7.3
wsnp_Ex_c658_1294440	5B	168	1.67E-04	0.05	T/G	28,4	21,1	7.3
wsnp_BF473658B_Ta_2_1	5B	168	1.69E-04	0.05	T/C	21,2	28,4	7.2
wsnp_Ex_c658_1295291	5B	168	1.85E-04	0.05	T/C	20,1	29,4	9.3
wsnp_Ku_c23836_33776356	5B	168	3.03E-04	0.05	A/G	28	21,2	6.8
wsnp_Ex_c658_1294003	5B	168	3.61E-04	0.05	T/C	28,4	21,1	7.3
wsnp_Ku_c57172_60417550	5B	168	4.02E-04	0.05	A/G	28,1	21,1	7
wsnp_Ex_c7173_12319519	5B	168	4.29E-04	0.05	A/C	21,2	28,4	7.2
wsnp_Ex_rep_c67549_66173636	5B	168	8.11E-04	0.04	A/G	21,1	27,6	6.5
wsnp_Ex_c5217_9237399	5B	169	1.78E-04	0.05	T/C	21,2	28,4	7.2
wsnp_Ex_rep_c66921_65344887	5B	170	2.84E-04	0.06	A/G	26,1	19,4	6.7
wsnp_BQ166999B_Ta_2_1	5B	174	9.56E-05	0.06	T/G	19,4	25,5	6.1
wsnp_Ex_c20988_30107609	5B	174	0.00133	0.04	A/G	27,2	19,9	7.3
wsnp_Ku_c11721_19085513	5B	175	7.67E-05	0.06	A/G	26,1	19,4	6.7
wsnp_Ra_c13646_21523723	5B	176	3.10E-05	0.06	A/G	20,1	25,5	5.4
wsnp_Ex_c13496_21243167	5B	178	4.12E-04	0.05	A/G	19,7	27,3	7.6
wsnp_Ku_rep_c103274_90057407	5B	213	0.00141	0.04	A/G	21,4	25,2	3.8
wsnp_CAP7_c90_52035	7B	41	1.54E-04	0.05	T/C	21,2	32,3	11.1
wsnp_be352570B_Ta_2_2	7B	45	2.09E-06	0.08	T/C	21,1	33,4	12.3

Table 4-5 (cont'd)

wsnp_CAP8_c3593_1773371	7B	45	4.84E-06	0.07	T/C	21	33,9	12.9
wsnp_Ex_c2539_4733110	7B	51	0.0017	0.04	A/G	20,2	26,7	6.5
Mexico 2012								
wsnp_Ex_rep_c66562_64849366	1A	71	0.00271	0.03	T/C	10.4	8.9	1.5
wsnp_Ex_c1767_3341220	2A	79	4.43E-04	0.03	A/G	13.3	8.8	4.5
wsnp_be498599A_Ta_2_2	2A	93	0.00447	0.00	A/G	8.9	13.4	4.5
wsnp_BE406351A_Ta_2_2	2A	113	8.65E-04	0.03	T/C	14.2	9.1	5.1
wsnp_BE403597A_Ta_2_1	2A	116	9.14E-04	0.02	A/G	14.2	9.1	5.1
wsnp_Ex_c28204_37349164	2A	119	0.00147	0.02	T/C	13.7	8.9	4.8
wsnp_Ex_rep_c69816_68774932	3A	35	0.0034	0.02	A/G	7.5	10.3	2.8
wsnp_JD_c940_1381378	5A	184	0.00209	0.04	T/G	9.9	11.6	1.7
wsnp_BG313770A_Ta_2_3	7A	20	0.00414	0.01	A/G	8.9	10.6	1.7
wsnp_Ra_c250_526345	7A	82	0.0015	0.02	A/G	14.8	9	5.8
wsnp_Ra_c26491_36054023	7A	105	0.00422	0.04	A/G	17.2	9.2	8
wsnp_Ku_c13905_22034406	2B	73	5.09E-05	0.02	A/G	8.4	12.5	4.1
wsnp_BF202681B_Ta_2_2	2B	94	0.00392	0.03	A/C	13.8	8.9	4.9
wsnp_CAP8_c303_286918	2B	122	1.44E-04	0.04	T/G	8.9	14.3	5.4
wsnp_Ra_c2842_5399988	2B	126	3.27E-05	0.03	T/C	9	20.1	11.1
wsnp_Ex_c46576_52042185	2B	167	9.41E-05	0.01	T/C	10	8.3	1.7
wsnp_Ku_c48694_54811376	2B	220	1.65E-04	0.02	T/C	14.6	9	5.6
wsnp_Ex_c11246_18191079	3B	63	4.14E-04	0.02	A/C	5	10	5
wsnp_Ex_c4888_8713275	3B	70	0.00339	0.03	A/G	10.2	7.4	2.8
wsnp_JD_c5067_6187376	3B	83	3.07E-04	0.02	T/C	9.1	14.9	5.8
wsnp_Ex_rep_c108114_91468537	3B	100	3.17E-04	0.02	T/C	9.1	11.8	2.7
wsnp_Ex_c3130_5789888	3B	132	9.71E-06	0.01	T/C	8.8	11.4	2.6
wsnp_Ra_c69_149394	3B	132	4.69E-05	0.02	T/C	11.1	8.9	2.2
wsnp_BE443187B_Ta_2_1	5B	146	0.0018	0.01	A/C	11.3	9.2	2.1
wsnp_Ex_c48257_53217539	5B	163	2.86E-04	0.02	T/C	11.5	9.1	2.4
wsnp_Ex_rep_c67549_66173636	5B	168	2.63E-05	0.04	A/G	13	9	4

Table 4-5 (cont'd)

wsnp_Ex_c658_1293780	5B	168	3.50E-05	0.02	A/C	13.2	9	4.2
wsnp_Ex_c17882_26646153	7B	32	8.32E-05	0.01	T/C	9.2	14.2	5
wsnp_BF474552B_Ta_1_1	7B	32	8.56E-05	0.01	A/G	14.2	9.2	5
wsnp_CAP8_c3593_1773371	7B	45	0.00386	0.04	T/C	9.2	13.1	3.9
wsnp_Ku_c8712_14751858	2D	139	6.88E-05	0.04	T/C	10.1	6.1	4

Table 4-6. Association analysis for fusarium head blight severity of the wheat association mapping panel using MLM model. Mexico. 2011-12.

Marker	Chr.	Position (cM)	P-value
Mexico 2011-12	NS		
Mexico 2011			
wsnp_Ex_c11860_19030807	7B	74	8.74E-06
wsnp_RFL_Contig3854_4205716	7B	78	7.81E-05
wsnp_RFL_Contig2167_1484520	7B	85	1.81E-05
Mexico 2012			
wsnp_Ex_c55735_58127324	2B	242	5.00E-06

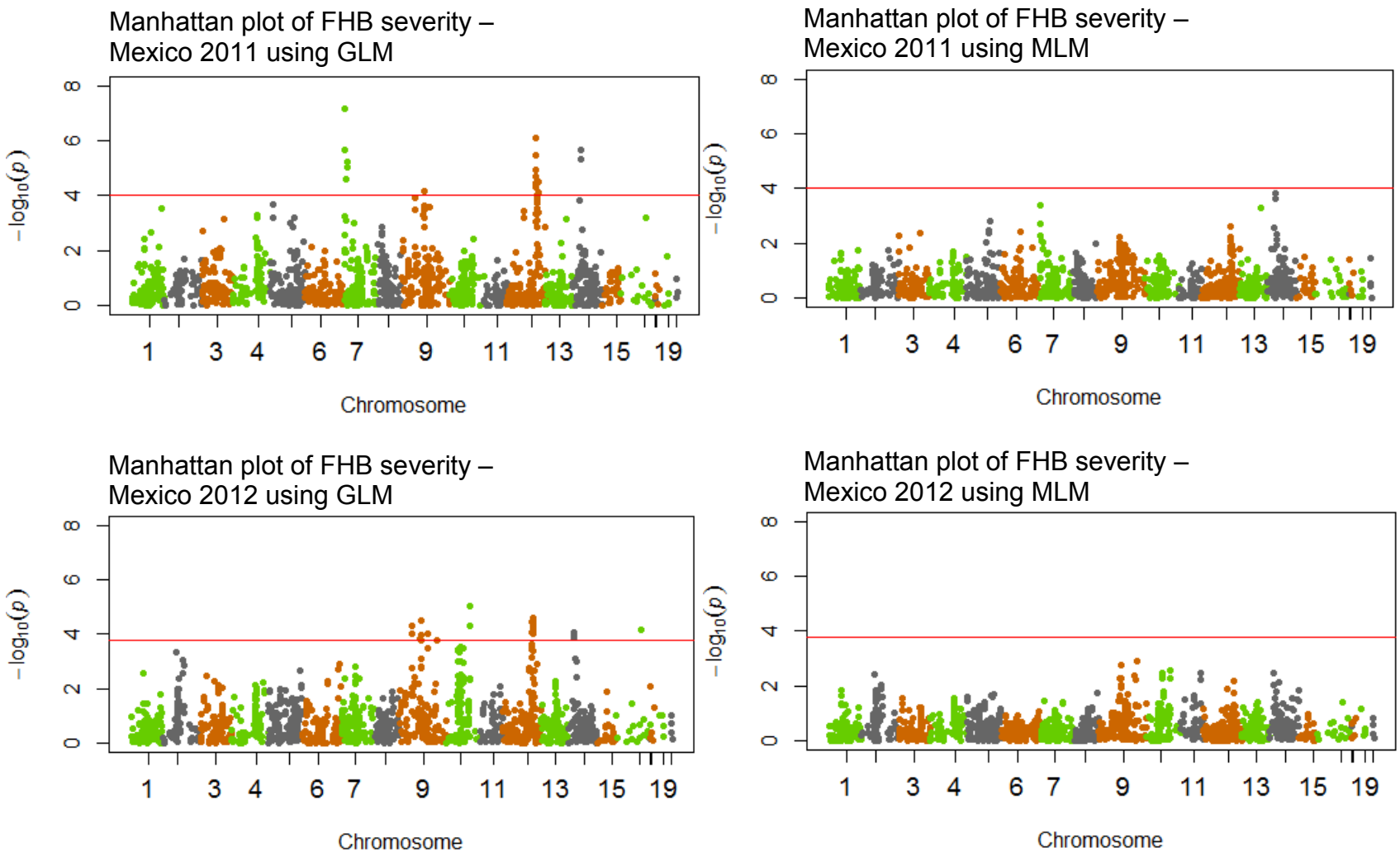

Figure 4-3. Manhattan plots of the association analysis for Fusarium head blight severity in the wheat association mapping panel using GLM and MLM. Mexico 2011 and 2012.

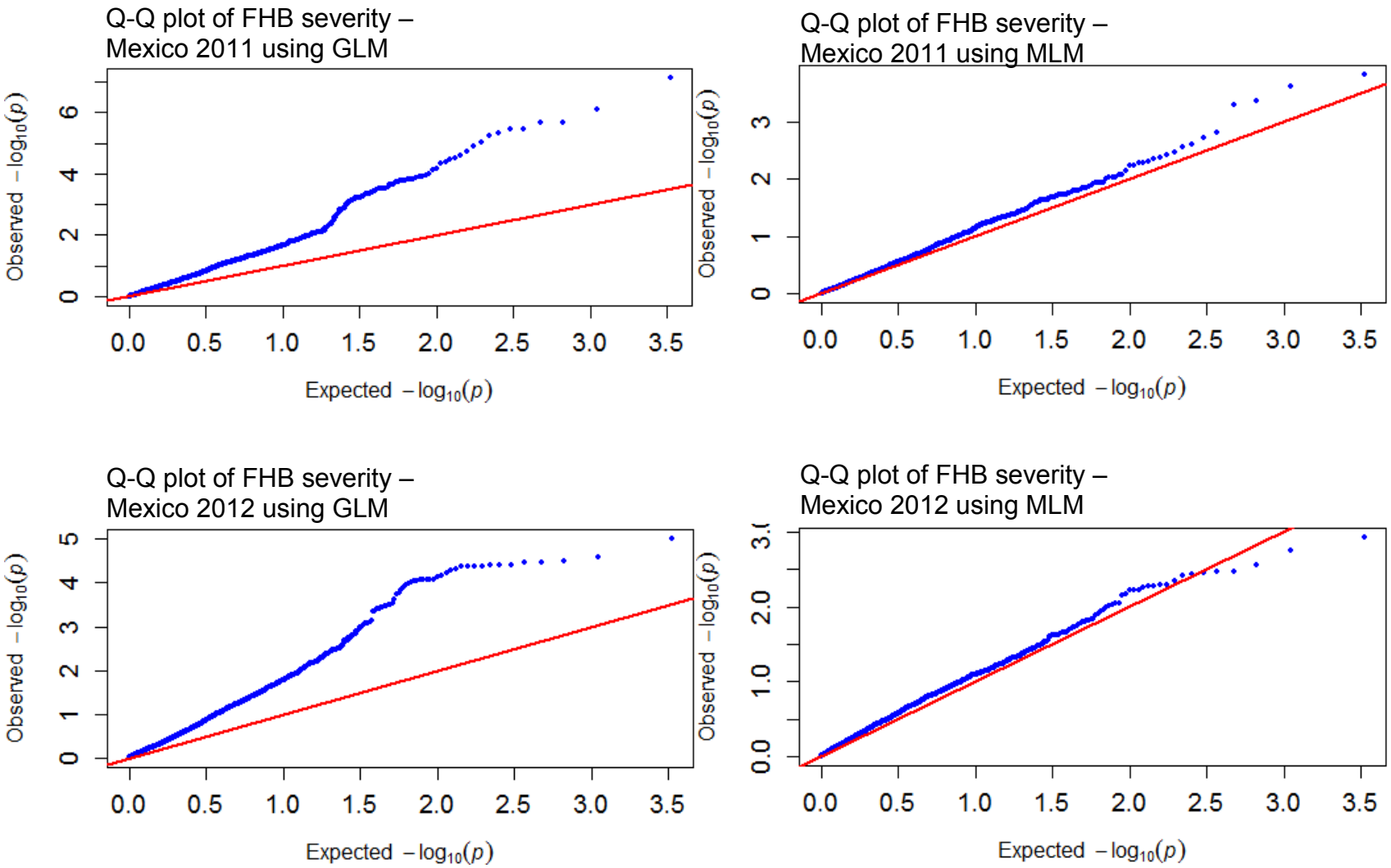

Figure 4-4. Q-Q plots of the association analysis for fusarium head blight severity in the wheat association mapping panel using GLM and MLM. Mexico 2011 and 2012.

Germplasm evaluation

The wheat AMP includes wheat accessions with high levels of resistance to FHB. In table 4-7, the top 25 accessions showed reduced percentage of severity (<7.0%), evaluated in two years under high disease pressure and adequate environmental conditions provided at El Batan. The Structure analysis (Chapter II) separated the wheat accessions in three sub-populationss. From the top 25 FHB resistant genotypes, there were 10 genotypes from sub-population 1, 10 genotypes from sub-populations 2, and five genotypes from sub-populations 3. In the other hand, from the bottom 25, most of the susceptible wheat accessions were assigned to sub-population 1 and 3, with eight and 11 accessions respectively. In the case of sub-population 2, six wheat accessions were located in the bottom 25.

Table 4-7. Top 25 and bottom 25 accessions based on FHB severity (%) in the wheat AMP with sub-populations classification. Mexico, 2011-12.

Acc. Number	Pedigree	FHB 2011 Severity (%)	FHB 2012 Severity (%)	Sub-population
	Top 25			
250	ATTILA/HEILO (b)	2	1	3
157	SUMAI #3	3	1	1
181	WBLL1/FRET2//mazar 99*2/3/GONDO	3	1	2
131	KAUZ//ALTAR 84/AOS/3/MILAN/KAUZ/4/HUITES/5/SHA3/SERI//SHA4/LIRA/6/KAUZ//ALTAR 84/AOS/3/MILAN/KAUZ/4/HUITES	4	3	131
213	SHA3/CBRD//TNMU/5/KAUZ//ALTAR 84/AOS/3/MILAN/KAUZ/4/HUITES	5	3	213
219	PRL/2*mazar 99//SRTU/3/PRINIA/PASTOR	5	3	2
249	ATTILA/HEILO (a)	5	1	3
118	FRANCOLIN #1/4/BABAX/LR42//BABAX*2/3/KURUKU	6	2	3
152	WBLL1*2/TUKURU//KRONSTAD F2004	6	1	3
167	WBLL1*2/4/YACO/PBW65/3/KAUZ*2/TRAP//KAUZ/5/GONDO	6	2	2
199	CBRD/FILIN	6	2	2
210	KETUPA*2/mazar 99/6/TURACO/5/CHIR3/4/SIREN//ALTAR 84/AE.SQUARROSA (205)/3/3*BUC/7/KAUZ//ALTAR 84/AOS/3/MILAN/KAUZ/4/HUITES	6	2	1
223	WBLL1*2/4/SNI/TRAP#1/3/KAUZ*2/TRAP//KAUZ/5/KAUZ//ALTAR 84/AOS/3/MILAN/KAUZ/4/HUITES	6	2	3
232	NG8675/CBRD//MILAN/7/CAL/NH//H567.71/3/SERI/4/CAL/NH//H567.71/5/2*KAUZ/6/mazar 99/8/CNDO/R143//ENTE/MEXI_2/3/AEGILOPS SQUARROSA (TAUS)/4/WEAVER/5/2*mazar 99	6	4	2
244	CPI8/GEDIZ/3/GOO//ALB/CRA/4/AE.SQUARROSA (208)/5/HAHN/2*WEAVER/6/SKAUZ/BAV92	6	1	2

Table 4-7 (cont'd)

132	PBW343/PASTOR*2/6/TURACO/5/CHIR3/4/SIREN//ALTAR 84/AE.SQUARROSA (205)/3/3*BUC	7	4	1
146	CHIBIA/WEAVER/5/KAUZ//ALTAR 84/AOS/3/MILAN/KAUZ/4/HUITES	7	2	1
148	C80.1/3*BATAVIA//2*WBLL1/3/TOBA97/PASTOR	7	3	1
154	WHEAR/2*KRONSTAD F2004	7	1	1
160	GONDO/CBRD	7	1	2
162	PICUS/3/KAUZ*2/BOW//KAUZ/4/KKTS/5/HEILO	7	4	2
173	KAUZ//ALTAR 84/AOS/3/MILAN/KAUZ/4/HUITES/5/CROC_1/AE.SQUARROSA (205)//KAUZ/3/SASIA/6/KAUZ//ALTAR 84/AOS/3/MILAN/KAUZ/4/HUITES	7	1	1
182	PFAU/WEAVER*2//BRAMBLING/7/IVAN/6/SABUF/5/BCN/4/RABI//GS/CRA/3/AE.SQUARROSA (190)/8/PFAU/WEAVER//BRAMBLING	7	1	1
216	HEILO/7/IVAN/6/SABUF/5/BCN/4/RABI//GS/CRA/3/AE.SQUARROSA (190)/8/VORB/FISCAL	7	2	2
248	NING MAI 96035/FINSI//HEILO	7	1	2
	Bottom 25			
76	CAL/NH//H567.71/3/SERI/4/CAL/NH//H567.71/5/2*KAUZ/6/PASTOR/7/ACHTAR*3//KANZ/KS85-8-4/8/CAL/NH//H567.71/3/SERI/4/CAL/NH//H567.71/5/2*KAUZ/6/PASTOR	35	10	1
91	KBIRD//WBLL1*2/KURUKU	35	13	3
122	TACUPETO F2001//WBLL1*2/KKTS/3/WBLL1*2/BRAMBLING	36	10	3
134	FRANCOLIN #1/KIRITATI	36	14	3
28	QUAIU #1	38	10	1
54	INQALAB 91*2/KUKUNA*2//PVN	40	11	3

Table 4-7 (cont'd)

57	WAXWING/2*ROLF07	42	9	1
67	ATTILA*2/PBW65*2/4/BOW/NKT//CBRD/3/CBRD	42	8	3
98	ATTILA*2/PBW65//MUU #1/3/FRANCOLIN #1	42	11	3
277	SOKOLL//SUNCO/2*PASTOR	42	8	2
39	WBLL1*2/CHAPIO//HEILO	45	7	2
102	MUU #1//PBW343*2/KUKUNA/3/MUU	48	21	1
20	PBW343*2/KUKUNA/3/PGO/SERI//BAV92	49	10	3
46	BAV92//IRENA/KAUZ/3/HUITES*2/4/MILAN/KAUZ//CHIL/CHUM18	49	14	1
87	FRANCOLIN #1/5/HE1/3*CNO79//2*SERI/3/ATTILA/4/WH 542	50	7	3
159	FALCIN/AE.SQUARROSA (312)/3/THB/CEP7780//SHA4/LIRA	51	22	3
275	MILAN/DUCULA//SUNCO/2*PASTOR	51	13	2
53	FINSI/METSO//FH6-1-7/3/FINSI/METSO	52	10	2
158	GAMENYA	52	80	1
289	CROC_1/AE.SQUARROSA (205)//BORL95/3/KENNEDY/6/CNDO/R143//ENTE/MEXI_2/3/AEGILOPS SQUARROSA (TAUS)/4/WEAVER/5/2*JANZ	53	10	3
107	CONI#1/6/2*HPO/TAN//VEE/3/2*PGO/4/MILAN/5/SSERI1	54	13	3
58	WBLL1*2/5/CNO79//PF70354/MUS/3/PASTOR/4/BAV92	57	11	1
294	SOKOLL/FRAME	57	10	1
77	BAV92//IRENA/KAUZ/3/HUITES*2/4/YUNMAI 47	61	16	2
110	MUU/5/TRAP#1/BOW/3/VEE/PJN//2*TUI/4/BAV92/RAYON/6/MILAN/S87230//BAV92	64	16	2

Analysis of variance of Deoxinivalenol concentration

The ANOVA for DON concentration in Mexico detected significant differences among accessions in each year (Table 4-8). The mean was 3.8 ppm. The average for DON concentration in 2011 was 5.0 ppm and 2.6 ppm in 2012. In 2011, the DON concentration ranged from 0.2 – 16.3 ppm, meanwhile, in 2012 the DON concentration ranged from 0.1 – 12.7 ppm. The coefficient of variance was CV= 53.8%. Broad sense heritability of DON concentration was H^2= 0.51.

The data distribution showed skewedness to the left to low levels (Figure 4-5). The correlation on the two years of experiments on Mexico was 0.3 (Table 4-10).

Table 4-8. ANOVA for DON concentration of 297 wheat accessions in two years. Mexico 2011-12.

Sources of variation	Df	Mean Squares	F value	P-value
Year	1	810.1	193.0	<0.0001***
Accession	296	8.6	2.1	<0.0001***
Block/Group	8	29.8	7.1	<0.0001***
Error	288	4.2		
CV(%)=	53.8			
Mean (%)	3.8			
H^2= 0.51				

Table 4-9. DON concentration in the wheat Association mapping panel. Mexico, 2011-12.

Location	Year	Range (%)	Average (%)
El Batan – Mexico	2011	0.2 - 16.3	5
	2012	0.1 - 12.7	2.6

Table 4-10. Correlations and p-values in the wheat Association Mapping panel between Mexico 2011 and 2012 for DON concentration. Mexico 2011-12. All values were highly significant (P< 0.001).

	Mexico 2011	Mexico 2012	Mexico 2011-12
Mexico 2011	1		
Mexico 2012	0.29	1	
Mexico 2011-12	0.87	0.73	1

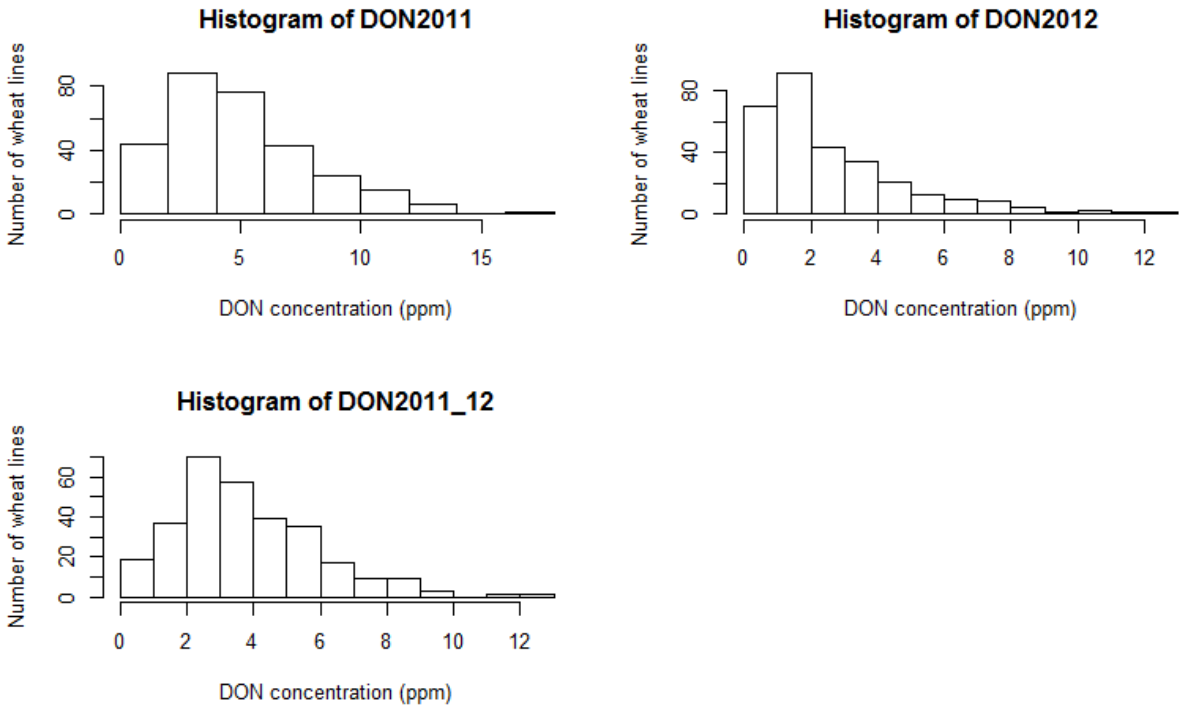

Figure 4-5. Distribution of DON concentration in the wheat AMP evaluated in Mexico 2011-12.

Association analysis for DON concentration

The association analysis conducted with the data collected from Mexico during 2011 and 2012 using the GLM method detected SNP markers significantly associated with DON concentration on chromosomes 1A, 3A, 5A, 7A, 2B, 5B, and 2D (Table 4-11). The analysis conducted with data collected on 2011 identied SNP markers located on

chromosomes 2B, 5B, and 2 D with the lagest effects. On chromosome 2B, one SNP marker located at 126 cM explained 5.2% of the phenotypic variance of the trait. The estimated effect of this SNP was 2.7 ppm. Other interesting region was located on chromosome 5B at positions 162 - 167. The effect of this region on the trait was 2.6 ppm. On chromosome 2B, one marker located at 139 cM explained 7.9% of the phenotypic variance and showed an effect of 2.5 ppm.

The association analysis conducted with data collected on Mexico 2012 detected 5 SNP markers associated with DON concentration. These markers were located on chromosomes 4A, 7A, 2B, and 2D. The regions found in this analysis were different from those found in 2011 exept for the SNP marker located on chromosome 2D at 139 cM. In this analysis, the QTL associated with this marker explained 6.3% of the phenotypic variance. The effect over the trait was 1.2 ppm.

The MLM model did not detected any significant SNP marker associated with DON concentration in the AMP.

Table 4-11. Association analysis for DON concentration of the wheat association mapping panel using GLM model. Mexico. 2011-12.

Marker	Chr.	Pos. (cM)	p-value	r2	Alleles	Allele 1 (ppm)	Allele 2 (ppm)	Effect (ppm)
Mexico 2011								
wsnp_Ex_c12123_19388313	1	148	1.19E-04	0.06064	A/G	5.4	4.4	1
wsnp_Ku_rep_c109724_94227136	1	149	2.38E-04	0.05649	T/C	5.4	4.5	0.9
wsnp_BQ167580A_Ta_1_1	3	123	6.96E-05	0.06341	T/C	3.7	5.2	1.5
wsnp_BE399966A_Ta_2_3	5	193	2.01E-05	0.07136	A/G	5.2	3.7	1.5
wsnp_Ex_c14219_22169892	7	11	6.30E-04	0.05044	A/G	5.6	4.3	1.3
wsnp_Ex_rep_c66476_64726880	7	13	3.13E-05	0.07097	T/C	4.4	6	1.6
wsnp_BG313770A_Ta_2_1	7	20	1.90E-04	0.05734	T/C	5.7	4.4	1.3
wsnp_Ex_c7776_13247654	9	5	4.63E-04	0.05161	T/C	4	5.3	1.3
wsnp_Ra_c2842_5399988	9	126	3.81E-04	0.05206	T/C	4.9	7.6	2.7
wsnp_Ra_c39562_47242455	12	70	5.41E-05	0.06538	A/G	4.4	5.5	1.1
wsnp_Ex_c5155_9140608	12	77	6.39E-04	0.04872	A/C	5.3	4.1	1.2
wsnp_Ku_c3102_5810751	12	97	2.54E-06	0.08499	A/G	5.5	3.5	2
wsnp_JD_c11594_12033647	12	162	2.84E-04	0.0431	A/G	4.8	7.4	2.6
wsnp_Ku_c15630_24304954	12	167	4.66E-04	0.0518	A/G	4.4	5.9	1.5
wsnp_Ex_rep_c66921_65344887	12	170	4.38E-04	0.05922	A/G	5.9	4.3	1.6
wsnp_BQ166999B_Ta_2_1	12	174	9.17E-04	0.04841	T/G	4.4	5.7	1.3
wsnp_Ku_c11721_19085513	12	175	2.20E-04	0.05771	A/G	5.9	4.4	1.5
wsnp_Ku_c8712_14751858	16	139	5.39E-06	0.07887	T/C	5.3	2.8	2.5
Mexico 2012								
wsnp_Ex_c1373_2628597	4	138	1.92E-04	0.05226	A/G	3	1.7	1.3
wsnp_Ex_c9971_16412345	7	154	1.40E-04	0.05728	C/T	2.1	2.8	0.7
wsnp_Ku_c13905_22034406	9	73	7.41E-05	0.05894	A/G	2.3	3.4	1.1
wsnp_Ra_c16822_25566950	9	73	8.58E-05	0.05691	C/T	2.9	1.3	1.6
wsnp_Ku_c8712_14751858	16	139	2.34E-05	0.06346	A/C	2.3	3.5	1.2

Table 4-12. Association analysis for DON concentration of the wheat association mapping panel using MLM model. Mexico. 2011-12.

	Marker	Chromosome	Position (cM)	P-value
Mexico 2011-12	NS			

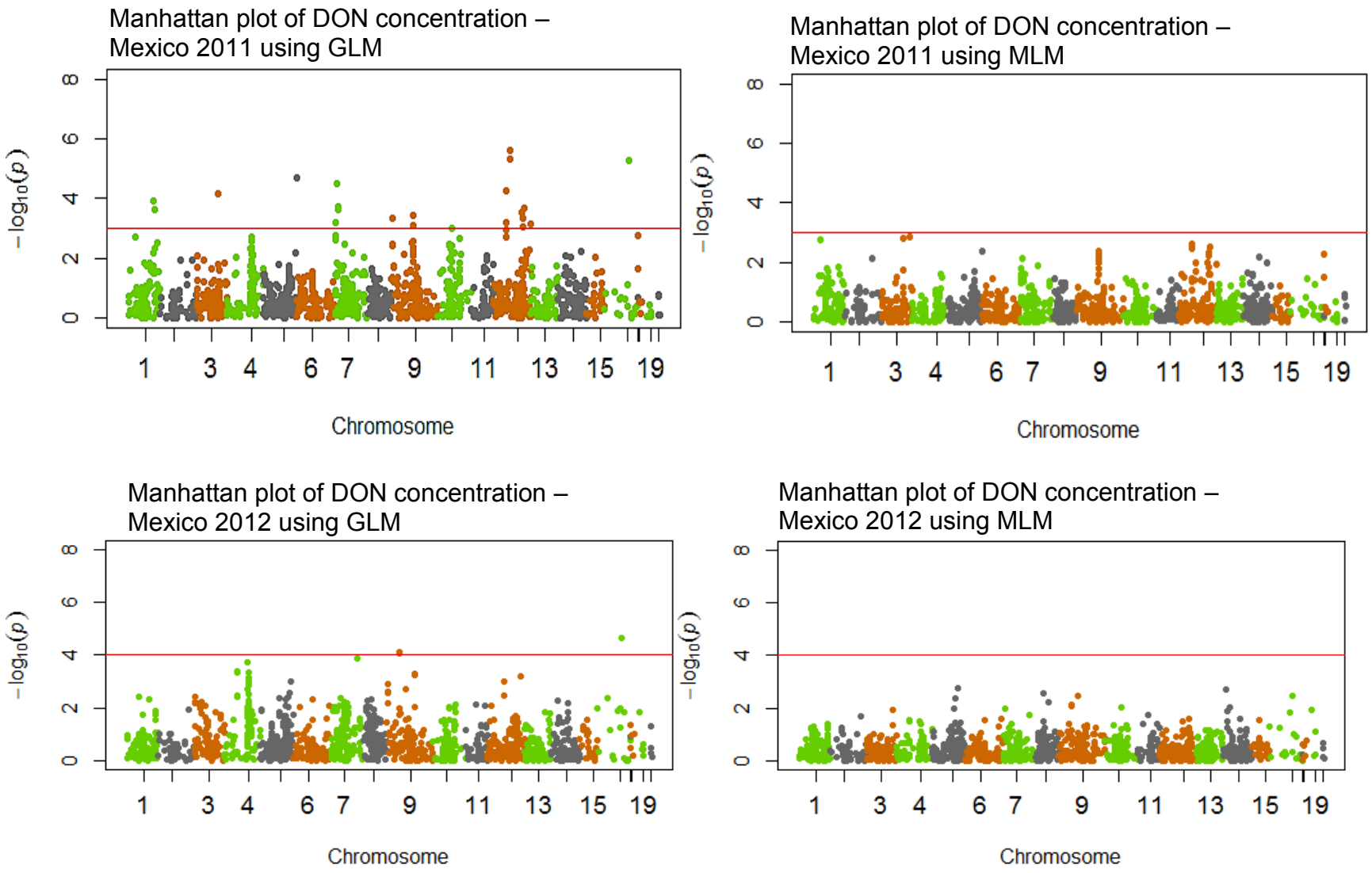

Figure 4-6. Manhattan plots of the association analysis for DON accumulation in the wheat association mapping panel using GLM and MLM. Mexico 2011-12.

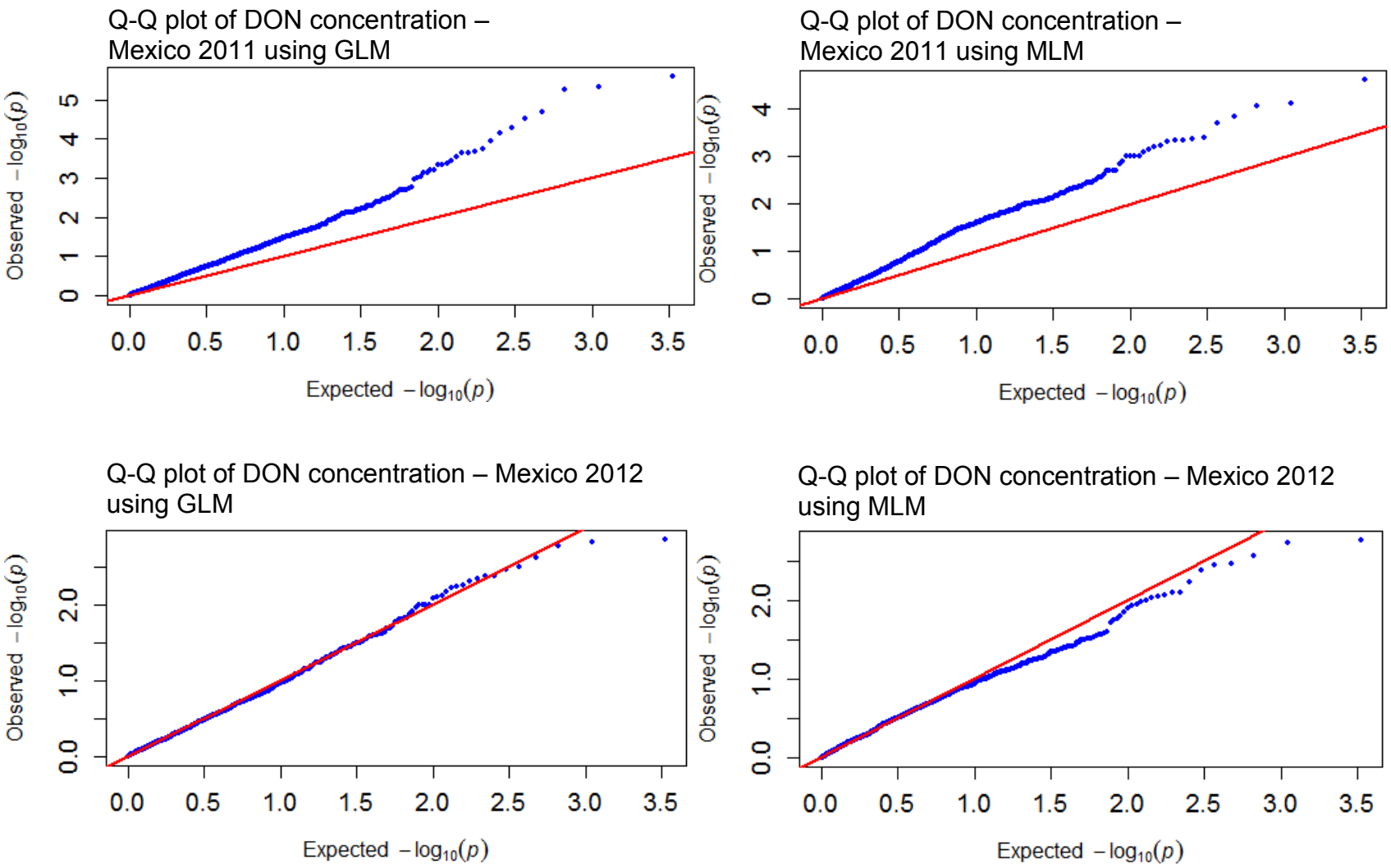

Figure 4-7. Q-Q plots of the association analysis for DON accumulation in the wheat association mapping panel using GLM and MLM. Mexico 2011-12.

Discussion

The Fusarium Head Blight data (percentage of severity and concentration of Deoxinivalenol in parts per million-ppm) were analyzed only from the experiments planted in Mexico. The weather conditions in Mexico ranged from 15 – 25^{o}C (Appendix E), which are ideal to the development of the disease. According to Schmale III and Bergstrom (2003) the optimum range of temperatures which favors the disease development are 15 - 30^{o}C. Additionally, the wheat AMP planted in El Batan - Mexico received additional irrigation from sprinklers which increased the relative humidity in the experiment.

The analyses of variance of these traits detected significant differences among locations, so the traits were analyzed independently as follows:

Statistical analysis FHB severity

In Ecuador, statistical analyses were not conducted with the data collected on 2011 and 2012 from the wheat AMP. The disease severity in 2011 was very low with an average of 3.8% in the whole population. More than 50% of the accessions did not show any symptoms in the spikes or seeds. It could be attributed to unfavorable environmental conditions for the development of the disease since wheat accessions possessing immunity to the disease are not expected (Miller and Greenhalgh, 1988; Snijders, 1994). In 2012, the disease was not present in the experiment, despite ground inoculations and the two inoculations at flowering time. Macroconidia require relative

humidity higher than 80% to germinate (Beyer et al., 2005) and Santa Catalina did not meet that condition in 2011 nor 2012.

In El Batan-Mexico the situation was different. The first year of evaluations (2011) was better in terms of disease severity. The average infection for the whole experiment was 22.4%. The disease severity for second year of study in Mexico (2012) was significantly lower compared with the first year. Even though the FHB severity ranged from 1.0 -80.0%, the average for the population was lower (9.6% of disease severity). The reason is that only one genotype (Gamenya) showed a high percentage of disease severity. The second accession more susceptible in 2012 was FALCIN/ AE.SQUARROSA(312) /3/THB/CEP7780//SHA4/LIRA with 22% of disease severity.

Low correlations were observed between FHB severity in Mexico 2011 versus 2012. Low correlations of FHB severity experiments conducted in the same location but different seasons are not uncommon (Somers et al., 2003). Accessions that were susceptible in Mexico 2011 (over 35% of FHB severity) were moderately susceptible in Mexico 2012 (5-22%) except from Gamenya (the susceptible control) with 80% of FHB severity in 2012.

Heritability estimates were low in the experiments conducted in Mexico. In the literature, the heritability for FHB traits is variable. Some studies such as Buerstmayr et al. (2000) or Miedaner et al. (2011) reported $H^2 > 0.7$; however, Verges et al. (2006) reported heritability values for FHB traits lower than 0.3. In this study, the low heritability resulted by the complexity of the trait and the GxE effect could result in slow progress in breeding for resistance.

Statistical analysis DON concentration

The data distribution showed skewedness to the left to low levels (Figure 4-3), giving the impression that most of the wheat accessions had low levels of DON. However, levels of 2 ppm of DON or higher are not accepted by the industry (van Egmond and Jonker, 2004). Considering that limit or threshold, in 2011, 85.5% of the wheat accessions exceeded 2.0 ppm, meanwhile, in 2012, 49.9% exceeded 2.0 ppm. The DON concentration was higher in 2011 due to climatic conditions which favored disease development. The same results were observed for FHB severity. The coefficient of variance was high CV= 53.8%. The reason for such high coefficients of variation might be the result of the reduced disease pressure observed in the experiment evaluated in 2012.The correlation between the two years of experiments in Mexico was low 0.3 (p value <0.001). It is not uncommon to find low correlation between DON concentration between two or more different seasons or between DON concentration and FHB severity (Bruins et al., 1993; McCormick et al., 2003). The reason for these observations could be caused by the high influence of the environment on the development of the disease and the various mechanisms of resistance that can be combined in the plant (Mesterházy et al., 2003; Somers et al., 2003). For example, Type I resistance can be more efficient with less relative humidity as occurred in 2012.

Germplasm evaluation

The evaluation of FHB severity and DON concentration in the wheat AMP allowed the identification of accessions with high levels of disease resistance to FHB (Table 4-7). The maximum percentage of FHB severity observed in these lines was 7%

in the evaluation conducted in 2011 in Mexico under high disease pressure. Based on the pedigree, it was possible to identify some wheat lines that are frequently present. For instance, there were 11 accessions developed from the synthetic wheat line ('ALTAR84/*Ae. squarrosa*) which has been previously used at CIMMYT to provide resistance to several biotic and abiotic constraints (Warburton et al., 2006) and was used to introgress Fusaium head blight resistance (Mujeeb-Kazi et al., 2001). Other ancestors frequently found in the list of the top 25 accessions was 'Heilo' (five times). Heilo, which showed resistance to yellow rust as well (Chapter III), was one of the parents in the last cross of most of the resistant accessions. 'Heilo' is also a wheat accession of special interest since it has high end-use quality and has two QTLs related with low-molecular weight glutenin subunits (Liu et al., 2010; McIntosh et al., 2012). Another accession found nine times in the pedigrees of the top 25 lines with resistance to FHB was 'Kauz' (nine times). This accession in commonly found in CIMMYT wheat lines, since it provides resistance to abiotic stresses and has improved nutrient use efficiency (N and P) and shows high yield in low and high input conditions in a wide range of different environments (Rajaram et al., 2002).

Association analysis of FHB severity

The association analysis conducted with data from Mexico 2011 and 2012 using the GLM method detected SNP markers significantly associated with FHB severity on chromosomes 1A, 2A, 3A, 5A, 7A, 2B, 3B, 5B, and 7B. Markers located on chromosome 2B and 5B were the same in the analysis conducted separately for each year.

When the MLM method was used, the analysis did not detect markers significantly linked to FHB severity in the data set from Mexico 2011-12. However, the individual analysis for each season using the MLM method detected markers on chromosome 7B (Mexico 2011) and 2B (Mexico 2012). MLM method is highly conservative compared with the general linear model (Yu et al., 2006) and this is the reason why in this study few SNP markers were detected using MLM.

Quantitative trait loci for FHB resistance have been mapped on every chromosome of the hexaploid wheat genome except on chromosome 7D (Buerstmayr et al., 2009). In this study, the association analyses with data collected from Mexico from 2011 and 2012 using the GLM method and the individual analysis from Mexico 2011 using GLM and MLM detected SNP markers significantly associated with FHB resistance on chromosome 7A position 8-9 cM. Several QTLs have been reported to be located on chromosome 7A. One of them was found in the Chinese source of resistance 'Wangshuibai' (Zhou et al., 2004). Following the report of the QTL discovered in 'Wangshuibai', two other QTL found in 'Frontana' (Mardi et al., 2006) and NK93604 (Semagn et al., 2007) were reported in the same chromosome 7A. The last report of a QTL located on chromosome 7A was a QTL discovered in 'Sumai 3' named as *Fhb7AC* was found near the centromere of chromosome 7A (Jayatilake et al., 2011). From all the QTLs reported previously, the only QTL located in the short arm of chromosome 7A was the QTL from 'Frontana', which is the region were the SNP markers were significant. This finding added to the fact that 'Frontana' was extensively used in CIMMYT germplasm to develop spring wheat lines with resistance to FHB suggested that the QTL found in this study could be the same QTL present in 'Frontana'.

Chromosome 5B was the other chromosome where several SNP markers were detected in the combined data set of Mexico 2011-12 and Mexico 2011alone using the GLM method. SNP markers were located in the distal region at 225 – 247 cM. QTLs in different regions of the long arm of chromosome 5 have been reported previously in winter wheat (Bourdoncle and Ohm, 2003; Klahr et al., 2007; Paillard et al., 2004) and spring wheat (Jia et al., 2005). One of these QTLs was detected in the cultivar 'Forno' which has been of interest, not only for FHB resistance and significant percentage of variation of the FHB severity explained (14.3%), but plant height or flowering time variation indicating linkage or pleiotropic effects (Buerstmayr et al., 2009; Paillard et al., 2004). Another source of resistance with a QTL detected on chromosome 5B is the Chinese landrace 'Wangshuibai' (Jia et al., 2005).

Association analysis for DON concentration

Even though, the number of studies conducted to detect QTLs controlling DON concentration are abundant, not many regions in the wheat genome have been identified compared with other traits such as FHB severity, incidence or spread (Buerstmayr et al., 2009). In this study, SNP markers significantly associated with DON concentration were found on chromosomes 1A, 3A, 4A, 5A, 7A, 2B, 5B, 7B, and 2D. In this study, chromosome 5A position 193 cM presented one marker significantly associated with DON concentration. In chromosome 5A one QTL has been reported in a population obtained by the cross Wuhan 1 x Nyu Bai (Somers et al., 2003). The QTL found in this study was discovered in the short arm of chromosome 5A and the source belongs to Chinesse germplasm. In other study (Jiang et al., 2007), a QTL located on

chromosome 5A was reported in spring wheat. One of the partent in this population was Veery, which is one of the most populat accessions from CIMMYT utilized as a source of multiple traits. Several QTLs associated with FHB severity have been reported in these chromosomes, but only one study has previously reported a QTL on chromosome 2D, present in 'Maringa' (Somers et al., 2003). The mechanism to control DON concentration has been elucidated by Lemmens et al. (2005), which found that DON was converted to DON-3-O-glycoside which is a less phytotoxic compound. The two possible ways proposed from the authors after this observation are that a gene encodes the enzyme DON-glucosyltransferase or regulates the expression of such an enzyme.

Several regions wich had effect over FHB severity and DON concentrations were detected. These regions were located on chromosomes 2B, 5B, and 2D. On chromosome 2B, SNP marker wsnp_Ra_c2842_5399988 located at 126 cM showed effect related with reduction on FHB severity and specially DON concentration with reduction of 2.7 ppm when the favorable allele was present. Similarly, SNP marker wsnp_ku_c15630_24304954 showed effect in the reduction of FHB severity and DON concentration. On chromosome 2D, SNP marker wsnp_ku_c8712_14751858 had effect on both traits with notable reduction on DON concentration (2.5 ppm) when the favorable allele was present.

Conclusions

The wheat AMP includes several wheat accessions with high levels of resistance to FHB. These accessions have shown allelic diversity for FHB resistance and are valuable sources of many genes to control FHB. Based on the pedigrees and the

classification of the wheat accessions in different sub-populations, it can be inferred that the allelic richness and potential contribution for breeding are not limited to FHB resistance but are valuable for many other traits.

Association mapping approach detected several regions associated with resistance to FHB severity and DON concentration. The number of regions and markers were drastically reduced when the MLM method was used instead of the GLM method. Special attention must be considered to this situation, which is commonly reported in the literature, and it will be important to validate these SNP markers and QTLs in mapping or breeding populations.

The wheat SNP chip is a valuable tool to conduct association mapping studies, but the reduced number of polymorphic markers detected in the D-genome in spring wheat populations needs to be addressed with the incorporation of additional markers in the D-Genome. For example, SSR markers that have been reported to be specific for D-genome.

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APPENDIX

Appendix: Temperature and precipitation. Mexico and Ecuador. 2011-12

Table 4-13. Temperature and precipitation data from Santa Catalina – Ecuador and El Batan - Mexico during 2011-12.

Location	Year	Months	Average temp. (°C)	Temp. max. (°C)	Temp. min. (°C)	Precipitation (mm)
Santa Catalina*	2011	February	11.3	19.6	3.8	206
		March	11.2	20.5	2.6	143.7
		April	11.1	19.9	2.5	262.2
		May	12.1	21.6	2	91.7
		June	12	20.6	2.2	61.5
	2012	February	11.1	18.6	4.5	227.3
		March	12.2	20.6	5	197.4
		April	11.1	23.7	3.2	219.3
		May	11.8	19.8	4.2	62.9
		June	11.8	21.2	2.6	10.2
El Batan	2011	Aug	17.6	25.7	9.6	66.1
		Sep	15.7	24.7	6.6	68.5
		Oct	15.0	25.1	4.9	94.6
	2012	Aug	16.2	22.6	10.9	75.6
		Sep	16.1	23.8	10.1	51.1
		Oct	15.1	25.6	5.3	9.3

* Data collected from the weather station of Santa Catalina Research Station
** Data collected from Wunderground.com ® (http://www.wunderground.com/weatherstation/WXDailyHistory.asp?ID=IESTADOD2)

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