International Journal of Agricultural Science Research Vol. 3(10), pp. 213-221, October 2014
Available online at http://academeresearchjournals.org/journal/ijasr
ISSN 2327-3321 ©2014 Academe Research Journals

Full Length Research Paper

Marker assisted evaluation for resistance to *Septoria tritici* blotch in Moroccan wheat recombinant inbred lines (RILs)

Diria Ghizlan[1]*, Abdelwahd Rabha[1], Labhilili Mustapha[1], Benchaba Mhamed[1], Gaboun Fatima[2], Arhbal Aziz[3] and Ibriz Mohammed[4]

[1]INRA, Biotechnology Unit, BP 415 boulevard de la victoire, Rabat, Morocco.
[2]INRA, Bioinformatic Unit, CRRA Rabat, Morocco.
[3]Agriculture and Marine Fisheries, Morocco.
[4]Department of Biology, University of Sciences Kenitra, Morocco.

Accepted 15 October, 2014

***Septoria tritici* blotch (STB), caused by *Mycosphaerella graminicola*, is the major foliar disease in temperate rainfall Moroccan growing wheat regions. An F9 recombinant inbred population from cross between resistant synthetic hexaploid wheat "syn6" and a susceptible cultivar Mehdia was evaluated for resistance to *septoria* during three years in Moroccan field conditions at Sidi Allal Tazi and Merchouch stations. 13 SSR markers associated to some Stb genes (Stb1, Stb2, Stb3, Stb4, Stb5, Stb6, Stb8 and Stb14) were applied on this recombinant inbred lines (RILs) population in order to find out the linkage between markers and resistance genes against this disease and to identify which of these known genes are effective. Only Xgwm389 marker linked to Stb2 had discriminate the septoria resistant lines from the susceptible marker. Most RILs that exhibited low *S. tritici* severity had 150 bp allele as the resistant parent "Syn 6" and the other lines having high disease severity had 133 bp allele present at Vernapolis, the considered carrier of Stb2, at Chinese spring and Mehdia susceptible parent. Suggesting that Stb2 gene is broking and there is another gene closely linked to Stb2 on the short arm of chromosome 3B confers resistance to Syn 6 and RILs population in Gharb region of Morocco.**

Key words: Disease resistance genes, *Mycosphaerella graminicola*, *Triticum aestivum*, RILs population, SSR markers, Stb genes.

INTRODUCTION

Septoria tritici blotch (STB) is one of the most important foliar diseases of wheat caused by the ascomycete *Mycosphaerella graminicola* (Fuckel) J. Schroët. STB spread is usually established through airborne ascospores that are discharged from wheat debris and deposited in young wheat crops in the fall. This is followed by rain splash driven spore dispersal during the growing season (Shaw and Royle, 1993; Goodwin, 2012). However, *M. graminicola* can reproduce sexually throughout the year, which provides the fungus with a mechanism to overcome adverse biotic or abiotic conditions (Codro et al., 1999; Goodwin, 2012). In Morocco, the sexual stage was also found (El Bakali et al., 2012).

S. tritici is a major disease of wheat in all wheat growing areas of the world when climatic conditions are favorable; this fungus can cause yield losses from 30 to 50% in susceptible wheat genotypes (Eyal et al., 1987). In Morocco, STB is a big problem in temperate rainfall wheat (*Triticum aestivum* and *durum*) growing regions and the yield production losses can exceed 50% under favorable conditions (Mazouz et al., 1995). Research for cultivars with better tolerance levels in Morocco resulted

*Corresponding author. E-mail: ghizlanediria@gmail.com. Tel: (+212) 662451755.

in the release of cv. Nesma in 1976 that was replaced by cv. Amal in 1993 which was more tolerant (Jlibene et al., 2002).

During the last decade, 18 major genes conferring resistance to the pathogen were identified and characterized (Ghaffary et al., 2011; Siḿon et al., 2012). They were: Stb1 (Adhekari et al., 2004c), Stb2, Stb3 and Stb4 (Adhekari et al., 2004a, b), Stb5 (Araiano et al., 2001), Stb6 (Brading et al., 2002), Stb7 (MacCartney et al., 2003), Stb8 (Adhekari et al., 2003), Stb9 (Chartrain et al., 2009), Stb10, Stb11 and Stb12 (Chartrain et al., 2005a, b), Stb13 and Stb14 (McIntoch et al., 2007), Stb15 (Arraiano et al., 2007), Stb17 (Simon et al., 2011) and Stb16, Stb18 (Ghaffari et al., 2011a, b). This number is limited. Moreover, the majority of these genes have narrow spectra of specificity towards *M. graminicola* isolates that represent current field populations in major wheat producing areas, and this limits their use (Arraiano and Brown, 2006; Chartrain et al., 2005b). Furthermore, *M. graminicola* is a heterothallic filamentous fungus with multiple sexual cycles during the growing season that defines its complex genetic population structure and influences disease management (Kema et al., 1996; Goodwin, 2007).

The wheat *M. graminicola* pathosystem complies with the gene-for-gene hypothesis where a pathogen effector interacts with a host target (Brading et al., 2002). Hence, the selection pressures that new Stb genes may exert on natural *M. graminicola* populations need a continuous effort to unveil key genes that control this disease (Zhang et al., 2001; Linde et al., 2002). The narrow diversity of wheat germplasm pushes breeders to use wild wheat progenitors as potential sources for the recovery of genetic diversity (Zhang et al., 2006; Raman et al., 2010). The production of synthetic hexaploid (SH) wheats goes back to the 1940s but is recently considered a strategic approach to exploit germplasm of wild wheat progenitors in commercial breeding programs (Xie and Nevo, 2008; Yang et al., 2009). Synthetics hexaploid wheat provides a rich source of genetic variation and can be readily hybridized with elite bread wheat cultivars and germplasm. Breeders have exploited these sources for resistance to a wide range of biotic and abiotic stresses (Adhikari et al., 2003; Arraiano et al., 2001; Mujeeb-Kazi et al., 2001; Xu et al., 2006; Friesen et al., 2009; Ghaffary, 2011a).

The conventional selection methods for Stb resistance can be very much improved by using genetic markers tightly linked with Stb resistance genes. The incorporation of the known genes in new cultivars could contribute to broadening the resistance to the pathogen (Goodwin, 2012; Raman and Migate, 2012). SSR markers, based on PCR amplification of microsatellites sequences (Simple Sequence Repeats) offer a much higher monolocus polymorphism than any other marker system (Röder et al., 1998). Such kind of marker should be also attributed to resistant genes by a genetic analysis of linkage between markers and resistant genes (Ganal and Röder, 2007). The selection accomplished in RILs wheat, with co-dominant SSR markers, offers the opportunity to rapidly stabilize the selected resistant individuals into homozygous estate.

In Morocco, recent resistance screening of 24 inscribe variety in Morocco indicated that the majority of these cultivars were highly susceptible to septoria (not published yet). Breeding for resistant cultivars is the best strategy to reduce the lost production and it is the most economical and efficient means of control. However, in Morocco little is known about genes conditioning resistance and how they are effective against the local population of the pathogen. In addition, there is still much work to do in relation to the incorporation of the genes in new cultivars broadening the resistance to the pathogen. In this work, we further investigate the potential of SH and derived breeding lines as sources of resistance to *M. graminicola* in breeding programs to *S. tritici*, and to identify which of the known genes are present and effective in our RILs population in Morocco.

MATERIALS AND METHODS

Plant materials

One hundred Recombinant Inbreed Lines (RILs) descendants and the both parents were used in this study. The RILs population was generated at the National Institute of Agronomic Research at the Biotechnology Unit Rabat, Morocco. The recombinant inbreed lines were obtained from a cross between “syn 6”, a resistant synthetic hexaploid bread wheat developed at CIMMYT by A. Mujeeb- Kazi and which has the pedigree (PGO CROC_1/ Ae. Squarosa (224)/3/ 2*BORL95), and cv. Mehdia, which is a susceptible Moroccan spring bread wheat genotype developed by the wheat breeding program at the National Institute of Agronomic Research, Morocco. The population was developed and advanced in green house through single seed descent by Dr. Diria Ghizlan of the Biotechnology Unit Rabat (INRA) and tested for phenotypic and marker analysis.

Septoria severity field evaluation

The 100 RILs (F9) and the parental lines used as controls were evaluated for resistance to *S. tritici* in Sidi Allal Tazi (SAT) and in Merchouch (MCH) Stations for three years. The climate in SAT station located in Ghab region is Mediterranean with an oceanic influence, with mild winter (22°C) and warm summer (37°C). The average annual rainfall in the SAT is 520 mm. The soils of the station are dominated by Dhess followed by shots. In MCH station, the climate is semi-arid with cold winter (-1°C) and a very hot summer (45°C). The average annual rainfall of the station is 396 mm. The soil station is dominated by the vertisol.

Table 1. SSR markers and primers that mark *Septoria* resistance genes used.

Markers	Resistance genes	Markers	Resistance genes
Xgwm335	Stb1	Xgwm146	Stb8
Xgwm389	Stb2	Xwmc500	Stb14
Xgwdm132	Stb3	Xgwm46	
Xgwm111	Stb4	Xgwm285	
Xgwm44	Stb5	Xgwm 499	
Xgwm369	Stb6	Xgwm344	

All lines were planted in double row of 2 m followed by an empty row per plot; the distance between rows is 30 cm and between seeds is 20 cm. The Moroccan genotype « Achtar » was used as sensitive local cheek. Sidi Allal Tazi Station was considered as reference station for assessing disease and was considered as septoria hot spot with serious plant diseases in recent years. Field testing often relies on natural inoculums either by windborne as cospores already present in the environment or by spreading infected straw from the previous season's crop as source of inoculums. However, we observed that pathogen overwinters in infected residue and is mainly dispersed by rain-splashed pycnidio spores (Shaw, 1987; Goodwin, 2012).

Scoring of *S. tritici* symptoms was performed combining Saari prescot scale (1975) and double digit scale (Eyal et al., 1987). The first digit (D1) represented the vertical disease progress ranging from 0-9 for evaluating the severity, and the second digit scale (00-99) (D2) represented severity measured as diseased leaf area in percentage (0 = 0% and 9 = 90%). Four plants in each replication were selected randomly to record the disease rating and their mean was applied. Data were transformed to the logit scale for statistical analysis using residual maximum.

DNA extraction

The healthy young leaves (one week old seedlings) were individually collected from the parents and the 100 lines of RILs and placed in 2 mL microcentrifuge tubes. The leaves were lyophilized for 72 h and stored at -80°C. For DNA extraction, 30 mg lyophilized leaves were ground to a fine powder with two stainless steel beads in grinder tissue. Total genomic DNA was isolated using a CTAB method with minor modifications (Saghai-Maroof et al., 1984). DNA quantity and quality were assessed with agarose gel and UV-Photometer. The Hexaploid wheat cultivars, Vernapolis, Ouasis, Nova prata, and Chinse spring harboring known resistance genes were used to analyze the RILs population.

SSR marker genotyping

For DNA amplification, 12 SSR pair of primers was used, 8 markers from them marked different *Stb* loci (Table 1). Primers that amplified at least one polymorphic band between parents were used to screen the RILs. PCR amplification followed the M13 tailed primer PCR method with the M13 oligonucleotide labeled with one of the four fluorescent dyes. 6-FAM, VIC, NED and PET added in the reaction mix were used. The PCR reaction was performed in 13 µl volumes including 25 ng of template DNA (3 µl), 1.5 U of Taq DNA polymerase (Home polymerase, Kansas University USA), 1× ASB PCR buffer, 2.5 mM $MgCl_2$, 200 µM of each dNTP, 250 nM of reverse regular primers and 200 nM of forward regular primers, 50 nM fluorescence-labeled M13 primer and 0.3% Tween20.

The reaction mixture was denatured at 95°C for 5 min, followed by 4 cycles at 96°C for 1 min, 68°C for 3 min with a decrease of 2°C in each subsequent cycle, and 72°C for 1 min; this was then followed by 4 cycles at 96°C for 1 min and 58°C for 2 min with a decrease of 2°C in each subsequent cycle, and 72°C for 1 min; 39 cycles at 96°C for 20 s, 50°C for 2 s and 72°C for 30 s; and a final extension step of 72°C for 5 min and 12°C for 5 min. After multiplexing PCR products labeled with four different fluorescent dyes, the electrophoresis was carried out on the Applied Biosystems ABI PRISM 3730 Genetic Analyzer (Applied Biosystems, Foster City, CA, USA). Gene Mapper software v 1.97 (Applied Biosystems) was used for fragment analysis and allele calling.

Statistical analysis

Analysis of phenotypic and genotyping data obtained from a set of 9 SSR markers previously mapped to a unique and single chromosome location had be done by the software XlSTAT and the statistical package Gensta for Windows 7th edt (Genstat 5 Committee, 2002). The RILs descendants were clustered by the weighted Neighbor Joining (NJ) method using the dissimilarity matrix. These analyses were carried out using the DARwin 5.0.148 software program (available at http://darwing.cirad.fr/darwin/Home.php).

RESULTS AND DISCUSSION

Septoria phenotyping

The *S. tritici* susceptible parent Mehdia characterized

Table 2. Descriptive statistics for Septoria field resistance.

Site	Mean of parents*		Means*		Min	Max	Standard deviation
	Syn 6	Mehdia	MPV	RILs			
SAT	31	92	63.5	59.55	11	96	29.51
MCH	11	92	51.5	35.66	11	96	29.79

(SAT: Sidi Allal Tazi station; MCH: Merchouch station; * mean of three years).

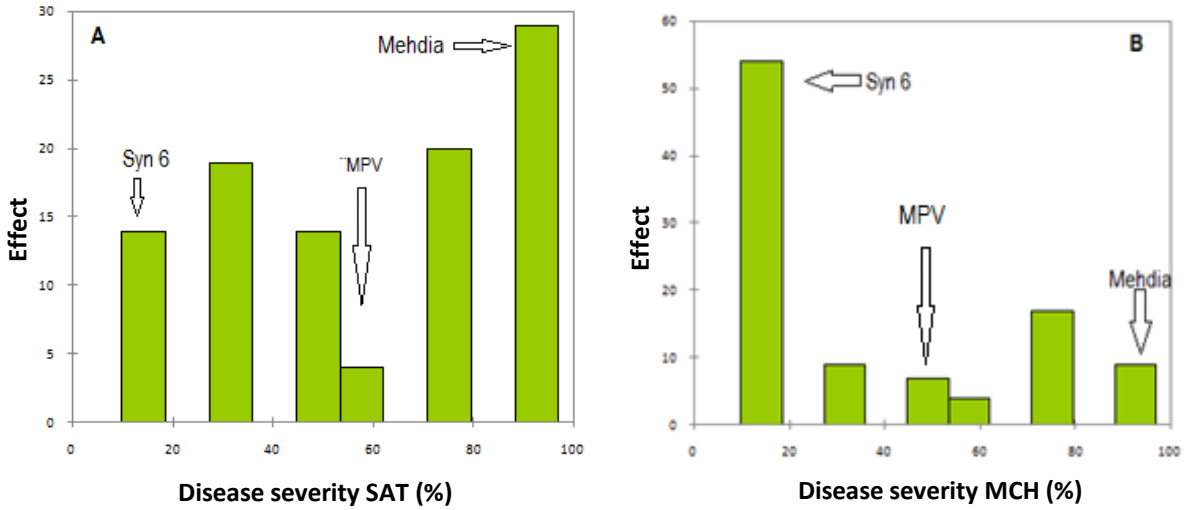

Figure 1. Histograms showing frequency distribution of the mean disease severity for Septoria tritici blotch (STB) reaction among 100 recombinant inbred lines (RILs) from the cross 'syn6' × 'Mehdia' evaluated during three years at (A) SAT station, and (B) MCH Station. Arrows indicate the mean value of both parental lines 'Mehdia' and 'Syn6' and mid-parent value (MPV).

with high severity levels exceeding 90% septoria tritici infection in both fields' evaluations, that is, SAT and MCH. The resistant septoria tritici parent syn6 had very low severity levels of 11 and 31% in MCH and SAT respectively during the three years of study. The RILs descendants varied from 11% to 96% in both stations. In SAT, Septoria severity level was higher with an average of 59.55%, while that in Merchouch station was 35.66% of the RILs (Table 2).

In SAT station of 100 descendants RILs evaluated, 33 were specifically resistant to highly resistant (Figure 1A), 14 were moderately resistant, 4 were moderately susceptible and 49 descendants were susceptible to highly susceptible. In MCH station, 63 descendant RILs were specifically resistant to highly resistant and 26 descendants were susceptible to highly susceptible (Figure 1B). Sidi Allal Tazi site is more favorable for pathogen development and selection because it is a humid agro ecological zone than in Marchouch site which is a favorable agro ecological zone. The high level of disease severity at SAT station is explained also by high variability genetic of *S. tritici* in Gharb region due to sexual and asexual pathogen multiplication (El bakali et al., 2012; Diria et al., 2014). Sidi Allal Tazi Station is considered as a reference station for assessing disease and is considered as *septoria* hot spot with epidemic level in recent years.

The cultivar 'syn 8' may therefore be a source of resistance to STB for wheat breeding. The resistant descendants RILs developed, harboring resistance genes and with height yield and good quality may therefore be a valuable source for wheat breeding especially in Mediterranean environments and in Morocco or may be proposed to the official catalogue for inscription.

SSR genotyping

Parents and some RILs were evaluated by 13 SSR pair of primers. The amplification products were specific for SSR marker Xgwm44 linked to Stb5 gene (Arriano et al., 2001), whereas Xgwm285 and Xgwm499 markers were monomorphic for the resistant and susceptible parents in the field. So we have no Stb5 gene in our resistance lines and the SSR markers Xgwm285 and Xgwm499 could not discriminate between resistant and susceptible lines. The nine primers that amplified at least one polymorphic band

Table 3. Alleles frequency of Xgwm369 marker linked to Stb6 in Mehdia cv. and Syn6 cv. Cross.

Alleles	Frequency	
	0	1
151 bp	0.96	0.04
172 bp	0.50	0.50
174 bp	0.85	0.15
205 bp	0.66	0.34

between parents were used to screen the RILs and to identify SSR markers associated with adult plant to *S. tritici* resistance and to know which Stb genes are effective in Gharb region in Morocco.

The SSR marker Xgwm335 linked to Stb1 mapped on chromosome 5BL at distance of 7.4 cM (Adhikari et al., 2004a) showed in this study three profiles. The first amplified three DNA bands (187, 232 and 234 bp) were present in Mehdia susceptible parent and also in Vernapolis. The second profile showed two alleles (187 and 232 bp). The third profile had only one allele (187 bp) and it showed that the genotypes of resistant parent Syn6, Chinese spring and Ouasis contained Stb1 gene (McIntosh et al., 2007). The first three alleles were amplified from both resistant and susceptible descends of RILs. It was suggested that Stb1 gene exists in Syn6 and in the RILs population, but Stb1 alone did not confer resistance to septoria in Gharb region in Morocco.

The Stb6 gene linked to SSR marker Xgwm369 is mapped on chromosome 3AS at 2 cM distance. Vernapolis and Chinese spring cultivars are known to have Stb6 gene (Chartain et al., 2005b; McIntosh et al., 2007). The SSR marker Xgwm369 showed four alleles (151, 172, 174 and 205 bp). Vernapolis, Chinese spring and Mehdia susceptible parent had unique allele (205 bp). 34% of the descendants of RILs had allele (205 bp) that means having Stb6 gene. The resistant parent Syn6 had two alleles (151 and 172 bp); only 4% and 50% of descendants of RILs had alleles 151 bp and 172 bp respectively. A new allele (174 bp) is present in 15% of descendants RILs but absent in the two parents (Table 3).

The results obtained for Stb6 were inconclusive because there are descendants with allele 205 bp and are resistant, and others having alleles 151 bp and 172 bp and are susceptible. That means the Stb6 gene exists in Mehdia and in 34% of RILs population but it is ineffective in Gharb region of Morocco.

The SSR marker Xgwmc500 is linked to Stb14 mapped on chromosome 3BS at 2 cM distance, and it confers resistance to Canadian isolate MG2 present in Salamouni cultivar (McIntoshet al., 2007) which showed four alleles (166, 172, 176 and 180 bp) in this study. Allele 180 bp is absent in both parents and allele 176 bp is present in both parents. Only alleles 166 bp and 172 bp are present in susceptible parent Mehdia but absent in resistant parent Syn6. This marker did not discriminate between resistant and susceptible RILs descendants. The both alleles (166 and 172 bp) are present in resistant and susceptible RILs descendants.

The SSR marker Xgwm146 is linked to Stb8 mapped on chromosome 7BL at 3.5 cM distance (Adhikari et al., 2003) showing two profiles; the first profile amplified two DNA bands (172 and 189 bp) in Syn 6 resistant parent and the second profile had one band (172 bp) in Mehdia susceptible parent and also in Vernapolis cultivar. The results obtained for Stb3 and Stb4 were inconclusive (Adhikari et al., 2004a, b).

The amplification products obtained for SSR marker Xgwm46 are monomorphic for one allele (162 bp) and polymorphic for allele 155 bp present in resistant parent and absent in the susceptible parent. Xgwm355 marker showed three alleles (122, 137 and 143 bp), the two first amplified in resistant parent and 143 bp amplified in susceptible parent. The both markers (Xgwm46 and Xgwm355) could not discriminate between resistant and susceptible lines. The alleles present in the resistant parent are also present in susceptible RILs descendants.

Consideration of genetic variation of *M. graminicola* population is essential to understand the virulence on the different cultivars. Differences in the population around the world could be attributed to variation in regular recombination, different migration patterns, and presence and importance of the sexual form. Sexual reproduction creates large numbers of genetically diverse isolates. Populations in this fungus are in genetic equilibrium as well as in drift migration equilibrium (Chen et al., 1994) attributed to a high rate of sexual recombination (McDonald and Linde, 2002; Zhan et al. 1998, 2004; El-Chartouni et al., 2011). Indeed, this ineffectiveness of these Stb genes is expected because *S. tritici* pathogen had a high genetic diversity and frequent sexual recombination in Morocco especially in Gharb region (Elbakali et al., 2012; Diria et al., 2014).

The SSR marker Xgwm389 is closely linked to Stb2 mapped on chromosome 3BS at 0.9 cM distance. In this study, this marker gives two alleles (133 and 150 bp). Vernapolis, Chinese spring, Nova prata and Mehdia had allele 133 bp which is being considered a carrier of Stb2 (Adhikari et al., 2004b). The second allele (150 bp) was present at the resistant parent “syn6” (Figure 2), at 48% of the descendants RILs with field resistance to *S. tritici* leaf blotch (Table 4). The cluster analysis based on septoria field reaction and SSR marker Xgwm389 data using weighted Neighbor Joining (NJ) method is presented in Figure 3. The dendrogram identified mainly two major clusters, first cluster consisted of descendants RILs with septoria field resistance and with allele 150 bp, second cluster consisted of descendants RILs with septoria field susceptibility and with allele 130 bp (Figure 3).

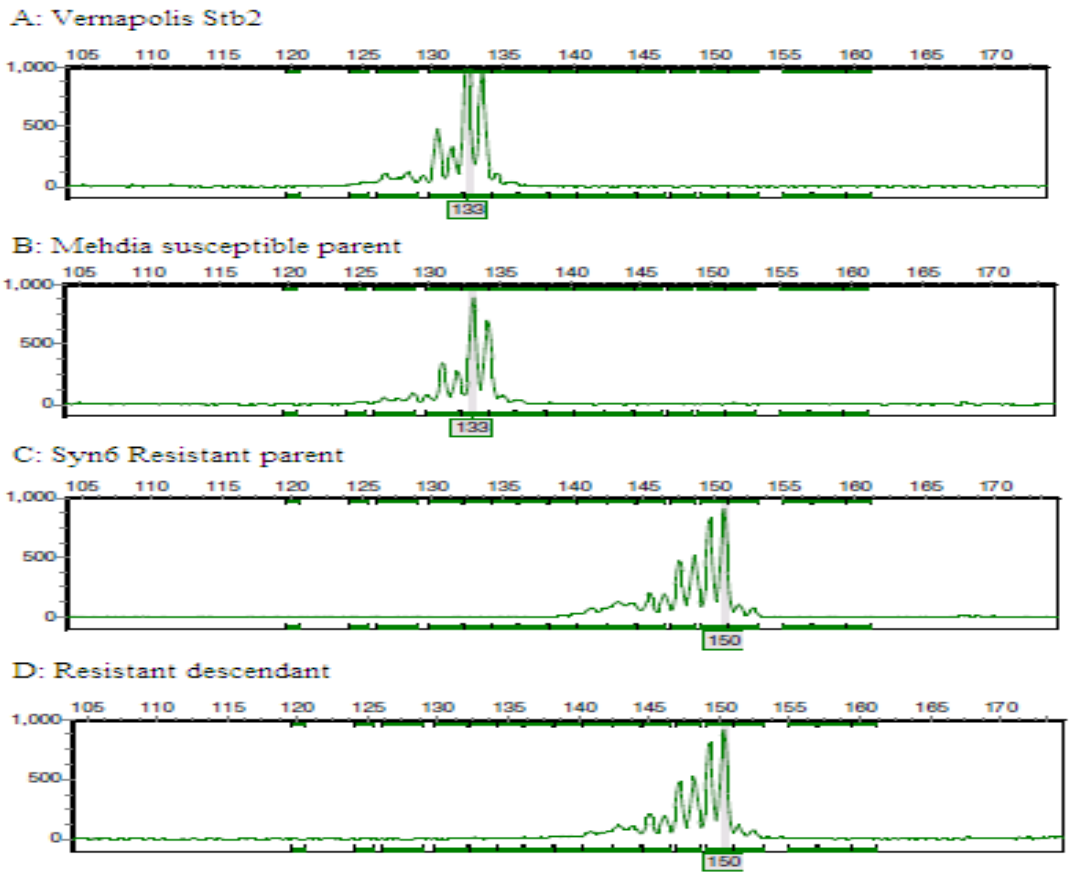

Figure 2. ABI generated electropherograms of SSR marker Xgwm389 on chromosome 3BS showing: (A) Polymorphisme: Vernapolis (Stb2); (B) Medhia (susceptible parent); (C) Syn6 (resistant parent); and (D) Resistant descendant.

Table 4. Alleles frequency of Xgwm389 marker linked to Stb2 in Mehdia cv. and Syn6 cv. Cross.

Alleles	Frequency		Mean	Standard deviation
	0	1		
133 bp	0.47	0.53	0.53	0.502
150 bp	0.48	0.52	0.52	0.502

Vernapolis harboring Stb2 gene showed the 133 bp allele like 52% of the susceptible lines that have the same allele (133 bp) (Table 4). It is suggested that Stb2 gene is present in our RILs populations, but it is broking and there is another gene closely linked to Stb2 on short arm of chromosome 3B conferring resistance to Syn6 and RILs population.

From the SSR markers testing and polymorphs between the two parents, only Xgwm389 markers had discriminated the septoria resistant lines from the susceptible. In all three years of field evaluation, most RILs that exhibited low *Septoria tritici* severity had 150 bp allele as the resistant parent “Syn 6” and most lines that had high disease severity had allele 133 bp present at Vernapolis (the considered carrier of Stb2), Chinese spring and Mehdia susceptible parent (Figure 3). However, it was suggested that Stb2 gene is broking but there is another gene closely linked to Stb2 on short arm of chromosome 3B conferring resistance to Syn6 and RILs population.

Conclusion

The analysis showed that there is another gene for

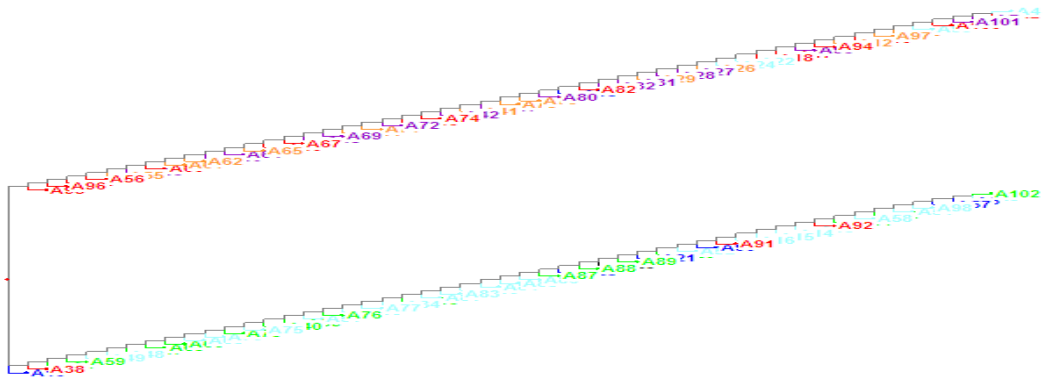

Figure 3. Dendrogram (NJ method) of 100 descendants RILs based on genetic distances calculated using data of Xgwm389 marker. Lines with orange color: high resistance; red color: resistance; purple color: moderate resistance; green color: very susceptible; blue light: susceptible; and blue color: moderate susceptibility; A101 resistant parent; A102 susceptible parent.

resistance to septoria on the short arm of chromosome 3B conferring resistance to the Moroccan races of *Septoria tritici* who is closely linked to *Stb2.* The synthetic hexploid wheat 'syn6' and the resistant descendants harboring a gene closely linked to Stb2 and Stb1 resistance genes may therefore be a valuable source of resistance to STB for wheat breeding especially in Mediterranean environments. In addition to *Stb2*, this chromosome arm contains also gene Sr2 for resistance against stem rust (Spielmeyer et al., 2003), and major quantitative trait loci (QTL) for resistance against *Stagonospora nodorum* blotch (Schnurbuschet al., 2003) and Fusarium head scab (Liu and Anderson, 2003) that could be exploited to develop linkage blocks with multiple resistances. This gene is closely linked to microsatellite marker Xgwm389, which can be used for marker-assisted selection and pyramiding this new gene with other resistance genes in the same chromosome for more durably resistant wheat.

ACKNOWLEDGEMENTS

The authors gratefully acknowledge Dr. Mujeeb-Kazi for supplying the seeds of the hexaploid synthetic lines of CIMMYT and Dr. Amri Ahmed (Head of Genetic Resources Section), and Dr. Kumarse Nazari (Cereal pathologist, Biodiversity Integrated Gene Management Program at ICRDA) for supplying seeds of 6th ISTTN12 with some Septoria resistance genes. The authors also acknowledge Dr. Guihua Bai, Kansas State University, for giving them the opportunity to do SSR analysis using ABI technology in USDA Small Grain Genotyping Lab with the assistance of Paul under Norman Borlaug Fellowship Program.

REFERENCES

Adhikari TB, Anderson JM, Goodwin SB (2003). Identification and molecular mapping of a gene in wheat conferring resistance to Mycosphaerella graminicola. Phytopathology, 93(9): 1158–1164.

Adhikari TB, Cavaletto JR, Dubcovsky J, Gieco JO, Schlatter A R, Goodwin S B (2004a). Molecular mapping of the Stb4 gene for resistance to *Septoria tritici* blotch in wheat. Phytopathology, 94(11): 1198–1206.

Adhikari TB, Wallwork H, Goodwin SB (2004b). Microsatellite markers linked to the Stb2 and Stb3 genes for resistance to Septoria tritici blotch in wheat. Crop Sci., 44(4): 1403–1411.

Adhikari TB, Yang X, Cavaletto JR (2004c). Molecular mapping of Stb1, a potentially durable gene for resistance to *Septoria tritici* blotch in wheat. Theor. Appl. Gene., 109(5): 944–953,

Arraiano LS, Chartrain L, Bossolini E, Slatter HN, Keller B, Brown JKM (2007). A gene in European wheat cultivars for resistance to an African isolate of *Mycosphaerella graminicola*. Plant Pathol., 56(1): 73–78.

Arraiano LS, Worland AJ, Ellerbrook C, Brown JKM (2001). Chromosomal location of a gene for resistance to *Septoria tritici* blotch (*Mycosphaerella graminicola*) in the hexaploid wheat 'Synthetic 6x'. Theor. Appl. Gene., 103(5): 758–764.

Arriano LS, Browm JKM (2006). Identification of isolate-specific and partial resistance to *Septoria tritici* blotch in 238 European wheat cultivars and breeding lines. Plant Pathol., 55: 726-738.

Brading PA, Verstappen ECP, Kema GHJ, Brown JKM (2002). A gene-for-gene relationship between wheat and *Mycosphaerella graminicola*, the *Septoria tritici* blotch pathogen. Phytopathology, 92(4): 439–445.

Chartrain L, Berry ST, Brown JKM (2005a). Resistance of wheat line Kavkaz-K4500 L.6.A.4 to *Septoria tritici* blotch controlled by isolate-specific resistance genes. Phytopathology. 95(6): 664–671.

Chartrain L, Joaquim P, Berry ST, Arraiano LS, Azanza F, Brown JKM (2005b). Genetics of resistance to Septoria tritici blotch in the Portuguese wheat breeding line TE 9111. Theor. Appl. Gene., 110(6): 1138–1144.

Chartrain L, Sourdille P, Bernard M, Brown JKM, (2009). Identification and location of Stb9, a gene for resistance to *Septoria tritici* blotch in wheat cultivars courtot and tonic. Plant Pathol., 58: 547–555.

Chen RS, Boeger JM, McDonald BA (1994). Genetic stability in a population of a plant pathogenic fungus over time. Mole. Ecol., 3(3): 209–218.

Chen RS, McDonald BA (1996). Sexual reproduction plays a major role in the genetic structure of populations of the fungus Mycosphaerella graminicola, Genetics, 142: 1119-1127.

Cordo CA , Sirńon MR , Perello AE, Alippi HE (1999). Spore dispersal of leaf blotch of wheat Mycosphaerella graminicola and Septoria tritici," in Septoria and Stagonospora Diseases of Cereals: A Compilation of Global Research, Van Ginkel M, McNab A, and Krupinsky J, Eds, pp. 98–101.

Diria G, Labhilili M, Bentata F, Abdelwahd R, Gaboun F, Mentag R, Amiri S, Ibriz M (2014). Genetic Diversity of Mycosphaerella graminicola in Morocco Using Amplified Fragment Length Polymorphism. J. Life Sci., 8(3): 211-222.

El-Chartouni L, Tisserant BC, Siah A. Duyme F, Leducq J B, Deweer C (2011). Genetic diversity and population structure in French populations of Mycosphaerella graminicola, Mycologia, 103(4): 764-774.

Elbekali AY, Siah A, Ramdani A, El-Chartouni L, Tisserant BC (2012). First report of the mating types mat1-1 and mat1-2 of *Mycosphaerella graminicola* in Morocco, J. Plant Pathol., 94(1): 243-247.

Eyal Z, Scharen AL, Prescott JM, Van Ginkel M (1987). The Septoria Diseases of Wheat: concept and methods of disease management. vi, 52 p. Mexico, DF (Mexico), CIMMYT.

Friesen TL, Chu CG, Liu ZH, Xu S, Halley S, Faris JD (2009). Host-selective toxins produced by *Stagonospora nodorum* confer disease susceptibility in adult wheat plants under field conditions. Theor. Appl. Gene., 118: 1489–1497.

Ganal MW, Röder, Varshney RK, Tuberosa R (2007). Microsatellite and SNP markers in wheat breeding, in: Genomics Assisted Crop Improvement, Genomics Applications in Crops, 2: 1-24.

GenStat Release 8 Committee, (2002). GenStat® for Windows Release 5·1. Oxford, UK: VSN International.

Ghaffary SMT, Faris JD, Friesen TL, Visser RGF, Vander Lee TA, Robert O, Kema GHJ (2011a). New broad-spectrum resistance to Septoria tritici blotch derived from synthetic hexaploid wheat. Theoretical and Applied Genetics. 24 (1) 125–142.doi:10.1007/s00122-011-1692-7.

Ghaffary SMT, Robert O, Laurent V (2011b). Genetic analysis of resistance to *Septoria tritici* blotch in the French winter wheat cultivars Balance and Apache. Theor. Appl. Gene., 123(5): 741–754, doi: 10.1007/s00122-011-1623-7.

Goodwin S (2007). Back to basics and beyond: increasing the level of resistance to Septoria tritici blotch in wheat, Australian Plant Pathology, 36:532-538.

Goodwin SB (2012). Resistance in wheat to Septoria Diseases caused by Mycosphaerella graminicola (Septoria tritici) and Phaeosphaeria (Stagonaspora) nodorum. Book: Disease Resistance in wheat; Chap. 8, ed.I. Sharma, CAB International, p. 322.

Jlibene M, Elalaoui AC (2002). Variétés de blé adaptées au Nord-Ouest du Maroc. Bulletin Transfert de technologie en agriculture N°88.

Kelm C, Ghaffary SMT, Bruelheide H, Röder MS, Miersch S, Weber WE, Kema GHJ, Saal B (2012). The genetic architecture of seedling resistance to *Septoria tritici* blotch in the winter wheat doubled-haploid population Solitär *x* Mazurka. Mol. Breeding, 29: 813–830. Doi 10.1007/s11032-011-9592-8.

Kema GHJ, Verstappen ECP, Todorova M, Waalwijk C (1996). Successful crosses and molecular tetrad and progeny analyses demonstrate heterothallism in Mycosphaerella graminicola. Current Genetics, 30: 251–258. doi: 10.1007/s002940050129

Linde C, Zhan J, McDonald BA (2002). Population structure of Mycosphaerella graminicola: From lesions to continents, Phytopathology, 92(9): 946-955.

Liu S, Anderson JA (2003). Marker assisted evaluation of Fusarium head blight resistant wheat germplasm. Crop Sci., 43: 760–766.

Mazouz H, Saadaoui M, Jlibene M, Lyamani A (1995). Importance des septorioses du Blé au Maroc, Al Awamia, 91: 63-69.

McCartney CA, Brûlé-Babel L, Lamari L, Somers DJ (2003). Chromosomal location of a race-specific resistance gene to *Mycosphaerella graminicola* in the spring wheat ST6. Theor. Appl. Gene., 107(7): 1181–1186.

McDonald CC, Linde C (2002). Pathogen population genetics, evolutionary potential, and durable resistance. Annu. Rev. Phytopathology, 40: 349-379.

McIntosh RA, Devos KM, Dubcovsky JV (2007).
Catalogue of gene symbols for wheat Supplement.http://wheat.pw.usda.gov/ggpages/wgc/upd.html.

Mujeeb-Kazi A, Cano S, Rosas V, Cortes A, Delgado R (2001). Registration of five synthetic hexaploid wheat and seven bread wheat lines resistant to wheat spot blotch. Crop Sci., 41: 1653–1654.

Raman H, Gustafson P (2010). Molecular breeding for aluminium tolerance in cereals. In: Costa de Oliveira A, Varshney R K (eds.), Root Genomics. Springer Verlag. Berlin, Heidelberg, Germany, pp. 251–288.

Raman H, Milgate A (2012). Molecular breeding for Septoria tritici Blotch Resistance in Wheat. Cereal

Research Communications, 40(4): 451–466. DOI: 10.1556/CRC.40.2012.4.1

Röder MS, Korzun V, Wendehake K, Plaschke J, Tixier MH Leroy P, Ganal MW (1998). A microsatellite map of wheat. Genetics, 149(4): 2007-2023.

Saari EE, Prescott JM (1975). A scale for appraising the foliar intensity of wheat diseases. Plant Disease Report, 59: 377–80.

Saghai-Maroof MA, Soliman K, Jorgensen RA, Allard RW (1984). Ribosomal DNA spacer-length polymorphisms in barley: mendelian inheritance, chromosomal location, and population dynamics. Proceeding of Natural Academic Sciences, USA. 81: 8014-8018.

Schnurbusch T, Paillard S, Fossati D, Messmer M, Schacher-mayr G, Winzeler M, Keller B (2003). Dissecting quantitative and durable disease resistance in Swiss winter wheat I. Septoria glume blotch resistance. Plant & Animal Genomes XI Conference, abstract P. 385.

Shaw MW (1987). Assessment of upward movement of rain splash using a fluorescent tracer method and its application to the epidemiology of cereal pathogens. Plant Pathol., 38: 35–43.

Shaw MW, Royle DJ (1993). Factors determining the severity of epidemics of Mycosphaerella graminicola (Septoria tritici) on winter wheat in the UK. Plant Pathol. 42: 882–899.

Simon MR, Ayala FM, Cordo CA, Röder MS, Börner A (2011). Molecular mapping of quantitative trait loci determining resistance to *Septoria tritici* blotch caused by *Mycosphaerella graminicola* in wheat. Euphytica Gene., 124(1): 125–142.

Simon MR, Cordo CA, Castillo NS, Struik PC, Börner A (2012). Population Structure of *Mycosphaerella graminicola* and Location of Genes for Resistance to the Pathogen: Recent Advances in Argentina. International Journal of Agronomy, vol. (2012), 7p. Doi:10.1155/2012/680275.

Spielmeyer W, Sharp PJ, Lagudah ES (2003). Identification and validation of markers linked to broad-spectrum Stem rust resistance gene Sr2 in wheat (*Triticum aestivum* L.). Crop Sci., 43: 333–336

Xie WL, Nevo E (2008). Wild emmer: genetic resources, gene mapping and potential for wheat improvement. Euphytica, 164: 603–614.

Xu SS, Cai X, Wang T, Harris MO, Friesen TL (2006). Registration of two synthetic hexaploid wheat germplasms resistant to Hessian fly. Crop Sci., 46: 1401–1402.

Yang WY, Liu DC, Li J, Zhang LQ, Wei HT, Hu XR, Zheng YL, He ZH, Zou YC (2009). Synthetic hexaploid wheat and its utilization for wheat genetic improvement in China. J. Gene. Genomics, 36: 539–546.

Zhan J, Mundt, CC, McDonald BA (1998). Measuring immigration and sexual reproduction in field populations of *M. graminicola*. Phytopathol, 88: 1330–1337.

Zhang P, Dreisigacker S, Buerkert A, Alkhanjari S, Melchinger AE, Warburton ML (2006). Genetic diversity and relationships of wheat landraces from Oman investigated with SSR markers. Genet Resour. Crop Evol, 53: 1351–1360.

Zhang X, Haley SD, Jin Y (2001). Inheritance of *septoria tritici* blotch resistance in winter wheat. Crop Sci., 41: 323-326.