Al-Azhar J. Agric. Res., Vol. 15 (Jone) 2013, pp. 1-15

Construction of Genetic Linkage Map and QTL analysis of Net Blotch Resistance in Barley

Abdel-Hadi I. Sayed[1], Shafik I. El-Morsy[1], Sami S. Adawy[2], Shafik D. Ibrahim[2]

[1]Department of Agricultural Botany, Faculty of Agriculture, El-Azhar University
[2]Molecular Genetics and Genome Mapping Department, Agricultural Genetic Engineering Research Institute (AGERI), Agricultural Research Center (ARC), Giza, 12619-Egypt.

ABSTRACT

Net blotch, caused by *Pyrenophora teres f. teres*, is one of the most devastating diseases causing significant losses in barley yield and quality. In the present investigation, two barley verities "AT4" (net blotch resistant) and "Femina" (net blotch susceptible) were used to develop a segregating F2 population. Linkage analysis and map construction were performed using Map Manager. The contracted genetic linkage map consisted of 85 markers including 45 AFLP, 11 SSR, 6 CAPS, 5 SCoT, 3 STS and 6 NBL. Linkage groups were assigned to individual barley chromosomes using the published map locations of the SSR markers as reference point. The produced map showed 7 linkage groups with 85 markers covered a total length of 1644.8 cM. The average length of linkage groups ranged from 77.7 to 739.4. Single point analysis was used to identify the genomic regions associated with net blotch in barley. A total of 14 QTL with a significance ranging from 0.01% to 5% were identified on 4 linkage groups (2, 4, 5 and 6). The most significant QTL was found on chromosome 6H. This QTL presents a promising opportunity for the strategic improvement of barley resistance to net blotch using marker assisted selection.

Key words: QTL; SSR; STS; AFLP; CAPS; Genetic map; *Hordeum vulgare*; Molecular Marker; marker assisted selection; net blotch resistance

INTRODUCTION

Barley (*Hordeum vulgare* L.) is the fifth important cereal crop species in crop production worldwide after maize, wheat, rice, and soybean. It is also a model species for genetic studies as it is an annual and diploid self-pollinating species and has a relatively short life cycle. Net blotch of barley, caused by the fungal phytopathogen *Pyrenophora teres Drechs.* f. *teres Smedeg.*, constitutes a serious constraint to barley production worldwide (Shipton *et al.*, 1973). Net blotch can cause significant yield loss and negatively affect the grain quality. Losses due to net blotch could reach 50% of yield with possible complete loss depending on cultivar susceptibility and environmental conditions (Steffenson *et al.*, 1996). Detecting of sources for resistance to net blotch and an understanding of their genetic background are very crucial in developing new resistant varieties to such disease. Resistance to net blotch is controlled by several genes and dependents on the source of resistance, the development stage and the pathotype used for testing (Svobodova *et al.*, 2011). Using molecular marker technology in barley represents a high efficiency tools for indirect selection and would enhance the efficiency and accuracy of screening for net blotch resistance. Furthermore, quantitative analysis has proven useful for locating

genes controlling complex traits and provides a more accurate estimation of gene location that qualitative analysis because of its lower sensitivity to even modest numbers of phenotypic mis-scores (Wright, 1998) and barley germplasm identification and classification (Struss and Plieske, 1998). The association between molecular markers and phenotypes is one of the most significant developments in the field of molecular genetics and molecular breeding and provide a substantial landmarks for elucidation of genetic variation and detection of genomic regions that is responsible for the trait, which in turn plays an essential role in the strategic improvement of barely using marker-assisted selection (Abu Qamar *et al.*, 2008; Agarwal *et al.*, 2008). Genetic maps have been used to identify markers for single gene and complex traits that are otherwise difficult and expensive to select for in plant breeding programs. Several genetic maps have been constructed and used in QTL analysis for net blotch resistance in barley (Stein *et al.*, 2007; Abu Qamar *et al.*, 2008; Adawy *et al.*, 2008; Gupta *et al.*, 2010; Cakir *et al.*, 2011; Svobodova *et al.*, 2011; Grewal *et al.*, 2012; Jian *et al.*, 2013; Prabin *et al.*, 2013). These studies indicate that there is a multigenic region controlling reaction to net blotch resistance genes and many QTL have been mapped in barley around the world. Furthermore, Several types of molecular markers, such as Amplified Fragment Length Polymorphism (AFLP), Simple Sequence Repeat (SSR), Cleaved Amplified Polymorphic Sequence (CAPS), Start Codon Targeted (SCoT) and Sequence Tagged Site (STS) have been used in mapping and QTL analysis in barley (Litt and Luty, 1989; Vos *et al.*, 1995; Konieczny and Ausubel 1993; Collard and Mackill, 2009; Olson *et al.*, 1989) respectively. The objectives of this study were to construct a genetic linkage map with chromosomal assignment to the linkage groups and to determine the most significant QTL for net blotch resistance in a barley mapping population derived from the intercross between AT4 (net blotch resistant) and Femina (net blotch susceptible) aiming to facilitate the breeding of barley through marker- assisted selection of cultivars characterized by net blotch resistant trait, which is controlled by multiple genes and difficult to handle in conventional breeding programs.

MATERIALS AND METHODS

Mapping population

Two barley varieties AT4 and Femina were provided from Agriculture Research Center (ARC) - Giza Egypt. These two cultivars were used to develop F2 mapping population (88 plants) from the interspecific cross between the Egyptian barley (AT4) and the German barley (Femina). The female parent was AT4. which is characterized by net blotch resistant and the male parent was Femina, which is characterized by net blotch susceptible. The F2 trials were grown in controlled conditions using one of the greenhouse stations in the Agricultural Genetic Engineering Research Institute - Agriculture Research Center (ARC) - Giza Egypt with two replications

Isolation of *Pyrenophora teres* pathogen, plantlet inoculation and phenotype scoring

Isolation, growing and harvesting of *Pyrenophora teres Drechs. f. teres Smedeg* conidia were done according Weiland *et al.*, (1999). Samples of infected barley leaves showing necrotic lesions, were collected in paper bags, marked for each examined location and transferred to the laboratory for isolating the pathogens. Small pieces (2-5mm) were cut from each sample and sterilized with sodium hypochlorite 1% for 2 min and dried between sterilized filter paper and placed onto potato dextrose agar plates (PDA) supplemented with streptomycin-sulfate (100 µg/ml). Sporulation was induced by incubating petri dishes under a black light blue fluorescent lamp emitting near ultraviolet light (NEC model FL8BL-B) with an alternating photoperiod (10 h light:14 h dark) at 19 ^{0}C for 3 d. Single conidia were then inoculated onto 3.9 %potato dextrose agar and incubated at room temperature for 7 d. For inoculum production, each isolate was grown separately on V-8 juice agar at 2^{0}C (Miller, 1955). After 7 days in culture, conidia were washed from the agar surface, filtered through a 330μm strainer and made up into an aqueous suspension containing 12,500 conidia/ml and 0.01% Tween-80. Culture collection was maintained on Potato Dextrose Agar (PDA) slants under paraffin oil. Morphological features including colony colour, mycelial type, production of vertical erect mycelial tufts (coremia), and production of conidiophores and conidia

were examined as described by Scott (1991). Plantlet inoculation was done according to Friesen *et al.*, (2006). Conidia were diluted to 4000 spores/mL, and 2 drops of Tween 20 (polyoxyethylene sorbitan monolaurate) per 100 mL of inoculum were added to reduce spore clumping. Plants were inoculated until a heavy mist was evident on all leaves, but inoculation ceased before runoff could occur. Plants were then placed in 100% relative humidity in the dark at 21 °C for 24 h, and then in a controlled growth chamber under photoperiod conditions of 12 h light: 12 h dark at 21 °C. Disease reactions were read at 7 d post-inoculation. The disease evaluations were done as described by (Tekauz, 1985) using a 1–10 scale.

DNA Markers

Analysis of segregation among the 88 F2 individuals was performed using 11 AFLP combinations, 18 SSR, 12 CAPS, 7 SCoT, 2 STS and 4 primers linked to net blotch.

SSR analysis

SSR assays were performed as described by Adawy (2007). Screening of segregation between the F2 individual plants was carried out using 18 SSR primer pairs (Table 1). The PCR reaction was conducted in 25μl reaction volume containing 1x PCR buffer, 1.5 2 mM $MgCl_2$, 0.2 mM of each dNTPs, 1mM of forward and reverse primers, 1 U Go-Taq Flexi polymerase (Promega) and 25 ng genomic DNA. Amplification was performed with a GeneAmp ®PCR system 9700 (Applied Biosystems, USA) and consisted of an initial denaturation step at 94°C for 5 min, followed by 40 cycles as follows: a denaturation step at 94°C for 1 min, an annealing step each primer for 1 min and an extension step at 72°C for 1.30 min; ending with an extension period of 72°C for 7 min.The PCR products were detected by electrophoresis on 2% agarose, 1xTBE gel stained with ethidium bromide and viewed under ultraviolet light (Fig. 1).

AFLP analysis

Eleven AFLP primer combinations (Table 2) were performed with minor modifications, according to the protocol of Vos *et al.* (1995) using AFLP® Analysis System II (Invitrogen, USA) (Cat.No.10483-022). Approximately 400 ng DNA of each of the 88 F2 individual plants was digested simultaneously with *Eco*RI and *Mse*I at 37°C for 2 hr. A small aliquot of the digested DNA was run on a 1.5% (w/v) agarose gel to check if the DNA digestion was complete. The digested samples were incubated at 70°C for 15 min to inactivate the restriction endonucleases. *Eco*RI and *Mse*I adaptors were ligated to the digested DNA samples to generate template DNA for amplification. The ligation products were diluted 10 fold in TE buffer and 5 μl added to preamplification reaction. Preamplification was carried out with 11-primer combinations each carrying one selective nucleotide in a thermocycler for 20 cycles set at 94°C denaturation (30 sec), 56°C annealing (60 sec) and 72°C extension (60 sec). The amplification products were diluted 50 folds in TE buffer and stored at -20°C. Selective AFLP amplification was carried out with *Eco*RI and *Mse*I primers each carrying three selective nucleotides and 5 μl of the diluted PCR products from the preamplification product. The PCR selective amplification temperature profile was as follows: one cycle at 94°C for 30 sec, 65°C for 30 sec, and 72°C for 60 sec; followed by 12 cycles of touchdown PCR in which the annealing temperature was decreased by 0.7°C every cycle until a 'touchdown' annealing temperature of 56°C was reached. Once reached, another 23 cycles were conducted as described above for preamplification. Two ml of the reaction product was mixed with an equal volume of formamide loading buffer (98% [v/v] formamide, 10 mM EDTA, 0.005% [v/v] of each of xylene cyanol and bromophenol blue), denatured by incubating at 92°C for 3 min and quickly cooled on ice. The products were analyzed on 6% (w/v) denaturing polyacrylamide gels. The gel was run at constant power (50–55 W) until the xylene cyanol was about two-thirds down the length of the gel. The gel was silver stained according to the protocol described by the manufacturer (Promega Corp., USA, Silver Sequence DNA Staining Reagents, Lot. No.171120) (Fig. 2).

SCoT analysis

SCot assay was performed as described by Collard and Mackill, (2009). Screening the segregation between the 88 F2 individual plants was carried out using twenty six primers, PCR amplification was performed in a total volume of 25 µl, containing 1x PCR buffer, 1.5 mM $MgCl_2$, 2.5mM dNTPs, 25 poml of primer, 1 U *Taq* polymerase (promega) and 30ng genomic DNA. Amplification was performed with a GeneAmp ®PCR system 9700 (Applied Biosystems, USA). A standard PCR cycle was used as follow: an initial denaturation step at 94°C for 5 min, followed by 40 cycles of 94°C for 1 min. 50°C for 1 min, and 72°C for 1.30 min; the final extension at 72°C was held for 7 min. All PCR amplification products were separated on 1.5% agarose gels in 1xTBE buffer stained with ethidium bromide and visualized under UV light. (Fig. 3).

CAPS analysis

Twelve primers were used in CAPS as described in (Abu Qamar *et al.*, 2008), PCR reactions were performed in a total volume of 25 µl and consisted of 40ng genomic DNA, 1×PCR buffer, 2.5 mM dNTPs, 1.5 mM $MgCl_2$, 1 u *Taq* polymerase (promega), and 20 pmol of forward and reverse primers. Amplification was performed in a GeneAmp ®PCR system 9700 (Applied Biosystems, USA) under the following program: 5 min at 94°C; 40 cycles of 40 sec at 94°C, 1min at 55°C, and 1min at 72°C; and extra extension for 7 min at 72 C. PCR products were produced as described above but were digested before being loaded onto the gel. Digestions were done by adding 1.5 µl of 10× buffer and 2 u of the restriction enzymes *Rsa*I, *Hae*III and *Eco*RV. The resulting 15 µl volume was incubated at 37°C for 2 h. Polymorphism was detected by separating the whole volume of treated DNA on 2% agarose gel (1×TBE) containing ethidium bromide, and visualizing it under a UV transilluminator. (Fig. 4).

STS analysis

Two STS primers were used in PCR amplifications according to (Abu Qamar *et al.*, 2008), which performed in a 25 µl reaction containing 1u *Taq* polymerase (promega), 1×PCR buffer, 1.5 mM $MgCl_2$, 2.5 mM dNTPs, 20 pmol of forward and reverse primers and 30ng of genomic DNA. The reaction amplified using PCR program that included denaturing at 94°C for 5 min, 40 cycles of 45 sec at 94°C, 1min at 55°C and 1 min at 72°C, and finally a 7min extension at 72°C. Detection of polymorphisms PCR products were run on 2% agarose gels to detect polymorphisms. (Fig. 5).

NBL analysis

Four NBL primers were used in PCR amplifications according to (Abu Qamar *et al.*, 2008) which performed in a 25 µl reaction containing 1u *Taq* polymerase (promega), 1×PCR buffer, 1.5 mM MgCl2. 2.5 mM dNTPs, 20 pmol of forward and reverse primers and 30ng of genomic DNA. The reaction amplified using PCR program that included denaturing at 94°C for 5 min, 40 cycles of 1 min at 94°C, 1 min at 50°C and 1.5 min at 72°C, and finally a 7min extension at 72°C. Detection of polymorphisms PCR products were run on 8% non-denaturing polyacrylamide gels (26.6 ml of 30% acrylamide solution, 62.7 ml water, 10 ml 10X TBE and 0.7 ml of 10% APS) to detect polymorphisms. (Fig. 6).

Genetic Linkage Map and QTL analysis

The markers that showed polymorphism between the parental lines were used to construct the genetic linkage map. For each marker, the F2 individuals were scored as '1' or '3' for presence of the parental band of the female parent (AT4) or the male parent (Femina), respectively, or '0' for missing data. Linkage analysis and map construction were performed by using Map Manager QTX14 (Manly and Cudmore 1997) using the Haldane function to convert the recombination frequencies to centiMorgans

(cM). The linkage groups were constructed using the "make linkage group" command with a minimum LOD score of 3.0 followed by ripple command for each linkage group to check the final order of markers. QTL analysis was performed as single point analysis (SPA) using QTL cartographer. A significance level 5%, 1%, 0.1% and 0.01% were used to declare a QTL.

RESULTS AND DISCUSSION

The population used in this study was useful for identifying loci controlling quantitative traits for Net Blotch because it was derived from an interspecific cross between genetically diverse parents exhibiting contrasting traits for Net Blotch resistance. From the survey of the two parents using 18 SSR, 11 AFLP primer combinations, 12 CAPS, 7 SCoT, 4 NBL and 2 STS, a total of 1104 major polymorphic bands were observed from SSR (102 bands), AFLP (834 bands), CAPS (48 bands), SCoT (85 bands), NBL (29 bands) and STS (6 bands) . Of these major bands, only 213 (19.3%) were polymorphic between the two parents, 30 for SSR, 133 for AFLP, 25 CAPS, 14 SCoT, 8 NBL and 3 STS (Tables 1, 2, 3, 4 and 5). For SSR, the number of polymorphic bands obtained by individual primer ranged from 2 for SSR-20 (ABC02222) and SSR-17 (GBM1423) to 10 for SSR-9 (XGWM129) with a mean of 5.6 bands. The primer SSR1 and SSR2 produced the largest number of polymorphic bands (4 in total) (Table 1). For AFLP, the number of bands generated by individual primer combinations ranged from 55 for 6/8 (*Eco*RI + AGG / *Mse*I +CTC) to 98 for 7/1 (*Eco*RI + ACC/ *Mse*I + CAA), with a mean of 75.8 bands. The primer combination *Eco*RI + ACC/ *Mse*I + CAA produced the largest number of polymorphic products (22 in total) (Table 2). For SCoT, the number of bands generated ranged from 8 for SCoT-20 to 15 for SCoT-9 with a mean of 12.1 bands. The primers SCoT-8 and SCoT-9 produced the largest number of polymorphic bands (3 in total) (Table 3). For CAPS, the number of bands obtained by individual primer ranged from 2 for CAPS-1(Bmag149), 3(MWG652), 4(MWG916), 8(CMWG2029) to 7 for CAPS-9(rbags39h18). The primer CAPS-9 (rbags39h18) produced the largest number of polymorphic bands (4 in total) (Table 4). For NBL, the number of polymorphic bands ranged from 3 for bar-23 to 12 for bar-22. The primers bar-22 produced the largest number of polymorphic bands (4 in total) (Table 3). For STS, the number of bands obtained were 2 for the primer Nb13 and 4 for the primer Nb 2.

Among the 194 polymorphic markers, 108 were unlinked and 85 were distributed on 7 linkage groups covering 1644.8 cM. The linkage groups ranged from 77.7 cM in linkage group 1 to 739.4 cM in linkage group 2(2H) with an average distance of 19.4 cM between loci (Figure 7). The distribution of markers, linkage group assignment and map coverage across the 7 barley linkage groups are summarized in Table (6). The biggest linkage group consisted of 36 marker loci covering 739.4 cM with 20.5 cM average distance in linkage group 2(2H) and the smallest were linkage groups 1 consisting of 4 marker loci with average distance of 19.4 cM, respectively. Linkage groups were assigned to individual barley chromosomes using the published map locations of the SSR markers as reference point (Table 1). The LG2, LG6 and LG7 were assigned to chromosomes 2H, 6H, and 7H respectively (Figure 7). Several genetic maps have been constructed and used in QTL analysis for net blotch resistance in barley (Steffenson *et al.* 1996; Stein *et al.* 2007; Abu Qamar *et al.* 2008; Adawy *et al.* 2008; Gupta *et al.* 2010; Cakir *et al.* 2011; Svobodova et al. 2011; Grewal et *al.* 2012; Jian *et al.* 2013; Prabin *et al.* 2013). These studies indicate that there is a multigenic region controlling reaction to net blotch resistance genes and many QTL have been mapped in barley around the world. The genetic map constructed in this work is different from the previously constructed maps as it consists of 6 different molecular markers (NBL, SSR, AFLP, STS, CAPS and SCoT) integrated together on one map. However, more markers are needed to produce more saturated genetic map for this population. The map constructed in this work was useful to determine QTL for net blotch resistance in the F2 barley population derived from the interspecific cross between AT4 and Femina. In this context, a total of 14 QTL with a significance ranging from 0.01% to 5% were identified on 4 linkage groups (2H, 4, 5 and 6H). Where, 3 QTL were identified on LG 2 (2H), 2 QTL on LG4, 1 on LG 5 and 8 on LG 6 (Figure 7). The most significant QTL was found on chromosome 6H and represented a large genomic region on chromosome 6H. This result is in agreement with several previous studies that used molecular markers to identify a region on chromosome 6H that harbors the net

blotch resistance genes. This work shows that multiple net blotch resistance genes exist at the locus on chromosome 6H (Friesen *et al.* 2006; Abu Omar *et al.* 2008). Moreover, Major resistance genes effective against net blotch disease have previously been identified on chromosome 6H by Cakir *et al.* (2003) and Manninen *et al.* (2006) using different resistant sources. Both Cakir *et al.* (2003) and Manninen *et al.* (2006) found that a major QTL on chromosome 6H accounted for 65% of the disease variation. From the work presented in this and previous study, It is possible to conclude that the 6H chromosome harbor a major genomic region that is responsible for net blotch resistance in barley. It is also possible to postulate that this region with the associated markers is a very promising rejoin for marker assisted selection for net blotch resistance in barley because the QTL detected in this rejoin was consistent across different types of population used in previous studies and effective against multiple pathotypes of *P. teres* f. *teres* (Manninen *et al.* 2006; Cakir *et al.* 2003; Friesen *et al.* 2006 and Abu Omar *et al.* 2008). Moreover, from the maps of Liu *et al.* (2010), and Kunzel *et al.* (2000), it can be concluded that Rpt5 is located at the very centromeric region of chromosome 6H. The centromere is located between HVM14 and HVM65 on the map of Liu *et al.* (2010), and the distance between the microsatellite markers is less than 10 cM. However, when the consensus barley map (Qi *et al.* 1996) is used to bridge the maps of Liu *et al.* (2010) and Kunzel *et al.* (2000), it can be concluded that this region covers more than half of the physical distance of chromosome 6H. Pierre *et al.* (2010) identified major QTL for net blotch on chromosome 6H, located in bins 2 and 6. The QTL located in bin 6 explained 19 to 48% of the phenotypic variation and the QTL located in bin 2 explained 25 to 44% of the phenotypic variation. While Gupta *et al.* (2010) detected QTL on chromosomes 3H and 6H using simple sequence repeat (SSR) markers. while Cakir *et al.* (2011) detected a new locus conferring resistance to *P. teres* f. maculata at both seedling and adult plant stages on the short arm of chromosome 6H and from the seedling testing against *P. teres f. teres*, five highly repeatable QTL were detected on chromosomes 2HS, 2HL, 3HS, 4HL, and 6HS.

Further population development, more saturated maps and allelism tests are needed to confirm the usage of the 6H rejoin in the breeding programs for selecting barley cultivars resistance to net blotch disease. Also, Friesen *et al.* 2006 identified QTL for net blotch on barley chromosome 4H. Although many major and minor resistance genes have been identified on 4H (Steffenson *et al.* 1996 and Raman *et al.* 2003). We have identified only 2 QTL on LG 4 but still there is no clear evidence that LG 4 in this map is the 4H barley chromosome. More anchored 4H specific markers need to be employed in this map to validate this assumption. On the other hand, Manninen *et al.* (2006) did not identify any QTL on 6H they reported the identification of QTL for net blotch in barley on chromosomes 1H, 2H, 3H, 5H, and 7H. This results does not agree with the results obtained in this and previous work. In this study, we designated 14 net-blotch resistance QTL with a significance ranging from 0.01% to 5% on linkage groups 2(2H), 4, 5 and a major QTL on 6(6H). Utilizing molecular markers and QTL mapping has proved to be a useful tool for locating chromosomal regions controlling genetic variation for net blotch resistance in barley. The results obtained from this work could be useful for the strategic improvement of barley through marker assisted selection for net blotch resistant cultivars.

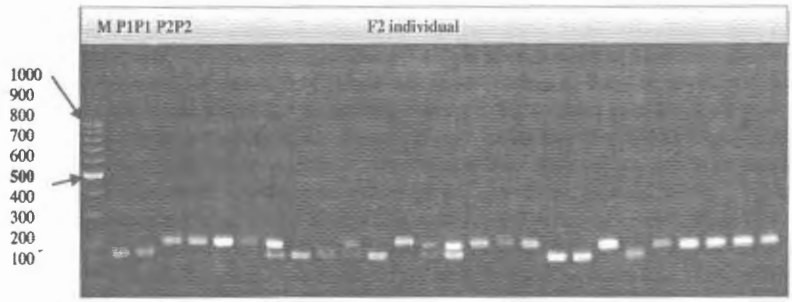

Figure (1): Segregation of SSR markers among the F2 population derived from the intercross between 'AT4' and 'Femina'. M is the DNA marker 100 bp plus ladder P1 (AT4) and P2 ('Femina').

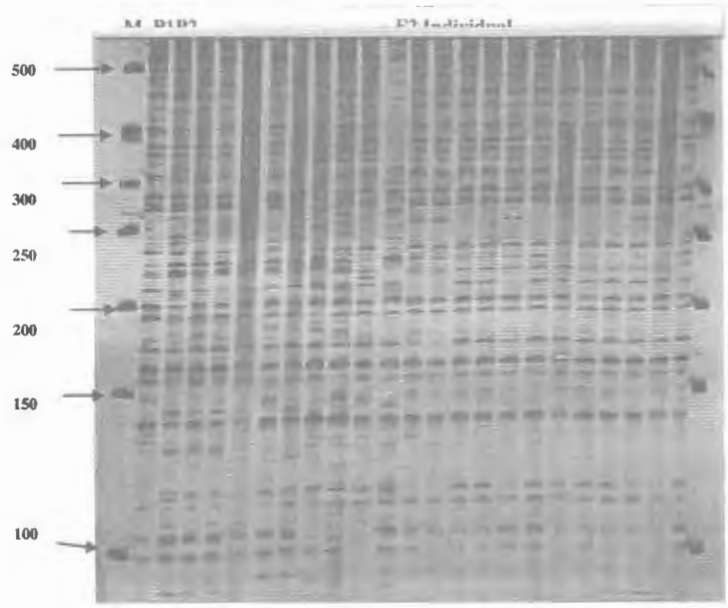

Figure (2): AFLP segregating pattern in F2 population from the cross of ('AT4') (P1) and ('Femina') (P2). The amplification was made using different primer combinations. M is the DNA marker 100 bp plus ladder.

Figure (3): SCoT agarose gel showing some polymorphic bands between the 2 parents P1 (AT4) and P2 (Femina) and the segregation among F2 individuals. M is the DNA marker 100 bp ladder.

Figure (4): CAPS agarose gel showing polymorphic bands between the 2 parents P1 (AT4) and P2 (Femina) and segregation among F2 individuals. The markers (M) used was 100 bp ladder.

Figure (5): STS agarose gel showing polymorphic bands between the 2 parents P1 (AT4) and P2 (Femina) and segregation among F2 individuals. The markers (M) used were DNA ladder 100 bp

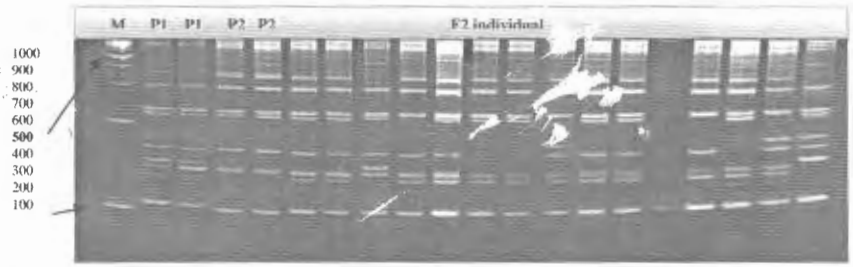

Figure (6): NBL acrylamide gel showing polymorphic bands between the 2 parents P1 (AT4) and P2 (Femina) and segregation among F2 individuals. The markers (M) used was100 bp ladder.

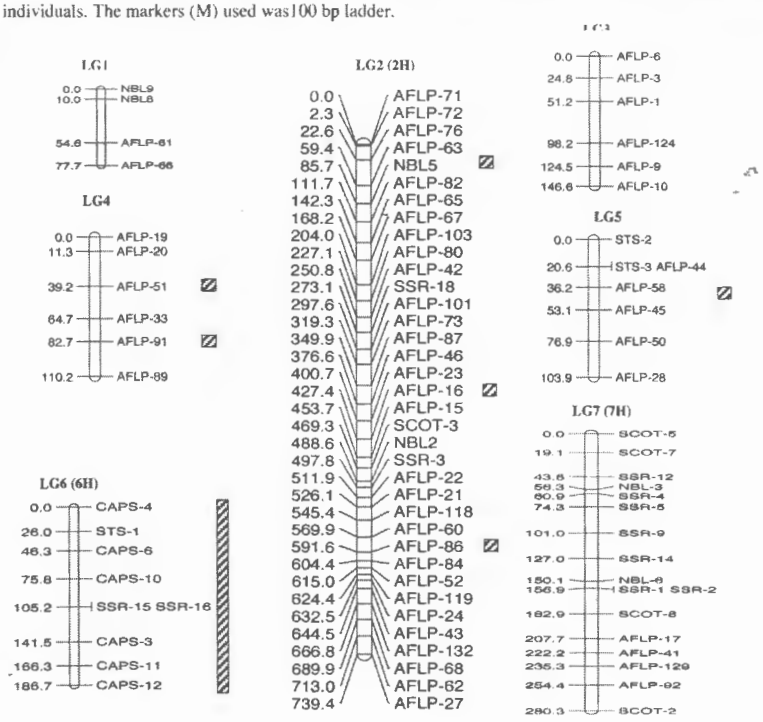

Figure (7): Molecular linkage groups of barley (intercross between 'AT4' and 'Femina') showing positions of QTL influencing net blotch. Map distances between adjacent markers are in cM.

Table (1): Primer code, Primer name, primer sequence, Chromosome and Marker size as detected by SSR.

Primer code	Primer Name	Primer sequence	*Chrom.	number of bands		% of polymerphism
				Total	Polymorphic	
SSR-1(140) ,2(220)	HVPRP	F.ATAACTACCAGATCACCTTCTGCC R.GGAACGAAGGGAGTATTA.AGCA	7H	8	4	50
SSR-3	HVCSG	F.CACTTGCCTACCTCGATATAGTTTGC R.GTGGATTCCATGCATGCAATATGTGG	2H	4	2	50
SSR-4	HVM4	F.AGAGCAACTACCAGTCCAATGGCA R.GTCGAAGGAGAAGCGGCCCTGGTA	7H	4	2	50
SSR-5	GBM1464	F. ATAGCCGTGCTCTTGCTCAT R. CAAGACCACCATTTGCATT	7H	6	2	33.3
SSR-6(320) ,7(600)	GBM1303	F. TCTTTTTGGAGGGGTTTCCT R. ATCATCTTCACGCTTCCTCC	7H	8	2	25
SSR-8	Xgwm333	F. GCCCGGTCATGTAAAACG R. TTTCAGTTTGCGTTAAGCTTTG	-	3	1	33.3
SSR-9	XGWM129	F.TCAGTGGGCAAGCTACACAG R.AAAACTTAGTAGCCGCGT	-	10	1	10
SSR-10(130) ,11(300)	Bmag 13	F. AAGGGGAATCAAAATGGGAG R. TCGAATAGGTCTCCGAAGAAA	3H	8	2	25
SSR-12	Xcfd39	F.CCACAGCTACATCATCTTTCCTT R.CAAAGTTTGAACAGCAGCCA	-	8	1	12.5
SSR-13(150) ,14(550)	MGB 318	F.CGGCTCAAGGTCTCTTCTTC R. TATCTCAGATGCCCCTTTCC	-	6	2	33.3
SSR-15(280) .16(700)	ABC02231 4	F. AGGCAAGTAGCAGCGAAGAC R. CTGCCATTGCTTGTCGTAGA	6H	4	2	50
SSR-17	GBM1423	F. ACAAATCCCCAAGCCAATCT R. CTTGCCTGTCAACGTCTTCA	6H	2	2	100
SSR-18	Bmac0144	F. TACGTGTACATACTCTACGATTTG R. ACTTATTCTGCATCCTGGGT	2H	3	1	33.3
SSR-19	Bmac0187	F. GCTCTCTCTCAGAAAAATGAA R. GAATTATTCTAGGGCTGTGAA	7H	8	1	12.5
SSR-20	ABC02222	F. TTGATGTGCAGCAAGTCTCC R.TCCTTAACCGCGATTCAAAC	6H	2	1	50
SSR-21	HVM60	F. CAATGATGCGGTGAACTTTG R. CCTCGGATCTATGGGTCCTT	3H	6	1	16.6
SSR-22	GBM1118	F. GACCGGAGCAGATCTTTGAG R. CTCCTCCTCCTCGGACTCTT	6H	8	2	25
SSR-23	HVGLUEND	F.TTCGCCTCCATCCCACAAAG R. GCAGAACGAAAGCGACATGC	1H	4	1	25
Total				**102**	**30**	**29.4**
Average				**5.6**	**1.6**	

*** The information for chromosomes assignments was obtained from www.graingenes.com**

Table (2): Selective nucleotides of AFLP primer combinations, number of total bands, polymorphic bands and percentage of polymorphism.

Primer Comb.	Selective nucleotides		Number of bands		% of polymorphism
	*Eco*R1	*Mse*I	Total	Polymorphic	
1/8	AAC	CTT	79	8	10.1
3/3	ACA	CAG	57	8	14.1
4/1	ACC	CAA	68	9	13.2
4/2	ACC	CAC	71	15	21.1
4/4	ACC	CAT	88	11	12.5
5/6	ACG	CTC	91	18	19.7
6/2	ACT	CAC	73	12	16.4
7/1	AGC	CAA	98	22	22.4
7/2	AGC	CAC	82	5	6.1
7/3	AGC	CAG	72	18	25
8/6	AGG	CTC	55	7	12.7
Total			**834**	**133**	**15.9**
Average			**75.8**	**12.1**	

Table (3): Primer name, primer sequence, number of total bands, polymorphic bands and percentage of polymorphism as detected by SCoT .

Primer Name	Primer Sequence	Number of bands		% of polymorphism
		Total	Polymorphic	
SCoT-7	ACAATGGCTACCACTGAC	13	2	15.3
SCoT-8	ACAATGGCTACCACTGAG	11	3	27.2
SCoT-9	ACAATGGCTACCACTGCC	15	3	20
SCoT-10	ACAATGGCTACCACCAGC	12	2	8.3
SCoT-17	CCATGGCTACCACTACCC	13	1	15.3
SCoT-20	CAACAATGGCTACCACGC	8	1	12.5
SCoT-22	CCATGGCTACCACCGCAC	13	2	15.3
Total		**85**	**14**	**16.2**
Average		**12.1**	**2**	

Table (4): Primer code, primer name, primer sequence, number of total bands, polymorphic bands and percentage of polymorphism as detected by CAPS.

Primer code	Primer name	primer sequence	Number of bands		% of polymorphism
			Total	Polymorphic	
CAPS-1	Bmag149	F. CAAGCCAACAGGTAGTC R. ATTCGGTTTCTAGAGGAAGAA	2	2	100
CAPS-2	MGB357	F. GCTCCAGGGCTCCTCTC R. AGCTCTCTCTGCACGTCCTT	4	2	50
CAPS-3	MWG652	F. AGCTGCTCGTTCTCGTTGA R. CACACCTTCTTCTTCCTCTT	2	2	100
CAPS-4	MWG916	F. GCGGACCAGATCAATATCGA R. CGACGTAGGGAAACACGCAT	2	2	100
CAPS-5	rbah58k07	F. AGAGAACCGGGCACCAAGA R. TGGCCTGCTCCTCATCACTG	6	1	16.6
CAPS-6	ABC02895	F. TGATCGGTCCAGTTCACCCA R. GGAATCGCAAGCACTACGGG	4	2	50
CAPS-7	GBS0468	F. TGAACATCAGTCAAACACCAACA R. CATCCTTCCTGACAGCTTAAACC	4	2	50
CAPS-8	CMWG2029	F. CCAGTTATCCGAATCCGGAA R. GTGGTCAGGTACATACGAAT	2	2	100
CAPS-9	rbags39h18	F. CACACCGCACACAACATACA R. TAAGCGTGTGTCATGGGAAA	7	4	57.1
CAPS-10	ABC06878	F. CGACAAGATGGTGGAGGAGT R. ACTTCGACAGGGAGGTCAAA	5	2	40
CAPS-11	cMWG679	F. TCAAGGCTAACCCCATGTTC R. CCCATGAAGATGAGTGCAT	6	2	33.3
CAPS-12	ABG458	F. CCCTTTCCTCCTCGTCCTTT R. CTTGAACCAAACGGCCTCTC	4	2	50
Total			**48**	**25**	**52.1**
Average			4	**2.1**	

Table (6): Distribution of molecular markers, assignment and centiMorgen (cM) coverage across the 7 linkage group of the genetic map used in QTL mapping.

Linkage Group	AFLP	SSR	CAPS	SCoT	STS	NBL	Markers		cM	cM/marker
							#	%		
LG1	2	-	-	-	-	2	4	4.7	77.7	19.4
LG2(2H)	31	2	-	1	-	2	36	42.3	739.4	20.5
LG3	6	-	-	-	-	-	6	7.1	146.6	24.4
LG4	6	-	-	-	-	-	6	7.1	110.2	18.4
LG5	5	-	-	-	2	-	7	8.2	103.9	14.8
LG6(6H)	-	2	6	-	1	-	9	10.5	186.7	20.7
LG7(7H)	4	7	-	4	-	2	17	20	280.3	16.5
Total	**54**	**11**	**6**	**5**	**3**	**6**	**85**	**100**	**1644.8**	**19.4**
average	**7.7**	**1.6**	**0.9**	**0.7**	**0.4**	**0.9**				

Table (5): Primer code, primer name, primer sequence, number of total bands, polymorphic bands and percentage of polymorphism as detected by NBL and STS.

Primer code	Primer Name	Primer Sequence	Number of bands		% of polymorphism
			Total	Polymorphic	
NBL					
Bar-21	Bmag173	F. CATTTTTGTTGGTGACGG R. ATAATGGCGGGAGAGACA	7	1	14.3
Bar-22	Bamg0381	F. TTTTATTATTGCATCTAGGGC R. TATCAAGATCATGACGTCTCA	12	4	33.3
Bar-23	Ebmac0623	F. CGAACATTGTCGTGTTAGTAA R. CTGTCATGCATAACCTATGG	3	1	33.3
Bar-27	Bmac0181	F. ATAGATCACCAAGTGAACCAC R. GGTTATCACTGAGGCAAATAC	7	2	28.6
Total			**29**	**8**	**27.5**
Average			**7.2**	**2**	
STS					
Nb-13	ABG388	F. GCACTGGCATAGTCTCACAA R. CGATGCTGGTTCGGTCATAC	2	1	50
Nb-21	ABC01719	F. GGAGACCTCCATCTTCGCCA R. GGCAGCGGAAAAACAACAGC	4	2	50
Total			**6**	**3**	**50**
Average			**3**	**1.5**	

Table (7): The most significant QTL detected by the single point analysis. This analysis fits the data to the simple linear regression model y = b0 + b1 x + e. The results below give the estimates for b0, b1 and the F statistic for each marker

Chrom.	Marker	b0	b1	2ln(L0/L1)	F(1,n-2)	pr(F)	Degree of Significance
LG2 (2H)	5	2.535	0.465	3.954	3.952	0.05	*
LG2 (2H)	18	2.023	-0.577	8.937	9.193	0.003	**
LG2 (2H)	27	1.934	-0.362	1.162	1.144	0.288	*
LG6 (6H)	1	2.18	0.863	25.634	29.082	0	****
LG6 (6H)	2	2.108	0.719	16.452	17.679	0	****
LG6 (6H)	3	2.077	0.791	20.138	22.114	0	****
LG6 (6H)	4	2.105	0.621	11.635	12.157	0.001	***
LG6 (6H)	6	2.129	0.601	11.182	11.653	0.001	***
LG6 (6H)	7	2.171	0.977	34.341	41.05	0	****
LG6 (6H)	8	2.017	1.395	91.647	157.664	0	****
LG6 (6H)	9	2.074	1.206	58.689	81.548	0	****
LG5	4	2.275	0.095	0.231	0.226	0.636	**
LG4	3	2.353	0.389	4.236	4.241	0.042	*
LG4	5	2.366	0.398	4.343	4.351	0.04	*

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تشييد خريطة جينومية وتحليل QTL لمقاومة التبقع الشبكي في الشعير

عبدالهادي ابراهيم سيد[1] ، شفيق ابراهيم المرسي[1]، سامي سعيد عدوي[2] ، شفيق درويش ابراهيم[2]

١- قسم النبات الزراعي – كلية الزراعة – جامعة الازهر
٢- قسم الواسمات الجزيئية والخرائط الجينومية – معهد بحوث الهندسة الوراثية الزراعية – مركز البحوث الزراعي

الملخص

التبقع الشبكي بسببة المسبب المرضي ***Pyrenophora teres* f. teres** وهو احد الامراض التي تسبب خسائر كبيرة لمحصول الشعير من حيث المحصول والجودة . في هذه الدراسة تم استخدام نوعين من الشعير (**AT4**) مقاوم للتبقع الشبكي و (**Femina**) حساس للتبقع الشبكي وذلك للحصول علي الجيل الثاني F2 للعشيرة . واجراء التحاليل وتشييد الخريطة الجينية باستخدام البرنامج (**Map Manager**) . حيث تم تشييد خريط الربط الوراثية باستخدام ٨٥ واسم التي تحتوي علي " ٤٥ واسم جزيئي AFLP و١١ واسم جزيئي SSR و٦ واسم جزيئي CAPS و ٥ واسم جزيئي SCoT و٣ واسم جزيئي STS و٦ واسم جزيئي NBL ". تم تعيين مجاميع الربط في خريط الربط الجينومية ككروسومات للشعير وذلك باستخدام الابحاث السابقة للخرائط الموقع عليها الواسم الجزيئي SSR . وظهرت الخريطة الوراثية ٧ مجاميع ارتباطية تحتوي علي ٨٥ واسم جزيئي حيث يبلغ طول المجاميع الارتباطية ١٦٤٤.٤ سنتيمورجان . ويتراوح طول المجاميع الارتباطية فيها ما بين ٧٧.٧ الى ٧٣٩.٤. وبستخدام التحليل الرقمي الفردي (SPA) لتحديد مناطق الجينوم المرتبطة بالتبقع الشبكي في الشعير. تم الحصول علي ١٤ QTL وذلك باستخدام التحليل الرقمي الفردي (SPA) تتراوح من ٠.٠١٪ الى ٥٪ حيث تم تحديدهم علي ٤ مجاميع ربط هما (٢ و ٤ و ٥ و ٦). حيث كانت اهم هذه QTL الموقعة علي المجموعه (٦) المعرفة بكروموسوم (6H). هذه QTL تعطينا الفرصة للتحسين الاستراتيجي لمقاومة الشعير للتبقع الشبكي باستخدام الانتخاب بمساعدة الواسمات.