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MAPPING OF QTLs CONTROLLING RESISTANCE TO LEAF STRIPE (*Pyrenophora graminea*) IN EGYPTIAN BARLEY

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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.

Genetic resistance to plant diseases is a major objective of most plant breeding programs. Determining the location of the genes/factors, controlling resistance can assist in the rapid and efficient development of new resistant varieties. The durability and stability of plant disease resistance is also a fundamental issue, as it is an important asset in new varieties. Leaf stripe disease on barley is caused by the seed-transmitted hemi-biotrophic fungus *Pyrenophora graminea* (anamorph *Drechslera graminea*). The disease causes severe yield reductions at high infection rates, especially in organic farming systems (Mueller *et al*., 2003). The fungal mycelia survive in seeds between the parenchymatic cells of the pericarp, and in the hull and the seed coat, but not in the embryo (Platenkamp, 1976). Leaf stripe disease is characterized by a

cold climate during the sowing season. In susceptible varieties, the disease causes brown stripes on the leaves, stunted growth and severe yield reductions (Tekauz, 1983; Porta-Puglia *et al.*, 1986). The fungus is unable to cause secondary infection through leaf-to-leaf transmission and therefore behaves as a true, seed-borne disease.

Detecting of sources for resistance to leaf stripe and an understanding of their genetic background are very crucial to develop new resistant varieties for such disease. Resistance to leaf stripe is controlled by several genes and dependents on the source of resistance. Genome research promises to promote continued and enhanced plant genetic improvement. As a world's leading crop and a model system for studies of many biological processes, genomics research of barley has advanced rapidly in the past few years. Genetic linkage maps are useful tools for studying genome structure, organization and evolution and to estimate gene effects of important agronomic traits, identifying introgression and for tagging genes of interest to facilitate marker aided selection (MAS) and map based cloning. Genetic linkage maps saturated with polymorphic molecular markers have already been generated for many species of economic and scientific interest including: barley (Rostoks *et al*., 2005; Stein *et al*., 2007; Adawy *et al.*, 2013), durum wheat (Diab *et al*., 2013; Patil *et al*., 2013; Kumar *et al*., 2013), cotton (Adawy *et al*., 2008 and 2013), maize (Stange *et al*., 2013), rice (Li *et al*., 2011; YueHui *et al.*, 2013), tomato (Gonzalo and van der Knaap, 2008; Shirasawa *et al*., 2010; Sim *et al*., 2012), and hexaploid wheat (Röder *et al*., 2004; Quarrie *et al.*, 2005; Paux *et al*., 2008; Bennett *et al.*, 2012).

The Simple Sequence Repeats (SSRs) are PCR-based marker and have proved to be useful in barley research as they offer reproducibility, multi-allelic nature, co-dominant inheritance, genome specificity, relative abundance, and good genome coverage (Varshney *et al*., 2005; Ganal and Roder, 2007). They have been used in the localization of genes to chromosomes (Roder *et al*., 2004), identification of quantitative trait loci (QTL) for yield and quality traits (Ganal and Roder, 2007). Meanwhile, the Amplified Fragment Length Polymorphism (AFLPs), developed by Vos *et al*. (1995). AFLP fragments are inherited in a Mendelian fashion as dominant or co-dominant markers, making the technique amenable to tracking inheritance of genetic loci in a segregating population. A new frontier type of genetic marker is start codon targeted (SCoT) polymorphism, which is a novel, simple and reliable gene targeted marker technique based on designed primers targeting conserved region surrounding the translation initiation codon ATG (Collard and Mackill, 2009). This type was successfully employed in development of QTL mapping in both durum wheat and barley (Adawy *et al.*, 2013; Diab *et al*., 2013).

The objectives of this study were to construct a QTL map with chromosomal assignment and to determine the most significant QTL controlling leaf stripe severity in a barley mapping population derived from the intraspecific cross between At29 (leaf stripe resistant) and At20 (leaf stripe susceptible) aiming to facilitate the breeding of barley through marker-assisted selection of varieties characterized by leaf stripe resistant trait, which is controlled by multiple genes and difficult to handle through conventional breeding programs.

MATERIALS AND METHODS

Mapping population

The mapping population used in this work was a segregating F_2 population of 60 individuals derived from the intraspecific cross between At20 and At29. The female parent was cv "At29," which is characterized by leaf stripe resistance and the male parent was cv "At20" which is characterized by leaf stripe sensitivity. The F_2 trials were grown in controlled conditions using one of the greenhouses in the Agricultural Genetic Engineering Research Institute (AGERI), Agriculture Research Center (ARC), Giza, Egypt.

DNA isolation

DNA was isolated from the two parents and the 60 F_2 plants using a DNAeasy Plant Mini Kit (Qiagen, Santa Clarita, CA).

Molecular markers

Four different types of molecular markers (13 LSL, 15 SSR, 18 SCoT and 10 AFLP primer pairs or primer combinations) were used to screen polymorphism between the two parental accessions (At20 and At29). Markers found to be polymorphic were then used to survey the segregation among the 60 individuals of the F_2 mapping population.

Simple sequence repeats (SSR) analysis

Forty-eight anchor microsatellite markers located on barley chromosomes were chosen from Grain Genes database and different published papers for analysis. SSR analysis was conducted as described by Hussein *et al.* (2006) and Adawy (2007). Analysis of segregation among the 60 F_2 individuals was performed using 15 anchor SSR primer pairs that showed a polymorphic pattern between the parents.

Start codon targeted (SCoT) analysis

SCoT analysis was performed as described by Collard and Mackill (2009). Initially, Forty-eight 18-mer SCoT primers were screened against the two parental varieties. Eighteen out of these 48 primers showed polymorphic pattern. Therefore, they were used to survey the segregation among the 60 F_2 individuals.

Amplified fragment length polymorphism (AFLP) analysis

The AFLP analysis was performed using the AFLP® Analysis System II (Invitrogen, USA) as described by Vos *et al.* (1995). Initially, 22 AFLP primer combinations (PCs) were tested on the two parental varieties. Among these, the best ten PCs were selected on the basis of the number of bands, clarity of pattern, and distribution on the gel. These were tested again on the parental varieties and the 60 F_2 individuals.

Leaf stripe linked (LSL) analysis

Thirteen markers linked to leaf stripe resistance were chosen from previously published papers for analysis (Tacconi *et al*., 2001; Bulgarelli *et al*., 2004; Biselli *et al*., 2010) on barley chromosomes. Some of these LSL markers were used as CAPS markers through cutting with restriction enzymes. Initially, all primers were screened against the two parental varieties. Six out of these 13 primers showed polymorphic pattern. Therefore, they were used to survey the segregation among the 60 F_2 individuals.

Phenotypes and traits measurements

The parents and F_2 mapping populations were evaluated for two morphological traits (disease severity and plant height).

Genetic linkage map construction and QTL detection

The 104 markers that showed polymorphism between the parental varieties were used to construct the genetic linkage map. Linkage analysis and map construction were performed using Map Manager QTX V.1.4 (Manly and Cudmore, 1997). The graphic representation of the linkage groups was created using Map Chart 2.1 (Voorrips, 2002). The association between phenotype and genotype was investigated using single point analysis (SPA), using Windows QTL Cartographer V. 2.5 (Wang *et al.*, 2007).

RESULTS AND DISCUSSION

An intraspecific F_2 population of 60 plants derived from the cross between the resistant variety “At29” and the susceptible variety “At20” to leaf stripe pathogen (*Pyrenophora graminea*) and was helpful for identifying loci controlling leaf stripe resistance and plant height.

From the survey of the two parents and F_2 population using 15 SSR, 10 AFLP primers combinations, 18 SCoT and 13 LSL, a total of 1098 major bands were observed (30 SSR, 821 AFLP, 218 SCoT and 29 LSL). Of these major bands, only 239 (21.7%) were polymorphic between the two parental varieties and among population giving 15 for SSR, 161 for AFLP, 50 SCoT and 13 LSL (Tables 1, 2, 3 and 4).

Molecular markers analysis

Different molecular markers including SSR, SCoT, LSL and AFLP were employed to identify markers that reveal differences between parents and among the F_2 individuals.

1. ***Simple sequence repeat analysis (SSR)***

Forty eight microsatellite primer pairs previously mapped the barely chromosomes (Grain Genes database) were used in this study. These primers were screened against parental varieties "At20" and "At29", in an attempt to detect polymorphic markers. Only 15 SSR primers (31.25%) showed polymorphism between the two parents. The analysis of segregation among the 60 F_2 individuals was performed using these 15 SSR polymorphic primers. These primers exhibited 15 SSR alleles (Table 1 and Fig. 1) with an allele size ranged from 120 bp to 700 bp.

2. Amplified fragment length polymorphism analysis (AFLP)

Among the twenty two AFLP primer combinations used to screen the two parents, only ten AFLP primer combinations (45.5%) showed polymorphism. The selected primer combinations, the total number of amplicons, polymorphic amplicons and polymorphism percentage are presented in Table (2). A total of 821 major AFLP bands were observed, 161 of these (19.6%) were polymorphic between the two parents. The number of amplicons/ primer combination ranged from 68 (E-AGC/M-CAG) to 98 (E-AGG/M-CTT). While, the number of polymorphic amplicons varied from 8 in primer combination (E-ACG/M-CTC) to 25 in primer combinations (E-AGG/M-CTT). The average number of polymorphic fragments per combination was 16.1.

3. ***Analysis of leaf stripe linked markers (LSL)***

Thirteen leaf stripe linked primers initially screened against parental varieties. These primers reported as linked markers to leaf stripe resistance genes (Tacconi *et al.*, 2001; Bulgarelli *et al*., 2004; Biselli *et al*., 2010). Some of these primers were developed as Cleaved Amplified Polymorphic Sequence (CAPS) markers through cutting with restriction enzymes. Six LSL of the 13 primers showed polymorphism between the two parents and consequently used in the analysis of segregation among the 60 F_2 individuals. These primers amplified a total of 28 amplicons including 15 monomorphic DNA fragments. While, 13 fragments were polymorphic (46.4%) (Table 3 and Fig. 3).

4. ***Start codon targeted analysis (SCoT)***

Among 48 SCoT primers initially screened against parental genotypes, only 18 SCoT primers (37.5%) showed polymorphism between the two parents and consequently used in the analysis of segregation among the 60 F_2 individuals. These primers amplified a total of 218 amplicons including 168 monomorphic

DNA fragments. While, 50 fragments were polymorphic, corresponding to a level of polymorphism of 22.9% (Table 4 and Fig. 4).

Linkage analysis and map construction

Two hundred thirty nine markers were used to construct the genetic map. Only 104 markers including (6 SSRs, 63 AFLPs, 7 LSL and 28 SCoT) were mapped in 8 linkage groups spanning 2099.4 cM of the barely genome. The resulting linkage groups were numbered as LG1 to LG8. The size of linkage groups varied from 117.8 cM for LG2 to 482.9 cM for LG6 with an average length of 262.4 cM. The number of markers located on each linkage group also varied from 6 to 28 markers. The distribution of markers, linkage group assignment and map coverage across the 8 barely linkage groups are summarized in Table (5). The main marker type contributing to this linkage map was AFLPs (60.5% AFLPs, 5.7% SSRs, 6.7% LSL and 26.9% SCoT). As shown in Table (5) and Fig. (5) some linkage groups were composed entirely of AFLPs (LG 1 and 3), whereas, others consisted of AFLPs and SCoTs (LG4), or AFLPs, SCoTs and SSRs (LG2 and 5). Additional markers are needed to provide bridges for joining unlinked markers.

Assignment of linkage groups to the chromosomes

Linkage groups were assigned to barley chromosomes according to published map locations of the SSR markers as reference point. Only 15 SSRs primers revealed polymorphic patterns between the two parents. Based on the presence of these SSR markers, only four linkage groups (LG) were assigned to chromosomes, where, LG2, LG5, LG6 and LG7 were assigned to chromosomes 2H, 5H, 6H and 7H, respectively. Five anchored SSR markers were used to assign the genetic linkage groups to barley chromosomes as for 2H (SSR-3), 5H (SSR-10 and SSR5), 6H (SSR-9) and 7H (SSR1). Nine SSR assigned markers were unlinked; representing 53% of the total SSR assigned markers used to construct the genetic map.

QTL analysis

A total of 40 QTL with significance ranging from 0.01% to 5% were identified for the two traits on all linkage groups except (LG3). These included 20 QTL for plant height LG4(6 QTLs), LG5(1 QTL), LG6(10 QTLs) and LG7(3 QTLs) and 20 QTL for Disease Resistance LG1(1 QTL), LG2(4 QTLs), LG4(5 QTLs), LG5(3 QTLs), LG6(1 QTL), LG7(5 QTLs) and LG8(1 QTL)) (Fig. 5). These 40 QTL were identified by using single-point analysis (SPA) (Table 6) which is the preferred method when the number of markers is not large enough which is the case in this study where only 104 markers were mapped on the 8 linkage groups or when complete genetic map is not available (Muhanad, 2003).

Development of genetic linkage map represents the first step towards tracing out the valuable alleles in a segregating population. The manipulation

of large number of genes will enables dissection of the complex traits to provide new ideas for efficient plant improvements, by natural selection or breeders; this depends on creating, evaluating, and selecting the right combination of alleles (Flavell, 1995).

Several barley maps were reported by using different molecular marker techniques. Sequence-related amplified polymorphism (SRAP) markers along with SSR markers were used to construct a barley linkage map with 175 loci covering 1,475 cM with a mean density of 8.7 cM per locus (Guo *et al*., 2013). Diversity arrays technology (DArT) were also used to cover 1,503 cM of barley genome with an average density of 2.33 cM (Rodríguez-Suárez *et al*., 2012).

Different molecular marker system (AFLP, SSR, CAPS, SCoT, STS and others) were used to develop 1644.8 cM. Barley map targeting net blotch resistance genes, caused by *Pyrenophora teres f. teres*, in an F_2 across between two barley varieties "AT4" (net blotch resistant) and "Femina" (net blotch susceptible) were used to develop a segregating F_2 population (Adawy *et al.*, 2013).

There are useful resistances loci to leaf stripe have been reported in the last few years. The first attempt was reported by Giese *et al.* (1993), they localized the 'Vada resistance' gene on barley chromosome 2 (2H). After a few years, Thomsen *et al.* (1997) mapped this qualitative resistance gene in variety 'Alf' on the long arm of chromosome 2 (2H) and proposed *Rdg1* as its designation and several sources of complete and quantitatively resistance found in European and non-European germplasm (Skou *et al.*, 1994; Pecchioni *et al.*, 1999).

By using STS and CAPS markers developed from RFLPs a qualitative resistance gene to *P. graminea*, named *Rdg2* and carried by a highly resistant six-rowed winter barley variety, has been mapped on the short arm of chromosome 7H (Tacconi *et al*., 2001). Gene confers resistance to *P. graminea* isolate Dg5 and has been mapped to the long arm of chromosome 2H, using a segregating population represented by 103 Recombinant Inbred Lines (RILs) of the cross L94 (susceptible) X Vada (resistant) and 194 RILs of the cross Arta (susceptible) X H. spontaneum 41-1 (resistant) (Biselli *et al.*, 2010).

In this investigation a genetic linkage map, for the first time, integrates SCoT marker technology in barley mapping for leaf stripe resistance. The early maps were based on restriction fragment length polymorphism (RFLP) markers (Tacconi *et al.*, 2001), while later the polymerase chain reaction (PCR)-based markers became dominant for genetic map construction, *e.g.* Amplified fragment length polymorphisms (AFLPs) (Biselli *et al.*, 2010). SCoT marker system used with other three user-friendly PCR-based marker systems (SSR, AFLP and LSL) to generate a genetic linkage map in cross between At20 and At29. Also, the constructed linkage map contains four

marker types that were not mapped collectively in any other barley maps. A genetic map constructed in 8 linkage groups consisted of 104 loci and spanning a total of 2099.4 cM with an average distance between loci of 262.4 cM.

Quantitative trait loci linked to leaf stripe

Twenty QTLs were defined by marker interval that included the LOD score peaks as responsible for leaf stripe severity (DS) this result is supported by the high portion of phenotypic variance.

Many linked loci (AFLP-11, AFLP-123, SCoT-34, AFLP-84 and SCoT-17) were located on barley chromosome 7H. In this content, our results agree with Tacooni *et al.* (2001). They developed a NIL population from the susceptible barley variety "Mirco" and the resistant barley variety "Thibaut" using RAPD, STS, AFLP and CAPS markers and reported a resistance QTL in chromosome 7H.

Another high number of Loci (AFLP-42, SSR-3, AFLP-46 and SCoT-1) were located on chromosome 2H , this is supported by a previous study on the "Vada" variety resistance to leaf stripe isolate Dg2 in which a major QTL was detected on chromosome 2H (Arru *et al*., 2002). Also, Pecchioni *et al.* (1996) identified a qualitatively acting factor conferring resistance to barley leaf stripe mapped on chromosome 2H. By using Restriction Fragment Length Polymorphism (RFLP) markers and based on Doubled Haploid (DH) barley lines derived from the F_1 cross between the variety "Proctor" (resistant) and the variety "Nudinka" (susceptible). Also, Arru *et al.* (2003) reported similar results by using a medium-density, molecular marker map derived from a 'Steptoe' (partially resistant) × 'Morex' (susceptible). There map demonstrates one QTL is common to the resistance for the two isolates, on the long arm of chromosome 2H, two QTLs are linked on chromosome 3H, a further two QTLs are isolate-specific for one isolate on chromosome 2H and for the other isolate on chromosome 5H. This is in agreement with (Biselli *et al.*, 2010), they mapped to chromosome 2H a major gene with its positive allele contributed by two segregating populations of L94 (susceptible) X Vada (resistant) and Arta (susceptible) X *H. spontaneum* 41-1 (resistant) detected for two different pathogen isolates.

Start Codon Targeted marker system was generally reproducible and revealed a high number of linked loci compared with other markers ,this was also supported in many plant species (Luo *et al.*, 2011; Bhattacharya *et al.*, 2013; Mulpuri *et al*., 2013), this encourage using SCoT to expand the quantitative trait loci identification on conserved region in plant genes surrounding the ATG translation start (or initiation) codon that has been well characterized in previous studies (Joshi *et al.*, 1997; Sawant *et al*., 1999).

By using BLAST tool provided by "The International Barley Genome Sequencing Consortium" database, SCoT-

21, SCoT34 and SCoT-35 were highly matched to atpase plasma membrane-type-like gene which responsible for pumping protons out of cells, and are essential for establishing and maintaining the crucial transmembrane proton gradient in plants and fungi (Justesen *et al*., 2013). SCoT-17 and SCoT-3 gave high similarity ratio with phosphatidylserine synthase 2-like gene which play crucial role as a structural component and involved in cell signaling (Sturbois-Balcerzak *et al*., 2001).

SCoT-1 gave a high similarity score with "E3 ubiquitin-protein ligase like", the importance of E3s is highlighted by the number of normal cellular processes they regulate, and the number of diseases associated with their loss of function or inappropriate targeting (Ardley *et al.*, 2005), SCoT-47 gave also high similarity ratio with udp-glucuronic acid decarboxylase, this enzyme participates in starch and sucrose metabolism and nucleotide sugars metabolism (Rubenstein *et.al.*, 1974).

On the other hand, SCoT-13 gave a high similarity ratio with "respiratory burst oxidase homolog protein" which involved in the generation of reactive oxygen species (ROS) during incompatible interactions with pathogens and in UV-B and abscisic acid ROS-dependent signaling and might be required for ROS signal amplification during light stress (Kwak *et al.*, 2003).

Seven Leaf Stripe Linked (LSL) markers out of 13 were mapped and only two were linked to quantitative pathogen resistance loci, although LSL-1 (NBS-1) was reported to be located on chromosome 7H (Biselli *et al.*, 2010). In this study, it has been separated in independent linkage group (LG8), this could be maintained by increasing mapped markers to fill gaps between the two linkage groups in future studies. On the other hand, LSL-5 (P-9) was successfully linked to chromosome 7H as confirmed previously by Bulgarelli *et al.* (2004). Most of LSL markers were concentrated on chromosome 7H (LSL-5, LSL-6, LSL-4 and LSL-2) which in high consistency with previous published results reported by (Bulgarelli *et al*., 2004; Biselli *et al*., 2010), also it highlights the link between chromosome 7H and leaf stripe pathogen resistance.

In previous studies, amplified fragment length polymorphism (AFLP) markers were identified as associated with the resistance gene (Arru *et al.*, 2002; Arru *et al*., 2003; Biselli *et al.*, 2010). Using of AFLP in this study was helpful to detect a high number of QTLs (AFLP-154, AFLP-42, AFLP-45, AFLP-46, AFLP-11, AFLP-123, AFLP-84, AFLP-125, AFLP-52 and AFLP-81) ,this support the idea of using this technique to identify more loci linked to pathogen resistance for leaf stripe and other plant pathogens.

Using SSR marker system for tracing pathogen resistance genes were reported in barley net blotch (Adawy *et al*., 2013), soil-borne viruses (Werner *et al.*, 2006) and genotyping (Macaulay *et*

al., 2001). In our study, the using of SSR was useful to detect two QTLs (SSR-3 and SSR-9).

QTL associated with plant height

Controlling of plant height is a highly important trait can be used for reducing yield loss caused by lodging and to increase harvest index (Bezant *et al*., 1996). Plant height show continuous variation and the inheritance of this trait is complex and usually supposed to include numerous genetic factors that interact with environmental conditions (Kjær *et al.*, 1995).

In this study, twenty QTLs for plant height were identified on four linkage groups (LG4, LG5, LG6 and LG7), with a high number of loci has been identified on chromosome 6H. Regarding this result, Teulat *et al.* (2001) identified major QTLs controls plant height on chromosome 2H, 3H, 4H, 5H, 6H and 7H, Chloupek *et al.* (2006) on 3H, 4H, 5H and 7H, while, Shahinnia *et al.* (2006) located one major QTL for plant height near to *uzu1* gene locus on chromosome 3H long arm. Several previous studies identified QTL for plant height expanded over barley genome. Qi *et al.* (1998) identified on 2H, 3H and 7H; while, Zhu *et al.* (1999) on 1H, 3H, 4H and 6H. Also, Kicherer *et al.* (2000) identified QTLs for plant height on 2H and 3H. Meanwhile, Marquez-Cedillo *et al.* (2001) on 2H, 3H, 4H and 5H. In addition, Yang *et al.* (2007) identified QTLs on 2H and 3H and Rao *et al.* (2007) on 2H and 3H.

Correlation between traits

In this study, plant height and disease resistance are possible to have similar locations. Shared genomic loci for agronomic traits have also been reported in barley in previous studies between yield and yield components; Biomass, tiller number in fertile and infertile, harvest index, plant height and spike length (Babaiy *et al.*, 2011), rain filling, malt protein, malt fragility, wort viscosity, diastatic power and ferment-ability (Bichoński and Śmiałowski, 2004) and heading date and plant height (Shahinnia *et al.*, 2006).

QTL results obtained from this work as some genomic regions were occupied by overlapped QTLs on linkage groups LG4 (SCoT-34 and SCoT-17) and LG6 (SSR-9) for both disease resistance and plant height.

According to Yang *et al.* (2007), our results could be caused by: (1) two strongly linked genes affecting different traits, (2) one single gene that produces a series of effects in related traits, (3) one gene affecting two or more independent traits, (4) two linked genes with effects in the same traits.

SUMMARY

Leaf stripe in barley, caused by *Pyrenophora graminea*, is an important seed-borne disease in organically grown as well as in conventionally grown Nordic and Mediterranean barley districts. In the present work, a QTL map for F_2 barley

mapping population derived from an intraspecific cross between At20 x At29 was constructed using 104 DNA markers (6 SSRs, 28 SCoTs, 63 AFLPs and 7 LSLs) distributed on 8 linkage groups and spanning 2099.4 cM of the barley genome. The size of linkage groups varied from 117.8 cM for LG2 (2H) to 482.9 cM for LG6 (6H) with an average length of 262.4 cM. Based on the used anchor SSR markers, only four linkage groups were assigned to chromosomes, where LG2, LG5, LG6 and LG7 were assigned to chromosomes 2H, 5H, 6H and 7H, respectively. Single point analysis was used to identify genomic regions controlling the leaf stripe severity and plant height traits. A total of 40 QTL were identified for the disease severity (DS) and plant height (PH) traits (20 QTL for disease severity and 20 QTL for plant height). This work represents the first genetic linkage map for barley population derived from an intraspecific cross between At20 and At29 showing chromosomal regions associated with disease severity and plant height traits in barley.

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Table (1): Primer name, primer sequence, chromosome and marker size as detected by SSR analysis.

Primer Code	Primer Name	Primer sequence (5`- 3`)	Chrom.	Marker size (bp)
SSR-1	Bmag914	F. GGGCAATATACAGTTCAACTC R. ATGAACTGGAGGCAGTAAATA	7H	180
SSR-2	Bmac0181	F. ATAGATCACCAAGTGAACCAC R. GGTTATCACTGAGGCAAATAC	4H	200
SSR-3	MGB391	F. AGCTCCTTTCCTCCCTTCC R. CCAACATCTCCTCCTCCTGA	2H	220
SSR-4	MGB318	F. CGGCTCAAGGTCTCTTCTTC R. TATCTCAGATGCCCCTTTCC	-	580
SSR-5	Bmag760	F. GTGATACATCAAGATCGTGC R. TCCCCAACACCAGTATCTA	5H	120
SSR-6	GBM1423	F. ACAAATCCCCAAGCCAATCT R. CTTGCCTGTCAACGTCTTCA	6H	200
SSR-7	Bmag210	F. ACCTACAGTTCAATAGCTAGTACC R. GCACAAAACGATTACATCATA	6H	150
SSR-8	HVM74	F. AGGAAGTCATTGCGTGAG R. TGATCAAGAATGATAACATGG	6H	130
SSR-9	cMWG2029	F. CCAGTTATCCGAATCCGGAA R. GTGGTCAGGTACATACGAAT	6H	240
SSR-10	Bmag222	F. ATGCTACTCTGGAGTGGAGTA R. GACCTTCAACTTTGCCTTATA	5H	180
SSR-11	ABC07920	F. CTGGCGCTATCAAAGGAAAC R. AAGTGCCTCCAGCCAAGTTA	6H	300
SSR-12	GMS149	F. ACCCTAACTCATGTTCT R. AAGGAACATAGCCAACTC	1H	420
SSR-13	MGB384	F. CTGCTGTTGCTGTTGTCGTT R. ACTCGGGGTCCTTGAGTATG	5H	700
SSR-14	EBmac0906	F. CAAATCAATCAAGAGGCC R. TTTGAAGTGAGACATTTCCA	4H	160
SSR-15	HVM68	F. AGGACCGGATGTTCATAACG R. CAAATCTTCCAGCGAGGCT	4H	210

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		Polymorphism%
	*Eco*R1	*Mse*I	Total	Polymorphic	
2/8	AAG	CTT	96	16	16.7
3/1	ACA	CAA	78	12	15.4
3/3	ACA	CAG	94	22	23.4
4/4	ACC	CAT	77	17	22.1
5/1	ACG	CAA	74	12	16.2
5/6	ACG	CTC	69	8	11.6
6/5	ACT	CTA	91	19	20.9
7/3	AGC	CAG	68	13	19.1
8/1	AGG	CAA	76	17	22.4
8/8	AGG	CTT	98	25	25.5
Total			821	161	19.6
Average			82.1	16.1	

Table (3): Primer name, primer sequence, chromosome No, marker size and restriction enzyme as detected by LSL (STS and CAPS markers linked) analysis.

Primer Code	Primer Name	Primer sequence	Chrom.	Marker size (bp)	Restriction Enzyme
LSL-1	NBS1	F. AGACTCACGCGTATGCCGATTCA R. CAGAACTGCCGCAGTGTAGTAGC	7H	340, 520	-
LSL-2	NBS2	F. CACCGCAGAAGAATGCCTACAAAACCCTGAGTCC R. CAAGGTAAGGATTGAGGAGAGC	7H	900	-
LSL-3	MWG2018	F. CACCATGCTCCCCGTCACCTACCTC R. CGTGAACCCGTCGCCCAAGTCTAAG	7H	300	-
LSL-4	ABG704	F. TCGCCTTCTTCAGATTCCTACCA R. ATTACCATGTCGATATTGTTTCCACTG	7H	500	*BamH1*
LSL-5	P9 (BV078153)	F. GCCTGCAAAATCATGCAGCAGCTCA R. CGCGCCAATCCGGGTTATCAAGAAG	7H	1300	-
LSL-6	P10 (BV078155)	F. CGTCAGAGTCGTCTGCATCAATTTCACG R. CGTGAACGCAAGCACTTCTTCATCCAAG	7H	330	-

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

Primer Name	Primer Sequence (5'- 3')	Number of bands		Percentage of Polymorphism
		Total	Polymorphic	
SCoT-1	ACGACATGGCGACCACGC	11	2	18
SCoT-4	ACCATGGCTACCACCGCA	10	3	30
SCoT-8	ACAATGGCTACCACTGAG	13	2	15
SCoT-13	ACCATGGCTACCACGGCA	12	5	42
SCoT-15	CCATGGCTACCACCGGCT	13	5	38
SCoT-20	CAACAATGGCTACCACGC	12	5	42
SCoT-22	CCATGGCTACCACCGCAC	14	3	21
SCoT-25	ACGACATGGCGACCGCGA	13	2	15
SCoT-26	ACGACATGGCGACCACGC	14	4	21
SCoT-27	ACCATGGCTACCACCGTC	10	2	20
SCoT-28	CAACAATGGCTACCACCA	12	1	8
SCoT-29	CAACAATGGCTACCACCC	10	4	40
SCoT-31	CAACAATGGCTACCACGA	12	2	17
SCoT-32	CAACAATGGCTACCACGC	12	2	17
SCoT-40	CAACAATGGCTACCACGT	13	2	15
SCoT-41	CAACAATGGCTACCAGCA	15	2	13
SCoT-44	ACCATGGCTACCACCGAC	10	1	10
SCoT-47	ACCATGGCTACCACCGCG	12	3	25
Total		218	50	22.9
Average		12.1	2.7	

Table (5): Distribution of molecular markers, chromosomal assignment and centiMorgen (cM) coverage across the 8 linkage group of the genetic map.

Linkage Group	AFLP	SSR	SCoT	LSL	Markers		cM	cM/marker
					#	%		
LG1	6	0	0	0	6	5.8%	118.9	19.82
LG2(2H)	3	1	2	0	6	5.8%	117.8	19.63
LG3	6	0	0	0	6	5.8%	120.8	20.13
LG4	11	0	4	0	15	14.4%	368.3	24.55
LG5(5H)	8	2	6	0	16	15.4%	356.0	22.25
LG6(6H)	19	1	7	1	28	26.9%	482.9	17.25
LG7(7H)	10	1	5	4	20	19.2%	411.9	20.60
LG8	0	1	4	2	7	6.7%	122.8	17.54
Total	63	6	28	7	104	100	2099.4	20.20
Average	7.9	0.8	3.5	0.9			262.4	

Table (6): The most significant QTL detected by the single point analysis. This analysis fits the data to the simple linear regression model $y = b_0 + b_1 x + e$. The results below give the estimates for b_0, b_1 and the F statistic for each marker.

Trait	Chrom.	Marker	b0	b1	-2ln(L0/L1)	F(1,n-2)	pr(F)	Significance
Disease severity	1	3	1.743	0.457	4.714	4.741	0.034	*
	2	2	1.693	0.511	5.981	6.080	0.017	*
	2	3	1.524	0.611	8.445	8.766	0.004	**
	2	5	1.576	0.538	5.907	6.001	0.017	*
	2	6	1.589	0.469	4.963	5.001	0.029	*
	4	1	2.183	0.674	4.329	4.340	0.042	*
	4	2	2.040	0.560	4.019	4.018	0.050	*
	4	3	2.254	0.928	12.766	13.752	0.000	***
	4	11	1.804	0.514	5.751	5.834	0.019	*
	4	13	1.940	0.745	11.793	12.598	0.001	***
	5	9	1.864	-0.659	9.549	10.005	0.002	**
	5	14	1.804	-0.514	5.751	5.834	0.019	*
	5	15	1.825	-0.475	4.651	4.675	0.035	*
	6	8	1.733	0.499	4.311	4.321	0.042	*
	7	2	1.892	0.483	4.216	4.222	0.047	*
	7	4	1.982	0.539	4.290	4.299	0.043	*
	7	6	2.073	0.677	6.933	7.104	0.010	**
	7	12	2.040	0.560	4.019	4.018	0.050	*
	7	15	1.882	0.564	6.260	6.379	0.014	*
	8	2	1.502	-0.449	4.031	4.031	0.049	*
Plant height	4	3	16.571	-2.571	9.047	9.439	0.003	**
	4	8	16.889	-2.071	5.708	5.788	0.019	*
	4	9	17.285	-2.496	12.681	13.650	0.000	***
	4	10	17.833	-1.572	5.174	5.223	0.026	*
	4	12	17.767	-1.854	7.324	7.530	0.008	**
	4	13	17.596	-1.648	5.206	5.257	0.025	*
	5	4	16.913	2.413	9.558	10.016	0.002	**
	6	5	18.200	-1.400	4.307	4.317	0.042	*
	6	6	18.200	-1.900	8.190	8.483	0.005	**
	6	7	18.200	-1.900	8.190	8.483	0.005	**
	6	8	17.989	-1.584	4.115	4.118	0.047	*
	6	9	18.089	-1.661	6.125	6.234	0.015	*
	6	11	18.407	-1.554	5.256	5.310	0.025	*
	6	16	17.866	-1.586	5.234	5.287	0.025	*
	6	17	17.444	-1.746	5.460	5.525	0.022	*
	6	19	14.625	-4.125	9.729	10.21	0.002	**
	6	22	17.358	-1.960	6.970	7.144	0.010	**
	7	14	17.222	-1.956	6.413	6.543	0.013	*
	7	17	17.783	-2.083	9.212	9.625	0.003	**
	7	18	17.783	-2.083	9.212	9.625	0.003	**

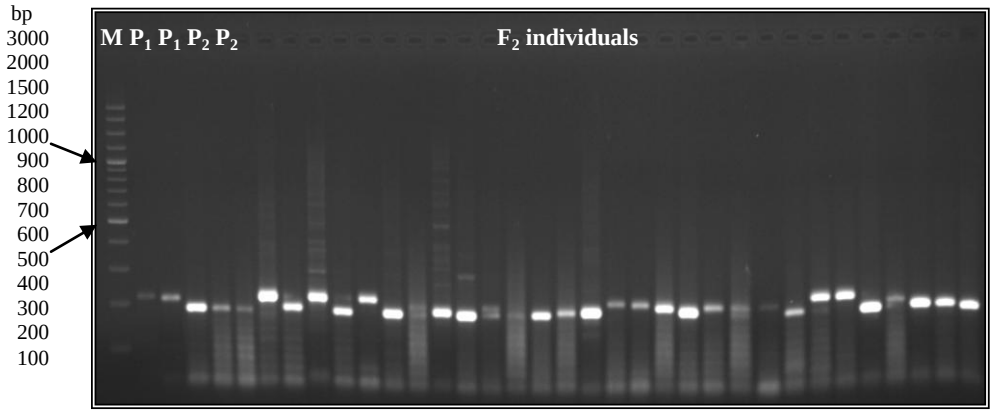

Fig. (1): SSR patterns of the two parents and F_2 Individuals derived from the cross "At20" and"At29" as revealed by primer SSR9 (cMWG2029). M is the standard DNA marker 100 bp Ladder, P1 (cv. At20) and P2 (cv.At29).

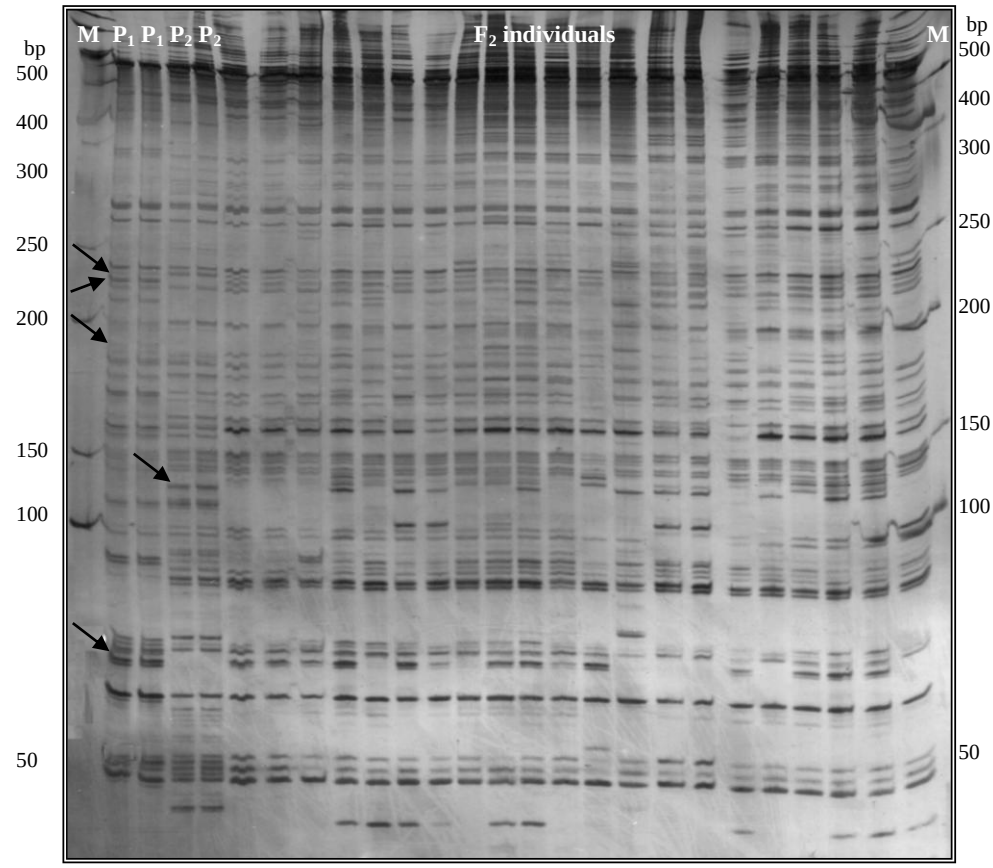

Fig. (2): AFLP patterns of the two parents and F_2 individuals derived from the cross "At20" and "At29" as revealed by primer combination ACA/CAG. M is the standard DNA marker 100 bp ladder, (P_1 P_1) (cv.At20) and (P_2 P_2) (cV.At29).

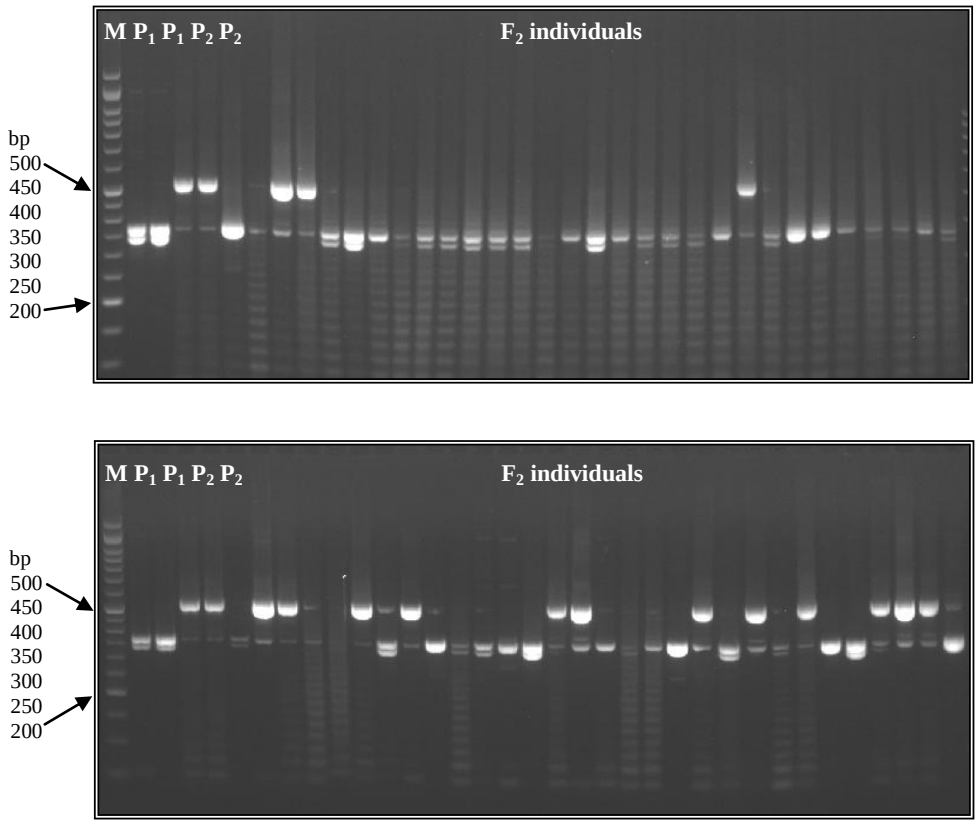

Fig. (3): Agarose gel showing some polymorphic bands between the 2 parents P_1 (At20) and P_2 (At29) and the segregation among F_2 individuals as revealed by primer LSL1 (NbS1). M is 50 bp DNA ladder RTU (Ready–to-use).

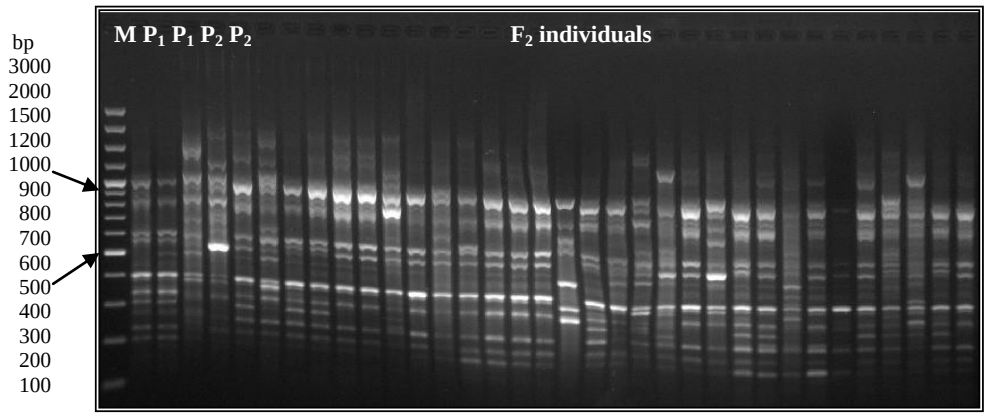

Fig. (4): SCoT patterns of the two parents and F_2 individuals derived from the cross At20 and At29 as revealed by primer SCoT-15. M is the standard DNA marker 100 bp ladder, P_1 (cv. At20) and P_2 (cv. At29).

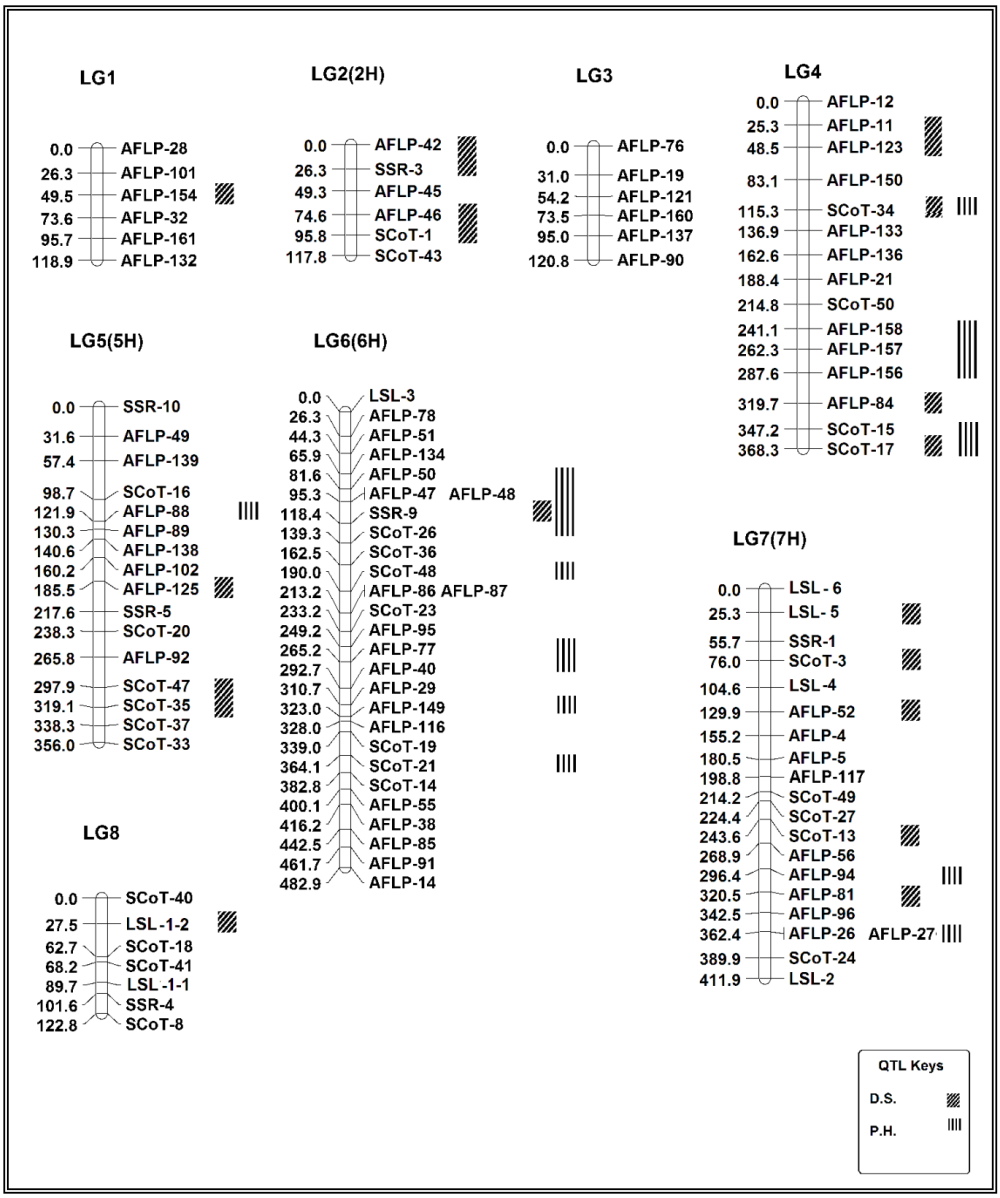

Fig. (5): Molecular linkage groups of barley (Intercross between At20 and At29) showing positions of QTL influencing plant height (PH) and disease severity (DS). Map distances between adjacent markers are in cM.