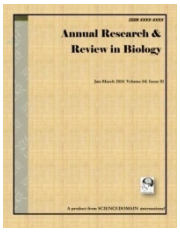

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QTLs Analysis for Important Quantitative Traits Related to Yield in Barley (*Hordeum vulgare L.*) Using RAPD Molecular Marker

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Authors' contributions

This work was carried out in collaboration between all authors. All authors read and approved the final manuscript.

Original Research Article

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ABSTRACT

Aims: QTL mapping not only presents useful information about number and loci of controlling genes, but also assist breeder to Marker Assisted Selection (MAS). The aim of this research is to show genetically map and complete the saturated maps and improving the barley cultivars by use of marker assisted selection.
Place and Duration of Study: This experiment was carried out in Agricultural Research Farm of Graduate University of Advanced Technology at Kerman province, Iran in 2004 - 2006.
Methodology: In this research random amplified polymorphic DNA (RAPD) markers was used on 111 F3 families derived from Afzal × Radical cross along with their parents were evaluated phenotypically and agronomically in a Randomized Complete Block Design (RCBD) with four replications. The 111 F3 families were evaluated by 13 selected primers among 40 primers which showed polymorphism in parental lines. Population linkage map

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was prepared using 140 RAPD band. Quantitative Trait Loci (QTLs) analysis was performed by interval mapping.
Results: Based on the results 2, 1, 1, 2, 1, 4 and 4 QTLs were recognized for the traits of tiller number, plant height, peduncle length, spike length, awn length, number of spikelet in spike and flag Leaf length, respectively and no QTL for node number.
Conclusion: The results showed relatively high R^2 values and low confidence limits for QTLs so that the relevant linkage between markers and QTLs can be used in breeding programmes.

Keywords: RAPD molecular marker; QTLs analysis; QTL mapping; barley traits related to yield.

1. INTRODUCTION

Yield is the most important trait and the one of great breeding objects in plants. For the improving yield we must attend to the traits that related to yield [1,2]. Molecular marker technology, in recent years, has created important changes in plant breeding [3] .Molecular markers especially markers based PCR prepared new possible for identify and improvement of quantitative traits for breeders as tools of genome identify and map preparation [4]. Plant breeders can select traits with high efficiency use saturated genetic map by molecular markers [5]. Mapping of quantitative traits loci (QTLs) is one of methods that in recent decade developed for genetically study of quantitative traits. In this method, investigate segregation of quantitative trait and molecular markers simultaneously, and number of genes, type of gene action, the rate of gene evaluated and location of QTLs on genome is identified [6]. One of the usual applications of molecular markers is marker assisted selection according to identify and use of markers that are linked to trait controlling genes. Anything linkage between molecular marker and gene location of controlling quantitative trait, increase efficiency and rate of selection. Kandamir et al. (2000) identified one QTL for the height of plant trait in chromosome number 3 [5]. Gerhard et al (2002) found 3 QTLs for the number of tiller, that illustrated 16.3 to 30.6 percent of phenotypic changes [7]. Da-wei Xue et al. (2010) reported a total of 31 QTLs were identified for the measured characters from two growth environments as waterlogged and well drained conditions. The phenotypic variation explained by individual QTLs ranged from 4.74% to 55.34%. Several major QTLs determining kernel weight, grains per spike, spikes per plant, spike length and grain yield were detected on the same region of chromosome 2H, indicating close linkage or pleiotropy of the gene(s) controlling these traits. Some different QTLs were identified under waterlogging conditions, and thus different markers may have to be used in selecting cultivars suitable for high rainfall areas [8]. Baghizadeh et al. (2007) reported quantitative trait loci (QTLs) that were mapped in an F3 population (90 F3 families) derived from a cross between two barley genotypes. A molecular marker linkage map of this cross had been constructed based on 71 random amplified polymorphic DNA (RAPD) markers. Seven agronomic traits: 1000 grain weight, spike length, plant height, number of spikelets per spike, awn length, number of grains per spike and number of spikes were measured in F3 population. A total of 28 QTLs were identified for 7 traits. Phenotypic variance explained by these QTLs varied from 12.5 to 48.9%. Mean environments could detect 11 QTLs, which 5 of them were new [9]. The aim of this research is to show genetically map and complete the saturated maps and improving the barley cultivars by use of marker assisted selection (MAS).

2. MATERIALS AND METHODS

This test performed in autumn 2004 on farm in Kerman province. In order to identify of inheritance method and evaluation of gene number controlling some of important traits that related to barely seed yield, first the two varieties, Afzal and Radical selected as crossing parents that have phonotypical differences to another. Generations F_1 to F_3 from this cross were obtained at years 2004 – 2006. The parents with F_1 to F_3 generations planted by four replications in Randomized Complete Block Design (RCBD). After complete maturity, 6 plants were selected from each line randomly. Sampling performed from F_3 families separately. Random harvesting from any family plants were done and put in plastic package separately and then were transferred to laboratory for measurement of morphological and agronomical traits. In this research, 8 quantitative and agronomic traits related to yield measured and investigated. Those were tiller number, plant height, peduncle length, spike length, number of spikelet in spike, length of flag leaf, awn length and node number. For any of the above traits six plants were selected randomly and the mean of data for any trait were evaluated. The second step was genotyping 111 families in F_3 barley generation used separately with parents (Afzal and Radical). For this purpose, those were planted in small pots with suitable soil. In each pot 15 seeds were planted averagely and pots were put in biotechnology laboratory. The leaf sample were prepared after 2 weeks from planting date (four leaf step) for DNA extraction.DNA genomic extraction from plant samples performed by dellaporta (1983) method [10]. Quantity and quality of the extracted DNA were determined by using DNA electrophoresis in 1% agarose gel, and spectrophotometry method [9]. For amplification of DNA, first 40 RAPD primers, manufactured by Sinagen Co, used on parents, Afzal and Radical, that 13 primers showed polymorphism in both parents and used on 111 families F3 (Table 1). Taq DNA Polymerase enzyme, dNTPs mixture, in addition to PCR buffer (10x), as well as $MgCl_2$ were prepared from Sinagen Co. 25 µl PCR mixture contained 1 µl of the DNA of prepared pattern with the concentration of 50 ng/µl, 2µl of $MgCl_2$ with 50mM concentration, 2.5µl of dNTP with 2.5mM concentration, 0.3µl of Taq DNA Polymerase with 5 unit/ µl concentration, 2µl of primer with 2µM concentration, 2.5µl of 10x PCR Buffer [KCl 500mM & Tris-HCl (pH = 8.4)] and 14.7µl of 2x distilled sterile water. The thermo cyclers utilized in the present study were of Master cycle models in simple and gradient types manufactured by Eppendorf Co. The amplification was carried out as follows: 1. Primary denaturing DNA was performed for 4 minutes at 94ºC (one cycle); 2. Forty cycles were carried out for 1 minute at 94ºC, Annealing of the primer to Single DNA in the optimum Annealing temperature pertaining to each primer for 1 minute, and extension of thermal primer for two minutes at 72ºC; 3. The extension was completed at 72ºC for eight minutes (one cycle). The samples are quickly removed from the device, subsequent to PCR which takes more than 4 hours, and are stored in the temperature of - 20ºC for a later experiment of electrophoresis. PCR was carried out in some randomly selected primers for a second time in order for the investigation of repeatability of RAPD bands to approve the clarity of bands [9,11].

PCR production were investigated by 1.5% agarose gel. RAPD bands were determined in gel documentation system [12]. Then based on present or absent band, genotypic matrix of data were prepared. Matrix containing 0 and 1 numbers and data related to 8 traits of 111 F3 families were interred to MAPMAKER software. MAPMAKER software designed linkage groups by maximum likelihood method. Then by use of MAPMAKER/Exp software with suitable LOD (3-7) and maximum of recombination ratio (0.5) designed linkage map. QTL mapping performed by MAPMAKER/QTL software and according to interval mapping. The threshold of LOD for QTLs identification considered as 2 equally and minimum of

chromosome walking considered as 2 CM. QTLs analysis performed for any of 8 traits investigated F_3 families separately.

Table 1. Sequences of 13 RAPD primers

Primer	Sequence
384 sinagen Co	5'-TGCGCCGCTA -3'
64 sinagen Co	5'-GAGGGCGGGA -3'
67 sinagen Co	5'- GAGGGCGAGC -3'
389 sinagen Co	5'- CGCCCGCAGT -3'
63 sinagen Co	5'- TTCCCCGCCC -3'
66 sinagen Co	5'- GAGGGCGTGA -3'
71 sinagen Co	5'- GAGGGCGAGG -3'
56 sinagen Co	5'- TGCCCCGAGC -3'
65 sinagen Co	5'- AGGGGCGGGA -3'
68 sinagen Co	5'- GAGCTCGCGA -3'
397 sinagen Co	5'- GGGCTGTGCC -3'
60 sinagen Co	5'- TTGGCCGAGC -3'
55 sinagen Co	5'- TCCCTCGTGC -3'

3. RESULTS AND DISCUSSION

Number and location of QTLs existence for any traits and value effect with action type of any QTLs are shown in Table 2. 140 markers according suitable LOD and maximum of recombination 0.5 put on 6 linkeage group (Fig.1.A-F). Length of these groups was variable from 120.2 CM in first linkage groups (Fig.1. A) to 481.5 CM in linkage groups 2 (Fig.1.B). These lengths obtained from the distance between markers and also between marker and QTLs. Length of map calculated equal 1748.7 CM by use of MAPMAKER software. Past reports about barley map length shows different numbers, for example, Behnam (1997) designed linkage map in barely that covered 680 CM [13]. Gerhard (2002) performed a study for identify QTLs crop traits in barely, produced genetically map that covered 1900 CM [7]. Helsingin (2000) prepared genetically map for barely by use of RAPD markers that covered about 650 CM, and Pawell et al. (1997) produced a map for barely that covered 800 CM [14,15]. For trait of tiller number was found two QTLs that put in linkage groups 3 (Fig.1.C) and 6 (Fig.1.F) separately. The first QTL in linkage group 3 localized by space of 3CM from 1538(389) marker and the second QTL that localized in linkage group 6 at space of 2.9 CM from 500(71) marker. These two QTLs considered total phenotypic variation (Table 2). Kandmir et al. (2000) found one QTLs for trait of tiller number. For plant height trait founded one QTL that considered 15.8% phenotypic changes, that localized in linkage group 3 that has 5.70CM space to link marker 1546(65) (Fig.1.C) and considered 15.8% from phenotypic variation (Table 2).

Table 2. The location of QTLs for agronomic traits in F3 population from crossing Afzal and Radical in Barley

Trait	D.E	A.E	R^2	LOD	Marker**	Loci*
Tiller number	1.322	-0.1	>50	>7	389(1589)	3(3.0)
	1.324	-2.06	>50	>7	(71)550	6(2.9)
Plant height	0.34	6.07	15.8	2.3	(65)1546	3(5.7)
Peduncle length	0.05	4.66	15	2.28	(65)1546	3(5.7)
Spike length	-3.24	-3.01	14.9	2.4	(64)700	2(3.5)
	-2.33	-2.42	20.7	2.9	(389)800	3(8.0)
	-2.87	-2.07	24.3	>7	(66)985	3(9.8)
Awn length	-1.73	2.17	22.3	>7	(389)475	3(5.3)
	-1.93	1.86	26.4	>7	(66)570	6(0.0)
	-3.85	-3.76	19.8	2.42	(384)375	3(8.0)
Number of spikelet in	-3.07	-2.82	23.5	2.26	(64)679	3(4.5)
spike	4.69	-2.71	26.7	2	(56)577	3(3.3)
	-6.41	-4.5	25.9	2.65	(65)971	3(4.0)
	8.28	9.6	38.1	6.22	(56)1760	2(3.2)
Flag leaf length	8.35	9.65	37.9	6.51	(63)965	2(6.0)
	9.44	9.57	35.7	5.84	(651)1546	2(4.0)
	7.39	9.7	41.9	>7	(65)971	3(5.7)
Node number	-	-	-	-	-	-

**:The number of out of parentheses show the linkage group and the number of in, is shown the space of QTLs to marker, **: The number of in parentheses is the primer name and the out number, is shown size marker, A.E: additive effect, D.E: dominant effect, R^2:explained phenotypic variance*

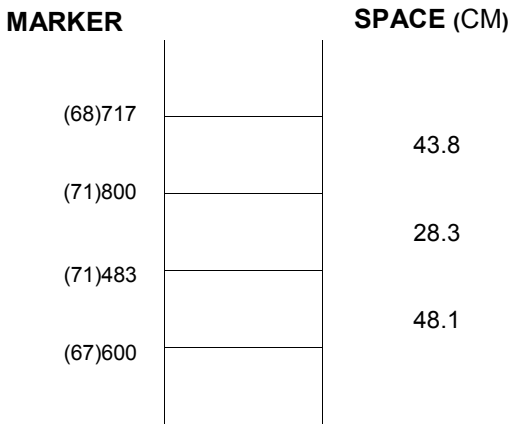

Fig. 1A. First linkage group

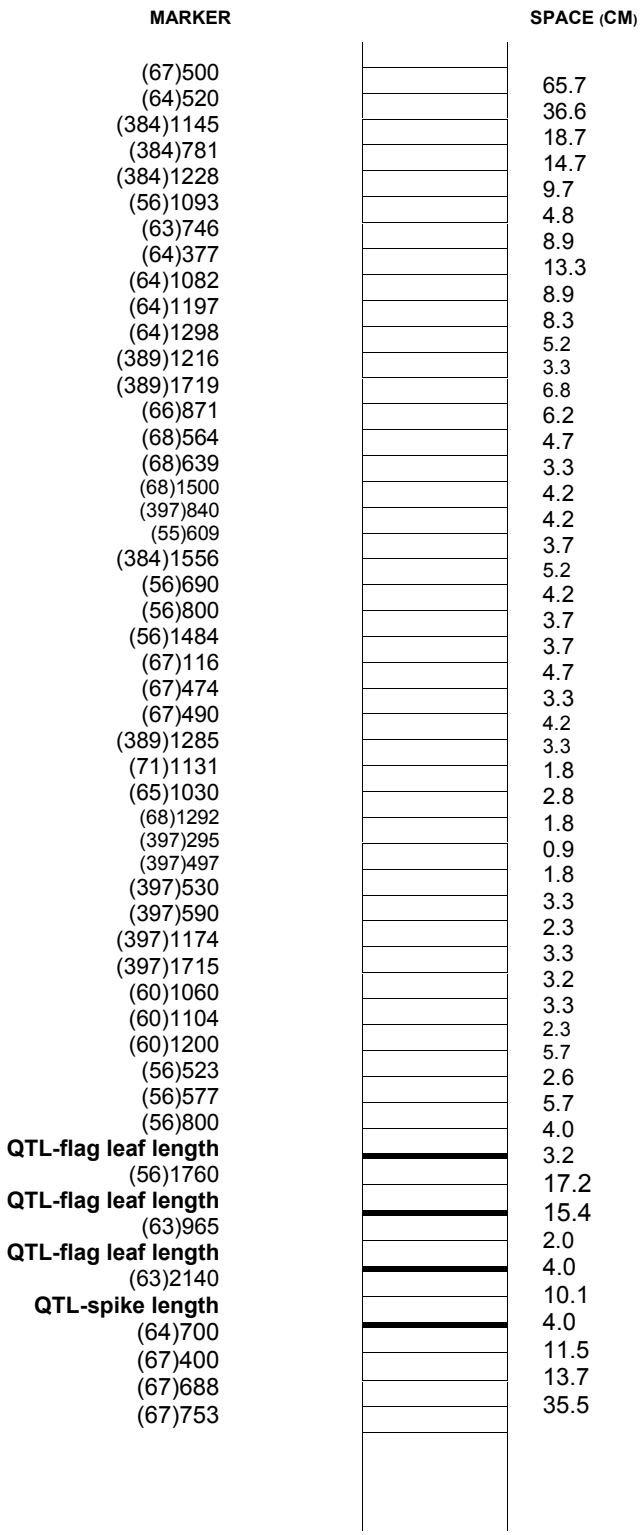

Fig. 1B. Second linkage group

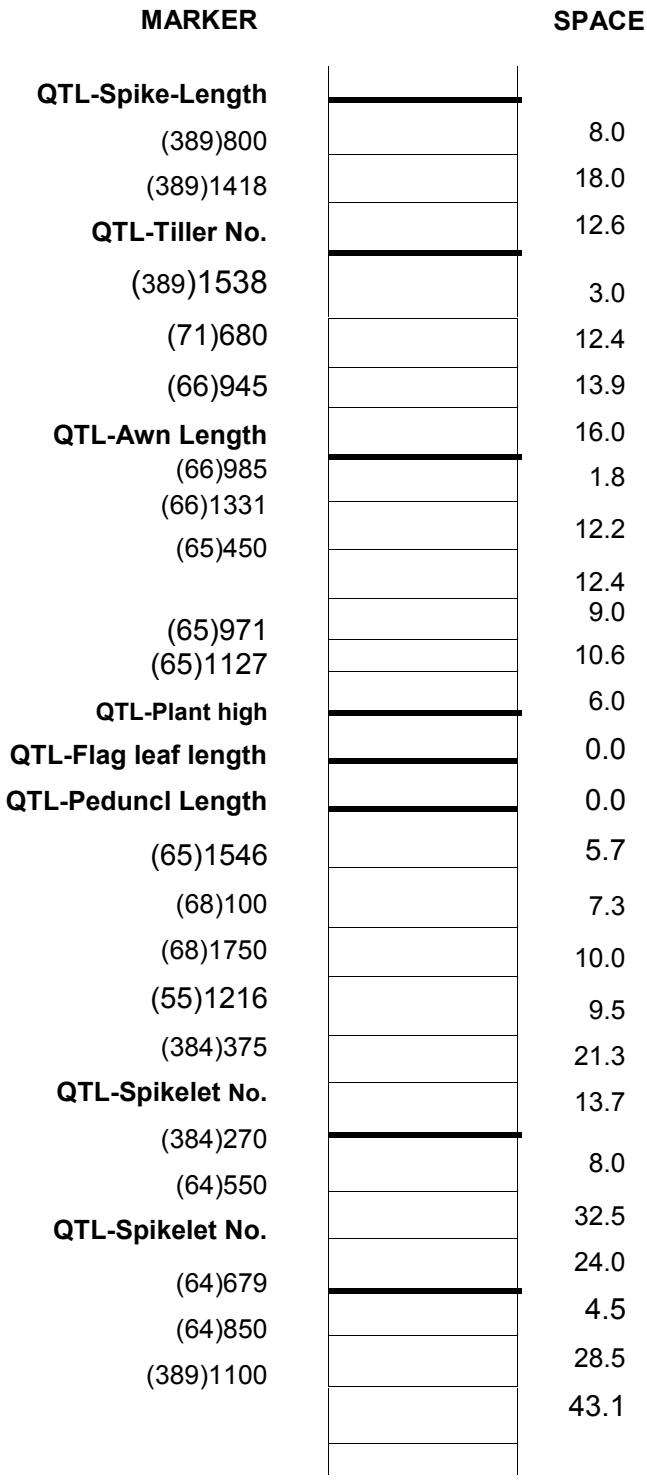

Fig. 1C. Third linking group

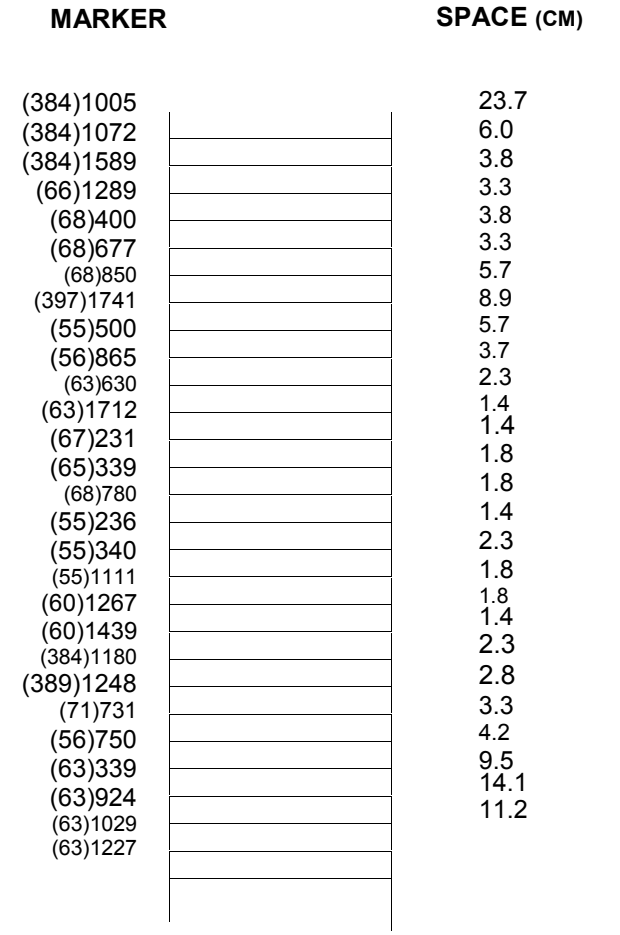

Fig. 1E. Fifth linkage group

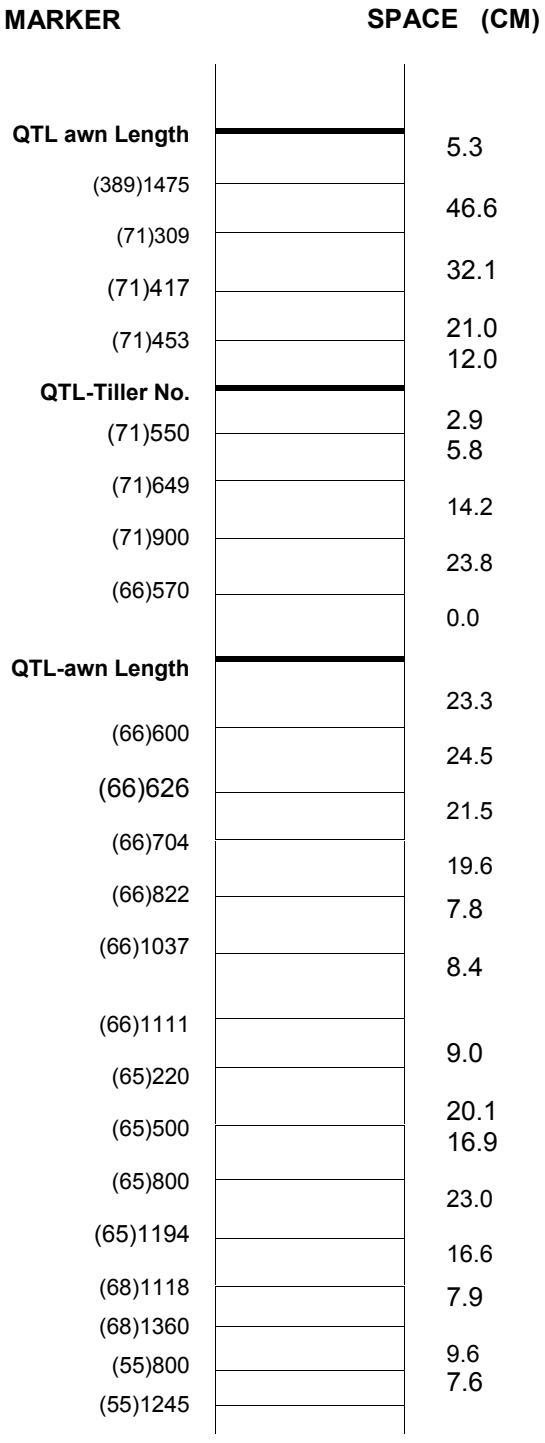

Fig. 1F. Sixth linkage group

kandemir et al. reported one QTL for plant height trait [5]. Helsingin (2000) found one to seven QTLs and Pawell et al. (2005) reported many QTLs number for plant height trait [14,15]. Peyghambari et al. (2005) reported 3 QTLs on 3H, 2H and 4H chromosomes for plant height trait [16]. For peduncle length trait was found one QTL that localized on linkage map 3 (Fig.1.C) and considered 15% from phenotypic variation and localized at 5.70 CM by 546(65) marker (Table 2). For spike length trait was found two QTLs that localized in linkage group 2 (Fig.1.B) and 3 (Fig.1.C) separately that first localized in 3.5 CM from (64)700 marker and considered 14.9% from phenotypic variation and another localized on linkage group 3 (Fig.1.C) and has space 8 CM to 800 (389) marker and considered 20.7% from phenotypic variation (Table 2).

For spike length trait Peyghambari et al. (2005) reported two QTLs on 2H and 3H chromosomes. For awn length trait found 3 QTLs that two QTLs localized on linkage group 3 and has 9.8 CM space to 985(66) marker and to 475(389) marker 3.5cm(Fig.1.C) and considered 23.3% to 24.3% phenotypic variation respectively and another QTLs considered 26.4% from phenotypic variation localized on linkage group 6 (Fig.1.F) and completely linked to 570(66) marker (Table 2) [16]. Gaj and Malozineski (1985) reported Existence many QTLs for awn length on chromosome 2. For trait of number of spikelet in spike found 2 QTLs localized in linkage group 3 that one of them has 8 CM space to link marker that is 375 (384) and considered 19.8% phenotypic variation the another localized 4.5 CM from 679(64) marker (Fig.1.C) and considered 23.5% from phenotypic variation (Table 2) [17]. Kandemir et al (2000) Bezant et al (1997) found one QTL for trait of number of spikelet in spike [5,18]. For length of flag leaf trait was found four QTLs that three QTLs localized in linkage group 2(Fig.1.B) that considered from 35.7% to 38.1% phenotypic variationand one QTLs localized in linkage group 3 (Fig.1.C) at the space of 5.7CM from 1546(65) marker and considered 41.9% phenotypic variation (Table 2).

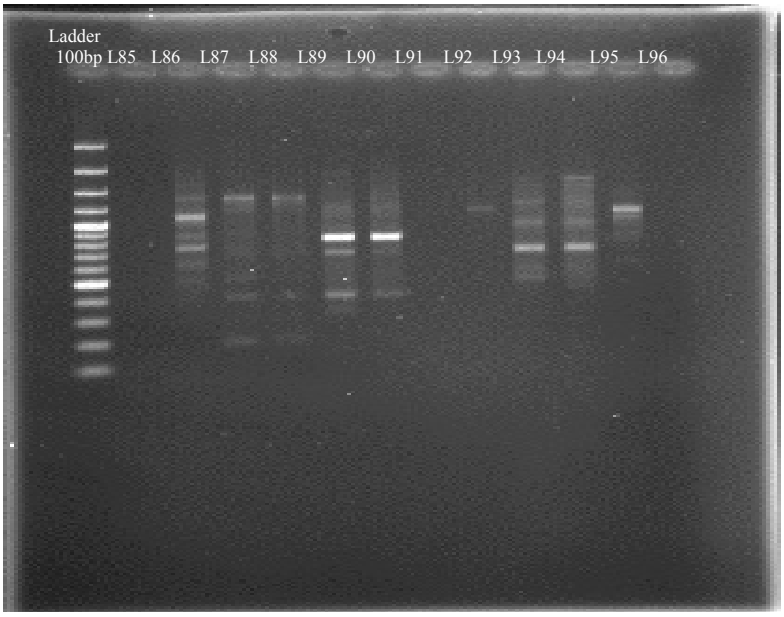

Fig. 2. DNA bands amplified from leaves of some understudied genotypes using397 primer of RAPD marker

4. CONCLUSION

The results showed this map covered 6 linkagegroup with 1748/7 centimorgan (CM) coverage and relatively high R^2 values and low confidence limits for QTLs so that the relevant linkage between markers and QTLs can be used in breeding programmes. As QTL mapping is very sensitive to errors in marker placement and between marker distances, to determine exact localization of the QTLs on the linkage map and better estimation of explained variation by these QTLs we recommend using of codominant markers to optimize the genetic linkage map.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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