Journal of Plant Molecular Breeding (JPMB)
Vol. 3/ Issue 1/ June 2015/ 1-17

Association analysis for traits associated with powdery mildew tolerance in barley [*Hordeum vulgare* L.] using AFLP markers

Zeinab Mohammadi[1], Atefeh Sabouri[1*] & Sedigheh Mousanejad[2]

1. Department of Agronomy & Plant Breeding, University of Guilan, Rasht, Iran.
2. Department of Plant Protection, University of Guilan, Rasht, Iran.

[*]Corresponding Author, Email: a.sabouri@guilan.ac.ir

Received: April 2015 Accepted: May 2015

Abstract

Association analysis is a useful method for evaluation of significant association between molecular marker and phenotype of trait. This study was performed to evaluate association between traits related with powdery mildew resistance and molecular markers. This investigation was performed using 77 barley genotypes and AFLP markers. In phenotypic evaluation, reaction of seedlings to powdery mildew was evaluated and the infection type and intensity were assessed based on 0-9 scale as the most important traits associated with resistance. Also in this study, the genetic diversity of genotypes was evaluated using seven combination primers *Eco*RI/*Mse*I. The average percentages of polymorphism and polymorphic information content were 92.37% and 0.43, respectively. General evaluation of the statistics of genetic diversity showed that among seven primer combinations, three combinations of E90-M160, E100-M160, and E100-M150 were higher value than others and had a more obvious effect in the detection and separation of barley genotypes. Association analysis was performed using four statistical models of GLM and MLM applying TASSEL software. In the complete MLM model, 33 markers showed significant association in the 5 percent probability level with traits and the highest coefficient of determination was related to marker E80-M150-3 that explained 14% of variations of infection intensity. E80-M510-3 and E80-M160-22 markers were showed significant association ($pr<0.05$) with both characteristic the severity and type of infection that can represent the effective role of this genomic region in resistance to powdery mildew. If the results are confirmed, it can be a suitable candidate for conversion to SCAR specific marker.

Keywords: AFLP marker, Association analysis, Barley, Fungal disease, Powdery mildew.

Introduction

Barley has an important role as a source of human food and is the most important nutrient following wheat, rice and maize.

Powdery mildew is one of the most important fungal diseases of barley which is caused by *Blumeria graminis* f. sp. *hordei* fungus. Powdery mildew reduces photosynthetic activity, increases respiration and transpiration, and reduces yield and the quality of harvested grain (20, 25).

In the past, improvement in quantitative traits of plants was performed with phenotypic evaluation but the

information of the controlling loci of these traits was not obtained. Today improvement of quantitative traits is performed mainly to identify their controlling loci in the genome. The rapid advances in molecular techniques (particularly in DNA markers) have initiated a new era in genetic studies. DNA markers have increasingly been used in genetic analysis due to their advantages namely, they are highly polymorphic, randomly distributed in genome, least influenced by environmental factors, neutral to selection, stable across different developmental stages and show different individuals in the DNA molecule (18).

Association analysis has been proposed as a method for locating the quantitative traits in recent years. A linkage disequilibrium-based method is the one which evaluating the relationship between phenotypic and genotypic data (11). In association analysis, linkage disequilibrium in natural populations and germplasm collections is used. The linkage disequilibrium is the non-random association between two markers, two genes or marker-gene (19). Association analysis is based on the use of molecular markers. One of the most important markers of DNA is the AFLP marker that was introduced by Vos *et al.* (27). In addition to human genetics, association analysis is used in animal and plant populations.

Association analyses between agronomic traits and SSAP markers were performed in 108 durum wheat genotypes by Rashidimonfared *et al.* (21). In this study, 10 primer combinations of SSAP produced 74 polymorphic bands. The relationship between six agronomic traits and 74 polymorphic markers was measured using stepwise regression. 32 SSAP markers showed significant association with at least one of the six agronomic traits.

In another study, in order to identify genomic regions associated with root traits, association analysis of 100 winter barley (including 50 six-row barley and 50 two-row barley) was performed using 3964 SNPs markers. In phenotypic evaluation, the traits of root dry weight, root volume, average diameter and average secondary roots were assessed. 15 QTLs were detected for traits using MLM model. SNP-2981 marker with maximum value of the log probability was associated with the number of secondary roots. Results of this experiment and the mapped agronomic traits in previous studies showed that most QTLs of root traits were related to traits such as yield, kernels per spike, heading date, lodging and plant height (3). Roy *et al.* (22) performed association analyses of 14 agronomic traits in 55 wheat genotypes using 20 microsatellite primer pairs. In this study, 519 polymorphic markers were generated. 131 SSR, 43 SAMPL and 166 AFLP markers showed significant association with at least one of the 14 agronomic traits.

In another study, association analyses of 48 rice genotypes were performed for the related traits with drought stress using SSR markers. 82 markers showed high correlation with the traits of root length, root dry weight and root

diameter. The markers RM170, RM572, RM318, RM3843, RM29, RM540, RM585 and RM36 were related to both root traits and yield under stress conditions (7).

Achleitner *et al.* (2) performed association analyses of 114 oats cultivars using eight AFLP primer combinations to identify markers associated with yield and yield components. Finally, 23 markers were introduced as markers with high potential and associated with complex traits for future breeding programs.

The present study was done to identify related markers with resistance controlling genes to powdery mildew that can be suitable to control diseases and reduce the utilization of fungicides. Utilization of resistant cultivars is the best way in order to adapt to the environment and protect human health.

Materials and methods

Plants materials

The plant materials consisting of 77 genotypes of barley were prepared from the Agricultural and Natural Resources Research Center of Golestan Province. The name and pedigree of barley genotypes are shown in Table 1.

This study was conducted with randomized complete block design in pots in greenhouse conditions in the Faculty of Agricultural Sciences, University of Guilan in 2013. Initially, samples contaminated with powdery mildew, were prepared. These samples were collected in June 2012 from the Varamin region and were stored in dry conditions in the Plant Protection Research Institute of Iran. Due to the presence of sexual stage of cleistothecium on the leaves of contaminated samples, which were able to survive under unfavorable environmental conditions for several years, the pathogen was activated and inoculated on the susceptible cultivar Afzal. The seedlings were placed at $20^{\circ C}$ in moist conditions. After sporulation of the pathogen on the leaves surface, spores were collected for contamination. Five seeds of each genotype were planted in plastic pots, 14 cm apart. Seedlings were inoculated with spores of the fungus in two-leaf stage. It should be mentioned that seeds of six genotypes did not germinate in some replications therefore increasing precision was eliminated. Thus in phenotypic evaluation, 71 genotypes were evaluated. Eliminated genotypes are: NB5, Jonoob, EBYT-W-89-17, EBYT-W-89-18, EBYT-W-89-19 and EBYT-W-89-6. After 12 days, the traits of the infection type and infection intensity were assessed based on 0-9 (23) scale. In order to normalize the distribution of experimental errors, data transformation was performed for the, infection type and infection intensity traits using equations (1) and (2), respectively.

$$\sqrt{X + 0.5} \quad (1)$$

$$\text{Arc sin}\sqrt{x + 0.5} \quad (2)$$

Table 1. Name and pedigree of studied barley genotypes in this research.

Number	Name or Pedigree	Number	Name or Pedigree	Number	Name or Pedigree
1	Youssef	27	EB-88-3	53	EBYT-W-89-17
2	Izeh	28	EB-88-4	54	EBYT-W-89-18
3	NB17	29	EB-88-5	55	EBYT-W-89-19
4	NB5	30	EB-88-7	56	EBYT-W-89-4
5	L4shori	31	EB-88-10	57	EBYT-W-89-5
6	Nimroz	32	EB-88-14	58	EBYT-W-89-7
7	Kavir	33	EB-88-16	59	EB-88-20
8	Prodogtive	34	EB-88-19	60	EBYT-W-89-8
9	Bahman	35	Bomi	61	39Motadel
10	36Motadel	36	Rihane	62	EB-86-17
11	31Motadel	37	Arass	63	EB-87-7
12	28Garm	38	Goharjow	64	EB-88-13
13	24Garm	39	Karoon	65	Dasht
14	21Garm	40	EB-88-2	66	Makouee
15	EC-84-10	41	Jonoob	67	Nosrat
16	45Motadel	42	Shirin	68	EC-83-17
17	EC-82-11	43	Torsh	69	EBYT-W-79-10
18	EC-81-13	44	Fajre30	70	MB-83-14
19	MB-82-12	45	W-82-5	71	W-79-10
20	EB-86-14	46	EBYT-W-89-2	72	EBYT-W-89-3
21	EB-86-6	47	EBYT-W-89-9	73	EBYT-W-89-6
22	EB-86-4	48	EBYT-W-89-10	74	EB-88-11
23	EB-86-3	49	EBYT-W-89-11	75	EB-88-6
24	EB-85-5	50	EBYT-W-89-13	76	EB-88-8
25	EB-87-20	51	EBYT-W-89-15	77	EB-88-9
26	EB-88-1	52	EBYT-W-89-16		

AFLP analysis

DNA extraction from the fresh leaves of samples was performed using the CTAB method described by Saghai-Maroof *et al.* (24). The quantity and quality of extracted DNA were determined using agarose gel of 0.8 percent. The AFLP method was performed according to Vos *et al.* (27) method. Six µl of extracted DNA was digested with restriction enzymes of *EcoR*I and *Mse*I for 3 hours at 37$^{\circ C}$. The DNA fragments were ligated to *EcoR*I and *Mse*I adopters for a period of 2 hours at 37℃ and 1 hour at 20℃. The samples of the previous stage were used for pre-amplification with the *EcoR*I and *Mse*I primers with one selective nucleotide. In this stage, the thermal cycles were 30 times with the program of 94℃ for 30 seconds, 60℃ for 30 seconds and 72℃ for 60 seconds. The products of pre-amplification stage were diluted 5:1 and then selective amplifications were done with 10 primers combinations with three selective nucleotides (Table 2) in touch down thermal cycle including three

stages of different temperatures. The PCR products were separated using polyacrylamid gel electrophoresis of 6% and were stained with silver nitrate. AFLP bands were scored as zero or one for absence or presence.

Table 2. Primer combinations in AFLP analysis.

*Eco*RI Primer		*Mse*I Primer	
Name	**Sequence**	**Name**	**Sequence**
E060	GACTGCGTACCAATTCAAG	M140	GATGAGTCCTGAGTAAAAC
E070	GACTGCGTACCAATTCAAT	M150	GATGAGTCCTGAGTAAAGA
E080	GACTGCGTACCAATTCACG	M160	GATGAGTCCTGAGTAAAGT
E090	GACTGCGTACCAATTCACT		
E100	GACTGCGTACCAATTCAGT		
E110	GACTGCGTACCAATTCATC		

Statistical analyses

The polymorphic information content (PIC) was calculated using equation (3) by Excel software:

$PIC_i = 2\ f_i\ (1\text{-}f_i)$ (3)

Equation above, PIC_i is the PIC of marker i, f_i is the frequency of presence of *i*th marker and $1\text{-}f_i$ is the frequency of absence of *i*th marker.

The marker index (MI) and the effective multiplex ratio (EMR) were calculated using equations (4) and (5) by Excel software:

MI= PIC×EMR (4)

EMR= $n_p \times \beta$ (5)

n_p and β are as:

n_p= The total number of polymorphic bands

β= Fraction of number of polymorphic bands to the total number of bands

Marker index and effective multiplex ratio were calculated by Excel software. other statistics of genetic diversity including the Nei's coefficient of variation and Shannon index were calculated using PopGene 32 (28) and PAST (14) software respectively.

The structure analysis and separation of population into subpopulations with different genetic structure were performed using STRUCTURE software. As previous information of population structure was not available, the number of subpopulations (K) was calculated with the simulation that was performed with 100000 Burn-in period and 100000 MCMC repetitions. The number of K was considered ranging from 2 to 10 and Evanno *et al.* (9) method was used to calculate the number of subpopulations. In this way, columns of K and LnP (D) were used for calculations and the mean L (K) and standard deviation (STD) of repetitions were calculated for each K. Then the subtraction of mean repetitions L'(K) was calculated for the adjacent groups from difference between upper group and lower group and the subtraction of L'(K) for adjacent groups was calculated as L''(K) values. Finally, the values of ΔK were calculated using equation (6). Also a bilateral chart of K and ΔK was plotted.

$$\Delta K = L''(K) / Stdev \quad (6)$$

The climax of the curve in bilateral chart of K and ΔK indicates the optimal number of K. Association analysis was performed between AFLP markers and phenotypic traits using four statistical models (Table 3) using TASSEL software.

Table 3. The four used statistical models for doing of association analysis of AFLP markers and phenotypic traits.

Model	Used data set
1: GLM[a]	Phenotype + AFLP
2: GLM	Phenotype + AFLP + Q[b]
3: MLM[c]	Phenotype + AFLP + K[d]
4: MLM	Phenotype + AFLP + K + Q

a: General linear model. b**:** Population structure data. c: Mixed linear mode. d: Kinship data obtained from general similarity of individuals in genetic background arising the kinship.

Results and Discussion

Statistics of genetic diversity

A total of 245 bands were generated out of seven primer combinations of *Eco*RI and *Mse*I, of which 227 bands were polymorphic and the average polymorphism obtained was 32.42 per polymorphic band. The average percentage of polymorphism in this study was 92.37 %. The high percentage of polymorphism obtained in this study showed that these markers can be used as powerful tools in the detection and separation of barley genotypes. Figure 1 shows AFLP banding patterns obtained from the amplification of primer combination of E100-M160 in barely as a typical example. Polymorphism information content for each primer combination separately is shown in Table 4. In this study, PIC index was variable between 0.39 and 0.48 with the average of 0.43.

Table 4. Genetic diversity statistics for seven primer combinations of AFLP.

Primer combination	Poly. Bands	Total. Bands	Poly. Percentag (%)	PIC	Marker index	Nei gene diversity	Shannon index
E80-M150	28	30	93.33	0.40	10.41	0.29	3.16
E90-M150	38	40	95.00	0.41	14.80	0.28	3.06
E100-M150	32	35	91.42	0.47	13.68	0.38	3.49
E110-M150	27	32	84.37	0.45	10.20	0.29	3.17
E80-M160	29	33	87.87	0.39	9.83	0.23	2.84
E90-M160	37	38	97.36	0.43	15.43	0.33	3.28
E100-M160	36	37	97.29	0.48	16.76	0.41	3.65
Total	227	245	646.46	3.05	91.11	2.25	22.65
mean	32.42	35	92.37	0.43	13.01	0.32	3.23

The highest rate of polymorphic information content was obtained in E100-M160, E100-M150 and E110-M150 primer combinations that were 0.48, 0.47 and 0.45 respectively.The polymorphic information content shows the resolution of a marker by the number of polymorphic alleles and their relative frequency in the population. Therefore, high values of PIC obtained

for mentioned markers show their high effectiveness in distinguishing genotypes in the present study. The diversity index of Nei was variable between 0.23 and 0.41. Furthermore, three combinations E100-M160, E100-M150 and E90-M160 had the highest values, respectively. The Shannon index was variable between 2.84 to 3.65 and the combination of E100-M160 had the highest value. The marker index was variable between 14.80 and 16.76. Also, the combination of E100-M160 had the highest value. This index considers the total number of bands and calculates potential of each primer to produce more bands on gel. General evaluation of the statistics of genetic diversity including the Nei's coefficient of variation, Shannon index and marker index showed that among seven primer combinations, three combinations of E90-M160, E100-M160, E100-M150 were the highest and in fact had a more powerful effect on distinguishing genotypes.

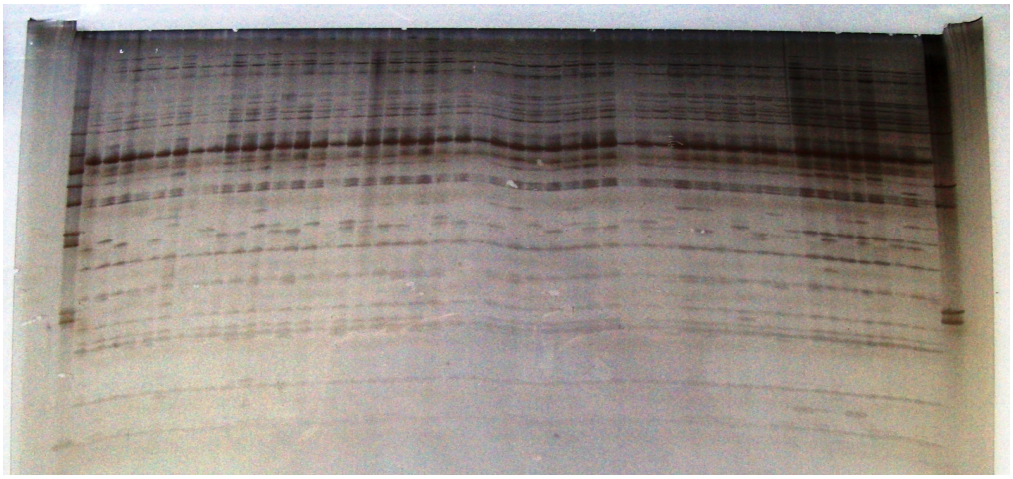

Figure 1. AFLP banding patterns obtained from the amplification of primer combination of E100-M160 in barely**.**

Structure analysis

The analysis of population genetic structure was performed using STRUCTURE software. Table 5 shows statistics to determine optimum K and Figure 2 shows a bilateral chart to determine the optimal number of K. Based on Figure 2, the climax of the curve is equal to 2. Therefore, the population structure is separable into two subpopulations with different genetic structures. Figure 3 shows inferred population structure by the STRUCTURE software. The assignment of individuals into sub-populations was performed using Spataro *et al.* (26) method. Also membership percentage for every individual in each group was calculated.

Table 5. Calculated statistics for optimum K using STRUCTURE software.

K	L(K)[a]	Stdev[b]	L'(K)[c]	L''(K)[d]	Δ K[e]
1	8327.79	6583.403	-18095.2	18391.43	2.793605
2	-9767.41	54.69084	296.23	-8054.24	147.2685
3	-9471.18	157.0086	-7758.01	-6152.94	39.18854
4	-17229.2	13557.57	-13911	21729.27	1.602741
5	-31140.1	29473.96	7818.32	-26914.1	0.913147
6	-23321.8	12344.15	-19095.8	14921.43	1.208786
7	-42417.6	30632.26	-4174.32	10429.37	0.34047
8	-46591.9	55448.22	6255.05	-2523.93	0.045519
9	-40336.8	59592.69	3731.12	32874.6	0.551655
10	-36605.7	29774.71	36605.72	-36605.7	1.229423

a: The mean of LnP(D) of repetitions for each K. b: The standard deviation (STD) of repetitions. c: $L(K)_n - L(K)_{n-1}$, d: $L'(K)_n - L'(K)_{n-1}$, e: $|L'(K)| / stdev$

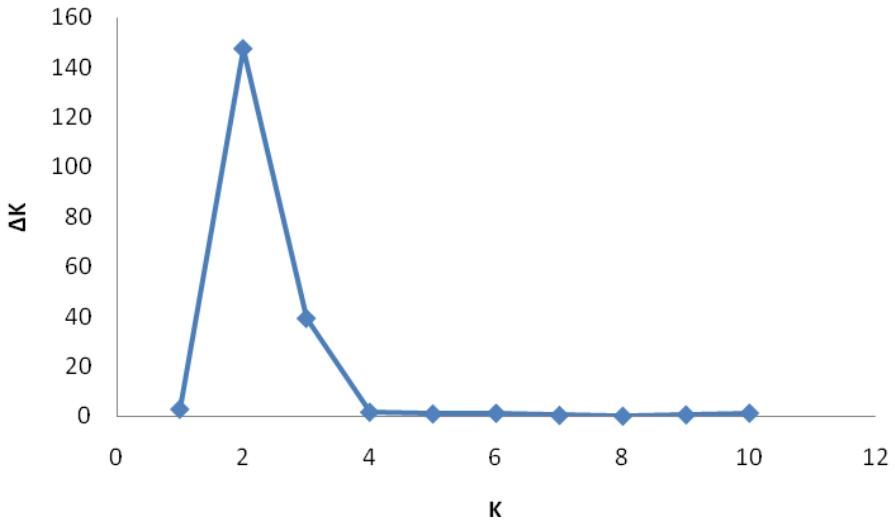

Figure 2. Bilateral chart to determine the optimal number of K. X axis: The number of sub-populations.Y axis: ΔK =.(L''(K))/Stdev. The method of calculation of ΔK are given in Table 5 and materials and method section.

Table 6 shows proportion of membership of each genotype in each of the two clusters. According to this method, the assignment of genotypes into groups is possible when that membership percentage of a genotype is 0.7 or more than 0.7 and if its membership percentage is less than 0.69, it is considered as a mixed genotype. Thus, 21 genotypes were assigned to group 1 and 35 genotypes were assigned to group 2. Furthermore, 21 genotypes were identified as mixed ones. Genotypes belonging to each sub-population had the most similarity in terms of allele frequencies and genetic structures and were different from the other groups.

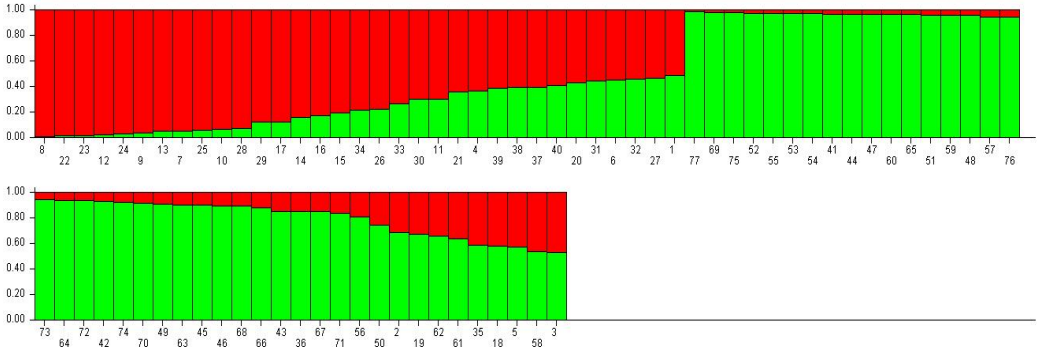

Figure 3. Inferred population structure out of STRUCTURE software obtained on the 227 AFLP markers data set, partitioned into K coloured segments at K=2. X axis: The genotypes numbers. The genotypes names with their numbers exist in the Table 1. Y axis: Proportion of membership of each genotype in each of the two clusters (sub-populations).

The spurious associations will be identified between marker and QTL. As association analysis was performed between molecular markers and traits regardless of population genetic structure, determining the genetic structure of populations and germplasm collections which is very important (10, 13).

Casas *et al.* (5) performed association analysis for 225 barley accessions (including the 175 SBCC accessions) using SSR markers. Considering the population structure in this study, the number of significant associations was reduced.

Association analysis

Association analysis was carried out using TASSEL software. The results are shown in Table 7. According to this table, in the first model, (GLM: G+P) 35 were identified for the disease severity and 3 markers were identified for infection type traits. In the second model, (GLM: G+P+Q), 36 markers showed significant association with the disease severity trait and one marker showed significant association with the infection type trait. In the model (MLM: G+P+K), 32 markers were identified for disease severity and 1 marker was identified for infection type traits. In the fourth model (MLM: G+P+Q+K), 31 markers were identified for disease severity and 2 markers were identified for infection type traits. In general, in the four mentioned models, 36 markers were identified for the disease severity trait and 3 markers for the infection type trait. In these four models, 13 markers were identified for the disease severity trait together that included: E80-M150-1, E80-M150-2, E80-M150-3, E80-

Table 6. Genotypes's membership based on extracted results of STRUCTURE software.

Name or pedigree	Percentage of group's membership 1	Percentage of group's membership 2	Name or pedigree	Percentage of group's membership1	Percentage of group's membership 2	Name or pedigree	Percentage of group's membership 1	Percentage of group's membership 2
Youssef	0.508	0.492	EB-88-3	0.535	0.465	EBYT-W-89-17	0.28	0.972
Izeh	0.31	0.69	EB-88-4	0.924	0.076	EBYT-W-89-18	0.028	0.972
NB17	0.467	0.533	EB-88-5	0.877	0.123	EBYT-W-89-19	0.025	0.975
NB5	0.633	0.367	EB-88-7	0.699	0.301	EBYT-W-89-4	0.189	0.811
L4shori	0.422	0.578	EB-88-10	0.556	0.444	EBYT-W-89-5	0.051	0.949
Nimroz	0.546	0.454	EB-88-14	0.54	0.46	EBYT-W-89-7	0.458	0.542
Kavir	0.944	0.056	EB-88-16	0.729	0.271	EB-88-20	0.038	0.962
Prodogtive	0.99	0.011	EB-88-19	0.785	0.215	EBYT-W-89-8	0.032	0.968
Bahman	0.961	0.039	Bomi	0.413	0.587	39Motadel	0.36	0.64
36Motadel	0.935	0.065	Rihane	0.147	0.853	EB-86-17	0.337	0.663
31Motadel	0.697	0.303	Arass	0.601	0.399	EB-87-7	0.093	0.907
28Garm	0.978	0.022	Goharjow	0.604	0.396	EB-88-13	0.058	0.942
24Garm	0.95	0.05	Karoon	0.611	0.389	Dasht	0.033	0.967
21Garm	0.841	0.159	EB-88-2	0.586	0.414	Makouee	0.115	0.885
EC-84-10	0.805	0.195	Jonoob	0.029	0.971	Nosrat	0.149	0.851
45Motadel	0.824	0.176	Shirin	0.067	0.933	EC-83-17	0.105	0.895
EC-82-11	0.876	0.124	Torsh	0.145	0.855	EBYT-W-79-10	0.015	0.985
EC-81-13	0.42	0.58	Fajre30	0.029	0.971	MB-83-14	0.083	0.917
MB-82-12	0.328	0.672	W-82-5	0.095	0.905	W-79-10	0.159	0.841
EB-86-14	0.568	0.432	EBYT-W-89-2	0.103	0.897	EBYT-W-89-3	0.058	0.942
EB-86-6	0.637	0.363	EBYT-W-89-9	0.03	0.97	EBYT-W-89-6	0.057	0.943
EB-86-4	0.984	0.016	EBYT-W-89-10	0.04	0.96	EB-88-11	0.074	0.926
EB-86-3	0.984	0.016	EBYT-W-89-11	0.087	0.913	EB-88-6	0.017	0.983
EB-85-5	0.966	0.034	EBYT-W-89-13	0.251	0.749	EB-88-8	0.054	0.946
EB-87-20	0.939	0.061	EBYT-W-89-15	0.036	0.964	EB-88-9	0.013	0.987
EB-88-1	0.778	0.222	EBYT-W-89-16	0.025	0.975			

Table 7. The results of association analysis between evaluated traits and AFLP markers using four statistical models.

	GLM: G+P			GLM: G+P+Q			MLM: G+P+K			MLM: G+P+Q+K		
Trait	**Marker**	**R^2**	**P**	**Marker**	**R^2**	**P**	**Marker**	**R^2**	**P**	**Marker**	**R^2**	**P**
II	E80M1501	0.130	0.0094	E80M1501	0.120	0.0138	E80M1501	0.100	0.0380	E80M1501	0.093	0.0161
	E80M1502	0.154	0.0037	E80M1502	0.146	0.0051	E80M1502	0.125	0.0178	E80M1502	0.118	0.0161
	E80M1503	0.170	0.0019	E80M1503	0.169	0.0020	E80M1503	0.141	0.0109	E80M1503	0.143	0.0161
	E80M1504	0.136	0.0075	E80M1504	0.129	0.0099	E80M1504	0.106	0.0317	E80M1504	0.102	0.0161
	E80M1505	0.136	0.0074	E80M1505	0.128	0.0102	E80M1505	0.107	0.0313	E80M1505	0.100	0.0161
	E80M1506	0.133	0.0083	E80M1506	0.124	0.0120	E80M1506	0.103	0.0345	E80M1506	0.099	0.0161
	E80M1507	0.131	0.0091	E80M1507	0.121	0.0131	E80M1507	0.101	0.0374	E80M1507	0.094	0.0161
	E80M1508	0.163	0.0026	E80M1508	0.150	0.0043	E80M1508	0.135	0.0132	E80M1508	0.126	0.0161
	E80M1509	0.130	0.0095	E80M1509	0.120	0.0135	E80M1509	0.100	0.0384	E80M1509	0.094	0.0161
	E80M15010	0.139	0.0066	E80M15010	0.127	0.0105	E80M15010	0.110	0.0285	E80M15010	0.099	0.0161
	E80M15011	0.148	0.0047	E80M15011	0.135	0.0078	E80M15011	0.119	0.0213	E80M15011	0.106	0.0161
	E80M15012	0.130	0.0095	E80M15012	0.120	0.0138	E80M15012	0.100	0.0384	E80M15012	0.093	0.0161
	E80M15013	0.130	0.0095	E80M15013	0.121	0.0133	E80M15013	0.100	0.0384	E80M15013	0.094	0.0161
	E80M15014	0.132	0.0086	E80M15014	0.120	0.0135	E80M15014	0.103	0.0352	E80M15014	0.094	0.0161
	E80M15015	0.139	0.0068	E80M15015	0.126	0.0108	E80M15015	0.109	0.0289	E80M15015	0.100	0.0161
	E80M15016	0.142	0.0060	E80M15016	0.128	0.0103	E80M15016	0.112	0.0261	E80M15016	0.102	0.0161
	E80M15017	0.156	0.0034	E80M15017	0.146	0.0051	E80M15017	0.127	0.0167	E80M15017	0.117	0.0161
	E80M15018	0.134	0.0080	E80M15018	0.124	0.0117	E80M15018	0.105	0.0333	E80M15018	0.097	0.0161
	E80M15019	0.133	0.0085	E80M15019	0.122	0.0130	E80M15019	0.103	0.0349	E80M15019	0.095	0.0161
	E80M15020	0.136	0.0076	E80M15020	0.127	0.0103	E80M15020	0.106	0.0318	E80M15020	0.100	0.0161
	E80M15021	0.141	0.0061	E80M15021	0.130	0.0094	E80M15021	0.112	0.0264	E80M15021	0.101	0.0161
	E80M15022	0.130	0.0093	E80M15022	0.120	0.0138	E80M15022	0.100	0.0378	E80M15022	0.093	0.0161
	E80M15023	0.136	0.0074	E80M15023	0.125	0.0115	E80M15023	0.107	0.0310	E80M15023	0.099	0.0161
	E80M15024	0.130	0.0095	E80M15024	0.120	0.0138	E80M15024	0.100	0.0383	E80M15024	0.093	0.0161
	E80M15025	0.130	0.0094	E80M15025	0.120	0.0138	E80M15025	0.100	0.0381	E80M15025	0.093	0.0161
	E80M15026	0.142	0.0059	E80M15026	0.130	0.0093	E80M15026	0.113	0.0258	E80M15026	0.102	0.0161
	E80M15027	0.133	0.0085	E80M15027	0.121	0.0130	E80M15027	0.103	0.0348	E80M15027	0.095	0.0161
	E80M15028	0.130	0.0095	E80M15028	0.120	0.0138	E80M15028	0.100	0.0384	E80M15028	0.093	0.0161
	E110M15013	0.064	0.0353	E110M15013	0.068	0.0293	E110M15025	0.069	0.0328	E110M15025	0.061	0.0161
	E110M15025	0.074	0.0228	E110M15025	0.060	0.0415	E110M15027	0.075	0.0265	E110M15027	0.073	0.0161
	E110M15027	0.098	0.0084	E110M15027	0.094	0.0097	E80M16022	0.074	0.0274	E80M16022	0.085	0.0161
	E80M16015	0.060	0.0403	E80M16015	0.062	0.0378	E100M16034	0.095	0.0444			
	E80M16022	0.083	0.0155	E80M16022	0.070	0.0270						
	E80M16024	0.059	0.0424	E80M16024	0.061	0.0388						
	E100M16034	0.103	0.0264	E80M16026	0.060	0.0402						
				E100M16034	0.090	0.0430						
IT	E80M1503	0.101	0.0281	E80M1503	0.117	0.0152	E80M1503	0.104	0.0325	E80M1503	0.114	0.0161
	E80M16022	0.057	0.0458							E80M16022	0.064	0.0161
	E100M16034	0.087	0.0476									

II: Infection intensity. IT: Infection type. R^2: Coefficient of determination. P: Significance probability level.

M150-4, E80-M150-5, E80-M150-6, E80-M150-7, E80-M150-8, E80-M150-9, E80-M150-10, E80-M150-11, E80-M150-12, E80-M150-13, E80-M150-14, E80-M150-15, E80-M150-16, E80-M150-17, E80-M150-18, E80-M150-19,E80-M150-20,E80-M150-21, E80-M150-22, E80-M150-23, E80-M150-24, E80-M150-25, E80-M150-26, E80-M150-27, E80-M150-28, E110-M150-25, E110-M150-27, E80-M160-22 and also one marker was identified for the infection type trait that was E100-M150-3.

E100-M160-34 marker in the three models (GLM: G+P), (GLM: G+P+Q), (MLM: G+P+K) furthermore, E110-M150-13, E80-M160-15 and E80-M160-24 markers in the two models (GLM: G+P) and (GLM: G+P+Q) showed significant association in 5 percent probability level for the disease severity trait. Also, the E80-M160-22 marker in models (GLM: G+P) and (MLM: G+P+Q+K) showed significant association in 5 percent probability level with the infection type trait.

The new method of MLM considers the information of population structure (matrix Q) and kinship data (matrix K) in association analysis. Initially, the matrixes Q and K for doing MLM method should be prepared and then be used in association analysis to control the false associations between makers and traits. The MLM model with matrixes of Q and K will lead to better results in comparison with other ways in which the Q matrix or K matrix are used alone. However, the Q matrix can be replaced with P matrix (principal component analysis) which in this way, the MLM method is executed similarly and can be suggested as a potential for replacement (1).

In the fourth model, including four data sets and the MLM model, E80-M150-3 and E80-M160-22 markers showed significant association in 5 percent probability level with both traits. Based on the results, the absence and presence of the band respectively are representative of the resistance and susceptibility of cultivars. The highest coefficient of determination related to E80-M150-3 marker was 14% that explained variations of infection intensity trait.

Dadras *et al.* (6) performed association analysis of yields and seven important agronomic traits including leaf area index, plant height, leaf number, fresh leaf yield, dried leaf yield, length and the width of leaf in tobacco using AFLP markers. They used four statistical models of association analysis. According to their results, the combination E110-M160-23 was simultaneously significant for the leaf area index and fresh leaf yield traits. Also nine primer combinations were identified with the MLM model and four data sets including phenotypic, genotypic data, Q-matrix and K-matrix were significant for several traits. They proposed that if the effectiveness of these regions in genetic control of these traits is confirmed, they can be suitable candidates for conversion into the SCAR specific marker.

Association analysis of 115 genotypes of barley using 10 traits and 10 microsatellite markers was performed by Ebrahimi *et al.* (8), and a total of 70

polymorphic alleles were identified. The maximum number of markers was found for a number of nodes and the minimum number of markers was found for leaf number and radical length. The highest and lowest coefficients of determination were related to the grain width and germination traits, respectively. According to the results, markers HVM20, Gmsoo3, Bmaco36 and HVHVA1 were in controlling regions of agronomic traits more than other markers that explained more variations of studied traits. Some of the markers were associated with several traits simultaneously, that is considering the existence of a significant correlation among morphological traits can be due to genetic linkage or pleiotropic effects. In order to understand this subject, preparation of segregation generations and linkage maps is essential. In another study, association analysis of 35 barley genotypes was performed using microsatellite markers for traits related to freezing tolerance by Gangkhanlou *et al.* (12). A total of 62 alleles showed significant association with changes of 12 traits among 13 traits evaluated. The traits of crown moisture and relative water loss obtained maximum (10 allels) and minimum (one allele) number of alleles. Zhang *et al.* (29) performed association analysis of 26 agronomical traits with 204 SSR markers and 94 maize inbred lines. Using structure analysis, five sub-populations were obtained. Furthermore, using MLM model, 39 loci showed significant association in the five percent probability level with 17 agronomic traits in two years.Three loci with plant height, four with days to flowering, five with the number of kernel rows, and three with hundred kernels weight showed significant association simultaneously. They expressed that these results can be useful in genetic improvement and molecular breeding of maize. In another study, association analysis of 103 wheat germplasm was performed using 76 SSR and 40 EST-SSR markers. In the evaluation of phenotypes 6 traits were assessed in three places during three years. In this study, six sub-populations were obtained by population structure analysis based on 49 SSR and 40 EST-SSR markers. A total of 10 SSR markers on chromosome 4A showed significant association with six agronomic traits using the MLM model and by taking the Q and K matrixes (17). In another study, association analysis was performed on 40 durum wheat genotypes and SSR markers. In this study, six agronomic traits were evaluated. According to the results, 14 markers showed significant association with evaluated traits. Markers wmc54, wms118 and wmc165 on chromosomes 3B, 5B and 3A showed significant associations with several traits respectively (4). In another study, association analysis on a set of 160 *Brassica rapa* was performed using AFLP markers. In phenotypic evaluation, the amount of phytate and phosphate in seeds and leaves and some morphological traits were studied. Based on structure analysis, four sub-populations were obtained. In association analysis, 170 and 27 markers showed significant association

with evaluated traits regardless of population structure and considering population structure (30).

As mentioned before, the fourth model including four data sets and MLM model, E80-M150-3 and E80-M160-22 markers showed significant associations in 5 percent probability level with both traits that can represent the effective role of this genomic region in powdery mildew resistance that may be due to genetic linkage or pleiotropic effects. If this experiment is performed in a few years and in several locations with different genotypes and these genomic regions are identified again, SCAR specific marker from these genomic regions can be provided. Julio *et al.* (15, 16) in their studies identified QTLs related to agronomic traits, leaf quality, chemical composition and characteristics of the smoke and QTLs associated with resistance to several diseases using QTL mapping in tobacco. They identified AFLP markers related to important QTLs. Some of these markers were converted to SCAR specific marker. They were also confirmed in recombinant inbred lines and doubled haploid populations.

Conclusion

According to the results, primer combinations of E90-M160, E100-M160 and E100-M150 achieved the highest amounts of the Statistics of genetic diversity. Thus, they can be used as suitable and powerful combinations in breeding programs of barley. Based on the Structure analysis, the genotypes were separated into two groups with different genetic structures and one group was identified as mixed genotypes**.** In the MLM model, with the consideration of population structure and kinship data, 33 markers showed significant association in the 5 percent probability level with traits. E80-M510-3 and E80-M160-22 markers were identified in the 5 percent probability level linked to both severity and the type of infection traits that can represent the effective role of this genomic region in resistance to powdery mildew. Linkage of two markers and loci controlling of traits studied can be confirmed by further research which can be a suitable candidate for conversion into SCAR specific marker.

References

1. Abdurakhmonov, I.Y. and Abdukaimov, A. 2008. Application of association mapping to understanding the genetic diversity of plant germplasm resources. Int J Plant Genom, 1-18.
2. Achleitner, A., Tinker, N., Zechner, E. and Buerstmayr, H. 2008. Genetic diversity among at varieties of worldwide origin and associations of AFLP markers with quantitative traits. Theor Appl Genet, 117 (7): 1041-1053.
3. Ataei, R., Mohammadi, V., Talei, A.R. and Naghavi, M.R. 2013. Association mapping for root characteristics in barley (*Hordeum vulgare*). Iran J Field Crop Sci, 44 (2): 347-357.
4. Bousba, R., Baum, M., Jighy, A., Djekoune, A., Lababidi, S., Benbelkacem, A., Iabhilili, M., Gaboun, F. and Ykhlef, N. 2013. Association analysis of genotypic and phenotypic traits using SSR marker in Durum wheat. OIIRJ, 3: 60-79.

5. Casas, A.M., Kopahnke, D., Habekub, A., Schwizer, G., Gracia, M.P., Lasa, J.M., Ciudad, F.J., Codesal, P., Moralejo, M.A., Molina-Cano, J.L., Igartua, E. and Ordon, F. 2006. Marker-trait association for disease resistance in the Spanish barley core collection. Eucarpia, Lleida, 81: 141-145.
6. Dadras, A.R., Sabouri, H., Mohammadinejad, G.h., Sabouri, A. and Shoai-Deylami, M. 2014 Association analysis, genetic diversity and structure analysis of tobacco based on AFLP markers. Mol Biol Rep, 41(5): 3317-3329.
7. Deshmukh, V.V. 2012. Genome-wide association mapping of drought resistance traits in rice (*Oryza sativa* L.). MSc. Biotechnology thesis. Tamil Nadu Agricultural University. India.
8. Ebrahimi, A., Naghavi, M.R., Sabokdast, M. and Moradi Sarabshali, A. 2011. Association analysis with microsatellite markers for agronomic traits in Iranian barley landraces. Mod Genet J, 6(1): 35-43.
9. Evanno, G., Reganut, E. and Goudet, J. 2005. Detecting the number of clusters of individuals using the software STRUCTURE: a simulation study. Mol Ecol, 14: 2611-2620.
10. Falush, D., Stephens, M. and Pritchard, J.K. 2003. Inference of population structure using multilocus genotype data: linked loci and correlated allele frequencies. Genet, 164: 1567-1587.
11. Flint Garcia, S.A., Thornsberry, J.M. and Buckler, E.S. 2003. Structure of linkage disequilibrium in plants. Annu Rev Plant Biol, 54: 357-374.
12. Gangkhanlou, A., Mohammadi, S.A., Moghaddam, M., Ghasemi Golazani, K., Shakiba, M.R. and Yosefi, A. 2012. Genetic diversity in barley as revealed by microsatellite markers and association of these markers by traits related to freezing tolerance. Seed Plant Improv J, 28(1): 101-114.
13. Gupta, P.K., Rustgi, S. and Kulwal, P.L. 2005. Linkage disequilibrium and association studies in higher plants: Present status and future prospects. Plant Mol Biol, 57: 461-485.
14. Hammer, Ø., Harper, D.A.T. and Ryan, P.D. 2001. PAST: Paleontological statistics software package for education and data analysis. Palaeontologia Electronica, 4(1): 1-9.
15. Julio, E., Denoyes-Rothan, B., Verrier, J.L. and Dorlhac de Borne, F. 2006a. Detection of QTLs linked to leaf and smoke properties in *Nicotiana tabacum* based on a study of 114 recombinant inbred lines. Mol Breed, 18(1): 69-91.
16. Julio, E., Verrier, J.L. and Dorlhac de Borne, F. 2006b. Development of SCAR markers linked to three disease resistances based on AFLP within *Nicotiana tabacum* L. Theor Appl Genet, 112: 335-346.
17. Liu, L., Wang, L., Yao, J., Zheng, Y. and Zhao, C. 2010. Association mapping of six agronomic traits on choromosome 4A of wheat (*Triticum aestivum* L.). Mol Plant Breed, 1(5).
18. Mohammadi, S.A. 2006. Molecular analysis of genetic diversity of perspectives. Ninth Iran Crop Sci Congr, 27-29 Aug, Tehran.
19. Mohammadi, S.A. 2008. New methods of genetic structure analysis of quantitative traits in plant. Tenth Iranian Crop Sciences Congress. Seed and plant improvement research institute, 18-20 Aug, Karaj.
20. Pesaraklu, S., Soltanloo, H., Ramezanpour, S.S., Nasrollah Nejad Ghomi, A.A., Kalate Arabi, M. and Kia, Sh. 2013. Genetic analysis of powdery mildew resistance (*Erysiphe graminis f. sp. hordei*) in some barley lines. J Plant Prod, 20(3): 49-69.

21. Rashidimonfared, S., Moradi, M., Hosseinzadeh, A.H. and Naghavi, M.R. 2008. Association analysis between main agronomic traits and SSAP markers in durum wheat samples. Mod Genet J, 3(2): 29-35.
22. Roy, J.K., Bandopadhyay, R., Rustgi, S., Balyan, H.S. and Gupta, P.K. 2006. Association analysis of agronomically important traits using SSR, SAMPL and AFLP markers in bread wheat. Curr Sci, 90(5): 683-689.
23. Saari, E.E. and Prescott, J.M. 1975. A scale for appraising the foliar intensity of wheat diseases. Plant Dis Rep, 59(5): 377-380.
24. Saghai-Maroof, M.A., Biyashev, R.M., Yang, G.P., Zhang, Q. and Allard, R.W. 1994. Extraordinarily polymorphic microsatillate DNA in barely species diversity, choromosomal location, and population dynamics, Proceed of the Nat Acad of Sci. USA, 91: 5466-5570.
25. Silvar, C., Dhif, H., Igartua, E., Kopahnke, D., Gracia, M.P., Lasa, J.M., Ordon, F. and Casas, A.M. 2010. Identification of quantitative trait loci for resistance to powdery mildew in a Spanish barley landrace. Mol Breed, (25): 581-592.
26. Spataro, G., Tiranti, B., Arcaleni, P., Bellucci, E., Attene, G., Papa, R., Spagnoletti-Zeuli, P. and Negri, V. 2011. Genetic diversity and structure of a worldwide collection of *Phaseolus coccineus* L. Theor Appl Genet; 122: 1281-1291.
27. Vos, P., Hogers, R., Bleeker, M., Reijans, M., Lee, T.V.D., Hornes, M., Frijters, A., Pot, J., Peleman, J., Kuiper, M. and Zabeau, M. 1995. AFLP: a new technique for DNA fingerprinting. Nucleic Acids Res, 23 (21): 4407-4414.
28. Yeh, F.C., Yang, R.C., Boyle, T.J.B., Ye, Z.H. and Mao, J.X. 1997. POPGENE, The User-friendly Shareware for Population Genetic Analysis. Edmonton, Molecular Biology and Biotechnology Centre, University of Alberta, Canada.
29. Zhang, Q., Wu, C., Ren, F., Li, Y. and Zhang, C. 2012. Association analysis of important agronomical traits of maize inbread lines with SSRs. Aust J Crop Sci, 6(6): 1131-1138.
30. Zhao, J., Paulo, M.J., Jamar, D., Lou, P., Eeuwijk, F.V., Bonnema, G., Vreugdenhil, D. and Koornneef, M. 2007. Association mapping of leaf traits, flowering time, and phytate content in *Brassica rapa*. Genome, 50: 963-973.

آنالیز همبستگی خصوصیات مرتبط با مقاومت به سفیدک پودری در جو [*Hordeum vulgare* L.] با استفاده از نشانگرهای AFLP

زینب محمدی[۱]، عاطفه صبوری[۱*] و صدیقه موسی‌نژاد[۲]

۱. گروه زراعت و اصلاح نباتات، دانشکده کشاورزی، دانشگاه گیلان

۲. گروه گیاهپزشکی، دانشکده کشاورزی، دانشگاه گیلان

*نویسنده مسوول: a.sabouri@guilan.ac.ir

چکیده:

تجزیه ارتباط به عنوان روشی برای تعیین نواحی ژنومی کنترل کننده صفات کمی و اقتصادی، نقش مهمی در اصلاح مولکولی گیاهان دارد. در پژوهش حاضر، شناسایی نشانگرهای مولکولی (هفت ترکیب آغازگری *Eco*RI/*Mse*I) مرتبط با صفات تیپ آلودگی و درصد آلودگی با استفاده از ۷۷ ژنوتیپ جو و نشانگرهای AFLP بررسی شد. در ارزیابی فنوتیپی، صفات تیپ آلودگی و درصد آلودگی به عنوان مهم‌ترین صفات مرتبط با مقاومت براساس مقیاس ۹-۰ بررسی شدند. هم‌چنین در این پژوهش تنوع ژنتیکی ژنوتیپ‌ها با استفاده از هفت ترکیب آغازگری بررسی شد. میانگین درصد چندشکلی و محتوای اطلاعات چندشکلی به ترتیب ۹۲/۳۷ درصد و ۰/۴۳ بود. بررسی کلی آمارهای تنوع ژنتیکی نشان داد که از بین هفت ترکیب آغازگری، سه ترکیب E90-M160، E100-M160 و E100-M150 نسبت به سایر ترکیبات مقادیر بالاتری را به خود اختصاص دادند و در حقیقت در تمایز ژنوتیپ‌ها نقش بارزتری ایفا نمودند. تجزیه ارتباط با استفاده از چهار مدل آماری GLM و MLM توسط نرم‌افزار TASSEL انجام شد. در مدل MLM کامل، ۳۳ نشانگر ارتباط معنی‌داری در سطح احتمال پنج درصد با صفات ارزیابی شده نشان دادند و بالاترین ضریب تبیین مربوط به نشانگر E80-M150-3 بود که ۱۴ درصد از تغییرات شدت آلودگی را توجیه کرد. نشانگرهای E80-M150-3 و E80-M160-22 در سطح احتمال پنج درصد پیوسته به هر دو صفت شدت و تیپ آلودگی شناسایی شدند که می‌تواند بیانگر نقش مؤثر این ناحیه ژنومی در مقاومت به سفیدک پودری باشد. در صورت تأیید نتایج مبنی بر تأثیر معنی‌دار این ناحیه ژنومی، این ناحیه می‌تواند کاندید مناسبی برای تبدیل به نشانگر اختصاصی SCAR باشد.

کلمات کلیدی: نشانگر AFLP، آنالیز همبستگی، جو، بیماری قارچی، سفیدک پودری.