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Original Article

Genetic and Phenotypic Variation for Flowering Time Genes in Barley (Hordeum vulgare)

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ABSTRACT

Objective: Flowering Time (FT) in cereals controlled by genes that had a main factor on plant development. **Methods:** Genetic and phenotypic diversity of four flowering time genes (*FT2, FT3, FT4, Ppd-H1*) in 19 genotypes of cultivated and wild barley was evaluated and a total of 107 alleles were amplified. Genotypes based on days to flowering time and molecular data were grouped into early, middle and late maturity. **Results:** Molecular data analysis showed that 83% of variety was observed within populations and 17% was related to among population. Neis the maximum and minimum genetic distance between early - late (0.629) and early–middle was (0.0224). The average of allelic polymorphism was 83.33%. Also, the results of Spearman correlation for markers the number of days to flowering indicated that Ppd-H1 marker has the most significant negative correlation with days to flowering that prefigures the dominant allele role of this marker to reduce the number of days to flowering. Generally, the results of this research showed that there is a high genetic and phenotypic variation for specific markers that affected flowering time in barley. Also, genotypes with dominant allele of Ppd-H1 (9bp, deletion) compared to other genotypes show earlier flowering in response to the condition of long days, that it is an important feature for selection of superior genotypes in response to the stress conditions at the end of season.

1.INTRODUCTION

Genetic diversity is the basis of plant breeding (Nevo et al., 1982). For begin appropriate breeding strategies, the breeder must have knowledge of plant genetic diversity to develop the breeding programs with more rigor (De et al., 1992). Today, molecular markers are able to modify investigation of quantitative traits in the classical breeding methods. When we be able to identify linked markers to genetically factors that controlled the quantitative traits, the possibility of selection in segregated generations will become easier and faster and the selection efficiency and genetic progress of selection will increase (Kikuchi et al., 2009). Barley (Hordeum Vulgare) is one of the most important cereals crops in the world that as a one of the first domesticated plants has important role in the human developmentally and preparing of foods. The barley breeding process for producing of genotypes with maximum productivity in different growth condition depends on creating diverse populations and selection of superior genotypes (Rahimiyan and Banayan, 1997). Plants were exposed to different biotic and abiotic Stresses during the growth

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season. Drought stress in barley leads to the reduction of the number of spike, grain number, biological yield, seed weight and etc which has negative effects on yield. Therefore, identification of early cultivars is very important for overcome drought Stress in cereals. Flowering appropriate time is an essential trait for reproduction and survival of plant species that lead to pollination, development, evolution and suitable transmittal of seeds. Plants have mechanisms that enable them to respond to environmental changes, such as day length and low temperature (Normohamadi et al., 1997). flowering in cereals winter such as wheat and barley induced by long days, in these cereals, vernalization and photoperiod pathways are more active in controlling of the flowering time. The numbers of PEBP genes (FT proteins) in cereals are 3-4 times higher than Arabidopsis that shows this gene family has much higher complexity and variety in cereals (Kikuchi et al., 2009). Ppd-H1 and Ppd-H2 genes are allelic diversity related to natural variation in response to the day length and photoperiodic. Ppd-H1 is a part of the plant circadian clock and it is the flowering inducer in long day condition (Turner et al., 2005). A recessive mutation in Ppd-H1 leads to delayed flowering in long day condition (Dvorak et al., 1998; Rahimiyan and Banayan, 1997). Also Ppd-H2 (FT3candidate gene) accelerates flowering in response to short day condition (Dvorak et al., 1998). It is reported that there is a large natural diversity in the first intron region of the FT1 gene which is linked with phenotypic variation number of days to flowering (Hemming et al., 2008; Yan et al., 2006). Indeed, response to the day length in wheat is controlled by three Ppd1, Ppd2 and Ppd3 genes which are located on chromosomes 2A, 2B and 2D respectively (Anderson and Reinbergs 1985; Bamforth and Martin1981). So, there are a lot of many genotypes of wild barley in Iran that must be evaluated for different purpose. In this research, some of cultivated and wild barley in the west region of Iran used in order to evaluate phenotypic and genetic diversity for some responsible gene in flowering time. The main objective of present study was identifying early maturation genotypes that can be used in breeding programs.

3. MATERIALS AND METHODS

In this study, 19 genotypes of wild and cultivated barley were used that had been collected from the cereals gene bank of Ilam University. (Table 1) Seeds were cultivated in pots with 15cm in diameter in greenhouse condition according to randomized complete block design with three replications. Greenhouse temperature was variable between 26-32 °C during the experiment. Genomic DNA was extracted from fresh and young leaves in 3 to 4 leaf stage according to Doyle et al (1987). The PCR was performed with 25 µl reaction volume and reagents as follows: 3µl genomic DNA, 1.8 µl magnesium chloride 20 mM, 1.8 µl dNTP 1 mM, 0.5 µl of each forward and reverse primer (concentration 10 Pico mole), 0.4 µl of Taq polymerase (5 units), 2 µl pcr buffer (10 x), and 15 µl double-distilled water. PCR amplification was performed as follows: initial denaturation at 94°C for 5 min, followed by 35 cycles (94°C, 30 seconds; 50°C, 45 seconds,and 72°C, 1 min) and a final elongation at 72°C for 5 min. The PCR product was analyzed by 1.5 ٪ agarose gel electrophoresis. The digestion reactions with NdeI and BstNI enzymes were performed in 15 µl final volumes that contain 5 µl of PCR productS, 2 µl buffers 10X, 1.5 µl of each enzyme, and 6.5µl double distilled water. The reaction mixtures Incubated at 37 °C for 30 minutes. The digestion reaction with SwaI enzyme was performed in 15 µl final volume that contains 5 µl of PCR products, 2 µl buffer 10X, 1.5 µl of each enzyme, and 14.5 µl double distilled water. The reaction mixtures Incubated at 30 °C for 30 hours. (Table 2) their Products with similar conditions of polymerase chain reaction products were Segregatied. Results were scored as zero (absent) and 1 (present) band for each marker. Simple analysis ANOVA of day to flowering phonological trait was performed on all genotypes. Then, the relation of the days to flowering traits associated with specific markers were examined by using Spearman correlation coefficient. Also, the genetic distance among and within populations, the percentage of polymorphic alleles, the observed number of alleles (Na), the number of effective alleles (Ne), the corrected heterogenous indices (UHe), Shannon index (I) and molecular variance analysis was performed by using of GenAlEx 6.41 software. Finally, clustering of genotypes based on phenotypic and genotypic data was drawn using MVSP3.22 software.

2. RESULTS

To be informed about the available genetic variety among 3 groups of early, medium growth and delayed the populations of cultivated and wild barley, distance and similarity matrix, was calculated by Nei corrected index. The obtained data shows that the maximum Nei genetic distance index exists between two groups of early - delayed (0.629) and the minimum distance exists between the medium growth and early groups (0.0224) (Table 3). In other words, medium growth genotypes have the most genetic similarity to early genotypes. Also, the results showed that the medium growth group shows the maximum polymorphic alleles (0.100) and early and delayed groups showed the minimum amount of polymorphic alleles (0.75) and the average percentage of polymorphic alleles was calculated 83.33 percent. Results show that maximum (0.420) and minimum (0.353) corrected genetic index was related to the medium growth and early group respectively. The maximum observed alleles number (2), the number of effective alleles (1.70), and the value of Shannon index (0.579) was related to the medium growth population. The minimum observed alleles number (1.5), number of effective alleles (1.62), and Shannon index (0.460), were observed in the delayed group (Table 4). In general, the medium growth had more allelic diversities and heterogeneous groups than the other two groups. Also,

the results of molecular variance analysis showed that there is a significant difference %5 among 3 groups which based on this information, 0.83 of the total variety is related to the variety within the cultivated and wild barley populations and 0.17 of the remaining variety is related to the variety among early, medium growth and delayed groups (Table 5). Correlation analysis using Spearman coefficient for days to flowering trait with specific marker show that Ppd-H1 has the highest negative and significant correlation (-0.496) at 5% level, which is in accordance with the fact that the presence of the dominant allele Ppd-H1 with reduction of the number of days to flowering in the studied genotypes. Also, a significant and negative correlation (-0.458), between FT3 marker and the days to flowering trait was observed which it may be due to the presence of spring genotypes among these groups which in their the FT3 (Ppd-H2) dominant allele leads to the reduction of the number of days to flowering. Also, there is a negative and insignificant correlation (-0.289) between FT2 marker and the days to flowering which implies the fact that the presence of FT2 allele is higher when Ppd-H1 dominant allele exists and leads to the reduction of the number of days to flowering. FT4 allele showed a week positive and insignificant correlation with the days to flowering trait which implies the unimportant role of this gene in flowering time occurrence. (Table 6) This result is consonant with Turner et al [11] study which showed that there is correlation between flowering time of barley varieties under the condition of day length and presence of Ppd-H1 dominant allele.

2.1. Cluster analysis based on molecular data

In this research, cluster analysis was performed by Jaccard genetic distance and UPGMA algorithm, and genotypes were classified into 6 groups. Cultivated genotypes 1, 6, 8,17,18,25,29,31,33 were placed in the first group which have Ppd-H1 dominant marker and FT3 gene. The cultivated genotypes 4, 14 and 35 were placed in the second group which has the recessive Ppd-H1 allele and F3 gene. Genotypes 7, 30, 20 and 46 with Ppd-H1 dominant marker and without FT3 gene were placed in the third group that these genotype belonged to wild barley. Finally, 28, 22 and 15 genotypes which have the recessive ppH1 allele and lack of FT3 gene are place in separate clusters. The comparison of molecular and phenotypic cluster analysis days to flowering trait showed a high variety in terms of the studied marker so that the medium growth genotypes are placed in more and separate clusters. Cultivated and wild genotypes were largely classified in terms of the studied marker (Figure 1).

2.2. Cluster analysis of phenotypic data

For quantitative variables, the numbers of clusters were selected by hierarchical decomposition method. In the first cluster, 1, 6, 7, 17, 18, 25 and 29 genotypes were placed that basedon days to flowering time are early genotypes. In the second cluster, 4, 15, 31, 33, 35, 8 , 20, 30 and 46 genotypes were placed that are show medium growth in terms of the days to flowering trait. In the third cluster, 14, 28 and 22 genotypes were placed that show delayed in terms of the days to flowering trait (Figure 2). The results obtained by the genotypes classification based on the molecular data greatly confirmed by phenotipical data.

CONCLUSION

Natural variation in photoperiodic response is the major factor that control flowering time, adaptation and grain yield. In this research, a high genetic diversity was observed among the studied population in terms of genetic structure of Ppd-H1. genotypes which contain dominant Ppd-H1 allele (9bp deletion) days to flowering decreased, but in the genotypes which contained recessive ppd-H1 (9bp insertion) the growth period was significantly roused which implies that recessive ppd-H1 allele has lower photoperiodic sensitivity to the condition of day length in comparison with dominant Ppd-H1 allele (sensitive to photoperiod). These results are consistent with Alqouda et al[1] and Zelotina et al[14]. Furthermore, Wang et al[12] reported that, the presence of FT2 marker and dominant Ppd-H1 in early species produced PCR product after digestion 2 alleles 328+206 by NdeI restriction enzyme, and this result is in consistant with our result. Indeed, in most of genotypes with the deletion of Ppd-H1 marker, FT2 marker is emerged as two bands of 328+206 and this marker the side of dominant Ppd-H1 allele reduce the of number days for flowering. Based on the obtained results from conducted analysis by GenAlex software, it is deducted that the medium growth group has the highest value of polymorphic alleles, also this group has the least difference with early group that according to the obtained results from molecular data of marker, this group has the highest number of dominant Ppd-H1 allele and has more tendency to early and the occurrence of medium growth phenotype in greenhouse. Therefore, Ppd gene can be used as suitable tools for selecting the immature barley varieties which are not able to adapt with non-desired cultivation in high risk agriculture regions (short vegetative period, exit the drought and hot dry summers). Furthermore, the presence or absence of dominant Ppd-H1 allele which determines earliness of the variety can be detected in laboratory by PCR processes and restriction enzyme which saves time in farm tests (Zlotina et al., 2013). also, these markers can be used in selecting the compatible varieties to the high risk climate conditions in the initial steps. In other words, it provides the possibility of predicting the growth season length for barley varieties in the first steps of plant development by using specific ppd markers and vrn (genes related to vernalization) and the possibility of indirect evaluation of important breeding features and also the possibility of selecting early and compatible varieties with different growth conditions.

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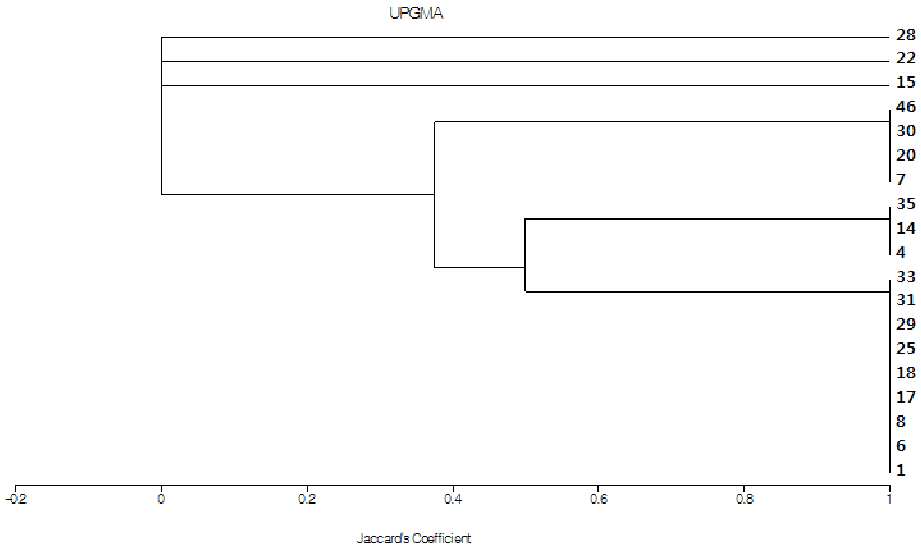

Figure 1- cluster analysis of molecular data based on four specific genes

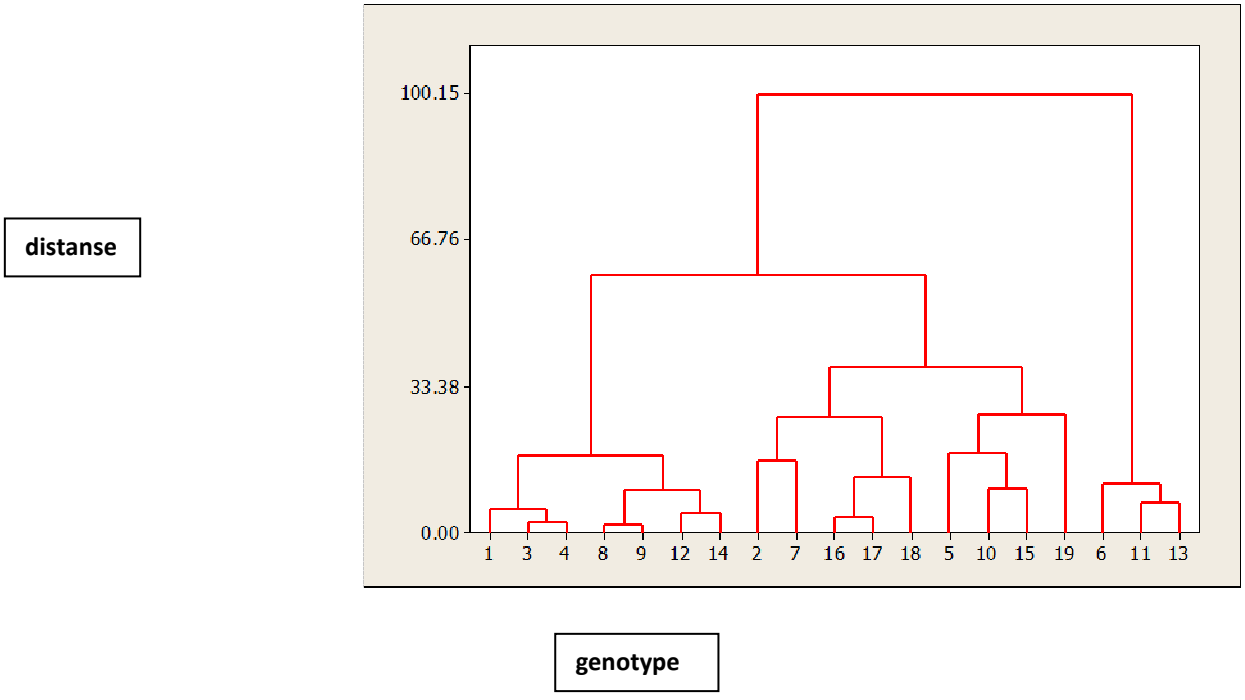

Figure 2- cluster analysis of quantitative data based hierarchical method.

Table1- Information of genotypes that used in this study

Row	Genotype codes	Used codes	Scientific name	Collection location
1	806	1	Hordeum vulgar	Ilam
2	1276	4	H.vulgar	kermanshah
3	1693	6	H.vulgar	kermanshah
4	895	7	H.vulgar	Ilam
5	1272	8	H.spontaneum	kermanshah
6	1667	14	H.spontaneum	lorestan
7	1275	15	H.vulgar	kermanshah
8	1808	17	H.vulgar	cultivar
9	859	18	H.vulgar	Ilam
10	850	20	H.spontaneum	Ilam
11	1260	22	H.spontaneum	Ilam
12	949	25	H.vulgar	Ilam
13	837	28	H.spontaneum	Ilam
14	853	29	H.vulgar	Ilam
15	918	30	H.spontaneum	Ilam
16	1809	31	H.vulgar	cultivar
17	1357	33	H.vulgar	Ilam
18	827	35	H.vulgar	Ilam
19	9	46	H.spontaneum	Ilam

Table2- Primers used in this study

Marker	Sequence 5-3	Tm	PCR product	Restriction enzymes
Ppd-H1	CCATGCTGCCAACTATGGTA	50	209	-
	TCCCAAAGTTCCTCTCTTTTCTC			
HvFT2	GGGTGCTTGAGATTGTCCAT	50	534	NdeI
	TCGTAGACGCATCTTTGTCG			
HvFT3	TTTTGCCCATCCTTAACACC	50	662	SwaI
	CTGATCCACCTTCCCTTTGA			
HvFT4	CGTTGAGATTGGTGGTGATG	50	554	BstNI
	GTACGGGGATGTTTGTACGG			

Table 3- Genetic distance (bottom diameter) and genetic similarity (over diameter) were calculated by Nei corrected index in cultivated and wild barley genotypes.

genotype	early	Medium growth	delayed
early	1	0.799	0.53
Medium growth	0.0224	1	0.884
delayed	0.629	0.123	1

Table 4- Premature genetic markers in the groups of early, medium growth and delayed genotypes.

Group	Samples	Na	Ne	UHe	I
early	7	1.75± 0.250	1.59±0.29	0.353±0.120	0.470±0.159
Medium growth	9	2±0	1.70±0.151	0.420±0.067	0.579±0.073
delayed	3	1.5 ±0.5	1.58±0.231	0.383±0.138	0.460±0.161
Average total	19	1.75±0.179	1.62±0.106	0.385±0.059	0.503±0.073

Table 5- Analysis of molecular variance(AMOVA) of system-specific markers

Sources of variation	Degrees of freedom	sum of squares	Mean square	Percent of the variance	PHiPT statistics	Possibility of significant
among groups	2	3.624	1.82	0.17	0.166	0.05
Within groups	16	13.429	0.839	0.83		
Total	18	17.053		0.100		

Table 6- Analysis of specific genes associated with days to flowering in the greenhouse conditions.

	Ppd-H1	FT2	FT3	FT4
FT2	0.0216			
FT3	0.185	0.185		
FT4	0.049	-0.185	0.131	
flowering time	0.496*-	-0.289	-0.458*	0.100