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The effect of Ppd-H1 gene's SNP 48 marker on heading dates in natural populations of barley from Middle East

[1]Abdul Wahid Baloch, [1]Ghulam Asghar Baloch, [1]Bakht-u-Nisa Mangan, [2]Imran Ahmad, [1]Weining Song

[1]College of Agronomy, Northwest A & F University, Yangling, Shaanxi, PR, China
[2]College of Horticulture, Northwest A & F University, Yangling, Shaanxi, PR, China

ABSTRACT

The flowering at an appropriate time for propagation and survival of any plant species is critical adaptive trait. Ppd-H1 is one of the most important gene which control photoperiod response in natural and landraces barley populations. In previous studies, the polymorphic site that is called SNP 48 marker of Ppd-H1 gene which is located in exon 6 of Ppd-H1, showed significant affects on heading dates in different barley cultivars. In the present study, a segment of 460 base pair successfully amplified and sequenced in 12 natural barley populations which belonged to different geographical areas of Israel and Jordan. Moreover, heading dates also observed among these wild barley populations under field conditions during the growing season of 2010-2011, the days to heading data exhibited a break of 15 days between early and late genotypes. On the basis of results obtained, the early and late genotypes all contained wild allele at SNP 48 and did not show any polymorphisms. It is concluded that there are other factors which are responsible for days to heading rather than only SNP 48 in wild barley populations.

Key words: *Ppd-H1 gene, Wild barley, Landraces barley, Polymorphism, Days to heading.*

Introduction

The major loci affecting the photoperiod response (Ppd genes) are in a collinear position on the short arm of the group 2 chromosomes in wheat and barley. In barley, dominant Ppd-H1 alleles confer early flowering under long days but have no effect under short days. Plants carrying the mutated, recessive ppd-H1 allele are late flowering. In other words, long days promote early flowering where as short days make flowering delayed. The Ppd-H1 is a pseudresponse regulator (PRR) which is most similar to PRR7 in Arabidopsis and was positionally cloned in barley by Turner *et al.,* (2005). Furthermore, Turner *et al.,* (2005) suggested that there is a single nucleotide polymorphism (SNP 22) situated in coding region of Ppd-H1 gene to be responsible for the allelic differences, resulting in recessive mutant form. However on the other hand Jones *et al.,* (2008), carried out an association study by using wider survey of landraces barley from different geographical areas of Europe and with much statistical support and on the basis of strong evidence found that SNP 48 which is extended from 2437 to 2449 base pair (Gen Bank: AY970701.1), situated in exon 6 of coding region better explains the causual basis of the Ppd-H1 mutation. They also found that barley varieties present in the north of Europe, where growing season is long because of the much rain are harboring a "T" (associated with late flowering at long days conditions) instead of a "C" (ancestral, associated with early flowering in long day condition), while SNP 48 harboring a "C" predominated in the south where the growing season is short because of drought. Moreover the study of Jones *et al.,* (2008) suggested that Ppd-H1 is the only locus conferring natural genetic variation in photoperiod response in barley. Recently, Lister *et al.,* (2009) have also reported similar results about latitudinal variation by studying photoperiod response gene in old European barley.

The heading time data of the Swedish landraces and modern varieties of barley showed that SNP 48 of Ppd-H1 gene has significant effect on heading date. Barley varieties carrying the "T" allele at SNP 48 flowered later than the one variety with the "C" allele which were sown together (Knoppel, 2009). So this study was carried out in order to know the genetic effect of a unique SNP 48 marker of Ppd-H1 gene on heading date in different wild barley populations from different region of Israel and Jordan which have not been reported yet and also to be familiar whether the latitudinal cline variation evolved from its ancestor's provenance or have evolved after introduction of barley into Europe.

Materials and Methods

Corresponding Author: Abdul Wahid Baloch, College of Agronomy, Northwest A & F University, Yangling, Shaanxi, PR, China

Plant materials:

An experiment consisting of 14 wild barley natural populations was conducted in the experimental field of Northwest A and F University, Yangling, Shaanxi, P.R. China during the growing season of 2010-2011. Each wild barley population consisted 3 rows of 2 meter long and 50 cm apart and was grown in rainfed conditions. At the time of flowering, the heading date was observed visually of each population under the filed condition. All wild barley populations selected from different geographical sites of Israel and Jordan i.e. north, centre and south of both countries (Table. 1).

Table 1: Country, region, genetic makeup of SNP 48 marker and days to heading of different wild barley populations.

population	Country	Region	Base at SNP 48	Days to heading
Mt. Hermon -222	Israel	North	“C”	190
TBBS-54	Israel	North	“C”	195
MT. Gilboa -3-3	Israel	Centre	“C”	180
Mehola-2-9	Israel	Centre	“C”	194
Sedge boker-4	Israel	South	“C”	185
Irbid Sal-46	Jordan	North	“C”	180
Jarash Om-4	Jordan	North	“C”	181
Amman Sahab-1	Jordan	Centre	“C”	195
Madaba-29	Jordan	Centre	“C”	188
Karak Mutah-5	Jordan	Centre	“C”	188
Maan Basta-9	Jordan	South	“C”	180
Showbak Gair-6	Jordan	South	“C”	180

DNA extraction:

Genomic DNA was extracted from a single green leaf of all wild barley populations according to Weining & Langridge (1991) protocol modified by Weining & Henry (1995) The young leaf was harvested from seedlings which were grown one and half months before in the field. The concentration of DNA was determined on a 0.1% agarose gel.

Primer, PCR and sequencing:

A pair of primer, Ppd-H1F (5’CCAACAGGCAGCAGCTAGAG 3’) and Ppd-H1R (5’ TTATTTCCACAGGGCTTGGTCAC 3’) were designed by using Primer premier 5 software to recognize a sequence in exon 6 of Ppd-H1 gene contains polymorphic site SNP 48. When used this primer pair in Polymerase Chain Reaction (PCR), these primers amplified a 460 bp fragment. PCRs were run on all DNAs samples for the region containing SNP 48 in duplicate. The PCRs were performed in 20 µl reactions containing 2µl Mg-free PCR buffer, 1.6 µl $Mgcl_2$, 1.6 µl dNTPs, 1 µl Of each primer (forward and reverse), 0.2 µl units of *Taq* polymerase and 1 µl BSA. The cycling parameters were: pre-denaturation for 3 min at 94 C^o, followed by: 40 cycles of denaturation for 1 min at 94 C^o, 30 sec at 60 C^o for annealing, 1 min for extension at 72 C^o and final extension of 10 min at 72 C^o. However, some PCRs sample showed faint band and for these samples the second round of PCR was carried out with almost similar reaction mixture except using 1 µl of first round reaction mix instead of DNA extract.

PCR products were run on 0.1% agarose gel; at 120 V for about 45 min which were subsequently stained with ethidium bromide and visualized under UV-light imagine system. Successfully amplified fragment resulted in band of approximately 460 base pair (Fig 2). All PCR products were sent to Sangon Company, Shanghai, China for direct sequencing in forward and reverse reaction using relevant PCR primers.

Data analysis:

Each sequence of barley genotypes was verified and checked for their homology using BLASTn (Altschul *et al*., 1990) through blast search engines (http://blast.ncbi.nlm.nih.gov/). The sequences of all barley populations were aligned using CLC Free Workbench 4 software (CLC Bio, Denmark).

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Results and Discussions

High variations observed for heading dates among different wild barley populations, with a break of 15 days. According to heading dates data, all wild barley populations can be divided into three different categories i.e. early, intermediate, and late. Out of 12 wild barley populations 5 populations namely Mt. Gilboa-3, Iribid Sal-46, Jarash Om-4, Ma'an Basta-9 and Showbak Gair-6 flowered earlier whereas only 4 wild barley populations such as Madaba-29, Karak Mutah-5, Sedge boker-4 and Mt. Hermon 222 bloomed comparatively later than earlier and can be considered as intermediate's one. However, some wild barley populations took more days for heading than early and intermediate's one, such as, TBBS-54, Mehola-2-9 and Amman Sahab-1 which can be grouped as late for days to heading (Table 1).

As far as SNP 48 is concerned, the region surrounding the site of SNP 48 was successfully sequenced in total 12 different wild barley populations which belonged to different geographical regions (north, center and south) from Israel and Jordan in order to know the genetic make up of SNP 48 marker of photoperiod response gene (Ppd-H1). Based on results obtained (Fig. 1), the SNP 48 contains only "C" for all wild barley populations and did not reveal any polymorphism. The results are beyond the way, neither supporting the latitudinal variations regarding geographical origin of genotypes and nor in agreement with days to heading data where early and late genotypes should have different bases at SNP 48 of Ppd-H1 gene despite same. In this regard, Jones *et al.,* (2008) and Lister *et al.,* (2009) reported that European landraces and old barley show clear latitudinal variations, the southern landraces contain cytosine where as northern ones possess thiamine at SNP 48 of Ppd-H1 gene.

The difference in heading dates clearly indicated that the marker SNP 48 of Ppd-H1 gene did not affect significantly on heading dates in wild barley populations, it probably makes differences in heading dates in barley cultivars as it was reported by Knoppel (2009) where responsive (Ppd-H1) cultivars are quite earlier and non-responsive (ppd-H1) cultivars delayed in flowering. According to Karsai *et al.,* (2008) that there is a number of other cues that trigger flowering in barley such as changes in light intensity and the vernilization requirement. For vernilization plants require an extended period of low temperatures before flowering can occur and is controlled by Vrn locus. Winter varieties of cultivated barley require vernilization, whereas spring growth habit plants lack this requirement (Laurie *et al.,* 2005). Almost all of the wild barleys have winter growth habit (Takahashi *et al.,* 1968). So from our results, it can be concluded that there may be other factors which strongly effects on heading dates in wild barley populations rather than SNP 48 only.

In this study, the analysis of Ppd-H1 gene among wild barley populations from different geographically areas did not show any latitudinal partitioning of alleles as this has been seen in European landraces. It clearly indicates that the ancestral provenance had not any partitioning of alleles at Ppd-H1 gene and it might be originated after the introduction of barley into Europe. The previous study of Jones *et al.,* (2008) reported that the non-responsive (ppd-H1) populations of wild barley are present in Fertile Crescent, it further suggest that mutation was happened in certain population of a particular site and consequently the non-responsive (ppd-H1) had arisen. Later on, these genotypes were spread to different regions of the world by different sources.

In addition, this study also sheds light on the spread of non-responsive genotypes from Fertile Crescent to Europe. Earlier study by Jones *et al.,* (2008) reported that European landraces barley originated from East of Fertile Crescent (Iran) rather than West Fertile Crescent (Israel-Jordan region) though their study has already proved by detailed analysis of recombinant break points. In this regard, Jones *et al.,* (2008) used 21 and the present study included more 12 populations and in total there were 33 different populations from Israel-Jordan region but there were only 3 mutant types which reported by Jones *et al.,* (2008); however, from Iran Jones *et al.,* (2008) used only 20 different populations and found 4 mutant types. It indicates more variations at SNP 48 occurs in Iranian wild barely and our study ultimately supports to earlier findings (Jones *et al.,* 2008) that European landraces barley originated from East Fertile Crescent because most of the European Landraces contain mutant types. Furthermore, many populations from Israel have already been sequenced for SNP 48 marker and found some mutations but populations from Jordan have not been studied yet. In current study, different populations were taken from Jordan but we did not found variations in these populations at SNP 48. The likely explanation is that, perhaps SNP 48 varies in Israeli region rather than Jordan region.

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