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CROP RESEARCH

Diversity of Dagestan Barleys for the Duration of the Period between Shooting and Earing Stages and Alleles in the *Ppd-H1* and *Ppd-H2* Loci

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Abstract—The duration of the shooting–earing period of 265 barley samples from Dagestan was studied. During the 3 years of study at the Dagestan Experimental Station of VIR (Derbent), fast-ripening samples k-15008 and k-15013 were identified. Evaluation of spring forms in the northwestern region of the country made it possible to identify sample k-15027, which had a high rate of development over 2 years. It was found that Dagestan barleys are strongly influenced by growing conditions; that is, they have a high rate of response. Vernalization temperatures, short photoperiod, and high temperatures during the growing season contribute to fast barley ripening. Using molecular markers, the allelic diversity of genes *Ppd-H1* and *Ppd-H2*, which are involved in the control of the duration of the shooting–earing period, was investigated. Most samples of local forms of barley carry dominant allele *Ppd-H2*, which causes early earing under short photoperiod. Translocation of the studied barley group to unusual conditions of northwest Russia leads to significant delay in plant development.

Keywords: barley, early ripeness, photoperiodic sensitivity, molecular markers

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INTRODUCTION

Dagestan has attracted the attention of many prominent researchers due to its distinctiveness, originality, and diversity of wild and cultivated plants. It was in Dagestan during expeditions of the 1930s that N.I. Vavilov was looking for beardless forms of barley, which were needed to confirm his law of homologous series in hereditary variation. One of the important features that determine the adaptive potential of the culture and the width of its ecological and geographical distribution is the rate of development. In mountainous areas of the republic, the preferred cultivars are those with a short growing season; in other regions, breeding was carried out for the combination of productivity and a fairly long growing season.

The collection of the Vavilov All-Russia Institute of Plant Genetic Resources (VIR) contains 282 barley samples from Dagestan; the information about the adaptive value of them is fragmentary, and polymorphism for genes controlling the time of earing in this group has not been investigated. The study of a fragment of the collection for 2 years showed that local Dagestan barleys are mainly midripening [1]. Based on the DNA polymorphism data, we structured the intraspecific diversity of 253 samples of this culture. Molecular markers made it possible to reveal significant polymorphism of Dagestan barleys and heterogeneity of most forms [2]. Phenotypic screening identified four sources of weak photoperiodic sensitivity: k-14891, k-18178, k-21812, and k-23785, which are supposed carriers of gene *eat8*. Molecular analysis in plants of sample k-14891 revealed a new, previously undescribed mutation in the sense *eat8* gene sequence caused by deletion of a single nucleotide [3].

The earing time in barley is determined by genes that control the type of development, weak sensitivity to photoperiod, and proper ripening. The development type is determined by three pairs of genes: *sh, Sh2*, and *Sh3* (subsequently referred to as *Vrn-H1, Vrn-H2,* and *Vrn-H3*). Genes *Sh2* and *Sh3* are epistatic with respect to the dominant allele *Sh*, and allele *sh* has a similar effect on recessive alleles *sh2* and *sh3* controlling the winter type of development. Genes *Sh*, *Sh2*, and *Sh3* are localized in chromosomes 4 (4H), 7 (5H), and 5 (1H), respectively [4, 5]. Genes *Vrn* con-

trol the need of plants for vernalization to initiate heading and, consequently, are involved in the regulation of the rate of barley development.

D.A. Laurie et al. [6, 7] identified five major genes and nine quantitative trait loci (QTL) that control the heading time in barley. Among them are genes *Ppd-H1* and *Ppd-H2* (*photoperiod response*), which are localized in chromosomes 2H and 1H, respectively, and control the genes controlling the response to vernalization, *Vrn-H1* and *Vrn-H2*, whose localization coincides with the position of genes *Sh* and *Sh2* identified previously. *Vrn-H1* and *Vm-H2* have been identified in cultivated varieties, while allele *Vrn-H3* is rarely observed. Dominant allele *Ppd-H1* controls rapid response to an increase in the duration of the photoperiod and early earing under long photoperiod. Heading delay under long photoperiod is caused by recessive allele *ppd-H1.* Dominant allele *Ppd-H2* under short photoperiod accelerates the start of heading, while the recessive allele delays it. On the background of expression of the genes that control the type of development and photoperiodic response of plants, the development rate is significantly affected by genes *eps*, which actually control maturity, or earliness *per se*.

Earliness and weak sensitivity to photoperiod is also controlled by genes *Eat5*, *Eat6*, *eat7*, *eat8, eat9*, and *eam1O* (*early maturity*), which are localized in chromosomes 5H, 2H, 6H, 1H, 4H, and 3H, respectively [8]. Dominant gene *Eat8* is the ortholog of the gene regulating the sensitivity to photoperiod in *Arabidopsis thaliana* [9]. Mutation in *Eat8* probably leads to the formation of a defective protein and, as a consequence, photoperiod insensitivity in the plant and early maturation.

H. Jones et al. [10] examined the nucleotide polymorphism of dominant and recessive alleles of locus *Ppd-H1* in 87 local varieties of barley and identified SNP significantly associated with the phenotype—earing periods under long and short photoperiod. Subsequently, M.M. Zlotina et al. [11] developed a CAPS-marker to identify dominant and recessive alleles. R. Kikuehi et al. [12] found that dominant allele *Ppd-H2* encodes a functional phosphatidylethanolamine-binding protein HvFT3, whereas a significant part of the coding sequence was lost in carriers of recessive allele *ppd-H2*.

Molecular markers have a limited use in identification of the allelic composition of loci *Ppd-H1* and *Ppd-H2* in Russian barley varieties. Thus, in 91 cultivars of spring barley approved for use in Russia and Belarus, the allelic status of genes *Vrn-H1*, *Vrn-H2*, *Vrn-H3*, *Ppd-H1*, and *Ppd-H2* was analyzed. Cultivars with dominant allele *Ppd-H1* were significantly ahead of other genotypes in terms of the rate of development during cultivation under long daylight hours, whereas carriers of this allele in the studied sample amounted to only 9%. *Vrn* gene alleles also significantly influenced the duration of the shooting–earing periods in the studied cultivars. Among the genotypes carrying identical alleles of genes *Ppd-H1* and *Ppd-H2*, carriers of the allelic combination *Vrn-H1vrn-H2Vrn-H3* started heading earlier than genotypes with a different combination of *Vrn* gene alleles [11].

The purpose of this work is to study the phenotypic diversity of Dagestan barleys for the duration of the shooting–earing period and evaluate the allelic status of genes *Ppd-H1* and *Ppd-H2.*

METHODS

Field experiments were carried out in 2012–2014 at the Dagestan Experimental Station of VIR (DOS VIR, Derbent district) and Pushkin laboratories of VIR (Saint-Petersburg). DOS VIR is located in the south-planar zone of Dagestan with a dry subtropical climate. Weather conditions, which differed in moisture and temperature conditions in the years of studies, generally favored the cultivation of barley. The conditions of the northwestern region (VIR) are characterized by a transition of the maritime climate into moderately continental. Winter is moderately cold and summer is moderately warm and humid.

In the collection nurseries of DOS VIR, we studied 265 samples of barley; in VIR fields, we estimated spring forms. We were using agricultural methods conventional for the area of research. Samples were seeded by hand in the third week of October (DOS WRI) and in the second half of May (VIR). Each sample was sowed in a plot area of 1 m^2, row spacing of 15 cm, and length of the row of 1 m. In the study of the collection, we used Methodical Guidelines for the Study of the World Barley and Oats Collection [13]. The appearance of full shoots was marked by the date when leaves that unfolded in the upper part were appearing on the soil surface in more than 75% of the plants in the plot. Earing was considered complete when approximately 75% of the plants were heading. We compared the length of the shooting–earing period in 70 spring forms studied within 3 years at the two points (six samples). For a correct comparison of earliness of the samples of the winter (DOS VIR) and spring (VIR) sowing times, we calculated the indicator called "excess of the shooting–earing period of the sample over its minimum value in the sample" (PPVK).

Using the molecular markers, we evaluated the allelic status of genes *Ppd-H1* and *Ppd-H2* in 193 local barley samples, as well as 27 cultivars and breeding lines. Total DNA was isolated from 7-day-old seedlings (2–10 plants of each sample) by the method D.B. Dorokhov and E. Kloke [14] with some modifications [15]. Alleles of genes *Ppd-H1* and *Ppd-H2* were identified using the molecular markers based on polymerase chain reaction (PCR). Amplification was carried out in a reaction mixture of 15–25 μL, which contained genomic DNA (50–100 ng), 1x reaction buffer

Table 1. Sequence of primers

Primer	Nucleotide sequences (5'–3')	Annealing t°	Reference
Jones5-F	GATGGATTCAAAGGCAAGGA	60°C	[10]
Jones5-R	CGTTAGAGCCCTGCTTCATC		
HvFT3-F	GTCCTCCTCCAGTATATGTC	60°C	[12]
HvFT3-R	CTACTCCCCTTGAGAACTTTC		
HvFT3-F4	GGATGGATCGGATTATTATTGTATG		
HvFT3-R1	CTGCACATTATTTGTGAT		

without $MgCl_2$, 1.5–3 mM $MgCl_2$, 0.2 mM of each nucleotide, 250 nM of each primer, and 1 unit Tag-polymerase (Dialat). PCR was carried out in an amplifier MyCycler (BioRad). The list of primers used in the work is shown in Table 1. Amplified fragments were separated by electrophoresis in 2% agarose gel in 1xTBE buffer. Gels were stained with ethidium bromide and photographed under ultraviolet light. Sizes of the fragments were estimated using the DNA marker FastRuler™ SM1113 (Fermentas).

Alleles of gene *Ppd-H1* [11] were identified by restriction analysis of the amplified fragments in a total volume of 22 μL: 2.2 μL 10X Buffer Tango, 0.8 μL restriction enzyme MspI (10 units/μL) (Feimentas), and 4 μL distilled water free of nucleases were added to 15 μL PCR product. The reaction mixture was incubated at 37°C for 16 h. The products were separated by electrophoresis in 3% agarose gel.

RESULTS AND DISCUSSION

Over the 3 years of study at the DOS VIR, we identified fast-ripening (the shooting–earing period at the underwinter sowing is from 124 to 162 days) samples k-15008 and k-15013 with a low rate of response; the sample k-18186 showed earliness in 2012 and 2013, while k-11439, k-15252, and k-23831 showed earliness in 2013 and 2014. In the conditions of VIR, we can note only sample k-15027, which proved to be fast ripening (the shooting–earing period at the spring sowing is 30–32 days) in 2013 and 2014.

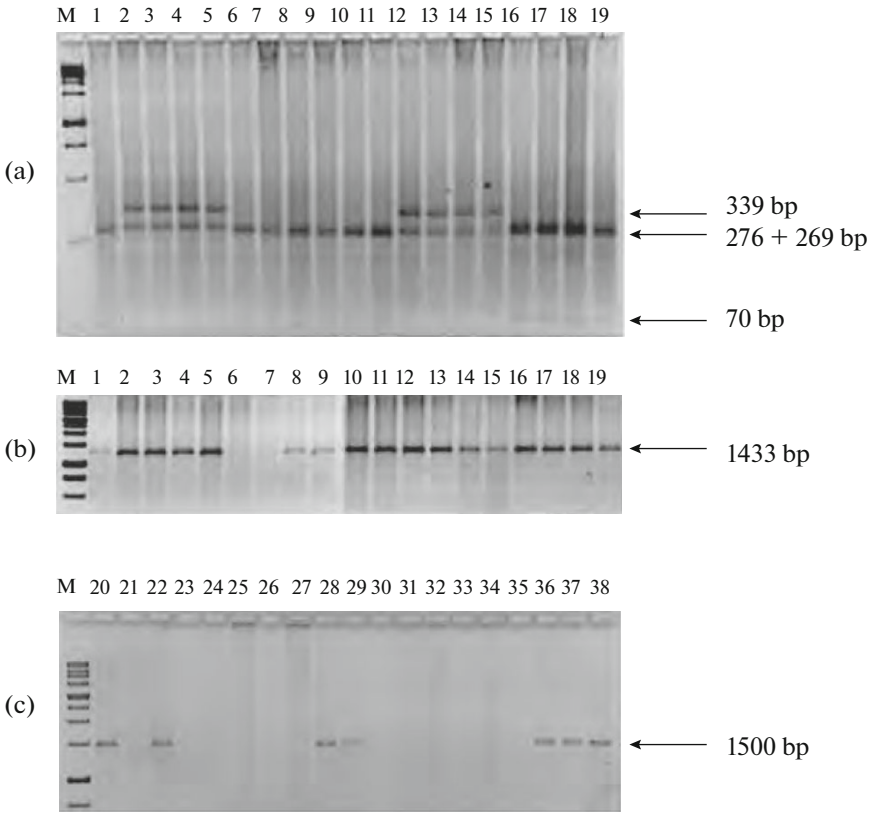
Dagestan barleys at the DOS VIR were ripening faster than those at the VIR: average PPVK over the 3 years in the studied samples of spring samples varied from 7.1 ± 0.4 to 24.2 ± 0.9 (VIR) and from 5.0 ± 0.4 to 6.1 ± 0.3 (DOS VIR). The significance of the differences was confirmed by Student's *t* test.

Environmental conditions affected the rate of development of samples at the VIR: PPVK significantly (*t* test ranged from 4.6 to 17.3) differed by the research years. The impact of the environment on the duration of the shooting–earing period at the DOS VIR was much less; only the samples of 2012 and 2013 ($t = 2.3$) were significantly different.

The molecular markers were used to identify alleles of genes *Ppd-H1* and *Ppd-H2* in local samples, as well as cultivars and lines from Dagestan (Fig. 1). Among the local samples, the most common combination was *ppd-H1/Ppd-H2*, which provides earliness under short photoperiod (122 samples). A combination of the two dominant alleles *Ppd-H1/Ppd-H2* characterized 55 samples; 16 forms have alleles *Ppd-H1/ppd-H2* and can be a source of earliness under a long photoperiod. Carriers of recessive alleles *ppd-H1/ppd-H2* were not found. The most common combination of dominant alleles in the cultivars and lines is *Ppd-H1/Ppd-H2* (ten samples), seven samples were characterized by combination *Ppd-H1/ppd-H2*, and another six samples by *Ppd-H1/Ppd-H2*. The combination of recessive alleles *Ppd-H1/ppd-H2* was detected in four samples. The morphologically uniform sample k-15008, identified over the 3 years for earliness in Dagestan, have a combination of alleles *ppd-H1/Ppd-H2*, that is, it is adapted to the conditions of short photoperiod.

Plants of another fast-ripening sample in Dagestan, k-15013, were carriers of dominant allele *Ppd-H1* and recessive allele *ppd-H2*, which may be due to its heterogeneity: k-15013 is represented by four species. Another polymorphic sample, k-15027, identified for earliness at the VIR under long photoperiod, has a combination of alleles *Ppd-H1/Ppd-H2*, which is typical of the samples adapted to the conditions of short photoperiod. In addition to heterogeneity of these forms, a possible explanation for the identified contradiction is the impact of genes *Vrn-H*, the research of allelic polymorphism of which we have not carried out. Local Dagestan barleys are dominated (90%) by carriers of dominant allele *Ppd-H2*; dominant allele *Ppd-H1* was observed in 29% of samples. During breeding, genotypes with dominant allele *Ppd-H2* are replaced by genotypes not adapted to local conditions that carry dominant allele *Ppd-H1*: among the cultivars and breeding lines, the carriers of dominant alleles of these genes account for 61 and 51%, respectively (Tables 2, 3).

Thus, we estimated the range of variability of Dagestan barleys for earliness and selected the material with a high rate of development. Under the conditions of the southern plane area of Dagestan, we identified fast-maturing samples k-15008 and k-15013; in



PCR analysis of genomic DNA of samples of local barley from Dagestan using markers developed for identification of: (a) dominant (276 + 269 + 70 bp) and recessive (276 + 339 bp) alleles of gene *Ppd-H1* [10, 11], (b) dominant allele (1433 bp) of gene *Ppd-H2* [12], (c) recessive allele (1500 bp) of gene *Ppd-H2* [12]. 1—k-23819; 2, 3—k-23820; 4, 5—k-23821; 6, 7—k-23822; 8, 9—k-23823; 10, 11—k-23825; 12, 13—k-23827; 14, 15—23830; 16, 17, k-25615; 18, 19—k-25616; 20, 21—k-17438; 22, 23—21772; 24, 25—k-21808; 26, 27—k-21817; 28, 29—k-23822; 30, 31—k-26290; 32, 33—k-26295; 34, 35—k-26297; 36, 37—k-28209; 38—k-28211.

the northwestern region of Russia, a high rate of development was characteristic of the sample k-15027. Dagestan barleys are strongly influenced by growing conditions, that is, have a high rate of response. Vernalization temperatures, short photoperiod, and high temperatures in the growing season contribute to its earliness. Using these identified forms in breeding will enhance the adaptive potential of modern varieties of barley in Dagestan and other regions of the Russian Federation.

Research of earliness and photoperiodic sensitivity of Dagestan barleys showed their affinity to growing in specific ecological and geographical conditions. Translocation of this group of barleys to unusual conditions of the northwest Russia leads to a significant delay in plant development. This is due to the allelic diversity of genes *Ppd-H1* and *Ppd-H2*, which are involved in the control of the duration of the shooting–earing period, typical of the local barley forms from Dagestan. Most of the local barley samples carry

Table 2. Distribution of alleles of gene *Ppd-H1* among Dagestan barleys

Group of samples	Lifestyle	Studied samples	Frequency of samples with allele *Ppd-H1*		Frequency heterogeneous forms
			Ppd-HI	*ppd-HI*	
Local forms	Winter	51	0.59	0.33	0.08
	Spring	190	0.22	0.74	0.04
Cultivars and lines	Winter	25	0.64	0.24	0.12
	Spring	8	0.12	0.88	0

Table 3. Distribution of alleles of gene *Ppd-H2* among Dagestan barleys

Group of samples	Lifestyle	Studied samples	Frequency of samples with allele *Ppd-H2*		Frequency heterogeneous forms
			Ppd-H2	*ppd-H2*	
Local forms	Winter	34	0.94	0.06	0
	Spring	169	0.89	0.09	0.02
Cultivars and lines	Winter	23	0.61	0.39	0
	Spring	5	0.60	0.40	0

dominant allele *Ppd-H2*, which leads to earlier earing under a short photoperiod.

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