Turkish Journal of Agricultural and Natural Sciences
Special Issue: 2, 2014

TÜRK
TARIM ve DOĞA BİLİMLERİ
DERGİSİ

TURKISH
JOURNAL of AGRICULTURAL
and NATURAL SCIENCES

www.turkjans.com

Investigation of the Cold Resistance of Two-row Winter Barley Cultivars (*Hordeum vulgare ssp. distichum* L.)

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Abstract

The cold resistance of eleven Bulgarian and European varieties of two-row winter barley (*Hordeum vulgare,ssp. distihum* L.) has been investigated in laboratory and natural field conditions. The laboratory trial has been conducted in Agricultural University - Plovdiv by express methods of Bojhanova and Petrova (2000) for determination of cold resistance based on the comparison of depression of root and shoot development in low temperatures. The accurate field trial from 2011 to 2013 was carried out on the experimental field of the Institute of agriculture – Karnobat. The differences in percentage of survived plants and the grain yield in natural climate conditions have been assumed. All investigated cultivars has been clustered in two major groups- "tolerant" and "susceptible", based on the analyses of the results. The correlation in cultivar`s behavior and their arrangement in identical groups in both methods prove the reliability of this express method and his possible use in the early stage of plant breeding process for barley tolerance to low temperatures. The best yielding cultivars with higher cold tolerance has been used in new breeding programs of winter barley.

Keywords: malting barley, cold resistance, plant breeding, express laboratory methods

Introduction

Major part of cultivated barley cultivars in Bulgaria for a long time were local selection. The last decades the seed market was opened and this created possibility for positioning of new European varieties. High yielding French, Belgian, Australian and other barleys now exceeds 30 % of our famers land. The full expression of their genetic potential is hard to achieve in such various and often risky soil and climate conditions as Bulgarians. Meantime some valuable biological and malting characteristics can be used in our breeding programs for selection of productive and qualitative malting barley with good seed characteristics and higher cold resistance.

Some of the primary risks in the meteorological conditions in our country are sporadic appearances of very low minimal winter air temperatures (often below -16 – 18^0 C) and reciprocal raising back with amplitude more then 20^0C. Such meteorological changes can be very risky for the crop. Draganov et al. (1986) and Lazarov (1986) claims that Bulgarian barleys has moderate requirements to the temperature regime in seeding and first stages of phenology development. During the winter after normal hardening they can tolerate air temperature from -10 to -12^0C, and up to -14^0C at the tillering node, for 24 hours without snow cover. Contemporary breeding achievements point as critical for the new breeding lines and cultivars -13.8^0 C (Vulchev et al, 2007), some has relatively good adaptive ability for quick regeneration after the winter period (Mihova et a., 2007) and such combining good cold resistance (up to -10 – 12^0 C) with high yield and good grain qualities (Dimova et al., 2005; Vulchev and Vulcheva, 2005). Despite this almost each year in different regions of the country we calculate degree of frost injury in barley crop. Increasing of barley cold resistance remains one of the major, still unsolved breeding problems.

Assessment of barley cold resistance and winter hardiness during the plant breeding process is in laboratory assays and field trials. Some laboratory methods includes cultivation in vegetation chambers with control of photoperiod, temperature and moisture and are quite expensive, till the field testing takes times and labor. Investigation of large number of breeding materials requires express, inexpensive but reliable methods well suited for screening of large number of samples. One of the most popular nowadays is the express method of Bozhanova and Petrova (2000), based on depression level of seedling and root development under stress conditions. It has been developed primary for hard wheat and indicated as suitable for grain crops (Ur et al., 2007). Researches with it has been made for common and hard wheat and other *Aegilops* crops. In previous research we established the possible use in 4-rows *(Hordeum vulgare ssp. tetrastichum L.)* and 6-rows *(Hordeum vulgare ssp. hexastichum L.)* (Marcheva and Koteva, 2013).

The present study aims at screening of cold resistance of Bulgarian and European 2-row winter barley *(Hordeum vulgare ssp. distichum L.)* cultivars implemented in practice and establishment of reliability of the results from the express laboratory method compared to the field investigated winter hardiness of plants.

Materials and Methods

The investigation has been conducted during the period 2011 – 2013 by laboratory and field trial. Object of this study are 9 Bulgarian and 3 European 2-row winter barley *(Hordeum vulgare ssp. distichum L.)* cultivars (Table 1). All of them are registered in National or European Variety List and are used by farmers. They differs in reaction to low temperatures. Results are compared to Obzor variety (one of the official standards for 2-row winter barley in Bulgaria) with cold resistance and winter hardiness group 3 by International scale (http://iz-karnobat.org and http://dai-gt.org).

In the experimental field of the Institute of agriculture – Karnobat from 2011 to 2013 has been determined winter hardiness of barley cultivars. Block method has been used – each variant has 4 replica in plots of 10 m^2. Optimal cultivation procedures as sawing in appropriate terms (5- 20 October) with 450 seeds/ m^2 and fertilizing with 10 kg/dka a.w. N are implemented. Winter hardiness is measured as percent of survived (in the end of March) from the total number of seedlings before the winter (end of November). The grain yield is presented as percent from the average standard Obzor, Emon, and Kaskadior 3 in kg per ha.

The natural fertility of the *Pellic Vertisol* in Karnobat experimental field combines medium content of total humus (2, 40 %), from weak acid to neutral reaction (pH in water 6. 5 – 6. 8), good reserve of available K_2O (34 - 40 mg/100 g), weak to medium reserve with mobile P_2O_5 (3. 5 – 7. 9 mg/100 g) and week reserve of mineral N (below 40 mg/1000 g).

Meteorological conditions during the vegetation period of winter barley are described by rainfall during the autumn (October and November), winter (December to February) and spring (March to June) period; average monthly air temperature during the critical for the crop winter months – December, January and February. Data were collected from MO – Karnobat dept of IMH – Sofiq.

Table 1. Evaluated 2-row winter barley varieties.

Variety	*Origin*	*Breeding institution*	**Winter hardiness**
Obzor	Bulgaria	Institute of Agriculture, Karnobat	** III group
Emon	Bulgaria	Institute of Agriculture, Karnobat	** III group
Kaskadior 3	Bulgaria	Dobrudzha Agricultural Institute, Gen. Tochevo	** IV group
Imeon	Bulgaria	Institute of Agriculture, Karnobat	** III group
Kuber	Bulgaria	Institute of Agriculture, Karnobat	** III group
Asparuh	Bulgaria	Institute of Agriculture, Karnobat	** III group
Orfey	Bulgaria	Institute of Agriculture, Karnobat	** III group
Lardeia	Bulgaria	Institute of Agriculture, Karnobat	** III group
Vanessa	Germany	Saatzucht Josef Breun, GDBR-Hersogenaurach	8 of 9 scale
Violetta	UK	LimaGrain	5 of 9 scale
Vicky	Sweden	Swedish Seed Association, Svalov	5 of 9 scale

Note: *9 scale where: 1 – strong winter hardiness, 9 – very low winter hardiness; ** method of Koh (1975) and international scale.

Laboratory testing with stress of low positive temperatures - 4 ^{0} C in four replica of developing seedlings and depression of their shoots and roots, relative to the development of control plants at 26 ^{0} C, has been proceeded (Bozhanova and Petrova, 2000). Coefficient of depression of shoot and root development has been calculated by the formula of Blum et al (1980) as $D_{\%}$ = [(A-B/A] x 100, where A is average shoot/root length in control at 26 ^{0} C, - B is average shoot/root length in stress at 4 ^{0} C. Seeds were Certified C 1 class 1 (collected under sieve 1.8 + 2.0 mm).

Results are analyzed by dispersion analyses (Dimova and Marinkov, 1999)

Results

Field winter hardiness

Meteorological conditions variations during the successive years of this trial are presented on Table 2. Vegetation rainfalls in 2012 and 2013 are relatively favorable for the growth and development of the crop.

They are close or slightly exceeding the average long term values for the same periods. The first year 2011 has some water deficiency, especially in the spring and early summer in heading and ripening of barley but it has no significant negative impact on the plants.

Table 2. Raifall and air temperatures during barley vegetation period in 2011 to 2013.

Period	Rainfall, mm			*Mean
	2011	2012	2013	
Autumn (October-November)	86	150	105	101
Winter (December – February)	118	182	197	128
Spring (March - June)	143	193	165	209
Vegetation (October - June)	347	525	467	438
Average monthly air temperature, °C				
December	3.2	3.9	1.7	3.2
January	1.5	-0.3	2.0	1.5
February	1.3	1.0	4.4	1.3

Note: * *monthly rainfall average for 1901-2010*; *Average air temperature for 1931-2010.*

The average air temperature during the winter vary (Table 3). In 2011 absolute minimal air temperature falls from -7.5^0 C to -10.0^0 C. Just once in the middle of December the temperature is crucial for barley – 15.2^0 C but combined with snow cover of 5.5 mm. The cold months January and February did not harm the crop as it already has hardened and did not froze. Damages were insignificant. The snow cover and crop condition protected the total expression and provided normal grain yield.

The air temperature in December 2013 is above zero and lower than the long term determined as this of January and February 2013 e higher and positive at all. Thus temperatures in 2013 has been warmer and safer for barley cultivation.

Table 3. Average air temperature in winter season of 2011, 2012 and 2013.

Month	Decade	Temperature, ^{0} C		
		2011	2012	2013
December	I	8.1	4.5	4.9
	II	-2.0	7.0	-0.7
	III	3.5	0.2	0.8
January	I	0.4	2.0	0.0
	II	4.3	0.2	3.9
	III	-0.3	-2.7	2.8
February	I	2.9	-4.6	5.7
	II	2.8	-1.3	2.9
	III	-1.9	2.8	4.7

The winter of 2012 was colder and risky for the crop and led the experiment to good evaluation of field winter hardiness. December was still warm, with average air temperature 3. 9 ^{o}C, and almost permanent vegetation which did now allowed normal hardiness of the plants. The next two months are colder than normal with minimal – 12^0 C in January and -15.2^0 C in February. The riskiest period (because of the duration and terms) is from 26th of January to 11th of February when the average air temperature was constantly low –up to – 13.6^0 C with very small snow cover of average 2.3 cm (0,5 to 3.0 cm).

Percent of winter killed plants varied in Bulgarian and European cultivars (Table 4). In relatively favorable winter temperatures in 2011 and 2013 all tested varieties shows good winter hardiness – 94 – 98% survived plants. In extreme conditions of 2012 variety Violetta and Vicky suffered severe with 24 and 29 % frozen seedlings.

Best winter hardiness is found for Asparuh and Kuber with not more than 5 % and 8 % lost. Varieties Obzor, Emon, Kasкadior 3, Imeon, Orfey and Lardeya are equivalent with 11 % to 16 % winter losses.

Analogical were the results for grain yield (Table 4). Varieties Violetta and Vicky had 14.2 % and 20.9 % lower grain yield than Obzor. Similar frost injuries – 19% were observed in Vanessa variety but the good spring crop recovery contributed for better yielding – 9.1 % lower grain yield then the standard Obzor. Combination of good winter hardiness and spring recovery of Asparuh and Kuber with higher genetic yielding potential contribute to the increase of obtained grains quantities – 10.6 % and 18.8 % above Obzor. All others are established as medium for the observed traits.

Table 4. Winter hardiness of barley in field conditions, expressed by survived plants (%) and grain yield (kg/ha) in 2012.

Variety	Survived plants %	Grain yield kg/ha	Relative to the standart yield, %
Obzor	89	7170	100
Emon	89	7080	98.7
Kaskadior 3	84	6870	95.8
Imeon	87	7040	98.2
Kuber	**95**	8500^{++}	**118.5**
Asparuh	**92**	7930^{+}	**110.6**
Orfey	87	7240	101.0
Lardeia	86	6780	94.6
Vanessa	81	6520^{-}	90.9
Violetta	76	6150^{--}	85.8
Vicky	71	5670^{---}	79.1

Significance at GD 5.0 +/-; 1.0 ++/--; 0.1 +++/---

Laboratory testing

Investigation of the barley genotypes reaction of cold stress shows higher depression of shoots than in roots for all tested varieties (Table 5).

Table 5. Laboratory assessment of barley cold resistance, expressed by depression of root and shoot growth.

Variety	Root length, mm Control at 26 °C	4 °C	Depression,%	Shoot length, mm Control at 26 °C	4 °C	Depression,%
Obzor	11.0	7.9	28.2	7.6	4.5	40.8
Emon	12.6	9.0	28.6	7.9	4.8	39.2
Kaskadyor 3	14.0	9.7	30.2	9.7	5.9	39.2
Imeon	11.7	8.7	25.7	12.4	7.0	43.5
Kuber	16.2	8.2	**49.4**	11.9	4.6	**61.3**
Asparuh	15.4	7.1	**53.9**	12.4	5.0	**64.9**
Orfey	10.2	8.4	17.7	8.4	5.1	39.3
Lardeia	14.7	9.0	38.8	9.5	5.0	47.4
Vanessa	12.6	9.4	25.4	10.9	6.5	40.4
Violetta	14.7	12.9	12.3	8.4	6.1	27.4
Vicky	18.2	15.7	13.7	7.9	5.9	25.3

According depression of shoot development varieties can be clustered in tree groups – Group 1 - Kuber and Asparuh (61.9 % and 64.9 %); second Obzor, Emon, Kaskadior, Imeon, Orfey, Lardeia and Vanessa (from 39.2 % to 47.4 %); the last one Violetta and Vicky (27.4 % and 25.3 %). The depression of root development reveals similar arrangement in clusters – tolerant genotypes - Kuber and Asparuh (49.4 % and 53.9 %); moderate reaction - Obzor, Emon, Lardeia, Kaskadior 3, Imeon and Vanessa (from 23.7 % to 38.8 %); sensitive are Violetta, Vicky and Orfey (12.3 % to 17.7 %).

Discussion

The analyses of field testing results of percent injured and survived plants related to number of autumn seedlings and their effect on the productivity determined Bulgarian varieties Kuber and Asparuh, selected in the Institute of Agriculture – Karnobat, as relatively “tolerant”, European varieties Vicky and Violetta as relatively “succeptible”. Kaskadior 3 (selection of Dobrudzha Agricultural Institute, Gen. Tochevo) and Vanessa (registered by Saatzucht Josef Breun, GDBR-Hersogenaurach) has lower winter hardiness but good spring recovery potential.

In laboratory conditions the depression of root and shoot development at low positive temperature show similar clustering. It has been established that lower percent of depression is related to weaker cold resistance and vice versa (Bozhanova and Petrova, 2000). Genotypes that cease intensive vegetation in autumn decrease of temperatures have better and quicker hardening and express better cold resistance in winter period.

Conclusion

Analyses of summarized data of field and laboratory testing reveal lower cold resistance of European varieties Violetta, Vicky and Bulgarian Orfey. Better results in these conditions were achieved in Obzor, Emon, Lardeia, Kaskadior 3, Imeon and Vanessa. Relatively high cold resistance has been found for Kuber and Asparuh.

Analogical results of field trial and laboratory test and genotypes clustering in similar groups, prove the practical application of this method for screening of low temperatures tolerance of large number of barley breeding lines in early stage of breeding procedures.

Results from this and previous study (Marcheva and Koteva, 2013) on cold resistance of 2 row and 6 - row winter barley varieties allows us to recommend the express method of Bozhanova and Petrova for wide use in barley breeding programs for assessment of cold and winter hardiness.

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