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Evaluation of some barley varieties concerning seed germination under different drought stress

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Abstract: Given that drought is the main environmental factor, which limits the crop productivity, the improvement of drought tolerance is considered as an important goal in barley breeding programs. The objective of this study was to assess the influence of different level of osmotic stress on seeds germination of 23 winter barley cultivars, in order to select the best suitable parents to be used in different breeding programs, and also to identify varieties which can be used efficiently for the establishment of this crop under water deficit in soil. Dana, Adi and Esterel varieties showed a good tolerance to osmotic stress, considering that these three varieties proved to be the most tolerant at stress levels of -0.5 and -1.0 MPa. The good stress tolerance at 1.5 MPa of 'Salemer' and 'Plaisant' varieties was associated with below-average germination for the other two stress levels. 'Ulla' and 'Hauter' varieties recorded high values of germination under stress-free conditions or at a low stress level but their germination was strongly affected by the increase of stress intensity. Considering that varieties with different origins showed a high similarity in terms of their reaction to different levels of osmotic stress, it turns out that these varieties possess different combinations of genes involved in tolerance to this stress, thus highlighting their potential use in barley breeding programs.

Key words

barley, drought, germination, osmotic stress.

Under the climate changes from last decades drought stress is the main factor limiting the yield and productivity of different crops, including barley. Given that drought stress can occur in any growth stage of the plants, it is important to test the tolerance from early stages starting with seed germination.

Drought is quantified as a reduction in water potential and considered as an osmotic threat [13]. Evaluation of different barley genotype for yield stability pointed out a high genetic plasticity under drought stress conditions [8; 9].

The process of dehydration of the cell, which is manifested by the exit of water from the cells, also called plasmolysis state, occurs when the external solution is more concentrated than the internal solution. Plasmolysis occurs in plants under drought conditions or when the soil solution becomes hypertonic. The analysis of variability and genetic base of germination under drought stress can improve barley growth and yield. An effective method to evaluate the drought stress at germination is to simulate high osmotic stress [10]. In the lab, by using a hypertonic solution that induces water stress with polyethylene glycol (PEG), sucrose, mannitol, it is possible to test the drought tolerance of plants at the stage of seed germination [16]. Drought simulated by different graduations of the osmotic value affects the germination ability of the seeds, so the higher the osmotic value of the solution used as the germination medium, the more the seeds germination ability is reduced. It is advisable to test the germination under normal and stress conditions in order to estimate the reduction due to drought stress [22].

Drought tolerance is a very complex trait which is usually accompanied by heat or other abiotic stresses that which causes different morphological and physiological changes [7; 18]. Drought tolerance-associated traits have quantitative inheritance, so that many genes with minor effect are involved [14]. Through the exposure of barley seedlings to osmotic stress only a minor QTL was detected [1; 19], while Xue et al (2019) found that a QTL controlling barley seedling growth under one abiotic stress can also respond to another stress.

Germination supports later the seedling growth, thus having an important role in plant development and finally on yield achieving [5]. Drought simulated by different graduations of the osmotic value affects the germination ability of the seeds, so the higher the osmotic value of the solution used as the germination medium, the more the seeds germination ability is reduced. Germination speed is more affected by drought stress than germination percentage [6]. Seeds priming had a significant positive effect on barley seeds germination under drought stress [11]. The uptake of sodium in barley seeds, allow them to germinate more rapidly under conditions of lower water potential [23].

Given the autumn rainfall deficit from last decade, after sowing barley encounters with drought

stress during seed germination and early growth stages. The clarification of barley drought tolerance during seed germination could be useful for improving the performance of other cereals under these stress conditions [17]. Rapid germination is an important trait enhancing crop establishment of barley under variable environmental conditions [15].

The objective of this study was to assess the influence of different level of osmotic stress on seeds germination of 23 winter barley cultivars, in order to select the best suitable parents to be used in different breeding programs, and also to identify varieties which can be used efficiently for the establishment of this crop under water deficit in soil.

Material and Method

The research was performed in Department of Genetic Engineering, Faculty of Horticulture and Forestry, Banat's University of Agricultural Sciences and Veterinary Medicine Timisoara. The biological material was composed of 23 winter barley varieties with ecologically and genetically differentiated.

The experiment was organized in a completely randomized block design with 5 replications and 100 seeds per replicate. The seeds of each cultivar were placed in sterile Petri dishes, on two layers of absorbent paper moistened with each PEG solution. The PEG solutions of different osmotic potential (-0.5 MPa; -1.0 MPa; -1.5 MPa) have been prepared by mixing different quantities (calculated according to Michel and Kaufman, 1973) of PEG 6000 (Riedel de Haen, AG, Germany) in deionized water used as a control (0 MPa). Germination was conducted in a seed germinator Wise Cube WGC-450 Incubator (Witeg Labortechnik GmbH, Germany), under a constant 20 ^{0}C in the dark. The germination percentage was recorded after seven days from setting up the experiment.

The comparison of varieties under different treatments was performed using analysis of variance and Multiple Range Test according to Ciulca (2006). The significances of differences were expressed using symbols and letters, considering as significantly different the varieties without common letters. To make possible the display in a single graph of the germination of each cultivar under each osmotic potential, were used the basic principle of the biplot technique [4] and GGE biplot method [21]. The clustering of cultivars was carried out using the unweighted pair group method with arithmetic mean (UPGMA), with NEIGHBOR program of PHYLIP package, version 3.5c.

Results and Discussions

The analysis of variance (Table 1) indicates the existence of significant differences between the barley varieties in terms of germination ability in different PEG solutions, amid a small and insignificant influence of the environmental conditions at the level of experimental design. The increase of the osmotic stress level had a considerable influence on the variability between barley varieties, so they showed differentiated reactions proportional to the change of the osmotic pressure.

Table 1

Analysis of variance for the germination percentage of barley seeds in different PEG 6000 solution

Source of variation	DF	PEG 6000 (0 MPa)			PEG 6000 (-0.5 MPa)		
		SS	MS	F	SS	MS	F
Totală	114	2371.81			3407.07		
Replications	4	27.77	6.94	0.59	84.58	21.15	1.49
Varieties	22	889.40	40.43	3.41**	1366.28	62.10	4.38**
Erorr	88	332.11	11.86		396.91	14.18	
Source of variation	DF	PEG 6000 (-1.0 MPa)			PEG 6000 (-1.5 MPa)		
		SS	MS	F	SS	MS	F
Totală	114	14344.38			9975.96		
Replications	4	408.42	102.11	1.64	210.07	52.52	1.53
Varieties	22	8245.12	374.78	6.01**	6541.10	297.32	8.69**
Erorr	88	5490.83	62.40		3010.79	34.21	

In terms of seeds germination under stress-free conditions (0 MPa), the studied varieties registered average values between 85.49% for 'Plaisant' and 96.03% for 'Ulla' variety, against the background of a low variability both between and within varieties. Most varieties (52%) recorded germination with values between 90 and 95%, followed by those (26%) with values below 90%. The varieties: 'Ulla', 'Hauter', 'Gerbe', 'Madalin' and 'Regal' showed a high germination ability of over 95% associated with a uniformity of seeds. Compared to the experience mean, the studied varieties did not achieve significant increases of germination. In contrast, for 'Salemer', 'Andrei' and 'Plaisant' varieties the values were significantly lower than the average (Table 2).

Table 2

Germination percentage of barley varieties in different PEG 6000 solutions (0 MPa; -0.5MPa)

Variety	Germination percentage (0 MPa)			Germination percentage (-0.5 MPa)		
	$\bar{x} \pm s_{\bar{x}}$	s %	Rank	$\bar{x} \pm s_{\bar{x}}$	s %	Rank
Dana	91.94±4.00 abcde	7.54	12	82.05±3.37 ab	7.12	2
Precoce	93.50±2.60 abcd	4.81	11	73.33±5.89 efgh	13.92	15
Adi	94.67±2.88 abcd	5.28	7	80.73±2.72 abcd	5.84	4
Mădălin	95.50±1.45 ab	2.62	4	76.20±3.82 bcdefg	8.67	9
Orizont	89.56±1.46 cdef	3.06	18	78.57±3.20 bcde	7.05	5
Andrei	87.10±5.72 ef	11.37	22	72.93±5.09 efgh	12.08	17
Regal	90.67±5.00abcdef	9.56	15	70.52±5.01 gh	12.31	22
Compact	91.17±3.92 abcde	7.44	14	75.85±4.21 cdefg	9.63	10
Gerbel	95.67±1.93 ab	3.50	3	72.27±3.34 fgh	8.01	18
Manitou	94.50±4.91 abcd	9.00	8	74.45±4.53 efg	10.55	13
Esterel	93.83±2.89 abcd	5.33	10	85.08±4.22 a	8.58	1
Majestik	94.83±3.46 abc	6.33	6	81.27±2.58 abc	5.50	3
Lyric	89.13±3.88 def	7.55	19	70.53±3.00 gh	7.37	21
Plaisant	85.49±3.38 f	6.98	23	74.35±3.67 efg	8.54	14
Hauter	95.83±4.23 a	5.73	2	76.67±3.99 bcdefg	9.02	8
Dina	91.50±4.24 abcde	6.02	13	72.16±4.11 fgh	9.86	19
Ogra	95.16±4.76 abc	8.67	5	75.08±4.71 defg	10.85	11
Salemer	87.66±5.20 ef	10.27	21	71.32±4.30 gh	10.45	20
Landi	89.06±4.82 def	9.37	20	77.44±2.83 bcdefg	6.34	7
Riniker	90.50±4.24abcdef	6.08	16	73.02±5.80 efgh	13.77	16
Pfyner	90.17±4.77 bcdef	9.16	17	67.43±3.28 h	8.42	23
Secura	94.16±3.35 abcd	6.17	9	77.59±4.02 bcdef	8.97	6
Ulla	96.03±3.76 a	6.78	1	74.96±3.82 defg	8.82	12
Exp. mean	92.07±0.62	3.39		75.38±0.84	5.58	
LSD	5%=5.66; 1%=7.57; 0.1%=9.92			5%=6.19; 1%=8.28; 0.1%=10.84		

Regarding the germination in PEG 6000 (-0.5 MPa) solutions, the studied varieties achieved values with an amplitude of 17.65% having the limits between 67.43% for 'Pfyner' and 85.08% for 'Esterel' variety, under a low inter-population and a medium intra-population variability. The highest share (48%) was represented by the varieties that have achieved germination between 70 and 75%, while 30% of the varieties achieved values of this trait between 75 and 80%, followed by the varieties (17%) with values over 80%. The highest values of germination under an osmotic stress of -0.5 MPa associated with a high drought tolerance were observed at the varieties: 'Esterel', 'Dana', 'Majestik' and 'Adi'. In the case of 'Pfyner', 'Regal' and 'Lyric' varieties, the low values of this trait indicate that the germination of their seeds can be affected by low levels of water stress in the soil. Compared to the experience mean, a significantly higher tolerance to the osmotic stress of -0.5 MPa was found only in the case of 'Esterel', 'Dana' and 'Majestik' varieties, while the germination of 'Pfyner' was significantly lower.

In case of testing the seeds germination under an osmotic stress of -1.0 MPa, the studied varieties showed values between 23.87% in 'Precoce' and 45.94% in 'Esterel', with an amplitude of 22.07%, under a middle variability both between and within varieties (Table 3). The highest frequency (66%) was registered by the varieties with germination ability between 30 and 40%, the rest of the varieties being distributed in two symmetrical classes. Under these conditions, the highest values of germination ability correlated with a high drought tolerance were achieved by the varieties: 'Esterel', 'Dana', 'Adi', 'Majestik', 'Ulla'. A high sensitivity to water stress associated with a low germination ability in PEG (14.3%) solution was observed for the varieties: 'Precoce', 'Lyric', 'Pfyner', 'Mantou', 'Gerbe'l. Compared to the experience mean, about one third of the varieties showed higher values of germination ability under an osmotic stress of -1.0 MPa, but a significant increase was recorded only by 'Esterel' variety. Also, from this point of view, no varieties with a drought tolerance significantly lower than the average of the experiment were observed. Also, from this point of view, no variety showed a drought tolerance significantly lower than the experience mean.

Table 3

Germination percentage of barley varieties in different PEG 6000 solutions (-1.0 MPa; -1.5MPa)

Variety	Germination percentage (-1.0 MPa)			Germination percentage (-1.5 MPa)		
	$\bar{x} \pm s_{\bar{x}}$	s %	Rank	$\bar{x} \pm s_{\bar{x}}$	s %	Rank
Dana	43.22±3.11 ab	16.09	2	28.46±1.44 abcde	11.31	5
Precoce	23.87±1.16 f	15.65	23	18.36±1.05 hi	12.79	21
Adi	41.08±2.57 abc	13.99	3	29.31±2.12 abc	16.17	3
Mădălin	31.29±1.27 cdef	9.08	14	22.99±1.17 cdefgh	11.38	11
Orizont	30.63±1.87 cdef	13.65	17	15.63±0.56 i	8.01	23
Andrei	30.25±1.42 def	10.50	18	20.87±0.83 fghi	8.89	18
Regal	31.08±1.69 cdef	12.16	15	22.40±1.62cdefghi	16.17	13
Compact	33.17±1.94 bcdef	13.08	11	27.03±1.74 abcdef	14.39	6
Gerbel	29.68±0.87 def	6.55	19	25.87±2.51abcdefg	21.70	8
Manitou	29.54±1.37 def	10.37	20	21.25±1.95 efghi	20.52	16
Esterel	45.94±2.75 a	13.39	1	21.10±1.47 fghi	15.58	17
Majestik	38.06±2.81 abcd	16.51	4	22.56±2.04cdefghi	20.22	12
Lyric	24.94±0.94 f	13.32	22	23.49±1.55 cdefgh	14.75	10
Plaisant	35.51±1.62 abcde	10.20	8	28.53±2.31 abcd	18.10	4
Hauter	33.19±1.41 bcdef	9.50	10	20.01±0.91 fghi	10.17	19
Dina	32.52±2.37 bcdef	16.30	12	26.53±2.14 abcdef	18.04	7
Ogra	31.89±2.55 cdef	17.88	13	24.71±1.26bcdefgh	11.40	9
Salemer	36.15±2.53 abcde	10.87	7	32.48±2.58 a	17.76	1
Landi	34.16±3.04 bcdef	19.90	9	17.68±0.78 hi	9.87	22
Riniker	30.88±2.34 cdef	16.94	16	21.81±0.88 defghi	9.02	14
Pfyner	27.30±1.82 ef	14.91	21	18.91±1.34 ghi	15.85	20
Secura	36.42±2.17 abcde	8.43	6	31.23±2.74 ab	19.62	2
Ulla	37.20±3.26 abcde	19.60	5	21.39±1.43 defghi	14.95	15
Exp. mean	33.39±1.12	16.10		23.59±0.93	18.86	
LSD	5%=10.76; 1%=14.23; 0.1%=18.38			5%=7.24; 1%=9.57; 0.1%=12.37		

Regarding the seed germination ability in solution of potential -1.5 MPa, the varieties achieved amplitude of 16.85%, with the limits between 15.63% at 'Orizont' and 32.48% at 'Salemer', amid a middle variability between and within varieties. Three quarters of the varieties achieved values between 20 and 30%, while only the 'Salemer' and 'Secura' varieties exceeded the limit of 30% for germination ability. The lowest values of seed germination under an osmotic stress of -1.5 MPa correlated with a high drought tolerance were observed at the varieties: 'Salemer', 'Secura', 'Adi', 'Plaisant', 'Dana'. An increased sensitivity to water stress assessed by germination ability was recorded by the varieties: 'Landi', 'Precoce', 'Pfyner', 'Hauter', 'Orizont'. Approximately 40% of the varieties achieved germination ability higher than experience mean, but only 'Salemer' and 'Secura' varieties registered a significantly higher drought tolerance. Also, only 'Orizont' variety showed a significantly lower sensitivity to water stress than the mean.

Given the reaction of barley varieties to different levels of osmotic stress (Table 4) it was observed that for Plaisant variety on the background of a lower value at the control, the seed germination was least influenced by this stress, recording a decrease from 11.14 to 56.96%. The seed germination of Pfyner, Gerbel and Ulla varieties was strongly affected by the low level of osmotic stress (-0.5 MPa). Under an osmotic stress of -1.0 MPa, it was found that Esterel, Dana and Plaisant varieties showed a good tolerance associated with a germination decrease of 47.89-49.98%.compared to control. Gerbel and Precoce were the most sensitive varieties to this level of stress, at which the germination decreased with 65.99-69.63%. Against the background of the osmotic stress intensification up to -1.5 MPa, it was observed that the germination of Salemer variety was the least affected, being followed by Plaisant variety. In the case of Hauter, Precoce and Ulla varieties, the germination of seeds under these conditions was reduced by approximately 75%.

Table 4

The effect of osmotic stress on germination of barley varieties

No	Variety	Germination (%)	Differences to control			Rank
		Control (0 MPa)	-0.5 MPa	-1.0 MPa	-1.5 MPa	
1	Dana	91.94	9.89	48.72	63.48	2
2	Precoce	93.50	20.17	69.63	75.14	23
3	Adi	94.67	13.94	53.59	65.36	5
4	Mădălin	95.50	19.30	64.21	72.51	18
5	Orizont	89.56	10.99	58.93	73.93	12
6	Andrei	87.10	14.17	56.85	66.23	7
7	Regal	90.67	20.15	59.59	68.27	14
8	Compact	91.17	15.32	58.00	64.14	8
9	Gerbel	95.67	23.40	65.99	69.80	22
10	Manitou	94.50	20.05	64.96	73.25	21
11	Esterel	93.83	8.75	47.89	72.73	4
12	Majestik	94.83	13.56	56.77	72.27	10
13	Lyric	89.13	18.60	64.19	65.64	15
14	Plaisant	85.49	11.14	49.98	56.96	1
15	Hauter	95.83	19.16	62.64	75.82	20
16	Dina	91.50	19.34	58.98	64.97	11
17	Ogra	95.16	20.08	63.27	70.45	16
18	Salemer	87.66	16.34	51.51	55.18	3
19	Landi	89.06	11.62	54.90	71.38	9
20	Riniker	90.50	17.48	59.62	68.69	13
21	Pfyner	90.17	22.74	62.87	71.26	19
22	Secura	94.16	16.57	57.74	62.93	6
23	Ulla	96.03	21.07	58.83	74.64	17

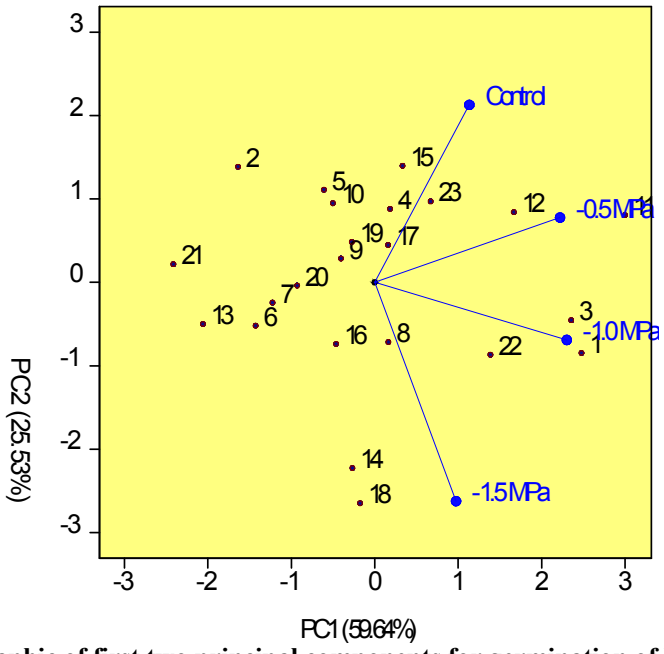

Figure 1. **Biplot graphic of first two principal components for germination of barley varieties under different osmotic stress**

The multivariate analysis (Figure 1) based on the first two principal components expressed 89.4% from the variability of the 23 varieties for for seed germination under different stress conditions. Depending on the projection of each genotype on the vectors of different stress levels, it was found that 'Dana', 'Adi' and 'Esterel' varieties showed a good tolerance to osmotic stress, considering that these three varieties proved to be the most tolerant at stress levels of -0.5 and -1.0 MPa. The good stress tolerance at 1.5 MPa of 'Salemer' and 'Plaisant' varieties was associated with below-average germination for the

other two stress levels. ‘Ulla’ and ‘Hauter’ varieties recorded high values of germination under stress-free conditions or at a low stress level but their germination was strongly affected by the increase of stress intensity.

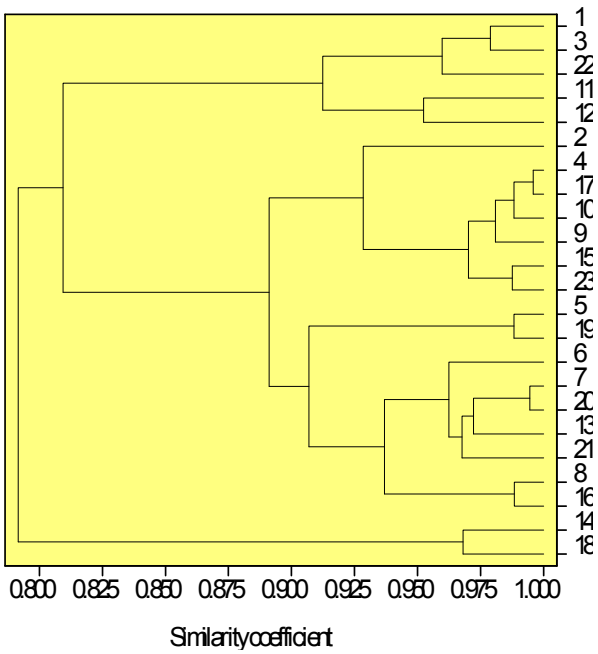

Figure 2. **UPGMA clustering of barley varieties based on their germination under osmotic stress**

Based on the dendrogram from Figure 2, it was observed that the varieties were grouped in four main clusters, with different structures. The first cluster includes ‘Dana’ and ‘Adi’ varieties, which exhibit approximately 98% similarity in terms of their reaction to the studied osmotic stress. Also, this cluster includes the ‘Esterel’ and ‘Majestik’ varieties, between which there is a similarity of approximately 95%, together with ‘Secura’ variety. The varieties of this first cluster recorded a good tolerance at different levels of osmotic stress. The second cluster consists of seven varieties that showed an average germination compared to the levels of -0.5 and -1.0 MPa associated with reduced germination under conditions of stress of -1.5 MPa. Between the varieties of this cluster (‘Precoce’, ‘Madalin’, ‘Ogra’, ‘Lyric’, ‘Gerbel’, ‘Hauter’ and ‘Ulla’) there is a similarity of 93-99%. The third cluster consists of 9 varieties characterized by a lower germination in conditions of osmotic stress, on the background of an intergenotypic similarity of approximately 91%. The varieties of the last cluster Plaisant and Salemer exhibit a similarity of 96%, showing the highest germination under conditions of a stress of -1.5 MPa.

Conclusions

‘Dana’, ‘Adi’ and ‘Esterel’ varieties showed a good tolerance to osmotic stress, considering that these three varieties proved to be the most tolerant at stress levels of -0.5 and -1.0 MPa. The good stress tolerance at 1.5 MPa of ‘Salemer’ and ‘Plaisant’ varieties was associated with below-average germination for the other two stress levels. As such, these varieties can be used efficiently for establishment of barley crop under conditions with soil water deficit. ‘Ulla’ and ‘Hauter’ varieties recorded high values of germination under stress-free conditions or at a low stress level but their germination was strongly affected by the increase of stress intensity.

Considering that varieties with different origins showed a high similarity in terms of their reaction to different levels of osmotic stress, it turns out that these varieties possess different combinations of genes involved in tolerance to this stress, thus highlighting their potential use in barley breeding programs.

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