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The effects of osmotic stress on seedlings growth of barley (*Hordeum vulgare*)

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Abstract Drought (water stress) and salinity are major problems facing agriculture all over the world. Plants are exposed to many types of abiotic stresses during their life cycle. The objective of this study was to determine if there were differential responses to osmotic stress on seedlings' growth dynamics in some barley cultivars. Fluid deficit was induced by polyethylene-glycol (PEG6000) solution, using control and 3 variants with different osmotic pressure (-2.72 Bars, -4.48 Bars, -7.35 Bars.). Determinations of seedlings growth were effectuate after a periods of 5/10/15/20 and 25 days from the induction of osmotic stress .Seedling growth was significantly reduced by , -7.35 Bars polyethylene glycol-induced drought stress.

Key words

barley, shoots groowth, polyethylene glychol

Barley is one of the most important cereal crops grown in many developing countries, where it is often subject to extreme drought stress that significantly affects production [1].

Among the different abiotic stresses, drought is most complex and devastating on a global scale [15].Water deficit (drought) at different stages of barley's life cycle, (from seed germination to maturity) may adversely affect the final yield of the crop. The effect of drought on the yield of cereals depends on the duration and the severity of the stress [16].

An appreciable genotypic diversity for shoot and root growth at early stages was reported in barley under favorable conditions or under other abiotic stress [12]. There is limited insight into morphological traits of shoot and seminal roots in wild barley at early plant growth stage under drought in a large population, although this species is mainly cultivated without irrigation in arid and semi-arid regions. Screening techniques based on physiological criteria should be rapid, simple and inexpensive, especially for the evaluation of large population [6]. One of the screening techniques based on physiological traits is the use of various osmotic to induce stress in plant tissues. The experiment for the present investigation was conducted in the laboratories of the Faculty of Horticulture and Forestry Timisoara, Department of the Genetic Engineering in agriculture. The 19 genotypes of Romanian and foreign barley were screened for tolerance to drought stress at the early seedling stage. The seeds of uniform size were weighted and surface-sterilized with 3 % (w/v) Sodium hypochlorite for 10 min and then washed thoroughly with deionized water. Seeds were transferred to sterile Petri dishes (100 mm in diameter) containing two layers of Whatman filter paper and were moistened with 10 mL distilled water Germination in mannitol and polyethylene glycol (PEG), measurements of root length or rooting depth, and the survival or growth of seedlings subjected to osmotic have been suggested for drought screening [5, 10]. Selection of tolerant cultivars has been considered as an economic and efficient means to improve drought tolerance [3,17]. A better understanding of mechanisms of adaptation to water deficit and maintenance of growth, development and productivity during stress periods would help the drought-tolerance breeding [3]. Nevertheless, drought tolerance is a complex trait resulting from the contribution of numerous factors. Among the several putative characters, water status parameters [13, 20] carbon isotope discrimination [13], roots and shoot characters [4, 8, 20], root-shoot partitioning [4, 18], early growth vigour [4, 8] are interesting traits for drought-tolerance evaluation.

The objective of this study was to determine if there were differential responses to osmotic stress on seedling s growth dynamics in some barley cultivars.

Material and Methods

(control) or (w/v) PEG 6000 solution. Petri dishes were placed in a growth chamber with a temperature of 20 ±2°C and a 12 h light/12 h dark photoperiod at 60% relative humidity. The experiment was conducted under normal (0 Bars) and drought stress (-2.72 Bars,-4.48 Bars, and – 7.35Bars) conditions created with the help of Polyethylene- glycol (PEG6000) by the method suggested by Michael and Kaufman (1973). After germination the plantules were grown on perlit cultures in same sizes of seed floats. Plants were kept in a greenhouse in a 14/10 hour day/ night at 20/22^{o}C night/ day temperature. The temperature was controlled by a

programmable air conditioner. Ten ml of distilled water or PEG solution was added to each culture pots under normal and drought stress conditions, respectively, after every 2 days to compensate the losses due to evaporation.
The data for seedlings' length (cm) determination was recorded on, 5, 10, 15, 20,and 25 days after sowing, for each variant of culture medium. Experimental data had been processed by statistical methods: the variance analysis and test t [2].

Results And Discussions

According to the F test, presented in table 1, studied varieties, the seedlings' age, the treatment with PEG as well as their interactions, had significant influence on the seedlings growth in barley crops.

Table 1

Analysis of variance on the effect of varieties, seedlings age and PEG's on seedlings' growt in barley

Source of variation	SP	GL	S^2	F Test
Source of variation	104085,47	3799		
Total	64,82	9	7,20	F=2,03*
Repetitions	1568,68	18	87,15	F=24,56**
Varieties	574,82	162	3,55	
Varieties error	60600,44	4	15150,11	F=6234,09**
Seedlings age	3880,32	72	53,89	F=22,18**
Varietie x seedlings age	1662,26	684	2,43	
Error for seedlings` age	20135,49	3	6711,83	F=2795,48**
PEG	1384,31	54	25,63	F=10,68**
Varietie x PEG	3637,80	12	303,15	F=126,26**
Seedlings age x PEG	4418,07	216	20,45	F=8,52**
Varietie x seedlings age x PEG	6158,46	2565	2,40	

Regarding the effect of varieties on the growth of seedlings have been obtained values between 8,02cm. at DH260/12 and 10,34cm. at Lyric genotype.

Comparing material with the Dana genotypes we can be observed that: genotypes Andrei, Regal, Compact, Lyric, recorded higher growth values, the results are statistically asured.

Table 2

The effect of barley varieties on shoots lenght

Cultivars	Average (cm)		Relative value(%)	Diference/significance
Orizont –Dana	8.67	8.65	100.23	0.02
Precoce - Dana	8,77	8,65	101,39	0,12
Adi - Dana	8,16	8,65	94,34	$-0,49^{00}$
Mădălin - Dana	8,40	8,65	97,11	-0,25
Andrei – Dana	9,16	8,65	105,90	0,51**
Regal – Dana	9,31	8,65	107,63	0,66***
Compact – Dana	9,83	8,65	113,64	1,18***
Djerbel – Dana	8,41	8,65	97,23	-0,24
Lyric – Dana	10,34	8,65	119,54	1,69***
Plaisant – Dana	8,91	8,65	103,01	0,26
Tas – Dana	8,08	8,65	93,41	$-0,57^{00}$
Secura – Dana	8,89	8,65	102,77	0,24
Dina – Dana	8,39	8,65	96,99	-0,26
DH19/1 – Dana	8,99	8,65	103,93	0,34
DH 254/10 – Dana	7,49	8,65	86,59	$-1,16^{000}$
DH 260/18 - Dana	8,51	8,65	98,38	-0,14
DH 260/12 – Dana	8,02	8,65	92,72	$-0,63^{000}$
DH 261/22 - Dana	8,25	8,65	95,38	$-0,40^{0}$

$LSD_{5\%}$=0,37 cm $LSD_{1\%}$=0,49cm $LSD_{0,1\%}$=0,63 cm

The seedlings length of the barley cultivars differed under the different osmotic potentials of PEG.

Regarding unilateral effect of PEG concentration on growth of barley seedlings (table 3), which showed values between 5.14 cm V3 version (-7.35 Bars PEG6000) and 11.50 cm Vo version (0Bars H2O).

The treatments applying through the hydric deficit induced had determined a significant decrease of seedlings' growth proportional with the differences level among these treatments. Our results are in accordance with results reported by [**19**], they showed that drought stress cause reduction of seedlings growth in beet too.

Table 3

The effect of PEG concentration on the seedlings' growth of barley

PEG solutions	Average (cm)		Relative values (%)	Difference / Significance	PEG solutions	Average (cm.)		Relative values (%)	Difference / Significance
V1 – V0	9,55	11,50	83,04	-1,95^{000}	**V2 – V1**	8,60	9,55	90,05	-0,95^{000}
V2 – V0	8,60	11,50	74,78	-2,90^{000}	**V3 – V1**	5,14	9,55	53,82	-4,41^{000}
V3 – V0	5,14	11,50	44,70	-6.36^{000}	**V3 – V2**	5,14	8,60	59,77	-3,46^{000}

$LSD_{5\%}$=0,14cm $LSD_{1\%}$=0,181cm $LSD_{0,1\%}$=0,23cm

According to presented data in table 4 it could observe as seedlings' age had a very significant influence on seedlings' growth to genotypes studied.

Table 4

The effect of barley seedlings` age on seedlings' growth

Seedlings` age	Average (cm)		Relative values (%)	Difference/Significance	**Seedlings` age**	Average (cm)		Relative values (%)	Difference/Significance
10 days – 5 days	6,33	2,24	282,59	4,09***	**20 days – 10 days**	11,64	6,33	183,89	5,31***
15 days – 5 days	9,84	2,24	439,29	7,60***	**25 days – 10 days**	13,44	6,33	212,32	7,11***
20 days – 5 days	11,64	2,24	519,64	9,40***	**20 days – 15 days**	11,64	9,84	118,29	1,80***
25 days- 5 days	13,44	2,24	600,00	11,20***	**25 days – 15 days**	13,44	9,84	136,59	3,60***
15 days – 10 days	9,84	6,33	155,45	3,51***	**25 days – 20 days**	13,44	11,64	115,46	1,80***

$LSD_{5\%}$=0,16 cm $LSD_{1\%}$=0,21 cm $LSD_{0,1\%}$=0,26 cm

From the point of view of seedlings' age influence on seedlings' growth (table 5) it observed that genotypes taken in study had different behavior during experimental period.

Thus 5 days from stress induction to DH 261/22 double haploid line registered the biggest growth (4.51 cm.), being followed by genotypes Tas, Secura, DH, DH 260/18, Lyric, and the lowest growth registered to Orizont , Precoce genotypes.

After 10 days from stress induction the highest growth registered to genotypes Compact, Djerbel, Secura, those being in the first places in top. In situation in which the hydric stress had activated a long period of time, 25 days, genotypes which had a higher growth were Plaisant, Djerbel, Tas, Secura, and the lowest growth registered to Orizont.

Table 5

Influence of seedlings` age on seedlings' growth in different genotypes of barley

No.	Genotypes	**5days**		**10 days**		**15days**		**20days**		**25 days**		Sum rank
		average	Rank	average	Rank	average	Rank	average	Rank	average	Rank	
0	1	2	3	4	5	6	7	8	9	10	11	12
1	**Orizont**	1,00	***19***	2,00	***19***	3,00	***19***	4,00	***19***	5,00	***19***	**95**
2	**Dana**	1,57	***16***	6,89	***5***	10,55	***7***	11,32	***9***	13,03	***14***	**51**
3	**Precoce**	1,11	***18***	6,24	***9.5***	10,79	***5***	11,25	***11***	13,85	***7***	**41**
4	**Adi**	1,73	***14***	6,02	***12***	10,59	***6***	11,30	***10***	14,21	***6***	**48**
5	**Mădălin**	2,30	***10***	5,35	***17***	9,71	***12***	10,72	***17***	12,72	***15***	**71**
6	**Andrei**	1,65	***15***	5,98	***13.5***	10,01	***10***	11,10	***14***	13,26	***11***	**50**
7	**Regal**	1,44	***17***	5,94	***15***	12,25	***1***	12,59	***5***	13,57	***8***	**46**
8	**Compact**	1,94	***12***	7,56	***1***	11,49	***3***	12,28	***6***	13,25	***12***	**34**
9	**Djerbel**	1,75	***13***	7,51	***2***	11,87	***2***	13,22	***2***	14,79	***2***	**21**

0	1	2	3	4	5	6	7	8	9	10	11	12
10	**Lyric**	2,46	***5***	5,98	***13.5***	10,54	***8***	12,72	***4***	10,35	***18***	**35**
11	**Plaisant**	2,45	***6***	7,19	***4***	11,06	***4***	16,19	***1***	14,83	***1***	**16**
12	**Tas**	3,07	***2***	6,67	***7***	9,01	***14***	11,56	***8***	14,27	***5***	**36**
13	**Secura**	2,49	***3***	7,45	***3***	9,10	***13***	6,98	***18***	14,40	***4***	**41**
14	**Dina**	2,24	***11***	6,81	***6***	10,08	***9***	12,02	***7***	13,31	***9***	**42**
15	**DH19/1**	2,32	***8***	6,24	***9.5***	8,91	***15***	11,19	***13***	13,30	***10***	**46**
16	**DH 254/10**	2,31	***9***	6,52	***8***	8,56	***16***	13,07	***3***	14,51	***3***	**39**
17	**DH 260/18**	2,48	***4***	4,66	***18***	6,71	***18***	11,02	***16***	12,59	***16***	**72**
18	**DH 260/12**	2,37	***7***	6,20	***11***	9,73	***11***	11,22	***12***	13,05	***13***	**54**
19	**DH 261/22**	4,51	***1***	5,73	***16***	7,30	***17***	11,04	***15***	11,51	***17***	**66**

$LSD_{5\%}$=0,71cm, $LSD_{1\%}$=0,94cm, $LSD_{0,1\%}$=1,20cm

Table 6

Influence of PEG concentration on seedlings' growth in different genotypes of barley

No.	Genotypes	**V0**		**V1**		**V2**		**V3**		Sum
		average	Rank	average	Rank	average	Rank	average	Rank	ranks
1	**Orizont**	12,20	***6***	9,15	***12***	8,76	***9***	4,57	***13,5***	**40.5**
2	**Dana**	11,55	***10***	9,95	***8***	8,61	***11***	4,48	***15***	**44**
3	**Precoce**	11,68	***9***	10,10	***5,5***	9,04	***5,5***	4,25	***16***	**36**
4	**Adi**	10,87	***14***	8,93	***16***	8,65	***10***	4,18	***17***	**57**
5	**Mădălin**	10,37	***16***	9,13	***14***	8,20	***14***	5,89	***3***	**47**
6	**Andrei**	12,55	***3***	9,34	***10***	9,04	***5,5***	5,71	***5***	**23.5**
7	**Regal**	12,08	***7***	10,49	***3***	9,38	***3***	5,27	***9***	**22**
8	**Compact**	12,60	***2***	10,21	***4***	8,84	***7***	7,67	***1***	**14**
9	**Djerbel**	9,83	***19***	9,96	***7***	9,18	***4***	4,66	***12***	**42**
10	**Lyric**	13,60	***1***	11,25	***1***	9,66	***1***	6,85	***2***	**5**
11	**Plaisant**	12,44	***4***	8,99	***15***	8,82	***8***	5,39	***8***	**35**
12	**Tas**	10,09	***18***	10,10	***5,5***	9,42	***2***	2,72	***19***	**44.5**
13	**Secura**	12,29	***5***	9,31	***11***	8,22	***13***	5,74	***4***	**33**
14	**Dina**	11,49	***11***	9,15	***13***	8,35	***12***	4,57	***13,5***	**49.5**
15	**DH19/1**	11,88	***8***	10,68	***2***	8,15	***15***	5,26	***10***	**35**
16	**DH 254/10**	10,94	***13***	8,03	***19***	6,94	***19***	4,06	***18***	**69**
17	**DH 260/18**	11,08	***12***	9,37	***9***	8,02	***17***	5,57	***7***	**45**
18	**DH 260/12**	10,34	***17***	8,55	***18***	7,99	***18***	5,18	***11***	**64**
19	**DH 261/22**	10,55	***15***	8,67	***17***	8,08	***16***	5,69	***6***	**54**

$LSD_{5\%}$=0,64cm $LSD_{1\%}$=0,85cm $LSD_{0,1\%}$=1,08cm

Concerning the concentration influence of PEG on seedlings' growth it could be observed from table 6 that: the biggest growth registered to species Lyric, Regal, Compact, Andrei and the lowest growth registered to double haploide lines.

Drought has a large influence on plant growth during germination, vegetative and the reproductive stages. At each stage, it acts as a constraint to crop productivity. However, drought occurring at the early developmental stages has been largely neglected in studies of drought tolerance. Significant differences were observed between water treatments studied. Similar differences were reported in several species on roots and early seedling traits (Dhanda *et al*., 2004; Szira *et al*., 2008).

Our results emphasized the existence of appreciable differences in seedlings traits in barley grown under drought stress conditions. We observed reduction in seedling traits in the drought stress condition .Several studies have reported the effect of water stress on shoot and root length in wheat (Hafid *et al*., 1998b; Guedira *et al*., 1997; Dhanda *et al*., 2004) in oat (Murphy *et al*., 1982), cultivated barley (Szira *et al.,* 2008) and wild barley (Lu *et al*., 1999).

Conclusions

The modifying of osmotic pressure determined significant differences concerning seedlings' growth; the growth decrease being proportional with osmotic pressure increase.

Foreign genotypes presented values of growth relative bigger in comparison with Romanian genotypes in the investigated periods. The double-haploide lines taken in the study were more affected by osmotic stress

Aknowlegement

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