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Tolerance of grass pea (*Lathyrus sativus* L.) genotypes to osmotic stress under in vitro conditions

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Original paper

Abstract: Osmotic stress resulting from cell dehydration is caused by water deficit, and leads to the disruption of many cellular functions. The effect of osmotic stress was assessed on seedling and callus parameters of six grass pea (*Lathyrus sativus*) genotypes under *in vitro* conditions. Osmotic stress was induced in the agar media by adding sucrose concentrations corresponding to –4.5 and –8 bar. The growth and morphological traits of *in vitro* seedlings and calli were evaluated 12 and 30 days after exposure to osmotic stress, respectively. In seedlings, genotypes differed significantly for most traits, while the osmotic potential significantly influenced on the dry weight of the rootlet, as well as the fresh weight of the plumule and seedling, and the dry weight of plumule. Rootlet, plumule, and seedling dry matter decreased under osmotic stress. In turn, the higher dose of sucrose led to a reduction in seedling growth of the genotypes. In callus, the genotypes varied significantly for callus fresh and dry weights, as well as final callus size. The effect of osmotic potential was significant on callus fresh weight and relative water contents of the calli. Based on the tolerance indices, ICARDA-I can be recommended as a osmotic stress-tolerant genotype.

Keywords: Callus; dry matter; drought; mesocotyl; seedling.

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Introduction

Grass pea (*Lathyrus sativus* L., Fabaceae), is a legume crop that is used as food and feed. It is a diploid species with chromosome number 2n=2x=14 (Arzani, 2006). The grass pea is a legume that grows in poor fertile and rain-fed soils. Among the various abiotic stresses, drought is one of the major reasons for losses in crop productivity. Against rapid progress in new research strategies for developing crop varieties having low water requirements are few (Huang et al., 2012). Grass pea is a crop highly tolerant to the stress caused by different abiotic stresses (Patto et al., 2006). The grass pea in comparison with other legumes has some morphological traits related to drought tolerance such as low width of leaves and the developed roots. However, research about the mechanisms and rate of drought tolerance in this crop is limited (Anderson et al., 2004).

Environmental factors, such as drought, impose stress on plants and leas to significant crop losses worldwide and a situation that will be exacerbated by climate change. Drought stress reduces leaf size, stem development, and root multiplication, thus reducing water-use efficiency (Flexas and Medrano, 2002). Drought is the most important environmental stressor, especially in arid and semiarid regions, where it impacts affect the growth and development of plants (Lonbani and Arzani, 2011). Plants have developed various mechanisms to tolerate drought stress, including physiological, biochemical, and molecular processes. These mechanisms involve osmotic adjustment and the maintenance of the plant's water status through efficient water use (Hoseini and Arzani, 2023). Osmotic adjustment is widely recognized as a key adaptive strategy for coping with drought stress, enhancing plant productivity (Yu et al., 2024). Osmotic stress conducts to the generation of reactive oxygen species (ROS) which prevent the normal functioning of cells by inactivating or degrading proteins, lipids, and DNA (Blokhina et al., 2003). The application of plant cell and tissue culture is an effective methodology to shorten the plant breeding programs for achieving high yield cultivars resistant to environmental stresses (George et al., 2008).

In this study, tolerance of several high-yielding grass pea genotypes to drought stress was investigated. The objective was to evaluate the grass pea genotypes for drought stress under in vitro conditions, specifically focusing on osmotic-stressed seedlings and calli.

Materials and Methods

This research was carried out at the Laboratory of Plant Production and Breeding Department, Faculty of Agriculture, University of Maragheh, East Azerbaijan Province, Iran. Six grass pea genotypes screened for drought tolerance under field conditions were used in this study. These genotypes originated from the International Center for Agricultural Research in the Dry Areas (ICARDA) were used in this study. Based on the field primary screening on 26 genotypes (data not shown), six tolerant grass pea genotypes (including a Local local check and five ICARDA genotypes namely ICARDA-I, ICARDA-II, ICARDA-III, ICARDA-IV, ICARDA-V) were exposed to the osmotic stress under *in vitro* conditions. The genotypes were exposed to osmotic stress -4.5 and -8 bar that were determined by vant Hoff equation (Piwowarczyk et al., 2014) induce by sucrose supplemented media. The experiment was carried out as a factorial arrangement based on a completely randomized design with four replications. Teratments were six genotypes and two osmotic levels. The experiment was conducted in two experimental groups; a) applying osmotic stress to seedlings *in vitro* conditions and b) applying osmotic stress to the calli of mesocotyl explants under *in vitro*.

The basal medium used in this experiment was the Murashige and Skoog (1962). In this experiment, proline (0.3 g/l), casein (0.3 g/l), and yeast extract (0.1 g/l) were used as growth stimulants to improve the culture status. Ascorbic acid (vitamin C, 50 mg/l) and citric acid (75 mg/l) were added to the culture medium for preventing explants necrosis. In addition to organic supplements, auxin growth regulators (2,4-D and NAA), cytokinin (BAP and Kin) were also used in the medium.

First, the ½ MS medium was prepared by the addition of BAP 10 ppm. After sterilization with autoclave, The sterilized seeds were planted in the media using forceps. The media were transferred to

a germination device at 26+ 2° C with a light intensity of 0.750 lux. After 7-10 days, sterile grown seedlings were ready for explants preparation. Ten seeds of six grass pea genotypes were sown on media containing different sucrose concentrations and cultivated for 14 days in controlled conditions in terms of light, humidity, temperature.

Explant culture for callus induction and evaluation of osmotic tolerance threshold under stress conditions

MS medium supplemented with organic supplements was distributed among sterile disposable Petri dishes. After 24 hours, mesocotyls were obtained from sterile seedlings and placed on the medium. To retain moisture and to control the contamination of the medium and explants, the Petri dishes were tightened with parafilm. They were stored in a dark room at a temperature of 26+2 °C. After the induction of callus subcultures were performed after 30 days for all samples.

After a preliminary experiment and analyzing the results related to the osmotic stress threshold using probit analysis, the osmotic potential (–8 bar) induced with 6% sucrose was determined as the suitable osmotic stress treatment for screening genotypes (data not shown). While, the –4.5 bar osmotic potential was found as the treshhold of osmotic stress and hence used as the control in this study. Then, The research then continued with two experimental groups; imposition of osmotic stress on seedlings under in vitro conditions and on calli.

Osmotic stress imposition on seedlings under in vitro

The seeds were cultured in glass bottles containing ½ MS medium without hormones and placed in a germinator for three days. Seedlings were transferred to a new medium for screening a stress threshold. The new medium was Full-MS with organic supplements as well as 10 mg/l BAP, in two osmotic potentials of -4.5 bar (control) and -8 bar (dry threshold concentration). After transferring the seedlings to a new medium, they were placed in a germinator at a temperature of 25+2 °C under the light.

For seedlings traits assay and, after each culture, the bottles were transferred again to a germinator at 26°C and 1200 lux light intensity. Finally, 7 days after culture, seedlings were evaluated in terms of fresh and dry (24 h at 72 °C), weight of seedling, rootlet and plumule numbers, number of nodes, length of rootlet, plumule, and seedling length, the diameter of rootlet and plumule, number of nodes and the number of leaves.

Osmotic stress imposition on callus samples

The culture medium for this experiment was prepared as full-MS with the addition of organic supplements and growth regulators (2 mg/l 2.4-D, 2 mg/l NAA). It was then divided into two parts, including 3% sucrose (-4.5 bar) and 6% sucrose (-8 bar. The calli derived were transferred to the medium under sterile conditions and grown inside the germinator under darkness conditions. A piece of callus from each genotype was cut into five sections and cultured on the MS medium. After culture, they were closed with parafilm to prevent contamination and moisture loss.

For assaying the *in vitro* traits; Petri dishes were visited every few days for any type of contamination. Then, the explants were evaluated once every five days for callus size and callogenesis index (explants produced callus per total number of explants). After 30 days, fresh and dry (48 hours at 72ºC) callus weight (mg), length and diameter of rootlet and plumule (mm), number of nodes and shoots, the relative growth rate of callus (RGR), and the relative water content (RWC, %) were evaluated.

Osmotic stress tolerance-related indices were calculated using seedling and callus dry weight to select the most tolerant genotypes. Tolerance index (TOL) [Rosielle and Hamblin, 1981], Mean productivity (MP) (Rosielle and Hamblin, 1981), Stress tolerance index (STI) (Fernandez George, 1992), Geometric mean productivity (GMP) (Fernandez George, 1992), Harmonic mean (HARM) (Fernandez George, 1992), Stress susceptibility index (SSI) (Fischer and Maurer, 1978), Relative decrease index (RDI) (Bidinger et al., 1987), Stress susceptibility percentage index (SSPI) (Mousavi et al., 2008), Stress non-stress production index (SNPI) (Mousavi et al., 2008) and Index of tolerance based on RGR (INTOL) (Soheilikhah et al., 2013) were evaluated as well.

Data Analysis

Analysis of variance and mean comparisons (Duncan's multiple range test) were performed. For

classifying genotypes, cluster analysis by Ward's algorithm and Euclidean distance coefficient was employed. Statistical analysis was done by SPSS and NTSYS softwares.

Results and Discussion

Seedling

Analysis of Variance

Genotypes varied significantly for the number of plumules, radicle diameter, seedling length, rootlet length, seedling length, rootlet length, dry weight of rootlet, fresh weight of plumule, fresh weight of seedling, dry weight of plumule, and dry weight of rootlet. Furthermore, the effect of osmotic potential on the dry weight of rootlet, fresh weight of plumule, fresh weight of seedling, and dry weight of plumule was significant. Genotype×osmotic potential interaction was non-significant on all traits except the number of nodes (Table 1). Fallahi et al. (2015) by applying osmotic potential using PEG on grass pea found that seed germination rate, stem length and root length decreased with increasing osmotic potentials. França et al. (2000) reported that drought stress reduced stem length in *Phaseolus vulgaris* cultivars. Drought stress reduced germination percentage and seedling growth parameters in black gram (Murillo - Amador et al., 2002; Pratap and Kumar Sharma, 2010). furthermore, reported that PEG-induced drought stress reduced the percentage and rate of germination and emergence of cowpea seedlings and also reduced the initial seedling growth.

Mean comparisons (Table 2) showed that genotype ICARDA-I had the highest recorded data for seedling length, rootlet diameter, number of plumules, number of leaves, fresh weight of plumule, fresh weight of seedling, dry weight of plumule, and dry weight of seedling. The highest rootlet length and dry weight of rootlet belonged to genotype ICARDA-V. The interaction effect of genotype×osmotic stress was significant only in shoot fresh weight. Genotype ICARDA-III × osmotic stress -8 bar had the highest shoot fresh weight.

Osmotic stress tolerance indices based on plumule dry weight

The estimation of osmotic stress tolerance indices based on plumule dry weight for six grass pea genotypes (the table is not inserted), the MP index, ICARDA-I has been defined as the most tolerant genotype due to its highest value and high yield in both stress and non-stress conditions. This index decreases in other genotypes, and ICARDA-III was more sensitive to stress. Rosielle and Hamblin (1981) showed that in most yield experiments, the correlation between MP and YS as well as MP and YP were positive. So, selection based on the MP index generally increases the yield of genotypes under both stress and non-stress conditions. GMP and STI indices had the highest value for ICARDA-I genotype. Considering, ICARDA-I was selected as the most tolerant genotype. Also, in other genotypes, these two indices decreased, and finally, ICARDA-III had the lowest value for both indices. Fernandez George (1992) used harmonic mean index (HARM) for the selection under stress conditions. Based on this index, ICARDA-I is selected as a tolerant genotype. The harmonic index data was decreased in other genotypes. In SNPI index (Mousavi et al., 2008), genotypes with higher values and in the SSPI index, genotypes with lower values are more tolerant against stress. So, according to SNPI, ICARDA-I was the most tolerant genotype and ICARDA-III was the drought-sensitive genotype. If the value of the RDI index Tsakiris and Vangelis (2004) is greater than one; the genotype is somewhat tolerant, and for the values less than one, the genotype is sensitive to drought stress. According to this index, the RDI of ICARDA-I, ICARDA-IV, and ICARDA-V genotypes was greater than one and are known as tolerant genotypes. This trait was higher in ICARDA-I than other genotypes. Other genotypes had less than one, which are known as drought-sensitive genotypes. Stress sensitivity index (SSI) is based on the performance of individual genotypes in both stress and non-stress environments, as well as the average performance of all genotypes in these two environments (Fischer and Maurer, 1978). High values of SSI indicate the sensitivity of genotypes to osmotic stress. Genotypes with low SSI had less yield changes and high stability under stress conditions than in non-stress environments. So, according to the SSI index; ICARDA-I was the most tolerant and ICARDA-III was considered the most sensitive genotype.

Table 1. Analysis of variance for the seedling traits of grass pea genotypes under in vitro osmotic stress conditions.

Source of Variation	df	MS							
		Number of rootlets	Number of nodes	Number of plumules	Radicle diameter	Plumule diameter	Seedling length	Plumule length	Rootlet length
Genotype (G)	5	34.88	0.50	38.04*	0.58**	0.22	7.34*	1.16	7.74**
Osmotic Potential (OP)	1	3.06	0.16	3.37	0.05	0.00	0.03	0.84	0.09
G*OP	5	6.37	1.96*	26.17	0.12	0.05	4.71	0.24	2.32
Error	12	15.88	0.83	12.04	0.11	0.17	3.16	1.14	1.56
CV (%)		12.05	24.3	3.1	17.6	10.01	15.6	29.5	27.1

* and ** are significant at 0.01 and 0.05 levels, respectively

Table 1 (continued)

Source of Variation	df	Dry weight of Seedling	Fresh weight of plumule	Fresh weight of Rootlet	Fresh weight of seedling	Dry weight of plumule	Dry weight of rootlet
Genotype (G)	5	243.34**	7555.00*	10.29.58	22473.57*	128.64**	71.46**
Osmotic Potential (OP)	1	237.37**	35420.17**	2115.93	42168.17*	315.37**	2.82
G*OP	5	27.27	4038.57	489.76	5015.37	20.17	6.93
Error	12	36.37	3546.33	7823.54	12650.25	12.20	9.68
CV (%)		15.1	27	30.1	21.1	14.1	19.6

Table 2. Mean comparison for the effects of osmotic stress on some seedling traits of grass pea genotypes.

Genotype	Rootlet length (cm)	Seedling length (cm)	Rootlet diameter (mm)	Number of plumule	Fresh weight of plumule (mg)	Fresh weight of seedling (mg)	Dry weight of plumule (mg)	Dry weight of rootlet (mg)	Dry weight of seedling (mg)
Local check	2.65b	6.5b	1.79b	15.5a	241.25a	367.75ab	28.25a	18.25ab	46.5a
ICARDA-I	2.9b	7.27ab	2.6a	14.25ab	257.5a	423.25ab	30.25a	13.75bc	44a
ICARDA-II	5.87a	9.62a	1.72b	9.75bc	236.5a	371.5ab	26.5ab	15.25bc	41.75a
ICARDA-III	5a	6.62a	1.44b	7.75c	136.75b	238b	14.75c	14bc	22.25b
ICARDA-IV	4.5ab	7.62ab	1.99b	9.75bc	237.25a	321.25ab	27.25ab	10.5c	37.75a
ICARDA-V	5.75a	9.37ab	1.72b	9.25bc	210.75ab	446.75a	21.75b	22.75a	44.5a

Same letter in Duncan grouping are not significantly difference (p≤0.01)

The lower values of the TOL index (Rosielle and Hamblin, 1981) indicate more tolerance of the genotype to stress. According to the TOL index, ICARDA-I was selected as the most tolerant genotype, whose performance in a stressful environment had low reductions than in a stress-free environment, i.e., the mentioned genotype had a reasonable yield stability. Also, the TOL index increased in other genotypes.

Callus

The genotypic effects on callus dry weight and fresh weight was significant. The osmotic potential effect was significant on callus fresh weight and RWC of callus. The genotype×osmotic potential interaction effect was significant on final callus size and callus growth rate (Table 3). A mean comparison showed that ICARDA-I had the highest callus dry weight (Table 4). Figure 1 shows the calli of a grass pea genotype after 14 days culturing mesocotyl explants under.in vitro osmotic potentials of – 4.5 and – 8 bar.

Osmotic stress tolerance indices for calli dry weight

In MP, GMP, STI, and HARM indices, the higher values show more resistance of genotype to osmotic stress. Accordingly, ICARDA-I had the highest value in the above indices which was considered as the most tolerant genotype to osmotic stress. Also, in other genotypes, the values of these indices decreased and finally, the control genotype was the most sensitive to osmotic stress. In the RDI index, the values greater than one are resistant and smaller than one is sensitive to osmotic stress. Again, ICARDA-I was chosen as the most tolerant genotype. The lower values of SSPI and TOL indices indicate resistance to stress. Based on these two indices, ICARDA-I genotype was characterized as stress-tolerant as well (Table 5). The first and most important effect of osmotic stress is impaired germination and poor seedling establishment (Harris et al., 2002). In a study on five chickpea cultivars, drought stress impaired germination and early growth of seedlings (Okçu et al., 2005). In addition, in alfalfa, germination potential, hypocotyl length, plumule fresh weight, rootlet fresh weight, plumule dry weight and rootlet dry weight decreased due to water deficiency (PEG), while rootlet length increased (Zeid and Shedeed, 2006).

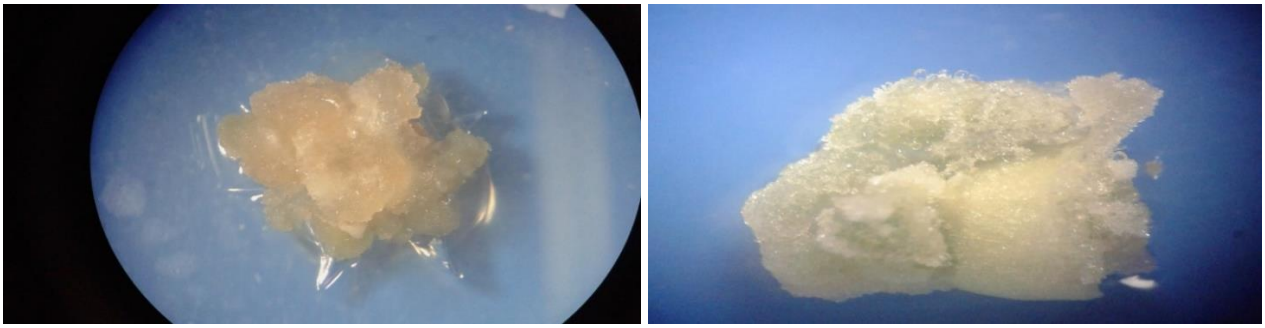

Figure 1. Callus of a grass pea genotype after 14 days of culture on media containing -4.5 and -8 bar sucrose.

Table 3. Analysis of variance for the *in vitro* traits of callus for the grass pea genotypes exposed to osmotic stress.

Source of Variation	df	MS				
		Calli fresh weight	Calli dry weight	Calli RWC	Final callus size	Callus growth rate
Genotype (G)	5	484.46**	15.74**	0.002	1.74**	0.0013
Osmotic Potential (OP)	1	286.73*	3.12	0.0086**	0.22	0.00006
G×OP	5	74.41	2.96	0.0006	1.84**	0.0025*
Error	24	62.73	2.86	0.0013	0.41	0.0008
CV (%)		24.22	47.70	4.09	6.54	35.74

Growth occurs through cell division, cell enlargement, and differentiation, involving genetic, physiological, environmental, and morphological events and their interactions. The quality and quantity of plant growth depend on the events that are affected by water deficit under drought stress (Taiz and Zeiger, 2006). Any disturbance in cell division, elongation, and development due to drought stress reduce plant height, leaf area, and overall plant growth (Kaya et al., 2006; Hoseini and Arzani, 2023). *In vitro* culture is commonly used to select drought-tolerant plants considering the differences between the response of plants to *in vivo* and *in vitro* conditions (Mohamed et al., 2000). Plant dry weight is one of the realistic criteria in determining the response of plants to various stresses such as drought, salinity, and metal toxicity (Talukdar, 2013). In bean plants subjected to drought stress using PEG, germination and seedling growth, were strongly affected and, it was concluded that inhibition of germination is mainly due to the osmotic effect of PEG (Garg, 2010). Drought stress in eight alfalfa reduced total plant growth and increased the ratio of dry weight that led to a significant reduction in the relative amount of leaf water potential (Lonbani and Arzani, 2011). Grass pea was subjected to drought stress using PEG, which showed that the plant height, stem fresh weight, root fresh weight, and leaf area decreased compared to the control (Jiang et al., 2013). In bean plants that assayed under drought stress and non-stress conditions; stem dry weight, root dry weight, and nodal dry weight were decreased under water deficit compared to the control conditions (Kabbadj et al., 2017).

Table 4. Mean comparison of callus traits between different grass pea genotypes under osmotic potential.

Genotype	Callus fresh weight (mg)	Callus dry weight (mg)	Final callus size
Local check	21.63c	1.9b	9.23b
ICARDA-I	45.83a	6.5a	10.40a
ICARDA-II	30.60bc	2.8b	10.16a
ICARDA-III	36.37ab	3.4b	9.35b
ICARDA-IV	24.37c	2.63b	10.17a
ICARDA-V	37.33ab	4.07b	9.24b

The different letters in each column indicate significant differences ($p \leq 0.01$) using Duncan's Multiple Range Test

Table 5. Osmotic stress tolerance indices of grass pea genotypes based on callus dry weight.

Genotype	TOL	SSI	SSPI	RDI	SNPI	HARM	STI	GMP	MP
Local check	0.06	-0.2	0.92	0.82	7.53	0.95	0.34	1.9	1.9
ICARDA-I	-3.27	3.73	-50.15	1.41	-10.87	3.05	3.75	6.29	6.5
ICARDA-II	0.66	1.18	10.15	0.67	5.45	1.38	0.73	2.8	2.8
ICARDA-III	-0.26	0.46	-4.15	0.91	-10.07	1.70	1.09	3.4	3.4
ICARDA-IV	-0.87	2.17	-13.23	1.18	-5.01	1.28	0.64	2.6	2.6
ICARDA-V	0.13	-0.17	2	0.82	16.05	20.3	1.56	4.1	4

Drought stress reduces cell elongation more than cell division rate and hence reduces plant growth by affecting various physiological and chemical processes such as photosynthesis, respiration, transport, and uptake of ions, as well as by interference in the metabolism of hormones (Jaleel et al., 2008).

Piwowarczyk et al. (2014) reported that with in vitro seed culture and stem propagation with BAP and PEG application; the number of branches per plant was significantly reduced, and drought conditions strongly affected the regeneration potential compared to the controls.

Cluster and principal components analysis

Figure 2 shows the grouping of studied grass pea genotypes based on seedling and callus traits. According to analysis, grass pea genotypes are divided into two separate groups. The first group included local and ICARDA-I genotype. The second group consisted of ICARDA-II, ICARDA-III, ICARDA-IV, and ICARDA-V genotypes. In other words, cluster analysis could separate the tolerant ICARDA-I genotype from others. Genotypes that located in the same group, are almost similar. In other words, they do not differ much in terms of the measured traits.

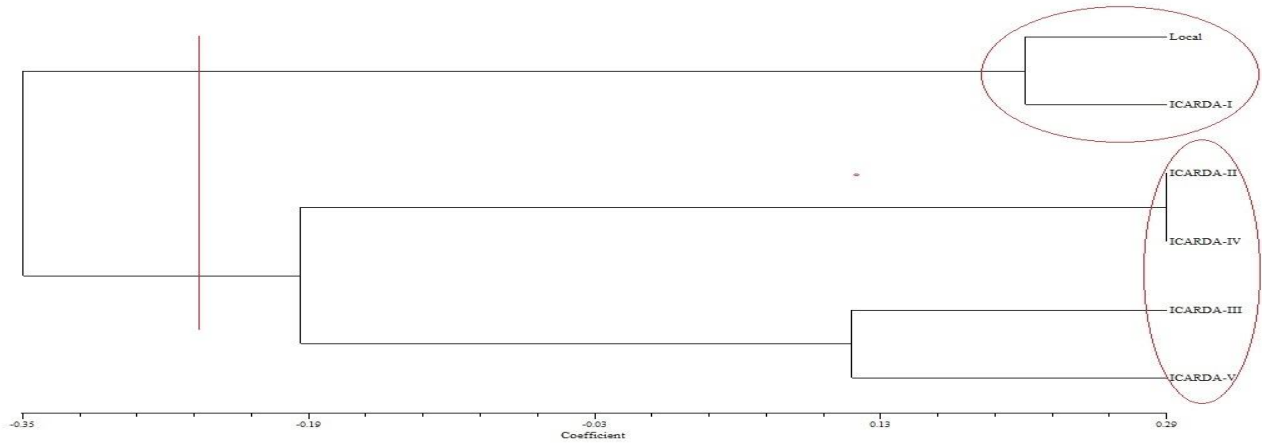

Figure 2. Cluster analysis of the six grass pea genotypes exposed to drought stress in in vitro conditions.

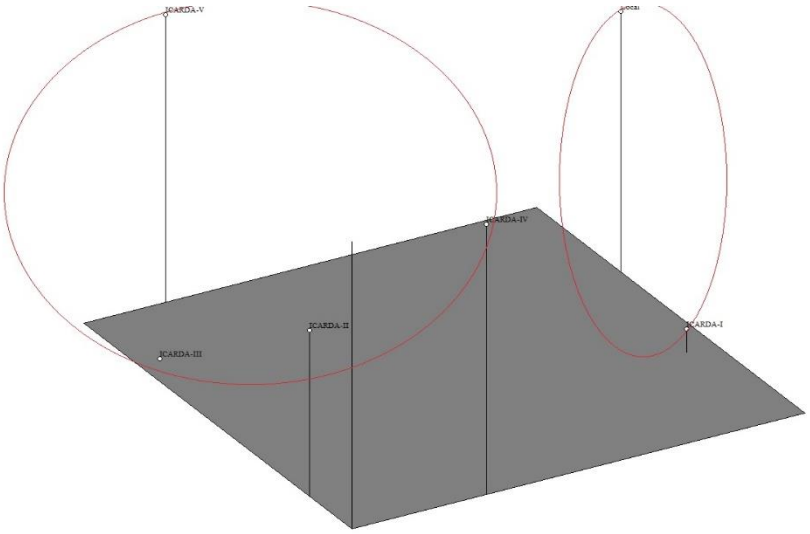

Figure 3. Three-dimensional scatterplot of grass pea genotypes (PCA) subjected to osmotic stress.

Principal components analysis for the grass pea genotypes showed that the first two components explained approximately 63.38% of variations. For medium explanation, these amount indicates relative efficiency of analysis. PCAs two and three-dimensional scatter plots of grass pea genotypes subjected to osmotic stress confirmed the results of cluster analysis (Figure 2) and genotypes were located in two groups as well. In a research by Soofinia et al. (2024), the first three main components of principal components analysis explained 82.08% of the total variation.

Conclusion

The dry weight of shoots was affected by osmotic stress but not the dry weight of the rootlet. Plant organs differed in their sensitivity to osmotic stress; shoots seem to be more sensitive than roots. The seedling indices; MP, GMP, STI, HARM, RDI, SNPI, SSPI, SSI, and TOL introduced ICARDA-I genotype as tolerant and ICARDA-III genotype as a sensitive one. Consistent with the finding of the seedling experimental group, callus indices including GMP, STI, HARM, and RDI identified ICARDA-I as the most tolerant genotype. Therefore, genotype ICARDA-I can be introduced as tolerant to osmotic stress under in vitro conditions. A remarkable reduction in the seedling dry weight was registered, which suggests a high impacts of osmotic stress on the grass pea genotypes. Likewise, evaluation of the osmotic stress tolerance indices resulted in a similar results; that, genotype ICARDA-I attained the higher tolerance related indices under osmotic stress. The osmotic stress tolerance screening criteria related to the callus and seedlings confirmed that ICARDA-I was the most osmotic stress-tolerant genotype under in vitro conditions. Thus, this genotype could be suggested for further studies in the genetic transformation and breeding programs.

Supplementary Materials

There is no supplementary material available for this article.

Author Contributions

Conceptualization, M.N., A.P.; methodology, A.A.; software, M.N.; validation, M.N., A.P. and A.A.; formal analysis, M.N..; investigation, M.N.; resources, S.S.; data curation, A.A.; writing—original draft preparation, A.P.; writing—review and editing, A.P..; visualization, X.X.; supervision, A.P.; project administration, A.A. All authors have read and agreed to the published version of the manuscript.

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Conflict of Interest Statement

The authors declare no conflict of interest.

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مقاله پژوهشی

تحمل ژنوتیپ‌های خلر (*Lathyrus sativus* L.) به تنش اسمزی در شرایط درون شیشه‌ای

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چکیده: اثر تنش اسمزی بر پارامترهای گیاهچه و کالوس شش ژنوتیپ خلر در شرایط درون شیشه‌ای مورد ارزیابی قرار گرفت. تنش اسمزی در محیط آگار با افزودن ساکارز ۴/۵- و ۸- بار القا گردید. ۱۲ و ۳۰ روز پس از اعمال تنش خشکی، صفات مورفولوژیکی گیاهچه‌ای در شرایط درون‌شیشه‌ای و کالوس به‌ترتیب مورد ارزیابی قرار گرفتند. در گیاهچه‌ها، اثر ژنوتیپ بر اکثر صفات و اثر تنش اسمزی تنها بر وزن خشک ریشه‌چه، وزن تر ساقه‌چه و گیاهچه و وزن خشک ساقه‌چه معنی‌دار بود. ماده خشک ریشه‌چه، ساقه‌چه و گیاهچه تحت تنش اسمزی کاهش یافت. بالاترین غلظت ساکارز منجر به کاهش رشد گیاهچه‌ای ژنوتیپ‌ها شد. در کالوس اثر ژنوتیپ بر وزن خشک و تر کالوس و اندازه نهایی کالوس معنی‌دار بود. اثر پتانسیل اسمزی بر وزن تر کالوس و RWC کالوس معنی‌دار بود. به طور کلی، قرار گرفتن در معرض تنش اسمزی باعث کاهش معنی‌دار طول ریشه‌چه و ساقه‌چه گیاهچه خلر نشد، اما بر وزن تر ساقه‌چه و گیاهچه تأثیر گذاشت. با توجه به شاخص های تحمل به خشکی، ژنوتیپ ICARDA-I به‌عنوان ژنوتیپ متحمل می‌تواند توصیه شود.

کلمات کلیدی: تنش اسمزی، کالوس، گیاهچه، ماده خشک، ، مزوکوتیل.

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