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In vitro salinity stress mediates grass pea genotypes' (*Lathyrus sativus* L.) responses

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Abstract: This study was carried out to determine the tolerance of grass pea genotypes to salinity stress at callus and seedling stages under in vitro conditions. The calli and seedlings of six selected tolerant genotypes based on the primary screening in the field were separately exposed to salinity treatments (0, 125 mM) in vitro. Salinity was imposed with NaCl during in vitro culture, and it significantly affected all seedling traits. Genotype of Iran had the lowest seedling dry weight and therefore was more sensitive to salinity stress. According to salinity tolerance indices for seedlings, genotype Greece-III was characterized as high-yield and relatively high–salt-tolerant genotype. Salinity significantly affected callus size, callus RWC, callus RGR, and callogenesis index. Calli fresh and dry weights were not affected by the treatments. For callus dry weight, genotype Greece-III had the highest mean; and the lowest mean belonged to Greece-I. The stress tolerance indices showed that the highest values belonged to genotype Greece-III, which showed high yield and yield stability and so reasonable salinity tolerance. Cluster analysis divided the genotypes into two separate groups. The first cluster consisted of Iran, Greece-II, and Greece-III genotypes, and the second cluster consisted of Bangladesh, Canada, and Greece-I genotypes. Cluster analysis potentially separated the tolerant and sensitive genotypes to salinity in terms of callus dry weight. Grass pea callus and seedlings were able to survive at 125 mM salinity. Salinity did not affect callus dry and fresh weights, but its effect was remarkable on seedling dry and fresh weights (55% less than control). Therefore, calli were reasonably salinity tolerant. The present study suggests that grass pea was reasonably tolerant to salinity and can survive under salinity conditions during the seedling and callus stages.

Key words: Callus, dry matter, mesocotyl, seedling

1. Introduction

Grass pea (*Lathyrus sativus* L.) is a seed legume that grows in semiarid and arid regions. It is an annual leguminous crop cultivated for animal or human consumption (Yadav and Mehta, 1995). The genus has several advantageous agronomic characteristics such as high grain-yielding capacity and high protein content of its grains (Croft et al., 1999). The legume produces economic yields under different environmental conditions and offers a high potential for use in marginal low-rainfall areas. Indeed, this has made it a popular crop in subsistence farming particularly in developing countries (Praveen et al., 1994). Grass pea had adapted to a range of environments around the world. It is a cool-season pulse grown in the Indian subcontinent, southern Europe, and northern Africa for forage and grain production for both livestock and human consumption (Campbell, 1997). It has cultivation potential in many challenging and divergent environments (Rao and Northup, 2011). It is a climate-resilient nutrient-rich legume crop grown in versatile agro-ecosystems (Kumar et al., 2013). The potential of grass pea as a health-promoting nutraceutical has recently been recognized despite the presence of a plant toxin called β-ODAP (Lambein et al., 2019).

Salt stress adversely affects plant survival, growth, and biomass (Xiong and Zhu, 2002). Salinity is one of the significant factors that limit crops' productivity. Therefore, the assessment of crop plants for their tolerance to salinity has become necessary (Hasegawa et al., 2000). Salinity can affect organogenesis behavior of in vitro cultures as well. When cells were subjected to salt stress, generally two detrimental effects were observed; an osmotic imbalance that causes water loss from cells due to the increase in the osmotic pressure outside cells, and the uncontrolled uptake of Na^+ ions, which imposes injuries on the cytoplasm (Miao et al., 2003). Plants exhibit a wide

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range of molecular, cellular, and whole plant responses upon exposure to salt stress (Hasegawa et al., 2000). These include morphological and developmental changes (such as life cycle, inhibition of shoot growth and the enhanced root growth) and the adjustments in ion transport and metabolic changes (Xiong and Zhu, 2002).

Tissue culture provides an opportunity to develop new germplasm adapted to the altering needs (Mathur et al., 2008). Plant regeneration through tissue culture has been widely used for the rapid and mass multiplication of legumes (Ochatt et al., 2013; Barpete et al., 2010). Roy et al. (1992) and Gharyal and Maheshwari (1983) succeed in regenerating grass pea plantlets from calli.

The objective of the present research was to assay the effects of NaCl-induced salinity on the growth responses of six grass pea genotypes cultured in vitro. Furthermore, we tried to develop an in vitro regeneration protocol for grass pea as a necessary first step for the upcoming biotechnology and breeding programs.

2. Materials and methods

This research was carried out at the Research Field and Laboratory of Plant Production and Breeding Department, Faculty of Agriculture, University of Maragheh, Maragheh, East Azerbaijan Province, Iran. The genotypes were provided by the International Center for Agricultural Research in the Dry Areas (ICARDA). Table 1 shows the origin of grass pea genotypes used in field salinity tolerance experiments. Based on the field primary screening on 26 genotypes (not published), six tolerant grass pea genotypes (Iran local check and five other genotypes) were exposed to salinity under in vitro conditions. These tolerant genotypes originated from Iran, Bangladesh, Canada, and Greece. The salinity levels were determined by probit analysis at (0, 25, 50, 75, 100, 125, and 150 mM NaCl) on local check genotype. According to the data analysis and means comparison, control and 125 mM NaCl salinity were determined to proceed the experiment mainly due to the highest tolerance and no significant fluctuations in the measured traits. In other words, 125 mmol NaCl was determined as the threshold concentration according to the preliminary results. The experiment was a factorial based on a completely randomized design with six genotypes and two salinity levels with four replications and five samples. The experiment was conducted in two ways: applying salinity to the seedlings under in vitro conditions, and salinity treatment on the callus with mesocotyl explant under in vitro conditions.

The seeds were surface sterilized (Sinha et al., 1983) and water imbibed overnight. Then, after the moisture around the seeds was dried, the seeds were placed in bottles with 15 mL MS medium (pH: 6–6.1, agarose 0.8%, growth supplements of vitamin C, citric acid, casein and proline in the amounts of 0.05, 0.075, 0.3, and 0.3 mg/L, respectively) (Piwowarczyk et al., 2016).

2.1. Seedling culture

For seedlings traits measurements and after each culture, the bottles were transferred to a germinator at 26 °C and 1200 lux light intensity. Finally, 7 days after culture, seedlings were evaluated in terms of the fresh and dry weights of seedling, rootlet, and plumule; the number of nodes; the length of seedling, rootlet, and plumule; the diameter of rootlet and plumule; and the number of new shoots.

2.2. Callus culture

For calli induction, a combination of 2 mg/L 2,4-D and 2 mg/L NAA was assayed on the mesocotyl explant. The number of explants used in each replication was 5 samples. Appropriate calli masses were cut once every 4 weeks and subcultured in a new medium containing the above growth regulator combination. After each culture, petri dishes were transferred to the culture chamber at 26 ± 2 °C under dark conditions. The obtained calli and seedlings in the medium containing; 2 mg/L BAP were used as culture sources. Thus, calli and mesocotyl explants were cut and five callus samples and five explants were placed into each petri dish.

For assaying the in vitro traits, petri dishes were visited every few days for any type of contamination. After applying the salinity levels, explants were evaluated once every 5 days for callus size and callogenesis index (number of calli induced in explants/total explants). After 30 days, fresh and dry (48 h at 72 °C) 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 percentage (RWC) were evaluated.

2.3. Tolerance indices for the seedling and callus culture

Salinity stress tolerance indices for the seedling and callus were calculated 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, 1992), geometric mean productivity (GMP) (Fernandez, 1992), harmonic mean (HARM (Fernandez, 1992), stress susceptibility index (SSI) (Fischer and Maurer, 1978), relative decrease in yield (RDI) (Bidinger et al., 1987), stress susceptibility percentage index (SSPI) (Moosavi et al., 2008), stress/non-stress production index (SNPI) (Moosavi et al., 2008), and index of tolerance based on RGR (INTOL) (Soheilikhah et al. 2013) were evaluated.

Growth was measured in terms of the fresh weight of callus and seedlings. The tolerance index of plantlets was calculated from the fresh weight (FW) by using the formula: (FW treated/FW control) × 100, and represented in percent tolerance (Wilkins, 1978).

Data were subjected to one-way analysis of variance (ANOVA) using SPSS (ver. 20) and were represented as

mean and standard error (SE). Duncan's multiple range test (Steel et al., 1996) was used for comparing the differences between the treatments ($p \leq 0.05$). Pearson correlation coefficients were computed. Cluster analysis based on Ward's minimum variance method and Euclidean distance measure with the trait means was performed to classify the genotypes.

3. Results and discussion

3.1. Initial field screening of genotypes

Twenty-six grass pea genotypes were exposed to six salinity levels (0, 25, 50, 75, 100, 125, and 150 mM) in the research field in 2017. The salinity was imposed with NaCl during the season and until harvesting time. Based on the primary screening, six tolerant genotypes and 20 sensitive genotypes were selected, of which the tolerant genotypes were used in the present study (Table 1).

3.2. Seedling

3.2.1. ANOVA

ANOVA (Table 2) for the seedling traits showed that salinity significantly affected all measured traits. Grain legumes are susceptible to salinity during seedling establishment (Kaya et al., 2003). The effect of salinity on seedling growth is seen in Figure 1. In addition, the genotypes' effect was significant on seedling fresh weight, fresh and dry weight of plumule, and the rootlet length and diameter. The interaction of salinity and genotype was nonsignificant on the traits. Total dry weight under in vitro culture is a dominant criterion indicating assimilates accumulation in plants and therefore reflects photosynthesis and respiration rate (Roy et al., 1992). Salinity induces great reductions in biomass production (Saha et al., 2015). Root growth potential is more susceptible and responsive to saline conditions than shoot growth. However, their

Table 1. The grass pea (*Lathyrus sativus* L.) genotypes used in field salinity tolerance experiment.

Genotype number	Origin	Tolerance	Genotype number	Origin	Tolerance
1	Bangladesh	S	14	Germany	S
2	Canada	S	15	Greece	S
3	Morocco	S	16	Greece	S
4	Ethiopia	S	17	Greece	S
5	Bangladesh	S	18	Greece	S
6	Pakistan	S	19	Canada	T
7	Hungary	S	20	Greece (Greece-I)	T
8	Bangladesh	S	21	Greece (Greece- II)	T
9	Bangladesh	S	22	Greece	S
10	Bangladesh	S	23	Greece	S
11	Greece	S	24	Greece (Greece-III)	T
12	Bangladesh	T	25	Afghanistan	S
13	Afghanistan	S	26	Local check (Iran)	T

T: Tolerant S: Sensitive

Table 2. Analysis of variance for the seedling traits of grass pea genotypes under in vitro saline conditions.

Source of variation	Df	MS								
		Rootlet dry weight	Plumule dry weight	Seedling dry weight	Rootlet fresh weight	Plumule fresh weight	Seedling fresh weight	Seedling length	Plumule length	Rootlet length
G	5	0.031	0.013*	0.012*	0.16	0.038*	0.016*	0.434	0.265	0.605*
S	1	0.084*	0.063**	0.075**	0.390**	0.413**	0.206**	29.24**	11.35**	4.155**
G×S	5	0.009	0.005	0.006	0.012	0.015	0.007	0.505	0.422	0.039
Error	34	0.022	0.003	0.004	0.020	0.013	0.006	0.854	0.448	0.234
CV (%)	-	50.85	49.49	58.23	46.52	34.13	30.90	31.36	37.60	41.25

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

Table 2 (Continued).

Source of variation	df	MS					
		Germination percentage	Plumule diameter	Rootlet diameter	RWC of seedling	Number of formed stems	Number of nodes
G	5	0.009	0.008	0.004*	0.073	3.076	1.493
S	1	0.630**	0.081**	0.027**	9.744**	84.24**	35.426**
G*S	5	0.118	0.004	0.001	1.773	1.435	0.647
Error	34	0.068	0.004	0.002	0.753	1.644	0.650
CV (%)	-	30.90	23.16	23.05	47.55	8.67	46.53

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

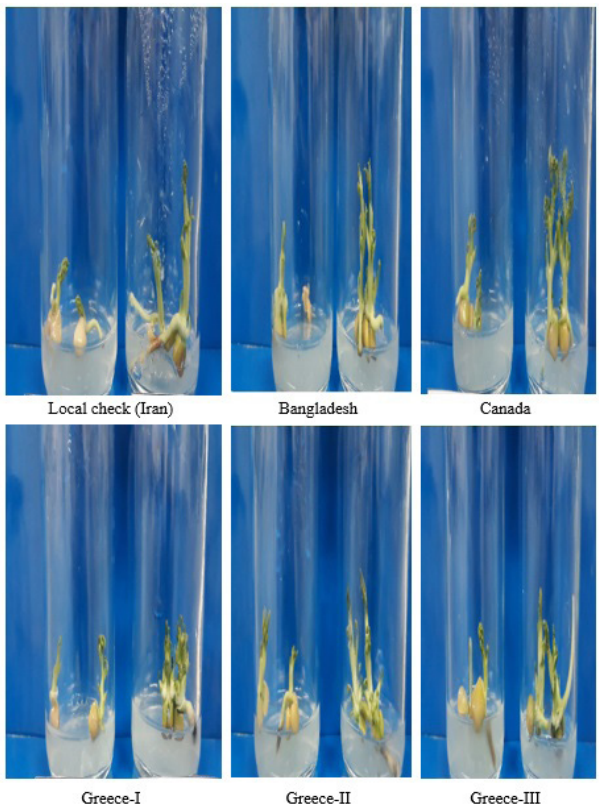

Figure 1. The effects of salinity on growth of grass pea genotypes in vitro (Left: 125 mM, Right: 0 mM, in every genotype).

growth-related traits are reasonable indicators of salinity damage.

Salinity responses are combinations of complex interactions between different morphological, physiological, and biochemical processes (Manchanda and Garg, 2008). Haileselasie (2012) used five salinity levels (0, 4, 6, 8, and 10 dSm^{-1} NaCl) on grass pea and reported that all growth traits, including shoot fresh and dry weights, shoot length, and root length, were decreased with salinity levels. In addition, salinity decreased grass pea biomass remarkably and the highest reduction was observed at 10 and 8 $dSm^{-1}$of salinity. Ahmed et al. (2014) surveyed the effects of 0, 5, 10, and 15 dSm^{-1} NaCl on grass pea and reported significant declines in germination percentage, root length, shoot length, and total dry matter. Therefore, their idea was that the response of grass pea to salinity varied depending on the genotype. The present experimental results and those of several former researchers (Yang and Zhang, 2005) showed that grass pea genotypes are relatively salt-tolerant.

The significant decrease in germination percentage in the present study was in agreement with the reports of Mahdavi and Sanavay (2007), who indicated that germination decreased by 50% in 197 mM NaCl. Further studies showed that germination percentage was not considerably affected at 50 and 100 mM NaCl in in vitro culture, but at 200 mM NaCl, these parameters were decreased significantly (Piwowarczyk et al., 2016). Germination inhibition under salinity conditions may be due to the intense external osmotic potential around the root, which impedes water uptake or imposes toxic effects of sodium and chlorine ions on seed germination or the combined results of both (Talukdar, 2011a). When salinity stress is applied to plants, ions are received at the membrane level or at the cell wall. Therefore, signal transduction initiates the salinity-related genes and stimulates several transcription factors to induce ion equilibrium (Song et al., 2019). The adverse salinity effects involve the prompt osmotic and chemical stress, resulting from the accumulation of harmful ions (Munns, 2002). In contrast, the prolonged adaptation behavior of plants to water shortage is an osmotic adjustment (Blum, 2017; Munns et al., 2020).

A significant decrease in the dry weight of seedlings (Figure 2) may be due to the combined effects of ionic and osmotic stress induced by NaCl. The significant reduction in dry weight of seedling, plumule, and rootlet with salinity was previously reported in grass pea genotypes (Talukdar, 2011b). The primary reason for the decrease in plant dry matter under salinity stress is the intensified Na^+ and Cl^- concentration which affects the selectivity of the membranes and the absorption of other essential elements by the plants (Shiyab, 2011). High salt concentration caused a remarkable decrease in biomass (Saha et al., 2015).

The significant decrease in the measured traits under salinity conditions (Table 2) agrees with the findings of Piwowarczyk et al. (2016), who reported that the shoot- and root-related traits were drastically reduced under salinity stress compared to nonstress conditions. They also reported that the root traits were more sensitive to the salinity than the shoots especially with high NaCl concentrations. In other research (Bandeoglu et al., 2004) on lentil, roots were less sensitive in this parameter than shoots. Similarly, Tsegaye and Gebrselassie (2014) reported that roots had less growth potential than shoots under salinity conditions. Rootlet and plumule lengths are predominant morphological markers in salinity tolerance responses. High salinity levels via the slowed water uptake may increase root and shoot lengths (Haileselasie, 2012). Talukdar (2011b) also reported a decrease in root and shoot elongation with different salinity levels on grass pea. Generally, the seedling, root, and shoot lengths were decreased significantly under salinity stress conditions in vitro (Piwowarczyk et al., 2016).

Water potential is the other significant physiological parameter under stressful conditions (Parida and Das, 2005). In this study, salinity had a significant effect on the relative water content of plants (Table 2). These results are similar to those obtained by Ghoulam et al. (2002), who reported the decreased seedling's relative water content in response to the salinity. They are also in line with the findings of Jamil et al. (2007), who reported a significant decrease in the relative water content at the salinity level of 170 mM, which can be due to the osmotic effect leading to the reduction in water uptake by the seedlings.

Mean comparisons (Table 3) revealed that the highest and lowest mean for seedling fresh weight, plumule fresh weight, seedling dry weight, and plumule dry weight were related to genotypes Greece-I and Iran (local control), respectively. Genotype Iran had the lowest seedling dry weight and therefore was more sensitive to salinity stress. For the root length and diameter, genotypes showed different behaviors.

3.2.2. Salinity stress tolerance related indices for seedlings

The highest values for TOL, GMP, RDI, and SNPI indices belonged to genotype Greece-III. Genotype Iran (control)

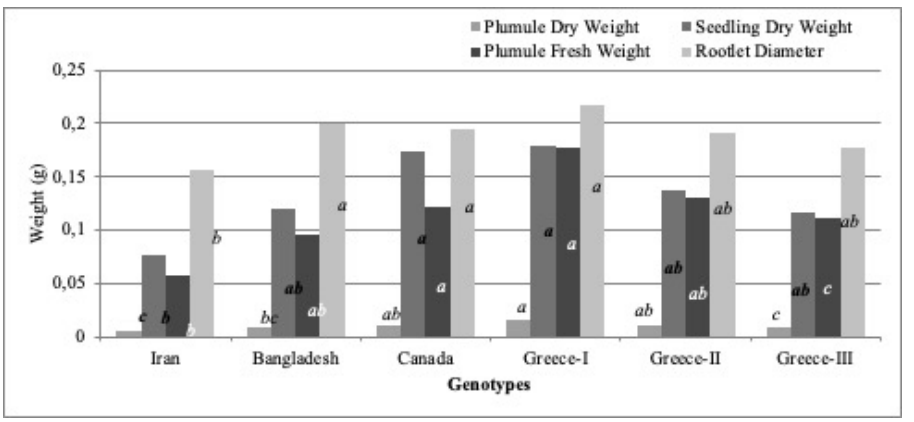

Figure 2. The effect of in vitro salinity stress (NaCl) on the seedling traits of grass pea genotypes.

showed the lowest SSI values and the least SSPI. The highest SSPI was recorded for genotype Canada, which also had a high MP index, indicating high yield potential and sensitivity to salinity stress. According to the SSI index, Greece-III was the most tolerant genotype to the salinity and its yield reduction was lower than the yield average of the total genotypes. In addition, genotypes Greece-II and Canada showed the highest SSI and the least RDI, respectively (Table 4). For STI and HARM indices, Greece-I had the highest values. In other words, Greece-I was tolerant to the salinity and had higher yields. However, unlike SSI, STI showed the lowest value for genotype Greece-III. It should be noted that the MP, HARM, and SNPI indices showed the lowest values for genotype Iran (Table 4). The SSI index selects the relatively low-yielding genotypes under nonstress conditions and the high-yield ones under stress conditions. The index varies between zero and one, and whatever this index was the higher, it shows higher susceptibility to the stress (Fernandez, 1992). Clarke et al. (1992) also expressed that the SSI index is based on selecting genotypes with higher yields and high tolerance to stress. TOL index determines low-yield genotypes under nonstress conditions and the high-yield ones with stress conditions. MP index shows genotype yield both under stress and nonstress conditions. This index shows high-yield genotypes under nonstress conditions and the relatively low-yield plants in stressful environments. The high STI index indicates high tolerance under stress conditions (Rosielle and Hamblin, 1981; Fernandez, 1992). Based on the arithmetic mean, the MP index will be a privilege whenever the yield differences between the stress and nonstress conditions are high. Geometric mean (GMP) is often used by breeders interested in partial yield under drought stress conditions. Under the stress, the amount of yield varies over the years in different fields (Fernandez, 1992). High value of harmonic index also shows the higher relative tolerance in cultivars under stress conditions. If the RDI is >1, the genotype is relatively stress-tolerant, and RDI<1 indicates a relatively stress-sensitive genotype (Fischer et al., 1998).

Since these indices have pros and cons, a reliable index is needed to understand the yield variations better to

Table 3. Mean comparisons with Duncan's multiple range test for the effects of salinity on growth-related traits of grass pea genotypes in vitro.

Genotype origin	Root length (cm)	Root diameter (mm)	Plumule fresh weight (g)	Plumule dry weight (g)	Seedling fresh weight (g)	Seedling dry weight (g)
Iran	1.29ab	0.157b	0.058b	0.0058c	0.087b	0.0769b
Bangladesh	1.06b	0.200a	0.095ab	0.008bc	0.1387ab	0.1193ab
Canada	0.53b	0.195a	0.121a	0.0113ab	0.2030a	0.1736a
Greece-I	1.01b	0.218a	0.177a	0.0161a	0.2000a	0.1794a
Greece-II	1.28ab	0.192ab	0.130ab	0.0111ab	0.1620ab	0.1375ab
Greece-III	1.62a	0.178ab	0.112ab	0.0079c	0.1500ab	0.1156ab

Genotypes with similar letters had no significant difference.

Table 4. Estimation of stress tolerance indices for the seedlings dry weight of six grass pea genotypes under in vitro conditions.

Genotype origin	TOL	MP	STI	GMP	HARM	RDI	SSI	SSPI	SNPI
Iran	0.034	0.0767	0.346	10.62	0.036	1.143	0.818	9.897	0.115
Bangladesh	0.106	0.127	0.816	9.77	0.052	0.731	1.343	31.221	0.133
Canada	0.159	0.186	1.732	10.14	0.076	0.715	1.363	46.640	0.191
Greece-I	0.079	0.179	1.885	8.98	0.086	1.142	0.818	23.114	0.269
Greece-II	0.120	0.137	0.942	10.56	0.056	0.700	1.382	35.244	0.139
Greece-III	0.001	0.116	0.822	11.77	0.058	1.766	0.023	0.352	0.666

Tolerance index (TOL), mean productivity (MP), Stress Tolerance Index (STI), geometric mean productivity (GMP), harmonic mean (HARM), relative decrease in yield (RDI), stress susceptibility index (SSI), stress susceptibility percentage index (SSPI), stress/non-stress production index (SNPI)

identify the relatively tolerant genotypes with stable yields. Therefore, the SNPI and SSPI indices were presented, which indicate relatively stable and high yield under stress and nonstress conditions (Moosavi et al., 2008). According to these indices, genotype Greece-III was characterized as high-yield and relatively high–salt-tolerant genotype.

3.3. Callus

3.3.1. ANOVA

Analysis of variance (Table 5) showed that salinity significantly affected callus size, callus RWC, callus RGR, and callogenesis index. Calli fresh and dry weights were not affected by the treatments more possibly due to the fact that the division and further growth of the calli cells were the same with control and saline conditions, which may be due to the availability of all the nutrients and growth elements in the medium.

The effect of salinity on callus growth is seen in Figure 3. The genotype effect was significant on fresh and dry weight of callus, callus RWC, callus RGR, and callogenesis index. The interaction of salinity and genotype had a significant effect only on the callogenesis index.

Mean comparisons (Table 6, Figure 4) revealed that the genotypes' response for each trait was variable. For callus dry weight, genotype Greece-III had the highest mean; and the lowest mean belonged to Greece-I.

In the present study, positive relationship was detected between callus dry weight with callus fresh weight (0.942**) and callus size (0.905**) (correlation table is not inserted). The correlation between callus fresh weight and callus size was positively significant (0.984*) and RGR of callus was negatively related with callogenesis index (–0.999*). There were no significant relationships among other traits.

3.3.2. Estimation of salinity stress-related indices for the callus dry weight

The stress tolerance indices (Table 7) showed that the highest values for MP, GMP, HARM, INTOL, STI, and SNPI belonged to genotype Greece-III, which showed high yield and yield stability and therefore reasonable salinity tolerance. Genotype Greece-III also showed the lowest value for SSI index, which underlined the high tolerance of this genotype to salinity stress. TOL and SSPI showed the highest value for Greece-III as well. In contrast, genotype Greece-I showed the lowest MP, GMP, and HARM indices. The lowest TOL and SSPI indices were observed for genotype Bangladesh. Genotype Greece-II also had the lowest INTOL, STI, and SNPI indices.

3.4. Cluster analysis

The studied genotypes were grouped based on the average of measured traits. Cluster analysis by Ward's minimum variance method and Euclidean distance measure divided the genotypes into two groups (Figure 5). The first cluster contained Iran, Greece-II, and Greece-III genotypes and the second cluster consisted of Bangladesh, Canada, and Greece-I genotypes. Cluster analysis correctly separated the genotypes in response to the salinity.

3.5. Plant growth parameters

Salinity did not affect callus dry and fresh weights. However, its effect on seedling dry and fresh weights was remarkable. The reduction percentage in both of seedlings fresh and dry weights was approximately 55% (Table 8). On the other hand, at 125 mM salinity, seedlings showed 55% reduction in salinity tolerance. Seedlings, unlike callus, displayed a reduction in growth rate. Seedling treated with 125 mM salinity showed 55% less tolerance than control ones. Dry matter production results from several physiological processes; the principal reason is low carbohydrates biosynthesis and availability (Hossain et al., 2009; Farooq et. al., 2009). NaCl salinity decreased the dry matter accumulation of seedlings, but did not change the callus dry weight (Tokarz et al., 2020). Thus, dry matter would be regarded as a tolerance biomarker for the abiotic stress factors (Piwowarczyk et al., 2016; Piwowarczyk et al., 2017). Crops respond to the salinity stress mainly via the changes in dry matter accumulation (Piwowarczyk et al., 2016; Okcu et al., 2005; Piwowarczyk et al., 2017; Eziz et al., 2017). Salinity interferes in plant growth and productivity by affecting seed germination, photosynthesis, nutrient uptake, and yield. For assessing the salinity tolerance, screening can be performed on the

Table 5. Analysis of variance for the in vitro calli traits of six grass pea genotypes exposed to salinity stress.

Source of variation	df	Callus size	Callus fresh weight	Callus dry weight	Callus RWC	Callus RGR	Callogenesis index
G	5	9.1	81.9**	6.6**	0.079**	2.08*	361.7*
S	1	64.4**	36.8	0.325	0.484**	4.24*	3066.4**
G×S	5	1.3	6.8	0.421	0.007	0.819	361.7*
E	34	4.0	24.9	0.087	0.019	0.784	136.2
CV %	-	21.19	12.67	13.94	1.5	8.85	12.71

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

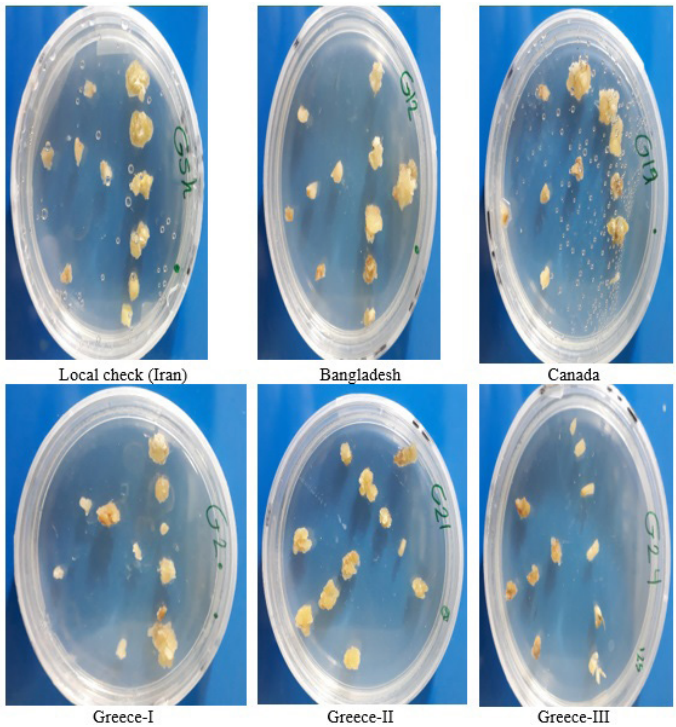

Figure 3. The effect of salinity on callus growth of grass pea genotypes in vitro.

Table 6. Mean comparisons with Duncan's multiple range test for the in vitro calli-related traits of six grass pea genotypes exposed to salinity stress.

Genotype origin	Callus fresh weight (g)	Callus dry weight (g)	RGR of callus (%)	RWC of callus (%)	Callogenesis index (%)
Iran	0.028ab	0.0023ab	14.14b	0.9186ab	83.3c
Bangladesh	0.0171b	0.0018bc	19.95a	0.8980c	89.5abc
Canada	0.0189b	0.0018bc	27.31a	0.9072bc	100.0a
Greece-I	0.0168b	0.0013c	17.78ab	0.9266a	97.6ab
Greece-II	0.026ab	0.0022abc	15.66ab	0.9197ab	95.8abc
Greece-III	0.0352a	0.0032a	13.03b	0.9057bc	85.4bc

Genotypes with similar letters had no significant differences.

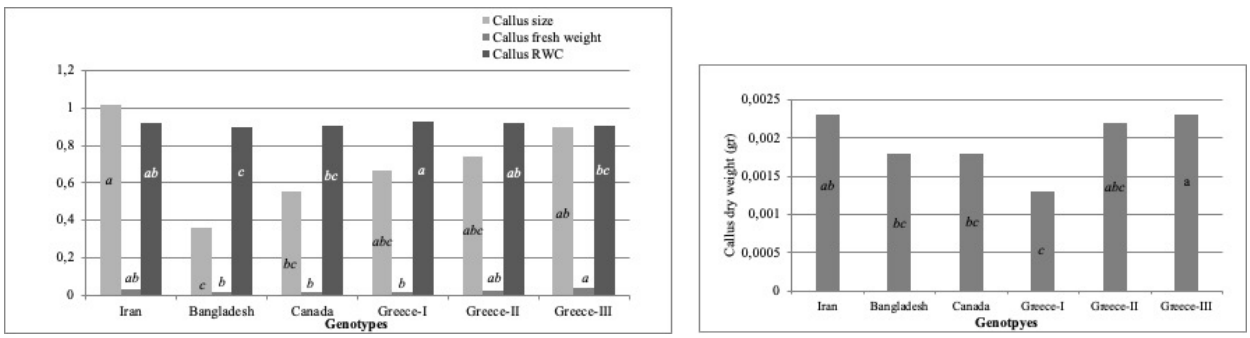

Figure 4. The effect of salinity stress in vitro on calli related traits of grass pea genotypes.

Table 7. Estimation of salinity tolerance indices for callus dry weight of 6 grass pea genotypes exposed to salinity stress in vitro.

Genotype origin	INTOL	TOL	MP	STI	GMP	HARM	SSI	RDI	SSPI	SNPI
Iran	0.640	–0.450	10.6	1.061	10.6	5.31	1.69	1.017	–2.209	–38.6
Bangladesh	0.409	–0.680	9.7	0.898	9.7	4.88	2.82	1.045	–3.339	–30.2
Canada	0.400	–0.430	10.1	0.967	10.1	5.07	1.69	1.017	–2.111	–36.9
Greece-I	0.640	–0.400	8.9	0.758	8.9	4.48	1.78	1.019	–1.964	–32.1
Greece-II	0.392	–0.340	10.5	0.048	10.5	5.27	1.28	1.007	–1.669	–42.0
Greece-III	0.989	0.740	11.7	1.303	11.7	5.884	–2.38	0.915	3.633	36.9

Index of tolerance based on RGR (INTOL), tolerance index (TOL), mean productivity (MP), stress tolerance index (STI), geometric mean productivity (GMP), harmonic mean (HARM), relative decrease in yield (RDI), stress susceptibility index (SSI), stress susceptibility percentage index (SSPI), stress/non- stress production index (SNPI)

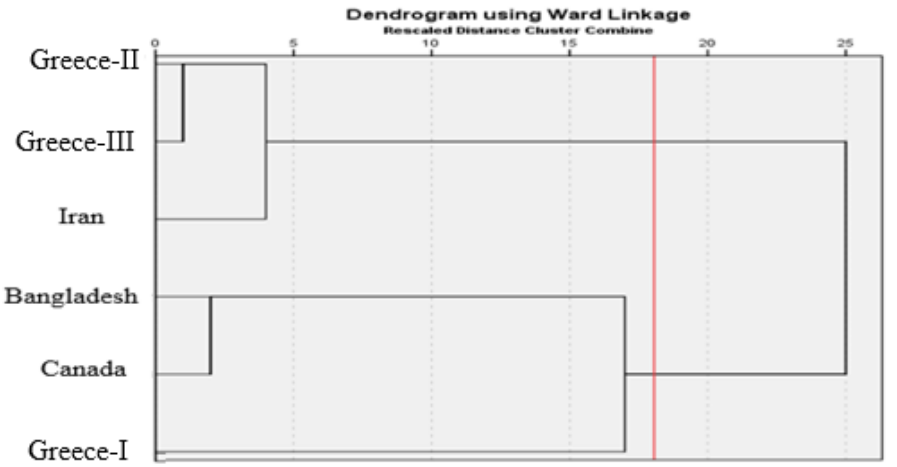

Figure 5. Cluster analysis of the six grass pea genotypes exposed to salinity stress.

Table 8. Tolerance index (percentage) of callus and seedlings of *Lathyrus sativus*. Callus and seedlings were treated with 0 and 125 mM of NaCl.

Salinity level (mM)	Seedlings			
	Fresh weight	%reduction	Dry weight	%reduction
0.00	0.25938	-	0.02100	-
125	0.11577	55.36	0.00954	54.57

basis of osmotic adjustment and biomass production. For decades, it has been known that salinity stress appears to be a polygenic and quantitative trait in nature and is dominantly regulated by several genes under diverse environments (Foolad, 2004).

4. Conclusions

The salinity tolerance of six preevaluated tolerant grass pea genotypes from the field conditions was assessed at the callus and seedling stages. Salinity affected seeds germination, probably due to the disturbances in water absorption under the high salt concentrations. However, a significant reduction in the seedling dry matter was registered, suggesting a low tolerance under saline conditions. Disorders affecting photosynthesis and resulting from water (osmotic) and chemical stress significantly impair the production of assimilates and their distribution in the plant. The results showed that genotype

Greece-III had the highest mean in most traits, especially in seedling dry weight. In this study, seedlings showed an imperceptible decrease in growth. Besides, evaluation of the salinity tolerance indices showed the same results; and in most indices, genotype Greece-III had the high tolerance and higher yields responses under salinity. It should be noted that only in STI and HARM indices, salinity tolerance of genotype Greece-I was recognized as superior. Genotype Greece-I had a high yield in nonstress conditions but not under saline conditions. It was found that Greece-III had a high yield and tolerance to the salinity of 125 mM among genotypes. The screening with the salinity treatment on callus and seedlings considering the tolerance indices showed that genotype Greece-III was the most salt-tolerant genotype and could be used in future studies for the genetic transformation and breeding programs.

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