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Original Research

Impact of priming on seed germination, seedling growth and gene expression in common vetch under salinity stress

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Abstract: Salinity is one of the most important abiotic stress factors that is expanding its influence because of global climate change and global warming. It causes gene expression changes, a reduction in seed germination and related characteristics, and poor seedling establishment in many crop plants by creating a lower osmotic potential in the seedbed and/or toxic ion effects in germinated seeds. In recent years, seed priming has been considered a promising strategy in modern stress management to protect plants against stress conditions. This study was conducted to elucidate the effects of osmopriming with polyethylene glycol 6000 (PEG-6000) on seed germination, seedling growth and gene expression in the common vetch (*Vicia sativa* L.) in different saline conditions. Common vetch seeds were primed with PEG-6000 solutions having different osmotic potentials (0.00, -0.50, -0.75, -1.00, -1.25, and -1.50 MPa) for 12 hours. Control (un-primed) and primed seeds were germinated and seedlings were grown in different saline conditions (EC= zero, 4, 8 and 16 dS m^{-1}). Furthermore, gene expression was compared in the primed seedlings in two different osmotic potentials (0.00 and -1.50 MPa) by microarray technology. Results demonstrated that germination percentage of common vetch seeds and seedling growth were diminished by high salinity. However, several priming treatments alleviated the adverse effects of high salinity on germination and early seedling growth of common vetch. The microarray showed that the expression of many genes in both stress and normal conditions was not significantly different.

Key words: Forage; Legume; *Vicia sativa*; Vigour; Gene expression.

Introduction

The ongoing global climate change causes extreme temperatures, drought and salinity, which are important stress factors in agriculture. These stress factors reduce the yield and cause unforeseeable crop loss in agriculture. Amongst these stress factors, salinity is one of the major environmental problems that restrict agricultural productivity, with adverse effects on germination, plant vigour and crop yield (1-3). Increased salinity around the world is expected to become a more serious problem for agricultural production in the next decades, especially in arid and semi-arid regions (4, 5). According to FAO estimates, currently more than 900 million hectares of land throughout the world, constituting about 6 percent of the world's total land, are affected by salinity (6). On the other hand, about 20 percent of the irrigated lands with high productivity worldwide are salt-affected (2).

Salinity stress causes changes in biochemical, physiological and metabolic functions in most plants. These changes negatively affect the plant performance and therefore the yield. Salt stress prevents or delays seed germination and seedling establishment by creating a lower osmotic potential in a seedbed and/or toxic ion effects in germinated seeds. Seed germination and seedling establishment are important, and are the weakest stages of crop plants against adverse environmental conditions (3, 7, 8). Inadequate/delayed seed germination and weak seedling growth cause meagre stand establishment in salt-affected soils. This is one of the main reasons for the low yield per hectare of field crops. Therefore, improving germination and early seedling development for good stand establishment could help to ensure high crop yield in salt-affected soils (3).

Improvement of salinity-resistant/tolerant genotype is one of the best remedies for protection from the adverse effects of high salinity and it can help to increase productivity in salt-affected soils (9-11). Breeding for salinity resistance/tolerance has been the focus of interest of many researchers and until today, considerable improvements in salt tolerance of certain important crop species such as barley, rice, pearl millet, maize, sorghum and alfalfa have been achieved (12-14). However, genetic progress in breeding for tolerance to this stress has been slow, as salt tolerance is complex, multigenic and quantitative in character and information is lacking on how most genes function in concert with other genes that may influence the mechanisms of salt tolerance (11, 15-17). In addition to genetic complexity, it is physiologically complex and has high interactions with environmental factors. When all these factors are considered together, breeding for salt tolerance is not an easy process (11, 18). Hence, the development of salt

tolerant genotypes by conventional breeding methods is time-consuming, labour-intensive and costly. Therefore, fast, easy and inexpensive approaches are necessary to prevent or alleviate the damage caused by salt stress in plants. In recent years, one of the most remarkable methods among these approaches is seed priming (19).

Seed priming is a pre-sowing treatment that partially hydrates seeds without allowing radicle emergence (20-22). In recent years, seed priming has emerged as a promising approach in modern stress management to protect plants against both biotic and abiotic stress conditions (19, 23, 24). Many researchers have shown that priming treatments increase seed germination percentage and provide faster and uniform germination in adverse environmental conditions in certain crops such as sunflower (25), wheat (26), barley (27), maize (28), rice (29), and vetch (30). Because of these positive effects, higher seedling emergence rate, better seedling growth and better stand establishment are achieved in the field. Thus, young plants can start the race one step ahead of adverse environmental conditions. There are different seed priming methods such as hydropriming, osmopriming, chemical priming, hormonal priming, biological priming, redox priming and solid matrix priming (24). Osmopriming, which is one of the more commonly used methods, is the soaking of seeds in aerated, low-water-potential solutions. Various chemicals are used to compose these low-water-potential solutions. Polyethylene glycol 6000 (PEG-6000), which is non-toxic for seeds, has a large molecular size and lowers water potential without penetrating into the seeds, is more commonly used as a chemical for lowering water potential (31).

While seed priming treatments have been widely used for at least two decades in vegetable and flower seeds (32), their use in field crops is more recent (3, 14, 20). Common vetch (Vicia sativa L.) originated from southern Europe and is now widely distributed in different regions of the world such as Europe, the Mediterranean basin, West and Central Asia, China, Eastern Asia, India and USA (33). The sowing area of vetches has decreased in the last 40-50 years. However, in recent years vetch cultivation has been increasing in the Mediterranean region and in other areas with a Mediterranean climate where average annual precipitation ranges from 250 to 350 mm. Common vetch is a forage legume that is moderately sensitive to soil salinity and is damaged by high salinity (34, 35). Certain pre-treatments before sowing can increase germination of common vetch (30, 36). Unfortunately, priming studies of common vetch under stress conditions are limited.

This study was conducted to investigate the effects of osmopriming with PEG-6000 at various concentrations on germination characteristics, early seedling growth and gene expression in common vetch in saline medium. It is aimed to contribute to higher and sustainable forage production in salt-affected lands by utilizing the results of the study.

Materials and Methods

This study was carried out at the Department of Field Crops, Faculty of Agriculture, Akdeniz University, Antalya, Turkey and Zagros Bioidea, Kermanshah, Iran. The common vetch cultivar, Gülhan, was used as seed material. Germination and seedling growth of the cultivar seeds primed with PEG-6000 were studied under salinity conditions.

Seed treatments: seeds were surface sterilized by dipping in sodium hypochlorite (5%) solution for 5 minutes and dried on filter paper. Surface-sterilized seeds were primed in aerated PEG-6000 solutions with osmotic potentials of zero (distilled water), -0.50, -0.75, -1.00, -1.25 and -1.50 MPa for 12 hours at 20 °C in the dark. Osmotic potential of PEG-6000 solutions was adjusted according to Michel and Kaufmann (37). After priming treatments for the stated durations, seeds were washed with tap water for 3 minutes and rinsed three times with distilled water. Thereafter, the seeds were dried at 27 °C on filter paper for 24 h up to their initial moisture content. Untreated dry seeds were taken as control.

Germination tests: 25 control and primed seeds for each treatment were placed in 90 mm (Ø) glass Petri dishes on a layer of filter paper (Schleicher & Schuell-blueband). Petri dishes were moistened with 10 ml of salt (NaCl; Merck-1064060250) solution and the dishes were sealed with parafilm to prevent evaporation. Four different salinity levels (EC= zero, 4, 8 and 16 dS m^{-1}) in salt solutions were used as germination media. After that, the seeds were incubated at 20 °C constant temperature, 70% relative moisture and a 14/10-hour light/dark photoperiod in a growth chamber for germination for 7 days. The seeds were considered as germinated when a radicle 2 mm long had emerged through the seed coat. Filter paper and salt solution were replaced with new one on the third day. The experiment was laid out according to a completely randomized design with three replications. The number of germinated seeds was recorded at 24-hour intervals, and recording continued until no further germination occurred. The following characteristics were calculated using germinated seed counts obtained at the end of the germination test.

Germination percentage (GP) was calculated for each treatment using the formula:

$$GP = \frac{Number\ of\ Germinated\ Seeds}{Number\ of\ Total\ Seeds}\ x\ 100$$

Mean germination time (MGT) was calculated according to the following equation (38):

$$MGT = \frac{\sum Dn}{\sum n}$$

where D is the number of days counted from the beginning of germination and n is the number of seeds, which were germinated on day D.

The coefficient of uniformity of germination (CUG) was calculated using the following formula (39).

$$CUG = \frac{\sum n}{\sum[(MGT - t)^2 n]}$$

where t is the time in days, starting from day 0, the day of sowing and n is the number of seeds completing germination on day t.

The germination rate (GR) was calculated as described by the Association of Official Seed Analysts (40) by the following formula:

$$GR = \frac{Germination\ percentage\ for\ days\ of\ first\ count}{Days\ of\ first\ count} + \cdots + \frac{Germination\ percentage\ for\ days\ of\ final\ count}{Days\ of\ final\ count}$$

Seedling growth: The germinated seeds were taken from the Petri dishes and placed in transparent PVC boxes (100x70x100 mm) with a layer of filter paper after every count, and 10 ml of the same salt solution was added for seedling growth. The seedlings were grown in these boxes until the 14th day at the same condition. Five seedlings were harvested in each box at the end of 14 days and the following traits were evaluated:

Seedling shoot and root fresh weights (mg seeding^{-1}) were measured. Seedlings were oven-dried at 65°C for 48 hours to determine seedling dry weight. Seedling shoot and root lengths (mm) were measured. The seedling shoot and root lengths were converted from mm to cm for seedling vigour calculation and vigour index were determined as follows (41):

Vigour Index (VI)= Germination Percentage (%)×Seedling length (cm)

In the current experiment, gene expression was investigated in the seedlings by microarray technology. Gene expression was conducted by the Zagros Bioidea Company. Furthermore, gene expression was compared in the primed seedling in two different osmotic potentials (0.00 and -1.50 MPa) by microarray technology in vetch (*Vicia sativa* L.).

Microarrays were designed by Imaxio (Clermont Ferrand, France; http://www.imaxio.com/index.php) which has been accredited by Agilent Technologies (Palo Alto, CA, USA; http://www.home.agilent.com/agilent/home.jspx). Based on 8623 annotated unigenes for Hypericum sp., 60-mer probes were designed using eArray software and custom 8 x 15K Oligo Microarray manufactured by Agilent. Value distribution allowed us to calculate and view the distribution of the values for the samples. Median-centred values were indicative that the data were normalized and cross-comparable.

Statistical analysis was carried out using Minitab-16 software for Windows. Multiple comparisons of main effects and their interactions were performed using least square means with Tukey's adjustment ($p<0.05$).

Results

The effects of priming treatments, salinity and interaction of priming treatment x salinity level of germination-growth medium on germination percentage (GP), mean germination time (MGT), germination rate (GR), shoot-root lengths (SL, RL), shoot-root dry weights (SW, RW) and vigour index (VI) of common vetch were significant ($p<0.01$). The coefficient of uniform germination (CUG) was also insignificantly affected by priming treatment but was significantly affected by salinity level of germination and growth medium ($p<0.05$) and interaction of priming treatment x salinity level of germination and growth medium ($p<0.01$). (Table 1).

Tukey multiple comparison test results showed that there were significant differences amongst the mean values of different salt levels for all properties examined in the study ($p<0.05$).

The results of germination properties suggested that the highest mean GP was obtained at zero and 4 dS m^{-1} salt levels, while lowest GP was determined at 16 dS m^{-1} salt level. The longest MGT was counted at 16 dS m^{-1} salt level, while the shortest was detected at 4 dS m^{-1} salt level. The highest and lowest CUG values were determined at the salt levels of 4 and zero dS m^{-1}, respectively. In the GR property, the highest value was obtained at 4 dS m^{-1} and the lowest value at 16 dS m^{-1} salt levels (Figure 1).

In the seedling development, measured values of shoot length and shoot dry weight, root dry weight and vigour index increased at 4 dS m^{-1} salt level and the highest values were determined at 4 dS m^{-1} salt level. The values of these properties were significantly decreased at higher salinity levels than 4 dS m^{-1} and the lowest values were determined at 16 dS m^{-1} salt level. In the root length, the longest roots were measured at zero salt level, while root length decreased as the salt level increased and the shortest root length was measured at

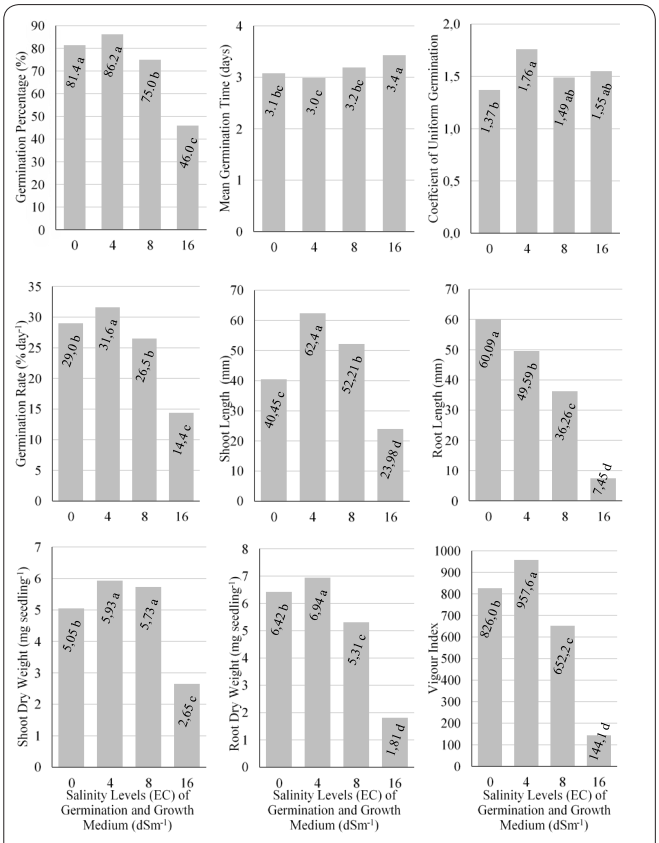

Figure 1. Effects of salinity levels on germination percentage (%), mean germination time (days), coefficient of uniform germination, germination rate (% day^{-1}), shoot length (mm), root length (mm), shoot dry weight (mg seedling^{-1}), root dry weight (mg seedling^{-1}) and vigour index of common vetch.

Table 1. Variance analyses of studied parameters.

Variation Source	GP[1]	MGT	CUG	GR	SL	RL	SW	RW	VI
Priming Treatment (T)	**	**	ns	**	**	**	**	**	**
Salt Concentration (S)	**	**	*	**	**	**	**	**	**
TxS	**	**	**	**	**	**	**	**	**

*, **, ns: Statistically significant at $p<0.05$, at $p<0.01$ and non-significant, respectively. 1 GP: Germination percentage, MGT: mean germination time, CUG: Coefficient of uniform germination, GR: Germination rate, SL: Shoot length, RL: Root length SW: Shoot dry weight, RW: Root dry weight, VI: Vigour index.

16 dS m^{-1} salt level (Figure 1).

The results demonstrated that differences amongst the means of priming treatments were significant for all traits except CUG. The highest GP was obtained in the control (un-primed seeds) and the seeds primed at -1.25 MPa osmotic potential. The lowest GP was determined in seeds primed at 0 MPa osmotic potential (hydropriming). The shortest MGT and the highest GR were determined in the control group (Figure 2).

In traits related to seedling development, it was determined that priming applications gave better results than the control. Mean values of shoot-root lengths and shoot-root dry weights and vigour index were determined to be higher for priming treatments than the control. The highest shoot length and shoot dry weight were obtained in seeds primed at -1.5 MPa osmotic potential. The longest root length was measured in seeds primed at -1.00 and -1.25 MPa osmotic potential. The highest root dry weights were recorded in seeds primed at -0.50, -0.75, -1.00, -1.25 and -1.50 MPa osmotic potential. Similarly, the highest vigour index values were determined in seeds primed at-0.75, -1.00, -1.25 and -1.50 MPa osmotic potential (Figure 2).

Significant priming treatment x salt level interaction showed that the effects of priming treatments varied according to the salt level of germination and growing medium. GP of seeds primed at -1.00 and -1.50 MPa osmotic potential were higher than the control for zero dS m^{-1} salinity level. GP of seeds primed at -1.00 and -1.25 MPa osmotic potential was higher than the control for 4 dS m^{-1} salinity level. GP of seeds primed at -0.75 and -1.25 MPa osmotic potential was higher than the control for 16 dS m^{-1} salinity level. However, GP of all priming treatments was lower than the control for 8 dS m^{-1} salinity level. MGT was significantly higher for all priming treatments compared to the control at all salinity levels. While some priming treatments increased CUG compared to the control at zero and 8 dS m^{-1} salt levels, CUG was lower than the control at 4 and 16 dS m^{-1} salt levels. It was determined that the mean GR values of the control were higher than the mean values of the priming treatments at all salt levels (Table 2).

At all salinity levels, priming treatments significantly increased shoot-root length, shoot-root dry weight and vigour index compared to the control. Despite germination of seeds, no shoot and root development was observed in control (un-primed) seeds and seeds primed at 0 MPa for 16 dS m^{-1} salinity level of germination media. The highest SL was measured in seeds primed at -0.75 MPa, 0 MPa, -1.5 MPa and -1.00 MPa osmotic potential for zero, 4, 8 and 16 dS m^{-1} salinity levels, respectively. While the longest RLs were observed in seeds primed at -1.25 MPa osmotic potential for zero, 8 and 16 dS m^{-1} salinity levels and at -1.00 MPa osmotic potential for 4 dS m^{-1} salinity level. The highest SWs were recorded in seeds primed at 0 MPa osmotic potential for zero and 8 dS m^{-1} salinity levels, at -1.50 MPa for 4 dS m^{-1} and at -1.00 MPa osmotic potential for and 16 dS m^{-1} salinity levels. The highest RWs were determined in seeds primed at -1.25 MPa osmotic potential for zero and 8 dS m^{-1} salinity levels, at -1.50 MPa osmotic potential for 4 dS m^{-1} and at -1.00 MPa osmotic potential for 16 dS m^{-1} salinity levels. The highest VI was calculated in seeds primed at -1.00 MPa osmotic potential for zero and 4 dS m^{-1} salinity levels, at -1.25 MPa osmotic potential for 8 dS m^{-1} salinity level and at -0.75 MPa osmotic potential for 16 dS m^{-1} salinity level (Table 2).

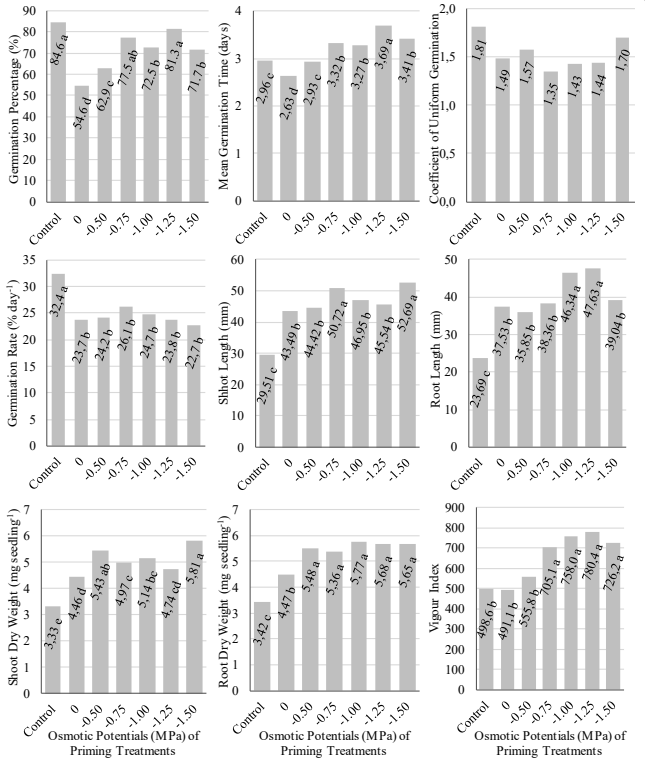

Figure 2. Effects of priming treatments with PEG-6000 germination percentage (%), mean germination time (days), coefficient of uniform germination, germination rate (% day^{-1}), shoot length (mm), root length (mm), shoot dry weight (mg $seedling^{-1}$), root dry weight (mg $seedling^{-1}$) and vigour index of common vetch.

Investigation of gene expression: Of the 8623 studied genes, expression of 5054 genes had no significant difference between seedlings in the two conditions (normal and saline), while 3569 genes showed a significant difference. Of these, 2783 genes in the saline and 786 genes in the normal conditions showed a more significant expression. Some genes were significantly up- or down-regulated in salinity condition in common vetch.

Some genes had a significantly higher expression in the saline and normal conditions. Gene expression data showed that ascorbate peroxidase (APX), glutathione-S-transferase (GST), thioredoxin (TXN), high affinity potassium transporter protein 1 (HAK1), salt overly sensitive 1 (SOS1), Metip1, Metip2, Metip3, Metip6, Metip7, Metip8, salicylic acid-dependent *PR-1,* salicylic acid-dependent *PR-5,* abscisic acid (ABA)-dependent *RAB-18, RD-29A,* ABA-inducible genes, *RD29A, RAB18*, AREB, TAS14, NCED3, CRK1, bZIP, WRKY, AP2, NAC and C2H2 zinc finger genes showed higher expression in seedlings in salinity condition. Some genes such as RBOH1, APX2, MAPK2, ERF5, MAPK3 and DDF2 were down-regulated in salinity condition.

The highest gene expression in the seedlings in salinity condition was found in SOS1, which showed an expression 119 times higher than in normal condition; and the most down-regulation was related to MAPK3, which showed an expression 154 times lower.

Discussion

Germination and early seedling development of

Table 2. Priming treatment x salinity level interaction for germination percentage, mean germination time, the coefficient of uniform germination, germination rate, shoot and root lengths, shoot and root dry weights and vigour index mean values of common vetch.

Osmotic Potential of Priming Treatment	Germination Percentage (%)				Mean Germination Time (day)				Coefficient of Uniform Germination			
	EC (dS m^{-1})				EC (dS m^{-1})				EC (dS m^{-1})			
	0	4	8	16	0	4	8	16	0	4	8	16
Control	93.3a-d*	93.3a-d	95.0abc	56.7ijk	3.09b-h	2.75f-i	2.89d-i	3.11b-h	1.08bc	2.71a	1.47abc	1.99abc
0 MPa	53.3jk	61.7g-j	65.0g-j	38.3kl	2.73f-i	2.68ghi	2.27i	2.83e-i	0.79c	1.33bc	1.91abc	1.95abc
-0.50 MPa	66.7f-j	86.7a-e	60.0hij	38.3kl	2.67hi	2.77f-i	3.19b-h	3.11b-h	1.60abc	1.42bc	2.18ab	1.09bc
-0.75 MPa	85.0a-f	78.3c-h	80.0b-g	66.7f-j	3.20b-h	3.00c-h	3.34b-g	3.74b	0.92bc	2.19ab	1.20bc	1.10bc
-1.00 MPa	100.0a	96.7abc	66.7f-j	26.7 l	3.03c-h	3.37b-f	3.51bcd	3.17b-h	1.74abc	1.05bc	1.08bc	1.83abc
-1.25 MPa	75.0d-i	98.3ab	88.3a-e	65.0g-j	3.52bcd	3.32b-h	3.49b-e	4.42a	1.60abc	1.79abc	1.38bc	1.01bc
-1.50 MPa	96.7abc	88.3a-e	70.0e-j	31.7 l	3.33b-h	3.05c-h	3.66bc	3.62bc	1.85abc	1.83abc	1.21bc	1.90abc

	Germination Rate (% day^{-1})				Shoot Length (mm)				Root Length (mm)			
	EC (dS m^{-1})				EC (dS m^{-1})				EC (dS m^{-1})			
	0	4	8	16	0	4	8	16	0	4	8	16
Control	36.6a	38.2a	35.4abc	19.3ghi	36.33ijk	48.54efg	33.17jk	-	46.72d-h	34.05gh	14.00 ij	-
0 MPa	19.4ghi	26.6c-g	34.3a-d	14.5hij	37.47h-k	70.83a	65.67ab	-	46.83d-h	55.33b-e	47.93c-h	-
-0.50 MPa	27.8b-g	35.8ab	19.9ghi	13.4ij	39.00g-k	63.67abc	52.17def	22.83l	54.00c-f	49.33c-g	30.92hi	9.17j
-0.75 MPa	31.5a-e	27.6b-g	26.1d-g	19.3ghi	44.50f-i	64.23ab	54.17cde	40.00g-k	57.00b-e	50.00c-g	36.43fgh	10.00j
-1.00 MPa	34.9abc	32.0a-d	23.1e-h	8.9j	42.50g-j	59.15bcd	44.17f-i	42.00g-j	72.00ab	63.67a-d	39.85e-h	9.83j
-1.25 MPa	22.1f-i	31.1a-e	27.1b-g	15.1hij	43.67f-i	60.15bcd	46.33e-h	32.00kl	79.10a	45.42e-h	53.83c-f	12.17j
-1.50 MPa	30.4a-f	30.2a-f	19.7ghi	10.4j	39.67g-k	70.25a	69.83a	31.00kl	65.00abc	49.33c-g	30.83hi	11.00j

	Shoot Dry Weight (mg seedling^{-1})				Root Dry Weight (mg seedling^{-1})				Vigour Index			
	EC (dS m^{-1})				EC (dS m^{-1})				EC (dS m^{-1})			
	0	4	8	16	0	4	8	16	0	4	8	16
Control	4.97fgh	4.83fgh	3.51jk	-	5.88 cde	5.60 de	2.19f	-	774.8def	771.6def	448.1hij	-
0 MPa	5.43d-g	5.63c-g	6.77ab	-	6.40 a-e	6.42 a-e	5.04e	-	449.1hij	775.5def	739.9ef	-
-0.50 MPa	5.67c-g	6.35bcd	6.07b-e	3.65 ijk	6.32 a-e	7.67 ab	5.95b-e	1.99 f	619.0fgh	979.5a-d	501.5g-j	123.0kl
-0.75 MPa	4.32hij	6.42bcd	5.83b-f	3.31 jk	5.97 a-e	7.37 abc	5.51e	2.59 f	866.1cde	894.5cde	724.6efg	335.2ijk
-1.00 MPa	4.98e-h	5.35d-h	5.52d-g	4.70ghi	6.47 a-e	7.42 abc	5.93b-e	3.27 f	1145.0ab	1187.7a	561.9f-i	137.5kl
-1.25 MPa	4.82fgh	5.23e-h	5.73b-g	3.19 k	7.30 a-d	6.43 a-e	6.66a-e	2.34 f	917.6b-e	1038.6abc	884.1cde	287.3jk
-1.50 MPa	5.20e-h	7.68a	6.68abc	3.69 ijk	6.60 a-e	7.70 a	5.85cde	2.44 f	1010.6abc	1056.1abc	705.5efg	132.8kl

*: No significant differences between means shared the same letter for each trait.

common vetch are critical stages that determine yield potential in adverse environments. Salinity is one of the main abiotic stress factors. Common vetch is a forage legume moderately sensitive to salinity. Therefore, germination and early seedling development of common vetch can be restricted by salinity. The results of this study demonstrated that high salinity adversely affected germination percentage, early seedling growth and related traits of common vetch. Salt stress prevents or delays seed germination and seedling establishment by creating a lower osmotic potential in a seedbed and/or toxic ion effects in germinated seeds.

The slightly saline germination medium (EC=4 dS m^{-1}) had no negative effect on the characteristics of the germination and seedling growth investigated in this study. Restrictive effects of salinity were observed at higher salinity grades (EC=8 and 16 dS m^{-1}), especially on seedling growth rather than seed germination. Therefore, root and shoot development was not recorded for control and hydropriming treatments at high saline germination medium (EC=16 dS m^{-1}). However, some priming treatments used in this study ensured the growth of roots and shoots of germinated seeds at moderate (EC= 8 dS m^{-1}) and high salinity (EC=16 dS m^{-1}) levels. These results showed that it could be misleading to evaluate only the germination percentage in such studies. Almansouri et al. (42) reported that germination percentage was not a valuable selection criterion for increase in salinity stress. In addition, the results of other previous studies demonstrated that high salinity affected seedling growth more than germination (43, 44). High salinity firstly reduces cell elongation and division due to the osmotic effect created around the root. High salinity secondly causes Na^+ toxicity and ion imbalance, which damages cells in the transpiring leaves. Hence, seedling growth declines in high salt-containing environments (45, 46).

In such studies, it is difficult to evaluate the results over many properties. For this reason, rather than deciding on one feature, it is necessary to propose a solution that takes into account a large number of features. Both the germination percentage and seedling growth values were used when calculating the vigour index. Therefore, it could be more appropriate to reach a judgment based on the vigour index values. As root and shoot development was not recorded for control and hydropriming (0 MPa osmotic potential) treatments in high saline germination medium (EC=16 dS m^{-1}) vigour index could not be calculated. Our results showed that priming treatments with PEG-6000 alleviated the detrimental effects of salinity and supported especially seedling growth in high saline germination medium. Previous studies by several researchers demonstrated that high salinity negatively affected germination and seedling growth in several plants. However, different priming treatments at appropriate doses and durations eliminated or mitigated these adverse effects of salinity (27, 47-50). The better germination and/or more vigorous seedling growth in the primed seeds could be attributed to the increase of antioxidant capacity, higher starch metabolism and higher nontoxic metabolite synthesis (24, 51, 52).

Observations showed that the expression of many genes was not affected by salt stress conditions. That is, their expression in both stress and normal conditions was not significantly different. Some genes have higher expression in stress conditions. This increase in expression is either a plant's response or its resistance to salinity. If it is proved that these genes cause resistance to salt stress, they can be transferred to susceptible species or cultivars. Some genes showed lower expression in salt stress conditions. In this case, it must also be considered whether their silencing causes resistance to salinity. In this case, it is possible to induce resistance to salinity in plants by silencing specific genes such as RNAi and with anti-sense techniques.

In conclusion, results demonstrated that high salinity decreased the germination percentage and early seedling growth of common vetch. However, priming treatment alleviated the adverse effects of high salinity on germination and early seedling growth of common vetch. Since both the germination percentage and the seedling growth were accounted for, we evaluated the results based on the vigour index, and concluded that vetch seeds could be primed at -1.25 MPa osmotic potential and at -0.75 MPa osmotic potential for moderate (EC=8 dS m^{-1}), and high saline (EC=16 dS m^{-1}) environments, respectively. In addition, it can be said that for such studies, instead of germination of seeds in Petri dishes, the seeds could be planted at the real sowing depth and that the results could then be evaluated based on seedling emergence characteristics, more confidently.

Conflicts of Interest

The authors declare no conflict of interest.

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