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Comparison of Biochemical Responses of Common Vetch (*Vicia sativa* L.) Seedling Organs to Salinity

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ABSTRACT

Background: Shoots and roots are autotrophic and heterotrophic organs of plants with different physiological and biochemical functions under stress conditions. The metabolites involved in tolerance enhancement differed between roots and shoots. In this study, the biochemical changes occurring in shoot and root organs under salt stress and the level of these changes were investigated. However, these changes in shoot and root organs were compared.

Methods: Seeds of common vetch were sown and subjected to 14 days of salt stress in basal MS medium containing 100 mM NaCl. In shoot and root tissue, biochemical parameters such as antioxidant enzymes activities (GR, APX, SOD and CAT), malondialdehyde (MDA) content and proline accumulation were determined.

Result: Results of the study indicated that the activities of antioxidant enzymes (SOD, CAT (except in shoot), GR and APX), MDA and proline accumulation enhanced by salt stress in both organs. On the other hand, morphological parameters decreased in both tissues. It seemed that antioxidant enzyme activities more active in root tissues. However, proline accumulation was found higher in shoot tissues than root tissue, while MDA content was higher in root tissue than shoot tissue. The present investigation provides essential information for the antioxidant components of the shoot and root organs of vetch seedlings under salt stress.

Key words: Antioxidant enzymes, *In vitro* culture, Malondialdehyde (MDA), Proline, Salt (NaCl) stress.

INTRODUCTION

Soil salinization is one of the most severe environmental problems in reducing plant growth and yield (Orak and Ateş, 2005; Desokya *et al*., 2018). About 80 million ha of arable lands worldwide affected by salinization and these areas continue to expand (Munns and Tester, 2008; Flowers *et al*., 2010). In arid and semi-arid regions, the situation is getting worst due to water scarcity. The higher evapotrans piration rates, aggravating the effects of salinity causes more water-loss in plants as a result of high temperature (Abdelgawad *et al*., 2016).

Different responses such as morphological, physiological, biochemical and molecular changes can induce in plants by salt stress (Abdelgawad *et al*. 2016; Ambede *et al*. 2012; Abreu *et al*. 2013). Stress conditions can increase the generation of reactive oxygen species (ROS). ROS harm biological systems due to formation oxidation for lipids, proteins, deoxyribonucleic acid and carbohydrates (Kavas *et al*., 2013). The antioxidant system protects plant cells from these harmful effects of ROS (Farooq *et al*., 2009; Pıwawarczyk *et al*., 2017). These systems considering antioxidant enzymes such as ascorbate peroxidase (APX), superoxide dismutase (SOD), glutathione reductase (GR) and catalase (CAT), however, incorporate low-molecular-mass antioxidants such as proline (Dugasa *et al*., 2019).

Common vetch (*Vicia sativa* L.) is an annual winter growing leguminous forage plant containing high protein, minerals and vitamins for livestock (Ertekin *et al*., 2017). Plants differ in their responses to salt, including common vetch (Orak and Ateş, 2005). Although the large extent of studies concerning salt stress (Orak and Ateş, 2005; Ertekin *et al*., 2017; Bilgili *et al*., 2011; Ertekin *et al*., 2018) for morphological parameters, there has been little study (Kusvuran *et al*., 2015) to date of the biochemical analyses of common vetch seedlings that grow under salt stress.

Plants are likely to react to adverse environmental conditions not only by the exchange of biomass of the shoot and root organs but also by altering the metabolic activity of these organs. We hypothesized that different organs may respond differently to salt stress and trigger specific defense mechanisms. We, therefore, conducted the present investigation to reveal the levels of the biochemical responses and compared to biochemical responses of shoots and roots of vetch seedlings under salt stress conditions.

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MATERIALS AND METHODS

Surface sterilization of seeds, salt stress treatment and culture conditions

Vetch seeds were rinsed with a 50% commercial bleach

solution (containing 5% sodium hypochlorite) for 20 minutes. After washing 3 times with distilled water, the seeds were moved to MS (Murashige and Skoog, 1962) basal medium containing 3% sucrose and 0.6% agar. Salt stress was induced by sodium chloride (NaCl) at a concentration of 100 mM to the MS medium basal. The cultures were maintained at 24±1°C with 16-hour photoperiod and 8 hours of dark, light provided with florescent lamps at an intensity of 27 µmol m^{-2} s^{-1}. Physio-biochemical analyses were performed in the shoot and root tissues that were formed in 14 days after inoculation of the seeds on the medium.

Morphological Parameters

Length (cm), wet-dry weight (g) of root and shoot organs were measured in 10-day seedlings. For measurement of dry weights, samples placed in a drying oven at 105°C for 3 h. The ratio (%) of dry weight/wet weight used for calculation of the dry matter (DM). Morphological parameters were measured in three replicates (10 seedlings per repetition) for each (control and salt) treatment.

Biochemical analysis

Determination APX activity

Ascorbate peroxidase (APX) activity in shoot and root tissues of treated and control plants were determined by a spectrophotometric assay (Cakmak and Marschner, 1992; Cakmak *et al*., 1995). 0.5 g fresh tissue was homogenized with liquid nitrogen and the powder was suspended with 8ml of a 50-mM phosphate buffer solution (pH 7.6). The suspension was centrifuged at 15,000 rpm for 15 minutes at ±4°C and supernatant was used for the enzyme assay. Assay solution contained a 50-mM phosphate buffer containing 0.1 mM of EDTA, 12 mM of H_2O_2 containing 10 mM of EDTA, 0.1 ml of 0.25 mM of L-ascorbic acid and enzyme extract, in a final volume of 1 ml. The decrease in ascorbate concentration was recorded at 290 nm (E = 2.8 mM^{-1} cm^{-1}) with Schimadzu UV-mini 1240 spectrophotometer. The blank solution is prepared with an assay mixture without adding enzyme extract.

Determination SOD activity

Superoxide dismutase activity determination was done according to the spectrophotometric method of Çakmak and Marschner (1992) and Çakmak *et al*. (1995). 0.5 g of the shoot and root tissues were homogenized with liquid nitrogen and the powders were suspended in 8 ml of suspension solution containing 50 mM (pH: 7.6) phosphate (P) buffer. The assay medium consists of a 50 mM (pH: 7.6) phosphate (P) buffer, the enzyme extract (25 to 100 µl) 0.5 ml of 50 mM Na_2CO_3 (pH 10.2), 0.5 ml of 12 mM of L-methionine, 0.5 ml of 75 µM of p-nitro blue tetrazolium chloride (NBT) and 10 µm of riboflavin. The decrease in NBT (nitro blue tetrazolium chloride) by O_2^- under light was recorded at 560 nm with Schimadzu 1240-UV mini spectrophotometer. The blank solution was prepared with an assay mixture without adding enzyme extract.

Determination GR activity

Glutathione reductase activity was determined according to the method of Çakmak and Marschner (1992) and Çakmak *et al*. (1995). 0.5 g fresh shoot and root tissues of control and treated plants were homogenized with liquid nitrogen and the powder was suspended with 8 ml of a 50-mM phosphate buffer solution (pH 7.6). The GSSG-dependent oxidation of NADPH was monitored by a decrease in absorbance at 340 nm (E = 6.2 mM^{-1} cm^{-1}). The assay mixture contained a 50-mM phosphor buffer (pH 7.6), 0.1 ml of 0.5 mM of oxidized glutathione (GSSG), 0.1 ml of 0.12 mM of NADPH and enzyme extract, in a final volume of 1 ml. The blank solution was prepared with an assay mixture without adding enzyme extract.

Determination of CAT activity

Catalase activity was determined according to the method of Çakmak and Marschner (1992); Çakmak *et al*. (1995). 0.5 g fresh shoot and root tissues of control and treated plants were grounded with liquid nitrogen by using cold mortar and pestle. The powders were then suspended in a suspension solution containing 8 ml of a 50-mM phosphate buffer solution (pH 7.6). The suspension centrifuged at 15,000 rpm for 15 minutes at ±4°C. The supernatant part used for the enzyme assay. Assay medium contained a 50 mM phosphate buffer (pH 7.6), 0.1 mM of EDTA, 0.1 ml of 100 mM of H_2O_2 and enzyme extract, in a final volume of 1 ml. The decrease in absorbance of the rate of H_2O_2 was recorded at 240 nm (E = 39.4 mM^{-1} cm^{-1}) by using Schimadzu UV-mini 1240 spectrophotometer. The blank solution was prepared with an assay mixture without adding enzyme extract.

Measurement lipid peroxidation (MDA content) and proline

The amount of malondialdehyde (MDA) was determined by Lutts *et al*. (1996). According to this method, 200 mg fresh sample (root and shoot tissues) was taken from frozen samples at -80°C, 5 ml of 0.1% acetic acid (TCA) was added and this mixture was centrifuged at 12500 rpm for 20 minutes. 3 ml supernatant was taken from 5 ml extract and 3 ml TCA containing 20% thiobarbutyric acid (TBA) (w/v) was added. The absorbance values of 532 and 600 nm were read on the spectrophotometer (Shimadzu UVmini-1240). The amount of MDA in shoot and root tissues was determined as µmol g^{-1} FW.

Proline analysis was performed by Bates *et al*. (1973). Fresh shoot and root samples (approximately 0.5 g) were homogenized with 3% sulfosalic acid. The filtered samples were reacted with ninhydrin in a water bath set at 100°C for 1 hour and the samples were taken to ice bath and the reaction was completed. After cooling, the medium was extracted with 4 ml of toluene. The pinkish-red sample monitor at 520 nm (Shimadzu UVmini-1240). The units of µmol g^{-1} FW was expressed for the content of proline in shoot and root tissue.

Plant tissue culture and biochemical analyzes were carried out in 2019 at the laboratories of the biotechnology institute of Ankara University (Ankara, Turkey).

Statistical analysis

The study was designed with a completely randomized block design with 3 replications. The Independent-Samples t-test of SPSS 22 was performed to analyze data.

RESULTS AND DISCUSSION

Under salt stress conditions, a significant decrease was observed in length, fresh-dry weights and dry matter of shoot by approximately 10.15%, 11.70%, 14.81% and 3.41%, respectively (Table 1). On the other hand, antioxidant enzymes activities (SOD, GR and APX) were significantly ($p<0.01$) increased by 20.70%, 10.65% and 6.46%, respectively, while interestingly, unlike these enzymes, catalase (CAT) activity significantly decreased by 28.26% when compared to control (Table 2). Compared to control, the MDA and proline contents of the shoot were also significantly ($p<0.01$) increased by 110.58% and 374.74% respectively, in the shoot of salt-treated vetch seedling.

The salt treatment caused significant reductions by 20.77%, 9.52%, 60.00% and 56.04% in length, fresh-dry weights and dry matter of root of salt-treated vetch seedling, respectively (Table 3). On the other hand, compared to control, the activities of SOD, CAT, GR and APX were increased by 57.97%, 200.96%, 4.84% and 6.40%, respectively, in the root of salt-treated vetch seedling (Table 4). However, the contents of MDA and proline were increased by 263.28% and 265.72% in the root of salt-treated vetch seedling when compared to control (Table 4).

This study aimed to reveal the level of biochemical responses of the shoot and root tissues of vetch seedling to salt stress. However, some morphological parameters of shoot and root were evaluated. The present findings showed that the morpho and biochemical responses of different seedling (shoot and root) organs of common vetch to salt stress were different. In terms of morphology, the typical adverse effect of salt stress on plants is that the reduction of growth due to the restriction of cell elongation (Bandeoğlu *et al*., 2004). Similarly, the shoot and root of vetch seedling showed diverse responses in terms of growth parameters under salt stress. In support of our observation, many studies have shown that there was significant adverse effect of salinity on the growth of seedling organs of the plant (Bandeoğlu *et al*., 2004; Talukdar, 2011; Tsegay and Gebreslassie, 2014; Piwowarczyk *et al*., 2016).

There some biochemical becomes changes in plants under stress conditions. One of them is the antioxidant defense mechanism that is preserving at the biochemical levels in plants from adverse effects of stress. Antioxidant

Table 1: Changes in morphological parameters of the shoot of vetch seedling under salt stress (100 mM NaCl).

	Length (cm)		Fresh weight (g)		Dry weight (g)		Dry matter (%)	
	Cont.	Salt	Cont.	Salt	Cont.	Salt	Cont.	Salt
	5.81	5.22	0.94	0.83	0.27	0.23	29.26	28.26
t value	5.731**		3.017*		3.474*		3.196*	

*,** Significant difference ($p< 0.05$ and $p< 0.01$) compared to control.

Table 2: Changes in biochemical parameters of the shoot of vetch seedling under salt stress (100 mM NaCl).

	APX		CAT		GR		SOD		MDA		Proline	
	(µmol min^{-1} mg^{-1} FW)				(U min^{-1} mg^{-1} FW)				(µmol g^{-1} FW)			
	Cont.	Salt	Cont.	Salt	Cont.	Salt	Cont.	Salt	Cont.	Salt	Cont.	Salt
	66.55	70.85	289.31	207.55	167.73	185.6	372.82	450.03	6.52	13.73	4.91	23.31
t value	8.655**		6.424**		5.577**		6.164**		2.665*		56.581**	

*,** Significant difference ($p< 0.05$ and $p< 0.01$) compared to control.

Table 3: Changes in morphological parameters of the root of vetch seedling under salt stress (100 mM NaCl).

	Length (cm)		Fresh weight (g)		Dry weight (g)		Dry matter (%)	
	Cont.	Salt	Cont.	Salt	Cont.	Salt	Cont.	Salt
	3.85	3.05	0.63	0.57	0.05	0.02	8.94	3.93
t value	2.858*		7.603**		3.534*		4.133**	

*,** Significant difference ($p< 0.05$ and $p< 0.01$) compared to control.

Table 4: Changes in biochemical parameters of the root of vetch seedling under salt stress (100 mM NaCl).

	APX		CAT		GR		SOD		MDA		Proline	
	(µmol min^{-1} mg^{-1} FW)						(U min^{-1} mg^{-1} FW)		(µmol g^{-1} FW)			
	Cont.	Salt	Cont.	Salt	Cont.	Salt	Cont.	Salt	Cont.	Salt	Cont.	Salt
	67.12	71.42	74.96	225.60	177.86	186.48	190.98	301.71	2.56	9.30	4.26	15.58
t value	3.115*		99.340**		15.962**		4.051**		3.679**		16.928**	

*,** Significant difference ($p< 0.05$ and $p< 0.01$) compared to control.

enzymes are an essential part of this mechanism. Glutathione reductase (GR) ascorbate peroxidase (APX), superoxide dismutase (SOD) and catalase (CAT), are the main four enzymes of the defense system in plants. In the present study, activities of GR, APX, SOD and CAT, were evaluated in shoot and root tissues of vetch seedling. The results showed that SOD, GR and APX activities were increased under salt stress in seedling organs (Table 2 and Table 4). A similar observation was also reported by Bandeoğlu *et al*. (2004) in Lentil, Kusvuran (2015) in Hungarian vetch (*Vicia pannonica* Crantz.) and Beyaz (2019) in sainfoin. Moreover, as supporting our results, Ercan (2008) reported that GR and APX activity raised in leaves and roots of Lentil (*Lens culinaris* M.) seedlings, depending on their differing in salt tolerance. Drought stress and salt stress are known to be brothers and both cause oxidative stress in plants. In parallel with the results of this research, it has been reported in many previous studies (Beyaz (2019); Beyaz and Yıldız (2020) that antioxidative enzyme activities at different levels [cellular and organ (root and shoot)] increase in plants as a result of drought stress.

As mentioned above, according to the literature, the general tendency is to increase the activity of antioxidant enzymes under salt stress in plants. On the other hand, we found that CAT activity tended to decrease (28.26%) in shoot tissue under salt stress. Similar results were reported by Bandeoğlu *et al*. (2004) study in which CAT activity was decreased in leaves of Lentil (14-day-old) under salt stress (100 and 200 mM NaCl). Moreover, Khan *et al*. (2002) noted that activity of CAT was decreased in root tissue of rice under salt stress. In addition, de Azevedo Neto *et al*. (2006) reported that salt stress did not affect CAT activity in leaves while reduced it in root tissue of salt-sensitive maize genotype. Similarly, Ercan (2008) noted that CAT activity significantly decreases in shoots (approximately 60%) and roots (approximately 48%) of Lentil seedlings under salt stress application (150 mM NaCl). Under salt stress, the deactivation of the catalase enzyme may be depending on the inhibition of new enzyme synthesis (Feıerabend and Dehne, 1996) or photo-inactivation of catalase (Polle *et al*., 1997).

Proline is one of the most important osmo-protectant that protects cells from free radicals in plants under stress conditions (Bandeoğlu *et al*., 2004). Our results also demonstrated a marked increase in proline contents in the shoot (374.74%) and root (265.72%) tissue of vetch seedlings under salt stress. Bandeoğlu *et al*. (2004) argued that proline accumulation in shoot and root tissue of Lentil increased under salt stress. Khan *et al*. (2002) also reported that proline accumulation increased in root tissue that exposed salt stress, of rice. The evidences of the study clearly showed that proline accumulation is higher in shoot tissue than in root tissue.

The evidences of the study indicated that the salt stress caused increase of the level of malondialdehyde (MDA) contents in the shoot (110.58%) and root (263.28%) tissue of vetch seedling. In support of this observation, Bandeoğlu *et al*. (2004) reported that MDA content was increased in shoot and root tissue of Lentil seedling under salt stress. Moreover Abdelgawad *et al*. (2016) noted that under salt stress, MDA content was increased in root tissue of maize seedling.

Overall, according to our results, the growth was reduced higher in root tissue than in shoot tissues. Roots are the first organ to come into contact with salt stress, so their growth is less than that of shoots (Lazof and Bernstein, 1997; Abdelgawad *et al*., 2016). On the other hand, the results of the present study showed that the antioxidant enzymes (SOD, CAT, GR and APX) were higher in root than in shoot. However, the increasing rate of MDA was higher in root tissue than in shoot tissue. These results could be attributed to the initial and intense exposure of the root tissue to the stress factor. Otherwise, the accumulation of proline was higher in shoot tissue than in root tissue. It may speculate that the reasons for this phenomena were due to the lack of activity of antioxidant enzymes such as CAT to scavenge ROS in shoot tissue.

CONCLUSION

In nutshell, it was determined that salt stress (100 mM NaCl) caused significant biochemical changes in root and shoot tissues but the response of shoots and root tissues to stress conditions were different. When the biochemical responses (antioxidant defense content) of root and shoot tissues under salt stress were compared, it was determined that the quantitative increase of antioxidant defense mechanism elements (especially antioxidant enzyme activities) was mostly in the root tissue. As a result of this study, important biochemical basis scientific information which can be used in future breeding studies (such as biotechnological and/or bioengineering) for vetch -an important legume forage plant- has been revealed. In addition, the study may be used as a base for further analysis of differential effects and relative responses of vetch against various environmental stresses.

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REFERENCES

Abdelgawad, H., Zinta, G., Hegab, M.M., Pandey, R., Asard, H., Abuelsoud, W. (2016). High salinity induces different oxidative stress and antioxidant responses in maize seedlings organs. Front Plant Sci. 7: 276.

Abreu, I.A., Farinha, A.P., Negrão, S., Gonçalves, N., Fonseca, C., Rodrigues, M. (2013). Coping with abiotic stress: proteome changes for crop improvement. J. Proteom. 93: 145-168.

Ambede, J.G., Netondo, G.W., Mwai, G.N., Musyimi, D.M. (2012). NaCl salinity affects germination, growth, physiology and biochemistry of bambara groundnut. Br. J. Plant Physiol. 24: 151-160.

Azevedo Neto, A.D., Prisco, J.T., En´Eas-Filho, J., Braga De Abreu, C.E., Gomes-Filho, E. (2006). Effect of salt stress on antioxidative enzymes and lipid peroxidation in leaves and roots of salt-tolerant and salt-sensitive maize genotypes. Environ. Exp. Bot. 56: 87-94.

Bandeoğlu, E., Eyidoggan, F., Yucel, M., Oktem, H.A. (2004). Antioxidant responses of shoots and roots of lentil to NaCl-salinity stress. Plant Growth Regul. 42: 69-77.

Bates, L.S., Waldren, R.P., Teare, I.D. (1973). Rapid determination of free proline for water stress studies. Plant Soil. 39: 205-207.

Beyaz, R., Yıldız, M. (2020). Influence of peg-induced drought stress on antioxidant components of callus tissue of sainfoin (*Onobrychis viciifolia* Scop.) ecotypes. Legume Res. online first article. DOI: 10.18805/LR-556.

Beyaz, R. (2019). Biochemical responses of shoot and root tissues of sainfoin (*Onobrychis viciifolia* Scop.) to NaCl-salt stress under *in vitro* conditions. Turkish Journal of Agriculture - Food Science and Technology. 7(1): 110-114.

Beyaz, R. (2019). Biochemical responses of sainfoin shoot and root tissues to drought stress in *in vitro* culture. Legum. Res. 42(2): 173-177.

Bilgili, U., Budakli Çarpici, E., Asik, B.B., Çelik, N. (2011). Root and shoot response of common vetch (*Vicia sativa* L.), forage pea (*Pisum sativum* L.) and canola (*Brassica napus* L.) to salt stress during early seedling growth stages. Turk. J. Fıeld Crops. 16(1): 33-38.

Çakmak, I., Atli, M., Kaya, R., Evliya, H., Marschner, H. (1995). Association of high light and zinc deficiency in cold-induced leaf chlorosis in grapefruit and mandarin trees. J. Plant Physiol. 146: 355-360.

Cakmak, I., Marschner, H. (1992). Magnesium defficiency and higlight intensity enhance activities of superoxide dismutase, ascorbate peroxidase and glutathione reductase in bean leaves. Plant Physiol. 98: 1222-1226.

Desokya, E.S.M., Merwad, A.R.M., Rady, M.M. (2018). Natural biostimulants improve saline soil characteristics and salt stressed-sorghum performance. Commun Soil Sci. Plan. 49(8): 967-983.

Dugasa, M.T., Cao, F., Ibrahim, W., Wu, F. (2019). Differences in physiological and biochemical characteristics in response to single and combined drought and salinity stresses between wheat senotypes differing in salt tolerance. Physiol. Plant. 165: 134-143.

Ercan, O. (2008). Effect of drought and salt stresses on antioxidant defense system and physiology of lentil (*Lens culinaris* M.) seedlings. Middle East Technical University (Turkey), Master Thesis (126 Pp.)

Ertekin, I., Yilmaz, Ş., Atak, M., Can, E. (2018). Effects of different salt concentrations on the germination properties of hungarian vetch (*Vicia pannonica* Crantz.) cultivars. Turkish Journal of Agricultural and Natural Sciences (Turkjans). 5(2): 175-179.

Ertekin, I., Yilmaz, Ş., Atak, M., Can, E., Çeliktaş, N. (2017). Tuz Stresinin Bazı Yaygın Fiğ (*Vicia sativa* L.) Çeşitlerinin Çimlenmesi Üzerine Etkileri, Journal of Agricultural Faculty of Mustafa Kemal University. 22(2): 10-18.

Farooq, M., Wahid, A., Kobayashi, N., Fujita, D., Basra, S.M.A. (2009). Plant drought stress: effects, mechanisms and management. In: Sustainable Agriculture. [Lichtfouse, E., Navarrete, M., Debaeke, P., Ve´Ronique, S., Alberola, C. (Eds)], Pringer Netherlands. Pp: 185-212.

Feierabend, J., Dehne, S. (1996). Fate of the porphyrin cofactors during the light-dependent turnover of catalase and of the photosystem Ii reaction-center protein D1 in mature rye leaves. Planta. 198: 413-422.

Flowers, T., Gaur, P.M., Gowda, C.L.L., Krishnamurthy, L., Samineni, S., Siddique, K.H.M., Turner, N.C., Vadez, V., Varshney, R.K., Colmer, T.D. (2010). Salt sensitivity in chickpea. Plant Cell Environ. 33: 490-509.

Kavas, M., Baloğlu, M.C., Akça, O., Köse, F.S., Gökçay, D. (2013). Effect of drought stress on oxidative damage and antioxidant enzyme activity in melon seedlings. Turk. J. Biol. 37: 491-498.

Khan, M.H., Singha, K.L.B., Panda, S.K. (2002). Changes in antioxidant levels in (*Oryza sativa* L.) roots subjected to NaCl salinity stress. Acta Physiol. Plant. 24: 145-148.

Kusvuran, A. (2015). The effects of salt stress on the germination and antioxidative enzyme activity of hungarian vetch (*Vicia pannonica* Crantz.) varieties. Legume Res. 38(1): 51-59.

Lazof, D.B., Bernstein, N. (1999). The NaCl induced inhibition of shoot growth: The case for disturbed nutrition with special consideration of calcium. Adv. Bot. Res. 29: 113-189.

Lutts, S., Kine, J.M., Bouharmont, J. (1996). NaCl-induced senesence in leaves of rice (*Oryza sativa* L.) cultivars differing in salinity resistance. Annual Botany 78: 389-398.

Munns, R., Tester, M. (2008). Mechanisms of salinity tolerance. Annu. Rev. Plant Biol. 59: 651-681.

Murashıge, T., Skoog, F. (1962). A revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiol. Plant. 15: 473-497.

Orak, A., Ateş, E. (2005). Resistance to salinity stress and available water levels at the seedling stage of the common vetch (*Vicia sativa* L.). Plant Soil Environ. 51(2): 51-56.

Piwowarczyk, B., Tokarz, K., Kaminska, I. (2016). Responses of grass pea seedlings to salinity stress in *in vitro* culture conditions. Plant Cell Tiss. Organ Cult. 124: 227-240.

Polle, A., Eiblmeier, M., Sheppard, L., Murray, M. (1997). Responses of antioxidative enzymes to elevated Co_2 in leaves of beech (*Fagus Sylvatica* L.) seedlings grown under a range of nutrient regimes. Plant Cell Environ. 20: 1317-1321.

Talukdar, D. (2011). Flower and pod production, abortion, leaf injury, yield and seed neurotoxin levels in stable dwarf mutant lines of grass pea (*Lathyrus sativus* L.) differing in salt stress responses. Int J. Curr Res. 2(1): 046-054.

Tsegay, B.A., Gebreslassie, B. (2014). The effect of salinity (NaCl) on germination and early seedling growth of *Lathyrus sativus* and *Pisum sativum* var. Abyssinicum. Afr. J. Plant Sci. 8(5): 225-23.