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ORIGINAL PAPER

EFFECT OF APPLICATIONS OF DIFFERENT POTASSIUM (K^+) DOSES ON ANTIOXIDANT ENZYME ACTIVITIES IN PEPPER PLANTS UNDER SALT STRESS*

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Abstract

This study was carried out to determine the antioxidant enzyme activities in pepper (*Capsicum annuum* L. cv. Demre – long pepper) plants grown under salt stress and supplied different doses of potassium (K^+). The study was conducted under controlled conditions in a 16/8 h light / dark photo period, at 25°C and in a chamber with 70% humidity. After the seeds planted in pumice were germinated, seedlings with 2 true leaves were transferred into hydroponic culture containing the Hoagland nutrient solution. The K^+ concentrations in the Hoagland solution were calculated as 146 mg L^{-1}, and this level was used as a control. Other doses were set to be 20 mg L^{-1} lower and then 20 mg L^{-1} and 40 mg L^{-1} higher than the control dose (K1 = 126 mg L^{-1}, K2 = 146 mg L^{-1}, K3 = 166 mg L^{-1}, K4 = 186 mg L^{-1}). The salt stress level applied to the plants was induced by 100 mM sodium chloride (NaCl). Sampling for measurement and analysis was done on the 20th day of the salt application. The samples underwent the following determinations: Catalase (CAT), Ascorbate Peroxidase (APX) and Superoxide dismutase (SOD) relative to the total plant weight, and Antioxidative enzyme activities. The results indicate that K^+ applications in the K3 = 166 mg L^{-1} and K4 = 186 mg L^{-1} doses reduced the impact of salt stress and the plants were less severely affected. Their antioxidant enzyme activities values in comparison to the control and other doses of K^+ were quite low.

Keywords: antioxidative enzyme activities, pepper, potassium doses, salt stress.

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INTRODUCTION

Plants developed defence mechanisms against all kinds of abiotic (low and high temperatures, deficiency or excess of nutrients, air pollution, heavy metals, drought, salinity and radiation) and biotic (viruses, bacteria, fungi and pests) stress factors. Plants try to adapt to these stress conditions and continue their growth and development. In plants under stress, responses occur within the framework of genotypic characteristics, hence some plant species and varieties are less affected while others are fatally damaged by stress. In addition to this type of adaptability based on genetic traits, it is known that 3 factors: plant different growth periods, severity and genus, are also effective defence mechanisms in plants (Yasar 2003, Parida, Das 2005, Yasar et al. 2008*a*,*b*, 2010, Ashraf, Harris 2013, Acosta-Motos et al. 2015).

Soil is considered to be saline when electric conductivity (EC) of the soil solution reaches 4 dS m^{-1} (equivalent to 40 mM NaCl), generating an osmotic pressure of about 0.2 MPa and significantly reducing the yields of most crops (Muns, Tester 2008). As a consequence, ion toxicity leads to chlorosis and necrosis, mainly due to Na^+ accumulation that interferes with many physiological processes in plants (MUNS 2002). Under salt stress, the Na^+ uptake competes with the K^+ uptake, which results in excess of cytoplasmic Na^+ sequestration instead of Cl^- within the cell. Increased concentrations of Na^+ and Cl^- decrease the uptake of Ca^{+2}, K^+, and Mg^{+2} in a number of plants, and also affects the K^+/Na^+ ratio (Parida, Das 2005). In many studies that focus on the tolerance to salt stress in plants, it has been concluded that the K^+/Na^+ and Ca^{+2}/Na^+ ratios in different plant organs and the Na+ concentrations in plant tissues are important parameters (Aktas et al. 2006, Kusvuran et al. 2007, Dasgan, Koc 2009). Because their ionic diameters and electrical charges are very similar, K^+ doses were applied to the saline medium in order to reverse the competitive advantage in the uptake of K^+ and Na^+ ions in favour of K^+.

When the amounts of antioxidants and antioxidant enzyme activities that transform free oxygen radicals into harmless compounds in the face of stress are high, plants will be more resistant to stress-induced damage. Chloroplasts in the plant have antioxidative defence systems against toxic oxygen derivatives. Vitamin E, vitamin C, glutathione and carotenoids (beta-carotene and zeaxanthin) are the leading antioxidants (Karanlik 2001). Enzymes such as super oxide dismutase (SOD), ascorbate peroxidase (APX), glutathione reductase (GR), catalase (CAT) are known to be the most effective antioxidative enzymes in the destruction of free oxygen radicals (Cakmak, Marschner 1992, Cakmak 1994, Gossett et al. 1994, Yasar 2003).

This study was carried out to determine the antioxidant enzyme activities of pepper plants grown under salt stress and supplied different doses of potassium (K^+) to determine the occurrence of stress.

MATERIAL AND METHOD

In the study, the Demre pointed pepper variety (*Capsicum annuum* L. cv. Demre) was used as material. The experiment was conducted in a split air-conditioned climate chamber and hydroponic culture, where the normal atmosphere was provided. For this purpose, pepper seeds were planted in 40x25x5 cm foamed germination containers filled with fine-grained pumice sieved and irrigated with tap water. Germination pots were placed in a growth chamber with temperature of 25±2°C and humidity of 70-80%, and continued to be watered in a way that preserved the humidity of the pumice. After the seeds germinated, when the cotyledon leaves became horizontal and the first true leaves began to appear, irrigation was started with the Hoagland nutrient solution (Hoagland, Arnon 1938).

The seedlings which had grown second true leaves in the pumice environment were taken into hydroponic culture in 25x25x18 cm plastic tubs filled with the Hoagland nutrient solution. Pepper seedlings were placed in perforated plastic trays by wrapping them with small 9x2 cm sponge pieces. The trays were placed on the tubs so that the plant roots were in the nutrient solution. Aeration was done by immersing thin plastic hoses connected to an aquarium air pump into the nutrient solution. Salt applications were started to seedlings grown up to 4-5 real leaves in the hydroponic culture. For the seedlings to be treated with salt, NaCl was added to the Hoagland nutrient solution to provide 100 mM salt concentration. During the renewal of the solutions repeated every week, the salt applications were continued at the same concentration. Different doses of K^+ were administered in addition to the Hoagland nutrient solution with salt application. The amount of K^+ used in the normal Hoagland nutrient solution is 146 mg L^{-1}. However, in our practice, we started with a lower dose of 20 mg L^{-1} and applied as control = 146 mg L^{-1}, K1 = 126 mg L^{-1}, K2 = 146 mg L^{-1}, K3 = 166 mg L^{-1}, K4 = 186 mg L^{-1}. The mg L^{-1} values of all the nutrients in the nutrient solution are given in Table 1.

Sampling for measurement and analysis was done on the 20th day of the salt application. Total plant weight (g) and Antioxidative enzyme activities (catalase, ascorbate peroxidase, superoxide dismutase) were determined in these samples.

Determination of total plant weight

The total plant weight was weighed at 1/10.000 precision on digital scales in 4 repetitions.

Spectrophotometric enzyme activities

Typically, 1 g leaf material without the main midrib was homogenized in 10 mL 25 mm EPPS buffer (pH 7.8) containing 0.2 mm EDTA and 2% PVP.

Table 1

Nutrient solution contents used (mg L^{-1})

Elements	Aplication				
	1 control (mg L^{-1})	2 K1+salt (mg L^{-1})	3 K2+salt (mg L^{-1})	4 K3+salt (mg L^{-1})	5 K4+salt (mg L^{-1})
Nitrogen (N)	186	186	186	186	186
Phosphorus (P)	39	39	39	39	39
Potassium (K)	146	126	146	166	186
Magnesium (Mg)	25.5	25.5	25.5	25.5	25.5
Calcium (Ca)	200	200	200	200	200
Sulphur (S)	34	34	34	34	34
İron (Fe)	10.6	10.6	10.6	10.6	10.6
Manganese (Mn)	0.50	0.50	0.50	0.50	0.50
Boron (B)	0.205	0.205	0.205	0.205	0.205
Cupper(Cu)	0.015	0.015	0.015	0.015	0.015
Zinc (Zn)	0.055	0.055	0.055	0.055	0.055

The nutrient solution used was prepared according to HOAGLAND, ARNON (1938).

The centrifuges obtained after homogenized samples were centrifuged at 15 000 g for 15 min were used in enzyme analysis. Samples for determining enzyme activities were kept at +4°C until analysed on a spectrophotometer (Analytic Jena 40 model). The following were determined: SOD activity, according to the method of reduction of NBT (nitro blue tetrazolium chloride) by O_2 – under light, APX activity, oxidation of ascorbateat 290 nm (E = 2.8 mM cm^{-1}), GR activity at 340 nm (E – oxidation of 6.2 mM cm^{-1}), and NADPH by measuring CAT, H_2O_2 degradation rate at 240 nm (E – 39.4 mM cm^{-1}) – CAKMAK, MARSCHNER (1992).

Making evaluations

The treatments were arranged according to the complete randomized design with 3 replications, in which there were 15 plants in each repetition. The statistical analyses of the plant growth parameters and the data obtained were performed according to the Duncan multiple comparison test (P<005) using the SAS Insitue, (1985) package program.

RESULTS

Plant growth parameters

The pepper plants of Demre were subjected to salt stress, the total weights of the plants were measured at the end of the 20th day and the values obtained are given in Table 2. In the experiment, although the total plant weight of pepper plants to which salt stress and different potassium doses were applied differs from the control group, the lowest value was in the K1 application and the highest value was measured in the K4 application relative to the control group application.

Antioxidant enzyme activities

CAT, APX and SOD enzyme activities, which are antioxidative enzymes activated to destroy radical oxygen derivatives formed as a result of oxidative stress, were examined in pepper plants with different doses of potassium applied with salt application, and the obtained data are given in Table 2.

Table 2

Total fresh weights (g) of pepper plants taken from the applications and catalase, ascorbate peroxidase, superoxide dismutase enzyme activities (mol min^{-1} mg^{-1} F.W) in leaves of the plants

Application	Total plant weight	CAT	APX	SOD
Control	14.75 *A*	253.380 *D*	17.320 *D*	63.333 *D*
K1+salt	4.94 *E*	417.673 *A*	33.286 *A*	112.667 *A*
K2+salt	7.52 *D*	366.700 *B*	21.500 *B*	98.000 *B*
K3+salt	8.64 *C*	280.350 *C*	18.233 *C*	88.000 *C*
K4+salt	10.75 *B*	246.967 *D*	18.343 *C*	84.000 *C*

The difference between the averages followed by the same capital letter in the same column is insignificant at $P \leq 0.05$.

Significant changes were observed in the CAT enzyme activity of plants on the 20th day of salt application compared to the control plants. Regarding the activity of catalase enzyme, the highest value was obtained from K1 application, while the lowest value was found in K4 application, which was in the same range of values as in the control group (Table 2). With potassium applications, CAT enzyme activity increased to the highest level at the lowest K^+ dose and then started to decrease as the K^+ dose increased. In response to the highest dose of K4, it reached the same range as the control.

Considering the applications in terms of ascorbate peroxidase enzyme activity, the highest activity value in ascorbate peroxidase (APX) activity was after K1 + salt application, followed by K2 + salt, K3 + salt and K4 + salt application (Table 2).

When superoxide dismutase (SOD) enzyme was examined, it was observed that the enzyme activities increased with K + Salt applications, but this increase started to decline as the K + dose increased. While the highest SOD enzyme activity was observed in K1 + Salt application, the lowest SOD enzyme activity was obtained from K4 + Salt application after the control (Table 2).

DISCUSSION AND CONCLUSION

In the study investigating the morphological and biochemical effects of potassium (K^+) on the Demre pepper plant under salt stress, an attempt was made to determine whether the plants' tolerance to salt was affected by applying different doses of potassium to the Demre pepper plant with salt application. At the end of the study, total wet plant weight and antioxidative enzyme activities in plant leaves were examined.

On the 20th day of the application of different K^+ doses with 100 mM NaCl, the highest decrease in the total plant weight of pepper plants compared to the control was in response to the first dose of potassium. As the potassium dose increased, the plant growth was found to be closer to that in the control. It is observed that there were also very significant differences between the potassium doses in terms of the total plant weight. In the salinity studies conducted by YASAR et al. (2006, 2007, 2008, 2013*a*, 2016) on different species, it was seen that the total plant weight was an important parameter in determining the response to salt stress. In addition, while the first dose of potassium could not reduce the negative effect of salt, the 2nd, 3rd and 4th doses were respectively the positively effective doses. The results we obtained from our study showed that the growth and development of plants decrease due to the slowing of respiration of plants in salty environments. As a result of the deterioration in the respiratory system, i.e. the decrease in stomata motility, the formation of hormonal changes in the plant metabolism and a decrease in the plant's photosynthesis, thus a decrease in the formation of assimilates and a decrease in the growth and development of the plant were observed (CAKIRLAR, TOPCUOGLU 1985, YASAR 2003, 2007).

It was noticed that there were statistical differences between the applications of different potassium doses applied to pepper plants with salt in the enzyme activities of CAT, APX and SOD in the plant leaves. The highest activity in all three enzymes occurred in K1 + salt application, and enzyme activities decreased as potassium doses increased. Even in K4 + salt applications, CAT enzyme activity was in the same statistical range as the control. In the salt stress research conducted by many researchers with different species and varieties until today, it has been observed that the antioxidant enzyme activities of stressed plants generally increase in tolerant varieties depending on the genetic structure of a variety. They argued that the most

important reason why plants are not harmed by salt is that they protect the plant cell from the harmful effects of radical oxygen derivatives formed by the activation of antioxidative enzymes (Gosset et al. 1994, Hernandez et al. 1995, Shalata, Tal 1998, Sreenivasulu et al. 2000, Yasar 2003, Yasar et al. 2006, 2007, 2008, 2013*b* 2014, 2016). However, when we evaluate the results of antioxidant enzyme activities obtained from our study and the results of total plant wet weights, we can say that potassium protects the plants from the toxic effect of salt, especially in K3 + salt and K4 + salt applications, and that there is a decrease in the activities of antioxidant enzymes because the plants are not stressed or else stressed only slightly. Similarly, Tuna et al. (2017) applied potassium with different doses of salt in their study on tomato plant and found that when they applied salt alone, the activities of the antioxidant enzymes CAT, SOD and APX increased compared to the control, while the activities of these enzymes decreased in salt + K applications. However, enzyme activities increased when high salt doses were administered together with potassium. It is possible that potassium has another effect, as a result of which plants treated with high doses of potassium do not have chlorosis and damage due to salt, but their growth is not as high as that of control plants. Potassium restricts plant growth in order to keep metabolic activity under control, maintaining the plant at a level of the growth that can control it.

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