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Rise Potassium Content of the Medium Improved Survival, Multiplication, Growth and Scavenging System of *in Vitro* Grown Potato Under Salt Stress.

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IN VITRO grown potato cultivars shoots were used to understand how polyvinyl pyrrolidone (PVP) and three levels of potassium interact with two levels of salinity to determine Na^+ and K^+ contents, and antioxidant enzyme activities leading to control survival, multiplication and growth of cultured plant shoots. Explant survival and number of regenerants/explant decreased with increase of NaCl concentration but increased K^+ content of the culture medium from 20 mM to 30 mM improved explants survival frequency and the growth parameters estimated under relatively high salt stress (80 mM NaCl) in both cultivars. Also, number of regenerants was influenced by K^+ level where it was higher on medium containing 40 mM NaCl and 30 mM K^+ than other cultured on the same NaCl concentration with 20 mM K^+. The positive effect of 30 mM K^+ on the previous parameters was associated with increase shoot K^+ content leading to decrease Na^+/K^+ ratio and increase of some antioxidant enzymes (SOD, POX and CAT in Agria or SOD, POX and APX in Hermes) activity, especially under relatively high salt stress. PVP application increased K^+ content and activities of some antioxidant enzymes (CAT and APX) but decreased Na^+/K^+ ratio of shoots subjected to relatively high salt stress in Agria but under both salt stresses in Hermes. Consequently, sufficient potassium supply was necessary to conserve low Na^+/K^+ ratio and efficient scavenging system by antioxidant agent (PVP) application or increase the endogenous antioxidant enzymes activities leading to minimize the negative effect of salt stress on *in vitro* grown potato.

Keyword: Polyvinyl pyrrolidone, PVP, antioxidant enzymes, sodium potassium pump, *Solanum tuberosum*.

Sodium (Na^+) is one of the most abundant cation where it represents 3% of the earth's crust and 5% of seas or oceans. Soil salinity may be due to natural or man-made reasons, one of them is the irrigation with saline water. Every day, salinity affects between 2,000 and 4,000 ha of irrigated lands all over the world and turns them to unsuitable for economic crop production. Reclamation of soil salinity are very expensive and time consuming. Then, solutions are restricted between creation of salinity-tolerant lines and/or increase the production of existing lines in saline soil (Shabala, 2013).

Under K^+ deficiency, many glycophytic plant species tend to use Na^+ instead of K^+ as fertilizer to fulfill some metabolic steps (Subbarao *et al.,* 2003), it may be due to the chemical and structural similarities between Na^+ and K^+ (Amtmann and Sanders, 1999). Moderate and high concentrations of NaCl are detrimental to glycophytic plant species due to rise the osmotic stress associated with lower water potential leading to reduction of photosynthetic activity and yield (James *et al.,* 2002; Husain *et al.,* 2003). In addition, under the influence of salinity stress, entry of Na^+ results in leakage of K^+ ions which leads to depletion of K^+ in the cytosol and deprive some metabolic functions from their K^+ leading to cell death (Shabala, 2009).

Under field condition, depletion of K^+ is expected (Amtmann *et al.,* 2006) and it leads to reduction quantity and quality of the yield (Cakmak, 2010). Transport of sucrose from leaves to other plant organs was retarded under K^+ deficiency (Cakmak, 2005) leading to enhance oxygen photoreduction and production of reactive oxygen species-ROS (Cakmak, 1994, 2005).

In few minutes, accumulation of reactive oxygen species (ROS) under salt stress was reported (Hong *et al.,* 2009) but it up-regulate by ROS defense system which include enzymatic and non-enzymatic components. The enzymatic component includes several enzymes such as superoxide dismutase (SOD), catalase (CAT), guaiacol peroxidase (POX) and ascorbate peroxidase (APX) (Asada, 1999 and Mittler *et al.,* 2004). Also, the non-enzymatic part include several compounds such as glutathione, carotenoids and phenols (Mittler e*t al.,* 2004 and Scandalios, 2005).

Potato (*Solanum tuberosum* L.) is one of the most important crop after wheat, rice and maize (Moeinil *et al.,* 2011), it is used for human consumption and production of starch and alcohol. Variation in salt sensitivity was observed between different potato cultivars (Ahmed and Abdullah, 1979), Agria and Hermes were classified as a moderately salt sensitive cultivars (Rahnama and Ebrhimzadeh, 2005, Levy and Velleux, 2007). Application of K^+ with moderate concentration of Na^+ attained the desired electrical conductivity to improve the performance, yield of tomato plants (Dorais *et al.,* 2000). Alhagdow *et al.* (1999) used micropropagated potato to examine how K^+ levels in cultured medium affected Na^+ accumulation and control growth parameters. Potassium effect on Na^+ accumulation under salt stress and its impact on growth parameters, taking into account the role of antioxidants, still needs to be clarified. Thus, the aim of our studies was to know how the application of different K^+ levels and PVP affect viability, growth and expression of antioxtant enzymes under the influence of different NaCl concentrations of two potato cultivars. The experimental conditions and concentrations of both K^+ and NaCl were extensively controlled where *in vitro* shoot cultures were used.

Materials and Methods

Shoot cuttings of two cultivars (Agria and Hermes) of potato *(Solanum tuberosum)* were micropropagated on MS (Murashige and Skoog, 1962) basal medium supplemented with 0.0 mM NaCl, 1 mg/l benzyl amino purine (BAP) + 0.5 mg/l gibberellic acid (GA_3) (control; free salt medium). Cultures were incubated at 25 ± 2 ºC with 16/8 h day/night, irradiance of 100 µmol m^{-2} s^{-1} and 70% relative humidity.

To study the effect of K^+ and NaCl as well as polyvinyl pyrrolidone (PVP), nodal segments (about 0.8 cm) excised from the middle part of micropropagated shoots, where shoot tips and basal nodes were excluded. Segments were cultured on MS medium supplemented with 1 mg/l BAP + 0.5 mg/l GA_3 and different concentrations of NaCl (0.0, 40 or 80 mM) and potassium (6, 20 or 30 mM) without or with 200 mg/l PVP. Potassium concentration of the MS medium was adjusted by using KNO_3 and total nitrogen content of the medium was kept constant by using NH_4NO_3. In all experiments, grown plants on MS medium containing 0.0 NaCl and 20 mM K were considered as the control. Three replicates with totally 30 explants (10 explants/each) per each treatment were designed. Cultures were incubated under tissue culture room conditions for one month. Frequency of shoot survival, number of shoots/explant, length of shoot (cm), number of nodes/shoot and fresh mass/shoot cluster were estimated. The activities of four antioxidant enzymes (SOD, POX, CAT and APX) were determined in harvested fresh shoots. The Na^+ and K^+ concentrations were determined in dry shoot materials.

Determination the activities of antioxidant enzymes

For antioxidant enzymes assay, protein extraction was carried out using extraction of 0.5 g of fresh shoots. Plant materials were grinded in 3 ml extraction buffer at 4 ºC and centrifuged at 13000 rpm for 15 min at 4ºC. The supernatants were transferred to new tubes and used to estimate the enzyme activity. The used extraction buffer was consisted of 50 mM phosphate buffer (pH 7), 0.1 mM Na_2EDTA and 1 % (w/v) PVP.

Determination of superoxide dismutase (SOD) activity

To determination SOD (EC 1.15.1.1) activity in grown shoots under the influence of different conditions, the method described by Giannopolitis and Ries (1977) was used. The reaction was carried out in 3 ml reaction mixture consisted of 50 mM potassium phosphate buffer (pH 7.8), 13 mM methionine, 75 mM NBT, 100 mM EDTA, 2 mM riboflavin and 50 µl enzyme extract. Readings were recorded spectrophotometrically at 560 nm following the production of blue formazan. The unit of SOD activity was defined as the amount of enzyme that inhibits the nitrobluetetrazolium photoreduction (Extinction factor (E) = 10.3 mM cm^{-1}).

Determination of guaiacol peroxidase (POX) activity

POX (EC 1.11.1.7) activity in supernatant obtained from grown shoots under different conditions was measured according to MacAdam *et al.* (1992). The reaction was performed in the final volume of 3 ml reaction mixture consisted of 50 mM potassium phosphate buffer (pH 6.8), 0.1 mM EDTA, 5 mM guaiacol, 0.3 mM H_2O_2 (30%) and 25 µl supernatant. The absorbance was measured spectrophotometrically at 470 nm, where the increase in absorbance was due guaiacol oxidation (E = 26.2 mM cm-1). Enzyme activity was calculated as µM of guaiacol oxidized $min^{-1}g^{-1}$ fresh weight at 25 ± 2 ºC (Zhang, 1992).

Determination of catalase (CAT) activity

According to the method of Aebi (1984), catalase (EC 1.11.1.6) activity in supernatant of grown shoots under different conditions was estimated spectrophotometrically. Assay mixture (3 ml) consisted of 50 mM phosphate buffer (pH 7.0), 0.1 µM EDTA, 20 mM H_2O_2 and 25 µl enzyme extract was used. Catalase activity was described as the decrease in absorbance at 240 nm for 1 min due to H_2O_2 decomposition (E = 0.036 mM cm^{-1}). One CAT unit is the amount of enzyme necessary to decompose 1 µmol min^{-1} H_2O_2 under the above mentioned assay conditions.

Determination of ascorbate peroxidase (APX) activity

APX (EC 1.11.1.11) activity was determined according to Nakano and Asada (1981). APX activity was estimated in 3 ml assay mixture that contained 50 mM phosphate buffer (pH 7.0), 0.5 mM EDTA, 0.5 mM ascorbate, 0.1 mM H_2O_2 (30%) and 25 µl enzyme extract. The decrease in ascorbate concentration was followed by decline in absorbance at 290 nm for 1 min and activity was calculated using the extinction coefficient (E = 2.8 mM cm^{-1}) for ascorbate.

Determination of sodium and potassium in dry plant materials

Shoots obtained under the influence of different conditions were collected and dried in a hot air oven at 70 ºC for 48 hr. Dry shoots were ground to fine powder. For analysis, dry plant materials were digested using nitric-perchloric procedure as recommended by AOAC (1990). Five ml concentrated nitric acid (HNO_3) was added to 0.1 g dry plant sample in a 25 ml glass conical. The mixture was heated using sand bath for 30-45 min. After cooling, 2.5 ml of 70% perchloric ($HClO_4$) was added and the mixture was heated again until dense white fumes appeared. After cooling, 2 ml deionized H_2O was added and the mixture was heated again to release any fumes. The solution was cooled, filtered and transferred quantitatively to a 10 ml volumetric flask using deionized H_2O. Contents of Na^+ and K^+ in plant materials were determined using atomic absorption spectrometer (AAS; PerkinElmer, Analyst 400). Three replicates were analyzed for each treatment. Sodium or potassium concentration values were expressed as mg/g dw of plant sample.

Statistical analysis

In all experiments, three replicates with thirty explants for each treatment were done. Data were presented as means ± standard deviation (SD) as held by the method of Snedecor and Cochran (1980). The software type SPSS 16 was used to perform Analysis of Variance (ANOVA). The significance level was measured running a Tuky test; $P \leq 0.05$ was considered as significant.

Results

Both Agria and Hermes potato cultivars (Table 1 and 2) were successfully cultured and multiplied on MS medium supplemented with 20 mM K^+, 1 mg/l BAP and 0.5 mg/l GA_3 (Fig.1). Under these conditions, the number of regenerants/explant of Agria (5.67) was higher than that of Hermes (5 regenerants/explant). Regenerated shoots were easily subcultured without the appearance of brown colour at the base of explants. All the estimated parameters were decreased under K^+ deficiency (6 mM) in cultured medium. Application of PVP resulted in significant reduction in number of formed shoots in Agria and it was non-significant in Hermes. Also, PVP improved the shoot length, number of nodes/shoot and fresh weight/shoot cluster in both cultivars irrespective the K^+ content of the medium. On salt free medium, the positive response of PVP on shoot growth of Hermes (Table 1) was better than Agria (Table 2) where significant increase in fresh weight/ cluster was registered.

Explant survival was not influenced by K^+ deficiency on salt free medium but it was negatively influenced by K^+ deficiency in combination with moderate and relatively high salt stress. While explant survival and number of regenerants/explant decreased with the increase of NaCl concentration (Table 1 and 2), increase K^+ content of the cultured medium from 20 mM to 30 mM improved the frequency of explant survival under relatively high salt stress (80 mM NaCl) in both cultivars. Number of regenerants on medium containing 40 mM NaCl and 30 mM K^+ was higher than that of medium containing 40 mM NaCl and 20 mM K^+. Number of regenerants/explant was not influenced by application of PVP but frequency of explant survival was improved when explants were cultured on MS medium containing 40 or 80 mM NaCl and sufficient levels of K^+ (20 and 30 mM) especially in Agaria.

The estimated growth parameters were drastically reduced when potato shoots were cultured on medium containing low K^+ content (6 mM) in combination with moderate (40 mM) or high NaCl content (80 mM) in Agria or Hermes cultivars (Table 1 and 2). In comparison to growth parameter values under low K^+ content of the medium, availability of more K^+ improved the growth parameters especially under relatively high NaCl concentration. In both cultivars, application of PVP improved some of these growth parameters including shoot length and/or number of nodes/shoot leading to increase of the fresh weight/cluster when the plants were subjected to moderate or relatively high NaCl.

Fig. 1. *In vitro* multiplication of potato plants on MS medium supplemented with 1 mg/l BAP + 0.5 mg/l GA_3.

TABLE 1. Growth parameters of cv. Agria microshoots in response to NaCl and K concentrations in MS medium without or with 200 mg/l PVP. Values are mean ± SD.

Presence of PVP	Na/K conc. (mM)	Frequency of survival shoots (%)	No. of shoots/ explant	Length of shoot (cm)	No. of nodes/ shoot	F.W./shoot cluster (g)
Without PVP	0 /20	100	5.67 ± 0.58	5.33 ± 0.25	5.33 ±0.58	0.0632 ±0.015
	0/30	100	4* ± 1	5.17 ± 0.35	5 ± 0	0.0448 ± 0.001
	0/6	100	3* ± 0	4.7 ± 0.44	5 ± 0	0.0451±0.003
	40/20	100	3* ± 0	4.27*±0.49	5 ± 1	0.0421 ± 0.003
	40/30	96.67	4* ± 1	3.13*± 0.21	5 ± 0	0.0351* ± 0.008
	40/6	90	2.7* ± 0.6	2.83* ± 0.32	4 ± 1	0.0233* ± 0.003
	80/20	73.33	2* ± 0	1.93* ± 0.31	3* ± 0	0.0149* ± 0.003
	80/30	76.67	2*± 0	2.23* ± 0.06	4 ± 0	0.0159* ± 0.002
	80/6	60	1* ± 0	1.53* ± 0.23	2* ± 0	0.0140* ± 0.002
With PVP	0/20	100	3.67* ± 0.58	7.73* ± 0.68	8.67* ± 1.53	0.0812 ± 0.012
	0/30	100	3*± 0	5.43 ±0.25	6.33 ± 0.58	0.0465 ± 0.002
	0/6	100	3* ± 1	4.87 ± 0.31	5.33 ± 0.58	0.0458 ± 0.017
	40/20	96.67	2.3* ± 0.6	4.27* ± 0.31	5.67 ± 0.58	0.0471 ± 0.010
	40/30	100	2.7* ± 0.6	3.47* ± 0.35	4.67 ± 0.58	0.0464 ± 0.012
	40/6	96.67	3* ± 0	3.20* ± 0.26	4 ± 1	0.0309* ± 0.004
	80/20	86.67	2* ± 0	2.17* ± 0.06	4.67 ± 0.58	0.0175* ± 0.002
	80/30	93.33	1.7* ± 0.6	2.3* ± 0.17	4.67 ± 0.58	0.0163* 0.005
	80/6	53.33	1* ± 0	1.63* ± 0.37	4 ± 0	0.0153* ± 0.003

* indicate significant difference at $P \leq 0.05$ between growth parameter of microshoots grown on MS medium supplemented with 0/20 for Na/K concentrations and others grown on MS medium supplemented with the other concentrations of Na/K without or with PVP.

TABLE 2. Growth parameters of cv. Hermes microshoots in response NaCl and K concentrations in MS medium without or with 200 mg/l PVP. Values are mean ± SD.

Presence of PVP	Na/K conc. (mM)	Frequency of survival shoots (%)	No. of shoots/ explant	Length of shoot (cm)	No. of nodes/ shoot	F.W./shoot cluster (g)
Without PVP	0 /20	100	5 ± 0	5.9 ± 0.1	5 ± 0	0.1053 ± 0.013
	0/30	100	3.67* ± 1.15	5.57 ± 0.31	3* ± 0	0.0789* ± 0.01
	0/6	100	2.33* ± 0.58	4.93 ± 0.70	3* ± 1	0.0327* ± 0.0004
	40/20	76.67	2.33* ± 1.15	4.5* ± 0.1	4.33 ± 1.15	0.0415* ± 0.004
	40/30	70	4 ± 1.73	4.27* ± 0.05	3.33 ± 0.58	0.0369* ± 0.002
	40/6	53.33	2.67* ± 0.58	4.1* ± 0.1	3.33 ± 0.58	0.0359* ± 0.004
	80/20	53.33	2.33*± 0.58	3.43* ± 0.32	3* ± 0	0.0318* ± 0.003
	80/30	73.33	2* ± 0	3.6* ± 0.36	3.67 ± 0.58	0.034* ± 0.002
	80/6	63.33	1.67* ± 0.58	1.6* ± 0.1	2* ± 1	0.0167* ± 0.004
With PVP	0/20	100	4.33 ± 0.58	6.9 ± 0.66	5 ± 0	0.1272* ± 0.015
	0/30	100	3.67 ± 0.58	6.03 ± 0.29	5 ± 0	0.0864* ± 0.002
	0/6	100	3 ± 0	5.63 ± 0.05	4.67 ± 0.58	0.029*1 ± 0.002
	40/20	83.33	2.67* ± 0.58	4.4* ± 0.72	5 ± 1	0.0421* ± 0.001
	40/30	86.67	4.33 ± 0.74	4.33* ± 0.74	4 ± 1	0.0404* ± 0.002
	40/6	70	3 ± 0	4* ± 0.44	4 ± 0	0.0384* ± 0.002
	80/20	66.67	2* ± 0	2.77* ± 0.21	4 ± 0	0.0342* ± 0.009
	80/30	56.67	1.67* ± 0.58	3.1* ± 0.17	4 ± 0	0.0359* ± 0.008
	80/6	53.33	1.67* ± 0.58	1.77* ± 0.21	3.33 ± 0.58	0.0184* ± 0.002

* indicate significant difference at $P \leq 0.05$ between growth parameter of microshoots grown on MS medium supplemented with 0/20 for Na/K concentrations and others grown on MS medium supplemented with the other concentrations of Na/K without or with PVP.

On salt free medium, while shoot K^+ content increased with the increase of K^+ concentration in medium (Table 3), application of PVP increased K^+ and Na^+ contents of shoots cultured on MS medium with 30 mM K^+. Under moderate salt stress, Na^+ content of potato shoots was higher than that of control (salt free medium) and it was affected by the K^+ content of the medium. The highest content of Na^+ was detected when the medium containing the lowest content of K^+ (6 mM). Increase K^+ content of the medium to 20 mM decreased Na^+ content and Na^+/K^+ ratio of both cultivars. Furthermore, Na^+ content and Na^+/K^+ ratio of shoots cultured on MS medium containing 30 mM K^+ and 40 mM NaCl was higher than other cultured on MS with 20 mM K^+ and 40 mM NaCl in Agria but vice versa in Hermes. Also, under relatively high salt stress, high K^+ content improved the estimated growth parameters than those of plants grown on MS medium with 20 mM K^+ and 80 mM NaCl and it was associated with increase shoot K^+ content leading to decrease Na^+/K^+ ratio and increase the activity of some antioxidant enzymes (SOD, POX and CAT in Agria or SOD, POX and APX in Hermes). Under the influence of relatively high NaCl concentration (80 mM), the highest Na^+ content and Na^+/K^+ ratio was detected irrespective the K^+

level in the medium. Shoots cultured on MS medium with 30 mM K^+ and 80 mM NaCl had lower Na^+/K^+ ratio than others cultured on 20 mM K^+ and 80 mM NaCl in both cultivars. Application of PVP increased K^+ content but decreased the Na^+/K^+ ratio in Agria shoots subjected to relatively high salt stress. In Hermes, the positive effect of PVP was detected where the shoots were grown under moderate or relatively high salt stress.

TABLE 3. Sodium and potassiun contents (mg/g DW) of cvs. agria and hermes microshoots, in response to NaCl and K concentrations in MS medium without or with 200 mg/l PVP . values are mean ± SD.

Presence of PVP	Na/K conc. (mM) in medium	Agria			Hermes		
		Na Content (mg/g D.W.)	K Content (mg/g D.W.)	Na/K ratio	Na Content (mg/g D.W.)	K Content (mg/g D.W.)	Na/K ratio
Without PVP	0/20	0.29 ± 0.01	14.56 ± 0.05	0.019	0.22 ± 0.01	15.38* ± 0.03	0.014
	0/30	0.34 ± 0.006	21.16* ± 0.07	0.016	0.47 ± 0.01	19.14* ± 0.25	0.025
	0/6	0.45 ± 0.006	10.80* ± 0.06	0.042	0.56 ± 0.007	8.92* ± 0.11	0.063
	40/20	1.87* ± 0.006	15.89 ± 0.08	0.118	2.07* ± 0.02	9.38* ± 0.57	0.221
	40/30	2.36* ± 0.02	12.53* ±0.007	0.188	1.74* ± 0.01	13.93* ± 0.07	0.125
	40/6	2.92* ± 0.01	9.20* ± 0.07	0.317	2.69* ± 0.02	8.23* ± 0.08	0.327
	80/20	4.93* ± 0.07	12.89* ± 0.09	0.382	5.85* ± 0.12	9.29* ± 0.06	0.629
	80/30	5.166* ± 0.07	14.89* ± 0.25	0.347	4.38* ± 0.08	10.11* ± 0.03	0.433
	80/6	4.64* ± 0.03	6.20* ± 0.01	0.748	5.98* ± 0.03	6.66* ± 0.09	0.898
With PVP	0/20	0.35 ± 0.003	14.51 ± 0.04	0.024	0.92* ± 0.03	14.19* ± 0.17	0.065
	0/30	1.82* ± 0.28	23.95* ± 0.38	0.076	0.83* ± 0.02	22.87* ± 0.14	0.036
	0/6	0.29 ± 0.01	8.40* ± 0.04	0.035	1.04* ± 0.02	11.49* ± 0.36	0.091
	40/20	1.42* ± 0.003	10.33* ± 0.06	0.137	4.30* ± 0.19	22.33* ± 0.39	0.193
	40/30	1.91* ± 0.04	22.09* ± 2.56	0.264	3.11* ± 0.02	18.67* ± 0.47	0.167
	40/6	2.97* ± 0.01	8.85* ± 0.05	0.336	5.26* ± 0.007	23.75* ± 2.56	0.221
	80/20	4.75* ± 0.04	14.08* ± 0.10	0.337	5.37* ± 0.06	17.48* ± 0.21	0.307
	80/30	6.44* ± 1.02	21.55* ± 0.38	0.30	4.94* ± 0.06	15.57* ± 0.26	0.317
	80/6	5.15* ± 0.52	8.60* ± 0.13	0.599	4.44* ± 0.01	7.99* ± 0.05	0.556

- indicate significant difference at $P \leq 0.05$ between antioxidant enzyme activity of microshoots grown on MS medium supplemented with 0/20 for Na/K concentrations and others grown on MS medium supplemented with the other concentrations of Na/K without or with PVP.

On salt free medium, in Agria cultivar, K^+ deficiency resulted in increase of POX and CAT activities but decreased SOD and APX activities in comparison to those of control (Table 4). In Hermes, while K^+ deficiency resulted in increased POX activity but decreased CAT activities, SOD and POX were unaffected (Table 5). Increase K^+ content (30 mM) of the medium than control resulted in increase of POX activity in both cultivars. Generally, application of PVP increased the activities of CAT and APX in both cultivars.

Agria shoots cultured on MS medium containing moderate NaCl (40 mM) and potassium deficiency (6 mM) showed increase of POX and CAT activities (Table 4). Under relatively high salt stress (80 mM), cultured shoots expressed increase of SOD, POX and CAT irrespective the level of K. Under the influence of PVP and irrespective the K^+ content of the media, SOD, CAT and APX activities of Agria shoots were increased in comparison to those grown on MS medium without PVP.

TABLE 4. Activities of 4 antioxidant enzymes (Unit g^{-1} FW) of cv. agria microshoots, in response to NaCl and K^+ concentrations in MS medium without or with 200 mg/l PVP. values are mean ± SD.

Presence of PVP	Na/K conc. (mM)	SOD	POX	CAT	APX
Without PVP	0/20	1.24 ± 0.30	1.44 ± 0.16	0.29 ± 0.12	1.54 ± 0.14
	0/30	1.27 ± 0.12	3.89* ± 0.22	0.48 ± 0.21	1.87 ± 0.21
	0/6	0.75* ± 0.06	2.14* ± 0.18	0.42 ± 0.10	0.75* ± 0.21
	40/20	1.09 ± 0.06	1.93* ± 0.17	0.54 ± 0.18	1.87 ± 0.45
	40/30	1.34 ± 0.10	0.45* ± 0.02	0.30 ± 0.10	1.07 ± 0.21
	40/6	0.57* ± 0.09	2.66* ± 0.17	0.42 ± 0.10	1.26 ± 0.14
	80/20	1.23 ± 0.14	2.62* ± 0.29	0.60 ± 0.10	0.70* ± 0.14
	80/30	1.39 ± 0.11	1.64* ± 0.08	0.64 ± 0.10	0.75* ± 0.08
	80/6	2.05 ± 0.09	2.47* ± 0.04	0.60 ± 0.10	0.93 ± 0.16
With PVP	0/20	2.12 ± 0.05	0.36* ± 0.03	2.46* ± 0.21	2.24* ± 0.28
	0/30	1.49 ± 0.05	0.36* ± 0.03	3.24* ± 0.31	2.47* ± 0.16
	0/6	0.29 ± 0.03	1.18 ± 0.08	4.56* ± 0.10	3.92* ± 0.24
	40/20	1.74* ± 0.02	1.34 ± 0.24	7.56* ± 0.36	3.59* ± 0.21
	40/30	1.72* ± 0.11	0.70* ± 0.01	3.84* ± 0.21	2.75* ± 0.29
	40/6	1.36 ± 0.07	1.07 ± 0.13	5.40* ± 0.31	2.15* ± 0.16
	80/20	2.12* ± 0.05	0.63* ± 0.002	2.46* ± 0.21	2.24* ± 0.28
	80/30	1.49 ± 0.05	0.36* ± 0.03	3.24* ± 0.31	2.47* ± 0.16
	80/6	1.29 ± 0.03	1.18 ± 0.08	4.56* ± 0.10	3.92* ± 0.24

- indicate significant difference at $P \leq 0.05$ between antioxidant enzyme activity of microshoots grown on MS medium supplemented with 0/20 for Na/K concentrations and others grown on MS medium supplemented with the other concentrations of Na/K without or with PVP.

In Hermes, in comparison to that of control, POX and APX activities increased under the influence of moderate or relatively high salinity irrespective the K^+ level of the medium (Table 5). Combination between relatively high NaCl and K^+ deficiency expressed the highest values of SOD and POX activities. Under the influence of PVP, while the activity of two enzymes (POX and CAT) increased under moderate NaCl content of the medium, one only (CAT) increased under relatively high NaCl. On the other side, activity of SOD decreased under the influence of PVP.

TABLE 5. Activities of 4 antioxidant enzymes (Unit g^{-1} FW) of cv. hermes microshoots, in response to NaCl and K^+ concentrations in MS medium without or with 200 mg/l PVP. values are mean ± SD.

Presence of PVP	Na/K conc. (mM)	SOD	POX	CAT	APOX
Without PVP	0/20	2.35 ± 0.09	1.94 ± 0.08	1.26 ± 0.18	1.07 ± 0.16
	0/30	1.99* ± 0.08	2.42 ± 0.20	0.66 ± 0.21	0.98 ± 0.24
	0/6	2.35 ± 0.04	2.03 ± 0.14	0.90 ± 0.18	1.07 ± 0.16
	40/20	2.17 ± 0.09	2.15 ± 0.21	0.30 ± 0.10	1.54 ± 0.14
	40/30	1.19* ± 0.02	2.87* ± 0.06	0.60 ± 0.10	1.8*7 ± 0.35
	40/6	1.72* ± 0.05	2.36 ± 0.16	0.36 ± 0.18	1.89* ± 0.08
	80/20	2.61* ± 0.07	2.74* ± 0.16	0.24 ± 0.10	2.15* ± 0.08
	80/30	2.89* ± 0.06	3.70* ± 0.17	0.42 ± 0.10	1.93* ± 0.08
	80/6	2.91* ± 0.05	4.31* ± 0.13	1.08 ± 0.18	1.91* ± 0.77
With PVP	0/20	2.49* ± 0.02	2.28 ± 0.03	4.38* ± 0.45	2.66* ± 0.24
	0/30	1.83* ± 0.02	2.13 ± 0.30	3.30* ± 0.10	2.38* ± 0.14
	0/6	0.19* ± 0.02	1.57 ± 0.14	7.68* ± 1.99	3.97* ± 0.21
	40/20	1.74* ± 0.01	2.25 ± 0.06	1.56 ± 0.21	1.26 ± 0.14
	40/30	1.03* ± 0.01	3.74* ± 0.24	2.52 ± 0.18	1.73 ± 0.21
	40/6	1.49* ± 0.05	2.95* ± 0.18	4.80* ± 0.55	1.87* ± 0.21
	80/20	1.83* ± 0.05	2.84* ± 0.51	1.14 ± 0.21	1.53 ± 0.23
	80/30	1.25* ± 0.03	2.97* ± 0.34	2.28 ± 0.21	1.87* ± 0.16
	80/6	1.36* ± 0.04	3.71* ± 0.16	2.58 ± 0.10	2.38* ± 0.14

* indicate significant difference at $P \leq 0.05$ between element concentration in microshoots grown on MS medium supplemented with 0/20 for Na/K concentrations and others grown on MS medium supplemented with the other concentrations of Na/K without or with PVP.

Discussion

In vitro techniques was used to improve plant characteristics and/or understand physiological and biochemical aspects where distinct conditions was easily established and modified to fulfill the aim of the experiments. In this concern, Agria and Hermes potato cultivars were successfully cultured and multiplied on MS medium supplemented with 0.0 mM NaCl, 1 mg/l BAP and 0.5 mg/l GA_3 (control). The number of formed shoots in Agria was higher than that of Hermes. On salt free medium, while the application of PVP reduced the number of shoots, it improved growth parameter especially in Hermes. PVP was used to decrease oxidation of phenolic compounds leading to increase survival and growth of the *in vitro* cultured plants (Shimelis, 2015). Accumulation of phenols, leading to browning the base of explants, was not detected in potato under the applied condition. Consequently, stimulation of growth parameters by PVP may be due to stimulation of some metabolites leading to cell and shoot elongation. Promotion of shoot initiation and elongation by application of antioxidant agents such as PVP or GH was previously registered (Standardi and Romani, 1990).

Survival of explant was only influenced by K^+ deficiency when it was in combination with moderate or relatively high salt stress. Under K^+ deficiency, rice leaves expressed higher values of antioxidant enzymes than those of control and it protected rice seedlings against cadmium stress (Liu *et al.,* 2013). Explant survival and number of regenerants/explant decreased with the increase of NaCl concentration. When K^+ content of the medium was increased from 20 mM to 30 mM, the number of shoots/explant under moderate salt stress (40 mM) and frequency of explants survival under relatively high salt stress (80 mM NaCl) were improved in both cultivars. Consequently, plants overcome the oxidative stress and survive (Çiçek and Çakirlar, 2008). Combination of PVP and sufficient K^+ (20 and 30 mM) improved the frequency of explants survival on MS medium containing 40 or 80 mM NaCl especially in Agria. Survival of shoot tips impeded in alginate matrix was improved by PVP (Uchendu *et al.,* 2010).

Growth parameters were drastically reduced when potato plants were cultured on medium containing low K^+ content (6 mM) in combination with moderate or high NaCl in Agria and Hermes cultivars. Availability of more K^+ (30 mM) in cultured medium than that of control (20 mM) improved the negative effect of relatively high NaCl on growth parameters. These results were in contrast to those of Alhagdow *et al.* (1999), they found that increase K^+ concentration 50% higher than that of basal MS medium did not promote *in vitro* shoot growth. Salt stress induces ROS formation and cell membrane damage leading to leakage of cytosolic K^+ through the activation of K^+ efflux channels (Cuin and Shabala, 2007). In this work, potassium loss could be avoided by increase of K^+ content of the medium and it decreased the harmful effect of salt stress in both cultivars. Consequently, improvement in growth parameters under the influence of sufficient exogenous potassium application may be due to decrease of Na^+/K^+ ratios and increase the efficiency of ROS scavenge system as was reported by Soleimanzadeh *et al.* (2010). In both cultivars, application of PVP improved the estimated growth parameters.

Our results indicated that Na^+ content of the cultured shoots increased with the increase of NaCl concentration, and it was consistent with the results of others (Dionisio-Seseand Tobita, 2007 and Aghaei *et al.,* 2009). Under salt stress, Na^+ content of potato shoots of both cultivars was affected by the K^+ content of the medium. Under salt stress, shoots subjected for K^+ deficiency (6 mM) showed higher Na^+ content and Na^+/K^+ ratio of both cultivars in comparison to others subjected to normal or high K^+ contents. In addition, Na^+ content and Na^+/K^+ ratio of shoots cultured on MS medium containing 40 mM NaCl and the highest K^+ content (30 mM K^+) was higher than other cultured on MS with normal K^+ content (20 mM) in Agria but vice versa in Hermes. Under these conditions, plants may modulate flux of Na^+ cross membrane (Tester and Davenport, 2003). Furthermore, under relatively high salt stress, the highest K^+ content of the medium expressed lowest Na^+/K^+ ratio leading to increase the survival frequency and growth parameters but they were still significantly lower than control. Under these conditions, low affinity flux results in Na^+ accumulation in plants through specific carriers (Garciadeblas *et al.,* 2003), non-

selective ion channels (Amtmann and Sanders, 1999), and K^+ selective ion (Amtmann and Sanders, 1999 and Zhang *et al.,* 2010). Application of PVP increased K^+ content but decreased the Na^+/K^+ ratio under moderate salt stress in Agria, but under moderate and relatively high salt stress in Hermes. Selectivity for K^+ over Na^+ uptake controlled the plant damage under salt stress (Bar-Tal *et al.,* 1991).

Under environmental stresses, "redox homeostasis" was established when ROS production overwhelms the ability of cells to scavenge them leading to cell damages and inhibition of growth (Mullineaux and Baker, 2010). It was detected when potato shoots were subjected to 80 mM NaCl. Potato as well as other plants overcomes ROS by their detoxification by enzymatic and/or non-enzymatic mechanisms (Caverzan *et al.,* 2016). Enzymes such as SOD, POX, CAT and APX create balance between them to determine the acceptable cellular level of ROS (Scandalios, 2005 and Caverzan *et al.,* 2016**)**.

Under K deficiency and salt stress, the estimated parameters were severely reduced it may be due to accumulation of toxic Na^+. In addition, deficiency of K^+ reduced sucrose export from plant leaves (Cakmak, 2005), retarded CO_2 assimilation and enhanced ROS production. This situation created the need for detoxification of ROS by increase of some antioxidant enzymes. In this work, SOD, POX and CAT, and SOD, POX and APX activities in Agria and Hermes increased, respectively, to control the negative effect of ROS on estimated parameters. Increase the activities of SOD, CAT and POX under low K^+ condition (0.5 mM) was detected to overcome the toxic effect of H_2O_2 (Ahmad *et al.*, 2014).

Under sufficient K^+ content of the media, some antioxidant enzymes were higher under relatively high salt stress than those of control such as activities of POX and CAT, and SOD, POX and APX in Agria and Hermes, respectively. This work indicated that sufficient potassium was necessary to conserve low Na^+/K^+ ratio and active scavenging system by application of antioxidant agent (PVP) or increase the endogenous antioxidant enzymes leading to minimize the negative effect of salt stress on potato. In Arabidopsis roots, ROS obtained under the influence of salt stress activated the outward rectifying K^+ channels (Demidchik *et al.,* 2010) leading to reduction of K^+ in root tissues. In barely roots, Na^+-induced K^+ efflux via outward rectifying K^+ channels (KORC) was detected. Salt- tolerant plants were able to enhance the activity of plasma membrane H^+-ATPase leading to minimizing the K^+ leakage from the cytosol. Consequently, restriction the activity of NORC may be beneficial for plants grow in saline soil (Zepeda-Jazo *et al.,* 2008). In this work, efflux of K^+ due to salt stress may be compensated by increase the availability of K^+ in cultured medium. In addition, application of PVP was generally increased of SOD, CAT and APX in Agria or POX and CAT in Hermes in comparison to those of untreated shoots. Abbasi *et al.* (2014) reported that moderate salinity stress enhanced the activity of SOD, CAT, and POD in maize hybrids but they decreased at high salinity stress except CAT. In other work, APX and CAT activities increased in *in vitro* grown shoots of potato of

one cultivar and vice versa in other cultivar indicating the salt tolerant of the first one (Sekmen *et al.,* 2007 and Aghaei *et al.,* 2009).

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تحسين الإكثار المعملي، النمو وجهاز إزالة الشوارد الحرة لنباتات البطاطس المنماة معملياً تحت ظروف الإجهاد الملحي بواسطة رفع محتوي البوتاسيوم في الوسط الغذائي

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تم استخدام الأفرع الخضراء المنماة معملياً بمزارع الأنسجة لسلالتين لنبات البطاطس (الأجاريا والهيرمس) لمعرفة تأثير البولي فينيل بيروليدون وثلاثة تركيزات بوتاسيوم مع تركيزين من كلوريد الصوديوم علي دلالات النمو، محتوي النباتات من الصوديوم والبوتاسيوم وكذلك علي نشاط الإنزيمات المضادة للأكسدة. بزيادة تركيز الملح قلت النسبة المئوية وكذلك عدد النبتات الجديدة النامية/مستقطع نباتي، ولكن زيادة تركيز البوتاسيوم في الوسط الغذائي من ٢٠ إلي ٣٠ ملي مول حسنت النسبة المئوية ودلالات النمو تحت تأثير الإجهاد العالي من الملح (٨٠ مل مول) وذلك في كلا السلالتين. زيادة محتوي البوتاسيوم في الوسط الغذائي من ٢٠ إلي ٣٠ مل مول عمل علي زيادة عدد النباتات الجديدة النامية/مستقطع نباتي عند تركيز ٤٠ مل مول من الملح. التأثير الإيجابي لتركيز البوتاسيوم العالي (٣٠ مل مول) علي دلالات النمو السابقة كان مصحوب بزيادة محتوي البوتاسيوم بالأفرع الخضراء مما أدي لنقص لنسبة أيونات الصوديوم/البوتاسيوم وكذلك زيادة نشاط بعض إنزيمات مضادات الأكسدة (سوبر أكسيد ديسميوتيز، بيروأكسيديز والكاتاليز في سلالة الآجاريا أو سوبرأكسيد ديسميوتيز، بيروأكسيديز والأسكوربيت بيروأكسيديز في سلالة الهيرمس)، خاصة تحت التركيز العالي نسبياً من إجهاد الملح. تزويد الوسط الغذائي بمضاد الأكسدة البولي فينيل بيروليدون زود محتوي البوتاسيوم ونشاط بعض إنزيمات مضادات الأكسدة (الكاتاليز والأسكوربيت بيروأكسيديز) وفي نفس الوقت قلل نسبة الصوديوم/البوتاسيوم للأفرع الخضراء المنزرعة علي التركيز العالي من الملح في سلالة الآجاريا وللأفرع الخضراء المنزرعة علي كلا تركزي الملح في سلالة الهيرمس. وبناءاً علي ذلك، فإن الإمداد الكافي بالبوتاسيوم وإضافة عامل مضاد الأكسدة (بولي فينيل بيروليدون) كان من الضرورة بمكان للحفاظ علي إنخفاض نسبة الصوديوم/البوتاسيوم وإثارة جهاز إزالة الشوارد الحرة بزيادة نشاط إنزيمات مضادات الأكسدة الداخلية مما يؤدي إلي تقليل التأثير السلبي للإجهاد الملحي علي نباتات البطاطس المنماة معملياً.