

## Interactive effect of salinity (NaCl) and potassium (k<sup>+</sup>) on *in vitro* growth of micropropagated potato

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DOI: <https://dx.doi.org/10.12692/ijaar/28.4.11-22>

### ARTICLE INFORMATION

#### RESEARCH PAPER

Vol. 28, Issue: 4, p. 11-22, 2026

Int. J. Agron. Agri. Res.

Gul *et al.*

ACCEPTED: 14 April, 2026

PUBLISHED: 19 April, 2026

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### ABSTRACT

Environmental stresses are among the most limiting factors to plant productivity. Among these, salinity is one of the biggest problems of Pakistan due to its arid to semi-arid climate. As the area under cultivation is limited and cannot be increased, we must resort to extensive salt affected areas worldwide to ensure food security. To utilize these salt affected areas we must devise strategies to able the crop plants to tolerate salinity and give economical yields. To accomplish this, current research was planned to understand the scope of alleviating the salinity stress by potassium application, the effects of salinity on plant's morphology, ionic concentration in micropropagated plantlets of potato cv. Desiree. A two-way factorial experiment consisting of five levels of salinity (0mM, 25mM, 50mM, 75mM and 100mM NaCl) and three levels of Potassium (0mM, 10mM and 20mM KNO<sub>3</sub>) was designed. The maximum growth was observed in plants with 0mM both NaCl and KNO<sub>3</sub>. Results revealed that the plant growth parameters, i.e, shoot length, number of roots, leaf number and number of nodes were seriously affected by increasing level of salinity. The most prominent effects were observed at 100mM NaCl. Also the addition of KNO<sub>3</sub> alone without NaCl has not shown any remarkable positive effect on growth of the plants. The beneficial effects of potassium were more pronounced at 20mM than 10mM KNO<sub>3</sub>. Salinity causes accumulation of Na<sup>+</sup> ions in plants which affects the absorption of K<sup>+</sup> by competing at uptake sites due to similar nature of both Na<sup>+</sup> and K<sup>+</sup> and by disturbing the membrane integrity by production of ROS causing leakage of K<sup>+</sup>. In this way nutritional imbalance results which was shown to be alleviated by the addition of KNO<sub>3</sub>. From this experiment, it has been concluded that the addition of potassium with different concentrations of NaCl alleviates the harmful effects of salinity by improving plant's growth characteristics and K<sup>+</sup>/Na<sup>+</sup> ratio of plants grown under salt stress under *in vitro* conditions.

**Key words:** Salinity, Potassium, *In vitro*, Potato

## INTRODUCTION

Potato (*Solanum tuberosum* L.) an important commercial food crop of the world is an auto-tetraploid ( $2n = 4x = 48$ ) and belongs to the family *Solanaceae* (Badoni and Chauhan, 2010). It is an important vegetable and a good source of antioxidants (Chen *et al.*, 2007). In Pakistan potato production is seriously impeded due to poor farming practices, susceptibility to several diseases and pests, non-availability of disease free certified seeds, and soil salinity (Farhatullah *et al.*, 2002). From all of the above mentioned limiting factors, soil salinity is the major constraint for low potato production not only in Pakistan but all over the world.

Preliminary studies showed that soil salinity is one of the most important abiotic stresses limiting the productivity of agricultural system around the world (Mahajan and Tuteja, 2005). According to an FAO statistics (2013), globally 7% of the current irrigated land is salt-affected while in Pakistan 37% of total irrigated land is saline.

Literature reported that salt stress is a complex mechanism which effects many morphological and physiological traits and also the metabolic pathways (Pitman and Lauchli, 2002; Borsani *et al.*, 2003; Parida and Das, 2005; Cuartero *et al.*, 2006; Ahmad, 2010; Nabati *et al.*, 2011). It affects the plant in two ways: First by reducing the uptake of water and second by ion accumulation to toxic levels (Montoliu *et al.*, 2009). The former rapid osmotic phase limits the plant growth while the latter slow ionic phase causes leaf senescence (Munns and Tester, 2008; Wang *et al.*, 2013).

Salinity is a common feature of arid and semiarid lands; plants have evolved mechanisms to tolerate salinity (Munns and Tester, 2008). Plants have developed divergent strategies to combat salt stress, such as restricting  $\text{Na}^+$  uptake, activating  $\text{Na}^+$  exclusion or cellular compartmentalization of excessive  $\text{Na}^+$  into the vacuole, synthesis of harmonious products, adjustment of photosynthetic and energy metabolism, antioxidant enzymes accumulation, hormones regulation, and cell structure modification (Zhang *et al.*, 2011; Yang *et al.*,

2012; Wang *et al.*, 2013). Khrais *et al.* (1998) demonstrated that out of 130 potato cultivars screened in *in vitro* study six cultivars (Amisk, BelRus, Bintje, Onaway, Sierra and Tobique) are salt tolerant. Similarly Aghaei *et al.* (2008) reported that the response to salt tolerance is genotype dependent after *in vitro* screening 10 potato cultivars.

The solution to the salinity problem is challenging. Primarily, any attempt to desalinate soil and water could be extortionate especially in developing countries where funds and expertise are limited (Ashraf, 1994). The other feasible options would be to breed for salt tolerant cultivars (Collins *et al.*, 1990). Another approach is to alleviate the salinity damage to plants by supplying nutrients (Rubio *et al.*, 2010).

Potassium is a macronutrient which is essential for different plant physiological and metabolic activities. Under normal circumstances, plants require higher amount (100mM-200mM) of potassium compared to sodium (less than 1mM) so as to have a high cytosolic  $\text{K}^+/\text{Na}^+$  ratio, essential for maintaining osmotic balance (Lokhande *et al.*, 2010). Sodium competes with potassium under salt stress in three ways: on uptake sites at plasma membrane, through membrane dis-integrity and by plasma membrane depolarization (Liu *et al.*, 2019).

Salinity and low potassium availability are important environmental factors restricting plant growth and productivity. The interactive effects of salinity and potassium on growth of *Houttuynia cordata* was studied by Zou *et al.* (2012) who found that plant biomass production, ratio of root and shoot, root numbers, water content etc significantly declined in the combined effect of salinity and  $\text{K}^+$  deprivation, and increased with salinity and reported that supplementary Potassium under salt stress alleviate salinity by enhanced plant biomass production in *Houttuynia cordata*. Yousufinia *et al.* (2013) demonstrated that under field conditions different barley cultivars showed increased  $\text{Na}^+$  concentration

with increasing NaCl levels, whereas  $K^+$  concentration and  $K^+/Na^+$  ratio were decreased with rising of the NaCl level. Regulation of Potassium uptake can be used as a strategy to maintain desirable cytosolic  $Na^+/K^+$  ratio (Chinnusamy *et al.*, 2005). So adding Potassium under saline condition may act as ameliorative on the growth of potato plant.

Hussain *et al.* (2013) investigated that potassium sulphate in two concentrations (50 kg/ha and 100 kg/ha) when applied in wheat fields in salt affected soils significantly increased 14% and 30% grain yield and 35% and 54% dry matter respectively. Negative effects of salinity have been reversed in hydroponically grown endives when  $K_2SO_4$  was supplied in the nutrient solution @10mM under a salt stress of 40mM NaCl while potassium enrichment alone has no effect on plant biomass, leaf/root ratio, leaf fresh weight, leaf number, and root length (Tzortzakis, 2010).

Exogenous application of inorganic nutrients K and P along with IAA in maize were reported to reduce the negative effects of salinity and the most promising results were obtained by the combined application of IAA, K and P under 100mM NaCl (Kaya *et al.*, 2013). In an *in vitro* experiment the effect of addition of three concentrations of K i.e. 6mM, 20mM and 30mM under 40mM and 80mM NaCl concentrations on two potato cultivars (Sierra and Russet Burbank) was investigated and results revealed that the Na concentration was greater when 6mM K was used in the medium and decreased upon 20mM K concentration (Alhag Dow *et al.*, 1999). Similarly, Borsani *et al.*, 2001 found that in tomato, the salt-hypersensitive mutants were found to be defective in K uptake and had an impaired K nutrition. These results again highlight the critical importance of adequate K nutrition in alleviating the detrimental effects of salinity in plants.

Regulated *in vitro* systems are ideal for successful selection for salt tolerance in plants because *in vitro* screening tests for salt tolerance could be performed in the laboratory rapidly and reliably.

Moreover, a highly significant correlation exists between field experiment and *in vitro* growth parameters (Morpurgo, 1991). *In vitro* cultures can therefore provide an effective alternative to avoid soil or environmental complexities and to focus on the growth while studying plant response to imposed stress factors (Yaman *et al.*, 2020; Daneshmand *et al.*, 2010).

Salinity changes the nutritional requirements of the growing plant, both in type and amount of the nutrient (Hu and Schmidhalter, 2025). This nutritional imbalance requires insights of the interactive effect that exist among various elements in different plants at various concentrations of NaCl and their subsequential effect on growth parameters. By understanding this phenomenon, strategies could be designed to cope with the problem of salinity or to ameliorate or alleviate the effects of salinity. Keeping in view the above mentioned factors, the present research work was conducted to study the *in vitro* growth response of Potato cv. Desiree at various concentrations of NaCl and  $KNO_3$ .

## MATERIALS AND METHODS

The research work was carried out at Tissue Culture Laboratory, Hazara Agriculture Research Station Abbottabad. Elemental analysis was done at Laboratory of Agricultural Chemistry, Department of Agricultural Chemistry, University of Agriculture, Peshawar.

Thirty days old micropropagated plantlets of *Solanum tuberosum* cv. Desiree were selected for the experiment. The Murashige and Skoog (1962) basal media with 30g sucrose as carbohydrate source and 6.5g agar as solidifying agent,  $1.0 \text{ mg l}^{-1}$  Ca-pentothenate,  $0.25 \text{ mg l}^{-1}$  Gibberellic acid ( $GA_3$ ),  $100 \text{ mg l}^{-1}$  Myoinositol at pH 5.8 was used in this study. The different treatments consisted of fifteen combinations of NaCl (0, 25, 50, 75 and 100mM) and  $KNO_3$  (0, 10 and 20mM).

After aseptic inoculation, the cultures were maintained for about 30 days in the growth chamber at  $22 \pm 2^\circ\text{C}$  under a photoperiod of 16 h provided by cool fluorescent tube lights.

The design used was two-way completely randomized factorial. Six replicates per treatment combination were used. Two-way Analysis of Variance (ANOVA) was checked using “Statistix 8.1” software at  $p \leq 0.05$ . The results were further employed to least significance difference (LSD) test to check significance of interaction pattern within, among and between treatments. The plant elemental ( $\text{Na}^+$  and  $\text{K}^+$ ) analysis was done according to the procedures described by Munns *et al.* (2010). The plant samples were washed with distilled water after removing from the media, dried at  $70^\circ\text{C}$  and then grounded to fine powder using pastel and mortar.

The plant samples were digested in 0.5M  $\text{HNO}_3$ . For various samples, different volumes of dilute acid were used. For samples of 100mg, 10ml of acid, for 20–50 mg, 5ml and for <20 mg, 2.5 ml of dilute acid was used for digestion. All of these samples were covered and placed in an oven at  $80^\circ\text{C}$  for 1 h. The vials were inverted once during the heating and once after cooling to suspend the plant material. After cooling, the solid particles were allowed to settle at the bottom. The extract was filtered using Wattsman filter paper. It was further diluted with distilled water in the ratio 1:10.

For making standards of Na and K, dried salts NaCl and KCl respectively were used. For both salts a stock of  $50\mu\text{g/ml}$  was made which was further diluted to make standards of 0, 0.5, 1, 2.5, 5, 10, 15, 20 and  $25\mu\text{g/ml}$  of Na and K.

The samples were aspirated and the readings were recorded by using Flame photometer (PFP7, Jenway).

## RESULTS

Potato plantlets exposed to salt stress for 30 days exhibited stunted growth and chlorotic leaves accompanied by necrotic tips. The lower leaves were shed prematurely. In general, the plantlets seemed to show wilting. These effects increased as the salt concentration increased. Visual observations showed that both salts NaCl and  $\text{KNO}_3$  have variable effects on plant growth depending on the concentration in the medium.

## Shoot and root emergence

Data regarding shoot and root emergence as presented in Table 1 on third day of culturing showed that shoot emergence was shown by all treatments. The maximum shoot emergence was recorded in control (without NaCl and  $\text{KNO}_3$ ) in which 6/6 replicates showed shoot emergence.

In rest of the treatments 5/6 replicates showed shoot emergence except in treatment interaction of 100mM NaCl with 10Mm  $\text{KNO}_3$  in which 4/6 inoculated nodes showed shoot emergence.

However a few plants showed root emergence on third day. The maximum root emergence was recorded in control in which 3/6 plantlets showed root emergence while in treatment with 50mM NaCl and 20mM  $\text{KNO}_3$  only 1/6 plantlets showed root emergence on third day (Table 1).

Although shoot emergence was observed in all treatments interactions after one week, yet root emergence was seriously affected by various combinations of NaCl and  $\text{KNO}_3$  (Table 2).

Data in Table 2 showed that the maximum root emergence in control (0mM both NaCl and  $\text{KNO}_3$ ) was recorded in all the six replicates while with increasing NaCl to 50 mM concentration few replicates showed root emergence in interaction with all concentrations of  $\text{KNO}_3$  whereas with further increase in salinity level of the media i.e 75 and 100mM; the ameliorative effect of  $\text{KNO}_3$  has not been further effective and no root emergence was recorded even after one week (Table 2).

## Shoot length

The effect of various concentrations of NaCl and  $\text{KNO}_3$  is very much evident from the data recorded after 15 days of culturing (Table 3). The highest mean shoot length (5.0 cm) was observed in control at 0mM concentration of both NaCl and  $\text{KNO}_3$  followed by plants grown at 20mM  $\text{KNO}_3$  without NaCl, i.e., 4.08 cm. The lowest mean value (1.98 cm) was observed in plants grown at 100mM NaCl

without KNO<sub>3</sub>. Generally it was observed that with increasing NaCl concentration the shoot length decreases. The effect of KNO<sub>3</sub> on shoot length is

ameliorative. However the statistical analysis reveals that the increase in shoot length caused by addition of KNO<sub>3</sub> is not significant (Table 3).

**Table 1.** Plantlets showing shoot and root emergence on 3<sup>rd</sup> day of culturing

NaCl	Growth parameters					
	Shoot emergence			Root emergence		
	KNO <sub>3</sub>			KNO <sub>3</sub>		
	0mM	10mM	20mM	0mM	10mM	20mM
0Mm	6/6	5/6	5/6	3/6	0/6	0/6
25Mm	5/6	5/6	5/6	0/6	0/6	0/6
50Mm	5/6	5/6	5/6	0/6	0/6	1/6
75Mm	5/6	5/6	5/6	0/6	0/6	0/6
100mM	5/6	4/6	5/6	0/6	0/6	0/6

**Table 2.** Plantlets showing shoot and root emergence after one week of culturing

NaCl	Growth parameters					
	Shoot emergence			Root emergence		
	KNO <sub>3</sub>			KNO <sub>3</sub>		
	0mM	10mM	20mM	0mM	10mM	20mM
0Mm	6/6	6/6	6/6	6/6	1/6	2/6
25Mm	6/6	6/6	6/6	1/6	2/6	4/6
50Mm	6/6	6/6	6/6	1/6	2/6	4/6
75Mm	6/6	6/6	6/6	0/6	0/6	0/6
100mM	6/6	6/6	6/6	0/6	0/6	0/6

**Table 3.** Effect of different concentrations of nacl and KNO<sub>3</sub> on growth of invitro grown potato plantlets after 15 days of culturing

NaCl	Growth parameters											
	Mean number of roots			Mean shoot length (cm)			Mean number of leaves			Mean number of nodes		
	KNO <sub>3</sub>			KNO <sub>3</sub>			KNO <sub>3</sub>			KNO <sub>3</sub>		
	0mM	10mM	20mM	0mM	10mM	20mM	0mM	10mM	20mM	0mM	10mM	20mM
0mM	4.16a	2bcd	3.16ab	5a	3.5abcd	4.08ab	7.16a	5.66abc	6abc	4.5a	3.66ab	3.73ab
25mM	1.5bcd	1.83bcd	2.5abc	3.28bcd	3.38bcd	3.73abc	5.33bc	6abc	6.33ab	3.16bcd	3.33abc	3.66ab
50mM	1.5bcd	1.66bcd	2.5abc	3bcd	3.05bcd	3.61abc	5.33bc	5.5abc	5.83abc	3bcde	3.16bcd	3.5ab
75mM	0.33d	1cd	2.16abcd	2.18cd	2.53bcd	3.11bcd	4.66bc	4.83bc	5.66abc	2de	2.66bcde	2.83bcde
100mM	0.16d	1cd	1.33bcd	1.98d	2.21cd	2.38cd	4.00c	4.23bc	4.5bc	1.83e	2de	2.16cde

Means followed by different letters in a column/row showed significant difference ( $p \leq 0.05$ )

Similar trend was observed in shoot length after thirty days. Data in Table 4 showed that the maximum shoot length was 6.01cm shown by plants grown without NaCl and KNO<sub>3</sub>. The minimum shoot length (2.00 cm) was recorded at 100 mM NaCl.

Final data on forty days of culturing was presented in Table 5. The data showed that the maximum mean shoot length remained the same in control (6.01cm) followed by the plants supplemented with 20mM KNO<sub>3</sub> with no NaCl i.e 4.95 cm, while the least value of mean shoot length was found to be 2.00 cm at 100mM NaCl with 0mM KNO<sub>3</sub> (Fig. 1M) and 2.38cm shown by plants grown at 75mM NaCl with

0mM KNO<sub>3</sub>. The value of mean shoot length at 75mM salinity interacted with 10mM KNO<sub>3</sub> is 2.63cm which is lesser than the value of plants grown at the same salinity level supplemented with 20mM KNO<sub>3</sub> (3.48cm). In other words, the addition of 20mM KNO<sub>3</sub> has alleviated the salt stress of 75mM. An increase in shoot length has been observed with the addition of KNO<sub>3</sub> showing ameliorative effect under different salinity levels but there is no statistically significant difference observed.

**Number of roots**

The data on number of roots after fifteen days (Table 3) revealed that plantlets grown without any NaCl and

KNO<sub>3</sub> showed maximum number of roots (4.16) followed by plants grown at 0mM NaCl and 20mM KNO<sub>3</sub> (3.16). The minimum numbers of roots were observed in plants grown at 100mM NaCl with 0mM KNO<sub>3</sub> (0.16). Generally it was observed that with increasing NaCl concentration badly affected number of roots. The effect of KNO<sub>3</sub> on number of roots is ameliorative as root number increased with increasing concentration of KNO<sub>3</sub>.

Similar trend was observed in number of roots after 30 days. The maximum root number was 4.33 shown by plants grown with 0mM NaCl and KNO<sub>3</sub>. The minimum value of

number of roots was found to occur at 100 mM NaCl (0.25). Plants grown at 75 mM and 100mM NaCl with or without KNO<sub>3</sub> concentrations differed significantly from those of 0 mM NaCl and KNO<sub>3</sub>. Final data on forty days of culturing showed that the minimum no of roots (0.46) at 100 mM NaCl differs significantly from plants grown at 0 mM and 25 mM NaCl without KNO<sub>3</sub> while the plants grown at 0 mM both NaCl and KNO<sub>3</sub> showed significant highest number of roots i.e 5.16. KNO<sub>3</sub> supplementation showed improvement in growth but no statistical significance in increase in number of roots was observed in most of treatment interactions (Table 5).

**Table 4.** Comparison of effect of NaCl and KNO<sub>3</sub> at different concentrations on *in vitro* growth of potato plantlets after 30 days of culturing

NaCl	Growth parameters											
	No pf Roots			Shoot Length (cm)			Number of Leaves			Number of Nodes		
	KNO <sub>3</sub>			KNO <sub>3</sub>			KNO <sub>3</sub>			KNO <sub>3</sub>		
	0mM	10mM	20mM	0mM	10mM	20mM	0mM	10mM	20mM	0mM	10mM	20mM
0mM	4.33a	3.16abc	3.5ab	6.01a	4.85ab	4.95ab	7.33a	6.83abc	6.85abc	6.16a	4.16b	4.33c
25mM	2.66abcd	2.83abcd	3.16abc	3.65bc	3.78bc	4.28abc	6.66abcd	6.16abcde	6.33abcd	3.95b	4.17b	4.33b
50mM	1.64bcde	1.83bcde	2.5abcd	3.55bc	3.78bc	4.21abc	5.33bcde	6.16abcde	6.2abcde	3.66b	3.72b	3.83b
75mM	0.33e	0.83cde	1.5bcde	2.38c	2.60c	3.28bc	4.66de	5bcde	5.73abcde	2.36b	3.24b	3.5b
100mM	0.25e	0.5de	1.33bcde	2.00c	2.40c	2.98bc	4.12e	4.75cde	4.83cde	2.23b	2.48b	2.88b

Means followed by different letters in a column/row showed significant difference ( $p \leq 0.05$ ).

**Table 5.** Comparison of effect of NaCl and KNO<sub>3</sub> at different concentrations on *in vitro* growth of potato plantlets after 40 days of culturing

NaCl	Growth parameters											
	No pf roots			Shoot length (cm)			Number of leaves			Number of nodes		
	KNO <sub>3</sub>			KNO <sub>3</sub>			KNO <sub>3</sub>			KNO <sub>3</sub>		
	0mM	10mM	20mM	0mM	10mM	20mM	0mM	10mM	20mM	0mM	10mM	20mM
0mM	5.16a	3.16abc	4.33ab	6.01a	4.87ab	4.95ab	7.5a	6.83abc	6.85abc	6.16a	3.66bcd	4.33b
25mM	2.16bcd	3.5ab	3.66abc	3.91	4.05abc	4.28abc	6.76abc	6.33abcd	6.34abcd	4.12bc	4.16bc	4.33b
50mM	1.5cde	1.83cde	2.5bcd	3.71bc	4.05abc	4.21abc	5.53bcd	6.66abcd	6.16abcde	3.66bcd	3.83bcd	3.83bcd
75mM	1cde	1.33cde	2cde	2.38c	2.63c	3.48bc	4.66de	5bcde	5.75abcde	2.88de	3.16bcde	3.5bcd
100mM	0.46e	0.8de	1.4de	2.00c	2.42c	2.98bc	4.16e	4.75cde	4.83cde	2.23e	2.48e	2.88de

Means followed by different letters in a column/row showed significant difference ( $p \leq 0.05$ ).

**No of leaves**

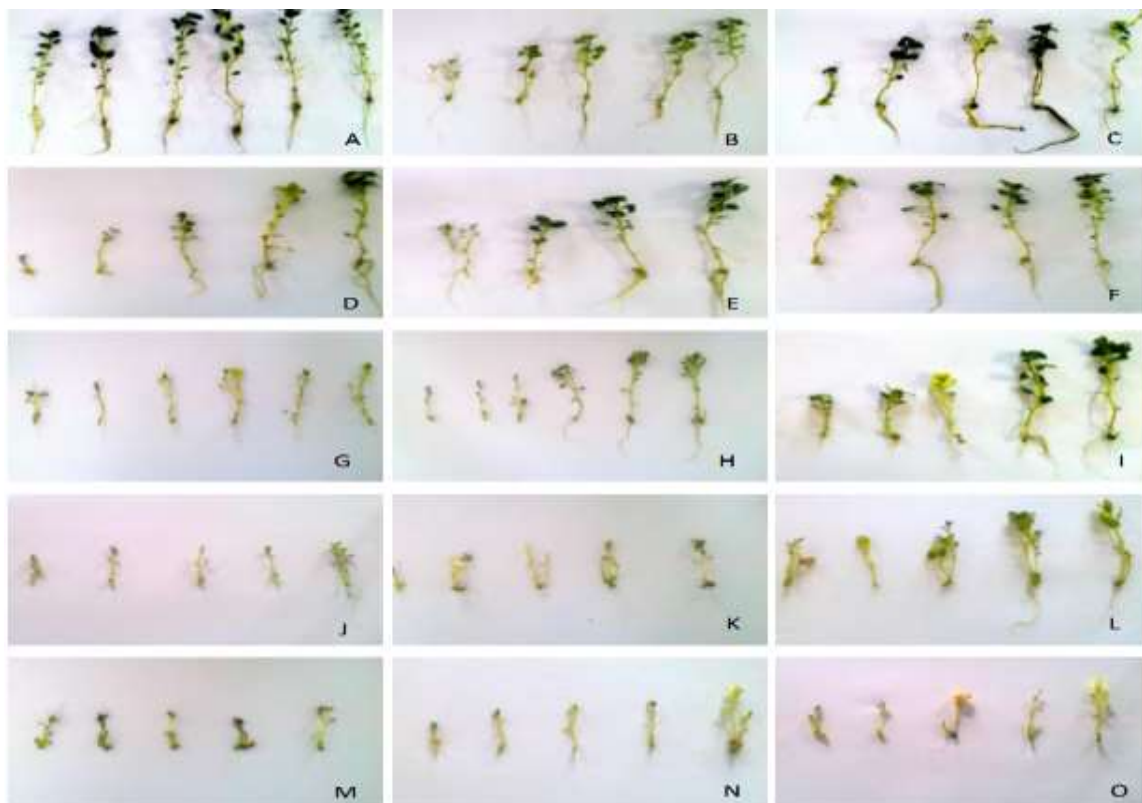
The data on number of leaves after fifteen days revealed that plantlets grown without any NaCl and KNO<sub>3</sub> showed maximum mean leaves number, i.e., 7.16 followed by plants grown at 25mM NaCl and 20mM KNO<sub>3</sub> (6.33) (Table 3). The minimum mean number of leaves (4.0) was observed in plants grown at 100mM NaCl without KNO<sub>3</sub> addition. Generally it was observed that with increasing NaCl concentration the leaf number decreased. The effect of KNO<sub>3</sub> on number of leaves is also

ameliorative. However the statistical analysis reveals that the increase in mean leaf number caused by the addition of KNO<sub>3</sub> is non-significant.

The maximum mean numbers of leaves after thirty days (Table 4) were 7.33 shown by plants grown with 0mM NaCl and KNO<sub>3</sub>. The minimum mean numbers of leaves were found to occur at 100 mM NaCl without any addition of KNO<sub>3</sub>, i.e., 4.12. Statistical analysis showed that no significant difference was found among treatments.

Final data recorded after forty days of culturing showed that the minimum number of leaves (4.16) at 100 mM NaCl and 0mM KNO<sub>3</sub> differs significantly from plants grown at 0 mM NaCl and 0 mM KNO<sub>3</sub> (Table 5; Fig. 1A)

but not from other treatments. KNO<sub>3</sub> supplementation showed improvement in growth. The maximum mean number of leaves, i.e., 7.5 were found at 0 mM both NaCl and KNO<sub>3</sub> concentration.



**Fig. 1.** Comparison of *in vitro* plants growth in different treatment interactions after forty days of culturing; A(0mM NaCl, 0mM KNO<sub>3</sub>); B (0mM NaCl, 10mM KNO<sub>3</sub>); C (0mM NaCl, 20mM KNO<sub>3</sub>); D (25mM NaCl, 0mM KNO<sub>3</sub>); E (25mM NaCl, 10mM KNO<sub>3</sub>); F (25mM NaCl, 20mM KNO<sub>3</sub>); G (50mM NaCl, 0mM KNO<sub>3</sub>); H (50mM NaCl, 10mM KNO<sub>3</sub>); I (50mM NaCl, 20mM KNO<sub>3</sub>); J (75mM NaCl, 0mM KNO<sub>3</sub>); K (75mM NaCl, 10mM KNO<sub>3</sub>); L (75mM NaCl, 20mM KNO<sub>3</sub>); M (100mM NaCl, 0mM KNO<sub>3</sub>); N (100mM NaCl, 10mM KNO<sub>3</sub>); O (100mM NaCl, 20mM KNO<sub>3</sub>)

### No of nodes

Generally, it was observed that with increasing NaCl concentration the number of nodes decreases. The effect of KNO<sub>3</sub> on number of nodes is also ameliorative. However statistically the increase in number of nodes caused by addition of KNO<sub>3</sub> is not significant. The maximum number of nodes (4.5) after fifteen days were shown by plants grown with 0mM both NaCl and KNO<sub>3</sub> concentration. The minimum mean number of nodes were found to occur at 100 mM NaCl (1.83) (Table 3). Table 4 presented no remarkable increase in the number of nodes after thirty days as compared with data of 15 days.

Final data after forty days of culturing showed that the minimum mean number of nodes (2.23) recorded at 100 mM NaCl with 0mM KNO<sub>3</sub> differs significantly from plants grown at 0 mM both NaCl and KNO<sub>3</sub> in which the mean number of nodes were found to be 6.16. KNO<sub>3</sub> supplementation showed improvement in overall plant growth (Table 5).

### Ionic content in plants

#### Na<sup>+</sup> concentration

Analysis of Variance showed that the means of treatment interactions for Na<sup>+</sup> concentration (Table 6) differed significantly from one another. The value of sodium ion

concentration by ANOVA showed that it is not only significant within its treatment but also influences K<sup>+</sup> concentration. The highest concentration of Na<sup>+</sup> was found to occur at 100mM NaCl with 0mM KNO<sub>3</sub>. Comparison by LSD showed that the concentration of Na<sup>+</sup> at 100mM NaCl with 0mM KNO<sub>3</sub> is significantly different from all other treatments. No significant difference in concentration of sodium ion was found at 100mM NaCl supplemented with

10mM and 20mM KNO<sub>3</sub>. Similarly three treatment interactions, i.e., 75mM NaCl with 10mM and 20mM KNO<sub>3</sub> and 50mM NaCl with 0mM KNO<sub>3</sub> have non-significant difference in the values of concentration of Na<sup>+</sup>. Regarding 0mM NaCl, all the treatment interactions supplemented with or without potassium were found non-significant in amount of sodium ions but significantly different from 25mM NaCl (Table 6).

**Table 6.** Comparison of effect of NaCl and KNO<sub>3</sub> at different concentrations on plant ionic (Na<sup>+</sup> and K<sup>+</sup>) content after 40 days of culturing

NaCl	KNO <sub>3</sub>					
	0mM		10mM		20mM	
	Na <sup>+</sup> (ppm)	K <sup>+</sup> (ppm)	Na <sup>+</sup> (ppm)	K <sup>+</sup> (ppm)	Na <sup>+</sup> (ppm)	K <sup>+</sup> (ppm)
0mM	40hij	218ab	32j	232a	34ij	220ab
25mM	51ef	196bcd	48fgh	204abc	41ghi	218ab
50mM	62cd	179cde	59de	192bcd	50fg	207abc
75mM	85b	165e	70c	183cde	66cd	195bcd
100mM	109a	151e	97b	163de	93b	174cde

Means followed by different letters in a column/row showed significant difference ( $p \leq 0.05$ ).

The data suggests that the concentration of Na<sup>+</sup> increased with increasing the NaCl concentration and decreased with the addition of Potassium (Table 6). Potassium application was effective and significantly decreased Na<sup>+</sup> concentration and increased K<sup>+</sup> concentration. The K<sup>+</sup>/Na<sup>+</sup> decreased with increasing salinity while increased with potassium application.

#### K<sup>+</sup> concentration

Statistical analysis of potassium ion concentration (Table 6) revealed that the means of the treatments are significantly different from one another. The minimum value of K<sup>+</sup> concentration was found to occur at 100 mM NaCl. The LSD test showed that the values of 0 mM, 10 mM and 20 mM KNO<sub>3</sub> are nonsignificantly different from one another. Moreover, the K<sup>+</sup> concentration in interaction with NaCl revealed that the values of K<sup>+</sup> concentration are insignificant at 0mM and 25mM NaCl, between 25mM and 50mM NaCl, at 50mM and 75mM and at 75mM and 100mM. However the concentrations of K<sup>+</sup> at 0 mM NaCl are significantly different from that of 75 mM and 100 mM NaCl. Similarly the concentration at 25 mM NaCl also differs significantly from 75 mM and 100mM NaCl (Table 6).

The results in Table 6 suggested that with increasing amount of salinity the concentration of K<sup>+</sup> decreases and that of Na<sup>+</sup> increases. The treatments in which potassium was added showed an increase in K<sup>+</sup> concentration while decrease in Na<sup>+</sup> concentration.

#### DISCUSSION

Crop production by artificial irrigation in many arid and semiarid regions of the world is exposed to salinity. Adequate regulations of mineral nutrients are needed to supplement and to sustain crop productivity of salt affected soils (Akhtar *et al.*, 2010; Mahar *et al.*, 2003).

The present study showed that salinity markedly reduced plant growth and plant biomass, confirming many previous findings that salinity reduces plant height (Agong *et al.*, 2004; Hajer *et al.*, 2006). Zhu (2002) reported that higher accumulation of Na<sup>+</sup> damaged plant metabolism and reduced plant growth. The treatments which showed better growth was due to their capability to selectively absorb K<sup>+</sup> over Na<sup>+</sup>. This has been confirmed by Munns and James (2003) that plants with lowest Na<sup>+</sup> concentration produced greatest biomass. These results are in conformity with Kaya *et al.* (2001) in which it was

concluded that potassium application reduced the  $K^+$  ion leakage in spinach grown under saline conditions. The leakage of potassium is due to production of reactive oxygen species (ROS) which damages the membranes allowing them to leak out. Moreover Potassium acts as a major osmoticum in vacuole for maintaining high tissue water content under stress condition (Marschner, 2012).

The deleterious effects of salinity on plant growth and physiology are associated with a number of other important factors, including nutrient imbalance, the toxic effects of ions (mainly  $Na^+$  and  $Cl^-$ ) and/or the oxidative damage resulting from imbalance between production of ROS and the antioxidant defense (Vital *et al.*, 2008).

Carden *et al.* (2003) also demonstrated that salt-tolerant barley plants accumulated higher  $K^+$  due to selective absorption of  $K^+$  and by a preferential loading of  $K^+$  rather than  $Na^+$  into the xylem. Numerous studies had shown that addition of  $K^+$  mitigated the undesirable effects of  $Na^+$  and improved  $K^+$  uptake of cucumber and pepper (Kaya *et al.*, 2001), olive (Chartzoulakis *et al.*, 2006) and juvenile mulloway (Doroudi *et al.*, 2006) and improved  $K^+/Na^+$  ratio under salt stress (Marschner, 2012; Carden *et al.*, 2003). It was suggested that the plant's tolerance response is characterized by distinctly higher  $K^+/Na^+$  ratio, which may be used as indicator of tolerance to salt stress.

Root growth was seriously affected by the saline media in our study. This is because under salt-stress, root growth is restricted by osmotic effects and toxic effects of ions, which results in lower nutrient uptake and inhibits the translocation of mineral nutrients, especially  $K^+$ . As a result of the similarities in physicochemical properties between  $Na^+$  and  $K^+$ ,  $Na^+$  could compete with  $K^+$  for major binding sites in key metabolic processes, including both low-affinity {e.g., non-selective cation channels (NSCC) and high-affinity (e.g. high-affinity  $K^+$  transporter (HKT)} transporters and could also disturb plant metabolism (Shabala and Cuin, 2008; Marschner, 2012).  $K^+$  deficiency can usually be observed under salinity stress. First, high levels of  $Na^+$  inhibit  $K^+$  activity in the soil resulting in a reduction of  $K^+$  availability.

Second,  $Na^+$  not only interferes with  $K^+$  translocation from root to shoot (especially in low  $K^+$  status) (Botella *et al.*, 1997), but also competes with  $K^+$  for uptake sites at the plasma membrane, resulting in lower  $K^+$  uptake. Third, salinity stress leads to plasma membrane dis-integrity and favors  $K^+$  leakage, resulting in a rapid decline in cytosolic  $K^+$  (Coskun *et al.*, 2010). Also, salinity induces significant membrane depolarization and favors  $K^+$  leaking through depolarization-activated outward-rectifying (KOR)  $K^+$  channels (Shabala and Cuin, 2008). Therefore, keeping cellular  $K^+$  content above a certain threshold and maintaining a high cytosolic  $K^+/Na^+$  ratio (either by retaining  $K^+$  or preventing  $Na^+$  from accumulating in the leaves) is critical for plant growth and salt tolerance. An increasing K supply corresponded with higher  $K^+$  accumulation in plant tissue, which reduced the  $Na^+$  concentration and resulted in a higher  $K^+/Na^+$  ratio. Members of high-affinity  $K^+$  transporter (HKT) family that mediate  $Na^+$ -specific transport or  $Na^+/K^+$  co-transport play a key role in plant  $Na^+$  tolerance mechanisms (Mian *et al.*, 2011; Platten *et al.*, 2006).

In the present study application of  $K^+$  significantly reduced the toxic effects of NaCl and improved plant growth in invitro potato plantlets. This was certified to antagonistic effect of  $K^+$  with  $Na^+$  (Lynch and Lauchli, 1984).

In conclusion, salinity stress reduced plant growth by affecting plant morphological characteristics, reducing relative water contents and membrane stability, decreasing photosynthetic activities, altering  $K^+/Na^+$  ratios and antioxidant activities. However, addition of potassium significantly alleviates harmful effect of salinity by improving the plant growth in terms of plant growth parameters and enhancing  $K^+/Na^+$  ratios.

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