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SALINITY IMPAIRS IONIC, PHYSIOLOGICAL AND BIOCHEMICAL ATTRIBUTES IN POTATO

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Salt stress is hampering plant growth and development especially in arid and semiarid regions due to enhanced evapotranspiration and underground brackish irrigation water. A pot experiment was therefore conducted to assess the malicious effects of salinity on two potato (*Solanum tuberosum* L.) cultivars namely N-Y LARA and 720-110 NARC. Various salinity levels (control, 2.5, 5.0, 7.5, 10.0 and 12.5 dS m^{-1}, developed with NaCl) were induced after 30 days of tuber emergence. Both the cultivars proved to be significantly ($p \leq 0.05$) affected by salt stress. However, N-Y LARA was less affected than 720-110 NARC. Salinity stress drastically reduced potassium (K^+) contents, protein contents, water relations and gas exchange attributes. However, sodium (Na^+) contents, Na^+: K^+ ratio, leaf electrolyte leakage, proline contents, melondialdehyde (MDA) contents and antioxidant enzymes activities like superoxide dismutase (SOD), catalase (CAT) and peroxidase (POD) were increased with increasing salinity stress. Conclusively, salt tolerance potential is cultivar dependent as both cultivars exhibited diverse performance vis-à-vis various studied attributes against different NaCl levels.
Keywords: Potato, salinity, Na^+: K^+ ratio, water relations, gas exchange, antioxidants.

INTRODUCTION

Agriculture is one of the most exposed sectors to vagaries of climate change (Malik, 2012). Around 20-25% of the world (Munns and Tester, 2008) and 26% of Pakistan's agricultural (Anonymous, 2010) irrigated lands are affected by salinity. Saline area is increasing day by day due to higher evapotranspiration that demand more irrigation and consequently, more salt accumulation in the root zone especially in arid and semiarid regions of the world (Iqbal *et al*., 2009; Mou, 2011). Predominately, salinization (50–80%) is caused by NaCl salt (Kaouther, 2012). Primary toxic ion is Na^+ as it not only impairs K^+ uptake but also interrupts regulation of stomata which eventually causes water loss. Na^+ enters in leaf apoplast through xylem stream and left behind as water evaporates. Na^+ mainly compete and occupies cations binding sites by reducing uptake and transport of Ca^{+2} and K^+ (Munns and Tester, 2008; Horie *et al*., 2012; Hasanuzzaman *et al*., 2013). Hyperosmotic accompanied with hyper ionic conditions enhance generation of reactive oxygen species (ROS) damaging the proteins, lipids, DNA and carbohydrates molecules, which weakens the plant defense mechanism and modifies membrane structures and its composition (Tuteja, 2007; Ismail, 2014; Jbir-Koubaa, 2014; Gao *et al*., 2015). Moreover, ROS intensifies MDA contents, deactivates enzymes, disrupts ions of normal cellular metabolism and enhances electrolyte leakage that causes programmed cell death (necroptosis) and reduced photosynthetic activity (Gao *et al*., 2015). Therefore, plants manifest various scavenging machineries like enzymatic antioxidant system (SOD, CAT, POD), the level of which is elevated during abiotic stresses (Gill and Tuteja, 2010; Choudhury, 2013; Ismail, 2014). It also affects plant water relations and gas exchange attributes together with metabolic toxicity, reduction in green pigments and thereby intervening photosynthetic activity (Ashraf and Harris, 2013; Gupta and Huang, 2014; Li *et al*., 2014). Potato (*Solanum tuberosum* L.) is a staple food for about 50% world's population and third largest crop in human consumption. It is fourth major crop with respect to area and production in the world. It provides high energy per unit land, water and time along-with valuable source of vitamins and minerals (Abhayapala *et al*., 2014; Gao *et al*., 2015). It is moderately salt-sensitive (Mitsuya *et al*., 2000) with 50% growth and yield reduction at 5 dSm^{-1} salt stress (Hmida-Sayari *et al*., 2005). However, tolerance level varies from cultivar to cultivar (Bruns and HechtBuchholz, 1990). Increasing saline area demands salt tolerant cultivars for sustainable potato production (Mou, 2011). Hence, it is necessary to understand salinity tolerance mechanism in potato, helpful in developing stress tolerant potato cultivars by using various modern techniques (Gururani *et al*., 2013). Considering this scenario, present experiment was conducted to assess adverse effects of salinity stress on ionic

imbalance, water relations, gas exchange, antioxidant enzymes and biochemical attributes of two potato cultivars.

MATERIALS AND METHODS

***Plant materials and experiment details*:** The study was carried out in the lath house at Institute of Horticultural Sciences, University of Agriculture, Faisalabad, Pakistan during autumn season, 2012-13. It was a pot (9 L, imperforated) experiment containing sand as growing medium. Two potato cultivars namely NY-LARA and 720-110 NARC were used in this study. Moreover, there were four replications and each treatment in every replication was comprised of three pots. Five tubers were planted in each pot. After the emergence of tubers, three plants in each pot were maintained for data collection. Half strength Hoagland solution was used as nutrient medium. Pots were irrigated according to need of plants by visual observing moisture status of sand. After 30 days of tuber's emergence, plants were subjected to six different NaCl concentrations i.e. 0.0, 2.5, 5.0, 7.5, 10.0 and 12.5 dS m^{-1}. To avoid osmotic shock to growing potato plants, salt concentrations were applied gradually in several steps (2.5 dS m^{-1} every two days). After 10 days of treatment application, fully expanded fourth leaf from the apex was used to measure data regarding various ionic, water relation, gas exchange, antioxidant enzymes and biochemical parameters.

***Ionic attributes*:** Leaf Na^+ and K^+ were determined by a method described by Yoshida *et al*. (1971) through flame photometer.

***Water relation attributes*:** Pre-dawn leaf water potential (Ψw) (-MPa) was determined with pressure chamber (manually tightened seal type, model 1000, PMS Instrument Company, Albany, USA) for which leaf was placed in gasket of pressure chamber (Model, 615, USA) to determine Ψw. The leaves used for Ψw were stored at -20°C to determine osmotic potential (Ψπ) (-MPa) by osmometer (Vapro-5520, Wescor Inc. U.S.A). Turgor potential (Ψp) (MPa), the difference between Ψw and Ψπ potential, was measured by using the equation: Ψp = Ψw - Ψπ.Water use efficiency (WUE), a ratio between photosynthetic rate (*Pn*) and transpiration rate (*E*), was measured by equation: Leaf relative water contents (LRWC) were calculated based on the method of Yamasaki and Dillenburg (1999) by following formula:

$$LRWC = \frac{FM - DM}{TM - DM} \times 100$$

***Gas exchange attributes*:** Gas exchange attributes (photosynthesis rate (*Pn*), stomatal conductance (*gs*) and transpiration rate (*E*) were assessed from intact leaves with an open LCi Portable Photosynthesis System (infrared gas analyzer) (ADC Bio-Scientific Ltd. Hoddesdon, Herts, EN11, England) from 10:30 am to 12:30 pm, operated at light intensity range of 674-943µmol m^{-2} s^{-1}, leaf surface area of 6.25 cm^2, ambient CO_2 concentration (Cref) range of 395-440 µmol^{-1}, temperatures range of leaf chamber (27.4-34.9°C) and surface (28.2-35.7°C), flow rate of air per unit leaf area (U) 200.78 µmol s^{-1}, ambient atmospheric pressure (P) of 995 mBar, H_2O partial pressure of 14.6 mBar and boundary resistance to H_2O at full flow (rb) was 0.17 m^2 s mol^{-1}.

***Membrane stability index*:** Membrane stability index (an indication of salt stress tolerance) was determined by measuring leaf electrolyte leakage (LEL) according to a method described by Farkhondeh *et al*. (2012) by using an equation:

***Antioxidant activities*:** For the estimation of antioxidant activities, fresh leaves (0.5 g) were grounded in an ice-cooled tissue grinder in 05 ml of 50 mM cooled phosphate buffer (pH 7.8). The homogeneous mixture was centrifuged at 15000 g for 20 min at 4°C. The supernatant was used for determining activities of the following enzymes. Superoxide dismutase (SOD) activity was analyzed according to the protocol of Giannopolitis and Ries (1977). Catalase (CAT) and peroxidase (POD) activities were estimated by the method of Chance and Maehly (1955).

***Biochemical attributes*:** Protocol of Lowry *et al*. (1951) was followed to measure total soluble protein contents in leaves. The proline contents (first biochemical marker under abiotic stresses) were calculated according to the method of Bates *et al*. (1973). The total phenolic and MDA contents were estimated by using the protocol of Julkunen-Titto (1985) and Heath and Packer (1968), respectively.

***Correlation matrix*:** Pearson Correlation Matrix of Na^+ with K^+, *Pn*, WUE, LEL, SOD, CAT, Proline and MDA was estimated by employing Statistix 8.1 software to evaluate the interdependence between the attributes.

***Statistical analysis*:** Research was executed in completely randomized design with four replications. Analysis of variance of all studied parameters was computed by using Statistix 8.1 software and comparison of means was done using Tukey HSD test.

RESULTS

Application of various NaCl levels (control (non-saline), 2.5, 5.0, 7.5, 10.0 and 12.5 dS m^{-1}) affected both the tested potato cultivars significantly ($p \leq 0.05$) which confirmed by using ANOVA and comparison of means through Tukey test. Statistical analysis revealed significant difference ($p \leq 0.05$) in interaction between treatment x cultivar. However, non-significant ($p \leq 0.05$) interaction in WUE between treatment x cultivar was observed.

***Ionic parameters*:** Minimum leaf Na^+ contents were

observed under non-saline conditions (control) while maximum was recorded at 12.5 dS m^{-1} NaCl, followed by 10.0, 7.5, 5.0 and 2.5 dS m^{-1} (Fig. 1A). It was observed that minimum Na^+ contents were shown in N-Y LARA (2.65 mg g^{-1} DW) under non-saline environment followed by 720-110 NARC (3.35 mg g^{-1} DW) at same NaCl concentration. On the other hand, the maximum Na^+ contents were recorded in 720-110 NARC (28.8 mg g^{-1} DW) at the highest salinity level (12.5 dS m^{-1}) followed by N-Y LARA (22.54 mg g^{-1} DW) at same saline treatment. On contrary, the highest K^+ contents was recorded under non-saline environment as compared to those grown under various levels of salt stress (2.5, 5.0, 7.5, 10.0 and 12.5 dS m^{-1}) in which K^+ contents continued to decrease with increasing salt stress (Fig. 1B)**.** Moreover, cultivar's comparison revealed that N-Y LARA showed maximum K^+ contents (62.1 mg g^{-1} DW) under non-saline environment while minimum K^+ contents (10.3 mg g^{-1} DW) were observed at salt stress level of 12.5 dS m^{-1} followed by 720-110 NARC (57.12 mg g^{-1} DW under control and 6.17 mg g^{-1} DW under 12.5 dS m^{-1} NaCl) (Fig. 1B). The results concerning Na^+: K^+ (Fig. 1C) revealed that maximum Na^+: K^+ was observed in 720-110 NARC (4.67) followed by N-Y LARA (2.19) at 12.5 dS m^{-1} NaCl level as compared to control.

***Membrane stability index*:** Membrane stability index is estimated by measuring leaf electrolyte leakage (LEL). It continued to increase with increasing salinity levels (control, 2.5, 5.0, 7.5, 10.0 and 12.5 dS m^{-1}). Results regarding LEL (Fig. 1D) elaborated that N-Y LARA exhibited less percentage increase in LEL i.e., 25.1% under 2.5 and 328.5% under 12.5 dS m^{-1} NaCl levels relative to control, whereas 720-110 NARC showed higher percentage increase of 31.9% and 390.6% in LEL under 2.5 and 12.5 dS m^{-1} NaCl levels, respectively.

***Water relation attributes*:** Application of various concentrations of salt (NaCl) stress (control, 2.5, 5.0, 7.5, 10.0 and 12.5 dS m^{-1}) resulted in reduced pre-dawn Ψw, Ψπ, Ψp and LRWC as presented in Figures 2A, 2B, 2C and 2D, respectively. N-Y LARA maintained the highest Ψw (-0.23 MPa), Ψπ (-0.89 MPA) and Ψp (0.67 MPa) under non-saline treatment as compared to 720-110 NARC which maintained the lowest Ψw (-0.32 MPa), Ψπ (-0.95 MPa) and Ψp (0.63 MPa) under control. In parameters like Ψw, Ψπ, Ψp and LRWC, N-Y LARA revealed 55.6%, 9.9%, 5.4% and 6.7% reduction, respectively, at 2.5 dS m^{-1} NaCl relative to control whereas, 345.6%, 46.1%, 55.1% and 41.9% reduction was observed at 12.5 dS m^{-1} NaCl level. However, 720-110 NARC exhibited 69.5%, 13.4%, 15.1% and 10.0% reduction in Ψw, Ψπ, Ψp and LRWC at 2.5 dS m^{-1} while 374.1%, 70.3%, 83.2% and 51.9% reduction in Ψw, Ψπ, Ψp and LRWC, respectively, was expressed under 12.5 dS m^{-1} salinity levels relative to control. Likewise, the highest WUE was observed in N-Y LARA (8.9%) at 2.5 dS m^{-1} salinity level followed by 720-110 NARC (7.8%) relative to control (Table 1). However, under 12.5 dS m^{-1}NaCl, minimum percentage reduction in WUE was exhibited by N-Y LARA (29.1%) followed by 720-110 NARC (35.35%) in comparison to control.

***Gas exchange attributes*:** Potato cultivars exhibited a decreasing trend in *Pn* rate, *E* rate and gs grown under 2.5, 5.0, 7.5, 10.0 and 12.5 dS m^{-1} salinity levels (Table 1). The highest *Pn* rate, *E* rate and gs were noted in N-Y LARA i.e., 10.58 (µmol m^{-2} s^{-1}), 4.96 (mmol m^{-2} s^{-1}) and 0.43 (mmol m^{-2} s^{-1}) respectively, followed by 720-110 NARC i.e., 8.71 (µmol m^{-2} s^{-1}), 4.6 (mmol m^{-2} s^{-1}) and 0.35 (mmol m^{-2} s^{-1}), respectively, under control. Likewise, 720-110 NARC exhibited less percentage reduction in *Pn* rate (22.93%), *E* rate (28.5%) and *gs* (24.8%) under 2.5 dS m^{-1} NaCl concentrations compared to control while the highest percentage reduction was observed at 12.5 dS m^{-1} NaCl in *Pn* rate (89.12%), *E* rate (83.1%) and *gs* (82.7%) concentrations relative to control. However, N-Y LARA showed minimum percentage reduction at 2.5 dS m^{-1} NaCl in *Pn* rate (14.32%), *E* rate (21.46%) and *gs* (18.1%) relative to control whereas higher percentage reduction in *Pn* rate (72.48%), *E* rate (61.36%) and *gs* (64.79%) was observed under 12.5 dS m^{-1} compared to control.

***Antioxidant activities*:** Salt stress induced a marked acceleration in SOD, CAT and POD activities in both the tested potato cultivars (Tables 2). In N-Y LARA, minimum SOD, CAT and POD activities were noted under non-saline conditions i.e., 1.12, 3.74 and 2.16 Units mg^{-1} protein, respectively while maximum activities were observed at 12.5 dS m^{-1} NaCl i.e., 2.66, 8.23, 4.39 Units mg^{-1} protein, respectively.

Table 1. Effect of salt stress on leaf photosynthetic activity (*Pn*), transpiration rate (*E*), water use efficiency (WUE) and stomatal conductance (*gs*) of potato cultivars.

Salt (dS m^{-1})	*Pn* (µmol m^{-2} s^{-1})		*E* (mmol m^{-2} s^{-1})		WUE (Pn/E)		*gs* (mmol m^{-2} s^{-1})	
	N-Y LARA	720-110 NARC	N-Y LARA	720-110 NARC	N-Y LARA	720-110 NARC	N-Y LARA	720-110 NARC
Control	10.6 a	8.7 c	5.0 a	4.6 b	2.1 b	1.9 cd	0.43 a	0.39 b
2.5	9.1 b	6.7 d	3.9 c	3.3 d	2.3 a	2.0 bc	0.35 c	0.26 e
5.0	6.0 e	3.3 h	3.1 d	2.1 g	1.9 c	1.6 ef	0.27 d	0.15 g
7.5	4.8 f	2.0 j	2.8 e	1.4 g	1.7 de	1.5 fg	0.20 e	0.11 h
10.0	3.7 g	1.4 k	2.4 f	1.0 i	1.6 ef	1.3 gh	0.18 f	0.08 i
12.5	2.9 i	0.9 l	1.9 g	0.8 i	1.5 f	1.2 h	0.15 g	0.06 j

Every value in above figures is the mean of 4 replicates. Means followed by different letter(s) within a same column are significantly different according to HSD (Tuckey) test (P≤0.05).

Table 2. Effect of salt stress on leaf total soluble protein contents, superoxide dismutase (SOD), catalase (CAT) and peroxidase (POD) activities of potato cultivars

Salt (dS m^{-1})	Protein (mg g^{-1} FW)		SOD (U mg^{-1} protein)		CAT (U mg^{-1} protein)		POD (U mg^{-1} protein)	
	N-Y LARA	720-110 NARC	N-Y LARA	720-110 NARC	N-Y LARA	720-110 NARC	N-Y LARA	720-110 NARC
Control	9.19 a	8.74 b	1.1 fg	1.0 g	3.7 fgh	2.8 h	2.2 h	1.7 i
2.5	8.46 b	7.49 c	1.4 ef	1.2 fg	4.5 ef	3.3 gh	2.7 f	2.0 h
5.0	7.58 c	5.41 f	1.8 cd	1.4 ef	5.9 cd	3.7 fgh	3.5 d	2.4 g
7.5	6.61 d	4.53 g	2.1 bc	1.6 de	6.4 bc	4.3 efg	3.8 c	2.6 f
10.0	5.83 e	3.92 h	2.4 ab	1.8 cd	7.2 ab	5.3 de	4.2 b	2.8 ef
12.5	5.14 f	3.29 i	2.7 a	1.9 cd	8.2 a	6.5 bc	4.4 a	2.9 e

Every value in above figures is the mean of 4 replicates. Means followed by different letter(s) within a same column are significantly different according to HSD (Tuckey) test (P≤0.05).

Table 3. Effect of salt stress on leaf proline contents, total phenolic contents and melondialdehyde (MDA) contents of potato cultivars

Salt (dS m^{-1})	Proline (µmoles g^{-1} FW)		Phenolic contents (mg g^{-1} FW)		MDA (µmole g^{-1} FW)	
	N-Y LARA	720-110 NARC	N-Y LARA	720-110 NARC	N-Y LARA	720-110 NARC
Control	6.8 g	6.1 g	11.4 i	10.9 i	0.36 h	0.49 gh
2.5	9.3 ef	8.4 f	16.8 g	13.5 h	0.43 h	0.75 ef
5.0	11.1 d	10.1 e	21.8 d	15.9 g	0.49 gh	0.98 d
7.5	13.2 c	11.8 d	24.8 c	17.9 f	0.61 fg	1.34 c
10.0	15.5 b	13.2 c	26.9 b	19.9 e	0.72 ef	1.51 b
12.5	19.2 a	15.2 b	28.7 a	21.5 d	0.87 de	1.77 a

Every value in above figures is the mean of 4 replicates. Means followed by different letter(s) within a same column are significantly different according to HSD (Tuckey) test (P≤0.05)

Table 4. Pearson Correlation Matrix of Na^+ with various ionic and physio-biochemical attributes.

	K^+	*Pn*	WUE	LEL	SOD	CAT	Proline	MDA
Na^+	-0.998*	-0.976	-0.949*	0.991**	0.999**	0.982**	0.986**	0.991*

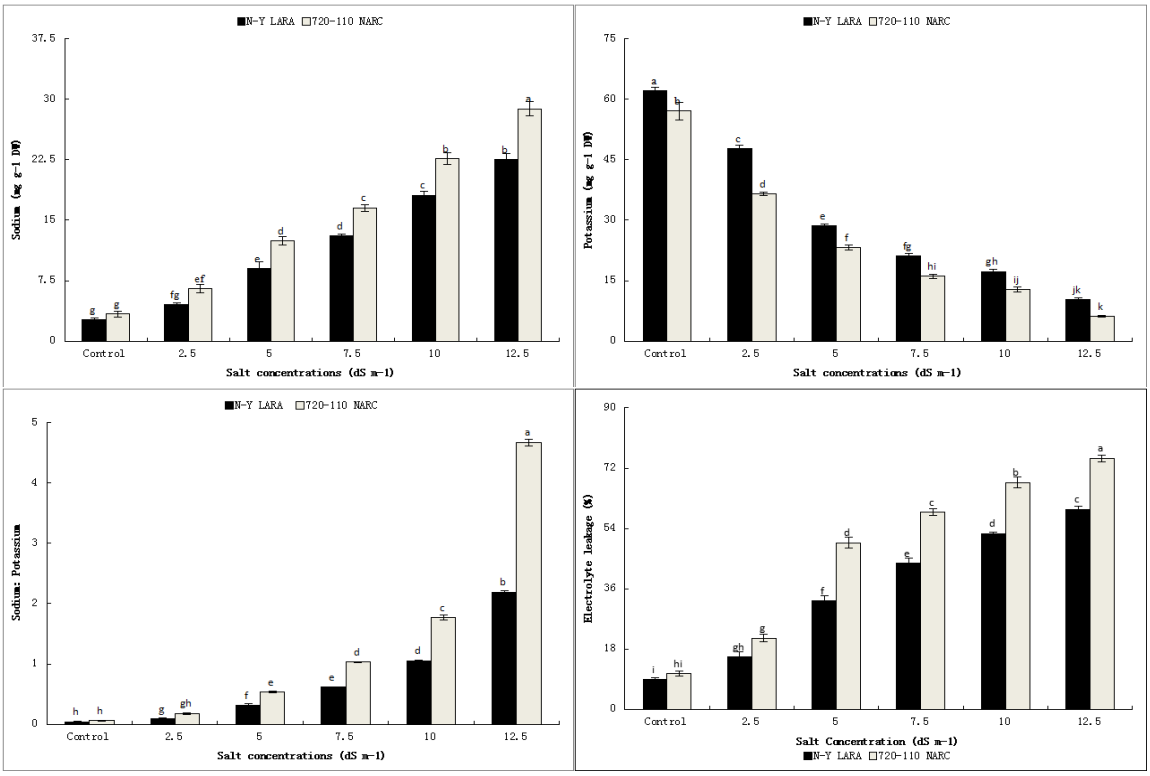
Values indicate Pearson's correlation coefficient. *significant (P < 0.05); **highly significant (P < 0.01). Na^+ (sodium contents), K^+ (potassium contents), *Pn* (Photosynthetic activity), WUE (water use efficiency), LEL (leaf electrolyte leakage), SOD (superoxide dismutase), CAT (catalase) and MDA (Melondialdehyde)

On the other hand, 720-110 NARC exhibited the lowest values under control for SOD, CAT and POD activities (0.99, 2.8 and 1.74 Units mg^{-1} protein, respectively) while maximum activities (1.94, 6.5 and 2.94 Units mg^{-1} protein, respectively) were observed at 12.5 dS m^{-1} NaCl.

***Biochemical attributes*:** Data regarding total soluble proteins revealed that salt stress distinctly reduced total soluble proteins in both the investigated potato cultivars (Table 2). Nevertheless, maximum and minimum total soluble proteins were noticed under non-saline and 12.5 dS m^{-1} salt stress level in N-Y LARA i.e. 9.1 and 5.1 (mg g^{-1} FW), respectively followed by 720-110 NARC (8.74 and 3.29 (mg g^{-1} FW) under same salt stress treatments. Furthermore, salt stress induced a significant elevation in proline, total phenolic and MDA contents in both the tested potato cultivars (Table 3). Minimum and maximum proline, total phenolic and MDA contents were observed under non-saline and 12.5 dS m^{-1} NaCl treatment in N-Y LARA i.e., 6.75 and 19.21 (µmol g^{-1} FW), 11.3 and 28.68 (mg g^{-1} FW) and 0.35 and 0.87 (µmol g^{-1} FW), respectively, followed by 720-110 NARC i.e. 6.11 and 15.24 (µmol g^{-1} FW), 10.9 and 21.55 (mg g^{-1} FW) and 0.49 and 1.77 (µmol g^{-1} FW), respectively, compared to control (Table 3).

DISCUSSION

Salt stress in plants negatively influences morphological, physiological and biochemical attributes. Plants have acquired different levels of tolerance and sensitivity based upon their types and adaptation (Abbas *et al*., 2015). Some



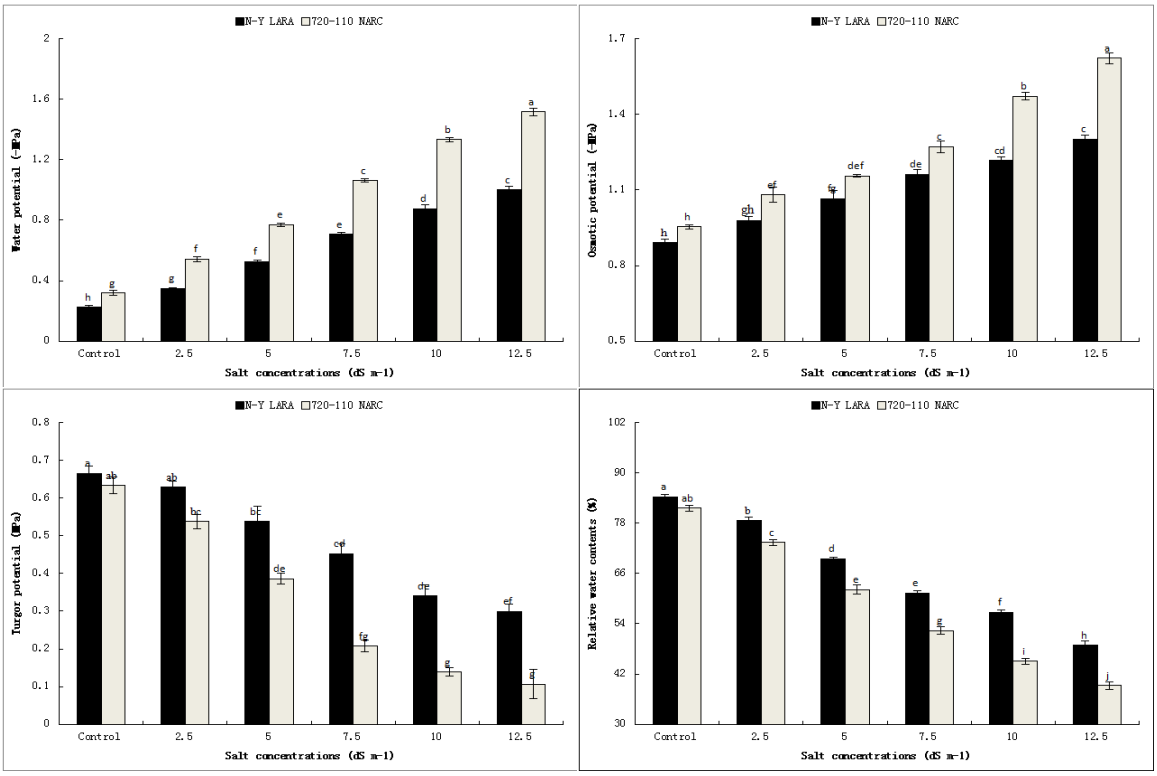
Every value in above figures is the mean of 4 replicates and vertical bars give standard error (SE) of the means. HSD (Tuckey Test) for cultivar and treatments were significant at $p \leq 0.05$.

Figure 1. Effect of salt stress on leaf (A) Sodium (Na^+) concentration (mg g^{-1} DW), (B) Potassium (K^+) concentration, (C) Na^+: K^+ and electrolyte leakage (LEL) (%) of potato cultivars.

plant species have developed modifications at cellular and sub-cellular levels by accumulating salts in their vacuoles more effectively in contrast to salt sensitive ones (Fidalgo *et al*., 2004; Aghaei *et al*., 2008; Maksimovic and Ilin, 2012). In present study, potato cultivar N-Y LARA proved to be tolerant as compared to 720-110 NARC based upon its physiology and biochemical attributes.

Salt stress reduces rhizosphere osmotic potential which becomes more negative with increasing salt levels leading to cell dehydration due to water efflux from cell (Rahnama *et al*., 2010; Amjad *et al*., 2014). In present study, higher Na^+ contents (Fig. 1A) and lowers K^+ contents (Fig 1B) were found in potato leaves under salinity stress. However, N-Y LARA revealed comparatively low concentration of Na^+ and greater K^+ contents as compared to 720-110 NARC. This trend might be due to genetic variability, root permeability to these ions (Akram *et al*., 2010; Mishra *et al*., 2013) and Na^+ distribution from roots to leave tissues in studied cultivars (Jaarsma *et al*., 2013). Moreover, Sodium ions (Na^+) on their way from roots to shoot via transpiration stream progressively buildup in vacuoles and later on shifts to cytoplasm of older leaves causing ionic toxicity and injury in cells with increased salt stress severity. Hence, elevated Na^+ level reduces K^+ uptake and depletes its contents in guard cells hindering stomatal regulation (Parvaiz and Satyawati, 2008). Na^+ accumulation enhanced Na^+: K^+ ratio which continues to increase with increasing salt stress (Aghaei *et al*., 2008; Maathuis, 2014; Zhang and Shi, 2013) as observed in present study (Fig. 1C). Besides, the correlation matrix table (Table 4) is elaborating that various variables are interlinked with each other. Na^+ had significant and highly negative correlation with K^+, *Pn* and WUE. Whereas, highly positive and significant correlation of Na^+ was noted with LEL, SOD, CAT, proline and MDA.

Likewise, during present study increasing salt stress resulted in reduced Ψw (Fig. 2A), Ψπ (Fig. 2B), Ψp (Fig. 2C) and LRWC (Fig. 2D). Accumulation of soluble salts in rhizosphere decrease soil Ψw compared to root cells (Tuteja, 2007). This reduces water influx to plant roots leading to physiological drought and buildup of solutes in roots and later on, in leaf cells which drops leaf cell's Ψw and Ψπ. This condition leads to hyperosmotic stress as leaves continue to transpire while water uptake from soil is reduced drastically. Hence, reduced water uptake and turgor maintenance result in osmotic stress, ionic imbalance and toxicity due to salt stress which in turn reduces cellular Ψp, LRWC, stomatal area and closure of stomata (Mishra, 2013; Gao *et al*., 2015; Farooq *et al*., 2015). Similarly, WUE, decreased under salt stress as



Every value in above figures is the mean of 4 replicates and vertical bars give standard error (SE) of the means. HSD (Tuckey Test) for cultivar and treatments were significant at $p \leq 0.05$.

Figure 2. Effect of salt stress leaf (A) Water potential (Ψw) (-MPa), (B) Osmotic potential (Ψπ) (-MPa), (C) Turgor potential (Ψp) (MPa) and (D) Relative water contents (LRWC) (%) of potato cultivars.

reported in present study (Table 1). Salinity stress results in reduced leaf area, osmotic stress, ionic toxicity, *gs*, *Pn* and *E* which in turn reduces WUE in plant species (Grewal, 2010; Odemiş and CaliSkan 2014). However, increased WUE at low salinity level as in present study at 2.5 dS m^{-1} NaCl (Table 1) may be attributed to increased functioning of aquaporin that enhanced membrane permeability to water and CO_2 in order to maintain plant cell water balance (Kaldenhoff and Fischer, 2003).

It is evident from the results that salt stress significantly inhibited *Pn* (Table 1), E (Table 1) and *gs* (Table 1) in the tested potato cultivars which is in accordance with the findings of Fidalgo *et al*. (2004) and Odemiş and Calişkan (2014). Salt stress influences gas exchange parameters through impaired intercellular CO_2 concentration (Romero-Aranda *et al*., 2001; Navarro *et al*., 2007), toxic ion's buildup in leaf cells, reduced canopy size, condensed *Pn* pigments, and altered activities of photosynthetic enzymes (Rahnama *et al*., 2010; Ashraf and Harris, 2013). Besides, salinity stress enhances ABA accumulation in stomatal guard cells due to salt initiated osmotic stress. Osmotic stress in turn reduces guard cell turgidity, narrows the orifice of stomata and leads to stomatal closure under severe salt stress (Wilkinson and Davies, 2002; Ashraf and Harris, 2013). Hence, salt induced physiological drought (Aghaei *et al*., 2008; Farooq *et al*., 2015) and closure of stomata reduces *gs* as observed in present study (Table 1). Additionally, *gs* is presumed to be the most affected attribute by salinity compared to other gas exchange parameters as *gs* is directly controlled by Ψw in roots and concentration of ABA in xylem sap (Tardieu *et al*., 1991; Akram and Ashraf, 2013; Odemiş and Caliskan, 2014).

Excessive accumulation of Na^+ in cytosol directly affects membrane stability through enhanced generation of MDA contents and leakage of electrolytes, which further aggravated with increasing salinity stress (Gao *et al*., 2015). Similarly, in present study, salt stress enhanced MDA contents (Table 3) and leaf electrolyte leakage (Fig. 1D). Maximum MDA contents and electrolyte leakage percentage were observed in 720-110 NARC relative to N-Y LARA cultivar. Salt stress intensifies ROS and inflicts oxidative stress that disturbs cytosolic metabolic activities (Zhu, 2001)

and increased leaf MDA contents (Gao *et al*., 2015). Thereby, enhances cellular damages like disintegration of membrane constituents and enhanced electrolyte leakage (Ismail *et al*., 2014). Although significant antioxidant enzymes like SOD, CAT and POD, production was observed under salt stress but their induction was not enough to eliminate ROS. It results into higher accumulation of MDA contents (Table 3) and cellular membrane damage (Fig. 1D) which further enhanced with increasing salt stress (Gao *et al*., 2015). Reduction in total soluble proteins were found in present study (Table 2) as reported by Aghaei *et al*., (2008) who identified reduction in total chlorophyll and protein contents.

Plant's cellular adaptive responses for salt tolerance involve acceleration of ROS scavenging antioxidant system such as SOD, CAT, POD (Mishra *et al*., 2013) and osmotic adjustment (e.g., proline, total phenolic contents) (Huang *et al*., 2013; Miljus-Djukic *et al*., 2013. In current study, enhanced production of antioxidant enzymes (SOD, CAT, POD) (Table 2) and osmolytes (Proline, total phenolic contents) (Table 3) were observed which continued to increase with increasing salt stress. ROS scavenging enzymes produce more during abiotic stresses. Antioxidant system (e.g., SOD, CAT, POD) maintains ROS to a less toxic level naturally within cell by converting ROS into water and oxygen. Superoxide dismutase (SOD) enzyme converts superoxide into H_2O_2 that is scavenged by CAT and peroxidases (POD) by converting it into water through Halliwell–Asada pathway (Asada and Takahashi, 1987; Chen *et al*., 2011; Bhattacharjee, 2012). Moreover, plants offset salinity stress by an accumulation of osmo-protectants like proline, that increases with increase in salinity and help to maintain water uptake, cell turgor, osmo-regulation and thereby normal physiological metabolism (Huang *et al*., 2013; Gao *et al*., 2015). Proline accumulated more in tolerant cultivar compared to sensitive one (Bojorquez-quintal *et al*., 2014) as observed in present study (Table 3). Total phenolic contents increased with increase in salinity (Table 3). Furthermore, there is a strong correlation between high concentration of total phenolic, antioxidants and abiotic stresses like salinity (Wahid and Ghazanfar, 2006; Noreen and Ashraf, 2009; Miljus-Djukic *et al*., 2013).

***Conclusions*:** Salt stress significantly affected ionic, water relation, gas exchange, antioxidant and biochemical attributes of potato. Moreover, genetic variations are found in potato cultivars as both the studied cultivars respond variably under salt stress. N-Y LARA proved tolerant cultivar than 720-110 NARC which testified as a salt sensitive cultivar due to its less tolerance mechanisms against salinity. Thus, N-Y LARA can successfully be grown in saline zones as it generated higher antioxidants, proline and total phenolic contents which continued to increase with increasing oxidative stress.

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