Research Article

Study of Wild and Transgenic Potato (*Solanum tuberosum* L.) Varieties in *In Vitro* Condition for Salt Stress Tolerance

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Abstract | Potato is amongst the important staple starchy food throughout the world but its salt sensitive. A large portion of irrigated land in major countries is affected by salinity or is likely to be salinized in near future. This study assessed the impact of various salt levels on morphology and biochemical attributes of a wild (Sante wild) and two transgenic (Sante 2 event and Sante 8 event) potato varieties in *in vitro* condition. These varieties were grown on Murashige and Skoog (MS) media subjected to (0, 25, 50, 75 and 100 mM) of Sodium Chloride (NaCl). Potato varieties showed an adverse *in vitro* growth response to all levels of salt in MS media, while plants of all the tested varieties were dead at concentrations more than 50 mM of NaCl in *in vitro* condition (75, 100 mM). Variation was observed in the level of tolerance to salinity in these potato varieties. Increasing NaCl (mM) level reduced the overall plant growth, number of shoots, number of nodes and leaves, root and shoot lengths (cm), fresh as well as dry root and shoot weights (g). In contrast, proline, Na^+ and K^+ (mg g^{-1} FW) contents increased, while total chlorophyll (mg g^{-1} FW) content in all the variants reduced in salt stress. Overall, maximum salt tolerance was exhibited by Sante 8 event at (50 mM) in *in vitro* condition, as compared to other potato varieties. Furthermore, Sante 2 showed slight resistance to salt stress and Sante wild resulted in poor growth which makes it the most salt sensitive variety among tested varieties. Transgenic Sante varieties of potato having gene *AtNHX1*, showed significantly more resistance than wild variety.

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Introduction

Potato has been one of the major fresh staple vegetables consumed around the world for a long time followed by maize, wheat, and rice (Spooner *et al*., 2005). The production of potatoes in 2019 from an area of about 17.34 million ha was 370.43 million tonnes worldwide with the average yield of about

21.36 thousand kg ha^{-1} (FAO, 2021). In seasonal year 2018-19 it was cultivated over an area of about 195.53 thousand hectares in Pakistan with a harvest of about 4.86 million tonnes (GOP, 2020) and average yield of 24.88 thousand kg ha^{-1} (FAO, 2021).

Potato is also a popular and third most produced and consumed crop in Pakistan after rice and wheat. Due to its diverse uses (raw and processed), nutrient capacity and cost affective potential, potato is consumed by billions of people throughout the world (Anwar *et al*., 2015). It is a good resource of carbohydrates, vitamins, proteins, minerals and fats (Zaheer and Akhtar, 2016).

Growing period of potato is comparatively shorter than other main crops with less labor input, making it an ideal choices for farmers. Its production in the country does not meet its potential as developed and neighboring nations. There are different biotic and a biotic factors which limit its potential production. The most important abiotic stresses include drought, salinity and low temperatures.

Salinity is one of the major threats which leads to significant physiological abnormalities in potato and results poor growth and yield (Rai *et al*., 2011). It also restricts the production of important food crops in various ways (Munns, and Tester, 2008). Plants are classified into glycophytes (plants sensitive to salt) and halophytes (plants which can survive salinity), based on the adaption to salinity, while majority belongs to the first category. An estimated 33% of irrigated and about 20% of total cultivated worldwide agricultural lands are prone to salinity and it is increasing at 10% annual rate due to different aspects, such as minimal precipitation, weathering of rocks, excessive surface evaporation, saline water irrigation and poor agricultural practices (Shrivastava and Kumar, 2015).

Whilst the problem of salinity is more common in arid regions, drought and salinity are said to be interlinked, occurring simultaneously (Katerji *et al*., 2003; Munns, 2005; Witzel *et al*., 2009; Solh and Ginkel, 2014). Comparatively, potato is considered moderately sensitive to salt (Backhausen *et al*., 2005; Shaterian *et al*., 2005), however, higher salt concentration may result in several abnormal changes (Bohnert *et al*., 1995).

Salinity affects plants in many ways at molecular, physiological and anatomical level (Guptha and Huang, 2014). Abnormal response may include, reduced photosynthetic pigmentation, protein, soluble sugars, fresh weight (Mosavi *et al*., 2018), micro tuber production (Li *et al*., 2018), plant height, leaf area, number of shoots and tubers, tuber size and weight (Zaman *et al*., 2018) as well as decreased ascorbate content (Fidalgo *et al*., 2004).

To survive in stresses like drought and salt, plants have multiple adaptive mechanisms, such as regulating growth, osmotic adjustments, ion-homeostasis and detoxification (Bohnert *et al*., 1995; Zhu, 2007). In reaction to salt stress, plant activates defense mechanism to survive such as rises the amount of free proline and the level of sugar and Na^+ accumulation in potato (Daneshmand *et al*., 2010; Jaarsma *et al*., 2013; Mosavi *et al*., 2018), rise in proline contents of tomato (Al-Hassan *et al*., 2015) and mannitol accumulation in potato (Askari *et al*., 2012). Level of potato tolerance in salt stress can vary with the variety and cultivar (Murshed *et al*., 2015; Biswas *et al*., 2017; Roy *et al*., 2017). Researchers have explored tolerance level of different potato cultivars and varieties in *in vitro* conditions as well *in vivo* (Zhang and Donnelly, 1997; Sudhersan *et al*., 2012; Biswas *et al*., 2017; Zaman *et al*., 2018).

Researchers have made efforts for improving salt tolerance capacity of potato to increase the production area. Plant breeding and biotechnological genetic alterations are some of the precise and long-lasting approaches to improve potato against stresses like drought and salinity (Cullins, 1991; Byun *et al*., 2007). Similarly, researchers have worked to improve quality of water to check uncontrolled leaks and floods and have formulated various strategies to check salt problems. However, these are time-consuming, laborious and expensive practices. Additionally, potato has a complex genetic makeup and a narrow germplasm, which makes it hard to develop varieties tolerant to salinity.

A biotechnological technique known as genetic transformation is used to introduce desired foreign gene from an organism (plant or animal) into the genome of agricultural crops, to improve their performance. Hence, genetically transformed plants are resistant to stresses like salinity, drought, insect pests and disease. Potato has overexpressed genes like *StDREB1* (Bouaziz *et al*., 2012), *mtlD* (Askari *et al*.,

2012) and *AtNHX1* (Wang *et al.*, 2013) against salt stress.

Researchers have emphasised on finding out the resistance mechanism of various plants to salt by using *AtNHX1* gene to improve growth and yield. Spontaneously, in drought and saline situations, plants adopt defence mechanisms in vacuole membrane. The Na^+ and H^+ antiporters, due to excessive salinity, can be adoptive mechanism and compartmentation of Na^+ in plant vacuoles. Elimination of Na^+ out of the cell trough cytosolic enzymes is another adoptive mechanism (Yamaguchi and Blumwald, 2005).

Arabidopsis thaliana plants have *AtNHX1*, which has vacuolar Na^+/H^+ antiporters (Aharon *et al.*, 2003; Sottosanto *et al.*, 2007), is used by researchers for developing transgenic salt tolerant plants such as in fescue (Tian *et al.*, 2006), peanut (Banjara *et al.*, 2012), sweet potato (Fan *et al.*, 2015), tomato (Rahman, 2017) and poplar (Yang *et al.*, 2017). Gene *AtNHX1* transmits the excessive of Na^+ and K^+ in the vacuole (Sottosanto *et al.*, 2007). This antiporter gene in plasma membrane and vacuole catalyzes the Na^+ and H^+ through the cell membrane. Hence, increasing vacuolar expression of Na^+/H^+ antiporter in plants leads to accumulation of proline and elevates Na^+, K^+ in leaves which gives plants a considerable degree of salt resistance and drought tolerance (Xu *et al.*, 2009). Studies were carried out on genetically modified plants having salinity resistance gene *AtNHX1* such as Arabidopsia (Apse *et al.*, 1999), brassica (Zhang *et al.*, 2001), tomato (Zhang and Blumwald, 2001) and maize (Yin *et al.* 2004). Likewise, *AtNHX1* gene expressed more salt tolerance than non-transgenic line (Wang *et al.*, 2013).

Conventionally, potato is produced by vegetative means, which is blamed for spread of various diseases for many generations. However, breeding can act as a vital function for improving varieties. Selection of resistant cultivar is important, but field trials are bound to irregular moisture, temperature fluctuations and salt distribution variations during growing season. In controlled tissue culture conditions, plant growth can be observed efficiently (Shatnawi *et al.*, 2004; Roumeliotis *et al.*, 2012). In tissue culture, disease free potato tubers can be produced from mother plants which can be regenerated from multiple shoots for mass production (Rabbani *et al.*, 2001). Similarly, meristem culture can eliminate virus. Hence, it is a useful technique for production of pathogen free potato plants (Zaman *et al.*, 2001). Scholars have also presented their *in vitro* studies on drought and salinity tolerance (Zhang and Donnelly, 1997; Khenifi *et al.*, 2011).

Climate change is one of the major challenges causing stresses in plants, including abiotic stress like salinity, which has decreased potato productivity (George *et al.*, 2017). Therefore, researchers recommend working on plants at the genetic level to enhance tolerance against saline conditions. Henceforth, this study was carried out on transgenic potato varieties (Sante) for screening of its salt tolerance and its morphological and biochemical reaction to salt stress during *in vitro* condition.

Materials and Methods

This research was carried out at National Institute for Genomics and Advanced Biotechnology (NIGAB) National Agricultural Research Center (NARC), Islamabad. *In vitro* culture was sub-cultured on Murashige and Skoog (MS) Media. Media was sterilized for 20 minutes in autoclave at 121°C with 15 psi pressure (Zaman *et al.*, 2018) and 4.43 g L^{-1} MS, with 1.0 mg L^{-1} Gibberellic acid, 30 g L^{-1} sucrose, 1.0 mg L^{-1} IBA and vitamins (Pyridoxine HCL, Thiamine HCL, Myoinositol and Nicotinic acid) 10 ml L^{-1} were supplemented.

Experimental treatments

The experiment designed as two factors factorial under Completely Randomized Design (CRD), was carried to check the level of tolerance of varieties (Sante wild, Sante 2 event and Sante 8 event) in 0, 25, 50, 75 and 100 mM NaCl. Uniform explants were taken from each event, after the 15 days of subculturing, and cultured on different media having five different levels of NaCl and a 5.8 pH was adjusted. However, in the highest concentrations (75 and 100 mM) of NaCl plants of all varieties were dead. Hence, statistical analysis was subjected to two factors: Varieties with three levels (Sante wild, Sante 2 event and Sante 8 event), and also NaCl Dose with three levels (0, 25 and 50 mM), having three replicates each. These potato cultures were kept for 4 weeks at 25 ± 2°C with 16 hours of fluorescent light (2500 lux) and 8 hours dark in growth room. The following growth parameters were analyzed to compare different potato varieties of salt tolerance.

Vegetative growth

For the study of growth behavior, data regarding number of leaves, shoots and nodes plant^{-1}; root and shoot lengths; fresh as well as dry root and shoot weights were recorded.

Biochemical analysis

For measuring proline content of potato plants, the process explained by (Bates *et al*., 1973) was employed and expressed in (μ mol. g^{-1} FW). The Sodium (Na$^+$) and Potassium (K$^+$) contents from leaves sample were measured by using the apparatus of flame photometer.

Total chlorophyll content (mg g^{-1} FW)

Process described by (Ling *et al*., 2011) was adapted to measure total chlorophyll contents of potato varieties using chlorophyll meter (SPAD-502, Minolta, Japan). These contents were measured after the 8 weeks of NaCl supplement in two different sunny days.

Statistical analysis

Two factors factorial design was used to statistically analyze the experimental data. Analysis of variance (ANOVA) was taken by Statistix 8.1 software (Statistix, 2006). Least significance difference (LSD) at P ≤ 0.05 was employed to compare different means (Zaman *et al*., 2018).

Results and Discussion

Potato varieties culture on MS media having various levels of NaCl, demonstrated considerably negative effect on plant morphological and biochemical parameters of all varieties. However, concentration higher than 50 mM resulted in the death of all plants in all varieties. Furthermore, potato explants stayed green for about one month without showing additional growth.

Number of leaves plant^{-1}

Potato varieties subjected to various level of NaCl showed that a mean (8.73) number of leaves were seen in Sante wild variety. Sante 2 event produced (10.53) leaves and Sante 8 event had maximum mean number of leaves (14.06) among tested varieties (Figure 2a). Increasing amount of NaCl in media significantly decreased leaves of potato plants. NaCl 50 mM concentration resulted in lowest significant mean number of leaves (14.11), followed by (18.55) at 25 Mm NaCl and (22.88) in control (Figure 2b). The lowest numbers of leaves (11.3) were seen at concentration in Sante wild at 50 Mm NaCl whereas; Sante 8 events showed the maximum non-significant number of leaves (28.0) (Figure 1a).

Number of shoots plant^{-1}

The NaCl stress showed a negative impact on the development of number of shoots plant^{-1}. With increasing salt stress, the numbers of shoots reduced (Figure 2b). The lowest mean number of shoots (2.77) were produced at 50 mM NaCl treatment and at zero salt maximum mean number of shoots were (4.22) (Figure 2a). Lowest number of shoots (2.3) was observed in 50 mM NaCl concentration in Sante wild, while highest number of shoots (5.3) was observed in Sante 8 event in control treatment (Figure 1b). Most salt sensitive variety which produced lowest number of shoot plant^{-1} (1.66) was Sante wild. Number of shoots produced by variety Sante 2 were (2.0), while highest number of shoots (2.53) were produced in Sante 8 event, showing NaCl tolerance amongst tested varieties (Figure 2a).

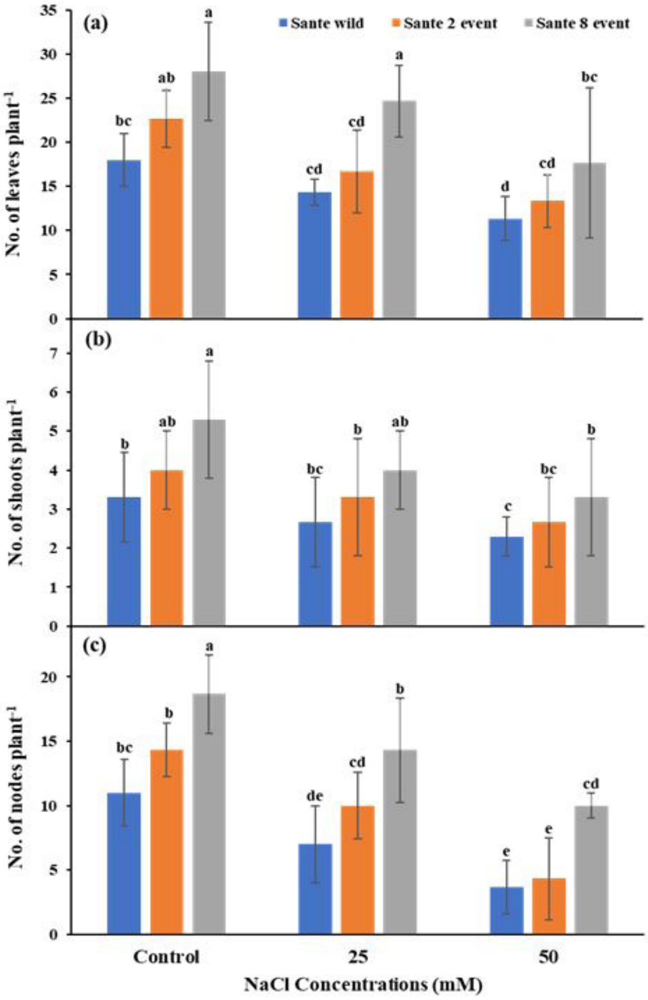

Figure 1: *Mean No. of leaves (a), No. of shoots (b) and No. of nodes (c) plant^{-1} of potato varieties treated with different levels of NaCl. Vertical bars indicate ± SE of means. Means not sharing similar letters are significantly different by LSD test at P ≤ 0.05.*

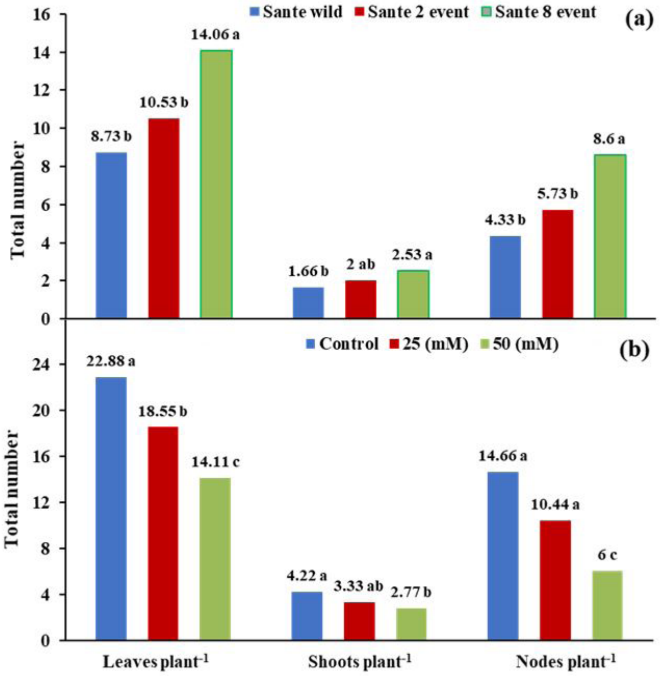

Figure 2: *Total means of various parameters of different potato varieties (a), and different NaCl concentrations (b). Means not sharing similar letters are significantly different by LSD test at P ≤ 0.05.*

Number of nodes plant^{-1}

All the three varieties of potato displayed negative effect in terms of nodes plant^{-1} in response to salt stress. Continuous decrease was seen with increasing salt stress. Significantly highest nodes plant^{-1} (14.66) were seen in zero salt treatment (Figure 2b). Sante wild at 50 mM NaCl concentration, resulted in lowest number of nodes plant^{-1} (3.66). However, significantly highest (18.66) nodes plant^{-1} were noticed in the plants of Sante 8 event which were not supplemented with salt (Figure 1c). Moderate resistance was shown by Sante 8 event variety to salinity, which produced significantly highest (8.6) nodes plant^{-1} followed by Sante 2 event (5.73) (Figure 2a). Whereas, Sante wild was the most salt sensitive variety which produce the lowest number of nodes plant^{-1} (4.33).

Root and shoot length (cm)

The results in (Table 1b) indicate that the minimum root length (2.06 cm) was found in 50 mM NaCl applied to Sante. In different potato varieties which were subjected to various levels of salt stress exhibited that the lowest mean root length (1.79 cm) was found in Sante wild which was sensitive to salt and Sante 8 event, maximum mean root length (3.86 cm) was seen, showing moderate resistance as compared to other tested varieties. Looking at different concentrations of NaCl, minimum root length (2.78 cm) was recorded on salt level of 50 mM which was significantly lower from all concentrations, while significantly highest root length (6.59 cm) was observed in control.

Data revealed that lowest shoot length (3.23 cm) was seen at 50 mM of NaCl in Sante wild followed by (4.50 cm) at 50 mM NaCl of Sante 2 event and in Sante wild (5.16 cm) at 25 mM NaCl. However, Sante 8 event at control showed the highest significant shoot length (18.66 cm). Amongst the tested varieties to sodium chloride, lowest significant mean shoot length (3.44 cm) was seen in Sante wild, while maximum significant mean shoot length (7.16 cm) was noticed in Sante 8 event which indicated moderate salt tolerance. Minimum (4.74 cm) and maximum (14.05 cm) mean shoot length was observed at 50 and 0 mM NaCl, respectively (Table 1a).

Fresh and dry root weight (g)

Results for fresh root weight plant^{-1} of potato varieties in different NaCl stresses in MS medium are presented in (Table 1c), which shows that a minimum fresh root weight (0.26 g) was recorded in Sante wild at 50 mM NaCl. Whereas, at zero salt stress, Sante 8 event had significantly highest fresh root weight (1.96 g). Similarly, in case of dry root weight, lowest weight (0.05 g) was seen in Sante wild at 50 mM NaCl treatment, whereas Sante 8 event resulted in maximum (0.15 g) dry root weight at control. In accordance with the result of mean fresh root weight (Table 1c), dry root weight of potato varieties was reduced with increasing level of NaCl (Table 1e). In different level of NaCl minimum fresh root weight (0.19 g) as well as minimum (0.04 g) dry root weight was calculated in Sante wild. Sante 8 event exhibiting maximum fresh (0.69 g) and dry (0.06 g) root weight.

Fresh and dry shoot weight (g)

Both fresh and dry shoot weight plant^{-1} of potato varieties subjected to various level of NaCl were adversely affected. A reciprocal decline in mean fresh (Table 1d) and dry (Table 1f) shoot weight was seen with increasing salt stress level. Non significantly lowest fresh and dry shoot weight were recorded at 50 mM NaCl in Sante wild, (0.25 g) and (0.06 g), respectively. Different potato varieties added with salt stress showed that (0.2 g) was the lowest fresh shoot weight and (0.04 g) was the lowest dry shoot weight noted in Sante wild. On the other hand, Sante 8 event displayed moderate tolerance among all assessed varieties in NaCl stress in terms of fresh (Table 1d) and dry (Table 1f) shoot weight.

Table 1: *Effect of various levels of NaCl on various aspects of potato varieties.*

Varieties	**NaCl concentrations (mM)**			**Mean**
	Control	**25**	**50**	
(a) Shoot length (cm)				
Sante wild	8.83 ± 0.76 cd	5.16 ± 1.50 ef	3.23 ± 0.75 f	3.44 C
Sante 2 event	14.66 ± 2.50 b	8.66 ± 1.50 cd	4.50 ± 1.80 ef	5.56 B
Sante 8 event	18.66 ± 3.05 a	10.66 ± 2.00 8c	6.50 ± 2.50 de	7.16 A
Mean	14.05 A	8.16 B	4.74 C	
(b) Root length (cm)				
Sante wild	4.23 ± 3.00 cd	2.66 ± 1.04 de	2.06 ± 1.40 e	1.79 B
Sante 2 event	6.25 ± 0.93 b	3.30 ± 1.15 de	2.30 ± 0.75 de	2.37 B
Sante 8 event	9.30 ± 1.80 a	6.00 ± 2.00 bc	4.00 ± 1.50 de	3.86 A
Mean	6.59 A	4.00 B	2.78 C	
(c) Fresh root weight (g) plant^{-1}				
Sante wild	0.40 ± 0.20 d	0.30 ± 0.10 de	0.26 ± 0.15 de	0.19 B
Sante 2 event	0.80 ± 0.20 bc	0.53 ± 0.20 cd	0.36 ± 0.15 d	0.34 B
Sante 8 event	1.96 ± 0.15 a	1.00 ± 0.55 b	0.50 ± 0.20 cd	0.69 A
Mean	1.05 A	0.61 B	0.37 C	
(d) Fresh shoot weight (g) plant^{-1}				
Sante wild	0.45 ± 0.11 c	0.30 ± 0.10 c	0.25 ± 0.14 c	0.20 C
Sante 2 event	0.98 ± 0.30 b	0.40 ± 0.10 c	0.33 ± 0.12 c	0.34 B
Sante 8 event	1.66 ± 0.37 a	0.79 ± 0.12 b	0.45 ± 0.16 c	0.58 A
Mean	1.03 A	0.49 B	0.34 C	
(e) Dry root weight (g) plant^{-1}				
Sante wild	0.10 ± 0.03 bc	0.08 ± 0.01 cd	0.05 ± 0.02 d	0.04 B
Sante 2 event	0.12 ± 0.03 ab	0.10 ± 0.02 bc	0.07 ± 0.02 cd	0.05AB
Sante 8 event	0.15 ± 0.05 a	0.11 ± 0.027 bc	0.08 ± 0.014 cd	0.06 A
Mean	0.12 A	0.09 B	0.06 C	
(f) Dry shoot weight (g) plant^{-1}				
Sante wild	0.11 ± 0.02 bc	0.07 ± 0.02 cd	0.06 ± 0.03 b	0.04 B
Sante 2 event	0.14 ± 0.05 ab	0.10 ± 0.03 bc	0.08 ± 0.01 cd	0.06 AB
Sante 8 event	0.18 ± 0.05 a	0.12 ± 0.06 b	0.10 ± 0.03 bc	0.08 A
Mean	0.14 A	0.09 B	0.08 B	
(g) Proline content (mg g^{-1} FW) plant^{-1}				
Sante wild	0.038 ± 0.014 f	0.84 ± 0.16 e	1.81 ± 0.25 d	0.53 C
Sante 2 event	0.054 ± 0.012 f	2.45 ± 0.27 c	2.98 ± 0.26 b	1.09 B
Sante 8 event	0.072 ± 0.020 f	2.71 ± 0.43 bc	3.72 ± 0.18 a	1.30 A
Mean	0.05 C	2.00 B	2.84 A	

± indicate SE of means. Means not sharing similar letters are significantly different by LSD test at P ≤ 0.05.

Proline content (mg g^{-1} FW)

A remarkable increase was seen in proline contents of Sante 8 event and Sante 2 event potato varieties than Sante wild (Table 1g). Unlike other parameters, proline content of potato varieties increased in parallel with increasing salt stress level. Sante 2 event exhibited an amount of (1.09 mg g^{-1} FW) proline. It was observed that in control minimum mean proline content (0.05 mg g^{-1} FW) accumulated, while at 50 mM of salt treatment, maximum mean proline (2.84 mg g^{-1} FW) was seen. Potato varieties subjected to various levels of salt showed that Sante wild was least tolerant to salt, which had significantly lower mean amount of proline (0.53 mg g^{-1} FW) and highest mean proline (1.30 mg g^{-1} FW) was noted in Sante 8 event which depicts moderate tolerance of Sante 8 event in saline conditions. In zero salt condition, Sante wild showed lowest (0.038 mg g^{-1} FW), whereas at 50 mM of NaCl treatment Sante 8 event exhibited higest (3.72 mg g^{-1} FW) proline content.

Sodium and potassium content (mg g^{-1} FW)

Under NaCl stress, sodium increased in the plants of potato varieties. Unlike Sante wild, plants of Sante 8 and Sante 2 event accumulated more sodium. Significantly lowest (1.38 mg g^{-1} FW) mean sodium in plant shoot was accumulated in followed by Sante 2 event and Sante 8 which accumulated (1.90 mg g^{-1} FW) and (2.31 mg g^{-1} FW) mean sodium, respectively (Table 2a). Lowest non-significant (0.78 mg g^{-1} FW) sodium was calculated in Sante wild at control treatment and significantly highest (5.80 mg g^{-1} FW) sodium accumulation was calculated in 50 mM NaCl treatment by Sante 8 event (Figure 3a). Potato varieties in zero salt resulted in lowest mean sodium (0.94 mg g^{-1} FW) in plant shoots. However, at 50 mM concentration of salt, significantly highest mean sodium content (4.77 mg g^{-1} FW) was found (Table 2b).

Table 2: *Total means of various parameters of different potato varieties (a), and different NaCl concentrations (b).*

(a) Verities			
	Sante wild	**Sante 2 event**	**Sante 8 event**
Sodium (mg g^{-1} FW)	1.38 c	1.90 b	2.31 a
Potassium (mg g^{-1} FW)	0.30 b	0.36 b	0.55 a
Chlorophyll (mg g^{-1}FW)	17.44 b	20.44 ab	23.28 a
(b) NaCl (Mm)			
	Control	**25**	**50**
Sodium (mg g^{-1} FW)	0.94 c	3.62 b	4.77 a
Potassium (mg g^{-1} FW)	0.45 c	0.63 b	0.95 a
Chlorophyll (mg g^{-1} FW)	45.30 a	35.61 b	21.04 c

Means not sharing similar letters are significantly different by LSD test at P ≤ 0.05.

Accordingly, slight increase was seen in potassium contents in the transgenic potato verities as compared with the Sante wild (Figure 3b). In no salt stress, lowest significant amount of potassium (0.34 mg g^{-1} FW) accumulated in Sante wild, while maximum significant (1.35 mg g^{-1} FW) accumulation was seen at 50 mM level of NaCl in Sante 8 event. Varieties with various NaCl levels showed that not significant lowest mean potassium (0.30 mg g^{-1} FW) was observed in Sante wild which was lower from (0.36 mg g^{-1} FW) of Sante 2 event and Sante 8 event showed highest potassium (0.55 mg g^{-1} FW), which indicates its moderate tolerance to salt stress (Table 2a). Potato plants subjected to no salt stress showed lowest significant potassium content (0.45 mg g^{-1} FW) than those subjected to 25 and 50 mM level of salt (Table 2b).

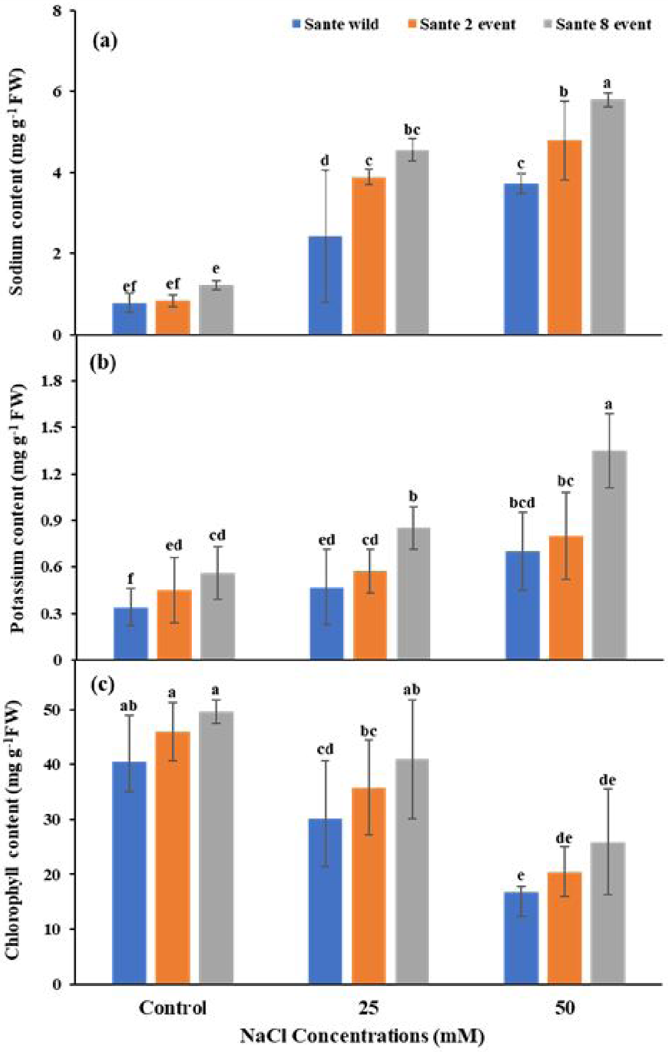

Figure 3: *Mean Sodium (a), Potassium (b) and Chlorophyll (c) contents of potato plants of different varieties treated with different levels of NaCl. Vertical bars indicate ± SE of means. Means not sharing similar letters are significantly different by LSD test at P ≤ 0.05.*

Total chlorophyll content (mg g^{-1} FW)

It is evident from the results of this study presented in (Table 2b) that total chlorophyll contents of transgenic and wild potato plants decreased in saline situation. The results of various levels of NaCl indicated that significantly lowest (21.04 mg g^{-1} FW) mean chlorophyll content was recorded at a salt level of 50 mM, while highest mean (45.30 mg g^{-1} FW) was calculated in control treatment. Sante wild, which showed poor results in this study, with various level of NaCl resulted in lowest mean chlorophyll content (17.44 mg g^{-1} FW) followed by Sante 2 event (20.44 mg g^{-1} FW). However, moderate tolerance was exhibited by Sante 8 event by showing the maximum mean (23.28 mg g^{-1} FW) (Table 2a). In comparison, Sante wild at 50 mM level of salt, showed minimum non-significant total chlorophyll content (16.73 mg g^{-1} FW). Nevertheless, highest non-significant amount of chlorophyll (49.53 mg g^{-1} FW) was reported when no salt was added in the media of Sante 8 event.

These findings indicate that transgenic Sante varieties of potato (having gene *AtNHX1*) have resisted salt stress at a moderate level. Researchers have explored that the expression of gene *AtNHX1* in plants leads to salt tolerance by incorporating it in peanut (Banjara *et al*., 2012), tobacco (Soliman *et al*., 2009) and sweet potato (Fan *et al*., 2015). Tobacco plants with expressing gene *AtNHX1* survived up to 150 to 300 mM of salt (Soliman *et al*., 2009). Smiler tolerance to salt was observed in tomato plants employed with *AtNHX1* gene from *Arabidopsis thaliana* (Rahman, 2017).

Growth of potato varieties was restricted when levels of NaCl increased in MS media in *in vitro* condition. However, transgenic varieties Sante 8 and 2 event resisted salt stress as compared to Sante wild. Accordingly, in a research potato showed reduces growth in response to salinity (Mosavi *et al*., 2018). Morphological parameters of potato plants were significantly reduced at 120 mM (Biswas *et al*., 2017) and 150 mM level of NaCl (Efimova *et al*., 2018). In accordance, as compared to wild type, genetically modified potato plants showed positive results at physiological, anatomical and molecular level (Shafi *et al*., 2017). Researchers found a drastic effect of salt on number of roots $plantlet^{-1}$ and roots length in *in vitro* condition. The viability percentage, fresh seedling weight and seedling height were decreased with increasing level of NaCl (Farhatullah and Raziuddin, 2002)

In accordance with our study, in an *in vitro* condition, increasing level of NaCl, reduced morphological development of different potato varieties. Two varieties Sultana and Taurus were reported to be salt tolerant (Murshed *et al*., 2015). Salt stress highly reduced shoot length of potato cultivars (Aghaei *et al*., 2008) Present results also agreed with a study where, Sante wild was found to be the most salt sensitive variety of potato even at 20 mM of salt, while Kroda variety of potato showed the most resistant at up to 60 mM of NaCl (Zaman *et al*., 2018). Similarly, increasing level of NaCl affected the development of root and depressed the growth of three potato cultivars in laboratory condition (Rahman *et al*., 2008). Anyhow, in contradiction to our findings in this study potato plants survived in 100 mM of NaCl but with different growth rate.

In terms of biochemical parameters, current findings were that sodium and proline contents of potato plants increased with the increasing level of salt, while potassium contents also slightly increased, but total chlorophyll contents decreased in salt stress. This observations of increasing amount of proline, agreed with the findings of similar research on potato (Mosavi *et al*., 2018). Accordingly, in transgenic *Arabidopsis*, significant increase in level of proline was reported in saline condition (Li *et al*., 2017). Our results agreed with studies on rice (Shekeela *et al*., 2016) and potato (Jaarsma *et al*., 2013), in terms of proline and sodium content but did not agree in terms of chlorophyll content in rice (Shekeela *et al*., 2016). Whereas, in coincidence to our findings, chlorophyll content was decreased in cherry tomato at high concentration of NaCl and level of proline was increased (Al-Hassan *et al*., 2015). This study did agree with a study focusing on salt tolerance of potato, in case of proline and sodium but did not agree in case of potassium content (Rahnama and Ebrahimzadeh, 2004), where potassium contents decreased while sodium and proline contents increased in all potato cultivars. In saline conditions, sodium increased in leaves, stems and tubers, however the potassium of stems and tubers rose but it declined in leaves (Ghosh *et al*., 2001).

In transgenic plants, overexpressing vacuolar Na^+/H^+ antiporter gene enable plants to grow in high salt concentration. Adoptive mechanism of plant in saline condition could be compartmentation of Na^+ in plant vacuoles or elimination of Na^+ out of the cell by cytosolic enzymes (Yamaguchi and Blumwald, 2005) along with maintaining higher K^+ concentration by lessening the inhibition of K^+ uptake (Xu *et al*., 2009). These findings establish that overexpression of *AtNHX1* in transgenic plants, improves salt tolerance in plants by rising Na^+ accumulation and keeping the K^+/Na^+ balance mainly in leaves.

Conclusions and Recommendations

On the basis of the above findings, it was established that Sante wild variety showed poor growth in *in vitro* saline condition as compared transgenic. Further studies at greenhouse and filed level are required to evaluate true potential of these transgenic events, which may lead to development of potato cultivars tolerant to salinity in near future.

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Novelty Statement

The selected variants were subjected to salt stress for first-time for their growth and biochemical attributes. Findings of the current study may provide a base for selection of potato variants that can be recommended for the areas having saline conditions.

Author's Contribution

Tahira Bibi and Kazim Ali: Devised the idea and supervised the research work.
Kalsoom: Conducted the research trial.
Nasir Shah and Muhammad Ibrahim: Provided practical assistance and helped in writeup of the manuscript.
Each author equally aided in analysis, feedback, revising and endorsing the manuscript.

Conflict of interest
The authors have declared no conflict of interest.

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