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IN VIVO ASSESSMENT OF SIX POTATO GENOTYPES FOR SALINE WATER TOLERANCE

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ABSTRACT

In vivo salt checking of six potato somaclones was conducted in Genetics Lab, Genetic and Genetic Engineering Department, Faculty of Agriculture, Benha University, Moshtohor 13736, Qaliuobia, Egypt under greenhouse conditions. Varied concentrations of saline solution (0, 20, 40, 60mM) were applied on potato. On mean basis, genotypes Diamant, and Spunta developed as the most salt tolerant genotypes with greatest plant height (48.6 cm), number of nodes (22.26), number of shoots plant-1 (3.47), leaf area (657.93 cm²); tubers No. plant-1 (5.66) and tuber weight plant-1 (38.65 g). Sante, and Lady Rosetta performed as moderately salt tolerant genotypes under salt stress. On the other hand Ditta, and Safrane was recorded as the most salt sensitive with minimum plant height (34.06 cm), number of nodes (18.53), number of shoots (2.93), leaf area (645.00 cm²), number of tubers (3.93) and tuber weight (19.10 mm). The results revealed that growth parameters of all varieties decreased as the salt stress level increased. The NaCl treatment generated an important loss of leaf greenness in treated plants, with significant decrease in chlorophyll content after salt treatment. This decrease was more significant in the two sensitive somaclones B, and E 21.5, and 27%. Protein content showed a significant decrease in all tissues of potato plants after salinity treatment, in the greenhouse conditions (Table 5). The highest decrease was noticed in sensitive somaclones B, and E 27.2, and 22.6% followed by moderate tolerant genotypes C, and D by 15.6, and 21.1% then tolerant somaclones recorded 19.1, and 12.6%. A high increase of the proline content was detected after salt addition in all somaclones ranged from 7.3 folds in somaclone F to 9.3 fold with somaclone B.

Keywords: *Solanum tuberosum*; potato; assessment; salinity; tolerance; Egypt.

Introduction

Soil saltiness is one of the most overpowering a-biotic stresses that seriously impede crop efficiency on the planet (Shrivastava and Kumar, 2015). It has exceptionally extreme and negative impact on development and advancement of harvest plants (Ma *et al.*, 2015 and Barnawal *et al.*, 2014). The expanded utilization of saline water in water system and tremendous industrialization caused greater saltiness and it has been accounted for up to 5 to 7% in the whole world (Ruiz-Lozano *et al.*, 2012). As per a gauge, 14 billion hectares of cultivable land and nearly one billion hectare of world land are saline influenced in arid and semi-arid territories (Aghaei, 2009). It has been accounted for that around 6% of all out arable land and about 20% inundated land in everywhere throughout the world, is saltiness influenced (FAO, 2008). This unfriendly circumstance of saltiness is expanding quickly and continuously 2050, over half of absolute arable acreage is relied upon to be saltiness influenced (Molla *et al.*, 2015 and Ashraf, 2004). Amel *et al.* (2015) announced more prominent decrease in development and yield of potato plants under saline condition. The report of Al-Hussaini *et al.* (2015) uncovered incredible misfortunes in crop development and yield of potato because of salt pressure. Solh and Ginkel (2014) announced that both saltiness and dry spell are interlinked crop yield restricting burdens that exist together in dry areas of the world. Meloni *et al.* (2003) and Allakherdiev *et al.* (2000) saw that soil

saltiness diminishes crop creation because of a few adjustments in plant's biochemical, sub-atomic and metabolic exercises and furthermore it rushes a few photosynthetic proteins that cause disintegration of cell film and its related structures. Saltiness additionally extraordinarily upsets pace of photosynthesis, breath and in plants causing genuine yield loses (Silva *et al.*, 2001; Zhang *et al.*, 2005; Fidalgo *et al.*, 2004). The higher measure of harmful salts of sodium in soil pulverizes plant roots causing water deficiencies, lack of plant supplements by upsetting particles take-up and transport. Amassing of Na⁺ and Cl⁻ causes ionic pressure, and meddling cell measures like cell division and chromosomal deviation prompts decreased plant development, improvement and harvest yield, and so forth. (Munns, 2002). Zhu (2007) additionally revealed radical yield decrease in a few harvest plants because of the saltiness stress. Potato (*Solanum tuberosum* L.) is a significant solanaceous palatable tuberous yield. It has become a modest wellspring of starch alongside nutrients, proteins and minerals (Anon., 2014). Potato is the fourth most significant beneficial food crop internationally after rice, wheat and corn and eighth most developed yield (FAO, 2008). It is developed in around 125 nations and more prominent than one billion populace uses it in its every day supper. It has been accounted for as profoundly salt touchy to poisonous salts of sodium and at 50 mM NaCl its development and advancement diminishes to half (Evers *et al.*, 2007).

Inordinate collection of chlorine and sodium in leaves may cause harmfulness and leaf consumes along the edges. Potato crop is exceptionally influenced when salts are applied at the hour of tuber development. Fidalgo *et al.* (2004) detailed decreased water content, influenced leaf stomatal conductance and happening rate in potato cultivar Desiree because of salt pressure. The ultra-structure of chloroplast changed essentially that gravely influenced photosynthesis, thus came about overabundance starch collection in leaves, decreased movement of nitrate reductase and low development and dry issue creation in tubers (Ghosh *et al.*, 2001). The current investigation was directed to screen potato assortments against saltiness so salt delicate cultivars might be for future hereditary improvement.

Material and Methods

Plant material and culture conditions

Six genotypes of potato namely; Diamant, Ditta, Sante, Lady Rosetta, Safrane, and Spunta (Table1) were obtained from Plant Biotechnology Department, National Research Center, Dokki, Giza, Egypt. Potato tubers germinated as

Table 1 : Names, and parentage of the six potato genotypes employed within these study

| S.N. | Somaclone name | Explant Source | Parentage |
|------|----------------|----------------|---|
| 1 | Somaclone A | Diamant | Tulner de Vries 64 30 8 x SVP 55 89 |
| 2 | Somaclone B | Ditta | Bintje x Quarta |
| 3 | Somaclone C | Sante | SVP Y 66 13 636 x SVP AM 66 42 |
| 4 | Somaclone D | Spunta | Cardinal x SVP VTn2 62 33 3 |
| 5 | Somaclone E | Safrane | Fanette x (Maris Piper x INRA 67.86.37) |
| 6 | Somaclone F | Lady Rosetta | Bea x USDA X 96 56 |
| | | | https://www.europotato.org/ |

Determination of chlorophyll and total soluble protein

Chlorophyll content was determined as described by (Arnon, 1949). Total soluble protein contents were determined according to (Bradford, 1976) with bovine serum albumin as standard.

Measurement of proline

Free proline content was determined using the ninhydrin method (Bates *et al.*, 1973).

Analysis of leaf lipid peroxidation

Plant lipid peroxidation was expressed as equivalents of malondialdehyde (MDA). The MDA content was determined as described by Dhindsa *et al.* (1981).

Statistical analysis

Statistical analyses were carried out by two-way classification of ANOVA to evaluate whether the means were significantly different, taking $P < 0.05$ as significance level.

Pairwise alignment done using the progressive alignment method, Guide tree created, AND was used to carry out a multiple alignment. Alignment from guide tree and phylogeny was done to Produce guide tree only.

Results and Discussion

Effect of NaCl stress on some growth parameters of potato:

(i) Plant height (cm)

There was a significant effect on the plant height of various potato varieties at different levels of NaCl (Figure 1).

described by (Aghaei *et al.*, 2009). In vitro grown plants were propagated by sub-culturing with an interval of 3 weeks. Propagation step repeated for several times until adequate number of plants acquired. Salt pressure was actuated by utilizing saline arrangements of 1M NaCl and five salt stress levels 0, 10, 20, 40, and 60 mM NaCl were applied as described by (Zaman *et al.*, 2018) in soil mixture. Data on different morphological development parameters were gathered and statistically analyzed in a CRD two factors (genotypes and NaCl treatments) with three replications.

In-Vitro culture of potato for determination of: chlorophyll, total soluble protein, Proline, and leaf lipid peroxidation

All the six potato genotypes were treated with NaCl as mentioned by (Zhang *et al.*, 2010). In vitro grown plants were sub-cultured on MS medium supplemented with 200 mM NaCl for 72 h. Chlorophyll, total soluble protein, proline, and leaf lipid peroxidation contents were measured. Data are mean \pm SE from three repetitions.

A negative effect was detected for plant height character of all potato varieties with each increased level of NaCl treatment. ANOVA for NaCl effect on plant height of utilized potato genotypes uncovered highly significant differences (Tables 2, and 3). The influence of saline solution applied to potato pots had badly affected in vivo growth and development of potato varieties that responded significantly to NaCl stress. On means basis, the highest plants were produced by variety Diamant (30.34 cm) followed by Spunta (29.32 cm), Lady rosetta (28.93), Sante (25.71) and Ditta (22.56). (Table 3). Safrane stood last (21.88 cm) and was recorded as the most NaCl susceptible variety. The various concentrations of NaCl likewise created noteworthy impact on the plant tallness It was reduced from 31.06 cm to 20.60 cm up to 60 mM NaCl i.e. greatest plant tallness (34.93 cm) in control and minimum (27.10 cm) in 60 mM NaCl (Table 2). Plant stature in crop plants reflects development and advancement because of the best possible capacity of photosynthesis and breath. Their development is hindered under such an ecological or initiated pressure. Potato being exceptionally helpless against salts, this development boundary was seriously influenced in this investigation. These discoveries are in solid congruity with those of Al-Hussaini *et al.* (2015) who announced diminished plant development, expanded injury to layer solidness and over all plant advancement in potato crop under salt pressure. Comparable discoveries were additionally revealed by Murshed *et al.* (2015) that NaCl decreased plant stature in potato plants at over 100 mM salt level. Mahmood *et al.* (2013) also detailed comparative impacts of soil saltiness on plants development because of gathering of Na^+ particles in leaves. Furthermore Askari *et al.* (2012) uncovered decrease

in plant stature of potato cultivar Agria because of salt pressure.

(ii) Number of nodes / plant

The analysis of variance demonstrated exceptionally noteworthy contrasts in number of nodes per plant among the genotypes and between salt levels also (Tables 2, and 3). The employed genotypes of potato at different salt intensification of NaCl performed diversely for number of nodes per plant (Figure 1). The plant development and growth of potato genotypes was cruelly decreased and the arrangement of new nodes was contrarily diminished by expanded degree of NaCl application (Table 2). Greatest number of nodes per plant was noted in genotype Sante (12.91) trailed by Lady rosetta (12.83), Spunta and Diamant (12.66) and Safrane (11.83) and Ditta (10.17) stood last. So also, various dilutions of NaCl influenced all potato genotypes with diminished nodes number per plant (Table 2). At control level of NaCl, most extreme number of nodes per plant (16.00) was recognized against least number of nodes (6.67) at 60 mM NaCl. Along these lines, numbers of nodes were incredibly diminished by rising the concentration of salinity as has been also reported by Murshed *et al.* (2015). Aghaei *et al.* (2009) researched potato varieties that displayed exceptionally low plant development and advancement due to NaCl stress. Comparable outcomes were accounted for by Mahmoud *et al.* (2009) and Etehadnia (2009) that nodes number per plant alongside tuber yield in potato was decreased at 75 mM NaCl stress and over all feeble plants were created under salt pressure.

(iii) Number of shoots / plant

The analysis of variance showed highly significant differences for number of shoots per plant between the genotypes (Table 3, and figure 1) and for salt stress concentrations as well (Table 2, and figure 1). A noteworthy decrease in shoot arrangement of potato genotypes was noted under different degrees of NaCl levels during this investigation. The raised degrees of NaCl fundamentally limited the quantity of shoots per plant. The accumulation of Na⁺ in the leaves halts photosynthesis that resulted in reduced plant shoot growth. On mean basis, variety Safrane developed maximum number of shoots per plant (1.74) followed by Sante (1.58), Diamant (1.50) and Lady rosetta (1.33). Ditta and Spunta produced minimum (1.16) number of shoots per plant. Likewise, the different levels of NaCl also affected all tested varieties in the development of number of shoots per plant. Maximum (2.03) number of shoots per plant was recorded at control concentration while minimum (1.00) number of shoots per plant was noted at 60 mM NaCl (Table 2). Shoot creation in crop plants is one of the indispensable results of development and advancement because of the correct elements of photosynthesis and breath. Their arrangement and development is hindered under such an ecological or incited pressure. Potato being highly vulnerable to salts; this growth parameter was severely affected. These results agree to the findings of Qayyum and Shoaib (2013) that reported very low growth and reduced number of shoots and branches in potato under salt stress condition.

(iv) Leaf area

In leaf area of potato genotypes, extraordinary decrease was likewise recorded because of expanded convergences of

NaCl stress. Development of leaves was contrarily influenced by NaCl application at specific fixations in the pots Figure 1. According to mean data, maximum leaf area was recorded in Spunta (540.90 cm²) followed by Diamant (499.19 cm²), Sante (454.61 cm²), Lady rosetta (448.08 cm²) and Safrane (396.78 cm²) (Table 4). Minimum leaf area was noted in Ditta (346.53 cm²) Table 3, and figure 1. Different levels of NaCl salt showed highly significant differences for all tested varieties. Maximum leaf area (621.83 cm²) was recorded in control against minimum (271.53 cm²) at 60 mM NaCl Table 2, and figure 1. These results are in strong agreement of Murshed *et al.* (2015) and Askari *et al.* (2012) who revealed decreased leaf zone in potato cultivars under salt pressure. Likewise Etehadnia (2009) contemplated impact of NaCl stress on potato genotypes and announced serious decrease in leaf territory alongside yield and development boundaries also. Arvin and Donnelly (2008) additionally announced comparable discoveries of leaf region decrease in potato cultivars under NaCl stress application.

(v) Number of tubers per plant

The different levels of NaCl caused significant variations in this parameter. Based on mean values, Diamant and Spunt produced maximum number of tubers per plant (5.25) (Table 4). Lady rosetta stood (4.67) third followed by Sante (4.58) and Safrane (3.24). Variety Ditta developed minimum (3.08) mean number of tubers per plant Table 3, and figure 1. Different levels of NaCl also significantly affected the process of tuber formation in tested potato varieties. Maximum tubers per plant (6.67) were noted at zero salt level and minimum (1.67) at 60 mM NaCl Table 3, and figure 1. Basically, roots elongated to low depth in the salinity affected soil for nutrients availability and absorption of water that results in the ultimate reduction of plant growth and low tuber formation. With increasing salinity, rooting was decreased in all cultivars and it was completely inhibited at 80 and 100 mM NaCl. This observation could be due to ion accumulation of Na⁺ in the roots as well. In crops the roots were reported to be amongst the first organs that are affected by salt stress. The present results confirm the findings of Munira *et al.* (2015) and Ghosh *et al.* (2001) who revealed decreased tuber yield in potato under salt pressure. Kirk *et al.* (2006) detailed extraordinary misfortune in yield and tuber arrangement in potato because of salt pressure application.

(vi) Tuber weight (g)

The analysis of variance (ANOVA) displayed highly significant results for tuber weight in the assessed potato varieties under NaCl stress (Table 1). The application of NaCl at various concentrations developed a negative impact as presented in Table 4. Tuber weight of all examined potato genotypes was definitely influenced because of use of saline water to prompt salt resistance. At each raised anxiety of NaCl an exceptionally huge derivation in tuber weight of potato cultivar was recorded. On the basis of mean, Diamant produced maximum tuber weight per plant (41.46 g) followed by Spunta (40.80 g), Sante (35.27 g), Lady rosetta (30.617 g) and Ditta (26.97 g). Safrane showed the least tolerance to NaCl application and developed 24.84 g mean tuber weight (Table 3, and figure 1). Higher NaCl levels reduced the tuber weight significantly in all investigated potato varieties. The potato plants at control level produced maximum (50.20 g) tuber weight per plant whereas, more

than 50% reduction was recorded at 10 mM and 20 mM NaCl level. At 40 mM NaCl further reduction of nearly less than 50% was noted and at 60 mM NaCl 75% reduction was recorded in tuber weight (Table 3, and figure 1). These findings agree to those of Ghosh *et al.* (2001) who reported harsh potato yield reduction and reduced tubers





plants due to salt stress. According to vegetative characteristics values of the six potato genotypes, they were divided as mentioned in Figure 1 into: saline tolerant genotypes (Somaclones, A and F), Somaclones C, and D moderate saline tolerant genotypes, while the remaining two Somaclones B, and E was saline sensitive genotypes.

Table 2: Effect of four NaCl concentrations on different plant growth parameters of potato plants grown under *in vivo* conditions.

| | Genotype | NaCl concentrations (mM) | | | | L.S.D _(0.05) |
|---|------------------------------|--------------------------|---------------------|---------------------|---------------------|-------------------------|
| | | Control | 20 | 40 | 60 | |
| 1 | Plant height (cm) | 31.06 ^a | 28.62 ^b | 25.51 ^c | 20.64 ^d | 1.94 |
| 2 | Num. of nodes/plant | 14.77 ^a | 13.16 ^b | 11.5 ^c | 9.28 ^d | 1.18 |
| 3 | Num. of shoots/plant | 2.03 ^a | 1.35 ^b | 1.27 ^b | 1.00 ^c | 0.23 |
| 4 | Leaf area (cm ²) | 525.53 ^a | 484.94 ^b | 422.55 ^c | 357.72 ^d | 34.23 |
| 5 | Num. of tubers/plant | 5.16 ^a | 4.66 ^b | 4.33 ^b | 3.22 ^c | 0.38 |
| 6 | Tuber weight/plant (g) | 42.20 ^a | 36.50 ^b | 31.62 ^c | 22.98 ^d | 3.86 |

Table 3: Effect of six potato genotypes on different plant growth parameters under *in vivo* conditions

| | Genotype | Genotype | | | | | | L.S.D _(0.05) |
|---|------------------------------|----------------------|---------------------|----------------------|---------------------|---------------------|---------------------|-------------------------|
| | | Diamant | Ditta | Sante | Lady Rosetta | Safrane | Spunta | |
| 1 | Plant height (cm) | 30.34 ^c | 22.56 ^a | 25.71 ^b | 28.93 ^c | 21.88 ^a | 29.32 ^c | 2.38 |
| 2 | Num. of nodes/plant | 12.66 ^b | 10.17 ^a | 12.91 ^b | 12.83 ^b | 11.83 ^b | 12.66 ^b | 1.44 |
| 3 | Num. of shoots/plant | 1.50 ^b | 1.16 ^a | 1.58 ^b | 1.33 ^{ab} | 1.74 ^b | 1.16 ^a | 0.28 |
| 4 | Leaf area (cm ²) | 499.19 ^{dc} | 346.53 ^a | 454.61 ^{cd} | 448.08 ^c | 396.78 ^b | 540.90 ^e | 41.92 |
| 5 | Num. of tubers/plant | 5.25 ^c | 3.08 ^a | 4.58 ^b | 4.67 ^b | 3.24 ^a | 5.25 ^c | 0.47 |
| 6 | Tuber weight/plant (g) | 41.46 ^d | 26.97 ^{ab} | 35.27 ^c | 30.617 ^b | 24.84 ^a | 40.80 ^d | 4.73 |

| S.N. | Genotype name | Phenotype | Saline Tolerance Grade |
|------|---------------|--|------------------------|
| 1 | Somaclone A |  | Tolerant |
| 2 | Somaclone B |  | Sensitive |
| 3 | Somaclone C |  | Moderate Tolerant |
| 4 | Somaclone D |  | Moderate Tolerant |



| | | | |
|---|-------------|--|-----------|
| 5 | Somaclone E |  | Sensitive |
| 6 | Somaclone F |  | Tolerant |

Fig. 1: Effect of four different salinity levels 0, 20, 40, and 60 mM on plant growth of the six employed potato genotypes

Effect of NaCl stress on chlorophyll contents

The NaCl treatment generated an important loss of leaf greenness in treated plants, with significant decrease in chlorophyll content after salt treatment. This decrease was more significant in the two sensitive somaclones B, and E 21.5, and 27%, In contrast, leaves of tolerant genotypes A, and F show less percentage of chlorophyll lose 18.9, and 17% respectively. Lowest decrease in the greenness recorded with the two moderate somaclones C, and D. These results indicate a potential relation between chlorophyll loses by saline water and degree of plant tolerance. Compounds or genes those are able to reduce damaging effects of various

stresses should be of great importance from both the theoretical and application points of view. The content of chlorophyll in tolerant genotypes Somaclones A, and F was higher than Moderate tolerant somaclones C, and D. Sensitive two somaclones show the lowest content of chlorophyll either in normal or NaCl stressed conditions (Table 4). Chlorophyll is the main component of photosynthesis. Photosynthesis has been suggested to be accompanied by S-nitrosylation of some proteins, an important protein modification involved in NO signaling (Lindermayr *et al.*, 2005).

Table 4: Effect of NaCl stress on chlorophyll, contents within six different potato genotypes *Solanum Tuberosum* L.

| Genotype name | Chlorophyll content ($\mu\text{g/g FW}$) | | |
|---------------|--|-------------|------------|
| | 0 mM NaCl | 200 mM NaCl | Decrease % |
| Somaclone A | 350.0 | 284.0 | 18.9 |
| Somaclone B | 218.7 | 171.7 | 21.5 |
| Somaclone C | 303.3 | 265.0 | 12.6 |
| Somaclone D | 318.0 | 276.7 | 13.0 |
| Somaclone E | 229.7 | 167.7 | 27.0 |
| Somaclone F | 353.3 | 293.3 | 17.0 |

Effect of NaCl stress on protein contents

Protein content showed a significant decrease in all tissues of potato plants after salinity treatment, in the greenhouse conditions (Table 5). The highest decrease was noticed in sensitive somaclones B, and E 27.2, and 22.6% followed by moderate tolerant genotypes C, and D by 15.6, and 21.1% then tolerant somaclones recorded 19.1, and 12.6%. The soluble protein content in tolerant genotypes A, and F was 4.5 and 4.8 mg/g FW higher than moderate genotypes C, and D 4.0, and 4.1 mg/g FW while the two sensitive somaclones B, and E gave lowest values 3.4, and 3.5 mg/g FW in unstressed conditions, respectively (Table 5). Salt stress induced reduction of soluble contents in

tolerant genotypes A, and F to 3.7 and 4.2 mg/g FW higher than moderate genotypes C, and D 3.4, and 3.2 mg/g FW while the two sensitive somaclones B, and E gave lowest values 2.5, and 2.7 mg/g FW in stressed conditions, respectively (Table 5). The reasons for the low protein content in the callus exposed to salt stress were due to the lack of effective conversion of amino acids to proteins and inhibition of the activity of certain enzymes in callus tissue (Bowler *et al* 1992; Soloman *et al* 1994; Mobaraky 2001). The low protein content is also due to high levels of salinity leading to an increase in Reactive Oxygen Species (ROS) production, which negatively affects protein synthesis (Makela *et al* 2003).

Table 5: Effect of NaCl stress on protein contents within six different potato genotypes *Solanum Tuberosum* L.

| Genotype name | Protein content (mg/g FW) | | |
|---------------|---------------------------|-------------|------------|
| | 0 mM NaCl | 200 mM NaCl | Decrease % |
| Somaclone A | 4.533 | 3.667 | 19.1 |
| Somaclone B | 3.433 | 2.500 | 27.2 |
| Somaclone C | 4.067 | 3.433 | 15.6 |
| Somaclone D | 4.100 | 3.233 | 21.1 |
| Somaclone E | 3.533 | 2.733 | 22.6 |
| Somaclone F | 4.767 | 4.167 | 12.6 |

Effect of NaCl stress on proline contents

Since osmoprotectants play important roles in plant response to salinity, proline content was measured in potato plants submitted to 0, and 200 mM NaCl treatments. A high increase of the proline content was detected after salt addition in all somaclones ranged from 7.3 folds in somaclone F to 9.3 fold with somaclone B (Table 6). Proline accumulation is the common characteristic in many plants under saline conditions (Storey *et al.*, 1977; Wyn Jones and Storey, 1978). Highest values of proline content between control plants recorded within the two tolerant genotypes A, and F 316.7, and 400 $\mu\text{g/g}$ FW followed by the two

moderate tolerant somaclones C with 263.3, and D 276.7 $\mu\text{g/g}$ FW and lowest values recorded by the most sensitive genotypes B, and E 190.3, and 223.3 $\mu\text{g/g}$ FW respectively (Table 6). The proline content was doubled several times as a result of exposure to the sodium chloride salt 200 mM for 72 h in all genotypes (Table 6). Highest contents of proline 400, and 316.7 $\mu\text{g/g}$ FW measured in the two tolerant somaclones F, and A respectively. The two moderate tolerant genotypes C, and D contain 263.3, and 276.7 $\mu\text{g/g}$ FW. While the least content 190.3, and 223.3 $\mu\text{g/g}$ FW was found in the most sensitive two somaclones B, and E consequently (Table 6).

Table 6: Effect of NaCl stress on proline contents within six different potato genotypes *Solanum Tuberosum* L

| Genotype name | Proline content ($\mu\text{g/g}$ FW) | | |
|---------------|---------------------------------------|-------------|----------|
| | 0 mM NaCl | 200 mM NaCl | Redouble |
| Somaclone A | 316.7 | 2805.3 | 8.9 |
| Somaclone B | 190.3 | 1776.7 | 9.3 |
| Somaclone C | 263.3 | 2400.0 | 9.1 |
| Somaclone D | 276.7 | 2535.0 | 9.2 |
| Somaclone E | 223.3 | 1913.3 | 8.6 |
| Somaclone F | 400.0 | 2916.3 | 7.3 |

Proline concentration in many salt tolerant plants has been found to be higher than that in salt sensitive ones. Petrusa and Winicov (1997) found that salt tolerance alfalfa plants rapidly doubled their proline content in the roots, whereas, in the salt sensitive plants the increase was low. Increased proline accumulation with increased sodium chloride concentrations is due to the deficiency of the activity of proline oxidase (Girija *et al.*, 2002). The increased accumulation of proline in the callus is for the purpose of increasing tolerance to salt stress (Ahmed *et al.*, 2009). The results of the study are consistent with many other researchers' findings (Amini & Ehsanpour 2005; Cardenas *et al.*, 2006).

The phylogenetic analysis revealed high sequence similarity of gene sequences belong to the two resistance plant genotypes Somaclone A, and Somaclone F to the gene StNOA1 of salinity tolerance compared to moderate tolerant genotypes (somaclones C, D) and sensitive genotypes somaclones B, E (Figures 2, and 3). Such sequence similarity could indicate saline tolerance in potato genotypes. Cultivars with low sequence similarity to salinity tolerance gene could have undergone unintended mutations through tissue culture process which employed to vegetatively propagate different genotypes. The other potential reason for such spontaneous genetic alteration is these breeding programs during cultivar selection.

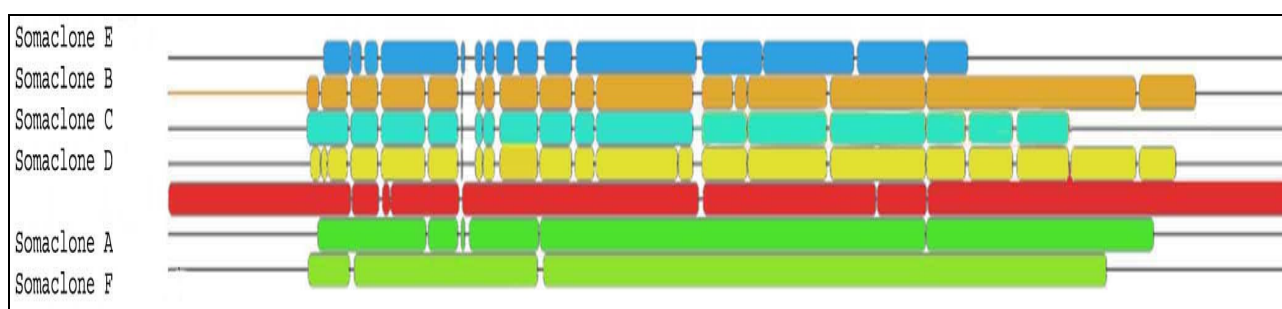


Fig. 2: Revealed the recovered gene sequences in addition to salinity tolerance gene StNOA1 and the sequence alignment of these sequences according to CLUSTALW tool.

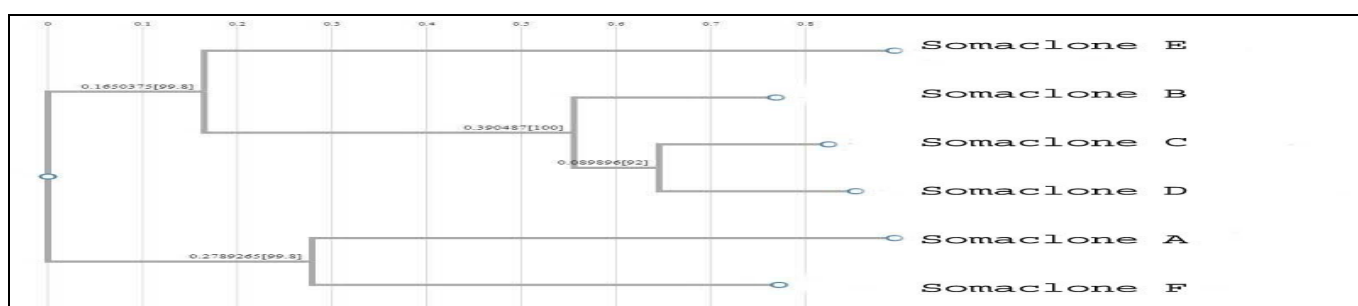


Fig. 3: Phylogenetic analysis of recovered gene sequences elucidate the relationship between the six current potato genotypes

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References

- Aghaei, K.A.; Ehsanpour, A. and Komatsu, S. (2009). Potato responds to salt stress by increased activity of antioxidant enzymes. *J. Integr. Plant Biol.* 51(12):1095-1103.
- Ahmed P.; Jeleel C.; Azooz M.; Nabi G.; (2009). Generation of (ROS) and non-enzymatic antioxidants during abiotic stress in plants. *Bot Res Int.*, 2:11-20.
- AL-Hussaini, Z.A.; Yousif, S.H.A. and AL-Ajeely, S.A. (2015). Screening four potato cultivars for salt tolerance. *Int. J. Agric. Biol. Res.* 5(2): 181-186.
- Allakhverdiev, S.I.; Sakamoto, A.; Nishiyama, Y.; Inaba, M. and Murata, N. (2000). Ionic and osmotic effects of NaCl-induced inactivation of photosystems I and II in *Synechococcus* sp. *Plant physiology*, 123(3): 1047-1056.
- Amel, M.B.; Hibal, G.; Boutheinal, M.H. and Abdehamid, E. (2015). Water use efficiency of potato crop irrigated under Tunisian climatic condition. *Sci. Agri.* 11 (1): 38-41.
- Amini, F. and Ehsanpour, A. (2005). Soluble protein, carbohydrates and Na^+/K^+ changes in two tomato (*Lycopersicon esculentum* Mill.) cultivars in vitro salt stress. *Am. J. Biochem. Biotechnol.* 1, 212-216.
- Anon (2014). The worlds healthiest foods. The George Mateljan Foundation. whfoods.org.
- Arnon, D.I. (1949). Copper enzymes in isolated chloroplasts: phenol oxidase in *Beta vulgaris*. *Plant Physiol.* 24: 1-15.
- Arvin, M.J. and Donnelly, D.J. (2008). Screening potato cultivars and wild species to abiotic stresses using an electrolyte leakage bioassay. *J. Agric. Sci. Technol.* 10: 33-42.
- Arvin, M.J. and Donnelly, D.J. (2008). Screening potato cultivars and wild species to abiotic stresses using an electrolyte leakage bioassay.
- Ashraf, M. (2004). Some important physiological selection criteria for salt tolerance in plants. *Flora.* 199: 361-376.
- Askari, A.; Pepoyan, A. and Parsaeimehr, A. (2012). Salt tolerance of genetic modified potato (*Solanum tuberosum*) cv. Agria by expression of a bacterial mtID gene. *Advances in Agriculture & Botany* 4(1):10-16. Biotechnology. CAB International Redwood Press UK. p. 340-351.
- Barnawal, D.; Bharti, N.; Maji, D.; Chanotiya, C.S. and Kalra, A. (2014). ACC deaminase containing *Arthrobacter protophormiae* induces NaCl stress tolerance through reduced ACC oxidase activity and ethylene production resulting in improved nodulation and mycorrhization in *Pisum sativum*. *J. P. Physiol.* 171: 884-894.
- Bates, L.S.; Waldren, R.P. and Teare, I.D. (1973). Rapid determination of free proline for water-stress studies. *Plant Soil*, 39: 205-207.
- Bradford, M.N. (1976). A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal. Biochem.* 72: 248-254.
- Cardenas, A.; Verda-Star, M.L.; Maiti, R.K.; Foroughbakhch, P.; Games, H.; Lozaano, M.L.; Gonzalez, M.A.; Diaz, G.G.; Hernandez, J.L. and Vallarta, M.R. (2006). Variability in accumulation of free proline on *in vitro* callus of four bean (*Phaseolus vulgaris* L.) varieties exposed to salinity and induced moisture stress. *Int J Exp Bot.*, 75:103-108.
- Dhindsa, R.S.; Plumb-Dhindsa, P. and Thorpe, T.A. (1981). Leaf senescence: correlated with increase leaves of membrane permeability and lipid peroxidation and decreased levels of superoxide dismutase and catalase. *J. Exp. Bot.* 32: 93-101.
- Etehadnia, M. (2009). Salt Stress Tolerance in Potato Genotypes. A Thesis submitted to the College of Graduate Studies and Research, University of Saskatchewan Saskatoon.
- Evers, D.; Overney, S.; Simon, P.; Greppin, H. and Hausman, J.F. (1999). Salt tolerance *Solanum tuberosum* L. Over expressing a heterologous somatin -like protein. *Biol. Plantarum*, 42: 105-112.
- FAO (2008). International year of the potato. www.Potato2008.org. Fidalgo, F.; A. Santos, I. Santos and R. Salema . 2004. Effects of long-term salt stress on antioxidant defense systems, leaf water relations and chloroplast ultrastructure of potato plants. *Ann. Appl. Biol.* 145: 185-192.
- Fidalgo, F.; Santos, A.; Santos, I. and Salema, R. (2004). Effects of long-term salt stress on antioxidant defense systems, leaf water relations and chloroplast ultrastructure of potato plants. *Ann. Appl. Biol.*, 145: 185-192.
- Ghosh, S.C.; Asanuma, K.; Kusutani, A. and Toyota, M. (2001). Effect of salt stress on some chemical components and yield of potato. *Soil Sci. Plant Nutr.* 47(3): 467-475.
- Girija, G.; Smith, B.N. and Swamy, P.M. (2002). Interactive effects of sodium chloride and calcium chloride on the accumulation proline and glycine betaine in peanut (*Arachis hypogea* L.). *Environ Exp Bot.*, 47:1-10.
- Kirk, W.W.; da Rocha, A.B.; Hollosy, S.I.; Hammerschmidt, R. and Wharton, P.S. (2006). Effect of soil salinity on internal browning of potato tuber tissue in two soil types. *Amer. J. Pot. Res.* 83(3): 223-232.
- Lindermayr, C.; Saalbach, G. and Durner, J. (2005). Proteomic identification of S-nitrosylated proteins in Arabidopsis. *Plant physiology*, 137(3): 921-930.
- Ma, Y.; Xu, T.; Wan, D.; Ma, T.; Shi, S.; Liu, J. and Hu, Q. (2015). The salinity tolerant poplar database (STPD): a comprehensive database for studying tree salt-tolerant adaptation and poplar genomics. *BMC Genomics.* 16(1):205.
- Mahmoud, M.H.; Bettaieb, T.; Harbaoui, Y.; Mougou, A.A. and Jardin, P. (2009). Differential response of potato under sodium chloride stress. *J. Biol. Sci.* 16: 79-83.
- Meloni, D.A.; Oliva, M.A.; Martinez, C.A. and Cambraia, J. (2003). Photosynthesis and activity of superoxide dismutase, peroxidase and glutathione reductase in cotton under salt stress. *Environ. Exp. Bot.* 49: 69-76.
- Molla, K.A.; Debnath, A.B.; Ganie, S.A. and Mondal, T.K. (2015). Identification and analysis of novel salt responsive candidate gene based SSRs (cgSSRs), from rice (*Oryza sativa* L.). *BMC Plant Biology.* 15:122
- Munira, S.; Hossain, M.; Zakaria, M.; Ahmed, J. and Islam, M. (2015). Evaluation of potato varieties against

- salinity stress in Bangladesh. Munns, R. 2002. Comparative physiology of salt and water stress. *Plant, Cell & Environment*. 25(2): 239-250.
- Munns, R. (2002). Comparative physiology of salt and water stress. *Plant, Cell & Environment*. 25(2): 239-250.
- Murshed, R.; Najlal, S.; Albiski, F.; Kassem, I.; Jbour, M. and Al-Said, H. (2015). Using growth parameters for in-vitro screening of potato varieties tolerant to salt stress. *J. Agri. Sci. Tech.*, 17: 483-494.
- Patell, R.M.; Prashera, S.O.; Donnellyb, D. and Bonnella, R.B. (2001). Effect of initial soil salinity and subirrigation water salinity on potato tuber yield and size. *Agric. Water Manag.* 46(3): 231-239.
- Petrusa, L.M. and Winicov, I. (1997). Proline status in salt-tolerant and salt-sensitive alfalfa cell lines and plants in response to NaCl. *Plant physiology and biochemistry (Paris)*, 35(4): 303-310.
- Qayyum, M. and Shoaib, K. (2013). Selection of potato (*Solanum tuberosum* L. cv. Cardinal) plantlets tolerant to in vitro salt and drought stress. *Pak. J. Biochem. Mol. Biol.* 46(1): 37-41.
- Ruiz-Lozano, J.M.; Porcel, R.; Azcon, R. and Aroca, R. (2012). Regulation by arbuscular mycorrhizae of the integrated physiological response to salinity in plants: new challenges in physiological and molecular studies. *J. Exp. Bot.*, 63: 4033-4044.
- Shrivastava, P. and Kumar, R. (2015). Soil salinity: A serious environmental issue and plant growth promoting bacteria as one of the tools for its alleviation. *Saudi J. Biol. Sci.*, 22:123-131.
- Silva, J.; Otoni, A.B.; Martinez, W.C.; Dias, C.A. and Silvam, P.M.A. (2001). Microtuberization of Andean potato species (*Solanum* spp.) as affected by salinity. *Sci. Horti.* 89: 91-101.
- Solh, M. and Ginkel, M. (2014). Drought preparedness and drought mitigation in the developing world's dry lands. *Weather and Climate Extremes*. 3:6266.
- Storey, R.; Ahmad, N.; Wyn, Jones R.G. (1977). Taxonomic and ecological aspects of the distribution of glycinebetaine and related compounds on plants. *Oecologia*, 27: 319-322.
- Wyn Jones, R.G. and Storey, R. (1978). Salt stress and comparative physiology in the Gramineae: Comparison of salt stress in *Spartina Xtownsendii* and three barley cultivars. *Aust. J. Plant Physiol.* 5: 839-850.
- Zaman, M.S.; Ali, G.M.; Muhammad, A. and Hussain, I. (2018). *In vivo* screening of potato (*Solanum tuberosum* l.) cultivars for saline water tolerance. *Journal of Agricultural Research (03681157)*, 56 (1).
- Zhang, B.; Wang, H.Q.; Liu, B.L.; Liu, J.; Wang, X.; Liu, Q. and Zhang, H.G. (2010). A potato NOA gene increased salinity tolerance in *Arabidopsis thaliana*. *African Journal of Biotechnology*, 9(36).
- Zhang, Z.; Mao, B.; Li, H.; Zhou, W.; Takeuchi, Y. and Yoneyama, K. (2005). Effect of salinity on physiological characteristics, yield and quality of microtubers in vitro in potato. *Acta Physiol. Pl.* 27:481-489.
- Zhu, J. K. 2007. *Plant Salt Stress*. John Wiley & Sons. Ltd.
- Zhu, J.K. (2007). *Plant Salt Stress*. John Wiley & Sons. Ltd.