Variability in salinity stress tolerance of potato (*Solanum tuberosum* L.) varieties using *in vitro* screening

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ABSTRACT

Salinity is one of the abiotic stresses that lead to an imbalance in the physiological processes of the plants and also affects potato growth and productivity in mainly semi-arid and growing areas. The accumulation of Na+ and Cl- ions in the cells is very toxic can influence all mechanisms and the enzymatic actions of the plants. *In vitro* screening of plant genotypes for osmotic stress represents a valuable tool as an alternative to field trials and can be applied based on osmotic stress tolerance. The main goal of this study was to reveal variability in salinity stress tolerance of potato varieties using *in vitro* screening. Stem cuttings consisting of a single node of different varieties were cultured on Murashige and Skoog (MS) medium supplemented with different concentrations of sodium chloride (NaCl) (0, 50, 100 and 150 mM). The differences among the plantlet length, number of branches, number of nodes, number of the leaflet, leaflet width, leaflet length, root length, number of the root, fresh plantlet weight, dry plantlet weight of all varieties were negatively influenced by all NaCl concentrations tested. Microtuberization and stolon growth of the varieties were also completely inhibited at high concentrations (100-150 mM). The Principal components analysis (PCA) was applied to the data matrix (15 morphological characteristics x 12 potato varieties) of the potato varieties. Also, a hierarchical cluster analysis (HCA) was used to identify the possible nearest and similarity of all morphological characteristics analyzed of the potato varieties. In grouping potato varieties, HCA and PCA results were found to be similar. We can speculate about the responses of morphological similarities of the potato varieties against salt stress. We concluded that Innovator and Kennebec are respectively the most salt-tolerant varieties. Hermes was moderately salt-tolerant and microtuberization capacity of Slaney was also high under salt stress conditions.

Index terms: Osmotic tolerance; microtuberization; plant toxicity.

RESUMO

A salinidade é um dos estresses abióticos que leva a um desequilíbrio nos processos fisiológicos das plantas e também afeta o crescimento e a produtividade da batata principalmente em áreas semi-áridas e em crescimento. O acúmulo de íons Na+ e Cl- nas células é muito tóxico e pode influenciar todos os mecanismos e as ações enzimáticas nas plantas. A triagem *in vitro* dos genótipos de plantas para o estresse osmótico representa uma ferramenta valiosa como alternativa aos ensaios de campo e pode ser aplicada com base na tolerância ao estresse osmótico. O principal objetivo deste estudo foi revelar variabilidade na tolerância ao estresse salino das variedades de batata ao utilizar a triagem *in vitro*. Estacas de caule constituídas por um único nó de diferentes variedades foram cultivadas em meio Murashige e Skoog (MS) suplementado com diferentes concentrações de cloreto de sódio (NaCl) (0, 50, 100 e 150 mM). As diferenças entre comprimento de plântula, número de ramos, número de nós, número de folhas, largura de folhas, comprimento de folhas, comprimento de raízes, número de raízes, peso de plântulas frescas, peso de plântulas secas de todas as variedades foram influenciadas negativamente por todas as concentrações de NaCl testadas. A microtuberização e o crescimento de estolões das variedades também foram completamente inibidos em altas concentrações (100-150 mM). A Análise de Componentes Principais (ACP) foi aplicada à matriz de dados (15 características morfológicas x 12 variedades de batata) das variedades de batata. Também foi utilizada uma Análise de Agrupamento Hierárquico (AAH) para identificar a possibilidade de proximidade e semelhança de todas as características morfológicas analisadas das variedades. No agrupamento de variedades de batata, os resultados de AAH e ACP foram semelhantes. Podemos especular sobre as respostas de similaridades morfológicas das variedades de batata contra o estresse salino. Concluímos que Innovator e Kennebec são respectivamente as variedades mais tolerantes ao sal. Hermes mostrou-se moderadamente tolerante ao sal e a capacidade de microtuberização de Slaney também foi alta sob condições de estresse salino.

Termos para indexação: Tolerância osmótica; microtuberização; toxicidade vegetal.
INTRODUCTION

Potato (*Solanum tuberosum* L.) with rich compounds providing high calories to humans and animals is the fourth largest stable crop grown under different climatic and soil conditions (Chandrasekara; Kumar, 2016). Salinity is estimated to be increased negatively impact plant growth, survival and crop yield as potato is one of the crops with limited abiotic stress tolerance (Gupta; Huang, 2014; Roy; Negrão; Tester, 2014; Zaman et al., 2015). Soil salinity affects plant development and growth and is one of the most critical worldwide problems that negatively limit crop productivity (Isayenkov; Maathuis, 2019). Approximately 8 million hectares of land (6% of the total land area) and 50% of irrigated lands throughout the world are under the threat of salinity (Kikuchi et al., 2015; Parihar et al., 2015; Charfeddine et al., 2019). Potato is intensively irrigated in many semi-arid and arid regions of the world, and this case may cause major salinity problems. Salt is extremely toxic for all plants and leads to Na⁺ and Cl⁻ toxicity (Maathuis, 2013; Flowers; Munns; Colmer, 2015).

Salt stress increases ionic stress, and osmotic stress in plant cells and salinity causes oxidative damage in plants by the production of reactive oxygen species (ROS) that are toxic to plant cells at high concentrations. Osmotic adjustment is one of the primary mechanisms that lead to tolerance against salt stress (Bündig et al., 2016). Potato is one of the moderately sensitive crops to salinity under *in vitro* and *in vivo* conditions, and the salinity stress has significant and disruptive impacts on potato tuber production. Potato tuber growth is markedly influenced by salinity, and the quality of the tubers might be drastically reduced (Katerji et al., 2003; Parihar et al., 2015; Jha et al., 2017).

The previous studies revealed that potato is more sensitive to salinity in the early growth periods (Levy, 1992; Nadler; Heuer, 1995). Therefore, improvement of salt-tolerant potato varieties against salinity is essential to facilitate potato production. Potato gives a complex response to salt stress at physiological, metabolic, and molecular levels as other important crops (Batelli et al., 2012; Shimazaki et al., 2016; Uranbey et al., 2017). Under salt stress conditions, many genes encoding complex protein structures are activated depending on the genetic characteristics of the plant species (Zhu, 2007; Çulha; Çakırlar, 2011; Jing et al., 2015). Many different metabolic and physiological changes also occur under salt stress, and gene and transcription factors are involved in these processes in potato (Batelli et al., 2012; Shimazaki et al., 2016; Uranbey et al., 2017).

The previous studies showed that salt stress drastically reduced tuber yield and yield components of field-grown potato (Ghosh et al., 2001; Elkhatib et al., 2006; Khalid; Aftab, 2016). Because a selection for salt-tolerant varieties in the field is time-consuming, cost-intensive and difficult to reproduce, *in vitro* cell and tissue culture techniques very fast, reliable and one of the modern methods for screening of potato varieties to salt tolerance (Murshed et al., 2015; Khierallah; Jawad, 2017; Hassanein; Salem, 2017; Uranbey et al., 2017; EL-Kazzaz; Ebad; Abd EL-Sadek, 2018; Jing-Wei et al., 2018; Raigond; Mehta; Singh, 2018; Al-Mahmud et al., 2018).

*In vitro* screening can be an alternative to efficiently select material for its reaction to salinity stress. *In vitro* microtubers in potato are valuable materials for disease-free, long-term preservation, and transport of genetic material, evaluation, and suitability of germplasm for *in vitro* selection (Uranbey et al., 2017). *In vitro* microtuberization is an advantageous method for physiological studies and selection of potato germplasm and also an important source of explants to evaluate potato plants for salt tolerance. There are limited researches about salinity effect on *in vitro* microtuberization of potato (Amerian; Esna-Ashari, 2011). The aim of this experiment was *in vitro* screening of potato varieties widely grown in semi-arid areas of the world for salt tolerance using morphological, growth and development parameters and to evaluate the effect of *in vitro* salt stress on microtuberization of these potato varieties.

MATERIAL AND METHODS

Plant material

This study was conducted at the Biotechnology Laboratory, Department of Field Crops, Faculty of Agriculture, Ankara University. Twelve potato varieties (Tokat 10/1, Tokat 6/24, Tokat Basciftcilik Beyazi (Tokat BCB), Tokat 3/161, Marabel, Slaney, Hermes, Granola, Burren, Kennebec, Lady Claire and, Innovator) widely cultivated in Turkey and the world, were screened for *in vitro* salt tolerance.

Tuber sterilization and sprouting

Tubers of investigated potato varieties were sterilized by a solution containing 2.5% NaClO for 20 minutes and then rinsed three times with sterilized water for 5 minutes, and then maintained at 24 °C for six weeks under the dark condition for sprout inducements. Stem cuttings consisting of a single node of the genotypes were
cultured on MS medium supplemented with different concentrations of NaCl (0, 50, 100, and 150 mM). Then, the tubers were kept at 4 °C in a solution containing 100 ppm GA₃ for 60 minutes for breaking the dormancy. Sprout tips at length 2-3 cm were chopped and sterilized by a solution containing 1.25% NaClO for 20 minutes, then washed three times with sterilized distilled water for 5 minutes. Then the meristic tissues at the ends of the shoots were cultured at 24 °C for six weeks before culturing in MS (Murashige; Skoog, 1962) media containing 3% sucrose under the condition of 16/8 h day and night photoperiod at 16000 lux light intensity. They reached a length of 1.0-1.5 cm after about four weeks. Newly generated potato plantlets of 6-8 leaves were achieved after 6-8 weeks.

**In vitro screening of plantlets and salt treatments**

Stem cuttings consisting of a single node of the potato varieties (0.5 cm length) with single-leaf in 4-6 weeks old plantlets were isolated and cultured on MS medium supplemented with different concentrations of NaCl (0, 50, 100 and 150 mM). Five explants were placed in each tissue culture vessel (OS140BOX, Duchefa Biochemie B. V, Holland) with three replicates for each NaCl concentration. The culture vessels were incubated under the condition of 16/8 h day and night photoperiod at 16000 lux light intensity. Shoot length, the number of nodes, the number of the leaflet, leaflet length, leaflet width, root length, the number of the root, fresh root weight, dry root weight, fresh plantlet weight, dry plantlet weight were determined after 10-12 weeks culture initiation. After 10-12 weeks, developed plantlets of the varieties were separated into roots and shoots, weighed for fresh mass (FM), and dried until constant weight in an oven at constant 70 °C for 48 h for dry mass (DM).

**Microtuber induction**

The second experiment was conducted to investigate the effect of salinity stress on microtuber induction. Single node stem segments (0.5 cm length) with single-leaf in 4-6 weeks-old plantlets excised and cultured on MS medium or MS medium containing 2.5 mg/L Kinetin, 60 g/L sucrose 0, 50, 100 or 150 mM NaCl, and 7 g/L agar for microtuberization studies. Five explants were placed in each tissue culture vessel (OS140BOX, Duchefa Biochemie B. V, Holland) with three replicates for each NaCl concentration. The culture vessels were incubated under the condition of 16/8 h day and night photoperiod at 16000 lux light intensity. The number of microtuber per plantlet, the total weight of microtuber per explant, dry weight of microtuber per explant were measured. After 12-14 weeks, developed microtubers, stolons, leaves and roots of the varieties were separated, counted, weighed for fresh mass microtuber (FMM) and dried until constant weight in an oven at constant 70 °C for 48 h for microtuber dry mass (DM).

**Statistical analysis**

Relative salinity tolerance of the 12 potato varieties was determined based on multivariate analysis of the relative means of the 12 growth parameters for each level of NaCl (0, 50, 100 and 150 mM). Means of the three microtuberization parameters were also evaluated for relative salinity tolerance. We used SAS software version 9.4 (SAS Institute Inc.) to analyze the recorded data, and the differences among the means were compared by Tukey’s multiple comparison tests (Düzgüneş; Kesici; Gürbüz, 1983). Principal components analysis (PCA) was carried out on the correlation matrix using the PCA subroutine of the IBM SPSS statistic 20 packages. The PCA was applied to the data matrix (15 morphological characteristics x 12 potato varieties) of the potato varieties. The data matrix of total morphological characteristics was rotated using the varimax rotation method.

**RESULTS AND DISCUSSION**

Although no morphological differences and relative deterioration were observed at all salt concentrations for all varieties tested, the plantlets of all varieties were significantly affected by NaCl concentrations. The differences among the plantlet length, number of branches, number of nodes, number of the leaflet, leaflet width, leaflet length, root length, number of the root, fresh plantlet weight, dry plantlet weight of all varieties were statistically influenced by all NaCl concentrations tested (P <0.01) (Table 1). There were also statistically significant interactions between salt concentrations and potato varieties (P <0.01) (Table 1).

We observed that the plant heights of all varieties were drastically decreased with increasing NaCl concentration. The highest plantlet length (9.91 cm) was determined in the control plantlets of Tokat 10/1. Plant lengths of Tokat 10/1 (7.06 cm), Tokat BCB (7.75 cm), Slaney (6.19 cm) were not affected by salt at 50 mM NaCl compared to those in control plantlets (Table 1). Moreover, plantlet lengths of all varieties were significantly affected by 100 mM NaCl concentration except Innovator. Our data are consistent with the studies reporting that increased salt concentrations negatively affect plant length and development of potato (Aghaei; Ehsanpour; Komatsu, 2009; Sudhersan et al., 2012; Zaman et al., 2015).
Table 1: Effect of different concentrations of NaCl in enhancement responses of potato under salt rates in the growth parameters, plantlet length (PL), number of branches (NB), number of nodes (NN), number of leaves (NL), leaf width (LW), and leaf length (LL).

| Cultivars | NaCl Doses (mM) | Variables |
|-----------|-----------------|-----------|
|           |                 | PL cm     | NB Plant⁻¹ | NN Plant⁻¹ | NL Plant⁻¹ | LW cm | LL cm |
| Innovator | 0               | 7.34 A-D  | 12.27 AB   | 10.27 B-D  | 17.47 B-D  | 0.82 A-E | 0.87 A-F |
|           | 50              | 7.02 A-E  | 12.00 A-C  | 8.87 A-G   | 14.20 B-H  | 0.95 A-B | 1.08 A-B  |
|           | 100             | 5.67 B-K  | 9.40 A-G   | 7.60 A-I   | 10.73 B-J  | 0.96 A   | 1.15 A    |
|           | 150             | 3.77 D-M  | 8.67 B-H   | 6.40 B-L   | 9.47 D-J   | 0.88 A-C | 1.03 A-C  |
| Granola   | 0               | 5.41 C-L  | 11.47 A-D  | 9.00 A-F   | 13.67 B-H  | 0.47 A-G | 0.62 A-H  |
|           | 50              | 2.48 F-M  | 7.07 D-K   | 6.13 B-M   | 9.87 C-J   | 0.24 A-G | 0.32 D-H  |
|           | 100             | 1.48 L-M  | 5.33 G-P   | 4.33 H-O   | 5.73 G-J   | 0.13 G   | 0.17 G-H  |
|           | 150             | 1.12 M    | 2.33 P     | 1.87 M-O   | 3.13 I-J   | 0.27 E-G | 0.53 A-H  |
| Marabel   | 0               | 5.84 A-I  | 13.67 A    | 11.00 A    | 19.33 B-C  | 0.37 G   | 0.57 A-H  |
|           | 50              | 3.52 D-M  | 9.00 G-A   | 6.13 B-M   | 11.00 B-J  | 0.38 A-G | 0.28 E-H  |
|           | 100             | 2.96 E-M  | 6.53 F-M   | 4.93 H-O   | 7.27 E-J   | 0.30 A-G | 0.31 E-H  |
|           | 150             | 2.50 F-M  | 4.87 G-P   | 3.40 D-O   | 5.53 G-J   | 0.37 G   | 0.49 A-H  |
| Kennebec  | 0               | 9.59 A-B  | 13.67 A    | 9.60 A-D   | 17.53 B-C  | 0.52 A-G | 0.84 A-F  |
|           | 50              | 6.40 A-G  | 11.40 A-E  | 9.13 A-E   | 16.27 B-E  | 0.67 A-G | 0.94 A-E  |
|           | 100             | 2.94 E-M  | 6.87 D-K   | 5.93 B-N   | 11.33 B-J  | 0.34 A-G | 0.51 A-H  |
|           | 150             | 1.83 M    | 3.87 P     | 3.00 D-O   | 8.33 D-J   | 0.27 E-G | 0.30 E-H  |
| Tokat 3/161| 0              | 5.83 A-I  | 10.66 A-F  | 9.47 A-D   | 19.13 A-C  | 0.46 A-G | 0.70 A-H  |
|           | 50              | 3.14 E-M  | 6.80 D-K   | 5.53 B-M   | 11.73 B-J  | 0.38 A-G | 0.64 A-H  |
|           | 100             | 1.58 K-M  | 4.13 H-P   | 2.80 K-O   | 6.53 F-J   | 0.45 A-G | 0.61 A-H  |
|           | 150             | 1.02 M    | 1.93 P     | 1.40 G     | 3.13 I-J   | 0.53 A-G | 0.69 A-H  |
| Hermes    | 0               | 6.57 A-F  | 11.47 A-D  | 9.73 A-C   | 27.40 A    | 0.65 A-G | 0.87 A-F  |
|           | 50              | 6.09 A-I  | 10.73 A-F  | 10.20 A-B  | 19.53 A-B  | 0.60 A-G | 0.79 A-H  |
|           | 100             | 3.55 D-M  | 9.13 A-G   | 7.47 A-J   | 10.07 B-J  | 0.43 A-G | 0.68 A-H  |
|           | 150             | 3.00 E-M  | 6.80 D-K   | 5.80 B-O   | 9.00 D-J   | 0.55 A-G | 0.90 A-E  |
| Tokat 10/1| 0               | 9.91 A    | 9.20 A-G   | 8.00 A-H   | 15.93 B-F  | 0.39 A-G | 0.61 A-H  |
|           | 50              | 7.06 A-E  | 7.33 I-J   | 5.80 B-O   | 12.60 B-J  | 0.47 A-G | 0.70 A-H  |
|           | 100             | 3.90 D-M  | 5.66 G-P   | 4.40 G-O   | 9.40 D-J   | 0.37 C-G | 0.45 B-H  |
|           | 150             | 3.72 D-M  | 5.33 G-P   | 4.27 H-O   | 8.27 D-J   | 0.40 A-G | 0.68 A-H  |
| Tokat BCB | 0               | 7.34 A-D  | 8.93 A-G   | 10.27 A-B  | 19.33 A-C  | 0.36 G   | 0.50 A-H  |
|           | 50              | 7.58 A-D  | 6.67 D-L   | 8.00 A-H   | 14.40 B-G  | 0.51 A-G | 0.62 A-H  |
|           | 100             | 1.97 I-M  | 1.80 M-P   | 1.93 G     | 3.60 I-J   | 0.15 G   | 0.23 E-H  |
|           | 150             | 1.68 M    | 1.53 P     | 1.67 M-O   | 2.60 I     | 0.36 C-G | 0.42 B-H  |
| Lady Claire| 0             | 4.74 D-M  | 6.47 F-N   | 5.27 C-O   | 11.20 B-J  | 0.59 A-G | 0.71 A-H  |
|           | 50              | 3.56 D-M  | 4.07 H-P   | 2.93 K-O   | 9.20 D-J   | 0.75 A-F | 0.87 A-F  |
|           | 100             | 2.38 G-M  | 2.53 P     | 1.87 M-O   | 7.33 E-J   | 0.37 C-G | 0.49 A-H  |
|           | 150             | 1.81 I-M  | 2.60 P     | 1.87 M-O   | 6.13 G-J   | 0.12 C   | 0.15 H    |

Continua...
Zhang and Donnelly (1997) also found low growth and development in potato at 75 mM NaCl. Similarly, (Rahman et al., 2008) also reported a decrease shoot length at 75 mM and 100 mM NaCl.

High concentrations of NaCl salt negatively affected the average number of branches in all varieties. The highest number of branches (12.27) was obtained in the control plantlets of Innovator. The number of branches of Innovator, Kennebec, Hermes, and Marabel was not affected by salt at 50 mM NaCl compared to those of control plantlets. Innovator showed high tolerance to 100 mM NaCl concentration in terms of the number of branches compared to those in other potato varieties. When the means of the number of branches of the varieties, including control plantlets, were evaluated in all salt concentrations, Innovator (10.58) gave the highest number of branches. These results are consistent with the results of many researchers who found a decrease in the number of branches with an increase in NaCl (Rahman et al., 2008; Qayyum; Shoaib, 2013; Ali et al., 2014; Kamil; Abdulhussien; Ali, 2016).

The number of nodes of all varieties was strongly influenced by different levels of NaCl (Table 1). The highest number of nodes was observed in the Hermes (8.30), unlike the lowest number of nodes was obtained in Lady Claire (2.98). The number of nodes of Innovator, Kennebec and Hermes was also not affected by salt at 50 mM NaCl compared to those of control plantlets. When the means of the number of nodes of the varieties including control plantlets were evaluated in all salt concentrations, the number of nodes ranged between 2.98-8.30 for all varieties. Similarly, (Aghaei et al., 2008; Mahmoud et al., 2009) reported that potato varieties were moderately tolerant of salt stress and all tested potato varieties showed overall stunted growth and the low number of nodes due to salt stress.

Leaflet formation of all potato varieties subjected to salt stress was delayed up to 1-2 weeks at higher salt concentrations (100 mM NaCl). In general, the number of leaflets, leaflet width and leaflet length of all varieties was repressed at high levels of NaCl. On the other hand, the highest leaflet width and length were measured in Innovator growth at 100 mM NaCl. When the means of the number of leaflets of the varieties including control plantlets were evaluated in all salt concentrations, the highest number of plant leaflets (16.50) was determined in Hermes, while the lowest number of plant leaflets (7.85) was seen in Burren. Innovator (12.97) and Kennebec (13.37) followed Hermes. These results are consistent with previous studies (Potluri; Prasad, 1993; Farhatullah; Raziuddin, 2002; Shaterian et al., 2005; Homayoun; Parisa; Daliri, 2011; Khenifi; Boudjeniba; Kameli, 2011). The reason for small size and a few numbers of leaflets under high NaCl levels may be due to the high osmotic pressure in the center of the multiplication. This is because most of the available energy is spent to resist

| Cultivars | NaCl Doses (mM) | Variables |
|------------------|-----------------|------------|
|                 | PL  | NB | NN | NL | LW | LL |
| Burren           |     |    |    |    |    |    |
| 0                | 5.49B | 6.20D | 5.07D | 10.73B | 0.34C | 0.49A |
| 50               | 4.53D-M | 4.80G-P | 3.73H-O | 8.20D-J | 0.63A-G | 0.77A-H |
| 100              | 2.76F-M | 3.47J-P | 2.73K-O | 6.13D-J | 0.32A-G | 0.45B-H |
| 150              | 2.15H-M | 3.00K-P | 2.00L-J | 6.33G-I | 0.26E-G | 0.37C-H |
| Tokat 6/24       |     |    |    |    |    |    |
| 0                | 6.62A-F | 7.87B-I | 7.00A-K | 14.13B-H | 0.51A-G | 0.60A-H |
| 50               | 5.00D-M | 5.27G-P | 4.53D-O | 10.07B-J | 0.72A-F | 0.89A-G |
| 100              | 2.92E-M | 3.87F-P | 3.47I-P | 8.47D-J | 0.41A-G | 0.48A-H |
| 150              | 1.55K-M | 1.40P | 1.33O-J | 3.93I-P | 0.31E-G | 0.39C-H |
| Slaney           |     |    |    |    |    |    |
| 0                | 9.49A-C | 6.67F-L | 5.80B-P | 12.40B | 0.81A-E | 0.99A-D |
| 50               | 6.19A-H | 5.27G-P | 4.40C-P | 11.60B-J | 0.88A-D | 0.95A-E |
| 100              | 1.93N-M | 1.73P-N | 1.47N-O | 4.73H-J | 0.48A-G | 0.66A-H |
| 150              | 1.93J-M | 1.40P | 1.40D-P | 4.13I-J | 0.40A-G | 0.53A-H |

Values not followed by the same letter are significantly different at $P < 0.01$ (Tukey's test) within the same parameter.
high pressure and create an osmotic balance within the cell for the metabolic process. (Smith et al., 1992) stated measurements of canopy and shoot responses to salinity provided an excellent indicator for plant yield potential under stress in tomato.

The number of roots and mean root length of all varieties were also negatively affected by high levels of NaCl (100 mM NaCl). The most extended root length was measured in Tokat 10/1 with 20.83 cm at control treatment, while the shortest root length was measured in Tokat 3/161 with 0.94 cm in 150 mM NaCl treatment. Compared to the other potato varieties, Innovator showed high tolerance to high NaCl concentrations in terms of root length. High salt concentration prevented the development of new roots. The root numbers ranged between 14.07 and 0.20 in Tokat BCB at control treatment and Granola at 150 mM NaCl treatment, respectively. The number of roots of Innovator, Marabel, Kennebec, Hermes, Burren, and Tokat 6/24 was also not affected by salt at 50 mM NaCl compared to control plantlets. When the mean of root numbers of the varieties including control plantlets was evaluated in all salt concentrations, the number of roots fluctuated between 3.28-7.93. The highest number of roots was determined in Kennebec. Unlike the lowest number of roots was obtained in Lady Claire (3.28). Similarly, the highest root length (14.94 cm) was also measured in Kennebec. Increased salt concentrations also led to a decrease in the root length. Naik and Widholm (1993) reported poor root development in potato above 100 mM NaCl. Also, (Evers et al., 1999) also stated low profile rooting in potato under salt stress conditions. Fresh plantlet weights of all varieties were strongly affected by different levels of NaCl (Table 2). When Fresh plantlet weights of the varieties including control plantlets were evaluated in all salt concentrations, the fresh plantlet weights fluctuated between 0.05 and 0.78 g. The maximum fresh plantlet weight was observed in Slaney (0.78 g) at 50 mM NaCl. Similarly, plant dry weight was not affected by salt at 50 mM NaCl compared to the control plantlets for Slaney and Kennebec. Figure 1 and 2 respectively indicate plantlet development/growth of the tolerant variety Innovator and the sensitive variety Granola.

**Table 2:** Effect of different concentrations of NaCl in enhancement responses of potato under salt rates in the growth parameters, root length (RL), number of the root (NR), fresh root weight (FRW), dry root weight (DRW), fresh plantlet weight (FPW), and plantlet dry weight (DPW).

| Cultivars | NaCl Doses | Variables | RL cm Plant⁻¹ | NR Plant⁻¹ | FRW g | DRW g | FPW g | DPW g |
|----------|------------|-----------|----------------|------------|-------|-------|-------|-------|
|          | mM         |           |                |            |       |       |       |       |
| Innovator| 0          | 19.28      | 8.80          | 0.16       | 0.01  | 0.28  | 0.03  |
|          | 50         | 16.82      | 9.07          | 0.19       | 0.008 | 0.39  | 0.04  |
|          | 100        | 12.53      | 7.53          | 0.08       | 0.002 | 0.33  | 0.03  |
|          | 150        | 11.16      | 5.13          | 0.03       | 0.001 | 0.27  | 0.03  |
| Granola  | 0          | 12.85      | 11.73         | 0.21       | 0.003 | 0.16  | 0.01  |
|          | 50         | 7.79       | 5.60          | 0.19       | 0.000 | 0.05  | 0.007 |
|          | 100        | 3.64       | 3.27          | 0.01       | 0.000 | 0.09  | 0.006 |
|          | 150        | 1.01       | 0.20          | 0.00       | 0.000 | 0.06  | 0.003 |
| Marabel  | 0          | 14.16      | 12.87         | 0.16       | 0.008 | 0.30  | 0.03  |
|          | 50         | 16.97      | 8.93          | 0.15       | 0.002 | 0.30  | 0.02  |
|          | 100        | 10.07      | 4.60          | 0.06       | 0.002 | 0.19  | 0.01  |
|          | 150        | 3.48       | 2.13          | 0.00       | 0.000 | 0.12  | 0.007 |
| Kennebec | 0          | 16.66      | 13.00         | 0.37       | 0.041 | 0.55  | 0.04  |
|          | 50         | 17.56      | 11.67         | 0.23       | 0.007 | 0.50  | 0.04  |
|          | 100        | 11.97      | 5.40          | 0.04       | 0.000 | 0.19  | 0.01  |
|          | 150        | 3.57       | 1.67          | 0.01       | 0.000 | 0.12  | 0.005 |

Continua...
**Table 2:** Variability in salinity stress tolerance of potato (Solanum tuberosum L.) varieties using in vitro screening.

| Cultivars | NaCl Doses | RL | NR | FRW | DRW | FPW | DPW |
|-----------|------------|----|----|-----|-----|-----|-----|
|           | mM         | cm | Plant^{-1} g | g | g | g |
| Tokat 3/161 | 0          | 19.51^{AB} | 8.20^{AH} | 0.31^{AF} | 0.01^{BE} | 0.19^{EH} | 0.02^{AC} |
|           | 50         | 6.38^{F,K} | 5.67^{CI} | 0.04^{FG} | 0.000^{E} | 0.13^{EH} | 0.008^{BC} |
|           | 100        | 5.49^{G,K} | 2.67^{EI} | 0.01^{G} | 0.000^{E} | 0.10^{FH} | 0.005^{BC} |
|           | 150        | 0.94^{K}   | 0.73^{IJ} | 0.00^{G} | 0.000^{E} | 0.09^{GH} | 0.005^{BC} |
| Hermes    | 0          | 15.18^{AG} | 8.20^{AH} | 0.06^{FG} | 0.0001^{E} | 0.20^{EH} | 0.01^{AC} |
|           | 50         | 7.53^{D,K} | 7.40^{AI} | 0.04^{FG} | 0.000^{E} | 0.21^{EH} | 0.01^{AC} |
|           | 100        | 7.72^{C,K} | 3.40^{EJ} | 0.01^{G} | 0.000^{E} | 0.12^{EH} | 0.005^{BC} |
|           | 150        | 7.26^{D,K} | 3.67^{EJ} | 0.01^{G} | 0.000^{E} | 0.14^{E,H} | 0.006^{BC} |
| Tokat 10/1 | 0          | 20.83^{A}  | 8.67^{AG} | 0.13^{D,G} | 0.007^{C,E} | 0.22^{E,H} | 0.01^{AC} |
|           | 50         | 10.64^{B,K} | 6.53^{EJ} | 0.10^{D,G} | 0.004^{DE} | 0.24^{D,H} | 0.01^{AC} |
|           | 100        | 11.82^{A,I} | 6.13^{EJ} | 0.04^{F,G} | 0.001^{E} | 0.16^{E,H} | 0.01^{BC} |
|           | 150        | 6.80^{E,K} | 5.60^{EJ} | 0.01^{G} | 0.000^{E} | 0.14^{E,H} | 0.006^{BC} |
| Tokat BCB | 0          | 19.69^{AB} | 14.07^{A} | 0.35^{A,E} | 0.024^{A-D} | 0.25^{D,H} | 0.02^{AC} |
|           | 50         | 9.25^{K}   | 4.87^{C,J} | 0.07^{F,G} | 0.003^{E} | 0.20^{E,H} | 0.01^{AC} |
|           | 100        | 4.13^{K}   | 0.87^{IJ} | 0.02^{G} | 0.000^{E} | 0.07^{G,H} | 0.005^{BC} |
|           | 150        | 0.99^{K}   | 0.27^{I}  | 0.00^{G} | 0.000^{E} | 0.08^{G,H} | 0.004^{BC} |
| Lady Claire | 0         | 15.61^{AF} | 5.27^{CI} | 0.08^{E,G} | 0.002^{E} | 0.12^{E,H} | 0.009^{BC} |
|           | 50         | 7.62^{C,K} | 4.13^{D,I} | 0.02^{G} | 0.000^{E} | 0.37^{B,F} | 0.07^{A} |
|           | 100        | 3.32^{K}   | 2.66^{IJ} | 0.09^{E,G} | 0.000^{E} | 0.14^{E,H} | 0.05^{AE} |
|           | 150        | 1.79^{K}   | 1.07^{IJ} | 0.00^{G} | 0.000^{E} | 0.04^{H} | 0.00^{C} |
| Burren    | 0          | 16.93^{A,D} | 7.60^{AJ} | 0.16^{D,G} | 0.009^{B,E} | 0.15^{E,H} | 0.01^{AC} |
|           | 50         | 15.47^{AG} | 5.00^{CI} | 0.13^{D,G} | 0.007^{C,E} | 0.18^{E,H} | 0.01^{BC} |
|           | 100        | 8.35^{K}   | 2.93^{IJ} | 0.05^{F,G} | 0.003^{E} | 0.18^{E,H} | 0.01^{BC} |
|           | 150        | 5.83^{E,K} | 1.73^{HI} | 0.02^{G} | 0.000^{E} | 0.14^{E,H} | 0.01^{BC} |
| Tokat 6/24 | 0          | 20.78^{A}  | 9.60^{AE} | 0.54^{AB} | 0.029^{AB} | 0.25^{D,H} | 0.02^{AC} |
|           | 50         | 17.10^{A,D} | 5.13^{CI} | 0.27^{B,G} | 0.017^{BE} | 0.27^{D,H} | 0.02^{AC} |
|           | 100        | 14.44^{A,G} | 3.20^{FJ} | 0.13^{D,G} | 0.006^{C,E} | 0.18^{E,H} | 0.02^{AC} |
|           | 150        | 1.52^{K}   | 0.33^{I}  | 0.00^{G} | 0.000^{E} | 0.09^{GH} | 0.005^{BC} |
| Slaney    | 0          | 10.17^{B,K} | 10.80^{A-D} | 0.44^{A-C} | 0.019^{B,E} | 0.58^{AB} | 0.03^{AC} |
|           | 50         | 15.10^{A,G} | 11.73^{AC} | 0.54^{A} | 0.026^{A,C} | 0.78^{A} | 0.06^{AB} |
|           | 100        | 4.18^{HK}  | 1.73^{HI} | 0.02^{G} | 0.000^{E} | 0.29^{C,H} | 0.02^{AC} |
|           | 150        | 1.79^{K}   | 1.40^{HI} | 0.01^{G} | 0.000^{E} | 0.14^{E,H} | 0.01^{AC} |

Values not followed by the same letter are significantly different at $P < 0.01$ (Tukey's test) within the same parameter.
**Figure 1:** Plantlet growth and development of Innovator variety at different NaCl concentrations after 4 weeks culture (A) Culture initiation from stem cuttings consisting of a single node of the variety (B) Plantlet growth/development of the control plants (C) Plantlet growth/development at 50 mM NaCl (D) Plantlet growth/development at 100 mM NaCl (E) Plantlet growth/development at 150 mM NaCl (F) Root development at different concentrations of NaCl (I) Control, (II) 50 mM NaCl, (III) 100 mM NaCl (IV) 150 mM NaCl.

**Figure 2:** Plantlet growth and development of Granola variety at different NaCl concentrations after 4 weeks culture (A) Culture initiation from stem cuttings consisting of a single node of the variety (B) Plantlet growth/development of the control plants (C) Plantlet growth/development at 50 mM NaCl (D) Plantlet growth/development at 100 mM NaCl (E) Plantlet growth/development at 150 mM NaCl (F) Root development at different concentrations of NaCl (I) Control, (II) 50 mM NaCl, (III) 100 mM NaCl (IV) 150 mM NaCl.
A Comparison of twelve varieties indicated that the fresh masses of the microtubers of Slaney were significantly higher than of all varieties at 50-150 mM NaCl concentrations (Figure 3A). Tokat 10/1, Tokat BCB, Lady Claire, Burren, Tokat 6/24, and Marabel just formed microtuber at 50 mM NaCl. However, Tokat 3/161 could not produce any microtuber including control plantlets (Figure 3B). Microtuber production drastically decreased as increasing salt intensity for all varieties as reported by Amerian and Esna-Ashari, 2011. The effect of NaCl on microtuberization frequency, the number of microtuber, and the mass of the obtained microtubers were genotype-dependent. Microtuberization of Slaney, Kennebec, Innovator, and Hermes was better and tolerant than other varieties. Slaney performed the best microtuberization capacity when compared to the other varieties in control and all salt concentrations. Salinity slightly influenced dry matter of the potato varieties and the data were not given. Salt decreased microtuberization by reduction of the water and CO₂ assimilation, osmotic imbalance, nutritional defects in plants as emphasized by (Chinnusamy; Zhu, 2003). Osmotic stress also reduces the yield power of water and salinity has suppressive effects on growth, development, and microtuberization of potato under in vitro and ex vitro conditions. High salt concentrations prevent intake of nutrients and water and most likely negatively affect microtuberization as indicated in the study.

The effects of NaCl concentrations on microtuber production of the twelve potato varieties were also examined. Potato varieties were negatively affected by NaCl concentrations at very different levels in terms of microtuberization capacity. The varieties gave very different microtuberization responses at different NaCl concentrations. Figure 4 also shows the presence of significant differences between the 12 potato varieties in the average number of microtubers. The initiation of microtuberization in all potato varieties was delayed up to 1-2 weeks at higher salt concentrations (at 100-150 mM NaCl). The frequency of microtuberization is the highest for control plantlets of varieties forming microtuber. MS medium containing various NaCl concentrations caused to weak and greenish microtuber for all varieties except Tokat 3/161. Slaney formed a microtuber at all NaCl concentrations and gave the highest number of microtubers per explant on MS medium. The highest NaCl concentrations (150 mM NaCl) inhibited microtuber formation and stolon growth in potato varieties and microtuberization capacity of the varieties decreased drastically at 100 mM and higher concentrations of NaCl except for Slaney. The variety also showed microtuberization performance at 150 mM NaCl. The lowest number of microtubers was obtained at 100 mM NaCl. However, the smallest sizes of microtubers were seen at 150 mM NaCl for Slaney. Microtuberization was achieved in Innovator, Kennebec, and Hermes at 100 mM NaCl. These varieties followed Slaney. However, the mean microtuber weights of these varieties were drastically reduced at 100-150 mM NaCl.

Principal components analysis (PCA) was carried out on the correlation matrix using the PCA subroutine of the IBM SPSS statistic 20 packages. The PCA was applied to the data matrix (15 morphological characteristics x 12 potato varieties) of the potato varieties. The data matrix of total morphological characteristics was rotated using the varimax rotation method. We performed principal component analysis (PCA) to identify the principal component that association with salt tolerance. The results have shown that two eigenvalues >2 explained about 72.4% of the total variance. The first principal component (PC1) accounted for 40.4%, the second (PC2) for 32.0%, and their loading (Figure 5A) and the score (Figure 5B) plots showed in Figure 5. The negative part of PC1 is related to the number of branches, the number of nodes and the number of leaves and the rest of the variables are related to the positive part of PC1. On the other hand, the negative part of PC2 is mainly related to fresh root weight, dry tuber weight per explant, number of tubers per explant and tuber weight per explant. The score plot of PC1 and PC2 shows some clustering of potato varieties. On the positive parts of PC2; Marabel, Tokat 10/1, Hermes, Innovator, and Kennebec varieties are grouped. Among these varieties, as it is shown on the positive parts of both PCs, Innovator and Kennebec varieties are closer than others. The negative part of both PCs; Tokat BCB, Tokat 3/161, Burren, Granola, and Lady Claire are close to each other. On the positive part of PC1 and negative part of PC2; Tokat 6/24 and Slaney are grouped.

A hierarchical cluster analysis (HCA) was also used to identify the possible nearest and similarity of all morphological characteristics analyzed of the potato varieties. The results of HCA, based on Ward’s method and Pearson correlation, are presented as a dendrogram in (Figure 6) according to the dendrogram, four major clusters were observed. These clusters contain:
Cluster 1: Granola, Tokat 3/161, Tokat BCB, Burren, Lady Claire, Tokat 6/24
Cluster 2: Slaney
Cluster 3: Innovator, Kennebec
Cluster 4: Marabel, Tokat 10/1, Hermes
Figure 3: Microtuberization of Slaney variety (A) at different NaCl concentrations culture (I) Control explants (II) 50 mM NaCl (III) 100 mM NaCl (IV) 150 mM NaCl, Microtuberization of Tokat 3/161 variety (B) at different NaCl concentrations (I) Control explants (II) 50 mM NaCl (III) 100 mM NaCl (IV) 150 mM NaCl.

Figure 4: Effect of salt (NaCl) levels on microtuberization number per explant (a and b) and weight per explant (c and d) in the potato varieties.
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Figure 5: Principal component analysis (PCA) for potato varieties based on the morphological characteristics (15 morphological characteristics x 12 potato varieties): a) the loading plot and b) Score plot.

In grouping potato varieties, HCA and PCA results were found to be similar. Regarding the groups and clustering seen in PCA and HCA, we can speculate about the responses of morphological similarities of the potato varieties used in this study against salt stress. Among the potato varieties, Granola clustered distinctly from the other varieties, showed different responses to salt stress. Innovator and Kennebec, clustered together showed high tolerance to high NaCl concentrations in terms of investigated parameters (Figure 5 and 6). According to the PCA and HCA results, we can speculate that Innovator and Kennebec are the most tolerant varieties, Marabel, Tokat 10/1 and Hermes are the moderate tolerant varieties.
CONCLUSIONS

In vitro microtuberization is a beneficial method for physiological studies and selection of potato germplasm. Salt stress affects morphological and physiological responses in plants including photosynthesis pathways. In vitro screenings of potato varieties is the most appropriate and short-term practice that can be carried out in the laboratory throughout the year. The results indicated that salt stress levels remarkably influenced all potato varieties compared to the control plant and there were noticeable differences among the potato varieties under salt stress conditions. Innovator and Kennebec are respectively the most salt-tolerant varieties in terms of microtuberization capacity and morphological characteristics. Moreover, Hermes was found to be moderately salt tolerant variety and microtuberization capacity of Slaney is remarkable under salt stress conditions. Our findings may contribute further research on the understanding of stress tolerance mechanisms at the cellular and molecular level and may also contribute potato-breeding programs for developing potato varieties with improved salt stress tolerance. Further study of the role of different genes and transcription factors in responding to salt stresses may also contribute to improving new varieties, salt- tolerant new varieties and deeper of understanding of its stress-tolerance signal-regulating mechanisms.

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