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Genetic Diversity Among some Potato Varieties For *In Vitro* Microtuberisation under Salinity Stress

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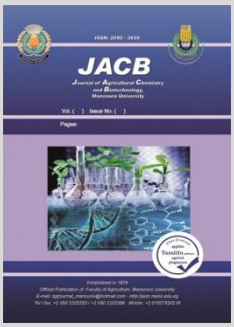

ABSTRACT

This study was carried out to investigate the tolerance to salinity stress, using three cultivated varieties. These cultivars were Diamant, Spunta and Cara. Single stem nodes were used as explant on MS medium supplemented by 0.0, 25, 50, 75 and 100 mM/L NaCl in a replicated experiment to assessment the salinity stress tolerance with respect to *in vitro* traits, which involved some morphological, physiochemical and microtuberisation traits. The results revealed that tests on the mean squares of genotypes were highly significant for all the studied morphological traits except for number of roots/plantlet, while it was significant for all studied physiochemical and microtuberisation traits, indicating the presence of real differences among the three cultivars under study. Both morphological and microtuberisation traits were highly influenced by levels of salinity, whereas highly significant difference was observed for these traits with no exception. The highest average of number of microtubers/plantlet was recorded for control (0.00 NaCl) with mean values 1.5, 2.0 and 1.80 for Spunta, Diamont and Cara cultivars, respectively and these values decreased with the increase NaCl concentrations. The heritability magnitude values were more than 50% for both number and weight of microtubers/plantlet, indicating that these traits are highly controlled by genotypes. From the results it could be concluded that microtubers induction ability is mainly controlled by genotypes as well as the interaction of genotypes by salinity stress levels. Also, the results recommended that *in vitro* selection for salinity stress is available for a suitable levels of sodium chloride ranged from25 to 100mM/L.

Keywords: *Solanum tuberosum*, microtuberisation, salinity stress, genotypic variance and heritability.

INTRODUCTION

Cultivated potato (*Solanum tuberosum L.*) is considered one of the vital crops in the world besides wheat, rice, and maize in global human nutrition. It's a food for about two-thirds of the world population, plays an appreciated role in a regional food shortage. Furthermore, potatoes have different industrial applications, such as processed into animal feed as well as alcohol and biofuels (Zhang *et al*, 2017). Potato tubers have high amount of starch, protein, fiber and vitamins (B1, B6, B9, C, and E) in addition to different minerals, antioxidants and various bioactive compounds such as carotenoids and phenolics (Devaux *et al*, 2021). Thus, it is considered one of the vital crops in terms of food security. The potato production globally in 2022 was approximately 375 million tonns. Whereas the highest production was recorded in China 95,5 million tons and India 56 million tons. While, in Egypt the potato production was 6,1 million tons (FAO,2023). Abiotic stress factors such as water deficit, salinity and temperature extremes cause about 30 billion dollars in losses to world agriculture (FAO, 2022).

The genetic improvement programs of Potato (*Solanum tuberosum* L .) seek ways to reduce a time and the impacts caused by biotic and abiotic stresses on the crops through improvement and/or production resistant or tolerant cultivars. Plant tissue culture techniques are used for production, conservation and improvement of most plant resources through an asexual process where clonal propagation is expected to produce a genetically uniform genotype. On the other hand, due to the high cost of importing seed tubers, as well as growing demand potato cultivation and expansion, the production of potato seed tuber in Egypt must be of higher priority. Somaclones were successfully used as a source of new potato clones with improved tolerance against drought stress (Albisk *et al*, 2012), salinity (Zeid *et al*, 2022) as one of biotechnological breeding tool for shorting period required to improve and/or produce effective potato lines with abiotic stress tolerance.

Salinity is one of the major factors which significantly influence physiological abnormalities in potato and subsequently negatively affect the growth and yield. The level of potato tolerance to salt stress can vary with different cultivars. Tissue culture techniques are very fast and efficient modern technique for screening potato genotypes to salt tolerance. Therefore, *in vitro* selection through screening could be alternative to traditional breeding methods for selecting genetic material according to its response to salinity stress. Thus, plant tissue culture techniques in assistance with traditional breeding methods have become recent approaches for achieving plants tolerant to abiotic stresses. Thus, the objectives of this study are to compare the ability of different potato cultivars for *in vitro* microtuberisation under salinity stress and to investigate the influence of genotypes and salinity stress levels on some *in vitro* traits related to microtuberisation process.

MATERIALS AND METHODS

In vitro micro-propagation studies were carried out during the period from 2022 to 2024 at the Tissue Culture Research Laboratory, Department of Genetics, Faculty of

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Agriculture, Mansoura University to investigate salinity tolerance stress, using three cultivated potato varieties.

Plant materials:

Three cultivated potato varieties belong to *Solanum tuberosum* L. were used as a source of the explants. These cultivars were Diamant, Spunta and Cara, which were provided by the Central Administration of Seed Certification, Ministry of Agriculture and Land Reclamation, Dokii, Egypt. Tubers from each cultivar were kept in the 4°C refrigerator for 7 days to break the dormancy and induction of sprouts. After 7 days cold treatment, tubers were kept in the dark for two weeks at 25 ± 2°C for the development of sprouts. Then, sprouts (2.0 cm) long and had about 5-7 nodes used as primary explants for the establishment of *in vitro* cultures. Sprouts of each variety were separated from the tuber and thoroughly washed under running tap water with detergent for 30 min. Under sterile conditions, sprouts were disinfected with immersing in 70% ethanol for 30 sec. followed by 5% sodium hypochlorite solution containing three drops of Tween20 as emulsifier per 100ml and stirring continuously for 20 minutes. Subsequently, the explants were rinsed three times by sterile di-distilled water to remove any traces of the hypochlorite solution. Under sterile conditions, the stereomicroscope was used to isolate meristem tips by removing the sprouts apical tips. The meristem tips were excised with two primordia leaves (0.25 mm length) and inoculated into test tubes (25 mm x 150 mm) contained 15 ml of the culture medium.

Culture media:

The culture medium was selected from the review of potato regeneration and composed of the basal salts and vitamins (Murashige and Skoog, 1962), which was supplemented with 30 g/l sucrose. This medium was used for culture initiation as well as the multiplication of small plantlets through single node stem culture. The prepared medium was adjusted to a pH of 5.7 before being autoclaved at 121°C for 20 minutes at 15 psi pressure. The microtuberisation medium used in this study was the same as initiation medium (Murashige & Shook, 1962) but supplemented by 80 g/l sucrose for direct microtuber development.

Salt tolerance assessment:

To obtain enough number of shoots, four times of subculture were made. Application of salt stress treatment is performed eight weeks later to increase uniformity, Murashige and Skoog (1962) basal medium supplemented with different concentrations of 0.0, 25, 50, 75 and 100 mM/L NaCl were used. Prepared medium was poured into jars (200 ml) . The experimental design used in this study was randomized complete block design (RCBD) with three replications. Each 4 jars with four single node stems represent one replication. The single node stems used to salinity tolerance study were applied according to procedure outlined by (Sharabash, 2001). All jars were incubated in the growth room at 27 ±1°C under 16 hours light of daily exposure to low light intensity (3000 lux) illumination. Application of treatment last up to 4 weeks. Then the vegetative parameters were recorded for all jars. Subsequently, single node explants from each plantlet in each treatment were transplanted to microtuberisation induction medium supplemented by the same NaCl concentrations. Also, the experimental design was RCBD, but with four replications, which each replicate was represented by four jars. Cultures were incubated at 20 ±2°C under darkness. After 7-8 weeks of darkness, microtubers were harvested and the number and weight of microtubers for each plantlet were recorded with respect to each NaCl concentration within each cultivar.

The data were recorded on the following morphological traits: Plantlet length (cm) (P.L), Shoot length (cm) (Sh.L), Root length (cm) (R.L), Number of roots/ plantlet (N.R/P) and Number of leaves/ plantlet (N.L/P) as well as relative water content (RWC%), which was recorded according to Barrs and Weatherley (1962). Also, the data were recorded on some physiochemical traits. These traits were proline content (mg/g), sodium content (ppm) (Na^+) and potassium content (ppm) (K^+). These traits were determined at Soil Department, Faculty of Agic., Mansoura Univ. according to Bates *et al* (1973) and Chapman and Pratt (1978) in addition to Chlorophyll A (C^a) (mg/g) and Chlorophyll B (C^b) (mg/g) were estimated for three samples of each treatment at Horticulture research Institute, ARC, Giza, Egypt, according to Lichtenthaler and Welburn, 1983.

Statistical analysis: In this study, different forms of analysis of variances were used to test significance difference between the NaCl concentration for each variety. In addition, the data combined over three cultivars and different concentrations were subjected to factorial analysis according to Singh and Choudhary (1985). To achieve the important goal of this study, several comparisons were made and the difference between any two means was tested for significance using Duncan's multiple range tests at 5% level of probability (Waller and Duncan, 1969). Estimates of genotypic, genotypic by environmental, environmental, phenotypic variances and heritability were determined according to the procedures outlined by Cockerham (1963) for all the studied traits.

RESULTS AND DISCUSSION

This research work was undertaken to investigate the microtuberisation ability through plantlets derived from *in vitro* culture of three potato cultivars (Spunta, Diamont and Cara) under salinity stress conditions. The resulted plantlets were *in vitro* evaluated for many traits to assess the ability of cultivars to produce salinity stress tolerant somaclones. The data obtained from the five levels of NaCl concentrations (0.0, 25, 50, 75 and 100 mM/L) of the three studied cultivars were subjected to combined analysis of variances and the mean squares of different sources of variances for morphological traits are shown in Table (1). The mean squares of genotypes were highly significant for the studied morphological traits except for number of roots/plantlet (N.R/P), indicating the presence of real differences among the three cultivars under study. Therefore, the planned comparisons between these cultivars in addition to further partition of total variance to its components are valid. On the other hand, treatments (salinity stress levels) and their interaction with genotypes were highly significant for root length (R.L), number of leaves/ plantlet (N.L/P) and ratio of water content (RWC%), indicating that these genotypes are highly influenced by the levels of sodium chloride concentration with respect to these traits. The results revealed that despite the treatments means squares of root length (R.L), shoot length (Sh.L) and number of roots/plantlet (N.R/p) are highly significant, their interaction with genotypes is not significant, revealed that the same behavior of the studied cultivars with different concentrations of sodium chloride with respect to these traits. However, the

physiochemical traits from the data over five levels of NaCl concentrations (0.0, 25, 50, 75 and 100 mM/L) were subjected to combined analysis of variances and the mean squares of different sources of variances are demonstrated in Table (2).

The obtained results revealed that replications mean squares were significant for chlorophyl content either a (C^a) or b (C^b), indicating that these traits are influenced by source of explant with respect to the different plantlets derived from each cultivar. Whereas the genotypes, levels and genotypes by levels interactions mean squares were significant for all studied physiochemical traits. This finding indicates that these genotypes are significantly influenced by the levels of sodium chloride concentration with respect to all traits (proline, sodium (Na^+), potassium (K^+), chlorophyl A (C^a) and chlorophyl B (C^b) contents). In addition, the comparison between means of cultivars as well as among means of studied salinity stress levels are valid and could be made. Analysis of variance for average number of microtubers per plantlet and average weight of microtubers per plantlet were made from the data combined over the five levels salinity stress and the resulted mean squares for each source of variance are demonstrated in Table (3). The results revealed that the genotypes, levels and genotypes by levels interactions mean squares were highly significant for microtuberisation traits. This finding indicating that these genotypes are significantly influenced by the levels of sodium chloride concentration with respect to average number of microtubers/plantlet and average weight of microtubers/plantlet. Therefore, the comparison between means of cultivars as well as among means of studied salinity stress levels are valid and could be made. In general, most of the previous results of analysis of variance related to some morphological, physiochemical and microtuberisation traits were in accordance with the results published by Mohamed (2021). In this respect, Anwar *et al.* (2010) indicated that the phenotypic analysis under salinity stress condition showed that regenerated and control plants under an established in vitro salinity screen system where media were supplemented with 0, 75, 150 and 200 mM of NaCl, high variance led to genotypes with different levels of sodium chloride. In addition, Mohamed *et al.* (2017), revealed that microtuberisation traits were highly influenced by genotypes.

Table 1. Combined analysis of variance and mean squares for the morphological traits over salinity stress levels

S.O.V	d.f	P.L (cm)	Sh. L cm)	R.L (cm)	N. R/P	N. L/P	RWC %
Replication	2	6.3	1.76	5.74	3.80	2.6	3.33
Genotype (G)	2	84.9**	54.27**	155.09**	1.87	48.5**	100.06**
Treatments (L)	4	566.7**	64.21**	261.97**	75.20**	382.0**	1093.44**
G x L	8	12.6	3.10	15.59**	0.28	19.4**	36.93**
Error	28	6.32	2.446	3.79	2.16	1.84	5.60

Where,*& ** are significant at 0.05 and 0.01 levels of probability, respectively.

Table 2. Combined analysis of variance and mean squares for physiochemical traits over salinity stress levels

S.O.V	d.f	Proline	Na^+	K^+	C^a	C^b
Replication	2	0.0005	0.010	0.010	0.68*	1.23**
Genotypes (G)	2	0.20**	0.79**	1.001**	6.59**	5.11**
Treatments (L)	4	0.16**	11.55**	2.01**	81.51**	39.35**
G x L	8	0.04**	0.07**	0.04**	1.83**	1.69**
Error	28	0.001	0.005	0.007	0.14	0.17

Where, * & ** are significant at 0.05 & 0.01 levels of probability, respectively.

Table 3. Combined analysis of variance and mean squares for microtuberization traits over salinity stress levels

S.O.V	d.f	Average number of microtubers/ plantlet	Average weight of microtubers /plantlet (mg)
Replication	3	0.02	2083.43
Genotypes (G)	2	0.71**	133315.38**
Treatments (L)	4	2.75**	34396.95**
G x L	8	0.09**	10213.96**
Error	42	0.02	970.85

Where, ** is significant at 0.01 level of probability.

Effect of Salinity Stress:

Regarding Spunta cultivar, the obtained results which presented in Table (4) revealed that plant length, shoot length and root length were not influenced by the low level of salinity (25 mM NaC1) and generally there were almost like control treatment. It could be observed that the highest average plant length, shoot length and root length were obtained at control treatment 26.0 cm, 12.0cm and 16.8cm, respectively and these values are not significand differed than the recorded values at salinity level of 25mM NaCl, while the treatment of 100mM achieved the lowest plant length , shoot length and root length with average means of 9.3cm, 5.6cm and 3.6cm, respectively. On contrast, the decrease in number of roots, number of leaves and relative water content were gradual parallel to the increase in the salinity level from zero to 100 mM NaCl. The control treatment achieved the highest number of root per plantlet (13.0), number of leaves per plant (24.6) and relative water content (81.9%), while the salinity level of 100 mM NaCl achieved the lowest number of roots per plantlet (5.0), number of leaves per plant (6.0) and relative water content with mean value of 53.7% (See Fig. 1).

The results presented in Table (5) showed that there was a significant difference between salinity level from zero to 100 mM NaCl with respect to the plantlets derived from *in vitro* culture of Diamont cultivar. There are no significant differences were detected for plantlet length, root length, average number of roots per plantlet and average number of leaves per plantlet between the control treatment and the low level of salinity (25 mM NaC1), but were significantly higher than other three levels of salinity stress. The highest values were obtained from Diamant cv. of plantlet length (29.6 cm), root length (21.3 cm) and average number of leaves per plantlet (21.3) at 25 mM NaCl. While the control treatment achieved the highest number of roots per plantlet (12.3). On the other hand, the salinity level of 100 mM NaCl recorded the lowest of plantlet length (10.0 cm), root length (5.8 cm), average number of roots per plantlet (5.0), average number of leaves per plantlet (10.0). However, the salinity level of 25

mM NaCl gave the better response after control regarding shoot length and relative water content (RWC) with means of 11.6cm and 66.5%, respectively. Control treatment gave the highest shoot length (15.5cm) and relative water content (83.2%), while the lowest values was obtained from Diamant cv. of shoot length (6.6 cm) and relative water content (61.4%) which were significantly lower than other studied salinity levels (See Fig. 2).

Table 4. Effect of salinity stress on Spunta cultivar for *in vitro* morphological traits

Treatments	P.L (cm)	Sh.L (cm)	R.L (cm)	N.R/P	N.L/P	% RWC
Control	26.0±4.0^{a}	12.0±2.0^{a}	16.8±3.8^{a}	13.0±2.6^{a}	24.6±2.5^{a}	81.9±2.4^{a}
25mM	25.3±1.5^{a}	11.0±1.0^{a}	14.3±1.1^{a}	10.3±1.5^{b}	18.0±2.0^{b}	71.3±7.2^{b}
50mM	19.6±2.5^{b}	10.6±3.5^{ab}	7.6±0.57^{b}	9.3±0.5^{bc}	10.3±1.5^{c}	60.2±0.6^{c}
75mM	13.0±1.7^{c}	7.3±0.5^{b}	5.6±1.1^{bc}	7.6±0.57^{c}	8.0±1.0^{c}	56.6±0.4^{c}
100mM	9.3±1.5^{dc}	5.6±1.1^{c}	3.6±0.57^{c}	5.0±1.0^{d}	6.0±1.0^{cd}	53.7±1.9^{c}

Where: Values are means ± SE (standard error).; Values with the same superscript letters are not significantly different from each other whilst, values with different superscript letters are significantly different at 0.05 level of probability.

Fig. 1. Spunta cv.'s plantlets after 30 days on salinity stress treatment. Where, S0, S1, S2, S3 and S4 are 0.0 , 25, 50, 75 and 100mM/L NaCl concentrations, respectively.

Table 5. Effect of salinity stress on Diamont cultivar for *in vitro* morphological traits.

Treatments	P.L (cm)	Sh.L (cm)	R.L (cm)	N.R/P	N.L/P	% RWC
Control	27.3±1.5^{a}	15.5±2.1^{a}	18.3±1.5^{ab}	12.3±1.5^{a}	20.6±1.1^{a}	83.2±0.9^{a}
25mM	29.6±2.5^{a}	11.6±1.5^{b}	21.3±3.2^{a}	11.0±2.0^{ab}	21.3±1.5^{a}	66.5±1.2^{b}
50mM	23.6±3.2^{b}	11.0±1.0^{b}	16.3±3.0^{b}	9.6±0.5^{bc}	16.0±1.7^{b}	58.1±1.6^{c}
75mM	12.3±3.7^{c}	8.0±1.0^{c}	7.0±1.0^{cd}	8.0±2.0^{c}	15.3±1.5^{b}	64.0±1.3^{bd}
100mM	10.0±1.0^{c}	6.6±0.5^{c}	5.8±1.7^{d}	5.0±1.0^{d}	10.0±1.0^{c}	61.4±1.5^{cd}

Where: Values are means ± SE (standard error).; Values with the same superscript letters are not significantly different from each other whilst, values with different superscript letters are significantly different at 0.05 level of probability.

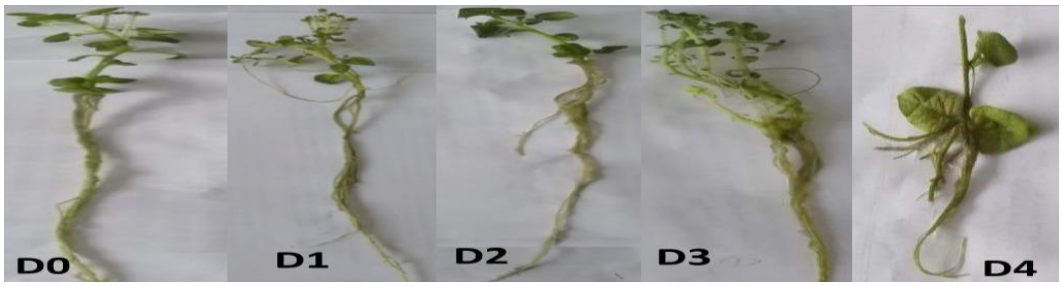

Fig. 2. Diamont cv.'s plantlets after 30 days on salinity stress treatment. Where, D0, D1, D2, D3 and D4 are 0.0 , 25, 50, 75 and 100mM/L NaCl concentrations, respectively.

In Cara cv., the results shown in Table (6) and Fig. (3) illustrated that no significant differences were detected between the low level of salinity (25 mM NaC1) and the control means for all morphological traits except for average number of leaves/plantlet and relative water content (RWC%), which were significantly higher towards control. The highest values were obtained from Cara cv. of plantlet length (25.3cm), shoot length (8.6cm), root length (12.1 cm) and number of roots per plantlet (12.0) with control treatment. While the level of salinity (100 mM NaCl) recorded the lowest of plantlet length (8.3 cm), shoot length (3.5cm), root length (4.0 cm) and number of roots per plant (4.3). On the other hand, there was significant decrease observed for average number of leaves per plantlet and relative water content (RWC) between salinity levels and control treatment. Control treatment gave maximum for average number of leaves per plantlet (25.0) and relative water content (79.9%), while the lowest value was obtained from Cara cv. for number of leaves per plantlet (7.0) and relative water content (48.4 %) with salinity level of 100mM NaCl compared to other treatments.

Table 6. Effect of salinity stress on Cara cultivar for *in vitro* morphological traits:

Treatments	P.L (cm)	Sh.L (cm)	R.L (cm)	N.R/P	N.L/P	% RWC
Control	25.3±1.5^{a}	8.6±0.6^{a}	12.1±1.0^{a}	12.0±1.7^{a}	25.0±1.0^{a}	79.9±1.4^{a}
25mM	23.3±4.5^{a}	8.3±1.5^{a}	11.6±3.0^{a}	9.6±2.5^{ab}	18.3±0.5^{b}	67.7±1.8^{b}
50mM	13.0±2.0^{b}	7.3±1.5^{a}	5.3±1.5^{b}	9.3±1.1^{ab}	10.0±1.0^{c}	58.2±0.5^{c}
75mM	9.3±1.1^{bc}	6.3±1.5^{a}	4.0±1.0^{b}	7.3±0.5^{bc}	8.3±0.5^{d}	53.4±1.5^{d}
100mM	8.3±1.5^{c}	3.5±0.5^{b}	4.0±1.0^{b}	4.3±0.5^{c}	7.0±1.0^{d}	48.4±1.0^{e}

Where: Values are means ± SE (standard error).; Values with the same superscript letters are not significantly different from each other whilst, values with different superscript letters are significantly different at 0.05 level of probability.

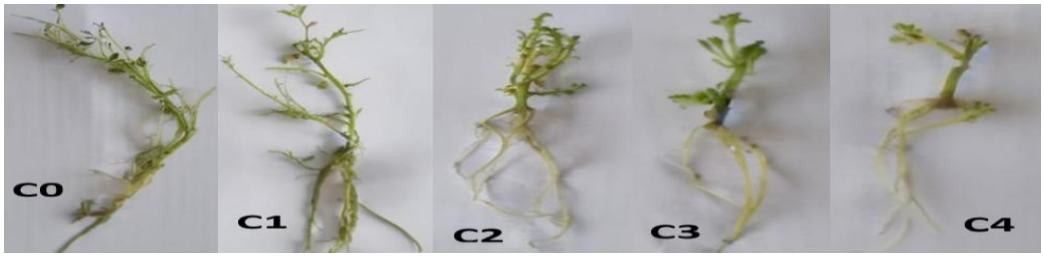

Fig. 3. Cara cv.'s plantlets after 30 days on salinity stress treatment. Where, C0, C1, C2, C3 and C4 are 0.0 , 25, 50, 75 and 100mM NaCl/L concentrations, respectively.

In the same trend, several investigations have been conducted on salt sensitivity potato genotype under pot and field conditions (Abdullah *et al.,* 2018) and *in vitro* condition (Ahmed *et al., 2020*), their studies revealed that the differences among the plantlet length, number of leaves, root length, number of roots, fresh plantlet weight, dry plantlet weight of the varieties were negatively affected by the studied NaCl levels tested.

The values of means and standard deviations (SD) for studied physiochemical traits with respect to four levels of salinity stress were calculated and the obtained results are presented in Tables (7, 8 and 9) for Spunta, Diamont and Cara cultivars, respectively. Regarding proline content, the highest value was recorded from Spunta cv. (Table 7) at the salinity level of 100 mM NaCl (1.28mg/g), while the lowest value was obtained at the control treatment with mean value of 1.15 mg/g. It could be noticed that the proline content in plantlet tissue is positively correlated with increased concentrations of salinity levels. In the same direction, it could be observed that the great increase of the sodium content (Na^+) that estimated by 3.44 ppm/g, attributed to the salinity level of 100 mM NaCl compared to 0.9 and 1.46 ppm/g for control and 25 mM NaCl, respectively. On the other hand, the control treatment gave the maximum potassium content (K^+) that estimated by 4.00 ppm/g against to 2.91 ppm/g at 75 mM NaCl level and 2.78 ppm/g at 100 mM NaCl level, which insignificant different. The higher NaCl senility levels significantly decreased chlorophyll a (C^a) and chlorophyll b (C^b) content in Spunta cv . Whereas the control treatment record the highest value of chlorophyll A (11.33mg/g), while the concentration of 25 mM NaCl has the highest value of chlorophyll B (5.89mg/g). On contrast, the NaCl concentration 100 mM NaCl gave the lowest value of chlorophyll A (5.04mg/g) and chlorophyll B (2.94mg/g). Results in (Table 8) showed that there was a significant difference in the interaction between salinity stress levels with respect to Diamant cv. The highest value of proline content was obtained at the salinity level of 100 mM NaCl with mean 1.59mg/g, while the lowest value was 0.93mg/g at the control treatment. Moreover, the increase in proline content was gradual parallel to the increase in the salinity level from zero to 100 mM NaCl. The effects of salt applications on sodium (Na^+), potassium (K^+) show significant difference among salinity treatments. Na Cl concentration 100 mM gave the maximum sodium content that estimated by 4.10 ppm/g, while the same treatment gave the lowest potassium content (K^+) that estimated by 3.36 ppm/g. Control treatment achieved the lowest sodium content (Na^+) of Diamant cv. that estimated by 1.13ppm/g. No significant differences were detected with the low concentration of salinity (25 mM NaC1) and generally it was almost similar to the control treatment on potassium content (K^+) that estimated by 4.30ppm/g and 4.42ppm/g, respectively. Salinity induces deficiency in chlorophyll A and chlorophyll B. Whereas, the control treatment gave the highest value of chlorophyll A (11.29mg/g), while there was no significant differences of chlorophyll B between the control treatment and the salinity stress level of 25 mM NaC1 that estimated by 7.09mg/g and 6.94mg/g, respectively. However, the salinity level of 100 mM NaCl gave the lowest value of chlorophyll A (3.36mg/g) and chlorophyll B (1.02mg/g). Regarding Cara cultivar, the obtained results of the effect of different salinity stress on physiochemical traits are presented in Table (9). The results cleared that there were significant differences among the studied salinity stress levels. Whereas, it could be observed that the great increase of proline content attributed to the treatment by 100 mM and 75 mM Na Cl levels, with means values of 1.25mg/g and 1.21mg/g, respectively while the lowest value was recorded at control treatment with mean value 0.99mg/g. Generally, sodium content (Na^+) of plantlets derived from Cara cv. increased with increasing salinity level as previously mentioned with Spunta and Diamont cultivars. The great increase of the sodium content (Na^+) that estimated by 3.80ppm/g, attributed to the treatment by 100 mM Na Cl concentration. In addition, the opposite direction was observed for potassium content (K^+) which gave the lowest potassium content of Cara cv. that estimated by 2.10 ppm/g, while control treatment achieved the lowest sodium content (1.08ppm/g) and the maximum potassium content (4.19ppm/g). Furthermore, the control treatment gave the highest value of chlorophyll A (10.21mg/g), and chlorophyll B (6.33mg/g). while the salinity level of 100 mM NaCl gave the lowest value of chlorophyll A (2.78mg/g) and chlorophyll B (0.99mg/g). In this respect, Allakhverdiev *et al.* (2000), Ahmed *et al.* (2020) & Farouk and Arafa (2018) concluded that the accumulation of Na^+ in cells is extremely toxic and can affect all plant mechanisms and enzymatic actions. Regarding Proline, Zhang *et al.,* 2021 demonstrated that proline is osmo-protectant, a protein preservative and cell differentiation control.

Table 7. Effect of salinity stress on Spunta cultivar for physiochemical trait

Treatments	Proline	Na^+	K^+	C^a	C^b
Control	1.15±0.03^{c}	0.90±0.07^{e}	4.00±0.07^{a}	11.33±0.11^{a}	5.86±٠.14^{a}
25mM	1.22±0.01^{b}	1.46±0.02^{d}	3.72±0.07^{b}	9.95±0.072^{b}	5.89±0.50^{a}
50mM	1.23±0.01^{b}	2.42± 0.09^{c}	3.43±0.02^{c}	8.88±0.11^{c}	4.59±0.27^{b}
75mM	1.26±0.02^{ba}	2.85±0.07^{b}	2.91±0.09^{d}	6.06±0.49^{d}	3.45±0.32^{c}
100mM	1.28±0.01^{a}	3.44±0.07^{a}	2.78±0.12^{d}	5.04±0.42^{e}	2.94±0.32^{c}

Where: Values are means ± SE (standard error).; Values with the same superscript letters are not significantly different from each other whilst, values with different superscript letters are significantly different at 0.05 level of probability.

Table 8. Effect of salinity stress on Diamont cultivar for physiochemical traits:

Treatments	Proline	Na^+	K^+	C^a	C^b
Control	0.93±0.10^e	1.13±0.09^e	4.42±0.04^a	11.29±0.76^a	7.09±0.55^a
25mM	1.31±0.02^b	1.84±0.07^d	4.30±0.07^a	9.83±0.83^b	6.94±0.85^a
50mM	1.47±0.03^c	2.63±0.07^c	3.85±0.10^b	6.84±0.55^c	4.28±0.51^b
75mM	1.49±0.01^b	3.46±0.05^b	3.48±0.12^c	3.7±0.40^d	2.50±0.45^c
100mM	1.59±0.02^a	4.10±0.05^a	3.36±0.10^c	3.36±0.32^d	1.02±0.002^d

Where: Values are means ± SE (standard error).; Values with the same superscript letters are not significantly different from each other whilst, values with different superscript letters are significantly different at 0.05 level of probability

Table 9. Effect of salinity stress on Cara cultivar for physiochemical traits:

Treatments	Proline	Na^+	K^+	C^a	C^b
Control	0.99±0.01^d	1.08±0.08^e	4.19±0.07^a	10.21±0.16^a	6.33±0.48^a
25mM	1.09±0.04^c	1.61±0.07^d	3.72±0.07^b	9.31±0.04^b	5.37±0.68^b
50mM	1.11±0.04^b	2.91±0.09^c	3.69±0.04^b	7.96±0.01^c	2.65±0.52^c
75mM	1.21±0.002^a	3.52±0.07^b	3.36±0.05^c	5.89±0.04^d	1.95±0.66^d
100mM	1.25±0.01^a	3.80±0.05^a	2.10±0.07^d	2.78±0.46^e	0.99±0.27^e

Where: Values are means ± SE (standard error).; Values with the same superscript letters are not significantly different from each other whilst, values with different superscript letters are significantly different at 0.05 level of probability

The values of means and their standard deviations (SD) for average number of microtubers per plantlet and average weight of microtubers per plantlet traits with respect to four levels of salinity stress were calculated and the obtained results are presented in Tables (10, 11 and 12) for Spunta, Diamont and Cara cultivars, respectively. The results presented in Table (10) as well as Fig. (4) revealed that the best mean value for average number of microtubers/ plantlet in Spunta cv. was observed at the control (1.5), which followed by 1.19 and 1.13 mean values for 25 and 50 mM NaCl levels, respectively with significant difference. The inferior number of microtubers/ plantlet mean values was recorded at the high levels of salinity with average mean 0.50 and 0.88 for 100 and 75 mM NaCl concentrations, respectively. The best mean value for average weight of microtubers/plantlet was recorded at 75mM NaCl (183.29mg), which was insignificantly different than the mean values of the control, 25 and 50mM with mean values 0f 153.04 mg, 173.05 mg and 178.0 mg, respectively. The lowest average of weight of microtubers/plantlet was observed at 100 mM level (116.63).

The results of microtuberisation traits for Diamont cv. with respect to the five levels of salinity are demonstrated in Table (11). Regarding average number of microtubers/plantlet, the highest mean value was recorded for control level (2.0) which significantly higher than other salinity levels with mean values of 1.56, 1.63, 1.13 and 0.75 for 25, 50, 75 and 100 mM NaCl concentrations, respectively. However, Diamont cv. recorded the highest average weight of microtubers/plantlet at level of 75mM NaCl (158.38mg) and significantly higher than all other studied levels with mean values of 116.16, 130.53, 121.10 and 84.83 mg for 0.00, 25, 50 and 100 mM Nacl, respectively (See Fig.5). Regarding Cara cv., the average means of microtuberisation traits was determined for each of salinity level and the obtained results are shown in Table (12) and Fig. (6). The results revealed that the highest average of number of microtubers/plantlet was observed in control with mean values 1.88, which insignificantly differed than the level of 25 mM NaCL (1.69). High value of average weight of microtubers/plantlet was detected at 75 mM NaCl level with mean values of 448.33mg, which greatly high significant than other studied levels with mean values of 251.87, 268.39, 286.45 and 139.45 mg for 0.0, 25, 50 and 100 mM NaCl levels, respectively. From the previous results of the microtuber induction rate of the studied cultivars, the same conclusion can be drawn, which indicates that increasing salinity levels is responsible for a decrease in the number of microtubers as well as increase in the microtubers weight. From these results, it can be recommended that the highest level of sodium chloride that can be tolerated is 75 mmol/L to be suitable for laboratory selection of salinity tolerance in potato varieties. In this respect, Ahmed *et al.* (2020) concluded that microtuberisation of the varieties were completely inhibited in the cases of high levels of sodium chloride (100-150 mM).

Table 10. Effect of salinity stress on Spunta cultivar for microtuberization traits

Treatments	Average number of microtubers/ plantlet	Average weight of microtubers/plantlet (mg)
Control	1.50±0.01^a	153.04±15.48^a
25mM	1.19±0.13^b	173.05±21.85^a
50mM	1.13±0.25^b	178.00±7.97^a
75mM	0.88±0.14^c	183.29±26.15^a
100mM	0.50±0.01^d	116.63±27.79^b

Where: Values are means ± SE (standard error).; Values with the same superscript letters are not significantly different from each other whilst, values with different superscript letters are significantly different at 0.05 level of probability.

Table 11. Effect of salinity stress on Diamont cultivar for *in vitro* microtuberization traits

Treatments	Average number of microtubers/ plantlet	Average weight of microtubers/plantlet (gm)
Control	2.00±0.20^a	116.16±2.88^a
25mM	1.56±0.13^b	130.53±9.23^a
50mM	1.63±0.14^b	121.10±10.22^a
75mM	1.13±0.14^c	158.38±17.30^b
100mM	0.75±0.01^d	84.83±3.29^c

Where: Values are means ± SE (standard error).; Values with the same superscript letters are not significantly different from each other whilst, values with different superscript letters are significantly different at 0.05 level of probability.

Table 12. Effect of salinity stress on Cara cultivar for *in vitro* microtuberization traits

Treatments	Average number of microtuber/ plantlet	Average weight of microtuber/plantlet (mg)
Control	1.88±0.14^a	251.87±15.20^a
25mM	1.69±0.13^{ab}	268.39±25.13^a
50mM	1.50±0.00^b	286.45±9.39^a
75mM	0.69±0.13^c	448.33±135.39^b
100mM	0.56±0.13^c	139.45±24.71^c

Where: Values are means ± SE (standard error).; Values with the same superscript letters are not significantly different from each other whilst, values with different superscript letters are significantly different at 0.05 level of probability.

Fig. 4. Spunta cv.'s microtubers after 7 weeks at microtuberisation induction medium. Where, S0, S1, S2, S3 and S4 are 0.0, 25, 50, 75 and 100mM/L NaCl concentrations, respectively.

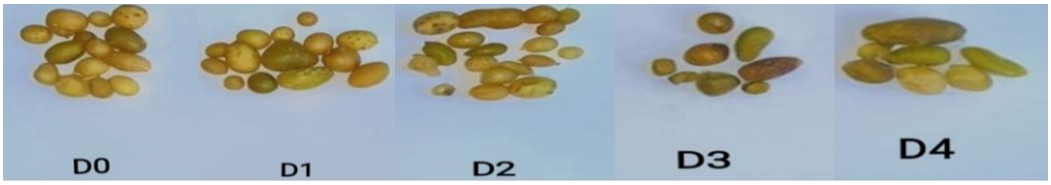

Fig. 5. Diamont cv.'s microtubers after 7 weeks at microtuberisation induction medium. Where, D0, D1, D2, D3 and D4 are 0.0, 25, 50, 75 and 100mM/L NaCl concentrations, respectively.

Fig. 6. Cara cv.'s microtubers after 7 weeks at microtuberisation induction medium. Where, C0, C1, C2, C3 and C4 are 0.0, 25, 50, 75 and 100mM/L NaCl concentrations, respectively.

Variance Components and Heritability:

The relative magnitudes of the variance components and heritability were estimated for all studied traits from the combined data over the five levels of salinity stress and the results are presented in Tables 13, 14 and 15 for morphological, physiochemical and microtuberisation traits, respectively. The results presented in Table (13) revealed that the genetic variance was high and positive for plantlet length, shoot length and root length. This finding is emphasized by the heritability values, which were more than 50% for these traits with percentages values of 53.5%, 76.8% and 64.18, respectively. On the other hand, the number of roots/plantlet, number of leaves/plantlet and relative water content (RWC%) were highly influenced by the levels of salinity, where the magnitude values of heritability were 0.83%, 8.4% and 5.60%, respectively. The genotype by levels interaction variations insure this finding, which were positive and higher than genetic variance for number of leaves/plantlet and relative water content (RWC), but it was lower in magnitude than genetic variance in the cases of plantlet length, shoot length and root length. Regarding physiochemical traits (Table 14), the values of genetic variance (σ^2g) were high in magnitude compared to the corresponding values of genotype by levels interaction (σ^2gL) in addition to error variance (σ^2e) in the cases of proline, sodium (Na^+) and potassium (K^+) content traits. These results were emphasized by heritability in broad sense which were 47.82%, 66.67% and 85.33%, respectively, indicating that these traits are mainly controlled by genetic factors. On the other hand, both chlorophyl a and b (C^a and C^b) had the opposite direction, which mainly influenced by salinity stress levels. The estimated amount of variance components and heritability for microtuberisation traits are presented in Table (15). The genetic variance (σ^2g) was high in magnitude compared to the corresponding values of genotype by levels interaction (σ^2gL) in addition to error variance (σ^2e) with respect to number and weight of microtubers/plantlet. These results are emphasized by heritability in broad sense which were 60.00%, and 70.86% for number of microtubers/plantlet and weight of microtubers/ plantlet, respectively, indicating that these traits are mainly influenced by genotypes (cultivars). These results are in agreement with the results obtained by Kadi *et al.* (2020) and Ahmed *et al.* (2020). They emphasized that genotypes (cultivars) and genotypes by medium composition highly influenced the microtuberisation.

Table 13. Variance components and heritability for morphological traits from the data combined over the levels of salinity stress

Variance components	P.L (cm)	Sh.L (cm)	R.L (cm)	N. R/P	N. L/P	% RWC
σ^2g	4.83	3.41	9.30	0.11	1.94	4.21
σ^2 gL	2.08	0.22	3.93	0.00	5.86	10.45
σ^2e	6.31	2.45	3.79	2.16	1.84	5.60
σ^2ph	9.01	4.44	14.49	0.83	8.41	16.53
H_b%	53.53	76.80	64.18	13.25	23.06	25.47

Where, σ^2g, $\sigma^2g\times L$, σ^2e , σ^2ph and H_b% are genotypic, genotype by levels, error and phenotypic variances as well as heritability in broad sense, respectively.

Table 14. Variance components and heritability for physiochemical traits from the data combined over the levels of salinity stress

Variance components	Proline	Na+	K^+	C^a	C^b
σ^2g	0.011	0.048	0.064	0.318	0.228
σ^2gL	0.012	0.022	0.009	0.564	0.505
σ^2e	0.001	0.005	0.007	0.136	0.167
σ^2ph	0.023	0.072	0.075	0.927	0.789
H_b%	47.82	66.67	85.33	34.30	28.90

Where, σ^2g, σ^2gL, σ^2e and H_b% are genotypic, genotype by levels, error and phenotypic variances as well as heritability in broad sense, respectively.

Table 15. Variance components and heritability for microtuberization traits from the data combined over the levels of salinity stress

Variance components	Average number of microtuber/ plantlet	Average weight of microtuber/plantlet
σ^2g	0.03	6155.07
σ^2gL	0.02	2310.78
σ^2e	0.02	970.85
σ^2ph	0.05	8708.49
H_b	60.00	70.68

Where, σ^2g, σ^2gL, σ^2e and H_b% are genotypic, genotype by levels, error and phenotypic variances as well as heritability in broad sense, respectively.

In conclusion, from all the previous results it could be concluded that microtubers induction ability is mainly controlled by genotypes as well as the interaction of genotypes by salinity stress levels. Also, the results recommended that *in vitro* selection for salinity stress tolerance is available as an alternative to field trials for a suitable levels of sodium chloride ranged from 25 to 100mM/L.

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الإختلاف الوراثى بين أصناف البطاطس لإنتاج الدرنات الدقيقة معمليا تحت ظروف الضغط الملحى

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الملخص

يهدف هذا البحث إلى دراسة أثر الإجهاد الملحي على بعض الصفات المعملية لبعض أصناف البطاطس باستخدام ثلاثة أصناف منزرعة وهي إسبونتا وديامونت وكارا بإستخدام العقد السابقية مفردة البرعم على بيئة MS مضاف اليها كلوريد الصوديوم بالتركيزات التالية ٠,٠ و٢٥ و٥٠ و٧٥ و١٠٠ مليمول/لتر قى تجربة عاملية لتقييم تحمل أصناف البطاطس للإجهاد الملحي فيما يتعلق بالصفات المعملية، والتي شملت بعض الصفات المورفولوجية والفيزيوكيمياتية والدرنات الدقيقة. وكشفت النتائج أن إختبارات المعنوية لمتوسط مربعات التراكيب الوراثية (الأصناف) كانت ذات دلالة إحصائية عالية لجميع الصفات المورفولوجية المدروسة باستثناء عدد الجذور للنبات، في حين كانت ذات دلالة إحصائية لجميع الصفات الفيزيوكيمياتية وخصائص الدرنات الدقيقة المدروسة، مما يشير إلى وجود فروق حقيقية بين الأصناف الثلاثة قيد الدراسة. وقد تأثرت كل من الصفات المورفولوجية وخصائص الدرنات الدقيقة بدرجة كبيرة بمستويات الملوحة، حيث لوحظت فروق ذات دلالة إحصائية عالية لهذه الصفات دون استثناء. وقد تم تسجيل أعلى متوسط لعدد الدرنات الدقيقة/النبات للمعاملة الكنترول (0.00 NaCl) بمتوسط قيم ١,٥ و٢,٠ و١,٨٠ لأصناف سبونتا وديامونت وكارا على التوالي، وانخفضت هذه القيم بزيادة تركيزات NaCl وكانت قيم معامل التوريث أكثر من ٥٠٪ لكل من عدد ووزن الدرنات الدقيقة/النبات، مما يشير إلى أن هذه الصفات تعتمد بدرجة كبيرة على صنف المصدر لمزارع الأنسجة (التركيب الوراثى). ومن خلال النتائج يمكن أن نستنتج أن القدرة على انتاج الدرنات الدقيقة يتم التحكم بها بشكل أساسي من خلال العوامل الوراثية وكذلك تفاعل التركيب الوراثى مع مستويات الإجهاد الملحي. كما أوصت النتائج بإمكانية االانتخاب المخبرى لتحمل الإجهاد الملحي لمستويات مناسبة من كلوريد الصوديوم تتراوح من ٢٥ إلى ١٠٠ مليمول / لتر.