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Production of Tomato Lines Tolerating to Salinity Using *In-Vitro* Culture Technique

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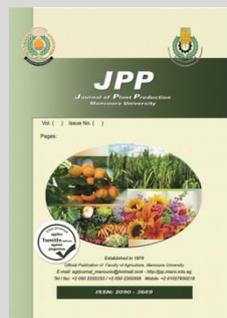


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ABSTRACT

Salinity is one of the major stress between the abiotic stresses for plants worldwide. This study aimed to investigate and evaluate variations in salt tolerance of four tomatoes genotypes (Super strain B, Castle Rock, Edkawy and Advantage II) under laboratory conditions using different concentration of NaCl. The cotyledon explants were cultured in MS media containing 0.5 mg/l BA and 0.5 mg/l Kin under different concentration of NaCl (0.0, 3.0, 6.0, 8.0 and 11.0 gm/L). Cotyledon culturability traits such as the percentage of each callus induction, plant regeneration, rooting, number of shoots, shoot length, roots number, root length and the plantlets number were measured to evaluate the salinity tolerance. All growth traits decreased with increasing of NaCl concentration in the culture media. The results suggested that the Edkawy genotype was the best for the percentage of plant regeneration (79.33%), percentage of rooting (54.66%), length of the shoot (7.46 cm), length of the root (5.73 cm) and callus fresh weight (2.72 gm) compared with the other genotypes under different levels of NaCl. Super Strain B and Castel Rock genotypes gave the highest shoots number and plantlets number (13.00, 13.06 and 4.66, 4.00 respectively). Advantage II genotype gave the lowest values for almost traits except the percentage of callus induction which was the best value (90%). Super Strain B genotype appeared the best in the percentage for callus induction (53.33%), percentage of plant regeneration (36.66%) and length of the shoot (4.66 cm) under the highest salt concentration (11.0 gm/L).

Keywords: Tomato, In-vitro Culture, Salinity Tolerant, NaCl



INTRODUCTION

Tomato, *Solanum lycopersicum* L., is one of the best and important vegetable crops in the worldwide for both production and harvested area. In 2017, among the top five tomato-producing countries after China, India, Turkey, and the United States was Egypt (FAOSTAT Food and Agriculture Organization of the United Nations, 2018). Fresh tomatoes, as a functional food, contain a considerable basic nutritional need of the human body because it has a conservable amount of minerals, and antioxidant compounds, such as polyphenols (Castagna *et al.*, 2013 and Htun *et al.*, 2020). High dietary intake of tomatoes can decrease coronary heart disease in consequence of decreasing Low-density lipid (LDL) Cholesterol levels (Silaste *et al.*, 2007).

On the other hand, there are abiotic and biotic stresses to reduce production of tomato under field conditions. High salinity is the most important constraint for crop production among abiotic stress (Zhang *et al.*, 2014). In the world, about 400 million hectares of land are affected by high salinity. Salinity affects every aspect of the morphology, physiology and biochemistry of plants and finally suppresses photosynthesis resulting in yield reduction (Flowers *et al.*, 2014 and Aazami *et al.*, 2010). As the high salinity levels in the cells of plant are because of osmotic and ionic stress combinations (Zhang *et al.*, 2014).

Salinity in water and soil is a universal threat to plant growth and development resulting in significant economic losses in the tomato crop (Albaladejo *et al.*, 2017). plants suffered from unfavorable effects as a result of osmotic

stress through salinity. Because high salinity caused a considerable reduction in water uptake from the soil, the osmotic balance of the affected plants is disrupted (Munns and Tester, 2008.). As a result, the lack of water leads to secondary consequences of salinity such as ionic imbalance and oxidative stress. To maintain ionic homeostasis and osmotic adjustment, Salt stress provokes several signaling pathways (Flowers *et al.*, 2014 and Zhang *et al.*, 2014).

Tomatoes is sensitive to moderate concentration of salinity in the soil. It is the best crop to study *in vitro* culture, salt tolerance and genetic studies Because of its low chromosome number i.e., $2n=2x=24$ and Because of extensive understanding of tomato genetics in the solanaceae family crops (Biswas *et al.*, 2017). Around the world, a successful methodology of in vitro plant cell and tissue culture techniques is being utilised to improve the genetics of tomato plants and select salt-tolerant cell lines. Tomatoes have been the subject of several *in vitro* studies in various applications (Biswas *et al.*, 2017 and Shanika and Seran, 2021).

The combination between the cytokines Kin (kinetin) and BA (benzyladenine) in the culture media was the best for Callus induction, plant regeneration and rooting compared with the combination between the cytokines and auxins (Ali *et al.*, 2012 and Ali *et al.*, 2017).

This study was done to investigate the following points;

- 1- Callus induction and regeneration ability from cotyledonary explants of four cultivated tomato genotypes under different NaCl concentrations.

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- 2- The effect of different NaCl concentrations on formation and number of shoots, roots and plantlets from cotyledon explants of four cultivated tomato genotypes.
- 3- The extent of genetic variability for salinity tolerance among different cultivated tomato genotypes using tissue culture techniques under different NaCl concentrations.
- 4- Assessing the possibility of obtaining salt tolerance tomato plants via tissue culture.

MATERIALS AND METHODS

This study was conducted at Tissue Culture Laboratory of Vegetable Research Department, Agricultural Research Center, Sakha Agricultural Research Station, Sakha, Kafr El-Shaikh Governorate, Egypt. Tomato Seeds were obtained from the Vegetable Research Department, Horticultural Research Institute, Agricultural Research Center, Egypt. The study was done in different steps:

- 1- *In vitro* seed germination of four tomato genotypes.
- 2- *In vitro* callus induction and plant regeneration under different level of NaCl (0.0, 3.0, 6.0, 8.0 and 11.0 gm/L) with four genotypes.

Chemicals

The following Chemicals were used in this study as; MS media, sucrose, agar, Kin (kinetin), BA (benzyladenine), NaCl (laboratory grade), sterilizing chemicals (sodium hypo chlorite NaOCl, 70% ethanol), absolute ethanol, ethanol (70%).

Culture media

Murashige and Skoog (1962) medium (MS) supplemented with 30 g / L sucrose and 8.0 g / L agar were used in this study. Two types of culture media were used as follows:

1. Hormone-free basal MS medium for raising of seedling to get explants.
2. MS medium supplemented with 0.5 mg/ L of BA and 0.5 mg/ L of Kin and different salt concentrations of NaCl (0.0, 3.0, 6.0, 8.0 and 11.0 gm/L) for callus induction and plant regeneration under salt stress treatment.

The medium pH was adjusted to 5.8 and subsequent autoclaving at 121°C and 15 psi for 20 min. The medium was then transferred into the culture room and cooled at 24°C temperature before being used.

Preparation of plant material

Seeds from 4 tomato genotype (Super strain B, Edkawy, Castle Rock and Advantage II) were grown *in vitro*. The seeds were washed with autoclaved water for 15 min and sterilized by soaking in Clorox (Sodium hypochlorite) solution for 10 min, followed by thoroughly rinsing in tap water. Then the seeds were immersed in 70% ethanol for 1 min and immersed in Clorox for 25 min followed by washing with sterilized distilled water. All the above-mentioned steps were carried out under a laminar airflow cabinet (Ali *et al.*,2012 and Shanika and Seran, 2021).

Seed germination for explant

Seeds were cultured in jars for germination in a hormone-free MS (Murashige and Skoog, 1962) basal medium containing 3% sucrose and 0.8% agar and the PH was 5.8. Ten seeds were cultured per culture jar. The cultures were incubated in the dark to four days; thereafter it was transferred to growth chamber under a16h/8h light/dark photoperiod with the illuminations of white

fluorescence light intensity of 2000–2500 lux and 70% relative humidity at 25±2 °C. Thereafter, seedlings olded three weeks after germination were used as explants source.

Explants preparation and inoculation

Three weeks after germination, the cotyledon explants (Ali *et al.*,2012) with 0.5 cm long were detached from the seedlings under aseptic conditions subsequently were placed (abaxial side) in jars containing MS medium supplemented with 30 g / L sucrose, 0.5 mg/ L of BA, 0.5 mg/ L of Kin and added with different salt concentrations of NaCl (0.0, 3.0, 6.0, 8.0 and 11.0 gm/L) for callus induction and plant regeneration under salt stress treatment (Ali *et al.*,2012 and Ali *et al.*,2017). The pH of the medium was adjusted to 5.8 and solidified by 8 g/ L agar. Six explants were cultured in every culture jar and jars were placed in maintained condition. The cultures were incubated in normal growth room conditions (16/8 light/dark regime) having the same light intensity and temperature as above for four weeks.

Callus induction and regeneration:

For callus induction, explant cultures were maintained in dark for the first two weeks, then allowed to grow under light for another two weeks. After four weeks, the callus induction and shoot regeneration were induced on the same media and sub-cultured in a fresh MS medium with the same ingredients (Ali *et al.*,2012).

The calli and regenerants were sub-cultured on the same media with salt treatment in different concentrations of NaCl. The incubation condition was same as mentioned earlier. After eight weeks of culture in different salt levels, the following parameters were recorded for each explant on the basis of callus fresh weight, the percent of explants forming callus, the percent of explants forming roots, the percent of explants forming shoot, shoots number, shoot length, roots number and the number of plantlets.

Statistical analyses

The experimental design was four genotypes × six NaCl concentrations. Three replicates were used and each treatment consisted of 18 explants cultured in a completely randomized design (CRD), as a factorial design. Differences among tomato genotypes were tested by the analysis of variance (ANOVA) and means significance differences were tested by Duncan's test at the 0.05 level of significance.

RESULTS AND DISCUSSION

In vitro selection for salinity tolerance: -

The present investigation was designed to study the possibility of obtaining salt tolerance tomato plants via tissue culture techniques. The inheritance of salinity tolerance in tomatoes was investigated using four tomato genotypes; i.e., Super Strain B, Castle Rock, Edkawy and Advantage II to determine the concentration of NaCl suitable for selection to salinity tolerance. The cotyledonary leaves were used as explants for induction of callus and subsequently regenerated plant on MS medium containing various levels of salt.

Callus growth and regeneration

The results showed that growth of callus and plant regeneration of tomato genotype appeared a dose -response via decreased with increasing salinity levels in the growth media. The effects of salinity on the percentage of callus induction and plant regeneration as shown in Table 1.

Table 1. Effect of different salt concentrations on the percentage of callus induction and regeneration traits of four tomato genotypes.

Genotypes	Callus induction percentage					mean	Plant Regeneration percentage					mean
	Concentration levels						Concentration levels					
	0.00g/l	3.00g/l	6.00g/l	8.00g/l	11.00g/l		0.00g/l	3.00g/l	6.00g/l	8.00g/l	11.00g/l	
Super Strain B	100 a	100a	90 b	90 b	53.33 d	86.66 b	100 a	100 a	70 b	66.66 b	36.66 d	74.66 b
Castle Rock	100 a	100a	100 a	70 c	46.66 e	83.33c	100 a	100 a	93.33 a	46.66 c	36.66 d	75.33 b
Edkawy	100 a	100a	100 a	100 a	46.66 e	89.33 a	100 a	100 a	96.66 a	66.66 b	33.33 d	79.33 a
Advantage II	100 a	100a	100 a	100 a	50 de	90 a	100 a	96.66 a	66.66 b	50 c	23.33 e	67.33 c
Mean	100 a	100a	97.5 b	90 c	49.16 d		100 a	99.16 a	81.66 b	57.5 c	32.5 d	

*Means with the same letter are not significantly different.

The obtained results indicated that all tested tomato genotypes were able to produce callus and regeneration shoot from callus under different concentrations of NaCl in the growth media. However, these abilities were different, depending on the genotype and the levels of salinity.

Regarding genotypes, the data indicated that the Advantage II genotype had the highest value (90%) for the percentage of callus induction followed by Edkawy genotype under the different concentrations of salt stress, while Castle Rock genotype gave the lowest value (83.33%). But Super Strain B was the best (53.33%) under 11.0 gm/L salt concentration.

On the other hand, the results revealed that the Edkawy genotype was the best in the percentage of plant regeneration trait (79.33%) if compared with the other genotypes under five different levels of salt concentrations (Figure 1). Meanwhile, both Super Strain B and Castle Rock genotypes had the highest value (36.66%) under the highest salt concentration. (11.0 gm/L), While Advantage II genotype had the lowest value (23.33%) for plant regeneration.

Concerning the salt concentration, all evaluated genotypes produced callus and regenerated shoot under

different levels of salinity. The percentage of both callus induction and plant regeneration rate decreased with an increasing salt concentration in all tomato genotypes for these.



Figure 1. Edkawy regenerated shoot

The shoots number and plantlets number:

As shown from the data presented in Table (2) The shoots number and plantlets number differed from salinity level to another. When NaCl level increased the shoots number and plantlets number was decreased.

Table 2. Effect of different NaCl concentration on the number of shoots and number of plants for all tomato genotypes tested.

Genotypes	Number of shoots					Mean	Number of plants					Mean
	Concentration levels						Concentration levels					
	0.00g/l	3.00g/l	6.00g/l	8.00g/l	11.00g/l		0.00g/l	3.00g/l	6.00g/l	8.00g/l	11.00g/l	
Super Strain B	30.33 a	18.00 c	8.66 de	5.33 g	2.66 h	13.00 a	7.66 a	5.33 cd	4.33 def	3.33 fg	2.66 gh	4.66 a
Castle Rock	27.66 b	19.33 c	8.66 de	6.00 fg	3.66 gh	13.06 a	6.66 b	5.33 cd	3.33 fg	3.00 g	1.66 hi	4.00 b
Edkawy	26.66 b	18.00 c	9.00 de	5.33 g	2.33 h	12.26 a	5.00 d	4.66 de	4.33 def	3.33 fg	1.66 hi	3.80 b
Advantage II	19.00 c	10.66 d	7.66 ef	4.66 gh	2.66 h	8.93 b	6.33 bc	5.00 d	3.66 efg	2.66 gh	1.00 i	3.73 b
Mean	25.91 a	16.5 b	8.5 c	5.33 d	2.83 e		6.41 a	5.08 b	3.91 c	3.08 d	1.75 e	

*Means with the same letter are not significantly different.

The highest shoots number and plantlets number were obtained by the cultivars Super Strain B and Castel Rock which had very closed values (13.00 ,13.06 and 4.66 ,4.00, respectively), while Advantage II cultivar had the lowest one for these traits (Figure 2).

Moreover, there are a wide range of differences among the tested genotypes under the same concentration of salt (Figure 3).



Figure2. Number of shoots (a) and the number of plants (b) for the cultivar Super Strain B.

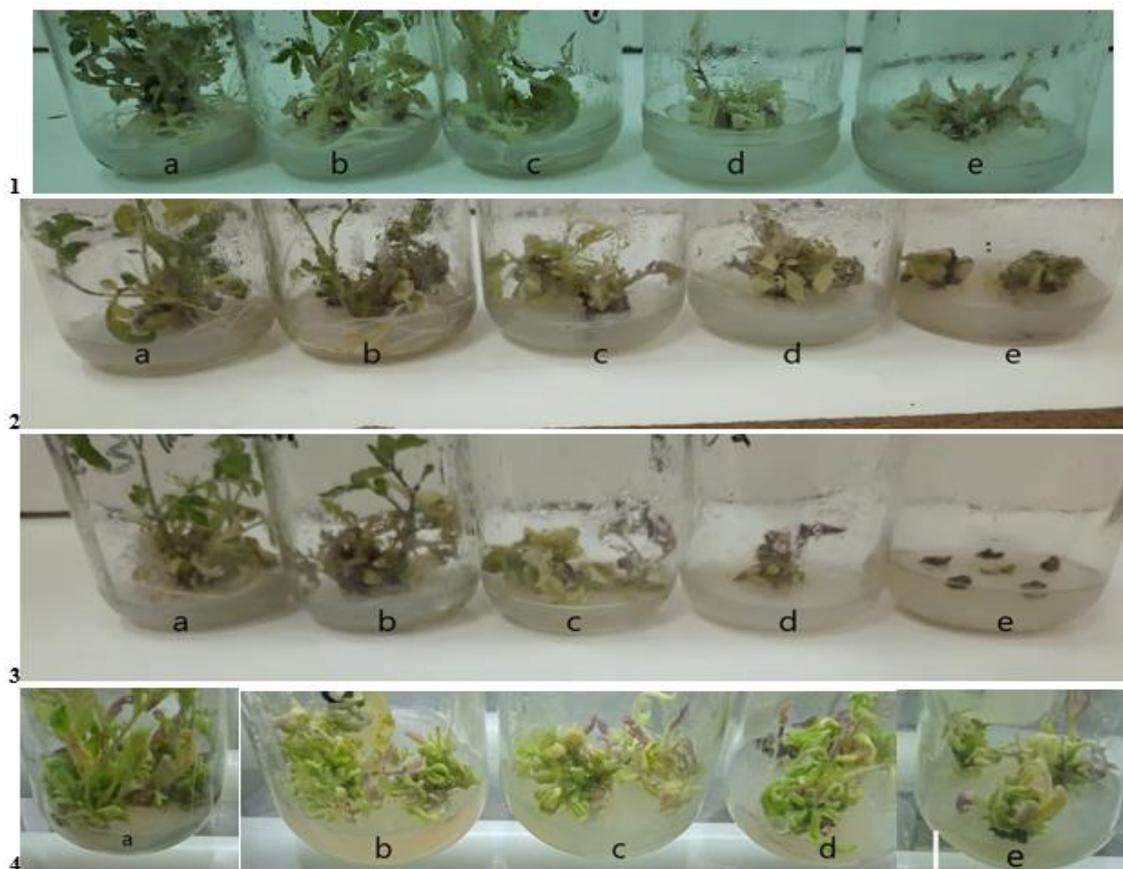


Figure 3. Effect of different levels of NaCl (a, b, c, d and e) on the number of shoots and number of plants in tomato cultivars.

Notes: Edkawy (1), Castle Rock (2), Advantage II (3) and Super Strain B (4), a (0.0 gm/L NaCl), b (3.0 gm/L NaCl), c (6.0 gm/L NaCl), d (8.0 gm/L NaCl) and e (11.0 gm/L NaCl).

The percentage of rooting rate

There were high significantly differences among genotypes for the percentage of rooting rate under varying levels of salt (Figure 4). The results appeared that Edkawy genotype exhibited the highest value (54.66%) of rooting followed by the Super Strain B (43.66%) while Castle Rock exhibited the lowest value (40.00%) for the same trait.

All tested tomato genotypes were able to initiate roots from callus under the concentrations of NaCl (0.0 and 3.0 gm/L) except the Advantage II genotype which didn't regenerate any roots under all different concentrations of NaCl.

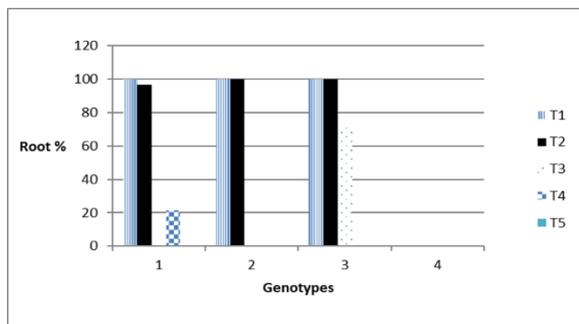


Figure4. Effect of different levels of NaCl (T1, T2, T3, T4 and T5) on the percentage of rooting rate t in all tomato cultivars.

Notes: Super Strain B (1), Castle Rock (2), Edkawy (3) and Advantage II (4), T1 (0.0 gm/L), T2(3.0 gm/L), T3(6.0 gm/L), T4(8.0 gm/L) and T5(11.0 gm/L).

In this regard, all genotypes didn't regenerate any roots under the higher salinity levels but the Super Strain B genotype was able to regenerate roots under the higher concentration of NaCl (8.0 gm/L) and Castle Rock genotype under the concentration of (6.0 gm/L).

The number of roots

The mean number of roots for the tomato genotypes under different levels of salt concentrations are presented in Figure (5). The results appeared that Super Strain B genotype had the highest value (4.80) for the mean number of roots followed by Edkawy (3.46) under different concentrations of salt stress. Meanwhile, Advantage II didn't regenerate any roots under the different concentrations of NaCl.

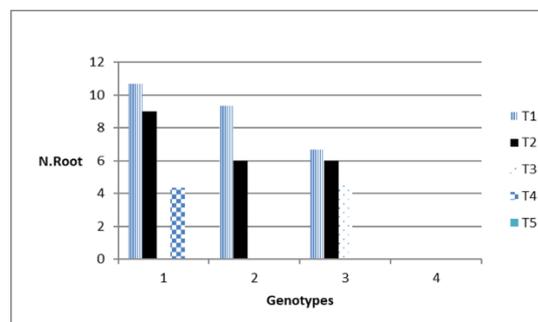


Figure5. Effect of different levels of NaCl (T1, T2, T3, T4 and T5) on the number of roots of tomato cultivars

Notes: Super Strain B (1), Castle Rock (2), Edkawy (3) and Advantage II (4), T1 (0.0 gm/L), T2(3.0 gm/L), T3(6.0 gm/L), T4(8.0 gm/L) and T5(11.0 gm/L).

Moreover, the other genotypes didn't initiate roots under the higher concentration of salinity except for Super Strain B and Castle Rock under the concentration of 8.0 gm/L and 6.0 gm/L, respectively for the same trait.

Shoot length

The effects of salinity stress on the shoot length grown *in vitro* from cotyledonary leaf explants are shown in Figure (6).

With regard to the genotype, Edkawy cultivar exhibited the highest value (7.46 cm) for the length of shoot followed by the other two genotypes Super Strain B and Castle Rock which had similar values (7.13 and 7.13 cm, respectively). Meanwhile, the Advantage II genotype gave the lowest value (5.53 cm) for the same trait.

But Super strain B genotype gave the best values (6.00 and 4.66 cm, respectively) under the higher concentration of salinity (8.0 and 11.0 gm/L NaCl) for the length of shoot.

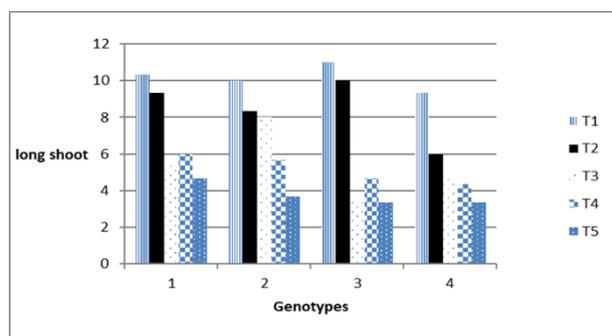


Figure 6. Effect of different levels of NaCl (T1, T2, T3, T4 and T5) on the length of shoot in all tomato cultivars.
Notes: Super Strain B (1), Castle Rock (2), Edkawy (3) and Advantage II (4), T1 (0.0 gm/L), T2(3.0 gm/L), T3(6.0 gm/L), T4(8.0 gm/L) and T5(11.0 gm/L).

In addition, shoots length decreased with the increased of NaCl levels (10.16, 8.41, 6.58, 5.16 and 3.75 cm for the five NaCl levels, respectively).

Root length

The length of roots for four tomato genotypes under the different levels of salt concentrations are presented in Figure (7). The results appeared that the length of roots in Edkawy genotype showed the best value (5.73 cm) followed by the Super Strain B which had very closed value with Castle Rock (4.73 and 4.53 cm, respectively) under the five different levels of salt concentrations. Meanwhile, the Advantage II genotype didn't regenerate any roots under the different levels of NaCl.

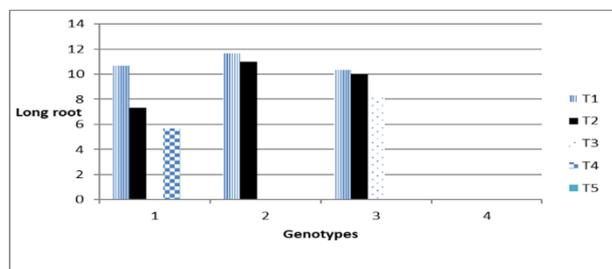


Figure 7. Effect of different levels of NaCl (T1, T2, T3, T4 and T5) on the length of roots for all tomato cultivars
Notes: Super Strain B (1), Castle Rock (2), Edkawy (3) and Advantage II (4), T1 (0.0 gm/L), T2(3.0 gm/L), T3(6.0 gm/L), T4(8.0 gm/L) and T5(11.0 gm/L).

On the other hand, all genotypes didn't initiate roots under the higher concentration of salinity except the Super Strain B under the higher level of salinity (8.0 gm/L).

Callus Fresh weight

Callus fresh weight was affected by increased NaCl level in the growth medium (Figure8). The highest weight of callus was found in Edkawy (2.72 gm), but the Super Strain B showed the lowest callus weight (1.77 gm) under the different levels of NaCl. Meanwhile, Castle Rock and Advantage II genotypes exhibited intermediate and very closed values (1.86 and 1.85, respectively).

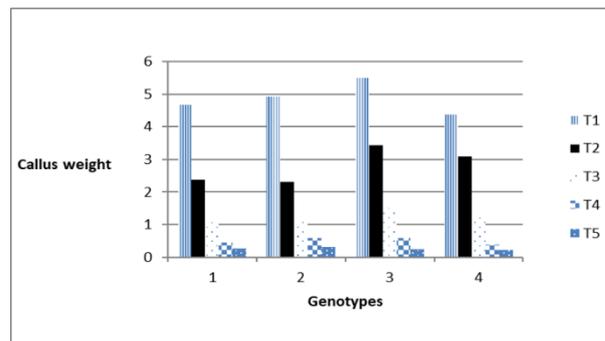


Fig.8. Effect of different levels of NaCl (T1, T2, T3, T4 and T5) on the fresh weight of callus in tomato cultivars

Notes: Super Strain B (1), Castle Rock (2), Edkawy (3) and Advantage II (4), T1 (0.0 gm/L), T2(3.0 gm/L), T3(6.0 gm/L), T4(8.0 gm/L) and T5(11.0 gm/L).

The fresh weight of callus showed a dose – response for decreased with the increased of NaCl levels (4.875, 2.796, 1.246, 0.531 and 0.255 gm, respectively).

Plant tissue culture techniques may offer the opportunity for rapid evaluation of germplasm for salt stress tolerance, in addition to being a valuable tool for salinity studies and generating salt tolerant plants (Zaki and Yokoi, 2016; Suliman *et al.*, 2020 and Mandeep *et al.*, 2021). Many studies have been carried out using various tissue culture methods (Rashed *et al.*, 2016).

This study, we determined the tolerance of four tomato cultivars to different levels of salinity stress *in vitro*.

The obtained results revealed that all tested tomato genotypes were able to produce Callus and regenerate shoots under various levels of NaCl in the growth media, as well as initiate roots from callus under some concentrations of NaCl (El-meleigy *et al.*,2004). These capacities were different depending on genotype and salt level. These results agreed with Mercado *et al.*, (2000), Rus *et al.*, (2000) and El-meleigy *et al.*, (2004). In this regard, callus derived from the cultivar Edkawy was more able to adapt to different salinity levels than other genotypes.

The results also showed that callus induction, shoot regeneration and root growth in these genotypes were decreased with increasing NaCl concentration in the growth medium. This agreed with Magdoleen *et al.*, (2011) and Mohammad, (2018). Several authors reported the use of NaCl as *in vitro* salinity screening in different plants (Vijayan *et al.* 2003, Zhao *et al.* 2009 and Aazami *et al.* 2010)

Callus was utilised by the majority of the researchers to assess its physiological and biochemical responses to salt stress. (Shibli *et al.* 2007; Ghane *et al.* 2014 and Alharby *et al.* 2016)

In this study, the growth of root was more affected by increasing NaCl levels in the culture medium than shoot growth. The length of roots was significantly decreased with an increased NaCl concentrations. These results are in agreement with Rashed *et al.* (2016). Therefore Cano *et al.* (1996) showed that root growth is the better characteristic for evaluating salinity tolerance of tomato genotypes.

The obtained results agreed with the previous findings concerning the physiological responses of tomato cultures to salt treatments (Rus *et al.*, 2000 and Magdoleen *et al.*, 2011). In this regard, Super Strain B was the best genotype for the number of regenerated shoots, the number of plants and the number of roots under the different levels of NaCl. Also, Super Strain B genotype was the best in the percentage of callus induction, percentage of regeneration and length of shoot under the highest salt concentration of NaCl (11.0 gm/L).

In addition, all the tested genotypes exhibited different responses differed from one genotype to another for salinity tolerance. Furthermore, there are differences among the genotypes under the same salt concentration. These results are in harmony with those obtained by Mohamed *et al.*, (2006); Plevnes *et al.*, (2007); Cayuela *et al.*, (2007) and Rashed *et al.*, (2016), who found that some genotypes surpassed the other genotypes under different levels of salt concentrations and all traits for all genotypes decreased as the concentration of NaCl increased.

In conclusion, differences among tomato genotypes for regeneration capacity under *in vitro* salt stress could be used as a rapid way to detect salt-tolerant genotypes. The length of shoot, the length of root, number of shoots, number of roots and number of plants revealed the possibility of ranking the tested tomato genotypes into salinity tolerance. All tested tomato genotypes were able to produce callus, regenerated shoot and plants under different concentrations of NaCl in the growth medium. However, these abilities were different, depending on the genotype and levels of salinity.

Hence, it was possible to obtain plants tolerant to high concentrations of salinity from the genotypes used in this study.

All genotypes didn't regenerate any roots under the higher salinity levels but the Super Strain B was able to regenerate roots under the higher concentration of NaCl (8.0 gm/L) and Castle Rock under the concentration of 6.0 gm/L.

So that, Super Strain B and Edkawy genotypes were more able for adaptation to different salinity levels than other ones and it was possible to obtain salinity tolerant plants from these genotypes.

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انتاج سلالات طماطم متحملة للملوحة باستخدام زراعة الأنسجة

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قسم بحوث تربية الخضر والنباتات الطبية والعطرية - معهد بحوث البساتين - مركز البحوث الزراعية - الجيزة

يعتبر إجهاد الملوحة أحد أهم الضغوط abiotic stress للنباتات في جميع أنحاء العالم. تهدف هذه الدراسة إلى تقصي وتقييم التباين الناشئ عن زراعة الأنسجة تحت مستويات مختلفة من تركيزات كلوريد الصوديوم والقابلية لتكوين نباتات متحملة للملوحة لأربعة تراكيب وراثية من الطماطم (*Solanum lycopersicum*) (سوبر استرين بي، كاسل روك، ادكاوي وأفانتج ٢) تحت ظروف المعمل باستخدام مستويات مختلفة من كلوريد الصوديوم NaCl. حيث تم زراعة بادئ الأوراق الفلجية على بيئة MS مضاف إليها منظمات النمو ٠,٥ مجم / لتر BA و ٠,٥ مجم / لتر من Kin مع مستويات مختلفة من كلوريد الصوديوم (٠,٠، ٣,٠، ٦,٠، ٨,٠، ١١,٠ جم / لتر). وقد تم التقييم على بعض صفات زراعة الأنسجة *Cotyledon (tissue) culturability traits* مثل النسبة المئوية لتكوين الكالوس، النسبة المئوية لتكوين متجددات نباتية جديده، النسبة المئوية لتكوين الجذور، متوسط عدد الفروع الخضرية، متوسط عدد الجذور، طول النبات، طول الجذر وعدد النباتات وذلك لتقييم تحمل الملوحة. حيث أظهرت النتائج انخفاض جميع قيم هذه الصفات مع زيادة تركيز كلوريد الصوديوم في بيئة الزراعة. وتشير النتائج المتحصل عليها من هذه الدراسة إلى أن التركيب الوراثي ادكاوي كان الأفضل في صفات النسبة المئوية لتكوين متجددات نباتية جديده (٧٩,٣٣٪)، النسبة المئوية لتكوين الجذور (٥٤,٦٦٪) وطول shoot (٧,٤٦ سم)، طول الجذر (٥,٧٣ سم) والوزن الطازج للكالوس (٢,٧٢ جم) مقارنة التراكيب الوراثية الأخرى المستخدمة تحت مستويات مختلفة من كلوريد الصوديوم. أعطت التراكيب الوراثية سوبر استرين بي وكاسل روك أعلى متوسط لصفات عدد shoot وعدد النباتات (١٣,٠٠، ١٣,٠٦، ١٣,٦٦، ٤,٠٠ على التوالي). أعطى التركيب الوراثي أفانتج ٢ أدنى متوسط قيم لكل الصفات تقريباً باستثناء صفة النسبة المئوية لتكوين الكالوس والتي أعطت أفضل قيمة (٩٠٪). كان التركيب الوراثي سوبر استرين بي هو الأفضل من حيث صفات النسبة المئوية لتكوين الكالوس (٥٣,٣٣٪)، النسبة المئوية لتكوين متجددات نباتية جديده (٣٦,٦٦٪) وطول shoot (٤,٦٦ سم) تحت أعلى تركيز من كلوريد الصوديوم (١١,٠ جم / لتر).