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Induction of *in Vitro* Sweet Potato Plant (*Ipomoea Batatas)* Tolerant to Sea Water

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ABSTRACT

This study aims to develop an efficient in vitro propagation protocol for the Egyptian sweet potato cultivar (Abees) (*Ipomea batatas)*, which confers seawater salinity tolerance using the modified exchangeable selection cycles system (ESC) which is an effective screening method for estimating and developing salt tolerance in tuber plants, providing an effective selection. Different seawater concentrations were 1000, 2000, 3000, 4000, 5000, 6000, 7000, 8000, 9000, 10000, 11000 and 12000 ppm. The variety (Abees) showed tolerance to seawater salinity at 3000 ppm. All plant growth rates were recorded after each exchangeable selection cycle. The modified exchangeable selection cycles provided an effective in vitro selection method for developing salinity tolerance and led to an increase in seawater salinity tolerances up to 10,000 ppm of the sweet potato cultivar (Abees) recording shoot length at 7.0 ± 0.70 cm, Number of leaves /shoot at 5.6 ± 0.5, root length at 13.4 ± 1.8 cm and number of roots/ shoot at 7.6 ± 0.5 and the analyses of proline content and total chlorophyll were performed. Exchangeable selection cycles allow crops to be grown and irrigated entirely with seawater when fresh irrigation water is limited. Ultimately, the selected *in vitro* sweet potato plantlets that thrive in saline seawater can contribute to the production of new varieties of salt-tolerant sweet potatoes.

Keywords: Salinity, sea water, sweet potato, exchangeable selection cycles

1. Introduction

Sweet potatoes are considered the seventh most important food crop in the world and fourth in developing countries (FAO, 1997). Sweet potatoes (*Ipomoea batatas*) are a nutrient-dense food, rich in complex carbohydrates, fiber and essential vitamins, especially vitamin A, C and several B vitamins. Its high antioxidant content, especially beta-carotene, promotes vision, immune function and skin health. In addition, sweet potatoes have a low glycemic index, which means they have a positive effect on regulating blood sugar levels, their ability to contribute to diverse diets, from boosting immune health to providing sustainable energy, makes it a key crop for global food security (Ke *et al.* 2016 and Xie *et al*. (2017). It is grown as a valuable source of industrial raw materials, animal feed, and human food in over 100 countries (Horton DE, 1987; Jarret and Florkowski, 1990). However, pests, diseases and environmental factors prevent the plant from reaching its maximum agricultural potential. Viral diseases are considered the main cause of low yield productivity (Wambugu, 1991 and Loebenstein *et al*., 2003) and the main cause of variety decline (Carey *et al.,* 1999; Gibson *et al.,* 1998 and Guo *et al.,* 2006). Climate change leads to extreme weather events such as high temperatures, severe droughts and soil salinization due to irrigation. Such extreme weather fluctuations are the main reason for the loss of agricultural productivity worldwide, reducing the average yields of most major crops by more than 50% and are considered a major threat to food security (FAO, 2012). The development of new methods and transfer technologies to produce pathogen-free clonal seeds can overcome this limitation and help unlock the significant yield potential of this crop. However, soil salinity is one of the abiotic factors affecting sweet potato production worldwide, including in Africa where it is a staple crop (Dasgupta *et al.,* 2008).

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The main objective of this study is to confer salinity tolerance to one of the advantageous local varieties in Egypt (Abees) by applying the successful system (Exchangeable Cycles of Selection) and estimating the development of the plants selected in vitro during the experiments to improve the new Egyptian line becomes sweet potato cultivar tolerant to sea salt salinity for future crops under climate change conditions.

2. Material and Methods

2.1 Explant preparation and Sterilization Condition

Sprouts were initiated on the tuber of sweet potato cultivar (Abees) by surface washing with tap water thoroughly and covered with a thin layer of wet cotton tissues, then incubated for two weeks under dark conditions and humidity up to 60%. The emerged sprouts were isolated and used as plant source material for *in vitro* experiments.

2.2 Sterilization

The initiated sprouts were isolated and washed twice with natural soap and then washed three times with tap water for 10 minutes and one time with sterilized distilled water. 20 % of Commercial Clorox (5.25% NaOCl) with 5 drops of Tween 20 were used for surface sterilization of the sprouts for 20 mins then the sprouts were rinsed with sterile distilled water Three times under aseptic conditions of laminar air fellow hood.

2.3 Sprouts Culturing

MS medium 4.4g/l (Murashige and Skoog, (1962), catalog No., 0222, Duchefa Com., Harrlem the Netherland) including vitamins and free of plant growth regulators were used for all cultures and 30 g/l sucrose with 8g/l agar for solidification were added to the medium. The medium pH was adjusted to 5.8. before autoclaving at 121°C and 1.1 kg/cm2 for 25 mins. The cultures were incubated for one month in a growth chamber at 22 ± 2°C under a photoperiod of 16/8 hours light/dark.

2.4 Induction of sweet potato cultivar (Abees)

The shootlets about 8-10 cm long were cut into nodal cuttings, each nodal cutting contained one axillary bud and was cultured in glass jars, each containing 20 ml MS medium (Murashige and Skoog, 1962) with vitamins in addition to 30 g/L sucrose, 7g/l agar for medium solidification and supplemented with 0.5 mg/L BA and 0.5 mg/l IBA for induction of sweet potato shoots and incubated for one month in a growth chamber at 22 ± 2°C under a photoperiod of 16/8 hours light/dark. For multiplication, the obtained shoots were segmented into nodal segments with 0.5 -1 cm length as a plant source material for multiplication, then cultured on the multiplication medium containing MS basal supplemented with BA at 0.5 mg/l and incubated for 3 weeks under growth chamber condition.

2.5 Critical concentration detection of seawater salinity

The obtained shoos from the micro-propagated were isolated and divided into node segments. Each segment had at least one node. They were cultured in glass vessels containing 20 ml MS basal medium in addition to 30 g/l sucrose, and 7 g/l agar to solidify the medium provided with different concentrations of sea salt (S9883 Sigma Aldrich, typical ion concentration of seawater) as shown in Table (1). The interchangeable selection cycle system of El Kazaz *et al.* (1990) and El Kazaz *et al.* (2016) was carried out with the corresponding modification optimized for sweet potato crops. To determine the critical salinity concentration of the sweet potato cultivar (Abees), nodal segments were cultured in all experimental treatments and incubated for 6 weeks in a growth chamber at 22 ± 2 °C under a photoperiod of 16/8 hours light/dark. At least 5 jars of each treatment were cultured, and the data of each treatment were recorded (cycle 1). The modified exchangeable selection cycles (ESC) system was carried out to determine the maximum salt concentration tolerated by the cultivar (Abees) after each experimental cycle. The modified exchangeable selection cycles were based on culturing the compatible plantlets at different salinity concentrations for six weeks to select the compatible plantlets and determine the first maximum salinity concentration, followed by a one-month subculture on free MS medium as a recovery period to reduce salinity stress (Cycle 1) then sub-culturing the sea salt tolerated plantlets obtained from the first cycle with the higher concentration for 6 weeks and so on as the second cycle (each cycle included exposure of the plants to the higher salt concentration for 6 weeks and the

recovery period for one month). Reinforce the tolerated plantlets etc. until the final critical point is reached where the plants could no longer survive and become yellow and dead. The last concentration tolerated by the plants was recorded and all data collected for all salt tolerance improvement cycles to create an appropriate statistical method to evaluate the results obtained by IBM®SPSS® [SPSS Inc; IBM Corporation, NY, USA] Statistics program, version 25 (2017), Windows. The data were tested for normality using the Shapiro-Wilk test (Shapiro and Wilk 1965; Razali and Wah, 2011). Data were entered into ANOVA with a P value of <0.05. The average values of all treatments were compared using the least significant difference as a post hoc test (Snedecor *et al.,* 1956), with a P value of <0.05 considered statistically significant (Alaa *et al.,* 2021).

Table 1: The Different Experimented Treatments of Salinity

Treatment	Salinity Concentration of Sea Salt (ppm)
S0	MS medium (free of Sea Salt)
S1	MS+1000
S2	MS+2000
S3	MS+3000
S4	MS+4000
S5	MS+5000
S6	MS+6000
S7	MS+7000
S8	MS+8000
S9	MS+9000
S10	MS+10.000
S11	MS+11.000
S12	MS+12.000

2.6. Proline determination

The Bates *et al*. (1973) method was conducted for estimation of free proline. Sulfosalicylic acid (3 percent v/v) was used to extract 1 g of leaf and stem samples, which were then filtered through Whatman 1 filter paper. 2 ml of the extracted solutions were boilined for 1 hour with 2 ml of both reagent glacial acetic acid and ninhydrin then four ml of Ice toluene was added. A spectrophotometer (LW Scientific, Model V325XS) was used to measure the proline content at 520 nm, and the result was expressed as µmoles/g of dry weight.

2.7. Total Chlorophyll analysis

Total chlorophyll content was estimated using the method of Lichtenthaler and Wellburn (1983). 0.5 g of Leaf samples were collected, cut into small pieces, and then macerated with 20 ml ice acetone 80% (v/v) for 24 h. An aliquot of 2.5 ml was discarded and then 2.5 ml ice acetone 80% (v/v) was added then centrifugation for 5 min at 5.000 rpm was conducted for the samples. An aliquot of 2.5 ml was extracted and 2.5 ml ice acetone 80% (v/v) was added.

3. Results and Discussion

Tissue culture techniques have proven effective in sweet potato propagation and allow for rapid propagation of plants with desirable traits. These methods help ensure genetic stability and uniformity, which is crucial for commercial cultivation, especially in areas with difficult environmental conditions. Sources include studies on salt tolerance in potato varieties (Meena *et al.,* 2022) and advances in tissue culture propagation (Bajpai *et al.,* 2019).

3.1 Sprouts sterilization and regeneration Sweet potato cultivar (Abees)

Sprouts emerged on the surface sterilized sweet potato tubers after two weeks and after one month, all sprout reached to the suitable length for starting the shoot induction experiments as observed in Fig (1). The surface sterilization was conducted firstly using washing the separated sprouts with natural soap twice then washing with tap water thoroughly before sterilization steps under aseptic conditions. This first step reduced the contamination greatly. After one month of incubation, the shootlets were regenerated from the nodal segments on the selected shoot induction medium (with 0.5 mg/l BA and

0.5 mg/l IBA), and primary callus was spontaneously initiated from the wounded nodal segments as well (Fig 2.A). All induced shoots were reached to the appropriated shoot length after the second sub-cultured on the same shoot induction medium. The different stages of induction and micropropagation of sweet potato cultivar (Abees) was shown in Fig 2. The multiplicities of sweet potato plants were produced by culturing on MS containing a low concentration of BA at 0.5 mg/l as shown in Fig (2.C).

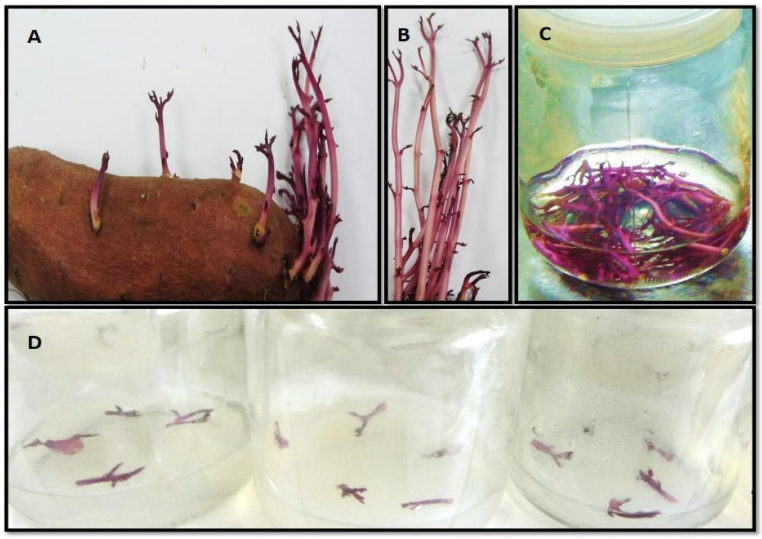

Fig. 1: Plant material A. The emerged sprouts on the sweet potato cultivar (Abees) after 2 weeks of incubation in dark conditions and up to 60% humidity. B. The separated sprouts of sweet potato. C. sterilization of the selected sprouts under Aseptic Conditions. D. The nodal segments cultured on the initiated medium.

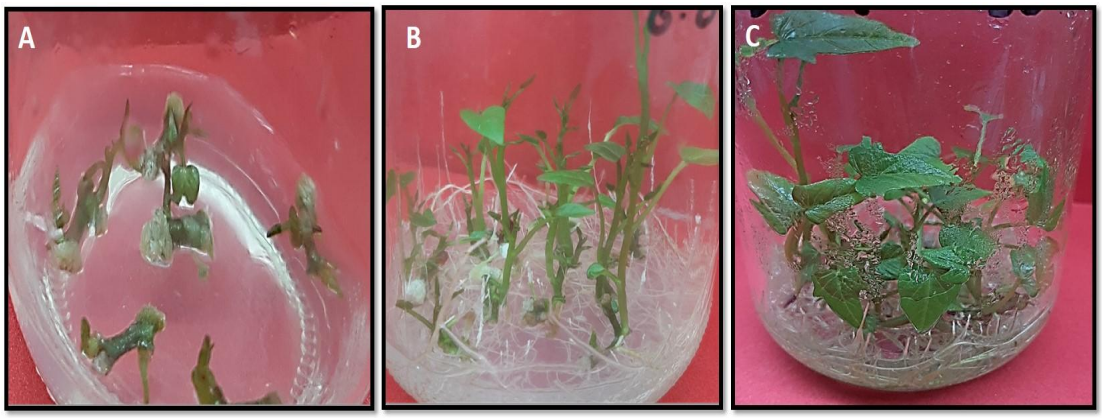

Fig. 2: Stages of *in vitro* micropropagation of sweet potato cultivar (Abees). A. shoot induced of nodal segments after cultured on the shoot induction medium. B shooting of plants. C. *in vitro* multiplication of sweet potato plants.

3.2. Exchangeable selection cycles of sweet potato cultivar (Abees)

The obtained shoots from the experimented nodal segments of sweet potato cultivar (Abees) showed tolerance to salinity of 3000 ppm of the experimented sea salt treatments (1000, 2000, 3000, 4000, 5000, 6000, 7000, 8000, 9000, 10.000, 11.000, 12.000 ppm) on the plantlets in the first selection cycle (Fig 3), based on the first selection, 3000 ppm was considered the start concentration, this result indicated that a critical point of tolerance is between 3000 and 4000 ppm of sea salt salinity. A previous study by Rodríguez-Delfín *et al.,* (2012) recorded the tolerance of sweet potato up to 2000 ppm in which different salinity treatments were tested to report that the increasing of the salinity levels significantly decreased the yield in two sweet potato cultivars (Huambachero and Untacip). The obtained shootlets from the first selection concentration (cycle 1) were cut into nodal segments and

cultured on free MS basal medium for month recovery, then were cultured as nodal segments to the second selection cycle (cycle 2) on higher sea salt treatments (4000, 5000, 6000, and 7000 ppm). The experimented explants were grown healthy and tolerated 6000 ppm of sea salt salinity whereas, unhealthy growth was observed with 7000 and 8000 ppm (Fig 4) which indicated that a new critical point of salinity tolerance is between 6000 and 7000 ppm.

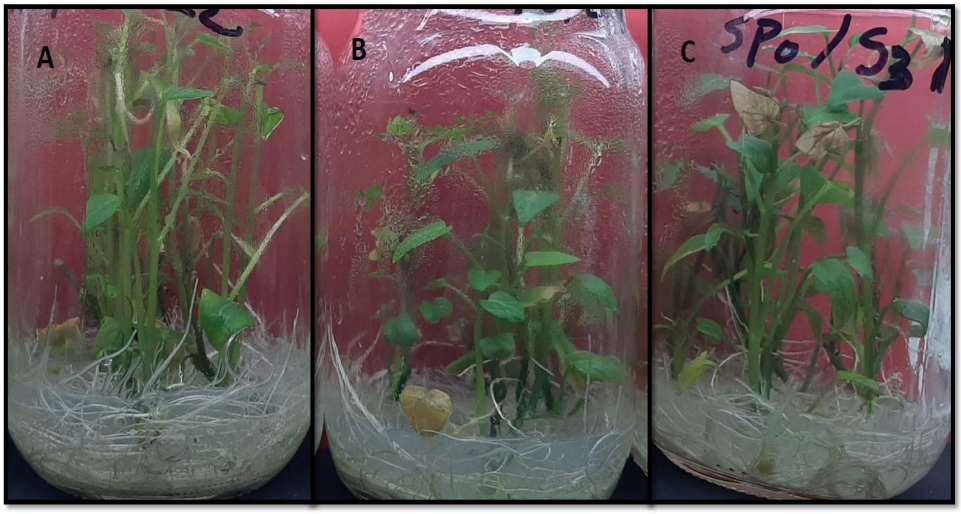

Fig. 3: Sweet potato plantlets produced from cycle 1 of ESC system A. The plantlets cultured on MS with 1000 ppm, B. The plantlets cultured on MS with 2000 ppm and C. The plantlets cultured on MS with 3000 ppm.

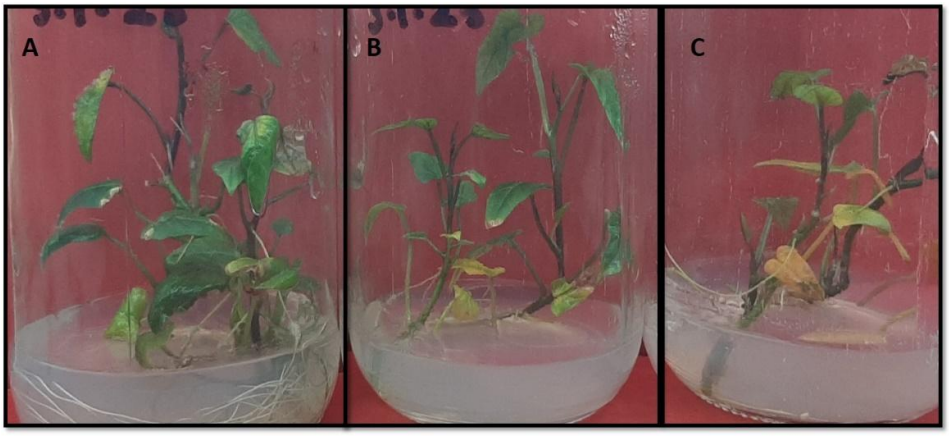

Fig. 4: Sweet potato plantlets produced from cycle 2 of ESC system A. The plantlets cultured on MS with 6000 ppm, B. The plantlets cultured on MS with 7000 ppm and C. The plantlets cultured on MS with 8000 ppm.

The shootlets that survived on 6000 ppm were transferred for growing on free MS basal medium for one-month recovery. The last cycle of selection (cycle 3) was carried out by culturing all tolerated shoots on medium supplemented with 8000, 9000, 10000, 11.000, and 12.000 ppm of sea salt treatments. The experimented explants were grown well and gave healthy shootlets that tolerated 10.000 ppm of sea salt salinity (Fig 5) whereas, with 11.000 ppm shoots couldn't grow healthy, and with 12.000 ppm their color turned to yellow and never grown general. This system developed the salinity tolerance of sea salt to the maximum concentration of 10.000 ppm without morphological changes. Notably, this abiotic stress could induce the stressed plantlets to release minor salt crystalline callus as observed in Fig (6) which is no longer released with each culture on free MS for recovery.

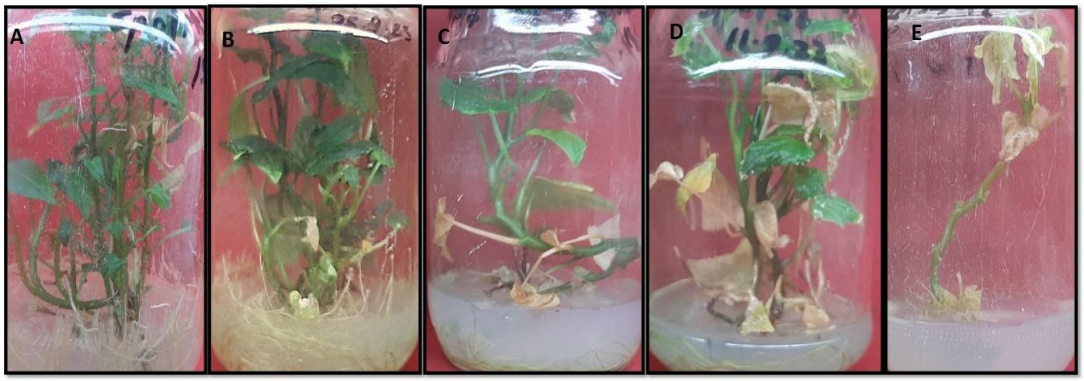

Fig. 5: Sweet potato plantlets produced from cycle 3 of ESC system A. The plantlets cultured on MS free, B. The plantlets cultured on MS with 9000 ppm and C. The plantlets cultured on MS with 10.000 ppm, D. The plantlets cultured on MS with 11.000 ppm and E. The plantlets cultured on MS with 12.000 ppm

Fig. 6: The abiotic stress induced the stressed plantlets to release minor salt crystalline callus on the surface of sweet potato leaves.

3.3. The influence of sea salt treatments on sweet potato plantlets during the exchangeable selection cycles (ESC).

Salt stress, a form of abiotic stress, plays an important role in determining the spread of sweet potatoes. Furthermore, salt stress is considered a major factor affecting the growth and productivity of sweet potatoes, especially in arid and semi-arid regions (Ahanger *et al.,* 2017). It is caused by the excess of Na+ and Cl-, which have a negative impact on plant growth and development, namely, low osmotic potential of the soil solution, nutritional imbalance, specific ion effect, and a combination of these factors (Tavakkoli *et al.,* 2010). The response of plants to salt stress mainly consists of numerous physiological and biochemical processes. The exchangeable selection system by El-Kazzaz *et al*. (1990) was established to confer sea water tolerance to different cultivars of potato (Jaerla, Cara, and Lady Rosetta) as reported in El-Kazzaz *et al.* (2016). This is the first study to apply this system to an Egyptian sweet potato cultivar. The tested nodal cuttings of sweet potato cultivar (Abees) in the first selection cycle showed tolerance to salinity of 3000 ppm of the sea salt treatments (1000, 2000, 3000, 4000 ppm) whereas they grew up healthy shootlets except those nodal cuttings tested at 4000 ppm showing color turned to yellow and grown unhealthy as seen in the obtained shootlets from the first selection cycle (3000 ppm) recorded shoot length (SL) at 4.8±0.37 and number of leaves/shoot (NL) at 5.9±0.4 and root length (RL)at 19.08±0.71 and root number/shoot (RN) at 14.4±1.20 (Figs 7, 10) were transferred as nodal cuttings on sea salt-free medium, and after one month of recovery subculture; they were transferred as nodal cuttings to the second selection cycle on the higher sea salt treatments (4000, 5000,

6000 and 7000 ppm) but low response observed to salinity treatment higher than 6000 ppm which recorded shoot length (SL) at 4.9±0.33 and number of leaves/shoot (NL) at 5.4±0.24 and root length (RL) at 16.08±1.28 and root number/shoot (RN) at 12.0±1.92. In general, *in vitro* shoot and root development of the selected sweet potato cultivar (Abees) plantlets was recorded under the different sea salt treatments of cycle 2 as shown in Fig (8).

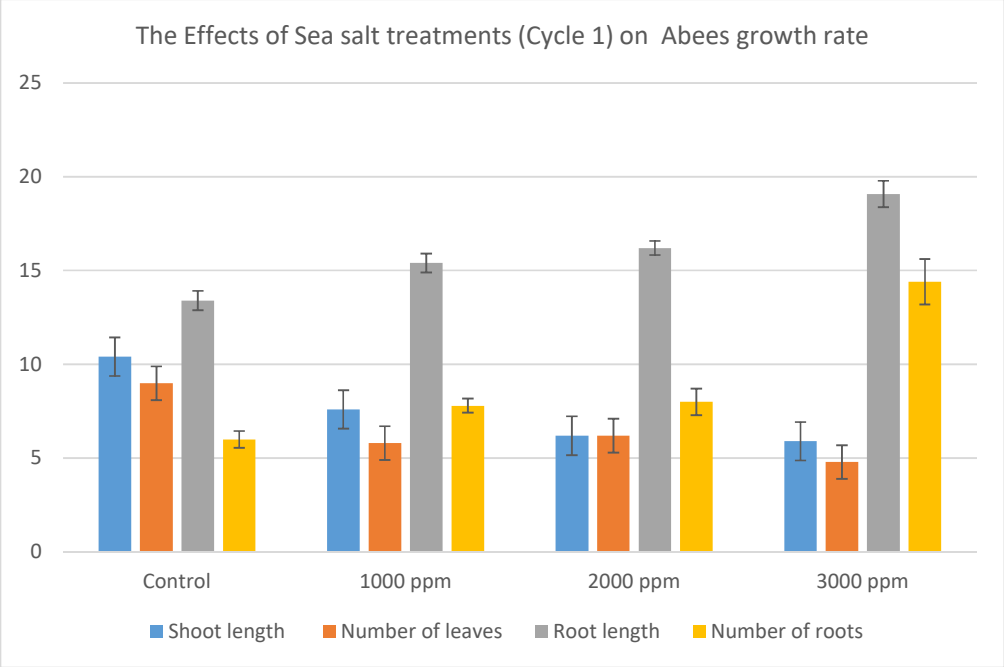

Fig. 7: Effects of the sea salt concentrations (1000, 2000, and 3000 ppm) compared to control without any sea salt concentration on the development of *in-vitro* sweet potato cultures after cycle 1.

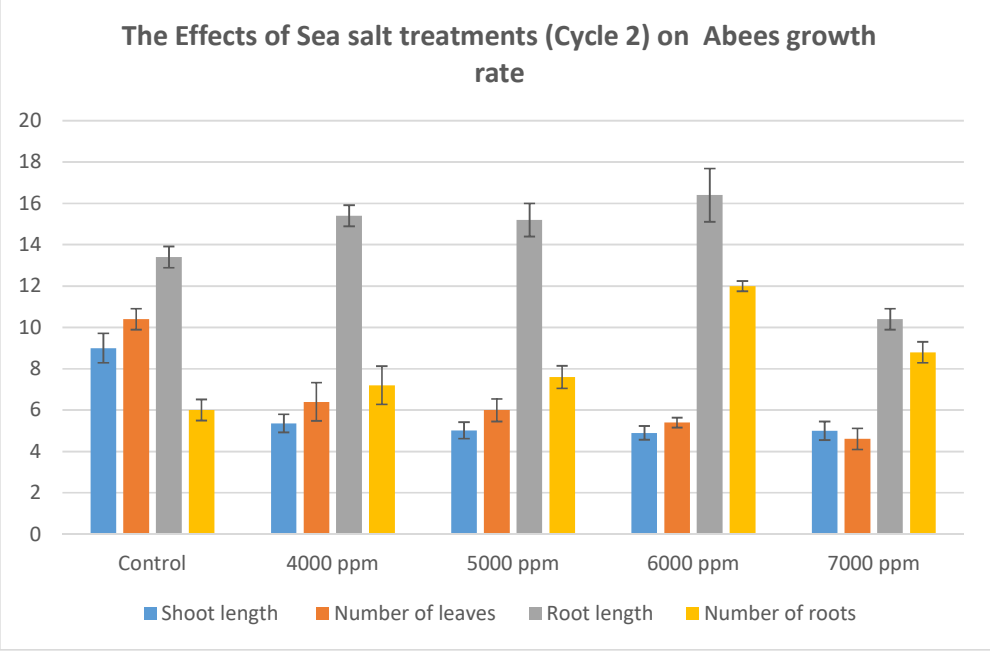

Fig. 8: Effects of the sea salt concentrations (4000, 5000, 6000, and 7000 ppm) compared to control without any sea salt concentration on the development of in vitro sweet potato cultures after cycle 2.

After one month of recovery on free sea salt medium, they were transferred as nodal segments to the third selection cycle on the higher sea salt treatments (8000, 9000, 10000, 11000, and 12000 ppm), no response was observed to salinity treatment higher than 10000 ppm which recorded shoot length (SL) at 7.0±0.70 cm and number of leaves/shoot (NL) at 5.6±0.5 and root length (RL) at 13.4±1.8 cm and root number/shoot (RN) at 7.6±0.5. The growth of the plants stopped with changing in color. In general, *in vitro* shoot and root development of the selected plantlets of sweet potato cultivar (Abees) was recorded under the different sea salt treatments of cycle 3 as shown in Fig (9). The results of the visual evaluation showed that there are differences in the response to salt stress between salt-tolerant and sensitive sweet potato varieties. In salt-sensitive varieties, growth was severely inhibited, wilting and yellowing symptoms appeared strongly after 14 days of salt stress. Most of the leaves dried up or the seedlings almost died. However, injury symptoms caused by salt stress appeared later in the tolerant cultivars, and leaf formation and elongation at the shoot tip were not significantly affected. These findings align with the findings of Farida *et al*. (2015) demonstrated that different sweet potato genotypes react differently to salt stress. They also discovered that the K+/Na+ content might be the cause of these variations. In maize, comparable outcomes were noted by Murat *et al*. (2016) with wheat crop, Begum *et al*. (2010) with Rice and by Chunthaburee *et al*. (2008).

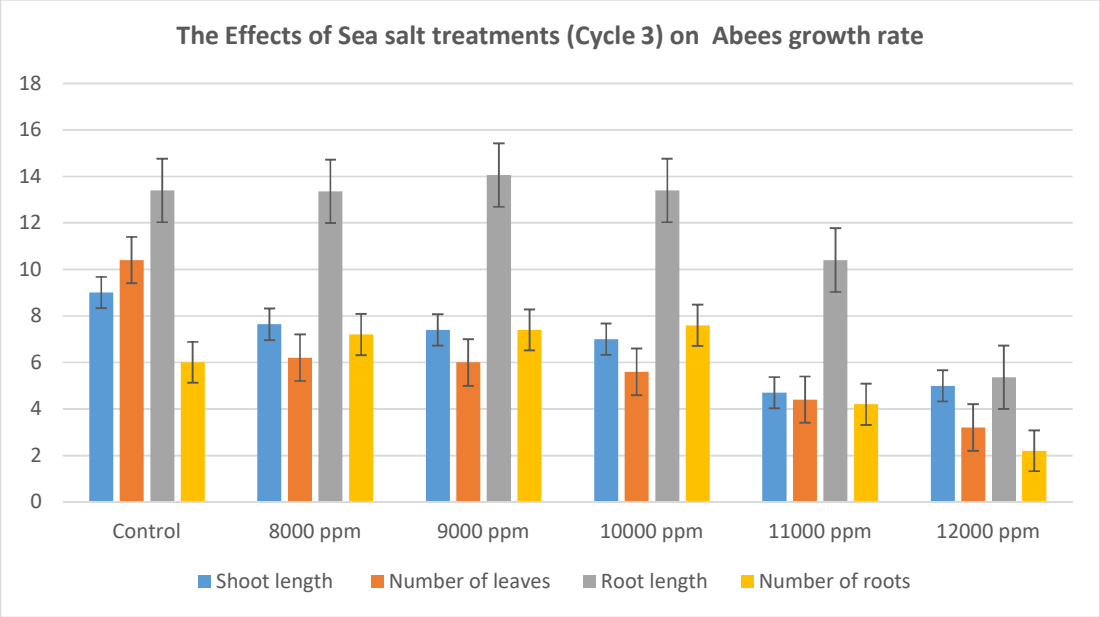

Fig. 9: Effects of the sea salt concentrations (8000, 9000, 10000, 11.000 and 12.000 ppm) compared to control without any sea salt concentration on the development of in vitro sweet potato cultures after the last cycle (cycle 3).

The data of the selected plantlets were recorded on the critical concentrations of the sea salt salinity treatments obtained from 3000 ppm in the first cycle, 6000 ppm in the second cycle and 10,000 ppm in the last cycle of sea salt salinity compared to the control as shown in Fig. (10) The tolerance strength of sea salt salinity increased after each cycle due to the adaptation of the selected plants to the higher salinity concentration gradually increasing, combined with recovery time during each exchangeable selection cycle. A significant result was recorded in terms of the development of the roots of the plantlets. Root length and number of roots increased under salinity in each cycle due to resistance to salt stress, as shown in Fig. (11).

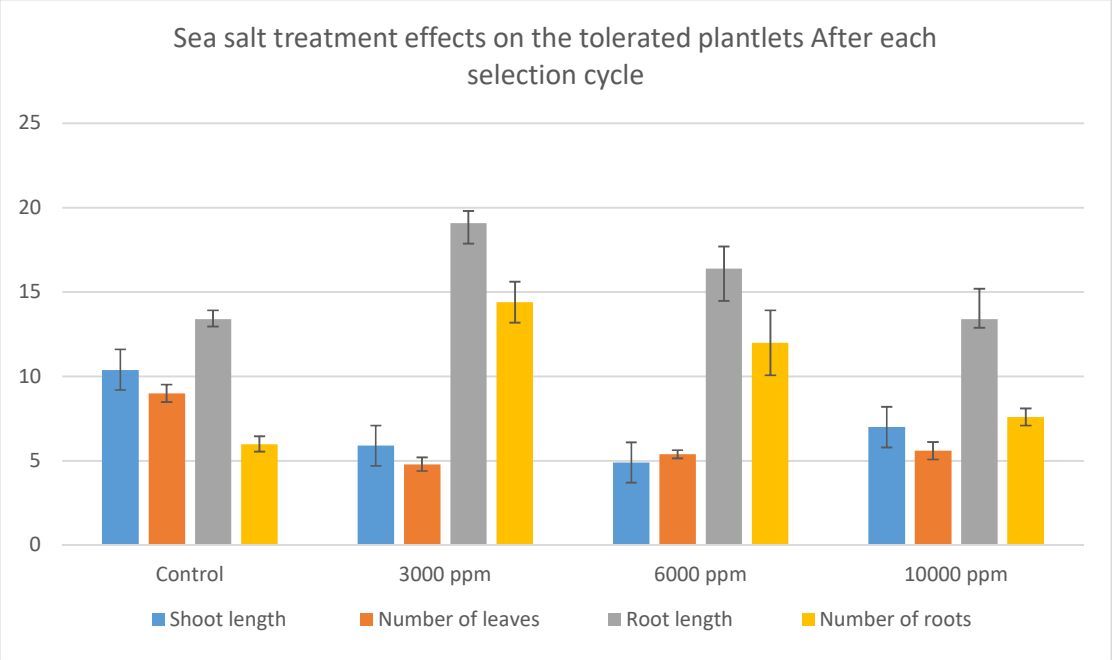

Fig. 10: The comparison between the growth rates of the selected plantlets after each selection cycle. The selected tolerated plants from cycle 1 at 3000 ppm, the selected tolerated plants from cycle 2 at 6000 ppm and the selected tolerated plants from cycle 3 at 10000 ppm.

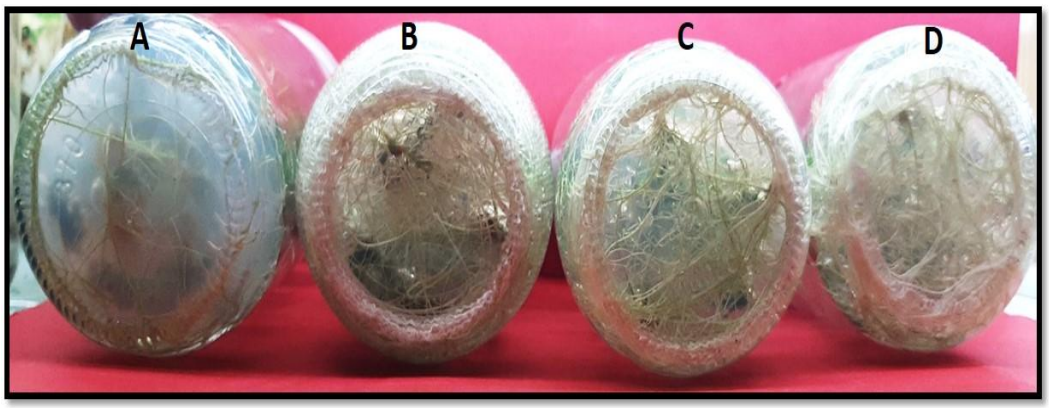

Fig. 11: Rooting of the selected plantlets after each selection cycle. A. control plants on free MS medium, B. The induced roots of the selected tolerated plants from cycle 1 at 3000 ppm, C. The induced roots of the selected tolerated plants from cycle 2 at 6000 ppm and D. The induced roots of the selected tolerated plants from cycle 3 at 10.000 ppm.

3.4 Effects of sea salt treatments on Proline contents and total Chlorophyll.

During the first phase of salt stress, plants accumulate fast-acting substances or small molecules and enzymes known as compatible solute, proline, peroxidase (POD), malondialdehyde (MDA), mannitol, and sorbitol-glycine betaine. The amount of accumulation of these small molecules and the activity of the enzymes determine the sweet potato resistance against salt stress (Rasool *et al.,* 2013). The total chlorophyll content decreased when plants were grown under sea salt stress conditions. The increase of salinity elevated the total chlorophyll when cultured on the higher concentration of sea salt in each exchangeable selection cycle (ESC) but decreased in the last cycle, recorded at 2.46, 2.74, and 1.35 mg/g with 3000, 6000, and 10.000 ppm, respectively. The increase of salinity increased significantly the total chlorophyll content in sweet potato cultivar 'Untacip' as studied by Rodríguez-Delfín *et al.* (2012). The proline content increased when elevating the salinity. In the leaves of the stressed plants recorded 15.21, 16.52, and 17.93 µmol/g with 3000, 6000, and 10.000 ppm respectively

while in the control plants, the value was 11.41 µmol/g as shown in Fig (12). During the first phase of salt stress exposure, proline accumulation in the leaves of the tolerant varieties (Xu-Zi-3 and Xu-28) was higher than that of the sensitive varieties (Yan-Zi-3 and Xu-22) as recorded by Mohamed and Qiang (2020). Strong accumulation of proline in tolerant cultivars helped reduce or delay the deleterious effects of salt stress (Lichtenthaler, 1998). Similar results were found by Gharsallah (2016), who clarified that in the first phase of salt stress, plants produce proline as a fast-acting substance to deal with stress. Furthermore, Zhang *et al.* (2017) found that plant tolerance was improved by increased proline and abscisic acid contents in leaf tissue during the first phase of salt stress in sweet potato. This study estimated and recorded the influence of sea salt stress on cultivar (Abees) of sweet potato and the conferred salinity tolerance that developed during the experiments using higher salinity concentrations through the modified exchangeable selection system.

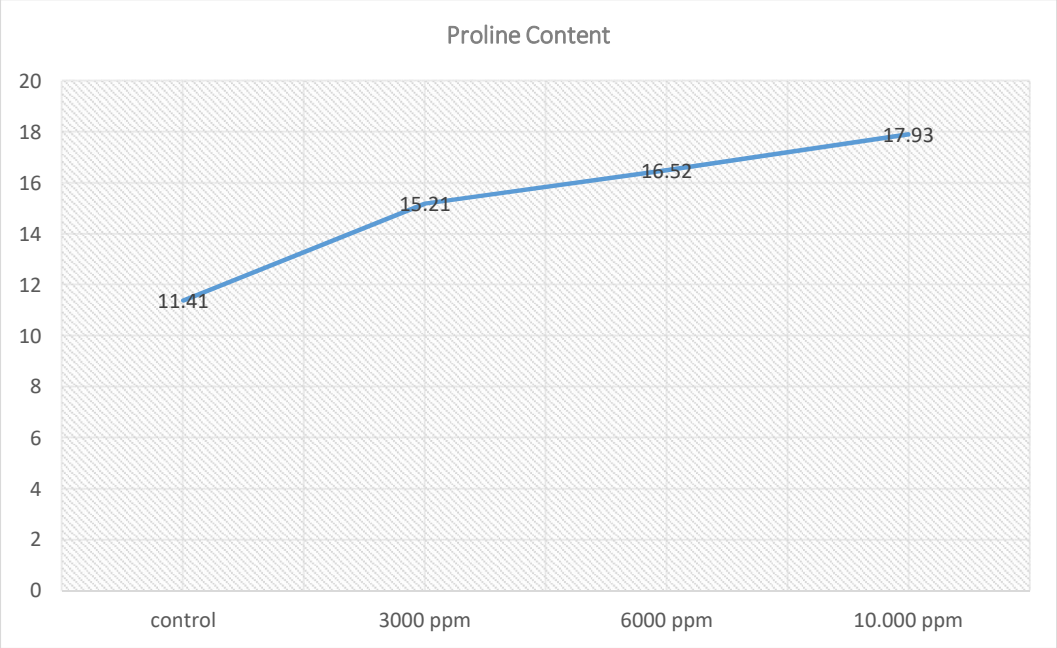

Fig. 12: The influence of sea salt treatments on the proline content in the *in vitro* tolerated sweet potato plants cultivar (Abees) after each cycle.

4. Conclusion

The epigenetic adaptation factor under salt stress conditions was presented in this study to improve a new sweet potato line by applying the system called exchangeable selection cycles which provides an effective screening method for estimating and developing salt tolerance in sweet potatoes. The in vitro sweet potato cultivar produced can tolerate sea salt concentrations between 9,000 and 10,000 ppm. These promising results present the possibility of selecting sweet potato plants that grow below seawater salinity. Through the exchangeable selection cycles, tuberous plants can be grown and developed gradually until they can be irrigated entirely with seawater when fresh water is scarce for irrigation.

5. Conflicts of interest

The authors declare there are no conflicts of interest.

6. Acknowledgement

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