Effects of Salt stress on banana (*Musa acuminata* L.) cv. Grandinin growing *in vitro*

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ABSTRACT

Salt is one of the most significant abiotic factors, which is a serious threat to crop yield worldwide, and it limits the growth and productivity of crops. Soil salinization is one of the most common abiotic stresses in agricultural production worldwide. Increasing salting area due to climate change and limited rainfall, the degraded area with saline soils has rapidly increased, which has led to great challenges facing global food security. Climate change-driven salinity stress increasingly threatens agricultural production, particularly for salt-sensitive crops like bananas (Musa spp.). Understanding the physiological and elemental responses of banana cultivars to salinity stress is crucial for developing mitigation strategies. This study investigated the in vitro effect of increasing NaCl concentrations (0, 500, 1000, 1500, and 2000 ppm) on the growth, physiological parameters, and element accumulation of banana (*Musa acuminata* L.) cv. Grandinin cultures. Banana plantlets were exposed to different NaCl concentrations in Murashige and Skooge medium for four weeks. Morphological characteristics, relative water content, chlorophyll content, elemental accumulation (N, K, P, Ca, Mg, Fe, Zn, Mn, Na, and Cl), and Proline content were measured. The results showed that NaCl stress significantly reduced plant growth parameters (shoot and root number, length, and formation percentage) and relative water content. Total chlorophyll content also decreased with increasing salinity. Conversely, Proline content and Na and Cl accumulation in leaves increased. Interestingly, the accumulation of essential elements (N, K, P, Ca, Mg, Fe, Zn, and Mn) decreased with increasing NaCl concentration. In conclusion, In vitro analysis revealed that *Musa acuminata* L. cv Grandinin exhibits sensitivity to NaCl-induced salinity stress, experiencing reduced growth, altered water balance, and disrupted chlorophyll metabolism. While Proline accumulation indicated a stress response, essential element uptake was compromised at higher salinity levels. These findings suggest the need for further research on salt-tolerant banana cultivars and improved irrigation practices to ensure sustainable banana production in the face of increasing salinity stress.

Keywords: Aseptic culture; salt stress; chlorophyll; proline; NaCl; plant growth parameters; banana.

INTRODUCTION

The banana (*Musa acuminata* L.) belongs to perennial monocotyledonous herbaceous plant of the order Zingiberales, which is not only the most widely consumed fruit but also the staple food of millions in many tropical countries and subtropical countries. Banana is grown in the humid tropics and subtropics of the Americas, Africa, and Asia, including Indonesia.. Although bananas are a popular food in Egypt, due to their high economic value, Egypt is not one of the world's largest banana exporters. The in vitro tissue culture technique involves the sterile culture of plant parts such as seeds, embryos, plant organs, explants, tissues, cells, and protoplasts in a container like a glass or bottle (Jain, 2010). Tissue culture can accelerate plant growth, and these plants are genetically uniform. The term "growth" refers to an increase in cell size, weight, and number. The height, wet weight, and dry weight of the plants can be used to calculate growth (Assani *et al*., 2001). Intensification, or expanding planting areas, is one method of increasing banana production in Egypt. However, Egypt's growing population hinders agricultural expansion, leading to a reduction in productive agricultural lands. The salinization of soil is a widespread environmental problem. Banana (Musa acuminata L.) is a salt-sensitive plant whose growth, development, and production are constrained by salt stresses. However, the tolerance mechanism of this salt-sensitive banana to salt stress is still unclear Salinization, the accumulation of dissolved salts in the soil due to anthropogenic processes, can be detrimental to plant growth. Because the osmotic pressure in the soil is higher than that in plant cells, high salt levels in the soil can cause plants to lose water and shrink (plasmolyze) (Yadav *et al*., 2011). In the process of plant growth, plants will inevitably face various biotic and abiotic stresses. In general, abiotic stress reduced crop yield by more than 50%, while biological stress reduced crop yield by less than 10% (Kreps *et al*., 2002). Salt is one of the most significant abiotic factors, which is a serious threat to crop yield worldwide, and it limits the growth and

productivity of crops. Soil salinization is one of the most common abiotic stresses in agricultural production worldwide. Due to climate change and limited rainfall, the degraded area with saline soils has rapidly increased, which has led to great challenges facing global food security (Godfray *et al.*, 2010). The growth and productivity of Musa spp are severely impacted by the gradual degradation of water resources and the erratic distribution pattern of annual precipitation amount (Abdel-Aziz *et al*., 2023). Improper soil salinity is one of several environmental pressures that can cause a loss of both vegetative potential and productivity. In Egypt, the low yield and output of bananas are attributed to the scarcity of water in this region and the global impact of various biotic and abiotic factors (Neumann, 1997). High salinity affects plant growth in three main ways: (1) by lowering the water potential in plants, (2) through the toxic effects of Na+ and Cl ions, and (3) by disrupting nutrient balance within the plant. Tissue culture, or in vitro tissue culture, has played a significant role in plant regeneration (Heslop-Harrison and Schwarzacher, 2007). This study investigates the in vitro responses of *Musa acuminata* L. cv Grandinin to salinity stress induced by different NaCl concentrations. Even at low salt concentrations, salinity limits plant vegetative and reproductive growth by causing widespread direct and indirect harmful effects (Shannon *et al*., 1994). Tissue injury is caused not only by the osmotic effects of salts, but also by specific toxic effects caused by Cl- and Na+ accumulation (Hasegawa *et al*., 2000). Bananas are grown in semi-arid climates where drought and salinity are common issues (Hassan *et al*., 2022). Salt tolerance varies greatly between plant genotypes (El-Hendawy *et al*., 2005). Some banana plantlet has been tested for salt tolerance in vitro (Mahmood *et al*., 2009). As the plant is very sensitive to salt stress, the yield of bananas reduced by half and the plant height decreased by about 75% under salt stress conditions (Yano-Melo *et al*., 2003). The goal of this study was to investigate how induced salinity levels of NaCl (0, 500, 1000, 1500, and 2000 ppm) affected physiological responses and element accumulation in banana (*Musa acuminata* L.) cv Grandinin cultures in vitro.

MATERIALS AND METHODS:

Plant material and experimental design:

This study was executed from 2019 to 2023 at the tissue culture laboratory of Horti-culture Dept., Faculty of Agriculture, and Al-Azhar University, where the suckers of *Musa acuminata* L Grandinin cultivars were taken from banana trees growing on a farm in the AL-Salhiyah region, Sharkia Governorate, Egypt.

Sterilization of explant:

The suckers were carefully washed in tap water to remove leaf sheaths and roots. The basal portion of the corm was then excised to a size of 12x12x15 mm. These explants were soaked in running tap water for 30 minutes, followed by a 30-minute soak in a cleaning solution while being continuously shaken. Detergent residues were then removed by washing with distilled water. The explants were then treated with a fungicide (SAAF + INDOFIL) for 30 minutes and finally rinsed with distilled water before being transferred to a laminar airflow chamber for further sterilization.

Preparation of culture media:

The explants were treated inside the laminar flow chamber with 70% ethanol for 2 minutes, and then washed with sterile water. Subsequently, the explants were treated for 5 minutes with 0.1% HgCl2 and rinsed three times with sterile water for 5 minutes each. The explants were cut to an 8x8x10 mm size, inoculated onto *Musa acuminata* L (MS) media supplemented in sterile conditions with agar at 7 g/L, sucrose at 30 g/L, NAA at 0.01 mg/L, and BA at 1.0 mg/L. They were then incubated in the dark for 21 days at 27±2 °C. The growth medium was replaced three times after 21 days, as the growth may be inhibited by phenolic compounds released into the medium. Current shoots of *Musa acuminata* L Grandinin cultivar were incubated at 27±2 °C with a 16-hour photoperiod for four weeks in the initial stage. After 30 days, uniformly developed explants were excised and re-cultured in the multiplication stage. Micro-shoots of *Musa acuminata* L Grandinin cultivar were cultured on MS media supplemented with sucrose at 30 g/L, agar at 7 g/L, and 1.0 mg/L each of BA and KIN. After another 30 days, uniformly developed explants were chosen and transferred to MS media consisting of 7 g/L agar, 1.0 mg/L BA, 30 g/L sucrose, and 0.01 mg/L NAA, with various concentrations of sodium chloride at 0.0, 250, 500, 1000, and 1500 ppm. The incubation conditions were kept the same as previously described in the multiplication stage, with the control group (0.0) using free MS media without NaCl.

Data collection and morphological characteristics:

Determination of morphological characteristics:

Growth of axillary shoots under salt stress evaluated after 30 days by measuring the increase in shoots number, shoot length (cm), roots number, and survival percentage was assessed based on the following equation: We used 9 plants for each treatment to estimate the survival percentage and the remainder of the morphological characteristics.

Shoot length (cm): summation of shoots length as cm / number of shoots.

Number of leaves per each shoot

Number of roots = calculated as the number of developed roots per each explants

Root length (cm): summation of roots length as cm / number of root.

Rooting %=[Number of rooted explants/ total number of cultured explants] X 100

Relative water content in leaf.

Relative water contents (RWC %) was implemented as previously reported Prior *et al* ., (1992), and was calculated using the following equation: To calculate the RWC, we used 9 leaves for each treatment. Leaf discs (about 1.0 cm in diameter) to minimize the area of chopped leaf surface/sample.

Relative water content (%) = (FW-DW)/(TW-DW) X100 (2)

Where: DW is the dry weight; FW is the fresh weight; and TW is the turgid weight.

Determination of photosynthetic pigments:

At the end of the experimental phase (30 days), the chlorophyll content was assessed using a portable chlorophyll meter (SPAD502, Minolta, Japan) as the SPAD unit; these units were then converted to mg m-2 as specified by Monje and Bugbee (1992) as follows:

Chlorophyll content (mg m-2) = 80.05+10.4(SPAD 502). Where: SPAD 502= chlorophyll meter reading (CMR).

Determination proline content.

The rapid colorimetric method was followed Bates *et al.* (1973) to estimate proline contents. Briefly, ninhydrin (1.25 g) was treated with glacial acetic acid (30 ml), followed by 6 M phosphoric acid (20 ml). The resulting mixture was heated until a clear solution was obtained and subsequently cooled and stored at 4°C (stable for 24h). Next, the explant material (0.5 g) was homogenized in 3% aqueous sulfosalicylic acid (10 ml), and subsequently filtered. The filtrate (2mL) was treated with freshly prepared acid ninhydrin (2mL), followed by glacial acetic acid (2mL). After the resultant mixture was heated at 100°C for 1 hour, the reaction mixture was poured into ice. The obtained mixture was subsequently extracted with toluene (4 ml) and vigorously mixed for 15-20 sec. The toluene phase was separated and the absorption of the chromophore was assessed by spectrophotometer at a wavelength of 520nm utilizing toluene as a reference blank. The concentration of Proline was finally evaluated from the standard curve and the fresh weight was defined.

Leaf nutrients:

Nitrogen (N) content was determined in leaf dry matter using the microkjeldahl method (Jackson & Sims, 1977). Phosphorus (P) concentration was determined colorimetric ally in leaf dry matter according to the method of Murphy and Riley (Murphy & Riley, 1962). Potassium (K) and sodium (Na) concentrations were determined in leaf dry matter using the method of Brown (Brown, J.G., 1946) by flame photometry. Calcium (Ca), iron (Fe), manganese (Mn), magnesium (Mg), and zinc (Zn) were measured in acid-digested solution using an atomic absorption spectrophotometer (AAS, AA4000, Spectrum-SP, Darmstadt, Germany) following the procedure described by Bouhlali *et al.* (2020). To measure Cl−, a chloridometer is typically used, which titrates the Cl− with Ag+ released from a silver wire according to the method of AWWA, APHA (1998).

Experimental design: The design of the present study followed a complete randomized block design with two factors, factor I: Sodium chloride (NaCl) treatments of various concentrations, i.e., 0, 500, 1000, 1500, and 2000 ppm and parameter and culture age repeated 3 times.

Statistical analysis

The analysis of variance was accomplished employing one -way ANOVA Co-stat software Stern, 1991 and means were compared using the Duncan test, a significant ($p \leq 0.05$).

RESULTS AND DISCUSSION:

Effect of Sodium chloride on Growth Morphological Parameters:

Data in Fig. 1&2&7 showed that exposing the *Musa acuminata* L. cv Grandinin cultivar to salt stress with NaCl significantly ($p \leq 0.05$) decreased the growth parameters compared to the unstressed *Musa acuminata* L. cv Grandinin. In general, there were significant differences between the stress treatments and the control. Adverse effects of salt stress on crops are nutrient limitations, ion toxicity, and oxidative and osmotic stresses (Shrivastava and Kumar, 2015). Salinity tolerance is under the control. Increasing NaCl concentration from 500 ppm to 2000 ppm led to a decrease in shoot number, shoot length, leaf number, root number, root length and root formation percentage, compared to the control. The maximum decrease occurred when the micro-plantlets of *Musa acuminata* L. cv Grandinin were cultured with MS media supplemented with 2000 ppm NaCl compared to unstressed and other treatments. Salinity greatly affects the growth of the banana plantlet *in vitro* culture. It was morphologically marked, especially in leaves and roots. This is may be due to the concentration of Na^+ ions which has inhibited high intake of K^+ ions into the plant. Normal plant cells have K^+ concentration higher than Na^+. The functions of the potassium ions among others are to maintain osmotic pressures in the cells, regulate the opening and closing of stomata, synthesize proteins and serve such as pyruvate kinase thus, the low concentration of K^+ in the cells causes chlorosis and necrosis.These results are in agreement with Bandita and Nibedita (2018), who showed that re-culturing the micro-shoots of Musa SPP. cv. Gaja Bantala on MS media supplemented with NaCl at 100, 200, 500, and 1000 ppm caused a reduction in root number compared to the control. The maximum decrease in root number occurred when the micro-shoots of Musa SPP. cv. Gaja Bantala were cultured on MS media supplemented with 1000 ppm NaCl compared to those of the control and other treatments. Similarly, Edriss *et al*. (2016) found that NaCl-induced salt stress ranging from 25 mM to 200 mM added to MS media reduced the morphological characteristics, such as shoot number, shoot length, leaf number, shoot fresh weight (g), shoot dry weight (g), survival percentage, callus formation, and root formation percentage, in all tested rootstocks, including some grape rootstocks, namely Salt Creek, Freedom, Dogridge, and Richter .

Effect of Sodium chloride on photosynthetic pigments:

Data in Figure 3 depict the responses of photosynthetic pigments. Increasing NaCl concentrations from 500 to 2000 ppm generally resulted in a decrease in photosynthetic pigments, like chlorophyll index, compared to unstressed *Musa acuminata* L. cv. Grandinin. Furthermore, the maximum reduction in all mentioned pigments was observed when the *Musa acuminata* L. cv. Grandinin plantlets were cultured on MS media supplemented with both 1500 and 2000 ppm NaCl compared to unstressed and other treatments. These findings are consistent with Sdek *et al*. (2017), who reported that MS media supplemented with NaCl at 0.0, 30, 60, 120, and 200 mM/L significantly decreased total chlorophyll content compared to the control. The maximum reduction was obtained when the micro-shoots of the tested cultivar were re-cultured on MS media supplemented with 200 mM/L NaCl compared to those of the control and other treatments. In plant photosynthesis, has important functions: one is to participate in the light absorption of precursor cells, and the other is to prevent the light oxidation of precursor cells (Muller *et al.*, 2001). Similarly, Abdrabboh *et al*. (2023) found that adding NaCl at 25, 50, 75, 100, 125, and 150 mM to MS media caused a reduction in photosynthetic pigments of some tested citrus rootstocks compared to the control. MS media supplemented with both 125 and 150 mM/L led to the maximum inhibition in chlorophyll a, b, total chlorophyll, and carotenoids in leaves of all tested citrus rootstocks compared to those of the control and other treatments. The decrease in chlorophyll during salinity stress is attributed to the diversion of glutamate, a precursor for chlorophyll and Proline, towards Proline production (Molazem *et al.*, 2010). Additionally, Oliveira *et al*. (2013) reported that chlorophyll a, b, and their ratio decreased in leaves when single nodes of some grape cultivars were cultured on MS medium supplemented with 100 mM NaCl. Changes in chlorophyll content are considered the most prominent biochemical response to salinity stress (Rao *et al*., 2007).

Effect of sodium chloride on the proline content:

Data in Figure 4 reveal that increasing NaCl concentrations from 500 to 2000 ppm in the growth medium led to a proline content increase in *Musa acuminata* L. cv. Grandinin compared to unstressed plants. The highest proline content was observed when *Musa acuminata* L. cv. Grandinin plantlets were cultured on MS media supplemented with

2000 ppm NaCl compared to unstressed and other treatments. These findings are consistent with Edriss *et al*. (2016), who studied the effect of NaCl at various concentrations (0.0, 25, 50, 75, 100, and 200 mM) on the proline content in leaves of four grape rootstocks. Proline content increased in all tested rootstocks as salinity increased. Significant differences were observed: Proline accumulation increased in all NaCl treatments compared to the control. Additionally, significant differences were found between rootstocks, with Salt Creek recording the highest Proline accumulation values at NaCl concentrations between 25 and 200 mM, followed by Freedom, Richter, and Dogridge. Similarly, Sdek *et al.* (2017) found that MS media supplemented with NaCl at 0.0, 30, 60, 120, and 200 mM/L caused a Proline content increase compared to the control. The highest Proline content was observed when the micro-shoots of the tested cultivar were re-cultured on MS media supplemented with 200 mM/L NaCl compared to those of the control and other treatments. Kafi *et al.* (2003) proposed that osmoregulation via cellulose solute accumulation, such as Proline, may be a mechanism for overcoming salt stress conditions (Sotiropoulos, 2007).

Effect of sodium chloride on relative water content:

The results in Figure 5 show the effects of salt stress on the relative water content (RWC) of *Musa acuminata* L. cv. Grandinin. Supplementation of the MS media with NaCl at 500, 1000, 1500, and 2000 ppm adversely affected the RWC of *Musa acuminata* L. cv. Grandinin compared to the control. The most significant negative effect was observed when plantlets were cultured on media supplemented with 2000 ppm NaCl compared to the control and other treatments. A high NaCl concentration in the growth medium has resulted in hyper ionic and hyper osmotic conditions in the plants. Excessive sodium ions in the medium will compete with potassium ions in order to be able to get into the plant. For plants susceptible to the condition of NaCl this condition will certainly influence their homeostatic system. An increased osmotic pressure in plants can lead to difficulty for water to get into the plant tissue. Eventually, the ability of the plant to grow will decrease. A plant's responses to salinity can inhibit its growth due to osmotic stresses; high osmotic pressure causes the inability of the plants to fully absorb water from the growth medium. In addition, there is also toxicity of ions or ion accumulation in the plant tissues. The number of ions can cause a disruption to the plant homeostatic process, resulting in a lot of proteins needed in the metabolism of the plants that are not well expressed (Munns and Tester, 2008). These findings align with Abdrabboh *et al*. (2023), who studied the impact of salt stress induced by varying NaCl concentrations (25-150 mM) on the RWC of various citrus rootstocks. Their results showed that RWC decreased in all tested rootstocks compared to the control when cultured on MS media containing any level of NaCl, regardless of the sodium chloride concentration or delivery method (25-150 mM NaCl, direct or shock treatment). The highest reduction in RWC occurred when Sour Orange and Volkamariana lemon shoots were cultured on the highest NaCl concentration (150 mM) compared to other rootstocks. Significant differences in RWC were observed among all citrus rootstocks when cultured on the same substrate.

Elements accumulation in leaf of the tested *Musa acuminata* L grandinin cv in relation to salt stress:

Data in Figure 6 show the effect of different NaCl concentrations (0.0, 500, 1000, 1500, and 2000 ppm) on the accumulation of various elements (N, Na, Mg, Cl, K, Ca, P, Fe, Zn, and Mn) in leaves of *Musa acuminata* L. cv. Grandinin. Clearly, increasing NaCl levels from 500 to 2000 ppm compared to the control decreased the accumulation of N, Mg, Ca, P, Fe, Zn, and Mn. The maximum reduction in the accumulation of all these elements was observed when *Musa acuminata* L. cv. Grandinin micro-shoots were cultured on MS media supplemented with 2000 ppm NaCl compared to the control and other treatments. These findings align with Edriss *et al.* (2016), who showed that MS media supplemented with NaCl at concentrations ranging from 25 to 200 mM led to a decrease in N, Na, Mg, Cl, K, Ca, P, Fe, Zn, and Mn compared to the control. Conversely, Na and Cl concentrations showed the highest values when micro-shoots of all tested grape rootstocks were re-cultured on MS media supplemented with higher levels of NaCl compared to the control and other treatments. The effect of K+ on osmotic potential balance depends on several factors, such as water content and cell wall elasticity (Mustard and Renault, 2004). Moreover, potassium is crucial for protein synthesis and stimulates photosynthesis (Buschman *et al*., 2000). An interaction between K+ and Na+ might be a key factor in determining the salinity tolerance of plants (Buschman *et al*.,

2000). The uptake of K+ is affected by Na+ due to their chemical similarities. As K+ is an essential nutrient for most terrestrial plants, transport systems with good selectivity for K+ over Na+ are considered important salt tolerance determinants (Amini and Ehsanpour, 2005).

CONCLUSION

This study demonstrates the susceptibility of *Musa acuminata* L. cv Grandinin to in vitro NaCl-induced salinity stress. Increasing NaCl concentrations significantly hampered plant growth, water balance, and chlorophyll metabolism. While Proline accumulation provided a stress response, essential element uptake was progressively compromised at higher salinity levels. Future research can pave the way for sustainable banana cultivation in the face of rising salinity challenges, safeguarding food security and agricultural livelihoods in affected regions.

REFERENCES

Abdel-Aziz, Hosny Abdel, Mohamed Sharaf, Magdy Omar, Ahmed Abou El-Yazied, Nada Ibrahim AlJwaizea, Shaimaa Ismail, Mohamed Omar, Khadiga Alharbi, Amr Elkelish, and Moataz Tawfik. "Improvement of Selected Morphological, Physiological, and Biochemical Parameters of Banana (Musa acuminata L.) Using Potassium Silicate under Drought Stress Condition Grown in vitro." PHYTON-INTERNATIONAL JOURNAL OF EXPERIMENTAL BOTANY 92, no. 4 (2023): 1019-1036.

Amini, F., Ehsanpour, A.A. 2005: Soluble proteins, proline, carbohydrates and Na+/K+ changes in two tomato Lycopersiconesculentum Mill.) Cultivars under in vitro salt stress. - Amer. J. Biochem. Biotechnol. 1: 212-216,

Assani, A., Haicour, R., Wenzel, G., Cote, F., Bakry, F. 2001: Plant regeneration from protoplasts of dessert banana cv. Grande Naine (Musa spp., Cavendish sub-group AAA) via somatic embryogenesis. Plant Cell Rep., 20: 482-488.

AWWA, APHA. 1998: Standard Methods for the Examination of Water and Wastewater, 18th ed. AWWA, APHA. Method 4500 Cl−.

Bandita, D., Nibedita, C. 2018: "Influence of NaCl Treatments on Micropropagation of Musa SPP. CV. Gaja Bantala" SSRG International Journal of Agriculture & Environmental Science 5.2 (2018): 59-64.

Bates, L.S., Waldren, R.P., Teare, I.D. 1973: Rapid Determination of Free Proline for Water-Stress Studies. Plant Soil 1973, 39, 205–207, doi:10.1007/BF00018060.

Behboudian, M.H., Törökfalvy, E., Walker, R.R. 1986: Effects of salinity on ionic content, water relations and gas exchange parameters in some Citrus scion—rootstock combinations. ScientiaHorticulturae, 28(1), 105-116.

Buschmann, P., Vaidynathan, R., Gassmann, W., Shroeder, J. 2000: Enhancement of Na+ uptake currents, time-dependent inward-rectifying K+channel currents, and K+ channel transcripts by K+ starvation in wheat root cells. Plant Physiology 122: 1387–1398.

Cavagnaro, J.B., Ponce, M.T., Guzmán, J., Cirrincione, M.A. 2006: Argentinean cultivars of Vitis vinifera grow better than European ones when cultured in vitro under salinity. Biocell, 30(1), 1-7.

Edriss, M.H., Baghdady, G.A., Abdrabboh, G.A., Abdel Aziz, H.F. 2016: April. In vitro responses of some grape rootstocks to salt stres. 3. In International Conference on Biotechnology Applications in Agriculture (ICBAA), Benha University, Moshtohor and Sharm El-Sheikh (pp. 5-9).

El-Hendawy, S.E., Hu, Y., Yakout, G.M., Awad, A.M., Hafiz, S.E., Schmidhalter, U. 2005: Evaluating salt tolerance of wheat genotypes using multiple parameters. European journal of agronomy, 22(3), 243-253.

Abdrabboh, G.A., Abdel-Aziz, H.F., Abd, A.A. 2023: *In vitro* screening for salt stress tolerance of certain citrus rootstocks through the exposure of their roots. Al-Azhar Journal of Agricultural Research.Article impress.

Godfray, H.C.J., Beddington, J.R., Crute, I.R., Haddad, L., Lawrence, D., Muir, J.F. 2010: Food security: the challenge of feeding 9 billion people. *Science* 327 812–818.

Hasegawa, P.M., Bressan, R.A., Zhu, J.K., andBohnert, H.J. 2000: Plant cellular and molecular responses to high salinity. Annual review of plant biology, 51(1), 463-499.

Hassan, I.F., Gaballah, M.S., Ogbaga, C.C., Murad, S.A., Brysiewicz, A., Bakr, B. M., Alam-Eldein, S.M. 2022: Does melatonin improve the yield attributes of field-droughted banana under Egyptian semi-arid conditions?. Journal of Water and Land Development.

Heslop-Harrison, J.S., Schwarzacher, T. 2007: Domestication, genomics and the future for banana. Ann. Bot., 100: 1073-1084.

Jain, S.M. 2010: In vitro mutagenesis in banana (Musa spp.) improvement. Acta Hort., 879: 605-614.

Kafi, M., Kamkar, B., Mahdavi, D.A., 2003: Crops' Responses to the Growth Environment (Doctoral dissertation, M. Sc thesis, University of Ferdowsi, Mashad, Iran (in Persian)).

Kreps, J.A., Wu, Y., Chang, H.S., Zhu, T., Wang, X., Harper, J.F. 2002: Transcriptome changes for Arabidopsis in response to salt, osmotic, and cold stress. Plant Physiol. 130, 2129–2141.

Mahmood, M., Rahman, Z.A., Saud, H.M., Shamsuddin, Z., Subramaniam, S. 2009: Responses of Banana Plantlets to Rhizobacteria Inoculation under Salt Stress Condition. American-Eurasian Journal of Sustainable Agriculture, 3(3), 290-305.

Molazem, D., Qurbanov, E.M., Dunyamaliyev, S.A. 2010: Role of proline, Na and chlorophyll content in salt tolerance of corn (Zea mays L.). American-Eurasian Journal of Agriculture and Environmental Science, 9, 319-324.

Monje, O.A., Bugbee, B. 1992: Inherent limitations of nondestructive chlorophyll meters: a comparison of two types of meters. HortScience, 27(1), pp.69-71.

Muller, P., Li, X.R., Niyogi, K.K. 2001: Non-photochemical quenching. A response to excess light energy. Plant Physiol. 125, 1558–1566.

Mustard, J., Renault, S. 2004: Effects of NaCl on water relations and cell wall elasticity and composition of red-osier dogwood (Cornusstolonifera) seedlings. Physiologiaplantarum, 121(2), 265-271.

Neumann, P. 1997: Salinity resistance and plant growth revisited. Plant, Cell & Environment, 20(9), 1193–1198.

Oliveira, D.A., Salvador, A.A., Smânia, A., Smânia, E.F., Maraschin, M., Ferreira, S.R. 2013: Antimicrobial activity and composition profile of grape (Vitis vinifera) pomace extracts obtained by supercritical fluids. Journal of biotechnology, 164(3), 423-432.

Prior, L., Grieve, A., Cullis, B. 1992: Sodium Chloride and Soil Texture Interactions in Irrigated Field Grown Sultana Grapevines. II. Plant Mineral Content, Growth and Physiology. Aust. J. Agric. Res. 1992, 43, 1067, doi:10.1071/AR9921067.

Rao, A.R., Dayananda, C., Sarada, R., Shamala, T.R., Ravishankar, G.A. 2007: Effect of salinity on growth of green alga Botryococcusbraunii and its constituents. Bioresource technology, 98(3), 560-564.

Munns, R., Tester, M. 2008: Mechanisms of salinity tolerance. Annu. Rev. Plant Biol., 59: 651-681.

Sdek, W.Z., Elazab, D.S., Aboul-Nasr, M.H., Elmahdy, T.K. 2017: Effect of Salinity and Drought Stress on Potassium Uptake in Musa Spp in vitro. Assiut Journal of Agricultural Science, 48 (3). 2017 May 1:198-211.

Shannon, M.C., Grieve, C.M., Francois, L.E. 1994: Whole-plant response to salinity. Plant-environment interactions. Marcel Dekker Inc., New York, NY, 199-244.

Shrivastava, P., Kumar, R. 2015: Soil salinity: a serious environmental issue and plant growth promoting bacteria as one of the tools for its alleviation. *Saudi J. Biol. Sci.* 22 123–131.

Sotiropoulos, T.E. 2007: Effect of NaCl and CaCl2 on growth and contents of minerals, chlorophyll, proline and sugars in the apple rootstock M 4 cultured in vitro. Biologiaplantarum, 51(1), 177-180.

Stern, R.D. 1989: CoStat-Statutical Software. California: CoHort Software (1989), Pp. 302, $76.00. Ex. Agric. 1991, 27, 87–87, doi:10.1017/S0014479700019232.

Vilarinhos, A.D., Piffanelli, P., Lagoda, P., Thibivilliers, S, Sabau, X., Carreel, F., D'Hont, A. 2003: Construction and characterization of a bacterial artificial chromosome library of banana (Musa acuminata Colla). Theor. Applied Genet., 106: 1102-1106.

Walker, R.R., Douglas, T.J. 1983: Effect of salinity level on uptake and distribution of chloride, sodium and potassium ions in citrus plants. Crop and Pasture Science, 34(2), 145-153.

Yadav, S., Irfan, M., Ahmad, A., Hayat, S. 2011: Causes of salinity and plant manifestations to salt stress: A review. J. Environ. Biol., 32: 667-685.

Yano-Melo, A.M., Saggin, O.J., Maia, L.C. 2003: Tolerance of mycorrhized banana (*Musa* sp. cv. Pacovan) plantlets to saline stress. *Agric. Ecosyst. Environ.* 95 343–348.

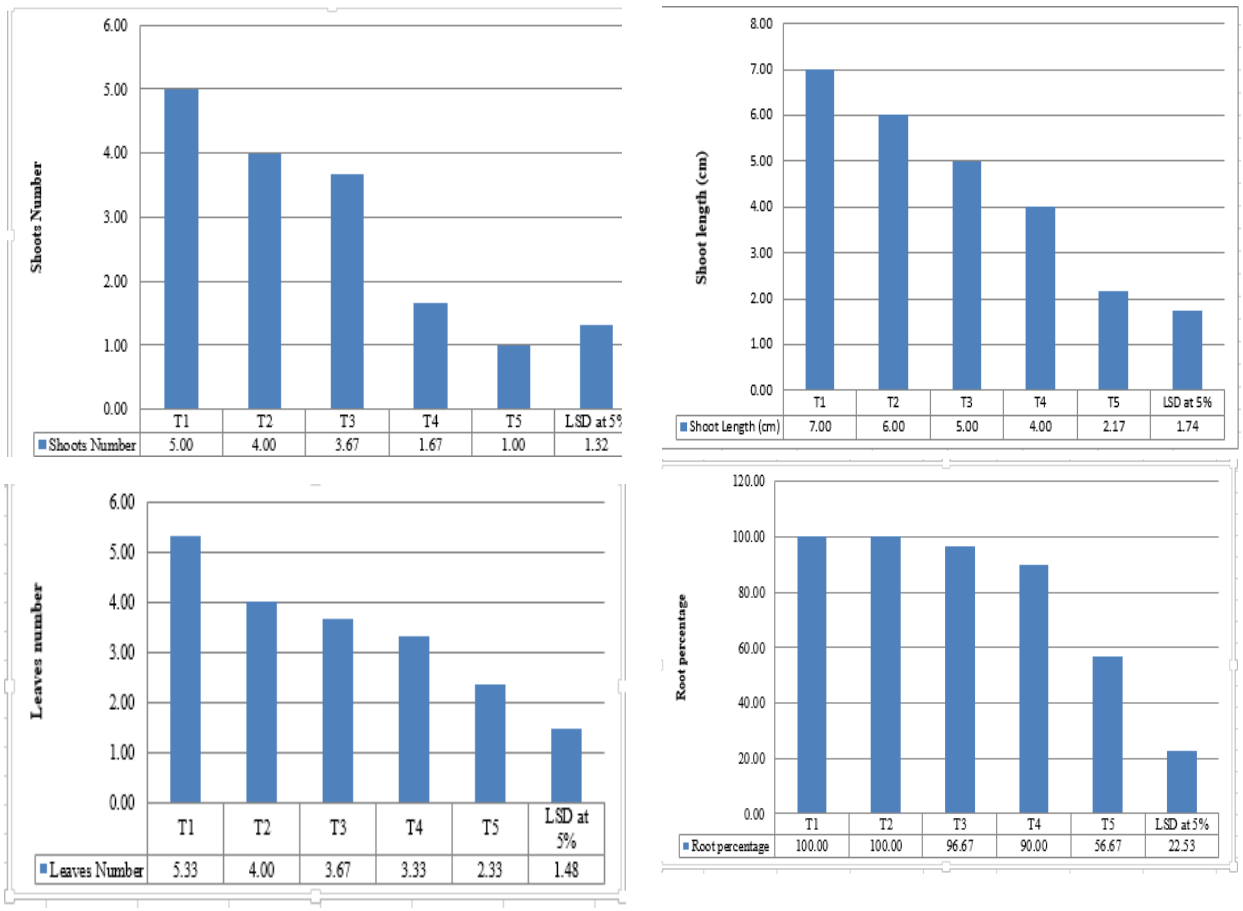


Figure 1: Effect of MS media supplemented with NaCl at different concentrations on shoot number, shoot length, leaf number and root formation percentage of *Musa acuminata* L Grandinin cv growing *in vitro*. T1: Control,T2 500 ppm (NaCl),T3: 1000 ppm(NaCl),T4: 1500 ppm(NaCl),T5: 2000 ppm (NaCl)

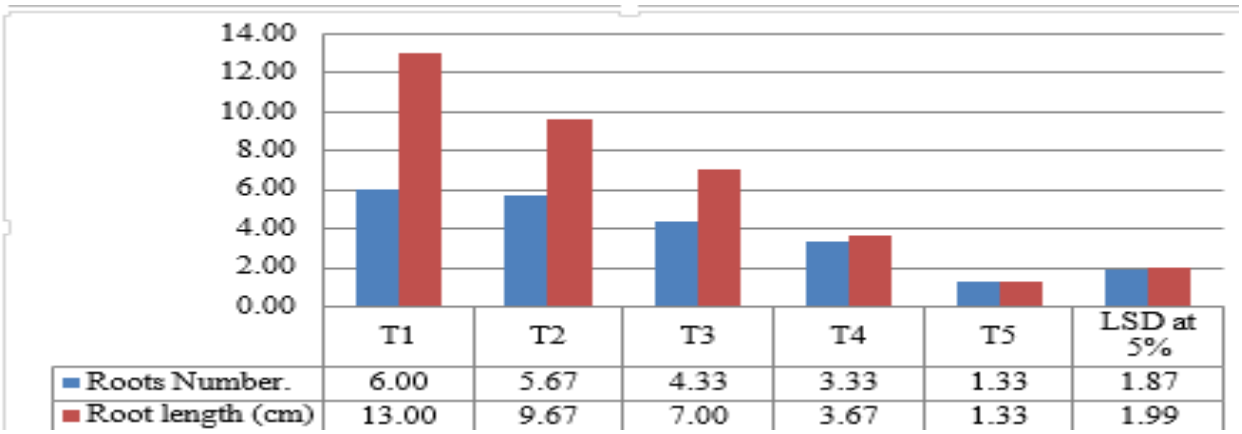


Figure 2: Effect of MS media supplemented with NaCl at different concentrations on root no. and root length of *Musa acuminata* L Grandinin cv growing *in vitro*. T1: Control,T2 500 ppm (NaCl),T3: 1000 ppm(NaCl),T4: 1500 ppm(NaCl),T5: 2000 ppm(NaCl)

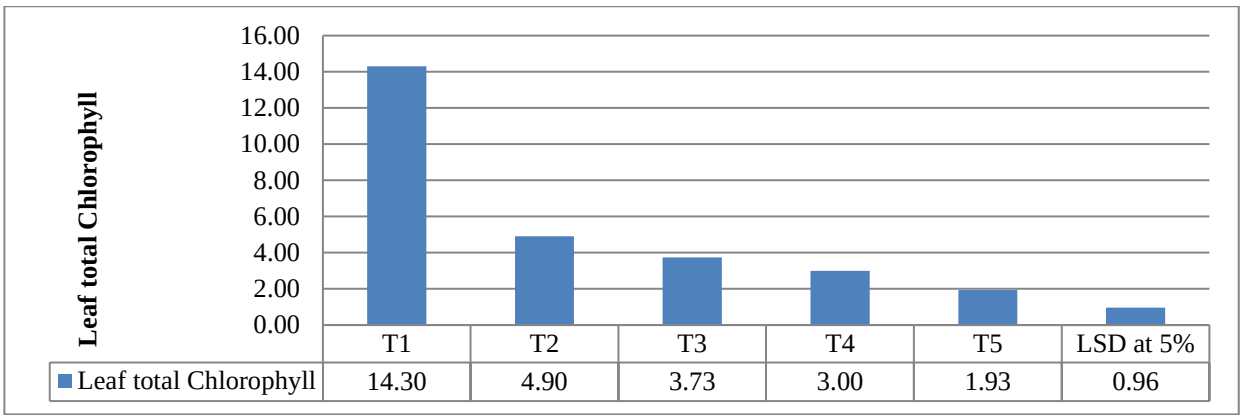


Figure 3: Effect of MS media supplemented with NaCl at different concentrations on leaf total chlorophyll content of *Musa acuminata* L grandinin cv growing *in vitro*. T1: Control,T2 500 ppm (NaCl),T3: 1000 ppm(NaCl),T4: 1500 ppm(NaCl),T5: 2000 ppm(NaCl)

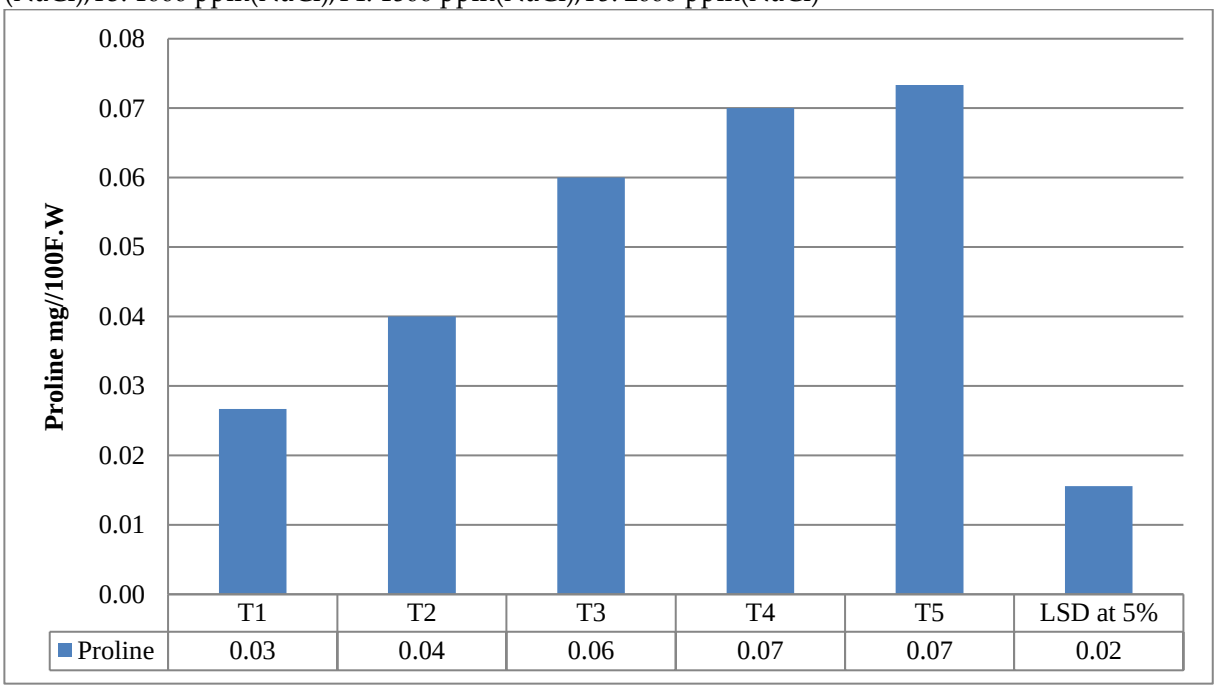
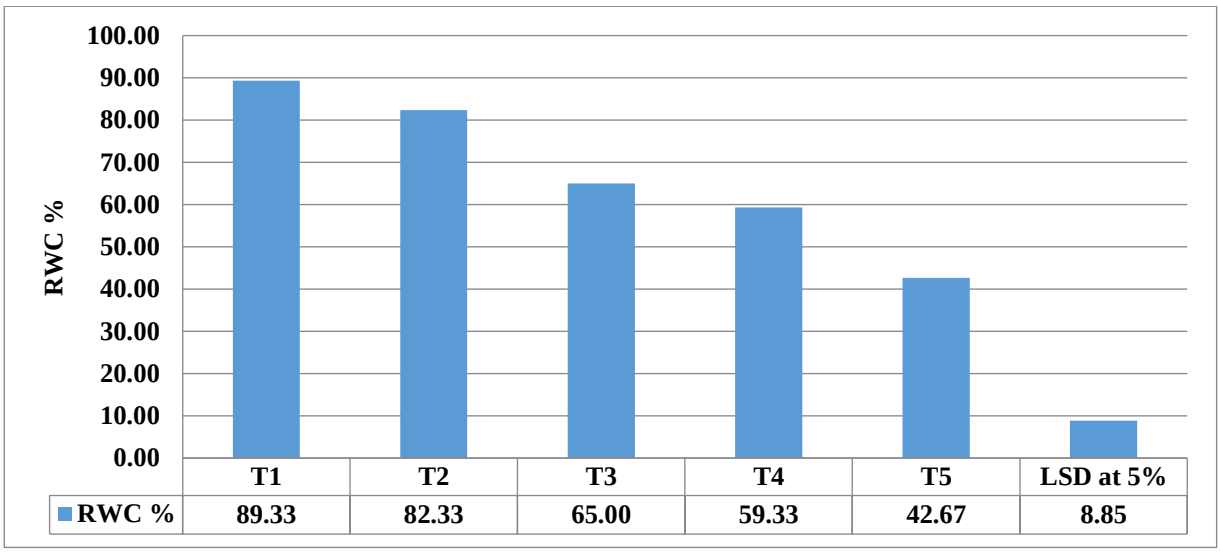
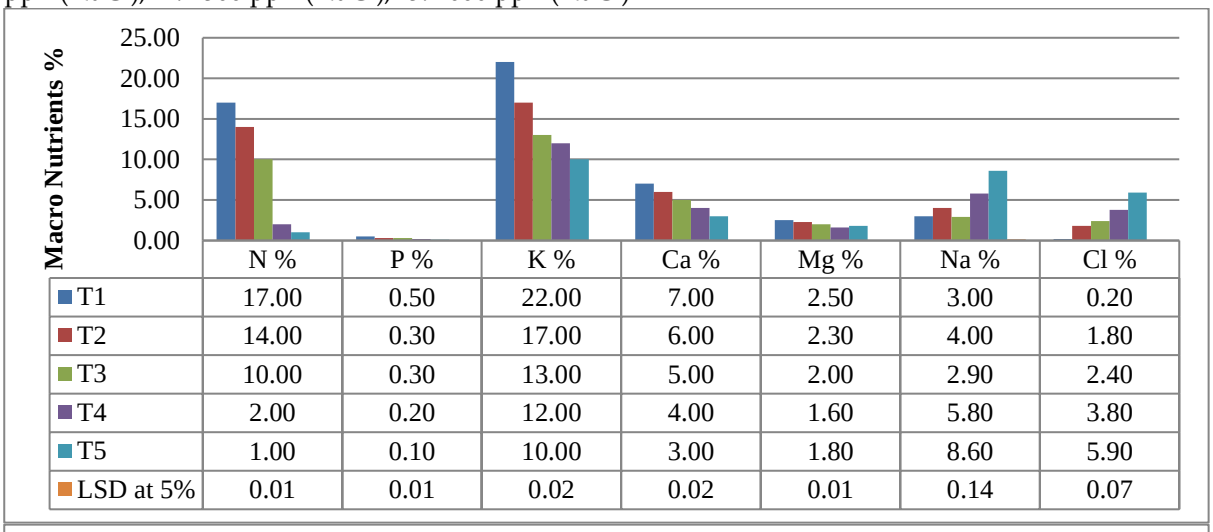


Figure 4: Effect of MS media supplemented with NaCl at different concentrations on proline content of *Musa acuminata* L grandinin cv growing *in vitro*. T1: Control,T2 500 ppm (NaCl),T3: 1000 ppm(NaCl),T4: 1500 ppm(NaCl),T5: 2000 ppm(NaCl)

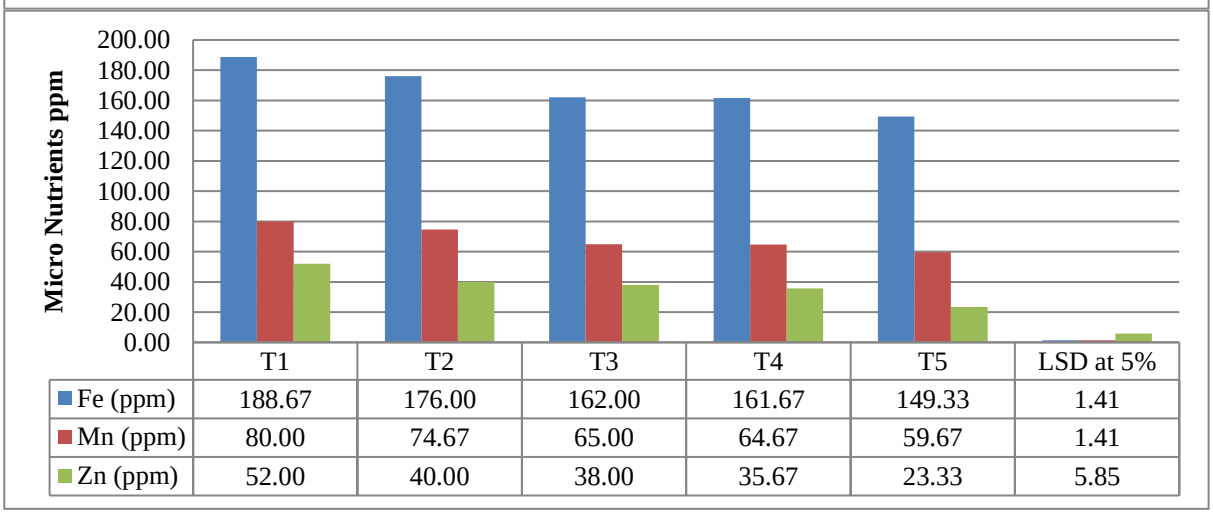


	T1	T2	T3	T4	T5	LSD at 5%
RWC %	89.33	82.33	65.00	59.33	42.67	8.85

Figure 5: Effect of MS media supplemented with NaCl at different concentrations on relative water content of *Musa acuminata* L grandinin cv growing *in vitro*. T1: Control,T2 500 ppm (NaCl),T3: 1000 ppm(NaCl),T4: 1500 ppm(NaCl),T5: 2000 ppm(NaCl)



	N %	P %	K %	Ca %	Mg %	Na %	Cl %
T1	17.00	0.50	22.00	7.00	2.50	3.00	0.20
T2	14.00	0.30	17.00	6.00	2.30	4.00	1.80
T3	10.00	0.30	13.00	5.00	2.00	2.90	2.40
T4	2.00	0.20	12.00	4.00	1.60	5.80	3.80
T5	1.00	0.10	10.00	3.00	1.80	8.60	5.90
LSD at 5%	0.01	0.01	0.02	0.02	0.01	0.14	0.07



	T1	T2	T3	T4	T5	LSD at 5%
Fe (ppm)	188.67	176.00	162.00	161.67	149.33	1.41
Mn (ppm)	80.00	74.67	65.00	64.67	59.67	1.41
Zn (ppm)	52.00	40.00	38.00	35.67	23.33	5.85

Figure 6: Effect of MS media supplemented with NaCl at different concentrations on accumulation some elements content of *Musa acuminata* L grandinin cv growing *in vitro*. T1: Control,T2 500 ppm (NaCl),T3: 1000 ppm(NaCl),T4: 1500 ppm(NaCl),T5: 2000 ppm(NaCl)

(A) :Mass production (B):Control (without NaCl) (C) :Treatment with NaCl

Figure 7: *In vitro* response of *Musa acuminata* L grandinin cv to salt stress.

تأثيرات تحمل الاجهاد الملحى على الموز صنف جراندنان النامى فى زراعة الأنسجة

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الملخص العربى

الملح أحد أهم العوامل الاجهاد البيئي التي تشكل تهديدًا خطيرًا للمحصول على مستوى العالم، كما أنه يحد من نمو وإنتاجية المحاصيل. وتعد عملية ملوحة التربة واحدة من أكثر الاجهادات البيئية شيوعًا في الإنتاج الزراعي حول العالم .ازدياد مساحة الملح تأثير الإجهاد الملحي الناجم عن تغير المناخ على إنتاج المحاصيل الزراعية بشكل متزايد، خاصة بالنسبة للمحاصيل الحساسة للملوحة مثل الموز .يعد فهم الاستجابات الفسيولوجية والعنصرية لأصناف الموز للإجهاد الملحي أمرًا ضروريًا لتطوير طرق التحمل. صممت هذه الدراسة التأثير المختبري لزيادة تركيز كلوريد الصوديوم (0، 500، 1000، 1500، و 2000 جزء في المليون) على نمو ومعلمات فسيولوجية وتراكم العناصر في زراعة الموز صنف جراندنان تعرضت شتلات الموز لتركيزات مختلفة من كلوريد الصوديوم في وسط موراشيجي وسكوك لمدة أربعة أسابيع. تم قياس الخصائص المورفولوجية والمحتوى النسبي للمياه ومحتوى الكلوروفيل وتراكم العناصر (النيتروجين والفسفور والبوتاسيوم و الكالسيوم والمغنيسيوم والكلور والصوديوم) ومحتوى البرولين. أظهرت النتائج أن الإجهاد الملحي قلل بشكل كبير من معلمات نمو النبات (عدد البراعم والجذور وطولها ونسبة التكوين) والمحتوى النسبي للمياه. كما انخفض إجمالي محتوى الكلوروفيل مع زيادة الملوحة. على العكس من ذلك، زاد محتوى البرولين وتراكم الصوديوم والكلور في الأوراق. ومن المثير للاهتمام أن تراكم العناصر الأساسية (النيتروجين والفسفور والبوتاسيوم و الكالسيوم والمغنيسيوم) والعناصر الصغرى (الحديد والزنك والمنجنيز) انخفض مع زيادة تركيز كلوريد الصوديوم. في الختام، كشف التحليل المختبري أن صنف جراندنان حساس للإجهاد الملحي الناجم عن كلوريد الصوديوم، حيث يعاني من انخفاض النمو واختلال توازن المياه واضطراب عملية التمثيل الغذائي للكلوروفيل. في حين أشار تراكم البرولين إلى استجابة للإجهاد، فإن امتصاص العناصر الأساسية قد تأثر عند مستويات ملوحة أعلى. تشير هذه النتائج إلى الحاجة إلى مزيد من البحث حول أصناف الموز المقاومة للملوحة وتحسين ممارسات الري لضمان إنتاج مستدام للموز في مواجهة تزايد الإجهاد الملحي.

الكلمات الاسترشادية: مزرعة معقمة , الاجهاد الملحى ,الكلوروفيل , كلوريد الصوديوم , الموز , قياسات نمو النبات.