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In Vitro Production of Cestrum Nocturnum L. Plants for Drought Tolerance

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Abstract

This experiment was carried out on *Cestrum nocturnum* plants at the tissue culture laboratory, Faculty of Agric. Saba Basha, Alex. Univ., during the 2019 and 2020 years to find a reliable protocol to investigate the micropropagation of different PGRs (KIN, BA and NAA) and to progress the plant's *in vitro* micropropagation ability under different levels of salinity. *In vitro*,MS culture media supplemented with BA and KIN at 0.25 mg/L, and NAA at 0.50 mg/L yielded the best results *in vitro* for both the shooting and rooting stages. For the enhancement of *in vitro* propagation ability under salinity stress, shootlets of *C. nocturnum* L. plants grown on MS media supplemented with 0.5 g/L NaCl + 5 or 10 g/L mannitol survived at 100%, but 91.0 and 89.36 percent, respectively, resulted from 1 g NaCl + 5 or 10 g/L mannitol, which nearly survival values were equaled with the control treatment.

Keywords: *Cestrum nocturnum,* Micropropagation, Salinity, Mannitol, PGRs

1. Introduction

Cestrum has around 300 species, the majority of which are native to warm subtropical and tropical regions. *Cestrum nocturnum* belongs to the *Solanaceae* family. Cestrum species are all evergreen, shadow-loving shrubs with toxic fruits (Maruyama, 1995). Because of its powerfully perfumed flowers at night, it is also known as Night blooming Cestrum, Lady of the Night, Queen of the Night, Night blooming Jessamine, and Night blooming Jasmine (Sultana and Bari Miah, 1992).

Tissue culture is a popular method of propagation in modern agriculture because it allows for the development of a large number of clonal plants from a small amount of starting material.Micropropagation is a relatively new technology and innovative method that has helped to overcome barriers to progress in the multiplication of elite species, and further advancements are expected (Ashish and Sharma, 2011). Plants are exposed to biotic and abiotic stresses as a result of environmental degradation, which limits their development and growth (Shao and Chu 2005). The primary abiotic factors that cause crop damage worldwide are salinity and drought (Vinocur and Altman 2005).

Rising salinity harms plants severely, causing growth inhibition, necrosis, decreased metabolism, and a decline in productivity and quality (Sivritepe and Eris 1999). Salt stress has a major effect on plant growth and development and agricultural productivity. The responses to salinity vary not only among the different ornamental plants but also among the different organs of a plant. Depending on osmotic upset, some physiological changes occur in stomatal conductance, transpiration, photosynthesis, chlorophyll content, and root and leaf activity. These changes may permit their use as parameters in salt tolerance screening (Kucukahmetler, 2002). Salinity stress causes an increase in reactive oxygen species (ROS) (O2; OH; H2O2; 1O2), resulting from oxidative stress that can damage DNA, generate lipid peroxidation, and inhibit enzymes (Gill and Tuteja, 2010). In addition to inorganic ions, amino acids, sugars, sugar alcohols, and organic acids can improve salt stress tolerance (Munns, 2005).

Mannitol accumulation occurs when plants are applied with low water potential (Patonnier *et al*., 1999), and this is controlled by inhibiting competing pathways and decreasing mannitol intake and catabolism (Stoop *et al*., 1996). The addition of chemicals to the growth media is the basis for these experimental setups. Similarly, NaCl is given to the medium to expose plants to salt stress, which is a combination of

osmotic stress (since NaCl decreases the medium's water potential) and Na+ toxicity, which is most noticeable at high NaCl concentrations (Munns and Tester, 2008). Plant tissue culture can be used to demonstrate how plants react to salt stress. *In vitro* propagation allows for more control than in vivo growth conditions and has the added benefit of being on a small scale, allowing for the monitoring of shoot and root responses in the presence of applied stress (Shibli *et al*., 1992). A range of factors, the most essential of which are PGRs (Taha *et al*., 2008), could influence many plants effectiveness with sequential micropropagation. Night Jasmine is a very important decorative plant. However, a low multiplication rate, sensitivity to salinity, and disease transmission all impede its cultivation in order to meet the demand for high-quality planting material for commercial cultivation. As a result, an effective *in vitro* micropropagation protocol is regarded as the best solution to this problem.The purpose of this study was to optimize a micropropagation strategy that used several PGRs (KIN, BA and NAA) and enhanced plant salt tolerance so that it could be grown in new places that were unsuitable for growing other plants.

2. Materials and Methods

The experimental work on *Cestrum nocturnum* L. plants was carried out at Saba Basha Tissue Culture Laboratory Technique, Faculty of AgricultureSaba Basha, AlexandriaUniversity from 2019 to 2020, to estimate and improve the plant's *in vitro* propagation potential under the effect of salinity stress.

2.1. Plant materials

Nodal explants of *Cestrum* plants were obtained from the mother plants from Horticultural Research Institute, Agriculture Research Center, Alexandria,and cleaned with running tap water for 1 hour. Explants were surface disinfected under sterile conditions with 70% alcohol for 1 minute, 15% (v/v) Clorox for 15 minutes, and mercuric chloride at 0.1 percent (w/v) for 5 minutes. The explants were rinsed five times with sterile distilled water after each disinfection treatment.Explants were cultured in MS medium (Murashige and Skoog, 1962) enriched with 2.5 percent sucrose and 0.7 percent agar (w/v) before being autoclaved at 1.2 kg/cm2 for 20 minutes. The pH of the culture medium was set at 5.6. Culturing was carried out in glass jars with 30 ml of medium placed vertically. Each treatment was three times reproduced and included five explants (for 15 explants per treatment). Jars were incubated in a growth room at 251°C and 80% humidity for 16 hours of daily light and 8 hours of darkness under a 2880 lux florescent light (40 mol-2S-1PPF).

2.2. In vitro experimental stages

Explants at the initiation stage were grown on solidified woody plant media for 35 days. PGRs were added to MS medium at various dosages to evaluate *in vitro* propagation performance: Benzyladenine at 0.25 and 0.50 mg/L with or without Kinetin at 0.25 mg/L and Naphthyl acetic acid at 0.25 or 0.50 mg/L. The *in vitro* growth (shooting and rooting) conduct of nodal explants attained from the most appropriate multiplication medium (MS + 0.25 mg/L BA + 0.25 mg/L KIN + 0.25 mg/L NAA) was examined under different concentrations of NaCl (0.0, 0.5, 1.0, 2.0, 4.0 mg/L), and two concentrations of Mannitol (5.0 and 10.0 g/L) were investigated in order to enhance *in vitro* growth capability under salt stress.

2.3. Chemical analysis

Plant material was collected after the multiplication stage, and photosynthetic pigments (chlorophyll a and b) and carotenoids were measured in shootlet tissues as mg/100 g fresh weight using a

spectrophotometer, according to the procedure developed by Saric *et al*., (1967), but proline was measured according to Bates *et al*., (1973).

2.4. Experimental design and data analysis

The data were statistically analyzed with a completely randomized design (C.R.D.) with three repetitions. A significant difference test at 0.05 (L.S.D 0.05) was used to compare treatment means.

3. Results

Table 1 and Figure 1 show the micro-shooting and rooting ability of *C. nocturnum* plants in response to varied plant growth regulator (Kin, BA, and NAA) concentrations. The MS media supplemented with KIN and benzyladenine at 0.25 mg/L and NAA at 0.50 mg/L produced the highest percentage of survived explants (99%), the longest shootlets (5.26 cm), and the highest root number (6.00) when compared to the control. While the number of shootlets (6.69) and leaves (10.17) were obtained from MS medium treated with BA at 0.50 + Kin and NAA at 0.25 mg/L, respectively. Data also demonstrated that when compared to other treatments, utilising a low concentration of BA (0.25 mg/L) mixed with Kinetin (0.5 mg/L) and NAA at 0.25 or 0.50 mg/L was preferred for increasing the micro proliferation ability of shootlets. This demonstrates the importance of all PGRs in achieving the internal hormone balance that gives rise to the best results.
The effect of salinity stress on micro-propagated plants The NaCl treatments had a substantial effect on the growth characteristics of *C. nocturnum*, such as survival rate, number of shootlets, shoot length, number of leaves, and number of roots. As shown in Table 2, Figures (2, 4 and 6), all *in vitro* growth characteristics, namely survival percent, shootlet number, and length/explant, leaves number, roots number, Chl.a., Chl.b., carotenoids, and proline content, decreased significantly and gradually as NaCl concentrations increased from 500 to 4000 ppm, with the exception of proline content, which increased significantly. When compared to the control, 500 ppm of NaCl did not significantly impair survival (98.66 percent) but did result in an increase in shoot number (5.42). At 4.0 mg/L NaCl, shoot length, leaf number, and shootlet number was reduced to about half of the control, while survival was reduced to 55.36 percent. As explants were treated with 4.0 mg/L NaCl, the number of roots decreased considerably from 0.5 to 2.0 mg/L NaCl when compared to the control treatment.

Table (1): Effect of different levels of KIN, BA and NAA (mg/L) and their combinations on the initiation stage of *Cestrum nocturnum* nodal cuttings cultured *in vitro* for 35 days.

Character			**Survival (%)**	**Shootles number/explant**	**Shootlet length(cm)**	**Leaves number/shootlet**	**Roots number**
PGR (mg/L)							
KIN	**BA**	**NAA**					
0.0	0.0	0.0	30.11	0.29	1.64	4.30	1.66
0.0	0.25	0.0	61.16	1.11	3.17	4.82	2.73
0.0	0.50	0.0	65.34	1.33	2.89	6.26	2.33
0.0	0.25	0.25	85.00	1.64	4.00	6.37	3.86
0.0	0.50	0.50	88.18	1.84	4.16	6.88	3.69
0.25	0.25	0.25	98.73	4.62	5.00	6.27	5.03
0.25	0.25	0.50	99.00	4.23	5.26	6.62	6.00

0.25	0.50	0.25	96.79	6.69	4.96	10.17	3.93
0.25	0.50	0.50	97.94	4.33	4.80	8.56	4.30
L.S.D.$_{0.05}$			3.85	0.15	0.35	0.51	0.45

1. Fig. 1 Micropropagation of *Cestrum nocturnum* under the effect of Kin, BA and NAA at 0.25mg/L.
2. Table (2): *In vitro* shooting, rooting and photosynthetic pigments (chlorophyll a and b), carotenoids of *Cestrum nocturnum* L. plants under the effect of salinity stress.

Salinity (ppm)	**Survival (%)**	**Shootlets number /explant**	**Shootlets length (cm)**	**Roots number**	**Leaves number**
Control	100.0	5.33	5.62	6.00	10.70
500ppm	98.66	5.42	3.35	3.31	7.50
1000ppm	87.00	3.45	1.72	1.48	6.68
2000ppm	72.87	3.23	1.67	0.33	5.75
4000ppm	55.36	2.25	1.39	0.00	3.55
L.S.D.$_{0.05}$	5.31	0.59	0.06	0.35	0.63

Fig. 2 Micropropagation of *Cestrum nocturnum* under the effect of salinity stress, only.

As illustrated in the Table of Contents (3) and Figure (3), the combination of mannitol and salt improved all aspects of personality. When shootlets were cultivated on MS media augmented with 0.50 NaCl plus 5 or 10 g mannitol, comparable to the control treatment, survival was 100%. In addition, the addition of 0.5 g NaCl with 10 g mannitol resulted in the highest results after the control for the number of shootlets, length of shootlet, and number of leaves (4.66, 5.27 cm, and 10.20, respectively). With rising salt concentrations, all metrics declined considerably. Even when 1 g of NaCl was added, the shootlets formed roots. However, using higher concentrations of 2 and 4 g inhibits root formation, as shown in

Table 3. These findings show that plants can thrive at concentrations ranging from 500 to 1000 ppm of NaCl, but greater concentrations induce poor growth or death. Mannitol combined with salt stress increased shootlet development.

Table (3): Alleviation of salinity stress on the *in vitro Cestrum nocturnum* L. under the effect of mannitol concentrations

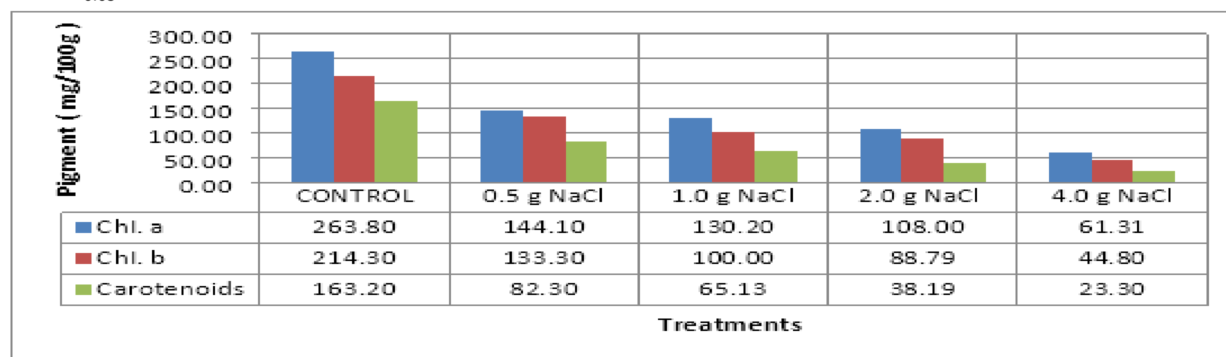
Characters Treatments	**Survival %**	**Shootlets number/explant**	**Shootlet length(cm)**	**Leaves number**	**Roots number**
Control	100.0	6.52	6.54	10.14	6.13
0.5g NaCl+5g mannitol	100.0	4.36	5.24	9.74	4.48
0.5g NaCl+10g mannitol	100.0	4.66	5.27	10.20	5.47
1.0 g NaCl+5g mannitol	91.0	2.99	2.09	8.86	3.41
1.0g NaCl+10g mannitol	89.63	3.38	2.14	9.24	3.55
2.0 g NaCl+5g mannitol	58.06	1.19	1.06	6.85	1.42
2.0 gNaCl+10g mannitol	68.10	1.23	2.10	7.41	1.64
4.0 g NaCl+5g mannitol	27.33	0.76	1.33	4.21	0.53
4.0g NaCl+10g mannitol	32.52	0.99	1.50	4.11	1.03
$L.S.D._{0.05}$	0.78	0.37	0.74	0.48	0.36

Fig. 3 Micropropagation of *Cestrum nocturnum* under the effect of salinity stress + mannitol.

Data showed that photosynthetic pigments under salinity stress (Fig 4) were affected by diverse levels of NaCl on the chlorophyll content of the leaves, with chlorophyll a and b. The salt level and chlorophyll a and b content had an inverse relationship. As the concentration of NaCl increased, the amount of

chlorophyll in the solution decreased. Chlorophyll a and b have the lowest amounts (61.13 and 44.80 mg/100g F.W. at 4.0 mg/L NaCl, respectively) as compared to the control (263.80 and 214.30 mg/100g F.W., respectively). Following the carotenoids content through salt stress exposure of explants, it appears from Fig. (4) that salt stress (NaCl) was an inhibitory agent for carotenoids creation in stressed leaves, where the carotenoids content was lowered. The lowest carotene content (23.30mg/100 g F.W.) was created at a NaCl concentration of 4000 ppm, while the maximum quantity (163.20mg/100 g F.W.) was produced in the control treatment. With increasing salinity, there were large and steady declines. The data in Fig. 5 show that mannitol concentration plays a role in pigment accumulation in cestrum shootlets produced under salt stress. Shootlets grown on MS culture medium supplemented with 0.5 g NaCl plus 5 g mannitol had the highest chlorophyll a and b levels (211.13 and 125.16 mg/100g F.W., respectively), as well as the highest carotenoids content (200.90 mg/100g F.W.).

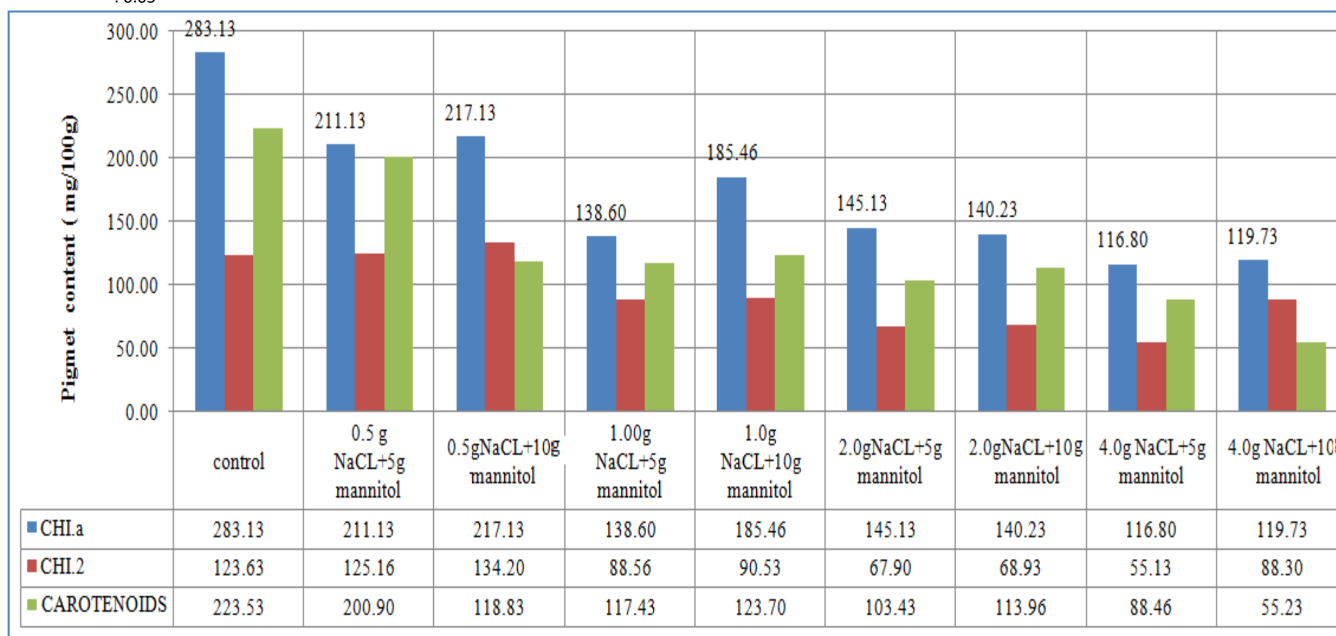
L.S.D. $_{0.05}$ Chl.a. 2.79 Chl.b. 9.08 carotenoids. 2.30



	CONTROL	0.5 g NaCl	1.0 g NaCl	2.0 g NaCl	4.0 g NaCl
Chl. a	263.80	144.10	130.20	108.00	61.31
Chl. b	214.30	133.30	100.00	88.79	44.80
Carotenoids	163.20	82.30	65.13	38.19	23.30

Fig. (4) The content of photosynthetic pigments (mg/100 g F.W.) in *C. nocturnum* shootlets is affected by salt levels.

L.S.D. $_{0.05}$ Chl.a. 17.65 Chl.b. 4.36 Caroteinods. 6.84



	control	0.5 g NaCL+5g mannitol	0.5gNaCL+10g mannitol	1.00g NaCL+5g mannitol	1.0g NaCL+10g mannitol	2.0gNaCL+5g mannitol	2.0gNaCL+10g mannitol	4.0g NaCL+5g mannitol	4.0g NaCL+10g mannitol
CHI.a	283.13	211.13	217.13	138.60	185.46	145.13	140.23	116.80	119.73
CHI.2	123.63	125.16	134.20	88.56	90.53	67.90	68.93	55.13	88.30
CAROTENOIDS	223.53	200.90	118.83	117.43	123.70	103.43	113.96	88.46	55.23

Fig (5). Photosynthetic pigments contents (mg/100gF.W) in *Cestrum nocturnum* L. shootlets under different salinity levels and mannitol concentrations.

Figure (6) depicts the response of proline content in Cestrum shootlets to salt stress. The highest proline concentration was 0.594 mol/mg. A minor increase was detected when the explants were grown on the greatest concentrations of NaCl, but a minor increase was detected when the explants were grown on the lowest concentrations of NaCl (4.0 mg/L), where the proline level was highest.The results shown in Fig. (7) showed that proline content in shootlet leaves ranged from 0.274 to 0.433 mol/mg in the control treatment and 2 g NaCl + 10 g mannitol, respectively, and that these variations did not reach statistical significance.

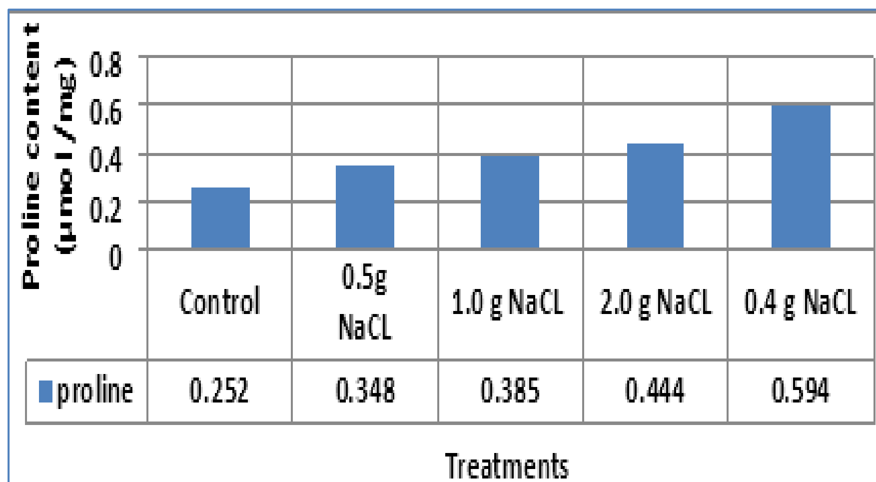


Fig (6) Proline content (mol/mg) in *Cestrum nocturnum* shootlets as affected by salinity levels.
L.S.D.$_{0.05}$:NS

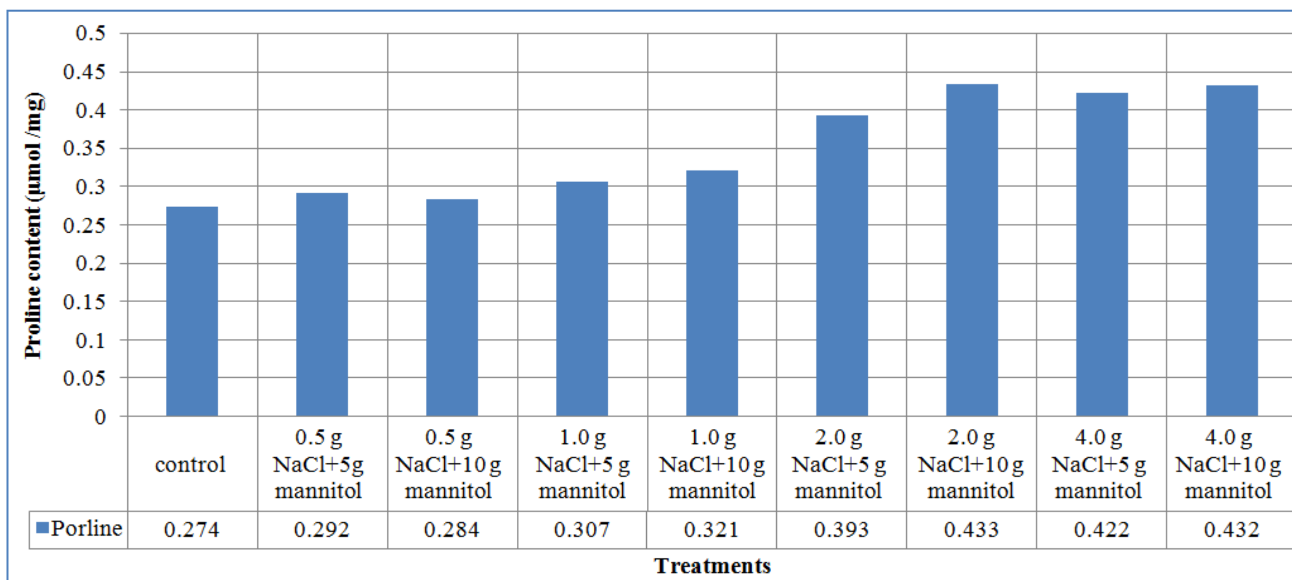


Fig (7). Proline content (mol/mg) in *Cestrum nocturnum* shootlets as affected by salt stress and mannitol levels.

Acclimatization stage

Cestrum micro-plants were carefully removed from the media and put in the greenhouse in small plastic pots with peat moss and sand medium (1:1). Finally, the plants were toughened by gradually lowering the humidity. After four weeks of transplanting, the success rate of acclimation reached 95% of the plants.

(Udin,2016) stated that plantlets of Cestrum were successfully transferred to pots and acclimatized (fig. 8).

Figure (8). Acclimatization stage of Cestrum plantlets grown on rooting medium of peatmoss and sand at (1:1, v/v) over four weeks.

4. Discussion

The addition of plant growth regulators (PGRs) to the culture media increased the *in vitro* proliferation of Cestrum shootlets in this investigation (Table 1). The obtained results agree withprevious studies (Rasheed, 2013; Udin, 2016) that indicated that varying concentrations of BA, NAA, and IBA had a substantial effect on *Cestrum nocturnum* bud sprouting. It was also reported that adding IBA to the culture medium encouraged bud break, particularly when BAP concentrations were low. Because of the high concentration of BAP used, the inhibition of adventitious meristem elongation may reduce the number of shoots (Borchetia *et al*., 2009).

In terms of the effect of salt stress on *in vitro* propagation ability (Table 2), salinity treatments significantly slowed the growth of apple rootstock explants. When the saline level was high: shoot length, shoot number, root number, rooting percentage, and length of roots were all reduced (Bahmani *et al*., 2012). Shahid *et al*. (2011)and Youssef *et al*. (2019) observed a decrease in shoot length as the NaCl concentration increased. This could be explained by insufficient photosynthesis produced by stomatal closure and a decrease in carbon absorption rate under salinity stress (Ben Ahmed *et al*., 2009). In terms of the data in Table 3, employing mannitol in conjunction with salt stress increased *in vitro* growth marginally. According to Abebe *et al*. (2003) and Matter *et al*. (2017), the increased growth performance of mannitol-accumulating calli and mature leaves was related to mannitol's additional stress-protective activities. However, the amount of mannitol accumulated in the callus and the new weight in the callus were insufficient to protect against stress *via* osmotic adjustment. Nelson *et al*. (2004) observed that water stress was reduced by mannitol when seeds of soybean (Glycine max L. Merril.) were submitted to sodium chloride.

Proline is a common organic compound that functions as an osmotic intercessor during salinity stress, an energy sink, a subcellular structural stabilizer, and even a stress-linked signal. It also helps with cell osmoregulation and protein protection during dehydration, and it can operate as an enzymatic regulator under stress conditions (Rontain *et al*., 2002).According to the results presented in Figs. (4 and 5), the pigments (chlorophyll a, b, and carotene) in cestrum leaf shootlets were drastically reduced when exposed to a high level of NaCl. The reaction of proline to salt stress (Figs. 6 and 7) could be attributable to these

osmoprotectants, which are important in lowering stress-induced cellular acidification, osmotic adjustments, and maintaining subcellular structures for recovery (Tan *et al*., 2008). Recent studies have also found that proline's protective function is based on preventing stress damage to the protein turnover machinery and upregulating stress-protective proteins (Khedr *et al*., 2003).
Notes rising proline accumulation with NaCl augmentation, the techniques plants have adapted to cope with stressful circumstances. The effect of salinity stress on proline accumulation in plant tissues, salt stress can cause changes in the organization of N-containing combinations, particularly proteins and free amino acids. The most well-known is proline accretion, which is commonly used as a salt stress indicator (Ashraf and Foolad, 2007). By increasing the free amino acids, we may be able to identify a resistance to salinity. Proline aids in the stabilization of subcellular structures (e.g. proteins and membranes), the caging of free radicals (Apel and Hirt, 2004), and the defense of cellular redox potential under stress situations (Ashraf and Foolad, 2007). Drought, for example, is imitated by adding osmotica, such as mannitol, sorbitol, or polyethylene glycol (Verslues *et al*., 2006), which lowers the medium's water potential. This makes it more difficult for plants to extract water, thus replicating what happens in dry soil.

Conclusion

MS culture medium augmented with benzyladenine at 0.25 mg/L, and Kinetin with indole butyric acid at 0.50 mg/L produced the greatest results for shooting and rooting characteristics of *Cestrum nocturnum* plantlets, and the growth has been increased by applying mannitol.

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