International journal of Advanced Biological and Biomedical Research
ISSN: 2322 - 4827, Volume 1, Issue 2, 2013: 197-210
Available online at http://www.ijabbr.com

Effect of Sodium Chloride on Tropane Alkaloids Accumulation and Proline content in *Datura metel* and *D. stramonium* callus cultures.

Raoufa Abdel Rahman[1], Sara E. Gomaa[2]*, Nader R. Abdelsalam[3], Hossam El-Din M.F. El-Wakil[3], Ahmed S. Khaled[3], Horya M. Hassan[2].

[1]Pharmaceutical Bioproducts Research Department, City of Scientific Research and Technology Applications, Borg-El-Arab, [2] Veg., Medicinal and Aromatic plants breeding Dept., Horticultural Research Institute (HRI), Agricultural Research Center (ARC). [3]Agriculture Botany Dept., Faculty of Agriculture (Saba Bacha), Alex. Univeristy, Egypt.

ABSTRACT

Plant tissue culture techniques were used to study the effect of NaCl on tropane alkaloids accumulation and proline content of both *Datura metel* and *Datura stramonium*. Callus cultures were established for both *Datura* species. However, MS medium supplemented with 1mg/l of both BA and NAA was the best for callus production in *D. metel*, while MS supplemented with 3 mg/l Kin and 1 mg/l 2, 4-D was the optimal medium for callus production in *D. stramonium*. Five NaCl concentrations (25, 50, 75, 100 and 125 mM) were used to test its effect on tropane alkaloids and proline contents in the produced callus. Total tropane alkaloids were extracted from both control and salt-treated calli and analyzed using HPLC. At 125 mM NaCl scopolamine and atropine concentrations recorded 8.5 and 11.5 folds higher than control of *D. metel*, while they recorded 2.5 and 3.5 fold higher than control of *D. stramonium* after one month of culture. Peroxidase activity was determined and results showed that *D. metel* recorded the highest enzyme activity at 100mM NaCl concentration, while *D. stramonium* callus showed the highest activity at 75mM NaCl. Proline content was increased by salinity in the rate of 0.326mM proline/25mM NaCl in *D. metel*, and in the rate of 0.1248 mM proline/25mM NaCl in *D. stramonium*. The results of this investigation showed that using plant tissue culture techniques to establish callus culture from both *Datura* species under NaCl stress is a powerful protocol for scopolamine and atropine accumulation improvement. **Key words:** In vito - Salt - NaCl - Datura metel - Datura stramonium - peroxidase HPLC - Scopolamine - Atropine - Proline

INTRODUCTION

The genus *Datura (Solanaceae)*, consists of annual and perennial herbs, shrubs and trees. Three species, viz, *Datura metel*, *Datura stramonium* and *Datura innoxia* are medicinally important **Warrier *et al.* (1994).** *Datura* plants, generally, are considered as a source of tropane alkaloids; hyoscyamine, atropine and scopolamine, which have been estimated to be between 0.06 and 0.5%. Furthermore, the seeds of *Datura metel* contain up to 30% fixed oil and about 0.2%

Corresponding Author E-mail: sara.emad@outlook.com

alkaloids **(Dewick, 1997).** Alkaloids, being anticholinergic agents, are used in medicine as antispasmodics, preoperative medication, analgesic, narcotics and in treatment of asthma, Parkinson's disease and motion sickness **(Pitta-Alvarez *et al.*, 2000).** The use of elicitors is one of the effective strategies employed to increase the production of important alkaloids in cell and organ cultures**.** On the other side, several functions are proposed for the accumulation of proline in tissues submitted to salt stress: osmotic adjustment, C and N reserve for growth after stress relief, detoxification of excess ammonia, stabilization of proteins and/or membranes and a scavenger of free radicals **(Silveira *et al.*, 2002).** However, Plants accumulate free proline in response to abiotic stresses such as increased levels of salinity, drought and low temperature **(Ashraf and Harris, 2005)**. In addition to the role of proline as an osmolyte for osmotic adjustment, it also stabilizes subcellular structures (membranes and proteins), scavenges free radicals, and buffers cellular redox potential under stress. Proline accumulation under stress has been correlated with stress tolerance of plants. The objective of this study was to enhance alkaloid accumilation of both *D. metel* and *D. stramonium via* tissue culture technique under abitotic stress and to screen thier resistability through peroxidase isozyme activity and proline content.

MATERIALS AND METHODS

Seeds of *Datura metel* and *Datura stramonium* were, kindly, provided by National Research Center, Cairo, Egypt. Small plants containing 6 - 8 leaves were used as a source of explants taken directly from the soil. Explants were sterilized according to **(Khlifa, 2008)**, then explants of *D. metel* were cultured on **Murashige and Skoog (1962)** medium (MS) supplemented with 1mg/l of both NAA and BA **(Raoufa *et al.*, 2008)**, while *D. stramonium* were cultured on MS-medium supplemented with different combinations of growth regulators i.e. BA, NAA, Kin or 2,4-D at different concentrations as shown in Table (1) to study their ability for callus induction. Light conditions were 16/8 (light /dark condition) through cooling inflourecens white lamped 3000 Lux.

Table 1. Media composition of the 12 treatments used for callus production for *D. stramonium.*

	Media treatment composition		
1.	MS + 1 mg/l (BA) + 1 mg/l (NAA)	7.	MS + 1 mg/l (Kin) + 1 mg/l (2,4-D)
2.	MS + 3 mg/l (BA) + 1 mg/l (NAA)	8.	MS + 3 mg/l (Kin) + 1 mg/l (2,4-D)
3.	MS + 5 mg/l (BA) + 1 mg/l (NAA)	9.	MS + 5 mg/l (Kin) + 1 mg/l (2,4-D)
4.	MS + 1 mg/l (BA) + 1 mg/l (2,4-D)	10.	MS + 1 mg/l (Kin) + 1 mg/l (NAA)
5.	MS + 3 mg/l (BA) + 1 mg/l (2,4-D)	11.	MS + 3 mg/l (Kin) + 1 mg/l (NAA)
6.	MS + 5 mg/l (BA) + 1 mg/l (2,4-D)	12.	MS + 5 mg/l (Kin) + 1 mg/l (NAA)

Growth rate was calculated according the following equation: $G_e - G_0$

Whereas: G_e = callus weight (gm) at the end of the 2^{nd}, 3^{rd}, 4^{th} and 5^{th} week respectively, G_0 = callus weight (gm) at the end of the prvious week i.e. 1^{st}, 2^{nd}, 3^{rd}, or 4^{th} week of culture. **Dung *et al.* (1981**)Levels of NaCl were added directly to the culture media before autoclaving at the final concentrations of 0, 25, 50, 75, 100 and 125 mM **(Ajungle *et al.*, 2009)**. Calli samples from each treatment were collected and dried in an oven for 3 days at 60°C and grounded. Alkaloid extraction method was carried out as described in **British Pharmacopoeia (1998)**. Atropine ($C_{17}H_{23}NO_3$) and Scopolamine ($C_{17}H_{21}NO_4.HBr.3H_2O$) standared samples were purchased from Sigma-Aldrich Company. HPLC Analysis of Tropane Alkaloids: Chromatographic conditions: Apparatus: Agilent Technologies 1200 series HPLC - Eclipse XD C-18 Detector according to **Huo *et al.* (2006)**. Mobile phase: 0.02M sodium acetate buffer and methanol (60:40). The buffer containing 0.02% triethanolamine and the pH was adjusted to 6.0 with acetic acid. Detection wavelength was 215 nm. Run took place for 6 min, flow rate was 1.0 ml/min and the column temperature was maintained at room temperature. Peroxidase was extracted in an extraction buffer as described by **Davis (1964).** Bands which were detected on the gel under cooling conditions, as described by **Manchenko (1994),** gel was incubated in staining solution i.e. 50 mM sodium acetate; 3-Amino-9-ethyl-carbazole (dissolved in a few drops of acetone; 3% H_2O_2) in the dark at room temperature or at 4°C until red-brown bands appears. Then gel was washed and fixed in 50% glycerol. Vertical slab gel electrophoresis unit (Hoefer Mighty small SE 245) was used. All glass plates were washed with dH_2O, then sterilized with 70% ethanol. Polyacrylamide gel was prepared as follows: The stacking gel solution was quickly poured over the two resolving gels and 10-well combs were used. Gels

were left to polymerize before loading samples. Proline content; was determined according to the method of **Bates *et al.* (1973**).

RESULTS AND DISCUSSION

Calogenesis of both *D. metel* and *D. stramonium*:

In case of *D. metel*, MS medium supplemented with 1mg/l of both BA and NAA was used for calogenesis as recommended by **Khlifa (2008)**. Among all tested treatments, MS supplemented with 3 mg/l Kin and 1 mg/l 2,4-D was the best medium composition for calogenesis. Figure (1) demonestrated that *D. metel* callus grew faster than *D. stramonium* callus after 4 weeks of culture. Both calli were green in color, *D. metel* callus was friable, while *D. stramonium* callus was compact in nature.

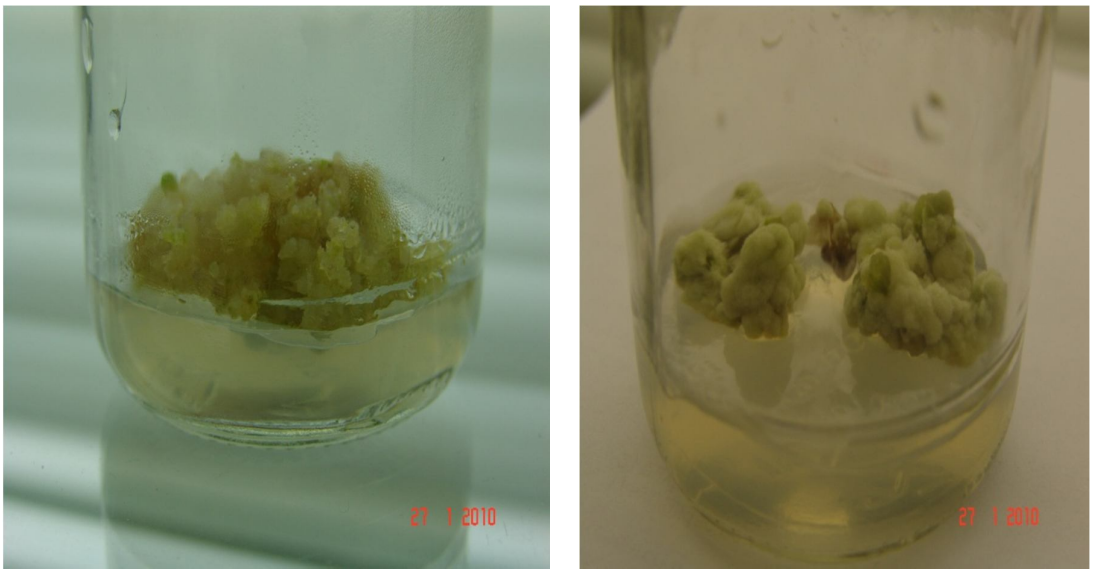


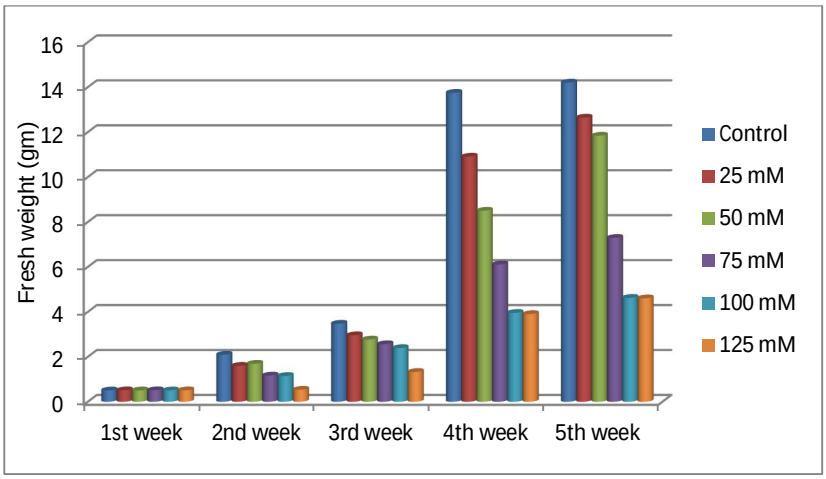
Figure 1. Callus induction of *D. metel* (left) and *D. stramonium* (right) after 4 weeks of cultivation.

In Vitro Salt Tolerance:

Data in Figure (2-a) expressed that *D. metel* control callus gained the highest fresh weight (14.203 ± 0.40 gm) followed by NaCl treated callus. Among the treated samples, callus treated with 25mM NaCl had the highest fresh weight (12.645 ± 3.775 gm) while callus treated with 125mM NaCl recorded the lowest fresh weight (4.603 ± 0.170 gm). The obtained results clearly showed that as NaCl concentration in the medium increased, the callus fresh weights decreased. In case of *D.*

stramonium, the obtained results showed that callus fresh weights of the control and treated samples were increased by increasing the growth period to reach the highest fresh weight (5.626 ± 0.810 gm) after 5 weeks of growth followed by NaCl treated callus (Figure 2- b).

(a)



(b)

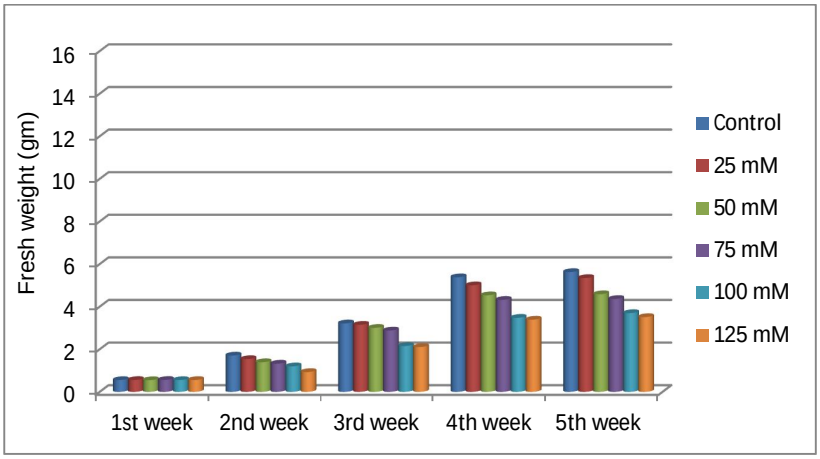
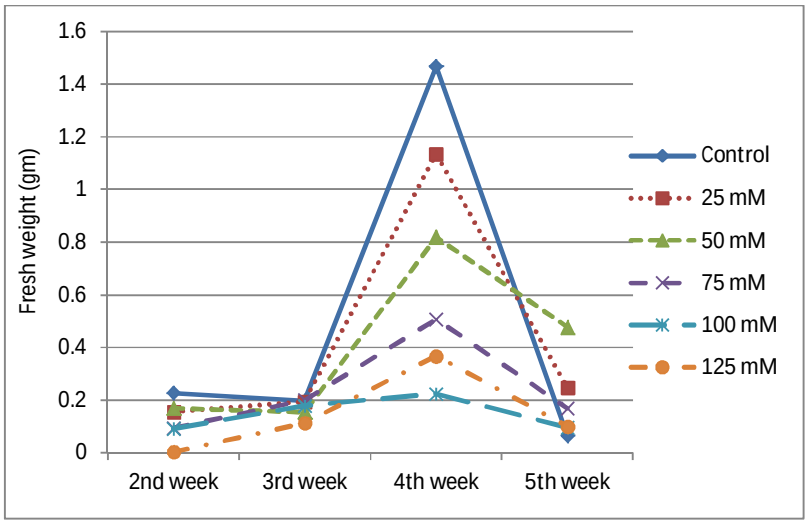


Figure 2. Effect of NaCl concentrations on *D. metel* (a) and *D. stramonium* (b) callus fresh weight.

The obtained results for both species clearly showed that callus fresh weights decreased by increasing NaCl concentrations. Concerning those of daily growth rates (gm/day), *D. metel* results in Figure (3-a) showed that the maximum values of growth rate (gm/day) were recorded at the end of 4th week from subculturing, the control callus weighed 1.465 gm then 1.134, 0.818, 0.507, 0.223 and 0.367 gm at NaCl concentrations of 25, 50, 75, 100, 125mM, respectively; while *D. stramonium*

recorded 0.309, 0.264, 0.218, 0.205, 0.189 and 0.183 gm and they were not the highest values among all the salt concentrations (Figure 3-b)

(a)



(b)

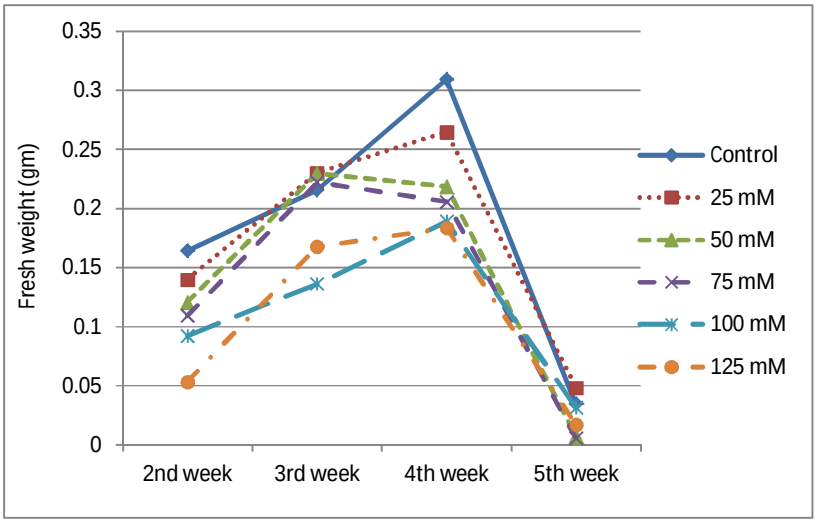


Figure 3. Average of calli cultures growth rate (gm/day) as influenced by NaCl concentrations in *D. metel* (a) and *D. stramonium* (b).

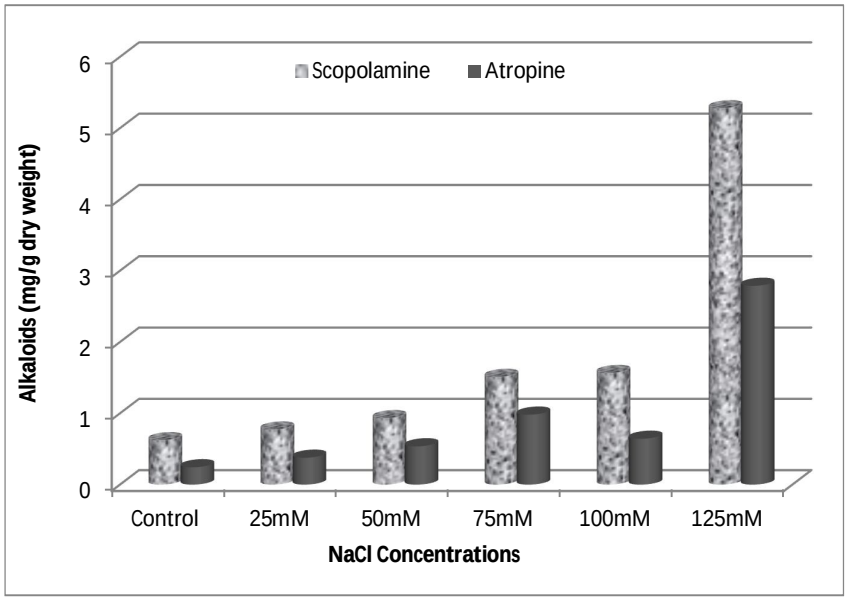
D. metel recorded the lowest growth rates in the 2nd week of subculturing as 0.227 gm for control and 0.156, 0.169, 0.093, 0.091 and 0.003 gm for the subsequent concentrations of NaCl. In general, significantly, the highest growth rates (gm/day) were recorded at the end of the 4th week of subculture. On the other hand, *D. stramonium* recorded the lowest growth rates in the 5th week of subculturing as 0.035 gm for control and 0.048, 0.007, 0.005, 0.031 and 0.170 gm for the subsequent concentrations of NaCl. However, the obtained results came in

agreement with those of **Smith (2009)** who reported that the rate of callus growth in many ways as a sigmoid curve in single-celled population. There are usually five stages for callus growth rate, a lag phase in which cells prepare to divide, a period of exponential growth in which cell division is maximal, a period of linear growth in which division slows down and cells enlarge, a period of decelerating growth and stationary (no-growth) period in which the number of cells is constant. The behavior of cells of callus tissue is different during each stage of growth, this might be a reason of the media influence or how long the callus remained at a particular stage.

HPLC Analysis of Tropane Alkaloids (Scopolamine and Atropine):

Total tropane alkaloids were extracted and data was analyzed by HPLC. Figure (4) showed that increasing in salt concentration in growth cultures was associated with tropane alkaloid accumulation. Notably, various concentrations of NaCl were efficient enough to stimulate the potential of callus for tropane alkaloid accumulation. It was observed that increasing the concentration of NaCl suppressed the growth of callus; however there was increase in the accumulation of both scopolamine and atropine. Results, clearly, showed that scopolamine and atropine values were increased in 125mM NaCl treated callus by 8.5 and 11.5 times in *D. metel* and 2.5 and 3.7 times in *D. stramonium*, respectively, in comparison to control. Among the different treatments, the highest scopolamine alkaloids values were 5.279 and 1.187 mg/g dw obtained due to the presence of 125mM concentration in the medium, while the highest values of atropine were 2.788 and 1.331 mg/g dw and obtained at the same salt concentration of *D. metel* and *D. stramonium* callus, respectively. So, one plant of either *D. metel* or *D. stramonium* could produce an average of (152.23 and 55.45 mg/g dw seeds) and (111 and 38.1 mg/g dw seeds), scopolamine and atropine, each in turn, after 4.5 month of cultivation in the field at the warm season of growth, while it possible to get the same amount of the previously mentioned alkaloids from (57.4 and 39.6 g dw) and (186.55 and 57.29g dw) of 125mM treated callus with NaCl after only one month in lab at any time of the year.

(a)



(b)

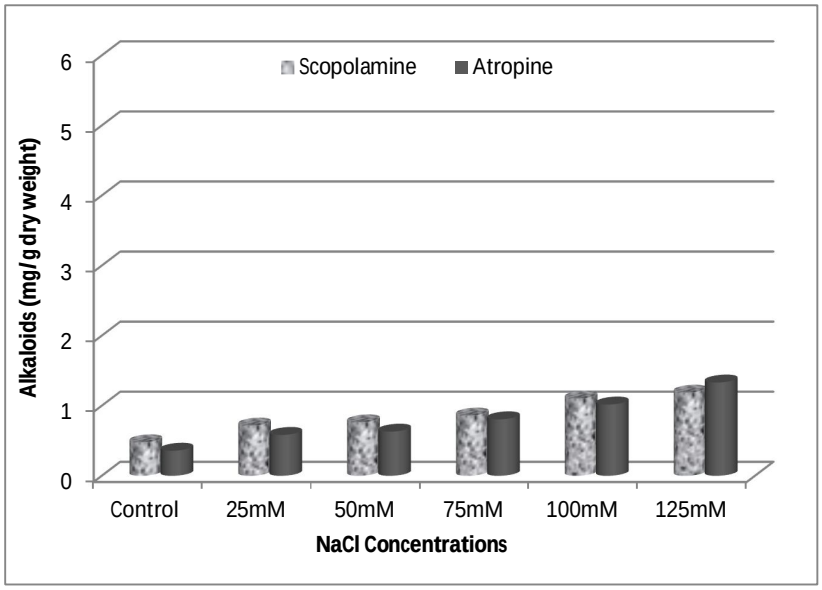


Figure 4. Scopolamine and atropine content (mg/gm dw) of *D. metel* (a) and *D. stramonium* (b) callus treated with different concentrations of NaCl.

Regression calculations showed that the increase or decrease in alkaloid content is due to the change in NaCl concentration. Results indicated, also, that scopolamine was increased in *D. metel* and *D. stramonium* by the rate of 0.125 mg/gm dw, and

0.0549 mg/gm dw, consecutively for each 25 mM of NaCl and atropine could increase by 0.160 mg/gm dw in *D. metel* and by 0.007 mg/gm dw in *D. stramonium*, consequently. The obtained results are in an agreement, more or less, with the results of **Ajungla *et al.* (2009)** who tested the possibility of various concentrations of NaCl to stimulate the potential of root cultures for accumulation of tropane alkaloids. They observed that increasing concentrations of NaCl suppressed the growth. However, there was an increase in the accumulation with increasing NaCl of scopolamine up to 129mM, whereas the increase was about 1.3 times more in comparison with the control one. They, also, stated that the addition of 172.4 mM NaCl was harmful for the growth as evident from a sharp decline in growth index. In this context, **Moons *et al.* (1997)** reported that the addition of NaCl into the culture medium increased the endogenous level of methyl jasmonate, which can stimulate the activity of enzymes involved in the biosynthesis of tropane alkaloids and consequently their increased accumulation. There is, currently, a 10-folds higher commercial demand for scopolamine than for hyoscyamine and atropine combined. So, it is of significance and importance to improve tropane alkaloids production especially the much more valuable scopolamine to meet the expansion of clinical need **(Zhang *et al.*, 2004).** So, it is worth mentioning that increasing levels of alkaloids are possible to be produced using aspetic conditions and that declining farm income, together with political and environmental considerations, are forcing scientists to consider other ways to avoid crop loss due to insect control, fertilizers, herbicides and fungicides **(Nix, 1985)**. Elicitors, which affect positively the release of secondary metabolites (as was observed in our research work), represent a valuable biotechnological strategy. It can be concluded that there are some metabolic disorders in the two test plants under salinization conditions. Fortunately these disorders include the accumulation of alkaloids for which these medicinal plants are generally considered **(Ahmed *et al*., 1989).**

Biochemical Studies:

Figure (5) illustrated qualitative and quantitative variation of peroxidase isozyme patterns in control and salt treated callus of the crude extract of the two *Datura* species (*D. metel* and *D. stramonium*). However, 31 bands were detected, 19 of them were polymorphic, which gave a polymorphism percentage of 61.3% in *D.metel* when treated with different concentrations of NaCl. Bands varied in their density between strong, intermediate and weak (2 bands for each) at the control lane, while, there were absent bands at the fifth location. Enzyme showed high activity at salt concentration of 100mM; band at the fifth loci was present as strong and faded away under 125mM concentration to appear as a weak band. Band number three appeared in all salt concentrations except for 25mM while band four was the common band in all salt treatments with the same strong density. All bands were detected in *D. stramonium* when treated with different salt concentrations of

NaCl. Bands were intermediate in the control lane, whereas in concentration of 25mM and 75mM, bands were strong. As for bands in concentrations of 50mM, 100mM and 125mM ranged between weak, intermediate, and strong which indicated that enzyme showed the highest activity in NaCl concentration of 75mM, then enzyme activity decreased again when salt concentration increased. Total number of bands was 30 bands. Percentage of polymorphism was 0% since all bands were present and no polymorphic bands appeared. Biochemical mechanisms underlying salt tolerance such as antioxidative enzyme activity and total soluble protein content may be more relevant criteria for step-wise improvement of salt tolerance in plants even in varieties **(Arshi *et al.*, 2002).**

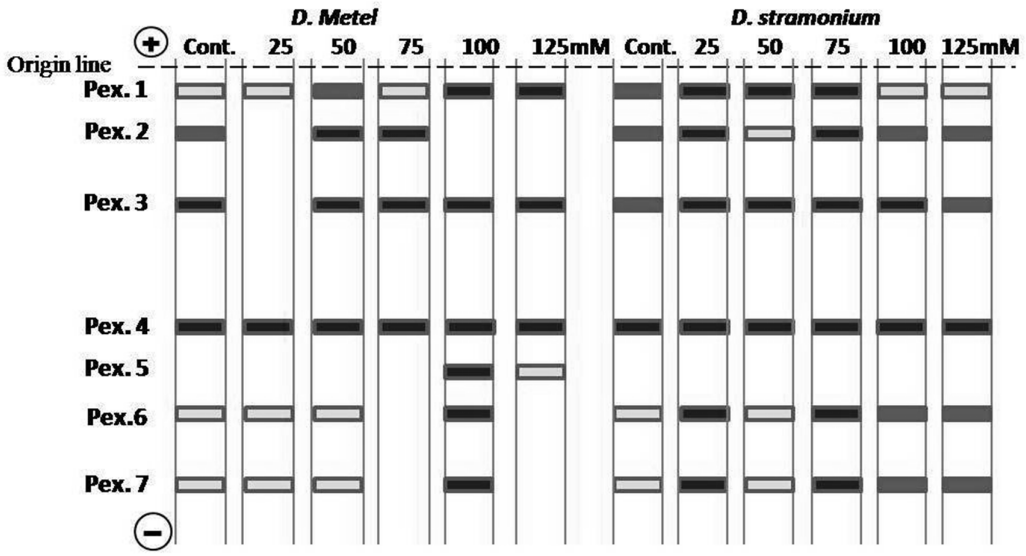


Figure 5. Zymograme of peroxidase isozyme among *D. metel* (left) and *D. stramonium* (right) under different NaCl concentrations.

Our results came, more or less, in alignment with **Rahnama and Ebrahimzadeh (2006)** results, who found that peroxidase isozymes had increased activity under salt stress in *Solanum tuberosum L.*The data of **Nagesh and Devaraj (2008)** illustrated that isozyme pattern of peroxidase showed over expression of all the five constitutive isozymes of peroxidase in French bean (*Phaseolus vulgaris*) under salt stress. It is obvious that salinity stress on plants may increase the isozymes activity of peroxidase isozymes and the number of bands. These results mean more gene expression for peroxidase isozymes genes under salinity conditions. The results observed that peroxidase banding patterns produce different results under different salt concentrations in the same species. Moreover the reaction to salinity of different genotypes was not the same and every genotype produced different banding patterns. These results are in accordance with **Weber *et al.* (1976)** who mentioned that plant isozymes were found to be correlated with resistance or susceptibility to some pathogens in plants. Also results came in agreement with **Ghallab (2007),**

who mentioned that peroxidase banding patterns showed different activity under different salt concentrations in the same genotype, also the band volume and band percentage for control are less than the salt treated samples and there are new bands appeared under salt treatment conditions.

Proline Content:

Data of figure (6) declare the relationship between the both variables. Proline was considered as the dependent one (Y) while the salinity concentration was determined as the independent variable (X) for the two selected species and it is obvious that proline content was increased by increasing salinity concentration of (NaCl). It is clear that increase or decrease in proline was due to the change in NaCl concentration. So, that if NaCl is increased by 25mM, proline will be increased by 0.326 mM and 0.1248 mM in *D. metel* and *D. stramonium*, respectively. The decrease or increase of proline induction due to salt stress indicated the susitability or resistance of the species to afford salt stress, so that, when the proline production is increased, this indicates that the genotype can afford salt stress and it is resistant to salt stress. This indicates that *D. metel* is more resistant to salt stress than *D. stramonium*. The disturbances in plant metabolism induced by salinization treatments affect generally the various metabolic pools of salt stressed plants. These changes in the contents of the various metabolites under salinization treatments may indicate an enhancement or retardation in the synthesis, accumulation or consumption of these cellular metabolites.

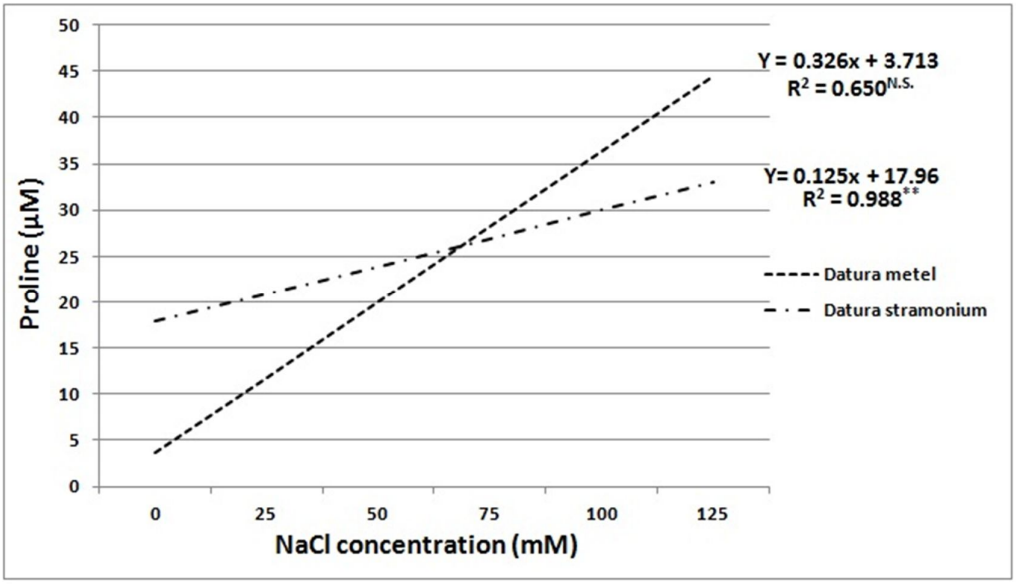


Figure 6. The regression between NaCl concentrations and the proline in *D. metel* and *D. stramonium* callus.

Result as of those **Stewart and Lee, (1974)** agreed that proline accumulation could play a role in osmoregulation by which suitable osmotic pressure is maintained within the living organisms**.** Moreover, this accumulation was regarded as an indicator for stress resistance and/or for stress damage **(Stewart and Hanson, 1980).** Similar results were also obtained by some other authors **(Reddy and Vora, 1985)** using some other economic plants. Such increase in amino acids contents could be regarded as an adopted defense mechanism of the plant to salinization. From the above results, it is recommended that growing the callus for the 4^{th} week is enough, because growth rate declines rapidly after that and this is considered a waste of chemicals, labour effort and time-consuming. Also, when callus treated with NaCl, *D. metel* showed the highest enzyme activity at 100mM salt concentration, while the highest activity for *D. stramonium* was detected at 75mM. This indicated that *D. metel* is more tolerant to NaCl than *D. stramonium*. Generally, it is obvious that the increase in total alkaloid contents in salt treated *D. metel* and *D. stramonium* goes parallel with the accumulation of proline. This could be due to the fact that ornithine, the precursor of tropane alkaloids **(Ahmed and Leffte, 1970)** and proline have the same precursor namely; glutamic acid. Therefore, it can be said that salinity can inhibit the transamination reactions and hence the glutamic acid is accumulated and transformed into other nitrogenous compounds such as proline and ornithine. Proline was accumulated and ornithine was further transformed into tropane alkaloid. At the biochemical level plants grown in saline soil shows increase in proline content, number of bands and activity of peroxidase isozymes and protein. This is due to the rise in gene expression under saline conditions. Our results showed that using plant tissue culture techniques to establish callus culture from both *D. metel* and *D. stramonium* plants and using sodium chloride as elicitor in plant culture medium is a powerful tool for scopolamine and atropine accumulation improvement. The protocol presented here can be used for the production of these medicinally important tropane alkaloids by the pharmaceutical industry, which will lead to economic considerations.Genetic modification of plants to improve their alkaloid induction is a possible way of increasing and improving the medicinal production.

REFERENCES

Ahmed A. and E. Lffte 1970. Biosynthesis of atropine moiety of hyoscyamine from 6-Nmethylornithine. Phytochem. 9: 2345-2347.

Ahmed, A.M., M.M. Heikal and R.M. Ali 1989. Changes in amino acids and alkaloid contents in *Hyoscyamus maticus* and *Datura stramonium* in response to salinization. Phyton, 29:137-147.

Ajungla, L., P. Patil, R. Barmukh and T. Nikam 2009. Influence of biotic and abiotic elicitors on accumulation of hyoscyamine and scopolamine in root cultures of *Datura metel* L. Indian J. of Biotech. 8: 317-322.

Arshi, A., M.Z.Abdin and M. Iqbal 2002. Growth and metabolism of senna as affected by salt stress. Biol. Plant., 45: 295-298.

Ashraf, M. and P.J. Harris 2005. Biotic Stresses "Plant resistance through breeding and molecular approaches". Book. Pp. 47:81.

Bates, L.S., R.P. Waldeen and I.D. Teare 1973. Rapid determination of free proline for water stress studies. Plant Soil 39: 205-207.

British Pharmacopoeia, B.P. 1998. The Stationary Office Dept., BP. KE 5833 Norwich, NR 3-IBR, 1: 709-711.

Davis, R.J. 1964. Disc electrophoresis 2-method of application to human serum proteins, The Plant J. 4 (2): 215-223.

Dewick, P.M. 1997. Medicinal Natural Products: a biosynthetic approach. John Wiley and Sons, Chichester, 274-278.

Dung, N.N., E. Szoki and G. Verzar-Petri 1981. The growth dynamics of callus tissue of root and leaf origin in *Datura innoxia Mill*. Acta. Botanica Academiae Scientiarum Hungaricae, 27: (4), 325-333.

Ghallab, Marwa M.A. 2007. Genetical and Biochemical studies on sugar beet plants induced by tissue culture. Ph. D. Thesies. Faculty of Agric., Alex Univ., Egypt.

Huo, S.G., X.X. Gu, S.Y. Wang SY and H.X. Li 2006. Determination of scopolamine and atropine in *Flos Daturae* by RP-HPLC. Zhongguo Zhong Yao Za Zhi. 31(13):1065-1067.

Khlifa, Heba D.A. 2008. Genetical and Biochemical Studies on *Datura metel* and its Extracts. MSc Thesis. Faculty of Agriculture, University of Alexandria, Egypt.

Manchenko, G.P. 1994. Handbook of Detection of Enzyme on Electrophoretic Gel. CRG Press-Boca Raton, FL. 553 p.

Moons A., E. Prinsen, G. Bauw and M.V. Montagu 1997. Antagonistic effects of abcisis acid and jasmonates on salt stress induced transcripts in rice roots, Plant Cell, 9: 2243-2259.

Murashige, T. and F. Skoog 1962. A revised medium for rapid growth and bioassay with tobacco tissue cultures. Physiol. Plant, 15: 473-497.

Nagesh, B.R. And V.R. Devaraj 2008. High temperature and salt stress response in French bean *(Phaseolus vulgaris).* Aust. J. Of crop Sci. 2(2): 40-48.

Nix, J. 1985. Farm Management Pocketbook, 16th ed. Wye College Farm Business Unit: Wye College Pub. 186.

Pitta-Alvarez, S., T. Spollansky and A. Giulietti 2000. The influence of different biotic and abiotic elicitors on the production and profile of tropane alkaloids in hairy root of *B. candida*. Enzyme Microb Technol. 26: 252-258.

Rahnama H. and H. Ebrahimzadah 2006. Antioxidant isozyme activities in potato plants (*Solanium tuberosum L.*) under salt stress. J. Sci. I. R. Iran. 17(3): 225-230.

Raoufa Abd El-Rahman, H.E. El-Wakil, A.E. Abou Gabal and H.D. Khlifa 2008. Production of Scopolamine and Hyoscyamine in Calli and Regenerate Cultures of *Datura metel* (L.). J. of App. Sci. Res.4(12): 1858-1866.

Reddy M.P. and A.B. Vora 1985. Effect of salinity on protein metabolism in bajora (*Pennisetum typhoides*) leaves. Indian J. Plant Physiol. 28: 190-198.

Silveira J.A.G., R.A. Viegas, I.M.A. Rocha, A.C.O. Monteiro-Moreira, R.M. Moreira and J.T.A Oliveira 2002. Proline accumulation and glutamine synthetase are increased by salt-induced proteolysis in cashew leaves. J. Plant Physiol.

Smith, R.H. 2000. Callus induction in plant tissue culture techniques and experiments. 2nd ed. Acad. Press, San Diego, California pp. 98-103.

Stewart, C.R. and A.D. Hanson 1980. Proline accumulation as a metabolic response to water stress. In: TURNER N. C. & KRAMER P. J., Adaptation of plants to water and high temperature stress, New York. pp. 173-189.

Stewart, C.R. and J.A. Lee 1974. The role of proline accumulation in haplophytes. Planta. 120: 279-289.

Warrier, P.K., V.P.K Nambiar and C. Ramankutty 1994. Indian medicinal plants: a compaendium of 500 species, Orient Longman, Hyderabad. 2: 164-172.

Weber, D., B. Clare and M.Stahmanm 1976. Ezymic changes associated with induced and natural resistance of sweet potato to *Ceratocystis fimoriata*. Phyto. 57 (4): 241-424.

Zhang, L., R. Ding, Y. Chai, M. Bonfill, M. Piaol, T. Xu, Y. Pi, Z. Wang, H. Zhang, G.Y. Kai, Z.H. Liao, X.F. Sun and K. Tang 2004. Engineering tropane biosynthetic pathway in *Hyoscyamus niger* hairy root cultures. Proc. Natl. Acad. Sci. USA 101: 6786-6791.