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REGULAR ARTICLE

IN VITRO PRODUCTION OF SOLASODINE ALKALOID IN *SOLANUM NIGRUM* UNDER SALINITY STRESS

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SUMMARY

The effect of Salinity stress on solasodine production by *Solanum nigrum* under tissue culture conditions has been investigated. Solasodine is steroidal alkaloid, alternative to diosgenin, which is used as a precursor for the commercial production of steroidal drugs. Salinity stress has been applied by adding NaCl to the culture medium MS, five concentrations were applied: 0.0 (control), 50, 100, 150, and 200 mM for 8 weeks. The obtained results show the possibility to increase solsasodine level production under salainity stress. However, the highest salinity stress concentration (200 mM of NaCl) has not significant when it is compared to (150 mM of NaCl) concentration. Positive correlations were observed between the NaCl levels and solasodine content accumulation, proline content and solasodine accumulation in Solanum nigrum calli. The solasodine production increased significantly as a result of increasing of NaCl concentrations. However, non-significantly differences were observed between 150 mM and 200 mM of NaCl on solasodine accumulation.

Key words: Salinity, Solasodine, *Solanum nigrum*, Murashige and Skoog (MS) medium

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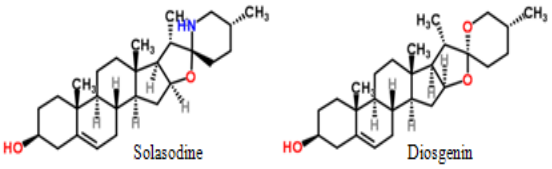
1. Introduction

Solanum genus comprises about 1400 species. *Solanum nigrum* is one of important medicinal plants and contains solasodine, a steroidal glycoalkaloid, which considered as potential alternative to diosgenin for commercial synthesis of various steroidal drugs (Fig.1). Solasodine have been reported to provide anticancer (Cham; 1994), toxic inhibitory to wide range of organisms (Roddick, 1996) and antiaccelerator cardiac activities (Krayer and Briggs; 1950). Solasodine alkaloid inhibits the acetylcholinsterase; a key enzyme in nerve impulse transmission (Roddick, 1989). Solasodine alkaloids reported recently as a new chemotherapeutic agent for treatment of cancer especially skin cancer (Cham, 2008).

Fig. 1. Chemical structure of Solasodine and diosgenin alkaloids



A number of analytical methods such as high performance thin layer chromatography (Trivedi et al., 2006), high performance liquid chromatography (Eanes and Tek, 2008), capillary electrophoresis (Kreft et al., 2000), gas chromatography (Laurila et al., 1999) and colorimetric method (Eltayeb et al., 1997) are available for determination of solasodine from its plant. Solasodine does not have a conjugated double bond in its structure. The nitrogen is protonated and forms complexes that are extractable into organic solvent like chloroform.

Salinity is one of major factors which can reduce substrate water potential, there by restricting water nutrients uptake by plants, and may also cause ionic imbalance and toxicity (Larcher, 1995; Lambers et al., 1998; and Houle et al., 2001). On the other side, proline accumulation in plant cells exposed to NaCl-stress is widespread phenomenon (Aspinall and Paleg, 1981; Chandler and Thorpe, 1986). However, proline

accumulation is correlated with growth inhibition induced by NaCl (Chandler and Thorpe, 1987; and Perez-Alfocean et al., 1994).

Much of recent research focuses on the higher plant response, physiological and metabolic processes under salt stress (Martinez et al., 1996, Ghoulam et al., 2002 and Girija et al., 2002). However, few data are available on solasodine alkaloid accumulation (Eltayeb et al., 1997). In this study, we induced *Solanum nigrum* calli to produce solasodine alkaloid under different levels of salinity stress.

2. Materials and methods

Solanum nigrum seeds

Solanum nigrum Seeds had been collected from wild plants in fayoum governorate (Egypt). Dried Seeds were rinsed with distilled water and cultivated on pots filled with heavy clay soil and irrigated by tape water. Ten days after germination, the seedlings were transferred to plastic containers (2 seedlings per container) filled with heavy clay soil and irrigated by tape water. The plants were then grown until the age of 30 days, then the apical buds separated and sterilized.

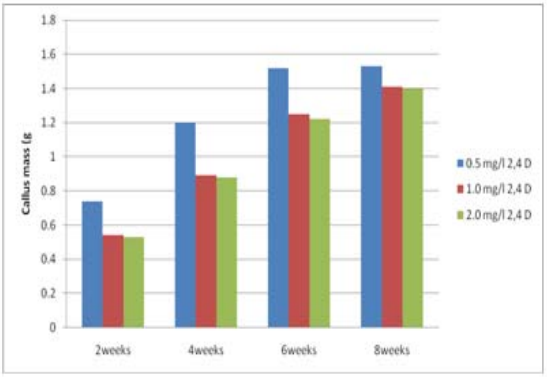
Plant materials sterilization

Different kinds of sterilization methods were tested to prevent the contamination, and the following method was chosen as the most suitable for the present study. Apical buds were washed with tape water, dipped in ethanol (70% v/v) for 10 seconds, soaked in a stirred sterilant solution for 12 min, and rinsed four times in sterile distilled water in laminar flow cabinet. The sterilant solution was consisted of sodium hypocloride containing 2% active Cl in addition of 10 drops of Tween 20. The preparation was made by diluting 40ml commercial pleach (CLOROX) with 5% active Cl in 160ml sterile distilled water.

In vitro cultivation

The apical buds were immediately cultivated in vitro on modified Murashige-Skoog (MS) medium supplemented with 0.4 mg/l thiamine HCl, 100 mg/l myo-inositol, kinetin (0.5 mg/l), 30 g/l sucrose and 7 g/l agar. The pH of the media was adjusted to 5.7 before sterilization (at 121°C for 20min). For maintenance and proliferation of callus MS medium supplemented with 2,4-D (0.5 mg/l) (best concentration examined for calli initiation during the period of study) (Fig. 2) and kinetin (0.5 mg/l) was used. The cultures were kept in a growth room at a temperature of 25°C light/20°C dark and provided with cool, white fluorescent light with a 16 h photoperiod (Fluorescent F40T12/WW/EG) lamp at a photon flux density of 100 mmolm2S1.

Fig. 2: *Solanum nigrum* Callus Growth (g) on Various Concentrations of 2, 4-D (mg/l) on MS Medium (Initial Weight of Callus: 1 g).



Salinity treatment

MS medium supplemented with optimized plant growth regulator levels was separately treated with 50, 100, 150 and 200mM NaCl. The pH of the medium was adjusted to 5.7 before autoclaving and cultures were inoculated. These cultures were regularly sub-cultured at the interval of two weeks on the same growth regulator and NaCl treatment. Solasodine quantification of each culture was made after 8 weeks of incubation.

After 15 days, the calli were transferred to MS media contain five levels of NaCl: 0.0 (control), 50, 100, 150 and 200mM. During the growth period, the incubated conditions were constant: relative humidity 80%, Temperature 25°C and 16/8 h light/dark cycle.

Total protein and proline contents

Three randomized calli tissue samples had been taken each 15 days from each

treatment (NaCl level). Free proline was extracted and determined spectro photometerically according to Bates method (Bates et al., 1973). 0.04 g dry weight of callus was homogenized with 3% sulfosalicylic acid and after 72 h that proline was released; the homogenate was centrifuged at 3000 g for 20 min. The supernatant was treated with acetic and acid ninhydrin, boiled for 1 h and then, the absorbance was determined by spectrophotometer at 520 nm. Contents of proline were expressed as mg /g DW. Total soluble protein content was determined according to the method of Lowry et al (1951).

Solasodine alkaloid estimation

Solasodine extraction and determination were carried out spectrophotometerically at 610 nm according to Eltayeb et al., (1997). Solasodine contents were expressed as mg/g DW.

Experimental design and statistical analysis

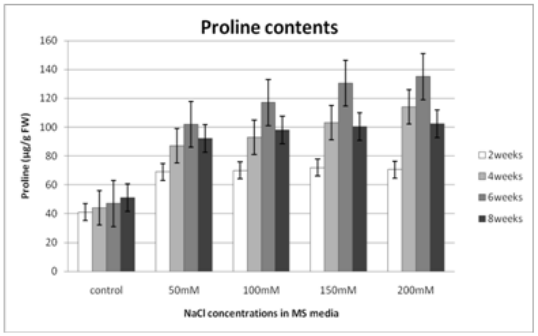
The data obtained from the experiments conducted twice with three replicates per treatment, were analyzed statistically by application of t-test. Sigmastat version 3.5 had been used.

3. Results

Growth regulator effect on calls mass: small concentration of NAA growth regulator has a great effect on *solanum nigrum* calls mass. Between the three concentrations; 0.5, 1.0 and 2 mg/l had been tested, the smallest concentration showed great ability to induce callus mass of *Solanum nigrum* than the two later concentrations Figure (2).

Proline contents: Figure (3) shows that the effect of NaCl stress on the callus proline contents, regardless the sampling time, the proline contents was increased significantly as a result of increasing NaCl concentrations in the culture medium from 0.0 till 200 mM when compared by the control. While at 8 weeks, the proline accumulation was slightly decreased than those observed at 4 and 6 weeks respectively. The maximum effect of NaCl on the accumulation of proline in calli, was at 150 mM of NaCl, the proline concentration was increased two fold than in the control.

Fig. 3: Effects of different NaCl concentrations on callus proline content in *Solanum nigrum*. Results are shown as Mean ± Standard error ($p<0.05$), obtained from three replicates.



Protein contents: the increase in NaCl concentration of culture medium has negative effect on total protein contents in the calli. The results present in figure (4) show that for all sampling times, NaCl treatments caused dramatically decrease of calli protein concentration, especially with 150 and 200mM of NaCl respectively, as compared with the control.

Fig. 4: Effects of different NaCl concentrations on callus protein contents in *Solanum nigrum*. Results are shown as Mean ± Standard error ($p<0.05$), obtained from three replicates

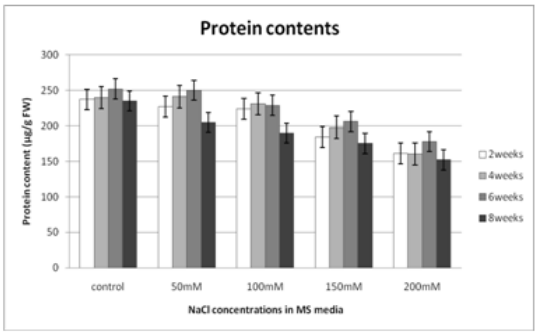
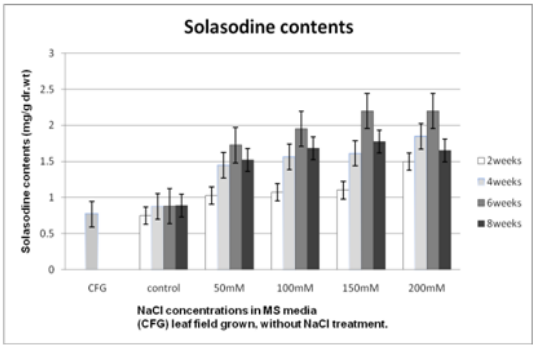


Fig. 5: Effects of different NaCl concentrations on callus Solasodine content in *Solanum nigrum*. Results are shown as Mean ± Standard error ($p<0.05$), obtained from three replicates



Solasodine contents: As well as the proline, the solasodine production, by the callus of *Solanum nigrum*, responds significantly to the increase of NaCl concentration in the culture medium. However, the effect of highest level of NaCl (200mM) on solasodine accumulation is remarkably lower than those of 150 and 100 mM respectively. The highest solasodine concentrations were observed at 6th week Fig. 5.

4. Discussion

Plant responses to NaCl stress have been studied intensively using anatomical, physiological, molecular and proteomic approaches (Wanget et al., 1997; Volkmar et al., 1998; Ephron et al., 1999; Dodd and Donavan 1999; Girija et al., 2002 and Peng et al., 2009). Generally, proline is known to play an important role as an osmoprotectant in plants subjected to hyperosmotic stresses such as drought and soil salinity (Delauney and Verma, 1993). Possible functional role of proline under stress conditions, as noted by Aspinall and Paleg (1981), may include (1) cutoplasmic osmoticum, (2) the hydration of polymers and (3) serving as nitrogen source compound during periods of inhibited growth. Many investigators found that amino acids accumulate in plant when exposed to salt-stress, mainly proline. In our study, we induced *Solanum nigrum* calli to produce solasodine alkaloid under salinity stress. So, to show the physiological effect of NaCl stress concomitant to solasodine alkaloid in *Solanum nigrum,* free proline and total protein had been estimated. The results showed that the free proline increased exponential with the increase in NaCl levels. In 150and 200mM of NaCl concentrations, the free proline had been greatly increased to 100% and 120% than its levels in control. The noticed increase in proline contents is due to; changes in proline metabolism profile under salinity stress, increased expression of proline synthetic enzymes breakdown of proline-rich protein (Tewari and Singh. 1991) and repressed activity of proline degradation ((Delauney and Verma, 1993; Peng et al.,1996).

Although an increase of proline and amino acids accumulation was observed in Cicer arietinum exposed to salt-stress (Soussi et al., 1998). However, opposite results were obtained by (Schobert and Komer, 1989 and Fougere et al., 1991). Our results also show that the increase in NaCl concentration stimulated the total protein accumulation in the callus.

In this study, proteins in *Solanum nigrum* calli were dramatically decreased during the period of study as a physiological response of salinity. It seems that the decrease in total soluble proteins during salinity stress was due to a severe decrease in photosynthetic rate in plants. Photosynthesis decreased under salinity stress (Lee et al., 2004), therefore, protein synthesis dramatically reduced or may be stopped. The decrease in total soluble proteins under drought stress was consistent with the findings of André Dias et al. (2004) in sorghum, Garg et al. (1997) in cluster bean and Surabhi et al. (2008) in mulberry (Morus alba L.) These investigators reported that salinity resulted in a decrease of some soluble proteins.

The solasodine is steroidal glycoalkaloids found in the family Solanaceae, especially genus Solanum. The results were shown that the NaCl stress stimulated the solasodine accumulation. However, positive correlations were observed (table 1). The response to NaCl stress was more remarkable after 4 and 6 weeks. Contrary, the increasing of NaCl from 150 to 200 mM has a negative effectjm solasodine production.

On the other hand, Salt treatments had had a great impact on solasodine content at callus level. The minimum content of solasodine (0.53mg/g dry wt.) occurred in leaves field-grown plants, whereas the maximum content (2.25mg/g dry wt.) was found in in vitro callus. It was obtained with 150mM NaCl supplemeted medium. We noted enhanced solasodine production in some cultures compared to field-grown plants and control treatment (without NaCl). However, the high NaCl level (200 mM) caused remarkably decrease in the solasodine accumulation in callus. This is

due to the great disruption in physiological processes under high salinity levels. Among various cultures, callus treated with 150mM NaCl contained the highest solasodine. Our results corroborate the earlier reports (Rocha et al.,2005) where enhanced alkaloid was noted with increased NaCl level. Various biotic and abiotic factors, used as elicitors, have been reported to increase secondary metabolite yield; application of NaCl enhances alkaloid production (Anitha and Kumari, 2006). In tomato hairy root cultures increased jasmonic acid was noted on 100mM NaCl amended media.28) The biosynthesis of solasodine starts from acetyl coenzyme (A), later converts to mevalonic acid, via mevalonic acid pathway, in which cholesterol, a key intermediate of solasodine, is synthesized. High salinity seems to enhance in vitro cholesterol production, which in turn increases solasodine in tissues, or the enhanced yield may be due to over expression of genes. As *Solanum nigrum* is an important and proven medicinal plant, the present protocol offers possibility of enhanced production of solasodine, using NaCl as an efficient and economical elicitor source.

5. Conclusion

The results shown that it is possible to increase the solasodine production by *Solanum nigrium* cultivated in vitro by increasing the NaCl concentration in culture medium. However, the high NaCl level (200 mM) caused decrease in the solasodine accumulation in callus. Total protein and proline contents were positively correlated with NaCl concentration. The best prominent effect of NaCl on solasodine and proline accumulations was 150 mM. While, the highest level of NaCl (200 mM) caused a decrease in solasodine production.

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