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Effect of Salt Stress on Physiological and Biochemical Characteristics in *Solanum nigrum L.*

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Abstract: ***Solanum nigrum L. is one of the important medicinal plant and contains solasodine, a steroidal glycoalkaloid, which considered as potential alternative to diosgenin for commercial synthesis of various steroidal drugs. In the present study, stem cuttings of uniform size were selected and treated with tap water (1700 ppm salts) and various concentration of NaCl such as 20mM, 40mM, 60mM, 80mM, and 100mM respectively, as experimental plants. Based on the findings it is concluded that Solanum nigrum can be cultivated under salt affected areas which could increase its production of secondary metabolites at the plant level especially at 80mM NaCl treated plants. Our results show that salt tolerance in Solanum nigrum L. depend greatly on the osmotic adjustment (proline, soluble sugars). Proteins were dramatically decreased at 100mM during the period of study as a physiological response to salinity. From the results of this investigation, it is clear that the greater amounts of amino acids, proline, soluble sugar, phenol and alkaloids accumulation is enhanced in the plants treated with 80mM NaCl. Since bore well water in our area being a hard water, where salt concentration ranges from 1700ppm to 8660ppm the plant like Solanum nigrum can with stand 1700ppm salts increase the production of secondary metabolities. It may be considered as stress tolerant, can be cultivated at commercial level to meet the ever increasing demand of medicine, as well as the pharmaceutical industries.***

Keywords: *Solanum nigrum*, *vegetative growth*, biochemical and phytochemical content and salt stress.

1. Introduction

Environmental factors influence the characteristics, composition, growth and development of individual plants and plant communities. When any of these environmental factors exceed the optimum tolerance of a plant, the result is stress to the plant, which in turn influences its developmental, structure, physiological and biochemical processes.

Environmental stresses strongly influence plant growth and development. Salinity is one of the most important of these stresses and can limit crop yield (Koca *et. al.*, 2007). High salt stress disrupts the homeostatic balance of water potential and ion distribution within a plant. Under high salinity, sodium toxicity may cause a range of disorders affecting germination, development, photosynthesis, protein synthesis, lipid metabolism, leaf chlorosis, and senescence. Salt tolerance is the ability to survive in soils with high soluble saline contents (Oncel *et. al.*, 2002).

Soil salinity is one of the major environmental abiotic stresses especially in arid and semi-arid regions and can severely limit plant growth and yield. Salinization of soils or waters is one of the world's most serious environmental problems in Agriculture. It is necessary to determine the environmental factors under which medicinal and aromatic plants give higher yields and better quality.

Today, 20% of the world's cultivated land and nearly half of all irrigated lands are affected by salinity (Rhoades and Loveday, 1990). Salt stress has become one of the most damaging environmental hazards to crop productivity all over the world (Ashraf and Ali, 2008). This adverse environmental condition impairs plant growth by both water deficit and ionic toxicity (Munns and Tester, 2008).Medicinal plants are rich in secondary metabolites like alkaloids, glycosides, steroids, flavonoids are potential sources of drugs. The question of subjecting medicinal herbs to modern scientific test has often been raised. Biosynthesis of secondary metabolites is affected strongly by salt stress resulting in considerable fluctuations in quality and quantity. There can be variation in the pattern of secondary product composition within the same plant. It is possible that the efficacy of the herb depend on the total effect of plant contents rather than the few chemical fractions separated from the herb. Such observation has prompted to select a plant *Solanum nigrum* L. for the changes in biochemical activities of under salt stress.

Solanum nigrum L. is one of the important medicinal plant and contains solasodine, a steroidal glycoalkaloid, which considered as potential alternative to diosgenin for commercial synthesis of various steroidal drugs. The leaves contain glycol alkaloids solamargine and solasonine. The juice of the leaves is used to alleviate the pain due to inflammation of the kidneys and bladder. Besides the leaves have diuretic antiseptic and antidysenrtic properties and also used in the treatment of sores and malaria.

2. Material and Methods

Estimation of photosynthetic pigments Photosynthetic pigments such as chlorophyll a, b, a & b and carotenoids were estimated following the method of Wellburn and Lichtenthaler (1984). The absorbance at 662 nm, 645 nm and 470 nm was measured for chlorophyll a, b and carotenoids respectively using an ELICO SL-171 Spectrophotometer.

Estimation of soluble proteins
The total leaf soluble protein was estimated by Lowry's method (1951). The absorbance was read at 650 nm with an ELICO SL-171 Spectrophotometer. The soluble protein content was estimated using Bovin serum albumin (BSA) as standard.

Estimation of Total Soluble Sugar

Total soluble sugar present in leaf was estimated using anthrone reagent (Jayaraman, 1981). The total soluble sugar present in the leaf was estimated from the Standard graph of glucose.

Estimation of free amino acid

Free amino acid was estimated by Ninhydrin method (Jayaraman, 1981). The color developed was measured at 550 nm using proper blank.

Estimation of proline

Proline was estimated by Bates *et al.*, 1973 method The coloured solution in the test tube was separated out and the absorbance was measured at 520 nm.

Estimation of peroxidase activity

Peroxidase activity was measured by the method of Addy and Goodman, (1972). The absorbance was measured at 420 nm. Estimation of Phenol and total alkaloids

Phenol was estimated by Mahadevan, 1996 methods The coloured solution in the test tube was separated out and the absorbance was measured at 520 nm. Total alkaloids was estimated by Manjunath method

3. Result and Discussion

The response of plants to salt stress varied in relation to the concentration of NaCl. The mean root length 7.78 cm was observed to be higher in tap water treated sample. There was a gradual decrease in root length than the shoot length as the concentration of NaCl increases. High levels of NaCl markedly affect root length. This could be due to excess of soluble salts in the soil that leads to osmotic stress, specific ion toxicity (Munns, 2003).

It has been observed that the survival percentage in 100mM treated plants showed 60% decrease when compared to control and tap water treated plants. The findings were inaccordance with the results obtained on *Majorane hortensis* and spearmint (Shalan *et al.*, 2006 andAl- Amier and Craker, 2007).

The leaves are highly sensitive to environmental stress. Leaf area revealed to be higher in control where as leaf area in tap water treated plant and 20mM NaCl treated plants had close similarity. The leaf area tends to decline in 100mM NaCl concentration. This could to be due to reduced cell division or expansion. Due to biotic stress from salt, the plant tries to cope with the situation by decreasing its leaf area, hence conserving energy (Das *et. al.*, 1900).

Chlorophyll

Salinity had more effect on Chlorophyll b content. The lowest pigments levels were observed in plants treated with 100mM NaCl. The salt stress leads to stomatal closure, which reduces Co_2 assimilation in the leaves and inhibit carbon fixation and lead to reduction in photosynthetic rate (Karthikumar *et al.*, 2007).

A decrease in photosynthetic pigment content of *Solanum nigrum* L. under salt stress. The result obtained in this study are in agreement with is one of the important metabolite pathways in plants. It is a targeted pathway during salt stress. Increased salinity levels reduced chlorophyll a and chlorophyll b contents and total chlorophyll. This could be attributed to the destruction of chlorophyll pigments and the instability of the pigment protein complex (Levit, 1980). It is also attributed to the interference of salt ions with the denovo synthesis of proteins, the structural component of chlorophyll, rather than the breakdown of chlorophyll Jaleel, *et al.*, 2007) Total chlorophyll content was found to be 2.1mg/g in control. Where as NaCl and tap water treated plants showed 1.14mg/g and this could be due to salinity effect which inhibited ions absorption involved in chloroplast formation. (Abd El-Wahab, 2006)

Carotenoids

Carotenoids and anthocyanin being an antioxidant, have the potential to detoxify the plants from the effects of reactive oxygen species.Salinity induced decline in carotenoids and anthocyanin as 99%, 88% in 100mM compared to control. The findings are in accordance with (Singh *et. al.*, 2008) in Maize.

Total soluble sugar

Increase in sugar content in the tolerant cultivar may facilitate osmotic adjustment in the plants (Ghoulam *et. al.*, 2002). This result also suggests that increased carbohydrate increased plant stress tolerance.

Table: 1 Effect of NaCl on growth analysis

Characters	Control	Tap H_2O treated	20mM NaCl treated	40mM NaCl treated	60mM NaCl treated	80mM NaCl treated	100mM NaCl treated
Shoot length (cm)	33.19±0.4(100%)	30.28±0.1 (91%)	27.85±0.66 (83.9%)	21.07±0.5 (63.4%)	19.12±0.4 (57.6%)	14.8±0.275 (44.5%)	12.2±0.5 (36.75%)
Root length (cm)	9.2±0.416 (100%)	7.78±0.27(84.5%)	6.58±0.303 (71.5%)	5.2±0.418 (56.5%)	2.12±0.46 (23%)	1.5±0.235 (16.2%)	1.17±0.2 (12.7%)
Leaf area (cm^2)	7.43±0.41 (100%)	6.7±0.270(89.7%)	6.69±0.418(88.4%)	5.7±0.303 (76.3%)	4.21±0.41(56.2 %)	3.62±0.235 (48.2%)	2.4±0.15 (32.1%)
Total Fresh Weight(g/seedling)	500±0.49 (100%)	454.6±0.4 90.9%)	433.66±0.6 (86.7%)	321.3±0.4 (64.2%)	204.6±0.4 (40.9%)	170.3±0.275 (34%)	116±0.4 (23.2%)

Total Dry weight (g/seedling)	106±0.49 (100%)	92.66±0.4(87.4%)	82.3±0.668 (77.3%)	61.6±0.47 (57.9%)	53±0.473(49.9 %)	43.3±0.275 (40.7%)	37±0.45 (34.7%)

The values in the parenthesis indicates percent activity. The values represents mean of 3 sample with their standard error (+/-)

Control- water 20mM,40Mm,60mM, 80mM,and 100mM

There was a gradual increase in concentration of sugar in salt (NaCl) treated from 20mM to 100mM. The prominent increase in sugar concentration (accumulation) was noticed as 38mg/g in 100mM treated sample, whereas the control and tap water treated samples showed 10.5mg/g and 16mg/g respectively. This was in confirmity with the earlier findings of Najafi *et al*.,2010 in *satureja hortensis.*

Protein and amino acids

Protein content in Solanum nigrumhad been significantly decreased along with increase of NaCl concentration.(Osman *et. al*., 2007).The concentration of protein showed gradual decline from 35% (20mM) to 90%(100mM).Whereas level of amino acids increased in salt stressed plants.(Fig.4). Proteins that accumulate in plants under saline conditions may provide a storage form of nitrogen that is re- utilized later (Singh *et al*., 1987) and may play a role in osmotic adjustment.

Secondary metabolites

Synthesis of secondary metabolites in plants was reported to be regulated and mediated by environmental factors like drought, light intensity and salt stress. The concentrations of various secondary plant products are strongly dependent on the growing conditions, especially under stress conditions.

Proline

Proline play an important role as an osmoprotectant in plants subjected to hyperosmotic stresses such as drought and soil salinity (Delanney and Verma, 1993). Proline occurs widely in higher plants and accumulation in large amounts than other amino acids. The results showed that free proline increased exponentially with the increase in NaCl levels. In 80mM and 100mM of NaCl concentrate, the free proline had been prominently increased to 10.4mg/g and 11.9mg/g than the control 2.23 mg/g. Remarkable increase in proline content under stress conditions could be due to changes in proline metabolism profile under salinity stress, with an increased expression of proline synthetic enzymes and breakdown of proline –rich protein (Tewari and Singh. 1991). Increase in proline content could be attributed to a decrease in proline oxidase activity in saline conditions.

Result of the present study are in agreement with earlier reports on proline accumulation under salt conditions in seedlings and mature plants (Das et al., 1990).

Phenols

Phenol value increases as the salt concentration increases. Phenole are synthesized in the leaves and than carried to other tissues and organs. In this study total phenols increased linearly as observed by (Ozyitgit *et al*., 2007).

Enzyme activity

All environmental stresses have been reported to lead to the production of reactive oxygen species that cause oxidative damage (Smirnoff, 1993). Studies reported increased activities of the antioxidative enzymes in plants under salt tress (Mittova *et al*., 2002).

Statistically significant decrease in catalase and peroxidase activity were observed in both tap water and NaCl treated plants. Increasing NaCl concentration resulted in increasingly lower caltalase activity (Fig.9). The activity of catalase, an important Scavenger of H_2O_2, showed a gradual decrease in both.

Total alkaloids

The results were shown that the NaCl stress stimulated the Total alkaloids accumulation. However positive correlations were observed upto 80 mM concentration. On contray the increasing of NaCl from 80mM to100mM has negative effect in Total alkaloids production. However high NaCl level 100mM caused decrease on alkaloids accumulation. The best prominent effect of NaCl on total alkaloids and proline accumulation was 80mM. Whereas the highest level of NaCl (100mM) treated plants caused a decrease in total alkaloids production. Our results correlate the earlier reports of (Jaleel *et al*., 2007) where enhanced alkaloid was noted with increased NaCl level.

As *Solanum nigrum* is an important and proven medicinal plant, the present protocol offers possibility of enhanced production of total alkaloids, using NaCl as an efficient and economical elicitor source.

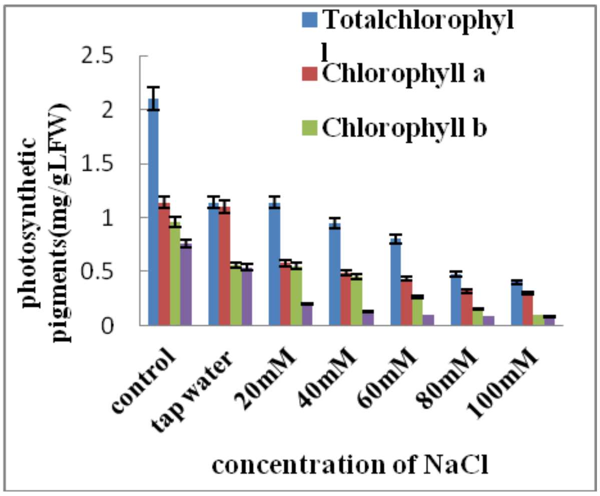

Figure 1: Changes in total chlorophyll content of *Solanum nigrum* plants treated with different concentrations of NaCl. The values are an average of 3 independent measurements.Mean ± SE, n=3

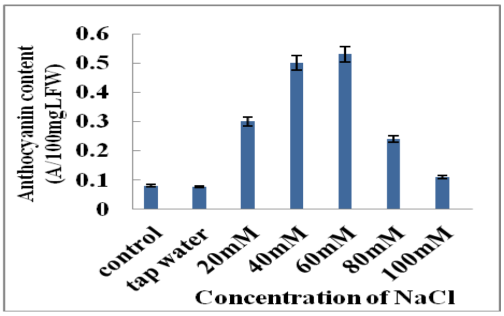

Figure 2: Changes in anthocyanin content of *Solanum nigrum* plants treated with different concentrations of NaCl. The values are an average of 3 independent measurements. Mean±SE, n=3

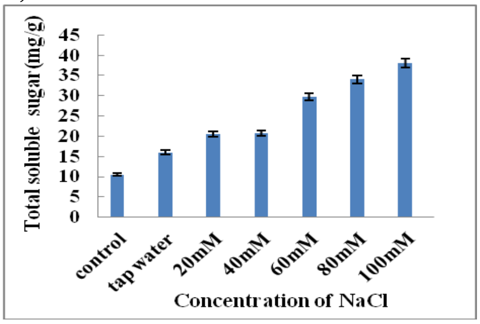

Figure 3: Changes in totalsoluble sugar content of *Solanum nigrum* plants treated with different concentrations of NaCl. The values are an average of 3 independent measurements. Mean±SE, n=3

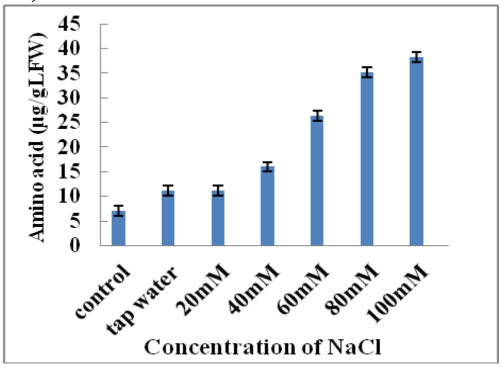

Figure 4: Changes in total amino acids content of *Solanum nigrum* plants treated with different concentrations of NaCl. The valves are an average of 3 independent measurements. Mean ± SE, n=3

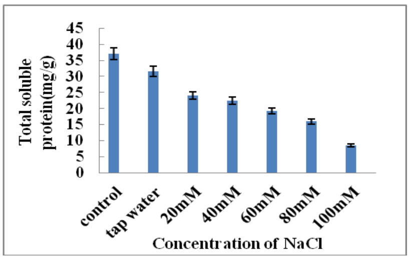

Figure 5: Changes in totalsoluble protein *content* of *Solanum nigrum* plants treated with different concentrations of NaCl. The values are an average of 3 independent measurements. Mean±SE, n=3

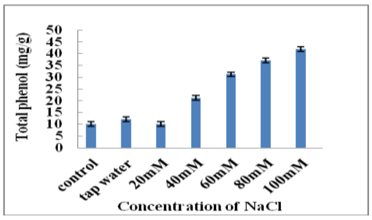

Figure 6: Changes in phenol content of *Solanum nigrum* plants treated with different concentrations of NaCl. The values are an average of 3 independent measurements. Mean±SE, n=3

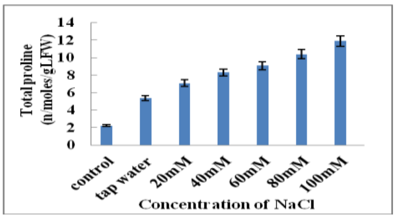

Figure 7: Changes in total proline content of *Solanum nigrum* plants treated with different concentrations of NaCl. The values are an average of 3 independent measurements. Mean±SE, n=3

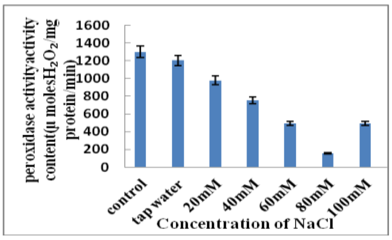

Figure 8: Changes in peroxidase activity content of *Solanum nigrum* plants treated with different concentrations of NaCl. The values are an average of 3 independent measurements. Mean±SE, n=3

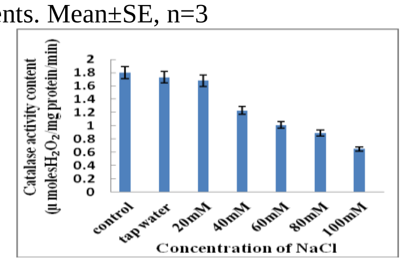

Figure 9: Changes catalase activity content of Solanumnigrumplants treated with different concentrations of NaCl. The values are an average of 3 independent measurements. Mean± SE, n=3

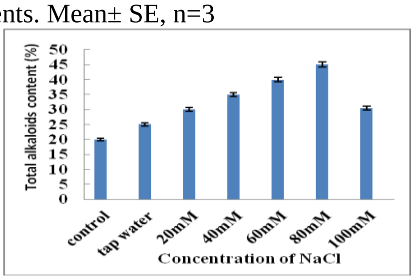

Figure 10: Changes in total alkaloids content of *Solanum nigrum* plants treated with different concentrations of NaCl. The values are an average of 3 independent measurements. Mean ± SE, n=3

4. Conclusion

Solanum nigrum L. may be considered as stress tolerant, can be cultivated at commercial level to meet out the ever increasing demand of medicine, as well as the pharmaceutical industries.

5. Acknowledgement

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