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EFFECT OF NACL SALINITY ON SOME ASPECTS OF NITROGEN METABOLISM OF *SIMAROUBA GLAUCA* DC. SEEDLINGS

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Abstract
A pot culture experiment was designed to study the effect of different levels of NaCl salinity on some aspects of nitrogen metabolism including nitrogen content, free proline content and activity of enzyme nitrate reductase of *Simarouba glauca* DC. seedlings. The nitrogen content in root, stem and leaf was decreased with increasing salt concentration, free proline content was linearly increased with increasing doses of NaCl salinity. The activity of enzyme nitrate reductase in leaflets of *Simarouba glauca* DC. seedlings was increased with increasing salt treatment. The results of the present investigation are discussed in this paper with recent and relevant literature.
Keywords: *Simarouba glauca* DC., seedlings, NaCl stress,Total nitrogen , crude protein, free proline, nitrate reductase.

Introduction

Globally for urbanization and industrialization additional land is utilized, which have domino effect on an overall decrease in agricultural and forest land cover, this leads to changes in environmental conditions. This change in environmental condition not only influences plant life but also affects the animal life. Yet, to fulfill the increasing basic needs of ever increasing human population, plants are the only source on the earth surface. But plants throughout their lifespan are frequently exposed to different kinds of stresses under both natural and agricultural conditions. Soil salinity is one of the important over increasing stress globally.

Salinisation of soil is common in arid and semi arid regions where the amount of rainfall is insufficient for substantial leaching. High concentrations of salts have detrimental effects on plant growth (Bernstein 1962, Taiz & Zeiger 2006, Ramoliya et al. 2004). Salinity is an ever-present threat to crop yield, especially in countries where irrigation is an essential aid to agriculture (Flowers, 2004). Now days the tree species such as *Prosopis, Sesbania, Gossypium* etc. have been employed as a remedy for salt affected soils. One of such tree species *Simarouba glauca* was first introduced by National Bureau of Plant Genetic Resources in the research station at Amravati in Maharashtra in 1966 (Juyal et al., 1991). In the present study, effect of salt concentration on *Simarouba glauca* has been investigated with respect to nitrogen content, free proline content and nitrate reductase activity of *Simarouba glauca* DC. Seedlings.

Materials and Methods

Simarouba glauca DC. edible oil tree and the freshly harvested seeds of *S. glauca* were purchased from Sri Sri Institute of Agriculture, Bangalore. Seeds of *Simarouba glauca* were sown in pots of size 2 ft. NaCl salinity treatment was given after the establishment of seedlings (6 months). The pots were watered alternately with tap water and NaCl solution of 4, 8, 12 and 16 ECe. These four concentrations were prepared according to standard procedure described in USDA Handbook-60. Control plants were irrigated with tap water only. The plants were treated with increasing NaCl concentration alternating with tap water once in a week to maintain the salt concentration in the pots and to restrict accumulation of salt and to compensate the loss of water by evapotranspiration from the plant surface. After 100 days of treatment plants were used for the biochemical analysis. The method described by Hawk *et al.* (1948) was employed for estimation of total nitrogen content of root, stem and leaves of *S. glauca*. Free proline was determined by following the method of Bates *et al.* (1973). The *in vivo* method of Jaworski (1971) was followed for determination of nitrate reductase activity **(EC 1.6.6.1)** in the leaflets of *S. glauca*. The standard curve was prepared with the help of different concentrations of KNO_2 and enzyme activity is expressed as µg of NO_2 liberated h^{-1} g^{-1} of fresh tissue.

Result and Discussion

A. Total Nitrogen and Crude protein

Effect of sodium chloride salinity on the total nitrogen and crude protein contents in root, stem and leaves of *S. glauca* is shown in Fig. 1. Perusal of the figure indicates that in control plants of *S. glauca* total nitrogen and crude protein contents are highest in stem followed by root and leaves. In *S. glauca* total nitrogen and crude protein contents are decreased in root, stem and leaves with increasing salinity.

Nitrogen provides molecular architecture which help in growth and development of plants. Nitrogen is important constituent of nitrate, nitrite, amino acids, amide, urea, ammonia, hexacosamines, quaternary ammonium compounds, proteins. Nitrogen is also a part of enzymes and co-enzymes which regulate many life processes (Leigh, 2003). The nitrogen requirement for optimal growth of the plants ranges from 2 to 5 % on dry weight basis which also depends on the plant species, developmental stages and organs (Marschner 1986). Crude protein content can be obtained from total nitrogen content of a plant tissue by multiplying it with 5.67.

The nitrogen metabolism is the major target of salt stress (Strogonov 1964). Excess of salt may induces alteration in nitrogen metabolism thereby leading abnormal plant growth and metabolism. There are several reports on decreased nitrogen content under salt stress. In *Acacia catechu* seedlings root, stem and leaf tissue decrease nitrogen content was noticed due to increase in soil salinity up to 10 dSm-1 (Ramoliya *et al.,*2004). Hirpara *et al.,*(2005) observed decline in nitrogen content in leaf, stem, tap rot and lateral roots of *Butea monosperma* seedlings due to salt stress. Vaghela *et al.* (2010) noticed decreased nitrogen content in root, stem and leaf tissues of *Butea monosperma* due to soil salinity. Nouman *et al.,*(2012) reported that root and leaf nitrogen content in *Moringa oleifera* was reduced due to salinity. Ali *et al.* (2013) reported decrease in nitrogen content in response to increasing salt treatment in leaves of *Simmondsia chinensis.* Sonar (2013) reported decline in nitrogen content due to 100 and 200 mM salinity in *H. tiliaceus* leaves. In seedlings of *Cassia fistula* L. the reduction in nitrogen content in root, stem and leaf tissues with increasing soil salinity was reported by Hardikar *and* Pandey *(*2011). In the present investigation the reduction in nitrogen content in the root, stem and leaves of *S. glauca* seedlings with increasing salinity was evident. But the values of nitrogen under saline condition are well maintained up to 16 ECe in *S. glauca* in stem and leaf while in root it is upto 12 ECe. Thus it indicates there is little disturbance in the nitrogen metabolism at higher level of salt stress in *S. glauca*.

B. Free proline

Effect of sodium chloride salinity on the proline content in leaflets of *S. glauca* is shown in Fig.2. It is clear from the figure that in control plants of *S. glauca* the proline content is lowest in comparison to treated plants. Proline content in leaflets of *S. glauca* seedlings is increased with increasing salinity treatments.

Proline is important amino acids that contribute to the structure of a protein molecule. Although the accumulation of proline occurs in all plant parts in response to stress but it is common phenomenon that the major site for proline biosynthesis and accumulation are leaves. Various biochemical reasons are involved in the accumulation of free proline as 1. Synthesis from glutamic acid and arginine 2. Decrease in the protein synthesis involving decrease in incorporation of amino acid like proline in the protein molecules. 3. Increase in breakdown of existing proteins. Accumulation of free proline is mainly from glutamate. Appreciable amount of proline in their free amino acid pool is present in several halophytes.

Proline accumulation takes place in a wide variety of plant species under various stress conditions and which plays an important role in adaptive mechanism (Aspinall and Paleg, 1981). An increase in proline content was noticed in the leaves of cashew under salt stress (Viegas, Barreto-de-Melo and Gomes da Silveira, 1999). Ahmad *et al.,* (2010a) noticed an increase in proline content in *Morus alba* under salt stress. An increase in proline content in leaves of *Pistacia vera* (Abbaspour 2012) and *Pistacia atlantica* (Benhassaini *et al.,* 2012) under salt stress. Sonar (2013) reported an increase in proline content in leaf tissue of *Hibiscus sabdariffa* in response to salt stress. According to him the *Hibiscus* species have got the capacity to accumulate proline under saline conditions and accumulated free proline can play a positive role in osmotic adjustment and in enzyme protection in the leaf tissue. In the present investigation there was a steady increase in proline content in leaflets under salinity stress. This is helpful in osmotic adjustment and in enzyme protection in the leaflet tissue under salt stress.

C. Nitrate Reductase (NR; EC. 1.6.6.2)

Effect of sodium chloride salinity on activity of enzyme nitrate reductase in leaflets of *S. glauca* is recorded in Fig. 3. It is evident from the figure that the enzyme activity increases with increasing NaCl treatments in leaf tissue. This increase is more prominent in 16 ECe NaCl treatment.

Nitrate reductase is a large metaloflavo-protein complex with multiple subunits with approximately 800 kDa mass. This enzyme catalyse the reaction of nitrite formation FROM nitrate (Ferrario *et al.,*1998). The enzyme was first isolated from *Neurospora* by Nason and Evans (1953). NADH acts as preferential electron donor for NR in higher plants. Other electron donors such as reduced methyl viologen, benzyl viologen, $FMNH_2$, $FADH_2$ and Dithionite are also involved under *in vitro* conditions (Beevers and Hageman, 1969; Notton and Hewitt, 1971).

There are some reports on influence of salt stress on the activity of nitrate reductase in plants. An increase in NR activity has also been noticed by Krishnamoorthy and Siddique (1985) in cowpea and Joshi (1987) in *Cajanus cajan* under salt stress conditions. Gaikwad (1995a) recorded an increase in the activity of NR in the leaves of *Amaranthus* species grown under saline conditions. Pandey and Agarwal (2002) recorded enhanced activity of NR in *Cassia angustifolia* seedlings only at 3 and 6 days after sowing and then declined subsequently due to salinity stress. In the present investigation salt stress caused an increase in activity of NR in leaflets of *S. glauca* species. This increase in NR activity is reflects in the reduction of nitrate content of *S. glauca* plants under salt stress condition.

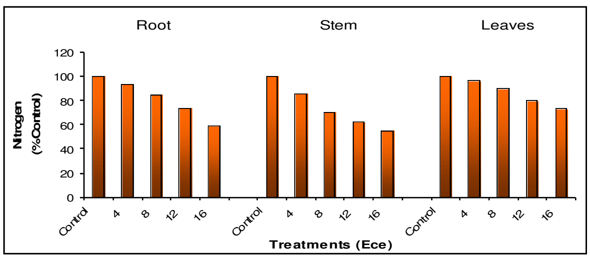

Figure 1. Effect of sodium chloride salinity on nitrogen content of root, stem and leaves of *S. glauca*

Control value:

Root- 3.13 g 100^{-1} g dry wt., **Stem**- 3.97 g 100^{-1} g dry wt., **Leaves**- 2.74 g 100^{-1} g dry wt.

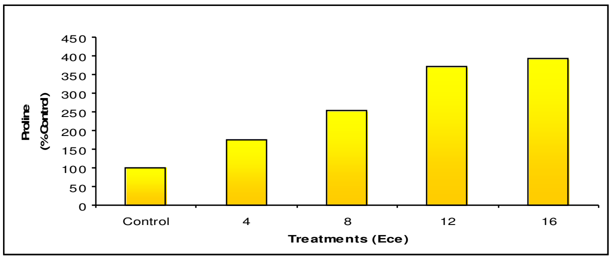

Figure 2. Effect of sodium chloride salinity on proline content of the leaflets of *S. glauca.*

Control value: 0.17 $g100^{-1}$g fresh wt.

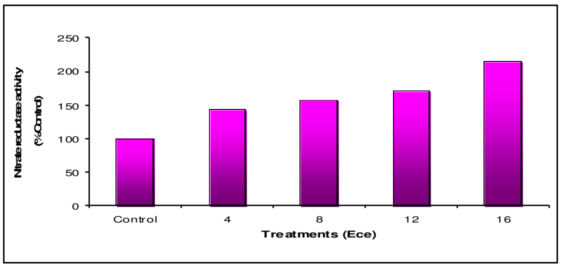

Figure 3. Effect of sodium chloride salinity on the activity of enzyme nitrate reductase in the leaflets of *S. glauca.*

Control value: 0.007 µg of NO_2 liberated h^{-1} g^{-1} fresh wt.

Conclusion

It can be concluded from the above investigation that, the nitrogen metabolism is not so altered by the salt concentration up to 12 ECe NaCl treatment, however, higher dose (16 ECe) caused slight alteration in nitrogen content so it reflects, minor alterations in nitrogen metabolism.

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