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EFFECT OF SALINITY ON BIOMASS, NITRATE REDUCTASE ACTIVITY AND FLAVONOID CONTENT IN *TEPHROSIA PURPUREA*

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ABSTRACT

Tephrosia purpurea or Sarpunkha belonging to family Fabaceae (Sub-family, Papilionaceae) is perennial woody herb, distributed in tropical and subtropical regions of the world .Plants are propagated through seeds. Plants have high economic value due to the presence of phytochemicals like flavonoids, alkaloids, carbohydrates, tannins and phenols, gums and mucilage, fixed oils and fats and saponins and lipids. Flavonoids have antioxidant and antimicrobial activity. *Tephrosia purpurea* Linn. is commonly known as wild indigo in English. In the present study, effect of different levels of NaCl concentration (50,100,200,300 ml) was investigated on biomass and some physiological parameters in sarpunkha. Salt stress causes gradual reduction in nitrate reductase activity and biomass of plants. But it has been observed that under salinity stress the level of flavonoid content increased. These results suggest that high flavonoid accumulation in Sarpunkha might be closely related to its tolerance ability.

KEYWORDS: Biomass, flavonoid, nitrate reductase, nitrogen fixation, phytochemical, tolerance

INTRODUCTION

Salinity is a harsh environmental factor that has major effect on plant. This effect can be seen in the quantity and quality of the plant (Zhu, 2002) .Plants exposed to salt stress undergoes changes in their metabolism with the changes taking place in their environment. In order to survive in salt stress condition, plants develop the network responses of physiological and biochemical defense mechanisms to protect themselves.

Nitrate reductase (NR, E.C.1.6.6.) is the first enzyme in the nitrate assimilation pathway which is very sensitive to NaCl concentration .In addition, the high level of Na+ also causes secondary responses in plants; consequently oxidative stress occurs, leading to cellular damage in the plant cell (Apel and Hirt, 2004).Thus stress induce production of reactive oxygen species (ROS) (Jaleel *et al.*, 2007; Ashraf, 2009). To prevent the potential cytotoxic effects of ROSs, the stimulation of antioxidant systems can assist in plant protection from oxidative stress. Plants have developed antioxidant enzymes such as superoxide dismutase, ascorbate peroxidase, glutathione reductase, catalase, peroxidase and non-enzymatic scavengers like glutathione, ascorbic acid, carotenoids and flavonoids which regularly maintain ROS balance within the cell (Vranova *et al.*, 2002; Dalmia and Sawhney, 2004). Therefore the present study aims to investigate the effect of NaCl stress on biomass, nitrate reductase activity and flavonoid content in *Tephrosia purpurea*.

MATERIAL AND METHODS

To study the effect of NaCl on nitrogen metabolism, seeds were collected from Botanical Garden of D.D.U Gorakhpur University campus. Seeds were dehusked, sterilized by 70% ethanol and washed with distilled water. Surface disinfected seeds were sown in earthenware pots (30x30cm) containing soil. These pots were irrigated with saline water containing 100mM, 200mM and 300mM NaCl, respectively. Water was applied in each pot daily to keep the sand moist and hence to maintain the salt level. Biomass, nitrate reductase and flavonoid contents were recorded at every ten days interval from day 35 up to day 65 with each salt concentration. Untreated plants were kept as control. Biomass of plants was calculated by drying of plants in an oven at 60 ± 2^0 to constant weight and their dry weight.

Nitrate reductase (NR) was assayed by the method of Streeter and Bosler *et al.*, (1972).Total flavonoid content was determined according to Change (2002).

Statistical analyses: This experiment was laid out in a completely randomized design (CRD) with three replications. Data were analyzed statistically by the Statistical Package for Social Sciences (SPSS) 16. One-way analysis of variance (ANOVA) was

performed to test the significant differences for all measurable variables.

RESULT

Biomass, NR activity and flavonoid content were estimated in *Tephrosia purpurea* grown under different NaCl concentrations and the results are presented .Biomass increased with plant age at every NaCl concentration level. Biomass increased in plants grown at 100mM NaCl concentration but at 200 and 300mM NaCl concentration significant decrease in biomass was observed as compared to control (Fig-1a). Nitrate Reductase activity increased up to 45 days but beyond that it decreased. The highest NR activity was recorded at 100mM on 45 th day of plant growth .NR increased significantly at 100mM, while it decreased gradually at 200or 300mM NaCl concentration compared to control (Fig-1b).Total flavonoid content increased with increasing salt concentration. Flavonoid accumulation was higher at 300mM NaCl concentration than control (Fig-1c).

DISCUSSION

Salinity is a primary constraint for plant growth and survival. In this study, we examined the effects of different concentration of NaCl on the performance of *Tephrosia purpurea.* Optimal growth occurred at salinities between 50mM and 100 mM. Plants gave better response at 100mM NaCl treatment than control. Increasing salinity (200 to 300 mM NaCl) caused a corresponding reduction in biomass accumulation. Biomass of *Tephrosia purpurea* up to 100mM NaCl concentration but at 200 and 300 mM NaCl concentration significant decrease was observed as compared to control. This was in accordance with Xianzhao *et al.*, (2013) who observed that at low salt stress, halophytes have a nutritional requirement for sodium and an optimal salt concentration during the process of growth., This may be an adaptation of halophytes to salt stress by accelerating growth in order to reduce the salt concentration (Mandak, 1999).

NR enzyme localized in the cytoplasm, is the first enzyme in the pathway of nitrate assimilation. It catalyses the reduction of nitrate to nitrite which is further reduced to ammonium by nitrate reductase in the chloroplast (Beevers and Hageman, 1983; Orcutt and Nilsen ,2000) have reported that increased Na^+ concentration decreases nitrate reductase activity by the inhibition of photosystem II which results in consequent breakdown of chlorophyll.However, in the present investigation, nitrate reductase activity increases in plants treated with 100mM NaCl concentration and decreases gradually with 200 and 300mM NaCl concentration Decrease in nitrate reductase is not due to direct inhibitory effect of salinity stress, it may be due to reduction in nitrate uptake. A similar observation was also made in

Rhizophora apiculata and *Acantus ilifolius* (Sivanadanam, 2012).

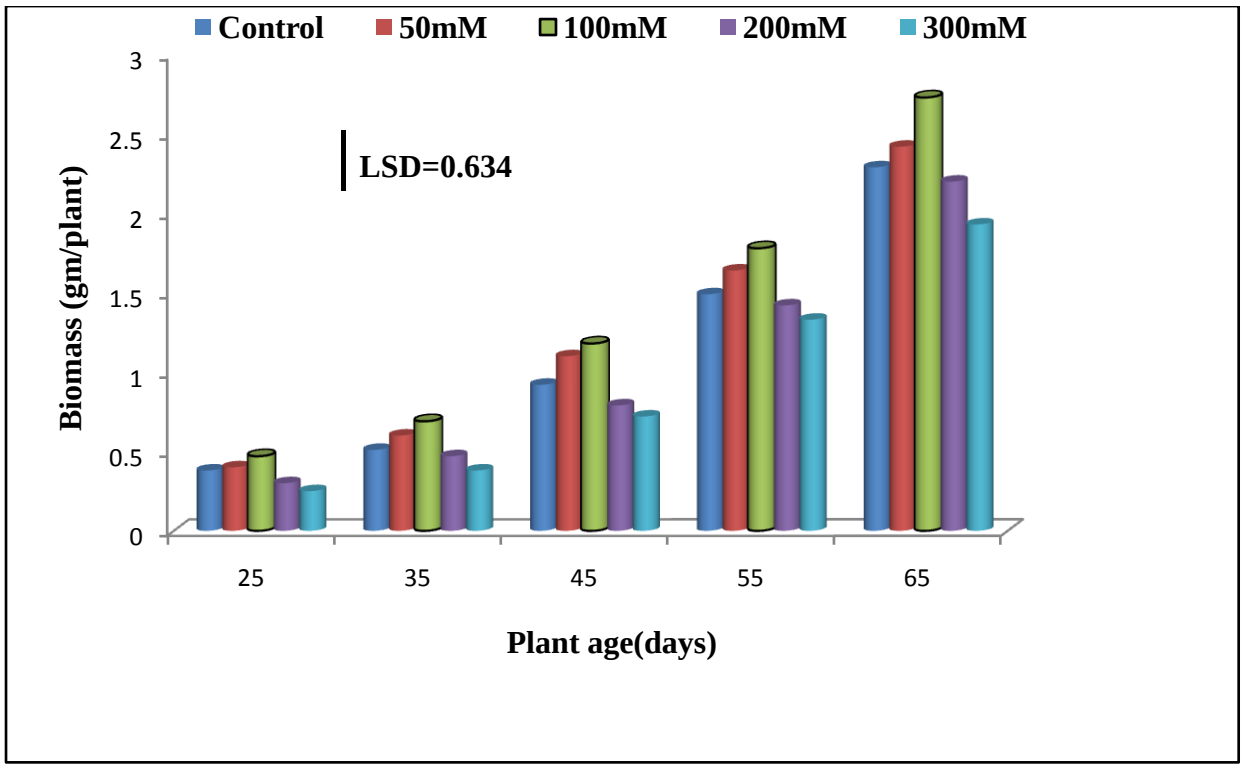

Fig 1a: *Tephrosia purpurea:* Biomass at different age of plant growth under different NaCl concentrations

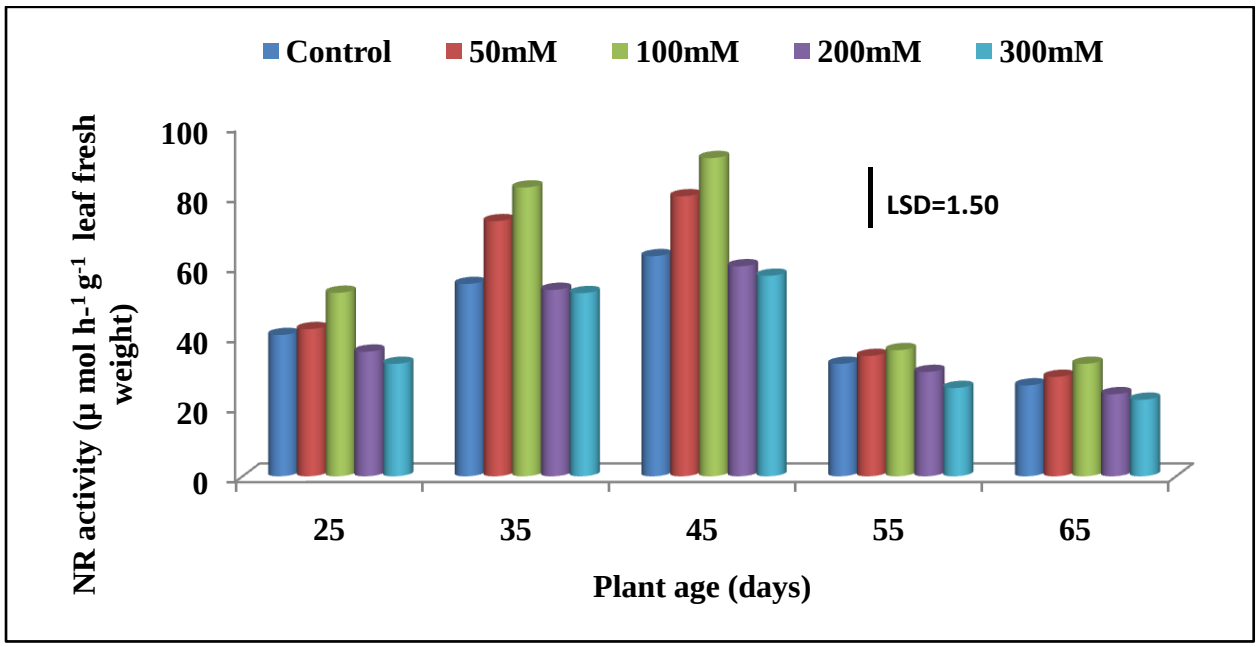

Fig 1b: *Tephrosia purpurea*: Nitrate reductase activity at different age of plant growth under different NaCl concentrations.

Flavonoid may have protective role under salt stress condition. Flavonoids are frequently induced by abiotic stress and play a major role in plant protection (Grace and Logan, 2000). In the present work flavonoid content increased with increasing salt concentration The result is in accordance with Chutipaijit *et al.*, (2009) who observed increase in flavonoid content in tolerant variety jasmine rice (KDML 105) and Sangyod (SY) of rice under salt stress and suggest the accumulation flavonoids may have an important role in osmotic adjustment, prevent the generation of oxygen radicals and protect the cells from damaging effects which lead to its resistance towards salinity.

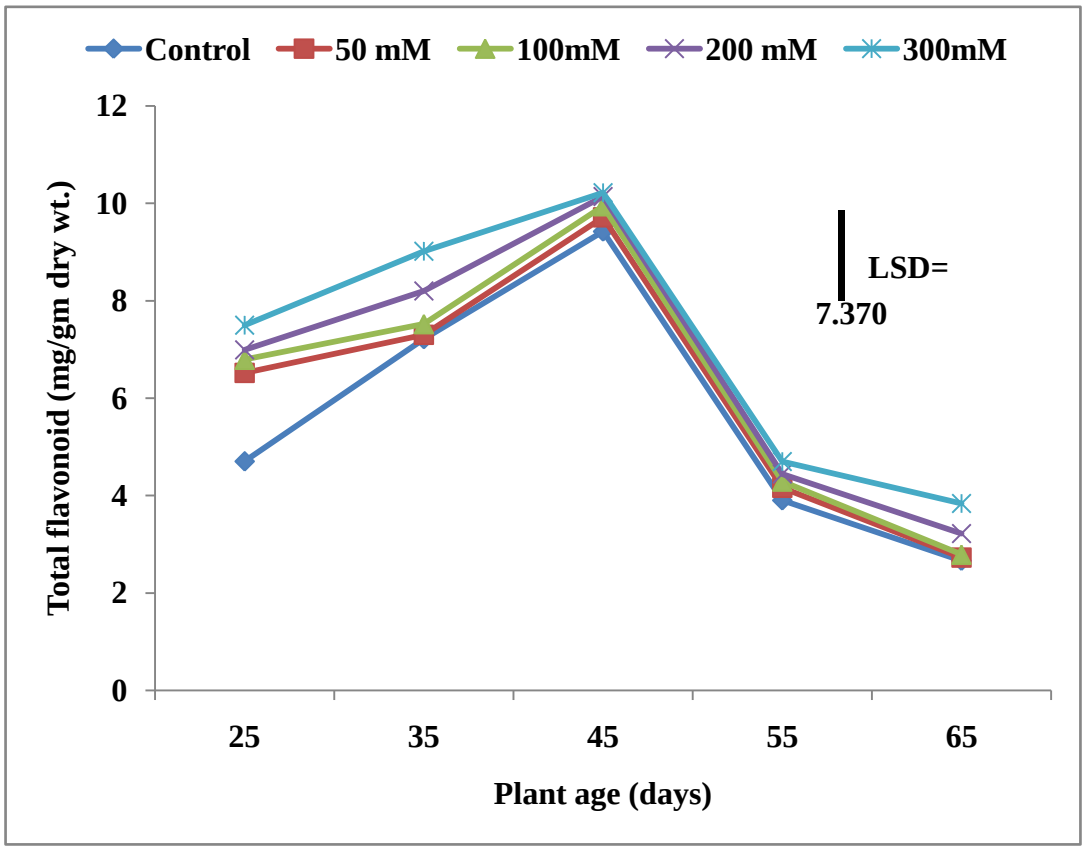

Fig 1c: *Tephrosia purpurea*: Flavonoid activity at different age of plant growth under different NaCl concentration

CONCLUSION

In the present study, it was observed that at lower salt concentration (100mM) the plant exhibited better growth as compared to control. However higher salt concentration had negative effect on plant growth .Hence, the plant is sensitive to higher level of NaCl concentration. Highest accumulation of NR activity at 100 mM indicates an adaptation mechanism by the plant to overcome osmotic stress. In contrast, accumulation of increased level of flavonoid at higher level of NaCl concentration is related with tolerant abilities of the plant.

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