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RESPONSE OF *TEPHROSIA PURPUREA* L. TO SALINITY STRESS IN RELATION TO GERMINATION, CAROTENOID CONTENT AND PROLINE CONTENT

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ABSTRACT

Salt stress is one of the major abiotic stresses limiting profitable crop production. The effect of salt stress on germination,carotenoid and proline content of *Tephrosia purpurea* was investigated Three different salinity level (50,100 and 200 mM)were used for germination experiment and distilled water as control. For pot experiment plants were treated with four different NaCl concentration (50,100,200,300mM).Normally irrigated plants were treated as control. Observations were recorded from day 25 up to day 65 at ten days interval. Result indicated that lower salt concentration (50mM) enhance seed germination but beyond it germination decreases. The result show that salinity increase carotenoid and proline content. The increase of proline with salinity increase shows the importance of the osmotic balance in low water potential condition. In general the results of the experiments showed that increase in production of proline as an osmotic regulatory mechanism for survival in high salinity levels.

Key words: Carotenoid, proline, salinity stress, seed germination, Water potential.

INTRODUCTION

Tephrosia purpurea or Sarpunkha belonging to family Leguminosae (Sub family -papilionaceae) the genus *Tephrosia* comprises between 300 to 400 species of annual and perennial woody herb, distributed in tropical and subtropical regions of the world (Willis 1973, Zhi and Pedley 2010). Plant 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 antioxidants and they have strong antimicrobial activity.

According to Ayurveda literature this plant has also given the name of wranvishapaka which means that it has the property of healing all types of wounds (Devprakash *et al* 2011). It has been used in Ayurvedic system, Siddha, Unani system of medicine for the treatment of various diseases. It has been reported to possess hepatoprotective and mast cell stabilizing effect in various experimental models (Sandhya *et al* 2010). The dried herb is effective as a tonic, laxative, and diuretic. It is also used in the treatment of bronchitis, bilious febrile attack, boils, ulcer, hypoglycemia, pimples, and bleeding piles, anthelmintic, alexiteric, and antipyretic.

Plant growth and productivity are greatly affected by environmental stresses such as dehydration, high salinity, low temperature and biotic pathogen infection. Salinity stress is one among the various environmental stresses. Soil salinity is a major factor limiting the crop production globally (Kumar *et al.*, 2010). Presence of any kinds of salt in growth environment increases osmotic pressure and water stress but salts toxicity are different in the process. Despite of the fact that sodium chloride is known as a salt with less toxic; however, it is one of the most common types of salts and consequently it is considered one of the most harmful salts (Bliss et al. 1984).

The salinity, by increasing the production of reactive oxygen species, such as super oxide (O_2-), hydrogen peroxide (H_2O_2) and hydroxyl radicals (OH^-), in the chloroplasts and other organelles, thus leading to disruption of cellular metabolism through membrane lipid peroxidation, protein oxidation, enzyme inhibition and damage to nucleic acids (Prakash *et al.*, 2011; Sabra *et al.*, 2012). Seed germination is usually the most critical stage in seedling establishment; determining successful crop production (Almansourie *et. al*., 2001; Bhattacharjee, 2008). Salinity is important factor limiting plant growth and delaying seed germination as well as final germination percentage (Rahman *et. al*., 2000).

Plants have improved complex mechanisms systems for adaptation to osmotic and ionic stress caused by high salinity, under the salt stress. One of the mechanisms is proline accumulation into cell. The role of proline in cell osmotic adjustment, membrane stabilization and detoxification of injurious ions in plants exposed to salt stress is widely reported (Kavi et al., 2005; Ashraf and Foolad, 2007).However, the significance of proline accumulation in osmotic adjustment is still debated and varies according to the species (Lutts et al., 1996; Rodriguez et al., 1997). Carotenoids (CAR) are responsible for quenching of singlet oxygen (Knox *et a*l 1985).

Although much work has been done on the effect of salinity on various aspects of crop plant growth and development, little information regarding salt tolerance of Tephrosia is known hence it is necessary to understand the response of *Tephrosia* to salinity stress.

In the present study the effects of different salinity concentrations on seed germination, carotenoid and proline content, in *Tephrosia* plants were investigated

MATERIALS AND METHODS

Mature fruits of *Tephrosia purpurea* were collected and seeds were separated. Seeds were then washed, dried and stored in plastic bags for six months

Seed Germination study:
Germination was observed by using Petri dishes with double filter papers. The Petri -dishes were arranged in a completely randomized block design with three replications. To control fungal infection during germination, seeds were surface sterilized by 0.1% $HgCl_2$ for one minute Seeds were mechanically scarified with the help of sand paper.

A total of 20 seeds were kept in each Petri -dish on double-layer Whatman paper, and 10 ml of appropriate solution or NaCl (50,100,200 and300mM) was added to each Petri- dish (Asgharipour and Rafiei, 2011).Distilled water was used as control. The seeds pretreated with NaCl solutions for 24 h at room temperature. The seeds were then drained, rinsed twice with distilled water, and were allowed to continue germination on moist double-layer Whatman paper in the dark .The Petri dishes were kept for 9 days. 5 ml of distilled water was added to the Petri dishes daily. Germination percentage was recorded.

Germination percentage:
The germination percentage (GP) was recorded up to 9 days of treatment .The radical and plumule emergence was taken as an index of germination .Germination percentage was

calculated by the following formula. (Cokkizgin and Cokkizgin, 2010; Tanveer *et. al*., 2010).

Germination percentage = No. of Seeds Germinated/Total No. of Seeds x 100

Seed vigor index:
Seed vigor index (SVI) was calculated according to Baki and Anderson (1973).The seedling length was measured in centimeters and SVI is calculated as follows:
SVI = [Seedling length (cm) x Germination Percentage (%)]

Plant growth-

Surface sterilized seeds of *Tephrosia purpurea* were inoculated with 96 hour grown culture of *Rhizobium* for 12 hour at 25°C to 30°C.The inoculated seeds were sown in earthenware pots (30×30 cm) containing sand. Two weeks after sowing the range of salinity treatments were added to the pots. The pots were irrigated with saline water containing 50mM,100mM,200mM and 300mM NaCl, respectively(corresponding E.C. was 4.42,9.85,21.8 and 32.5ds/m respectively) every week . Each pot was supplied with Hoagland's (1944) nutrient solution weekly. There were 3 replications for each treatment and the experiment was arranged in a complete randomized design. Water (250 ml) was applied to each pot daily to keep the sand moist and hence to maintain the salt level.
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Plant samples were collected from each set at 10 day interval starting from day 25 to day 65, were used for the study of chlorophyll content, crotenoid content and proline content.

Estimation of carotenoid content:
Carotenoid content in the leaf tissues (100 mg) of control and NaCl-treated seedlings were extracted in acetone and determined by the method of (Arnon, 1949). The absorbance of the extract was recorded at 480 nm.

Estimation of proline content :
Proline content of leaf tissues was estimated spectrophotometrically following the ninhydrin method described by Bates et al (1973) with minor modification. Powdered frozen tissues (200 mg) were homogenized in 3% of sulphosalicylic acid to precipitate protein. Samples were mixed, centrifuged at 8000 ×*g* for 15 min, and the supernatants were treated with glacial acetic acid and ninhydrin reagent [3% (w/v) ninhydrin in 60% cooling, the products were extracted with toluene by vortex mixing, and then the absorbance of the upper (toluene) phase was determined at 520 nm against a zero time blank. Proline concentrations were calculated using proline standards (0-100 μg mL–1) in identical manner

Statistical analysis:
The experimental design comprised complete randomized blocks (CRD) with three replicates. The results were evaluated by analysis of variance using the Statistical Analysis System software spss16.0, and treatments means were considered significantly different at $p<0.05$. Mean separation was evaluated by Least Significant Difference (LSD) test (Duzgunes *et. al.*, 1983).

RESULTS AND DISCUSSIONS

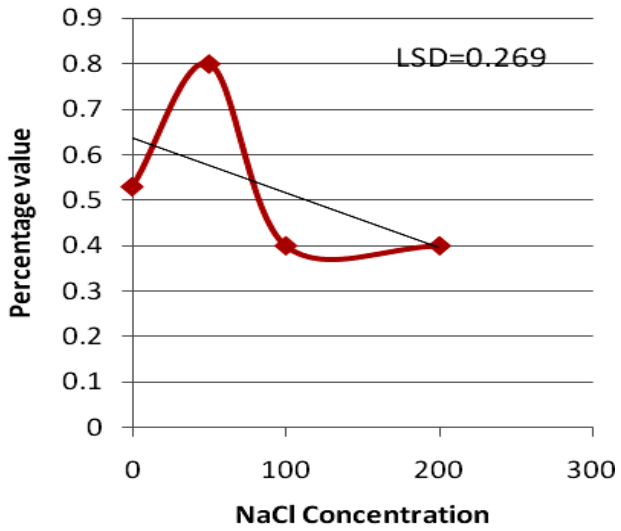
Germination percentage:
Maximum GP in 50 mM NaCl concentration, and minimum in 200 mM NaCl concentration was recorded .there is a steep increase in GP of the plant when the concentration was increased from control level to 50mM but GP was significantly affected by NaCl showing a decreasing trend from 50 mM to 200mM.(Fig 1A). The increase in salinity not only decreased the germination but also delayed the germination initiation (Hajer *et al* 1996).Inhibition or delay in germination under saline condition is due to an osmotic effects.(Gosset *et al* 1994,El-Baz, *et al*, 2003) which limits the uptake of water during seed germination.*(Flowers et al 1986*)

Seed vigor index:
The highest SVI was observed in the control whereas the lowest was noted at 200 mM NaCl concentration .The SVI increases at 50mM in comparison to control, but it shows decreasing trend when the NaCl concentration increases further.(fig 1B) .It means increased NaCl

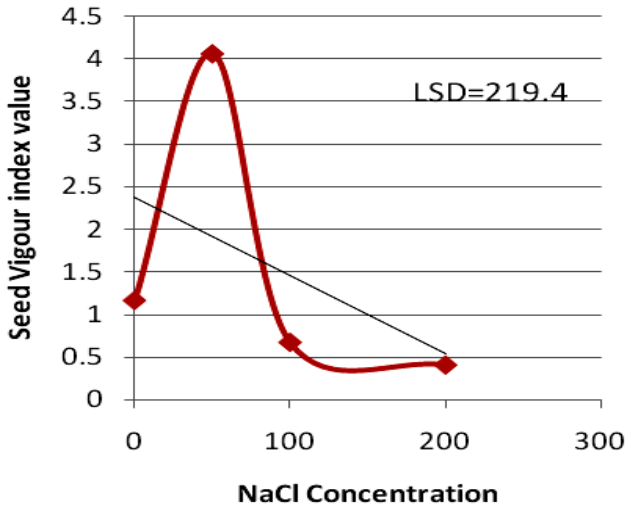
concentration after 50mM caused a harmful effect on the seed. Salt toxicity effects in plants are clearly visible in both root and shoot growth (Amzallag and Lerner, 1994; Mallaiah, 2013). The results indicated a decrease in trend in shoot height and root length as salinity increased. This is supported by the findings of Gill and Singh (1992). Similarly, Al-Mutawa (2003) reported that increased salinity also leads to decreased radical length.

Figure-1: Effect of salt stress on germination percentage and seed vigour index

A)



B)

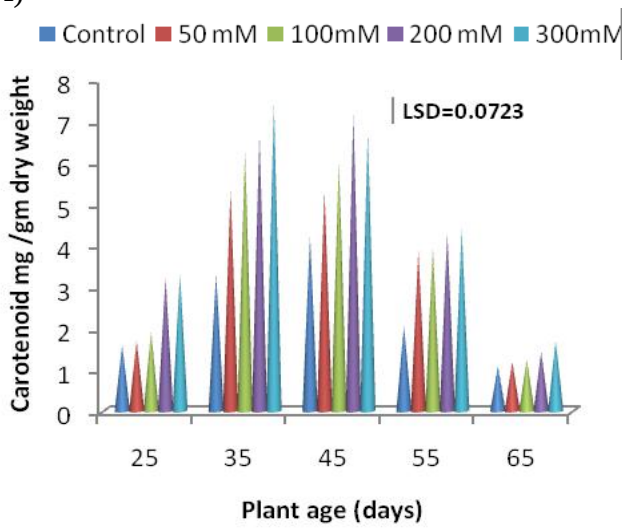


Carotenoid content:

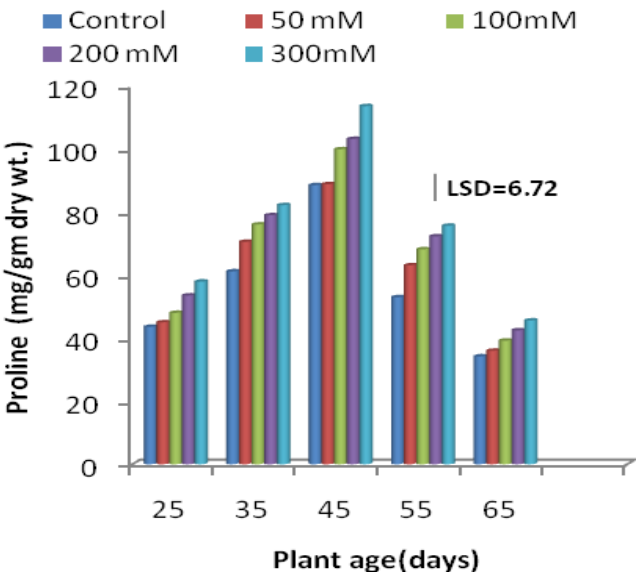
A significant variation in the carotenoid content in the leaves of the plants was observed at different NaCl concentration. Carotenoid content increased from day 25 to day 45 which was followed by a gradual decline .Carotenoid content increased with increasing salt concentration. Zia et al (2009) have also reported higher carotenoid content in salt tolerant hot pepper cultivars Carotenoids (CAR), being antioxidants, have the potential to detoxify the plants from the ill effects of ROS (Verma and Mishra, 2005). Carotenoids are responsible for quenching of singlet Oxygen (Knox and Dodge, 1985)

Figure-2:Proline content and carotenoid content at different age of plant growth in control and different concentration of NaCl

A)



B)



Proline content:
A significant variation in proline content in leaves of plant treated with different NaCl concentration was observed. There was a gradual increase in Proline concentration from day 25 to day 45 followed by a gradual decline. Maximum Proline content was observed at 45 DAS in plant treated with 300mM NaCl concentration. The results of the present study are in agreement with Zahra *et al*., 2010 in *Plantago ovata* under salinity stress. Proline is an organic solute known to be involved in osmoregulation which reduces the cell osmotic potential to a level to provide high turgor potential for maintaining growth (De Lacerda *et al*. 2005; Ashraf and Harris, 2004; Chaum *et al*. 2004).

CONCLUSIONS

This study demonstrated that in *Tephrosia*, seed germination varied according to the change in NaCl concentration. 50mM NaCl concentration had a positive effect and induced seed germination in comparison to control. At the germination stage, seeds were found to be sensitive to high-level salt concentration .Process of seed germination is sensitive to high salt concentration which reduces seed germination. From results it may be concluded that accumulation of carotenoid and proline content may act as osmoprotectants in *Tephrosia* under saline condition and it can be used as biochemical markers for salinity tolerance.

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