

IMPACT OF SALINITY ON SEED GERMINATION IN *TEPHROSIA PURPUREA* L.

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ABSTRACT

This study was done to investigate the effect of different level of NaCl concentration on the germination of *Tephrosia purpurea* L. seed. Different concentration of NaCl (50,100,200 and 300 mM) and distilled water (control) was experimented on the seed of this wild leguminous plant. The experiment showed that the Mean germination time (MGT), Germination index (GI), Coefficient of velocity of germination (CVG), Germination percentage (GP), and Seed vigor index (SVI) varied between 1.7 and 3.5 days, 1.91 and 1.00, 1.08 and 0.280, 80 and .60% and 4.06 and 0.40, respectively. There was no germination at 300 mM. Significant differences were found among NaCl treatments in terms of GI ($p < 0.05$), GP ($p < 0.01$), and SVI ($p < 0.01$). The plant showed positive response under treatment of 50mM NaCl concentration but beyond that it showed negative response in all examined parameters, except MGT. The max GI, GP, CVG, and SVI were observed at 50mM concentration and min at 200mM NaCl concentration. Correlation coefficients between all parameters were calculated and the consequences showed that MGT, GI, GP, CVG, and SVI had significant positive or negative correlation with each other.

Key words: Correlation, Salinity stress, Sodium chloride (NaCl), Seed germination, Wild legume.

INTRODUCTION

The genus *Tephrosia* L. or Sarpunkha is a wild legume, grows throughout India and Western Himalaya, belongs to family Leguminosae (Sub family-Papilionaceae) is a plant of high economic value due to the presence of phytochemicals like flavonoids, alkaloids, carbohydrates, tannins and phenols, gums and mucilage, fixed oils, fats, saponins and lipids. Flavonoids have antioxidants and strong antimicrobial activity. The plant also relieves dental pain, asthma, leprosy, arrests bleeding. According to Ayurveda, this plant is digestible, anthelmintic, alexiteric, and antipyretic. It is

alternative cure for the diseases of liver, spleen, heart, blood, tumours, ulcers, leprosy, asthma, poisoning etc. Leaves are tonic to intestines and a promising appetizer. They are also used in piles, syphilis and gonorrhea. Plants grow wild and are in abundance so they are helpful in checking the soil-erosion. Leaves are used as fodder. Moreover seeds can be used as substitute to coffee. Being a leguminous plant it enriches the soil by fixing atmospheric nitrogen.

Abiotic stresses affect plant metabolism, disrupt cellular homeostasis and uncouple major physiological and biochemical processes. Among abiotic stresses salinity is one of the

most severe problem worldwide in agricultural production. One third of the world's agricultural land is damaged and approximately 5% of 1.5b ha of cultivated land is affected by salt (Tabatabaei, 2006). Saline stress is one of the main factors limiting legume productivity in arid and semi-arid regions (Lluch *et al.*, 2007), and salinity has direct harmful effect on numerous plant species.

Salinity limits growth and development in plants. The response of plants to excess NaCl is complex and involves changes in their morphology, physiology and metabolism (Hilal *et al.*, 1998).

Salinity is known to retard plant growth through its influence on several facets of plant metabolism including osmotic adjustment, ion uptake. Enzyme activities, photosynthesis and hormonal imbalance. Although much work has been done on the effect of salinity on various aspect 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.

Seed germination is a crucial stage in the life history of plants and salt tolerance during germination is critical for the stand establishment of plants and grows in saline soils (Khan *et al.*, 2000). Several investigations have indicated that seeds of most species attain their maximum germination in distilled water and are very sensitive to elevated salinity at the germination and seedling phases of development (Gulzar *et al.*, 2003; Ghoulam and Fares, 2001). In this study, the effect of salinization on the germination of *Tephrosia* seeds was examined through laboratory experiment.

MATERIALS AND METHODS

Seed material:

Seeds were collected from wild plants of the previous year crop. Seeds were mechanically scarified with the help of sand paper. In *Tephrosia purpuria* (Linn.) Pers. germination was improved by scarification with sand

followed by presoaking in hot water at 50 C for five minute (Sundraraj et al 1971).



Control



50mM



100mM



200mM

NaCl Solutions and seed treatments:

To control fungal infection during germination, seeds were surface sterilized by 0.1% HgCl₂ for one minute (Ramakrishna *et al.*, 1991). and washed thoroughly with distilled water .

The seeds were then germinated in 120-mm-diameter sterilized Petri dishes. All Petri dishes were washed with tap water, followed by a rinsing with distilled water, and then were sterilized at 120°C for 15 min in hot air oven.

The Petri dishes were arranged in a completely randomized block design with three replications. A total of 20 seeds were put in each Petri dish on double-layer Whatman paper, and 10 ml of appropriate solution or NaCl(50,100,200 and 300mM) was added to each Petri dish (Asgharipour and Rafiei, 2011). Distilled water was used as control.

Subsequently, the seeds were imbibed in 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 new

Whatman paper in the dark. The Petri dishes were kept for 9 days. During this period, the Petri dishes were observed daily. Each day, 5 ml of distilled water was added to the Petri dishes.

Mean germination time:

The seedling stunted primary roots were considered as abnormally germinated. A seed was considered to have germinated when the radical reached a length of 10 mm (Goertz and Coons, 1989). The mean germination time (MGT) was calculated to assess the rate of germination (Ellis and Roberts, 1981) as follows:

$$MGT = \Sigma(Dn) / \Sigma n$$

where n is the number of seeds germinated on each day and D is the day of counting. Cotyledons were not included in fresh and dry weight comparisons.

Germination index :

Germination Index (GI) was calculated as described by the Association of Official Seed Analysts (AOSA, 1983) as:

$$G = \frac{\text{(Number of Germinated seeds in first Count/Days of first count)} + \dots + \text{(Number of Germinated seeds in final Count/Days of final count)}}{\text{Total number of seeds}}$$

Coefficient of velocity of germination:

Coefficient of velocity of germination (CVG) was evaluated according to Maguire (1962) as follows:

$$CVG = \frac{(G_1 + G_2 + \dots + G_n)}{(1 \times G_1 + 2 \times G_2 + \dots + n \times G_n)}$$

where G is the number of germinated seeds and n is the last day of germination.

Germination percentage:

The germinated seeds were counted daily according to the seedling evaluation procedure described in the Handbook of Association of Official Seed Analysts. The number of germinated seeds were recorded every 24 h (AOSA, 1990). Ten days after germination, the

germination percentage (GP) was obtained by dividing the number of germinated seeds in any Petri dish by the total number of seeds, multiplied by 100 (Cokkizgin and Cokkizgin, 2010; Tanveer *et al.*, 2010).

Seed vigor index:

Seed vigor index (SVI) was calculated according to Baki and Anderson (1973) as follows:

$$SVI = [\text{Seedling length (cm)} \times \text{GP (\%)}]$$

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

Mean germination time

It was observed during the study that there is significant difference in MGT after NaCl treatment Table 1. The highest MGT was observed at 200mM (3.5 days), and the lowest was observed at control state (1.7 days) in Table 2. The present results agree with those reported by Pujol *et al.* (2000), who observed that an increase in salinity induces both a reduction in the percentage of germinating seeds and a delay in the initiation of the germination process.

Correlation coefficient

All possible combinations were estimated and are shown in Table 3. The perusal of data shows that MGT has significant negative correlation with germination index. Relationship between the MGT and NaCl concentration of *Tephrosia* seeds has been shown in Fig 1 A.

It is mention worthy that at 50mM the germination enhanced in comparison to control, but beyond it the increase in salt concentration

did not support seed germination rather its effect is negative.

NaCl concentration when increased affected the CVG negatively. Considerable decrease is noted

Table. 1. Summary of analysis of variance for all the analyzed parameters

Source of variation		MGT	GI	CVG	GP	SVI
NaCl	3	1.701	0.538	0.429	0.107	8.548
Error	8	0.354	0.230	0.166	0.013	5.078
Coefficient percentage		30.51	35.04	93.80	36.92	156.00

*, ** Significant at 0.05 and 0.01 probability levels, respectively

Table.2. Salinity effects for all parameters on germination in *Tephrosia purpurea*

NaCl concentration	MGT	GI	CVG	GP	SVI
Control	1.7a	1.88a	1.08a	0.53	1.16
50 mM	2.7ab	1.91a	0.39a	0.80	4.06
100 mM	3.1ab	1.00a	0.32	0.40	0.66
200mM	3.5b	1.16a	0.28	0.40	0.40
LSD Value	1.3882	1.1189	0.9509	0.2690	219.46

Means followed by the same letter are not significantly different at $p < 0.05$ level (LSD test)

Table 3. Correlation matrix for analyzed variables

	MGT	GI	CVG	GP	SVI
MGT	1.00				
GI	-0.543*	1.00			
CVG	-0.576*	0.034*	1.00		
GP	-1.27*	0.476*	-0.140*	1.00	
SVI	-0.201*	0.038*	-0.128*	0.837**	1.00

*, ** Significant at 0.05 and 0.01 probability levels, respectively

Germination Index:

GI is affected by NaCl treatment (Significant difference). GI increased with 50 mM concentration in comparison to GI under control condition. Further GI decreases, when the concentrations were increased to 100 mM and above. The highest GI, was observed in NaCl concentration of 50 mM and it kept on decreasing when the concentration was increased thereafter, in Fig 1.B. The response of the plant from 50mM conc. and above is similar to the result reported by (Yan 2008).

Coefficient of velocity of germination:

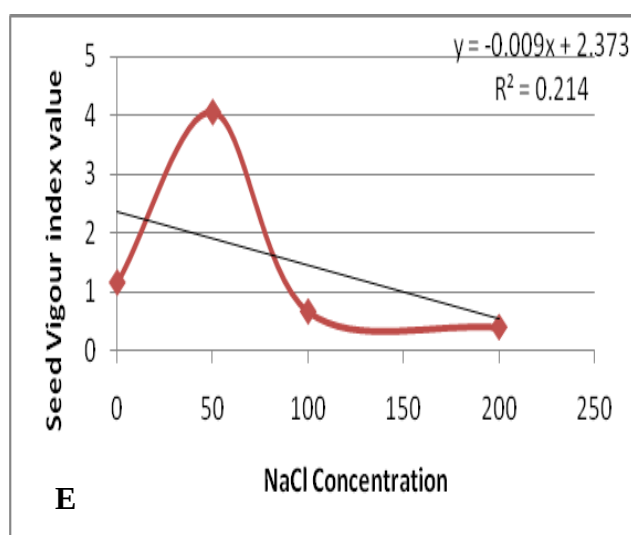
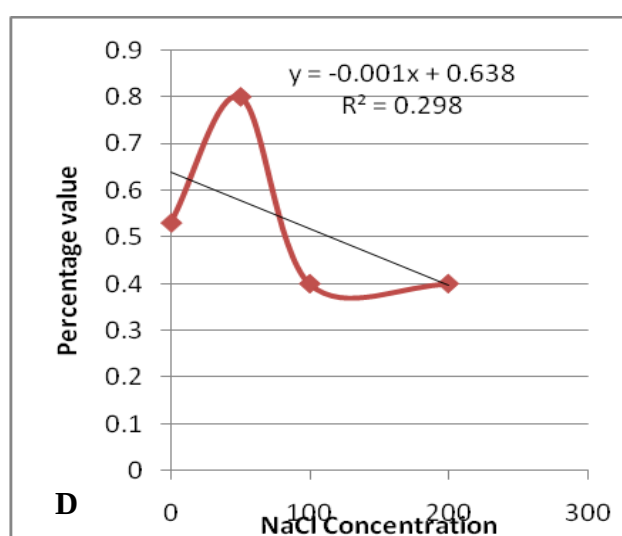
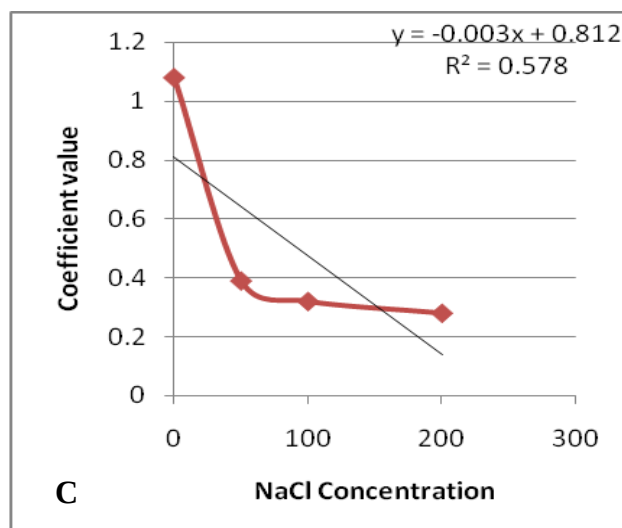
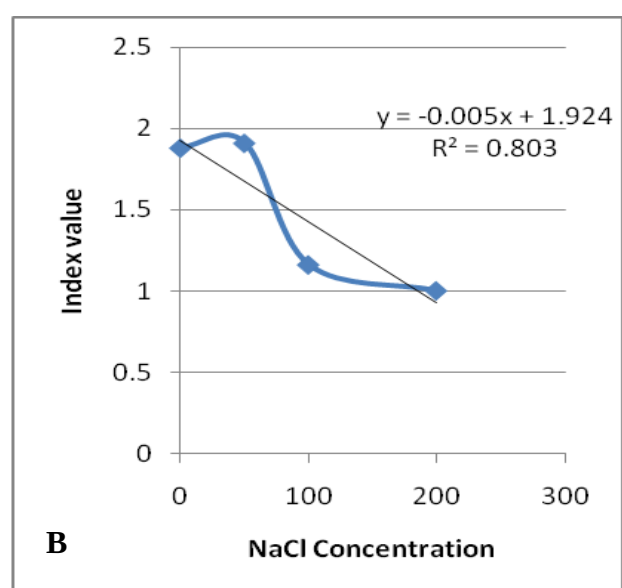
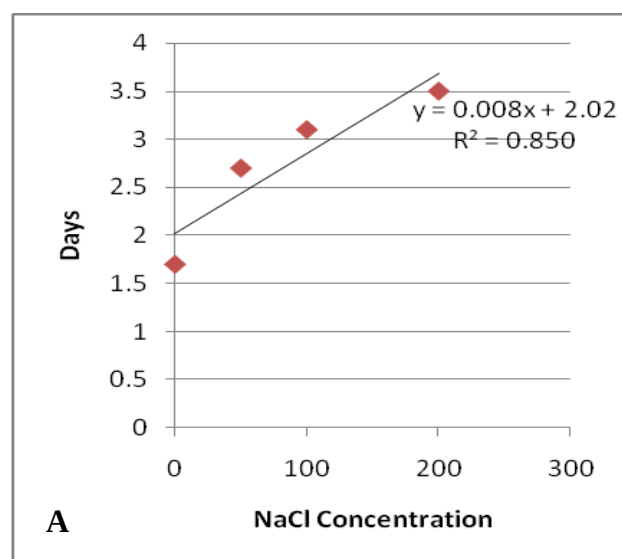
in CVG, depending on NaCl concentration. Fig. 1C shows CVG at different NaCl concentrations. These results are also in agreement with those reported by (Alihan Cokkizgin 2012).

Germination percentage:

Maximum GP in 50 mM NaCl concentration and minimum in 200 mM NaCl concentration was recorded (Table-3). Fig. 1D shows 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. 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).

Fig. 1.B. Relationship between
(A)- MGT and NaCl,
(B)- GI and NaCl
(C)-CVG and, NaCl,
(D)-GP and NaCl
(E)- SVI and NaCl concentration in *Tephrosia purpurea*



Seed vigor index:

Significant differences were observed between NaCl treatment and SVI. Furthermore, considerable decrease in SVI was observed,

depending on the increase in NaCl concentration. The highest SVI was observed in the control (1.16), whereas the lowest was noted at 200 mM NaCl concentration (0.400). Fig. 1E shows the relationship between SVI and NaCl concentration. The results indicate that all the parameters have highly significant correlation with SVI. The SVI increases at 50mM in comparison to control, but it shows decreasing trend when the NaCl concentration increases further. 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). 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.

CONCLUSION

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.. Furthermore, the correlation coefficient results indicate that all the possible combinations had a positive significant correlation with each other, except the MGT coefficient.

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