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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 from 1.7 to 3.5 days, 1.91 to 1.00, 0.28 to1.08, 60 to 80% and 0.40 to 4.06, 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. Maximum value of GI, GP, CVG, and SVI were observed at 50mM concentration and minimum at 200mM NaCl concentration. Pearson's correlation coefficients between all parameters were calculated and we observed that MGT, GI, GP, CVG, and SVI were significantly related with each other.

Keywords: *Wild legume, Salinity stress, Sodium chloride, Seed germination, Pearson's correlation.*

INTRODUCTION

The genus *Tephrosia* L. or Sarpunkha is a wild legume belonging to family Leguminosae (Sub family-Papilionaceae) and grows throughout India and Western Himalaya. It 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 and 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 problems 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 effects 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 growing 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 (Ghoulam and Fares, 2001; Gulzar et al., 2003). In this study, we examined the effects of salinization on the germination of *Tephrosia* seeds through laboratory experiments.

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).

NaCl Solutions and seed treatments

To control fungal infection during germination, seeds were surface sterilized by 0.1% $HgCl_2$ 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) (Fig. 1). Distilled water was used as control (Fig. 1).

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.

Figure 1. Germination of *Tephrosia purpuria* seeds in different NaCl concentrations.

Mean germination time

The seedlings with 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:

$$GI = \frac{Number\ of\ germinated\ seeds\ in\ first\ count}{Number\ of\ days\ of\ first\ count} + \ldots\ldots\ldots + \frac{Number\ of\ germinated\ seeds\ in\ final\ count}{Number\ of\ days\ of\ final\ count}$$

Coefficient of velocity of germination

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

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

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 24h (AOSA, 1990). Ten days after germination, the germination percentage (GP) was obtained by dividing the number of germinated seeds in a 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 = [Seedling length (cm) × 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 treatment 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 DISCUSSION

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) (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.

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

Source of variation	df	Mean of Square				
		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.* Means followed by the same letter are not significantly different at $p < 0.05$ level (LSD test).

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

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 purpuria* seeds has been shown in Fig. 2A. It is worthy to mention 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.

Table 3. Pearson's correlation matrix for analyzed variables.

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

*,** 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 (Fig. 2B). The response of the plant from 50mM conc. and above is similar to the result reported by (Yan, 2008).

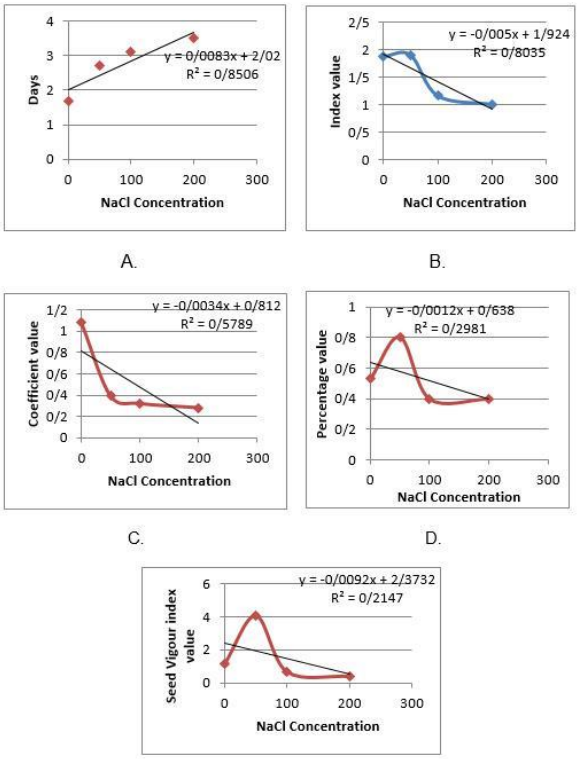

Figure 2. Relationships of NaCl concentration with (A) MGT, (B) GI, (C) CVG, (D) GP and (E) SVI in *Tephrosia purpurea*

Coefficient of velocity of germination

NaCl concentration when increased affected the CVG negatively. Considerable decrease is noted in CVG, depending on NaCl concentration. Fig. 2C shows CVG at different NaCl concentrations. These results are also in agreement with those reported by (Alihan Cokkizgin, 2012).

Germination percentage

We observed maximum GP in 50 mM NaCl concentration and minimum in 200 mM NaCl concentration (Table 3). Fig. 2D shows there is a steep increase in GP of the plant when the concentration 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 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

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. 2E shows the relationship between SVI and NaCl concentration. The results indicate that all the parameters exhibit 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 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 decreasing 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.

CONCLUSIONS

This study demonstrated that in *Tephrosia purpuria*, 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 significant positive association with each other, except the MGT coefficient.

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