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EFFECTS OF DIFFERENT LEVELS OF NaCl ON THE SEED GERMINATION OF *CYAMOPSIS TETRAGONOLOBA* L.

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Abstract

Effects of four different levels of NaCl (05, 10, 15, 20 dS/m) on the seed germination of cluster bean (*Cyamopsis tetragonoloba* L.) cultivars were investigated. Only distilled water was used in the control. The experiment was conducted in the Petri dishes under laboratory condition and laid out in CRD comprising five replicates. The results indicated that the germination index (GI), seed vigor index (SVI), germination stress index and seedling length (radicle and plumule) decreased with increase of salinity levels and germination percentage (GP) was reduced significantly at the highest level of salinity (200 mM). However, mean germination time (MGT) and time taken for 50% germination (T50) increased by increasing salinity levels. Correlation coefficient between all possible combinations was estimated and the results indicated that GP, GI, MGT, T50, and SVI had significant positive or negative correlation with each other.

Introduction

Salinity is one of the major environmental stresses that decrease the crop production particularly, in the arid and semi-arid areas of the world (Schleiff 2008). Total salt affected area of the world is about 7% (Musyimi *et al.* 2007) and it is mainly restricted to arid and semi-arid regions where land degradation and population growth are of major concern (Geissler *et al.* 2010). In Pakistan, nearly 10 M ha areas are affected by different levels of salinity and it consists of 12.9% of total land in the country. The problem of salinity in the irrigated area of Pakistan occurred due to inadequate drainage practices (FAO 2008).

Seed germination plays important role towards yield (Sanchez *et al.* 2014). To start with the process of germination of seeds, absorption of water is the first step that should occur under favorable environmental conditions (Tobe *et al.* 2001). Salinity of growth medium delays or prevents seed germination and later on seedling establishment and such conditions prevail mainly due to evaporation of water under high temperature. Germination percentage and seed vigor index are the important criteria for selection of salt tolerant cultivars (Bybordi 2010).

Cluster bean (*Cyamopsis tetragonoloba* L.) also known as Guar is an important summer legume crop in Asian countries (Rao and Shahid 2011). It is mostly grown in the arid and semi-arid regions and its uses are of multifarious nature as it is used for human consumption as well as forage for cattle (Satyavathi *et al.* 2014, Choy *et al.* 2015). Seeds of Guar contain Guar gum which is being used in wide range of industries such as textile, pharmaceutics, food industry, paint, and cosmetics (Mudgil *et al.* 2014, Jukanti *et al.* 2015). In view of the importance of cluster bean in the agrochemical industries and due to agrobotanical characters of this crop, this is gaining considerable attention to grow on abiotic stress hit areas (Ali *et al.* 2015). It is therefore, worthwhile to consider the problems caused by salinity in the production of this important crop and the main aim of the present study, was to examine the seed germinated and vigor indices under salinity stress.

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Materials and Methods

The seeds of cluster bean (*Cyamopsis tetragonoloba* L.) varieties (BR-90 and BR-2017) were collected from the Guar Research Station, Regional Agricultural Research Institute, Bahawalpur. The experiment was laid out in CRD with factorial arrangement. The study was conducted at the Department of Environmental Sciences, COMSATS University Islamabad, Vehari Campus. Thirty healthy seeds were placed in each Petri dish with filter paper Whatman No. 1 and salinity stress (05, 10, 15, 20 dS/m) were imposed by preparing the solution of NaCl salt. Distilled water was used as control. Seed germination was counted daily by following the method of Association of Official Seed Analysis (AOSA 1990). Following parameters regarding germination were recorded: From the second day, the germinated seeds of clusterbean were counted daily at 10:00 a.m. and seeds having redicle length of 2 mm were considered germinated. Germinated seed counting continued till the germination became constant and the resulted final counting considered as final germination percentage. The germination percentage (GP) was calculated according to the formula of ISTA (2009).

$$GP = \frac{Ni}{N} \times 100$$

where, *Ni* is the number of seeds germinated and *N* is the total number of seeds.

Time taken for 50% germination (T50) was calculated according to the following formula of Farooq *et al* (2005):

$$T_{50} = t_i + \frac{\left[\frac{N}{2} - n_i\right](tj - ti)}{n_j - n_i}$$

where, *N* is the final number of seeds germinated and *ni* and *nj* are the cumulative numbers of seeds germinated at adjacent counts at times *ti* and *tj* when $ni < N/2 < nj$.

Mean germination time (MGT) was calculated according to the equation of Ellis and Roberts (1981):

$$MGT = \frac{\Sigma Dn}{\Sigma n}$$

where, *n* is the number of seeds germinated on day *D* and *Di* is the number of days as counted from the beginning of germination.

In order that, from the second day to 7th day once in 24 hours germinated seeds were counted and germination index (GI) was determined following Maguire equation (1962):

$$GI = \sum_{i=1}^{n} \frac{Si}{Di}$$

Or

$$GI = \left[\frac{\text{Number of germinated seeds in first count}}{\text{Days of first count}}\right] + \ldots + \left[\frac{\text{Number of germinated seeds in final count}}{\text{Days of final count}}\right]$$

where, *GI* is germination index (number of germinated seed in each day); *Si* is number of germinated seeds in each numeration; *Di* is number of days till nth numeration and *n* is number of numeration times.

Germination stress index (GSI) is estimated by the formula of Bouslama and Schapaugh (1984).

$$GSI = \frac{\text{Germination speed at stress condition (PIs)}}{\text{Germination speed at control condition (PIc)}} \times 100$$

Seed vigor index (SVI) was calculated with the help of Abdul-Baki and Anderson method (1970):

Seed vigor index = GP × means of seedling length × 100

where, *GP* is germination percentage and seedling length is equal to radicle + plumule length. The radicle and plumule length were measured with the help of scale at the time of harvest.

Data were collected and analyzed statistically by using AVOVA and LSD test at 5% level of probability and was used to test the differences among mean values (Steel *et al.* 1997).

Results and Discussion

The data presented in Fig. 1a showed that effect of different salinity treatments on germination percentage (GP) was non-significant ($p > 0.05$) up to 100 mM. The higher levels of salinity (150 and 200 mM NaCl) decreased the GP significantly. Maximum GP was recorded in control treatment while minimum at highest salinity level (200 mM). Analyzed data indicated that germination percentage differ significantly in both the varieties (BR-90, BR-2017) of cluster bean at 200 mM salinity. However, non-significant difference was recorded at all the lower salinity treatments. High concentration of NaCl salinity (200 mM) led to a significant ($p < 0.05$) reduction of germination percentage by 19 and 13 in BR-90 and BR-2017, respectively when compared with the control treatment. Increasing concentration of NaCl in the growth medium may decrease the water potential (Bradford 1995) and it may decrease or inhibit the seed water absorption that initiates the seed germination process (Katembe *et al.* 1998, Fatima *et al.* 2018). The reduction in the absorption of water due to increased level of salinity in the growth medium may be due to imbalance of anion and cation which creates ion toxicity (Panuccio *et al.* 2014).

The analyzed data (Fig. 1c) regarding time taken for 50% germination (T50) was significantly ($p < 0.05$) increased with increasing the salinity levels at all the treatments. However, the difference between the cultivars (BR-90 and BR-2017) at each salinity treatment was found to be non-significant ($p > 0.05$). The highest salinity level of 200 mM increased the T50 value in BR-90 and BR-2017 by 57 and 58%, respectively when compared with control treatment. Due to salinity stress, the water absorption rate of seed decreased leading delays in the metabolic activation necessary for the emission of the radicle and subsequent mobilization of reserves in different seed parts (Jamil *et al.* 2006).

Mean germination time (MGT) increased with the increase of salinity levels in both the cultivars (Fig. 1b). At control treatment the MGT significantly ($p <0.05$) decreased as compared to highest salinity level (200 mM). Overall, non-significant difference of MGT was recorded at all salinity treatment levels for both BR-90 and BR-2017. High salinity treatment (200 mM) of NaCl increased mean germination time in BR-90 and BR-2017 by 26 and 37%, respectively when compared with control treatment (Fig. 1b). The results of present study correlate with those of Murillo-Amador *et al.* (2002) in cowpea and Okcu *et al.* (2005) in pea and they observed that NaCl delayed MGT. Similar results were reported by Mensah and Ihenyen (2009) that salinity stress delays the germination which may be due to reduction of absorption of water.

Fig. 1d showed that germination index (GI) decreased with the increase of salinity treatments. The difference between the cultivars (BR-90 and BR-2017) regarding GI was non-significant at all the salinity levels except at 100 mM in which cultivar BR-90 attained the higher value of GI as compared to BR-2017. The highest concentration of NaCl (200 mM) led to a significant reduction of GI by 50 and 42% BR-90 and BR-2017, respectively when compared with the control treatment. It has been observed that value of GI is very helpful to predict the emergence of seed (Wang *et al.* 2004) and it decreased under stress.

Germination stress index (GSI) decreased gradually with the increase in salinity levels. The highest GSI in both the cultivars (BR-90 and BR-2017) was observed at control treatment and the lowest was recorded at 200 mM salinity level. However, non-significant difference of GSI value was recorded in both BR-90 and BR-2017 at all salinity levels. Both the cultivars (BR-90 and BR-2017) showed the same gradual change in GSI by increasing the salinity treatment. High concentration of NaCl salinity reduces the GSI in BR-90 and BR-2017 by 36 and 44%, respectively when compared with control treatment. Germination stress index (GSI) had a significant positive correlation with the speed of germination and cultivars attaining higher value of GSI which can tolerate the stress conditions (Sapara 1991, Bahari and Bighdilu 2014).

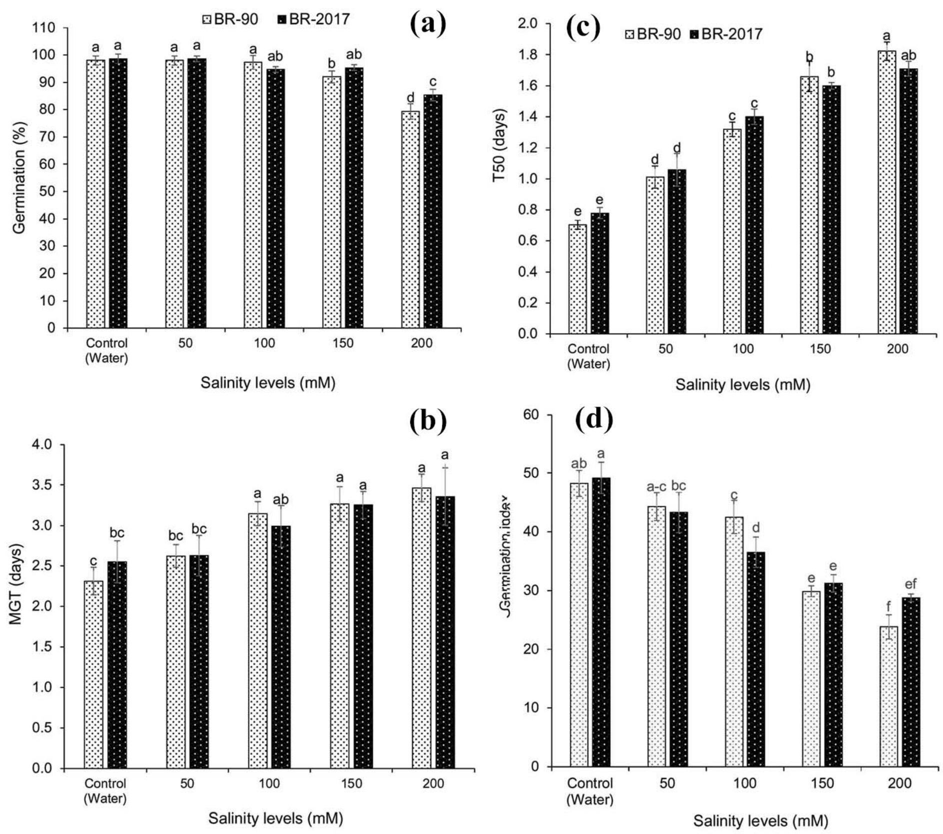

Fig. 1. Germination percentage (a), mean germination time (b), time taken for 50% germination (c) and germination index (d) of cluster bean seed sown under salinity levels. Values are mean ± standard error.

Seed vigor index (SVI) decreased significantly ($p < 0.05$) with the increase at all salinity levels. Data presented in Fig. 2a, showed that the highest SVI was observed at control treatment and it sharply decreased to minimum at the highest salinity level (200 mM) in both cultivars (BR-90 and BR-2017). The non-significant difference of SVI value was observed between BR-90 and BR-2017 at all the salinity treatments except in control where BR-90 attained the higher value as compared to BR-2017. Increase in concentration of NaCl salinity reduces the SVI in BR-90 and BR-2017 by 82 and 83%, respectively as against control treatment. Germination and seedling growth indices e.g. seed vigor are the most important criteria for selection of tolerant cultivars (Bybordi 2010) and salinity stress influences the seed vigour during germination of soybean seed (Khajeh-Hosseini *et al.* 2003).

According to the results presented Fig. 2c the radical length of cluster bean seedling gradually deceased by the increase of NaCl salt treatment in the growth medium as compared to control. Maximum radical length was observed at control level and minimum at 200 mM salinity. However, non-significant difference of radicle length was recorded both in BR-90 and BR-2017. The highest salinity level (200 mM) reduces the redicle length in BR-90 and BR-2017 by 78 and 75%, respectively when compared with control treatment.

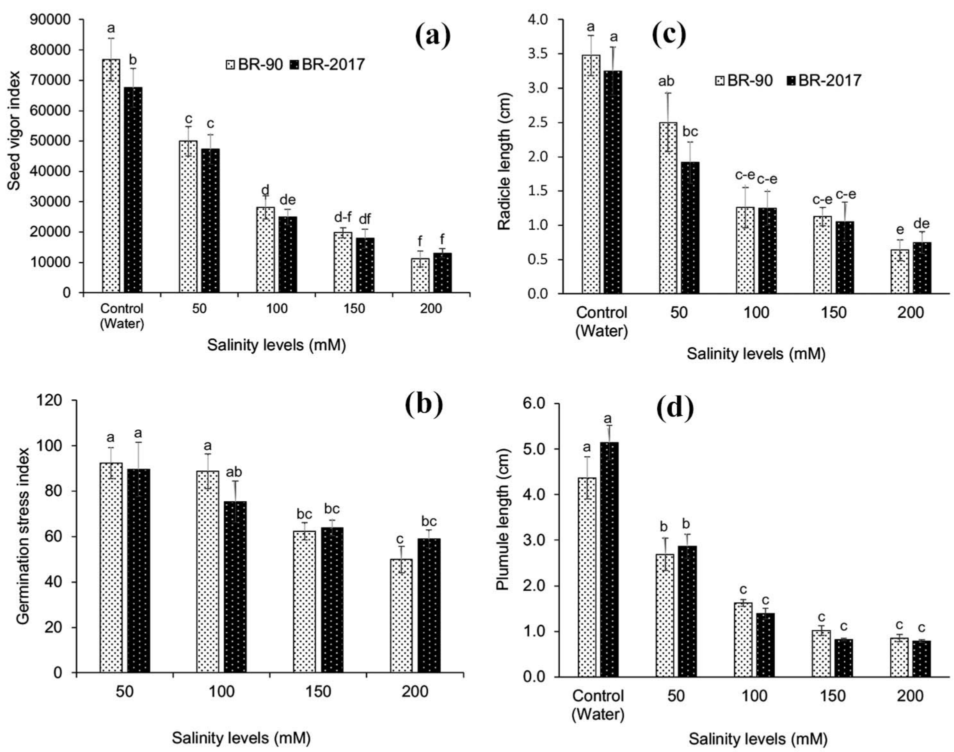

Fig. 2. Seed vigor index (a), germination stress index (b), radicle length (c) and plumule length (d) of cluster bean seed sown under salinity levels. Values are mean ± standard error.

Plumule length decreased with increasing salinity level in the growth medium. Plumule length decreased sharply up to 100 mM salinity treatment and then non-significant decrease was observed. High NaCl concentration (200 mM) reduces the plumule length of plants of BR-90 and BR-2017 by 84 and 82%, respectively when compared with control treatment (Fig. 2d). These results are in agreement Soltani *et al.* (2002) and Esechie *et al.* (2002) that root and shoot lengths were diminished by increasing the salinity stress in the growth medium. Eeffects of salinity stress on maize crop indicates that there is considerable variation among root and shoot length of cultivars. Furthermore, it was found that those having high root and shoot length result in high biomass and are considered salt tolerant (Akram *et al.* 2007, Akram *et al.* 2010).

Correlation analysis indicated that highly significant ($p < 0.05$, 0.98**) correlation was observed between T50 and MGT. It was also observed that SVI and GI have also highly significant relationship ($p < 0.05$, 0.92**). However, highly negative correlation was observed between GI with T50 and MGT. Highly negative correlation was also observed for SVI with MGT, T50 and GI (Table 1).

Table 1. Pearson correlation coefficient (r) for analyzed variables.

Parameters	GP	MGT	T50	GI	SVI
GP	1.00	-	-	-	-
MGT	–0.76*	1.00	-	-	-
T50	–0.79*	0.98**	1.00	-	-
GI	0.86**	–0.92**	–0.97**	1.00	-
SVI	0.70^{NS}	–0.97**	-0.98**	0.92**	1.00

*,** Significant at $p < 0.05$ and 0.01% levels, respectively. GP = Germination percentage, MGT = Mean germination time, T50 = Time taken for 50% germination, GI = Germination index and SVI = Seed vigor index.

In conclusion, it may be said that high salinity remarkably inhibited the seed germination, decreased the germination indices and also delayed the germination time in cluster bean. It is evident that 50 **-** 200 mM NaCl salinity significantly reduced the radicle and plumule length of cluster bean seedling.

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