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## SEED GERMINATION AND SEEDLING GROWTH OF PIGEON PEA (*CAJANUS CAJAN* (L.) MILLSPAUGH) AT DIFFERENT SALINITY REGIMES

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### ABSTRACT

Pigeon pea (*Cajanus cajan* (L.) Millspaugh) is one of the important leguminous crops of arid and semiarid region used traditionally as food, fodder and fuel. In this study the salinity tolerance of this crop was investigated at earlier phase of crop production. Experiments were conducted to find out seed germination and seedling growth at variable salinity levels 0, 0.05, 0.1, 0.15, 0.2, 0.25 and 0.3% sea salt under laboratory and greenhouse conditions. Results indicated that all investigated parameters were significantly affected by stress applied. A progressive decline in germination percent germination, rate of germination, coefficient of germination velocity and mean germination index were recorded to decline with increasing sea salt concentrations. However, mean germination time increased with increasing salinity. Similarly, under greenhouse conditions seedling emergence and shoot height were significantly reduced at 2.54 and 2.1  $\text{d.s.m}^{-1}$ , respectively. Present studies indicated that this crop is salt sensitive at germination stage.

**Keywords:** Pigeon pea, *Cajanus cajan*, germination indices, salinity, seedling growth.

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### INTRODUCTION

Scarcity of good quality water enforces the growers to irrigate the crops with low/moderately saline water at marginal lands which ultimately enhance soil salinity due to high evapo-transpiration (Azeem and Ahmad, 2011). Symbiotic N<sub>2</sub>-fixing crops are highly appreciated for their contribution in solving crucial problems of sub-tropical areas to fight against desertification and soil degradation. These plants acquire 90% of their nitrogen from the atmosphere while contribute up to 40% nitrogen to the soil and also considered as an excellent source of protein supply for human and animal consumption (Peoples *et al.*, 1995). However, these plants are sensitive to various environmental stresses particularly salt stress whereas few species are better to tolerate high drought condition (Zahran, 1999).

Pigeon pea (*Cajanus cajan* (L.) Millspaugh) of the Family Fabaceae is an important leguminous crop of semiarid areas, utilized as food, fodder and fuel. It can fix up to 200 Kg nitrogen. ha<sup>-1</sup>. year<sup>-1</sup> due to symbiotic association of *Rhizobium* with its deep penetrating roots (Bhattacharyya *et al.*, 1995).

The total cultivated area of Pigeon pea is about 6.22 million hectare and global annual crop production is around 4.74 million tones, whereas, total seed production of this crop is about 0.15 million tonnes (FAOSTAT, 2013). Its seeds are an excellent source of good quality protein (up to 24%) and foliage is used as animal fodder with high nutritional value (Pandey *et al.*, 2014). Besides being used as food and fodder this plant also have therapeutic value and it is used against diabetes, fever, dysentery, hepatitis, and measles (Grover *et al.*, 2002). It also use traditionally as a laxative and was identified as an anti-malarial remedy (Ajaiyeoba *et al.*, 2013). Despite its edible and economic importance, this crop is still underexploited in various parts of the world. Few studies about the nutritional quality, medicinal uses and drought tolerance of this species are available however salinity tolerance both at germination and seedling level is not well documented. Present study investigates the germination and seedling establishment of pigeon pea under various salinity regimes to find out its early growth response under saline conditions.

### MATERIAL AND METHOD

#### Seed germination experiment:

Seeds of *Cajanus cajan* were purchased from local market of Mirpurkhas, Sindh, Pakistan. Seed germination of *C. cajan* was carried out in petri plates under various sea salt concentrations. Their electrical conductivities mentioned in Table 1. All seeds were surface sterilized prior to germination, with 0.5% sodium hypochlorite for 2 minutes, rinsed three times with sterilized distilled water and imbibed with respective sea salt solution for 30 minutes. Ten seeds were placed in sterilized petri plates (9 cm diameter) containing filter paper soaked with respective sea salt solutions (10 ml) and placed in germination chamber (EYELA LTI-1000, Japan) at 28 ± 1°C in dark.

#### Germination percentage (GP)

Seed germination of *C. cajan* was recorded every 24 hours according to the seedling evaluation procedure up to seven days. The percent germination was calculated using the following formula (Cokkizgin and Cokkizgin, 2010).

$$GP = \frac{\text{Number of germinated seeds}}{\text{total number of seeds}} \times 100$$

#### Germination index (GI)

Germination index was recorded according to AOSA (1990) by using following formula

$$GI = \sum \frac{Gt}{Dt}$$

Where Gt is the number of germinated seed on day t, and Dt is the total number of days (01-07)

#### Coefficient of germination velocity (CVG)

Coefficient of germination velocity was calculated described by Maguire (1962).

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

Where, G represents the number of germinated seeds counted per day till the end of experiment.

#### Mean germination time (MGT)

Mean germination time was calculated by Ellis and Roberts (1981) by using following formula.

$$MGT = \frac{\sum(nd)}{\sum n}$$

Where 'n' is the number of germinated seeds in day d, whereas  $\sum n$  is the total germinated seeds during experimental period.

#### Germination Rate (GR)

Germination rate was determined according to following formula (Shipley and Parent, 1991).

$$GR = \frac{\text{Number of germinated seeds}}{\text{day}}$$

Where number of germinated seeds were recorded from 01 to 07days.

#### Seedling establishment experiment

Seedling establishment experiment was carried out in Biosaline research field, Department of Botany, University of Karachi. Surface sterilized seeds were imbibed in their respective sea salt concentration mentioned in seed germination experiment. The seeds were sown in small plastic pots filled with 1.5 Kg sandy loam soil provided with farm manure at 9:1 ratio (30% water holding capacity). Sea salt solutions of different concentrations mentioned above were used for irrigation. The electrical conductivity of soil saturated paste ( $EC_e$ ) was also determined at the end of the experiment (Table 1). Data on seedlings emergence was recorded and their height were measured after 14 days of salinity treatment. EC of the soil ( $EC_e$ ) was initially  $0.54 \text{ dS.m}^{-1}$ . It, however, after 14 days of irrigation with differentially saline water increased substantially (Table 1).

#### Statistical Analysis

Data were analyzed by using (ANOVA) and the significant differences between treatment means were examined by least significant difference (Zar, 2010). Fifty percent reduction of germination indices and seedling growth were calculated by using linear regression. All statistical analysis was performed using SPSS for windows version 14 and graphs were plotted using Sigma plot 2000.

## RESULTS

#### Germination percentage

The final percent germination related with salinity in accordance with Maas and Hoffman (1977) linear relative threshold response model as follows:

$$\% \text{ Relative Final Germination} = 100 - 200 (Ke - 0.05)$$

Where threshold salt concentration was 0.05% and  $K_e$  is the concentration of salts at which % relative final germination may be predicted. This model indicated 50% decline in % final germination at 0.30% salt concentration corresponding to  $EC_{iw}$ : 4.2 dS.m<sup>-1</sup>.

#### Germination rate

Rate of germination was significantly ( $p < 0.001$ ) decreased from first day to final (day 07) of observation and it was inversely correlated with sea salt concentration. High germination rate was recorded in control and low sea salt concentrations in early days of seed incubation compared to higher sea salt concentrations but the difference was reduced with increasing day of incubation (Fig. 1b).

#### Coefficient of velocity of germination

A progressive decline ( $p < 0.001$ ) in coefficient of germination velocity was observed with increasing salinity and fifty percent reduction was observed at 0.21% sea salt concentration ( $EC_{iw} = 3.19$  dS.m<sup>-1</sup>) (Fig. 2a).

#### Germination time

Mean germination time of seeds was increased significantly ( $p < 0.001$ ) with increasing sea salt concentrations. However, the difference was insignificant at lowest (0.05 %) salinity (Fig. 2c).

#### Mean Germination Index

Mean germination index was also significantly decreased ( $p < 0.001$ ) with increasing salt concentrations except for 0.05% salinity. Fifty percent reduction in mean germination index was observed at 0.188% sea salt concentration ( $EC_{iw} = 2.89$  dS.m<sup>-1</sup>) (Fig. 2d).

#### Seedling Emergence

Seedling emergence from soil was reduced significantly ( $p < 0.001$ ) with increasing salt concentration of irrigation water. Not a single seedling emerged from soil in  $\geq 0.25\%$  saline water irrigation. However, lower salinities (0.05%, 0.1%) showed slight decrease in seedling emergence with respect to controls. Seedling emergence related with salinity in accordance with a quadratic model as follows:

$$\text{Seedling Emergence (\%)} = 97.7751 + 44.344 \text{ salt} - 2221.5238 (\text{salt})^2 \pm 6.578; r=0.9810, F = 153.58 (p < 0.0001)$$

Fifty percent reduction in seedling emergence was noticed at 0.16 % sea salt concentration ( $EC_{iw} = 2.41$  dS.m<sup>-1</sup>). All seedlings which emerged from soil survived until 14 days of respective irrigation (Fig. 3a).

#### Shoot Height

Shoot height was measured after fourteen days of irrigation. Shoot length was significantly ( $p < 0.001$ ) decreased with increasing salinity. A lesser decline was observed in low salinity (0.05% and 0.1% sea salt irrigation) compared to controls while larger decline in shoot height was noticed from 0.15% sea salt concentration. Shoot height related with salinity as follows:

$$\text{Shoot height (cm)} = 9.116714 - 34.20286 \text{ salt} \pm 0.9221, r = 0.968, F = 1288.93 (p < 0.0001)$$

Fifty percent reduction in shoot height was estimated at 0.13 % sea salt concentration ( $EC_{iw} = 2.10$  dS.m<sup>-1</sup>) (Fig. 3b).

## DISCUSSION

Seed germination is the protrusion of radicle from the seed which is adversely affected by salinity stress (Kaymakanova, 2009). Salinity imposes the osmotic stress by accumulation of Na<sup>+</sup> and Cl<sup>-</sup> which decrease soil water potential that ultimately inhibits the imbibition process (Othman, 2005). Percent final germination behaved against salinity in accordance with linear threshold response model of Maas and Hoffman (1977). The germination of a salt tolerant desert legume, *Indigofera oblongifolia*, and a desert graminoid, *Pennisetum divisum*, are also reported to behave to salinity in similar manner (Khan and Ahmad, 1998; 2007). A decrease in germination percentage, germination rate, coefficient of velocity of germination and germination index was observed with increasing salinity levels. While increase in mean germination time showed delayed germination at higher salinity treatments. Higher sea salt (0.3%) shows 50% or more reduction in all germination indices as compared to control. Our results are parallel with the finding of other workers such as Kafi and Goldani. (2001) who found the same trend in chickpea at higher salinities. Pujol *et al.*, (2000) reported that increased salinity inhibit the seed germination as well as delay in germination initiation in various halophyte species. Similar response was also found in some other crops such as pepper (Khan *et al.*, 2009), sunflower (Vashisth and Nagarjan, 2010) and eggplant (Saeed *et al.*, 2014). Salt tolerance within species may vary at germination and other growth phases (Khan and Ahmad, 1998).

According to our results, *Cajanus cajan* appeared to be a salt sensitive in initial growth phase. It could be relatively more tolerant at later growth stages.

Salt stress delayed or either seized the metabolic activities during seed germination in salt sensitive and even in salt tolerant plants (Khan and Ahmad, 1998, Ali *et al.*, 2013). Salinity also imposed the oxidative stress due to overproduction of reactive oxygen species which may alter metabolic activities during germination, growth and developmental stages (Zhu, 2001; Munns, 2005; Lauchli and Grattan, 2007). In our study seeds of pigeon pea were unable to emerge above 0.25% sea salt concentration. Height of seedling was significantly affected by increasing salinity. Similar results were also reported in Indian mustered (*B. juncea*; Almansouri *et al.*, 2001), some *Brassica* species (Sharma *et al.*, 2013) and tomato cultivars (Jamil *et al.*, 2005). Growth retardation with increasing salinity may be due to reduced photosynthetic efficiency and inhibition of enzymatic and non enzymatic proteins (Tavakkoli *et al.*, 2011). Furthermore, salt stress also limit the DNA and RNA synthesis leads to reduced cell division and elongation during germination, growth and developmental stage.

Khan and Sahito (2014) mentioned that variation in salt tolerance may exist at species, subspecies and provenance level. Furthermore, the salt tolerance of a species may also vary at germination and growth phases (Khan and Ahmad, 1998; Ali *et al.*, 2013). Srivastava *et al.* (2006) suggested that the genetic variability influences the salinity tolerance e.g., the wild species like *Cajanus platycarpus*, *C. scaraboides* and *C. sericea* showed better salt tolerance than *C. cajan*. Wardill *et al.* (2006) also indicated the genetic diversity in *Acacia nilotica*. *C. cajan* in this study appeared to be a salt sensitive crop but relatively more tolerant at germination phase as compared to early seedling growth. However, Ashraf (1994) found that salinity tolerance of different varieties of *C. cajan* not varied at germination and early growth stages, whereas, at adult growth stage showed better salt tolerance. Therefore, it is suggested that its cultivation on marginal saline lands needs to check for its salt tolerance at later growth phase at species, subspecies and provenance level.

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Table 1. Electrical conductivities of different Sea salt solutions

Sea salt (%)	EC <sub>iw</sub> (dS.m <sup>-1</sup> )	*EC <sub>e</sub> (dS.m <sup>-1</sup> )
0	0.4	0.5
0.05	0.9	1.61
0.1	1.6	2.78
0.15	2.4	3.54
0.2	3.2	4.33
0.25	3.9	4.83
0.3	4.2	5.52

$$EC_e = 0.447 + 1.204 (\text{salt \%}) \pm 0.2797$$

$$R = 0.987, F = 723.01 (p < 0.00001)$$

\* Electrical conductivity of soil saturated paste determined after 14 days of saline water irrigation in pots.

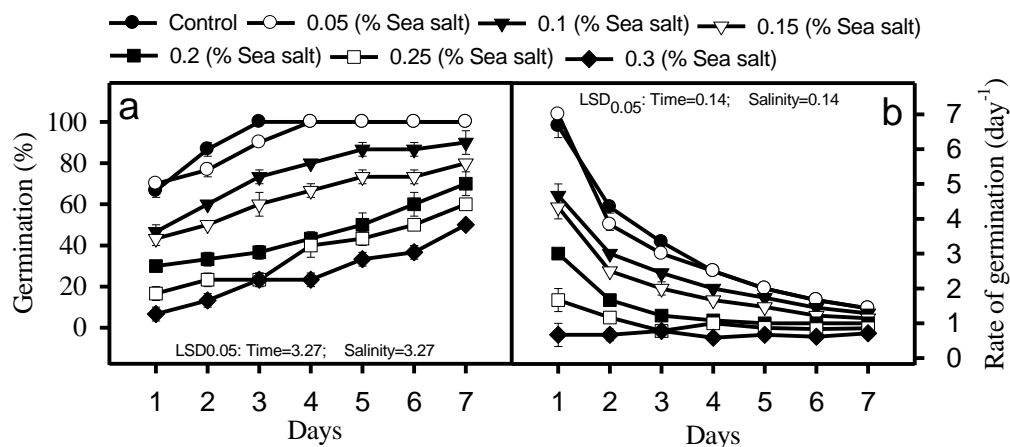


Fig. 01. Effect of different sea salt solutions on germination percentage and rate of germination of *C. cajan*

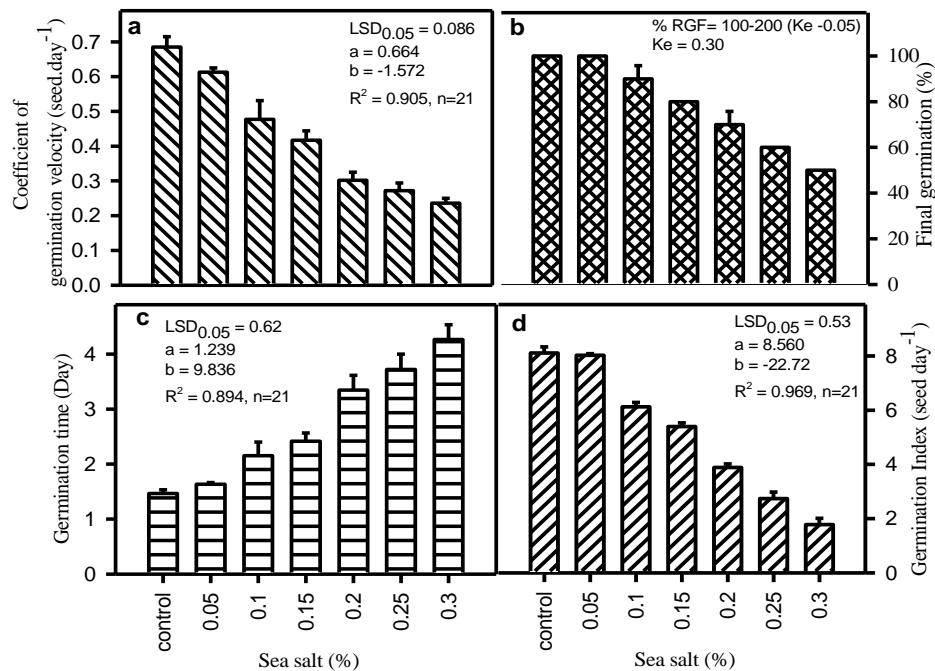


Fig. 02. Effect of different sea salt solutions on seed germination indices of *C. cajan*

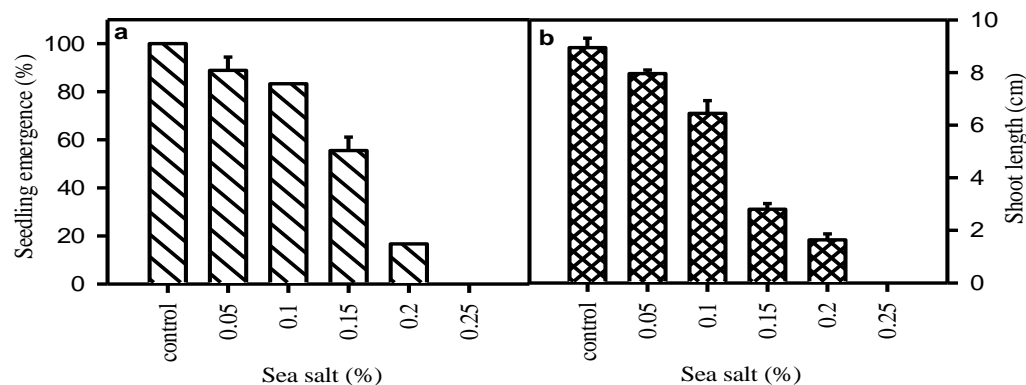


Fig 3. Effect of various sea salt solutions on seedling emergence (a) and shoot length (b) of *C. cajan*.

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