

## Effects of NaCl Stress on Seed Germination And Early Seedling Growth of Castor Bean (*Ricinus communis* L.)



### Botany

KEYWORDS : salinity, germination and castor bean.

**Nidhi Rajput**

Department of Botany, School of Sciences, IFTM University, Moradabad-244001

**Ashok Kumar**

Department of Botany, School of Sciences, IFTM University, Moradabad-244001

**A.K. Chaudhry**

Department of Botany, School of Sciences, IFTM University, Moradabad-244001

### ABSTRACT

*The castor bean (*Ricinus communis* L.) is an oilseed that produces excellent oil, it qualifies as important crop for energetic security. Salinity is one of the most environmental factors that affects different development stages of plants, especially germination stage. The objective of the research was to characterize the deleterious effects of salinity on seed germination and seedling growth of castor bean. The present study shows the effects of NaCl stress on seed germination and seedling growth of four castor bean cultivars (DSP-222, DSP-555, GCH-4 and GCH-7) at different levels. For this purpose, seeds of castor were grown in sand culture and treated with aqueous solutions of different salinities which were prepared by dissolving the salt of NaCl. (25, 50 and 75mM) and distilled water was used as a control. Traits such as germination percentage, length of shoot and root, fresh and dry weight of seedling and seedling vigour index (SVI) were evaluated. The analysis of variance (ANOVA) showed that NaCl stress had significant effects on germination percentage, shoot length, root length, fresh and dry weight of seedling at different salinity levels. It is evident from the experiment that cv. DSP 555 showed higher germination % and seedling growth at all salinity levels as compared to control.*

### INTRODUCTION

Castor is a cross-pollinated diploid ( $2n = 2x = 20$ ) plant belonging to the family *Euphorbiaceae* and genus *Ricinus*. Castor grows as an indeterminate annual or perennial depending on climate and soil types in tropical, sub-tropical and warm temperate regions in the world (Damodaram and Hegde, 2010). The world castor area and product are 1.26 million hectares and 1.14 million tonnes respectively, with a productivity level of 9.2 t/ha. In India it is grown in kharif season in about 7.9 lakh hectares with a production of 10.5 lakh tonnes and productivity of 1,339 kg/ha (FAO 2008). Many phytochemicals found in the plant tissue and seeds of castor have potential medicinal uses (Morris, 2004). Castor oil has been primarily used as purgative or laxative in traditional medicine to counter constipation, but the commercial interest in castor bean is mainly increasing because its seeds contain high amounts of a unique oil consisting of up to 94% of the fatty acid ricinoleic acid (12-hydroxy-cis-9-octadecenoic acid) (Scarpa and Guerci, 1982; Gong *et al.*, 2005).

Salinity is a source of environmental stress on the seeds of various species, including castor bean (Severino *et al.*, 2012). Castor bean is grown in most part of semiarid regions where salinity stress may affect germination and plant growth intensively (Pinheiro *et al.*, 2008). High salinity in irrigation water or soil is a common environmental problem affecting seed germination and plant growth. Salinity in fact causes both hyper-osmotic stress and hyper-ionic toxic impacts and the consequence can be plant demise (Hasegawa, 2000). Salinity stress involves changes in various physiological and metabolic processes, depending on severity and duration of the stress, and ultimately inhibits crop production. Salt stress induces reactive oxygen species (ROS) production and leads to oxidative damages. These toxic oxygen species may react with macromolecules and lipid components of membranes causing damage through lipid peroxidation resulting in increased permeability of the membrane and shows adverse effect on plants (Singh, 2004).

Germination is the first stage and one of the important and sensitive stages of the plant life cycle. It is important process in seedling growth (De Villiers *et al.*, 1994). Seed germination is also severely affected by salinity (Sholi, 2012). Increased salinity caused a significant reduction in germination percentage, germination rate, root and shoots length and fresh root and shoots weights (Jamil *et al.*, 2006).

Under salt stress, the plant increases the external osmotic pressure and as soon it increases, the shoot growth rate and new buds emergence significantly declines as well as the shoot dry weight is reduced (Munns and Termaat, 1986; Dadkhah, 2011). Salinity is responsible for delayed seed germination and establishment of seedlings (Bybordi and Tabatabaei, 2009) Growth and production of castor were also inhibited by high salinity (Na) in the either the irrigation water or in the soil (Silva *et al.*, 2008).

Great effort has been devoted to overcome the deleterious effects of salinity on plant. The objective of this research was to determine the effects of salinity on seed germination, seedling growth by seeds of castor bean cultivars during seed germination at various concentrations of NaCl, and selection of saline tolerant cultivars for breeding programs.

### Materials and methods

The pure line seeds of four cultivars of castor bean (*Ricinus communis* L.) were collected from the Dantiwada Seeds PVT. LTD. Ahmedabad (Gujarat) and Navbharat Seeds PVT. LTD. Near Gujarat Vidyapith, Ashram Road, Ahmedabad (Gujarat), for the experimentation to test their salt tolerance at germination and early seedling growth stage. The following varieties were collected for present investigation:

**Table-1**

S. No.	Varieties	Seeds Company
1	DSP-222	Dantiwada Seeds PVT. LTD. Ahmedabad (Gujarat)
2	DSP-555	Dantiwada Seeds PVT. LTD. Ahmedabad (Gujarat)
3	GCH-4	Navbharat Seeds PVT. LTD. Ahmedabad (Gujarat)
4	GCH-7	Navbharat Seeds PVT. LTD. Ahmedabad (Gujarat)

Sand culture experiment was conducted to test the salinity tolerance of 4 genotype of castor bean. Plastic pots, which were used in the present experiment, were thoroughly cleaned with detergent and then washed repeatedly with deionized water. The sand was sterilized in hot air oven 70 °C for 18- 36 hours. Five seeds were kept at equidistance in each plastic pots filled with sand and moistened with 300 ml of aqueous solution of differ-

ent salinity level. The distilled water was used as control. Saline solutions of different concentration viz. 0, 25, 50 and 75mM were prepared by dissolving NaCl salt in distilled water.

The seeds were allowed to germinate at room temperature in day and night ( $35 \pm 5^\circ\text{C}$ ). This experiment was set up in a randomized design with three replicates to eliminate the experimental errors. Water evaporated from the plastic pots was compensated by suitable quantity of distilled water.

The observations on length, fresh and dry weight of root and shoot were recorded at 10 days after germination (DAG). The dry weight of root and shoot was measured after keeping plant samples in hot air oven at  $(50-60)^\circ\text{C}$  and 36 hours. For this purpose, the sample were collected following completely randomized design considering three replicates and the data were subjected to statistical analysis for the variance (ANOVA) by Microsoft Excel Software. A critical difference (CD) was constructed when F-Test indicated statistically significant differences between genotypes using the method described by Bruning and Kintz (1977) at  $P=0.05$ . Percent reduction (PR) due to salinity stress in relation to the non stressed environment was also determined for length, fresh and dry weight of root and shoot. Seedling vigor index (SVI) was determined by the formula given by Abdul-Baki & Anderson (1973):

Seedling vigor index = Germination percent x (Shoot length + Root length).

## Results and discussion

### Effect of salinity on seed germination

The analysis of variance (ANOVA) showed that the percentage germination in all cultivars was significantly decreased with increasing salinity levels (25 to 75mM). The cultivar DSP-555 showed minimum reduction (12-50%) in germination percentage. Present study shows that germination percentage of castor bean cultivars was inhibited strongly particularly at highest level (75mM) of salt concentration (Fig. a). The results are similar to those reported by other researchers (Jeannette *et al.*, 2002; Saboor and Kiarostami, 2006; Al-Taisan and Wafa, 2010; Dkhil and Denden, 2010; Sedghi *et al.*, 2010; Sozharajan and Natarajan, 2014; Wahid *et al.*, 2014 and Mohammadizad *et al.*, 2014). This decrease in growth due to a reason of too much accumulation of  $\text{Na}^+$  (McConnel *et al.*, 2008).

NaCl is the predominant salt causing salinization and it is expected that plants have involved mechanisms to regulate its accumulation (Munns and Tester, 2008). NaCl affected seed germination by creating external osmotic potential which causes difficulties in absorption of the necessary water quantities for the germination process (Abdelly, 1992 and Xiao-Fang *et al.*, 2000). The salt treated seeds might developed osmotically enforced inhibition by salinity stress. It has been claimed that decrease in the water potential gradient between seeds and their surrounding media may be adversely affect the germination and subsequent metabolic events of seedling growth and development (Afzali *et al.*, 2006). Silva *et al.*, 2005 also suggested that increasing salinity delayed germination and reduced total emergence of castor seed. Lopes (2014) reported that the castor bean seeds had a reduction in germination percentage due to the decrease of  $\Psi_s$ , by the addition of NaCl in the solution of germination.

### Effect of salinity on castor bean shoot and root length (cm)

In the present investigation there is regular decline in the root and shoot length, with increase in salinity levels. According to the Fig. b and c, the cultivars DSP-555 showed minimum reduction in shoot and root length (11-25% and 9-24%) at all levels of salinity. The effects of salt applications on the shoot and root length of DSP-555, DSP-222, GCH-4 and GCH-7 cultivars

were determined to be statistically significant. Present findings demonstrated that the root length is less affected than shoot. Our results are in agreement with (Gupta and srivastava, 1989 and Neumann, 1995). Werner and Finkelstein (1995) reported that salt stress inhibits root and shoot elongation due to slowing down water uptake by the plant. Djanaguiraman *et al.*, 2003 were also suggested that most of the seedling parameter viz., germination, root length, shoot length, vigour index and dry matter accumulation were reduced by NaCl solution.

### Effect of salt on fresh and dry weight of shoot and root

Salt concentrations have differential effects on fresh and dry weight of shoot and root of all castor bean cultivars. It is cleared from graphs (Fig. d-g) that fresh and dry weights of shoot and root in all the cultivars were adversely affected by salinity. The cultivars DSP-555 showed minimum reduction in fresh weight of shoot and root (17-36% and 22-36%) at all levels of salinity. Similar pattern was also observed for dry weight of shoot and root. Minimum reduction (4-24% and 11-51%) was observed in cv. DSP-555. The cultivar DSP-555 was lesser affected than others. The effects of salt applications on the fresh and dry shoot and root weights of DSP-555, DSP-222 and GCH-4 cultivars were determined to be (statistically) significant at 75mM NaCl level. The results are similar to those reported by researchers (Shannon and Grieve, 1999; Essa and AlAni, 2001; Akbarimoghaddam *et al.*, 2011; Zani *et al.*, 2012 and Agarwal *et al.*, 2015). Giaveno *et al.*, (2007) reported that salt treatments affected root and shoot fresh weight. Janmohammadi (2012) were also suggested that the salt sensitivity of castor bean plant under salt stress conditions. This inhibition due to a lack of efficient activity of CAT and GPX probably lead to imperfect  $\text{H}_2\text{O}_2$  scavenging.

El-Bassiouny and Bekheta (2005) have shown that accumulation of ions in wheat plants grown in the presence of salt (14dS/cm) environment causes osmotic and pseudo-drought stresses leading to decrease of water absorption. The decrease of tissue water content resulted in reduction of cellular growth and development. Therefore, restriction of water absorption was one of the most important causes of stem and root growth decrease

### Effect of different salinity levels on seedling dry weight

Seedling dry weight in all castor bean cultivars was decreased significantly with increasing salinity levels. The highest seedling dry weight was found in the DSP-555 and lowest in GCH-4 at all salinity levels (Fig. h). The rate of reduction in seedling dry weight at these levels of salinity (25 to 75mM) in comparison with the control ranges 7.21-35.91%, 9.27-37.47% and 12.29-40.64% in DSP-555, DSP-222 and GCH-4 respectively. It is evident that cv. GCH-7 proved most sensitive and cv. DSP-555 proved tolerant to salinity in the terms of total seedling dry matter. Our results are also similar to the findings reported by Ashraf and Wahid, (2000) in corn, Rastegar and Kandi, (2011) in soybean and Hoque *et al.*, (2014) in maize. Ashraf (2002) mentioned that the reduction in seedling fresh and dry weight is due to decreasing water uptake by seedlings in salt stress presence. Mohamedin *et al.*, (2006) have also been reported that salinity induced water deficit hence the reduced the plant growth.

### Effect of different salinity levels on seedling vigor index

Increasing salinity caused significant reduction in seedling vigor index as compared to control. The maximum seedling vigor index was observed in DSP-222 at control treatment and minimum vigor index in GCH-7 at this level. In all four castor bean genotypes, seedling vigor index decreased with increasing salinity levels (Fig. i). The cultivars DSP-555, DSP-222 and GCH-4 showed gradual reduction in all salinity levels (25, 50 and 75mM) while in cultivar GCH-7, seedling vigor index drastically reduced at 25 and 50mM when it compared with control. Our results are in agreement with (Mensah *et al.*, 2006; Ozturk, 2008; Belaqqiz *et al.*, 2009; Ghanavati and Sengul, 2010; Cokkizgin, 2012; Kandil *et al.*

al., 2012 and Yogita *et al.*, 2014). Seedling vigour index is related to special impact of ions and reduction of environmental water potential in presence of salinity (Keshavarzi *et al.*, 2011)

**CONCLUSION**

It is concluded from the present findings that based on seedling dry weight and seedling vigor index, the DSP-555 variety is more tolerant rather than the other varieties.

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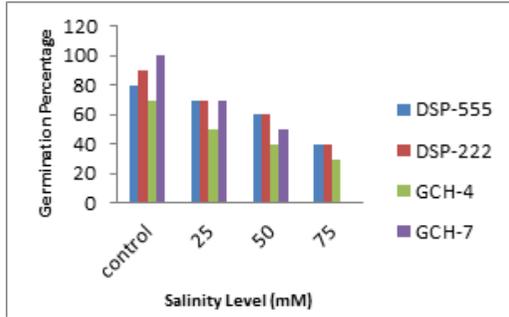


Fig.a. Effect of NaCl on Germination Percentage

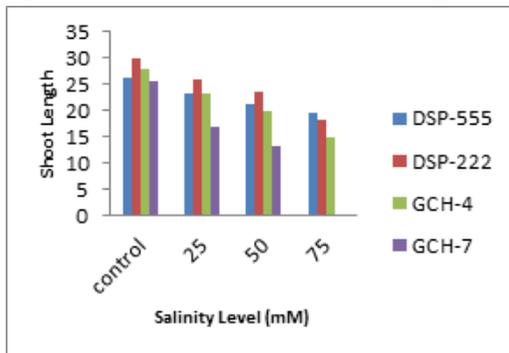


Fig.b. Effect of NaCl on Shoot Length

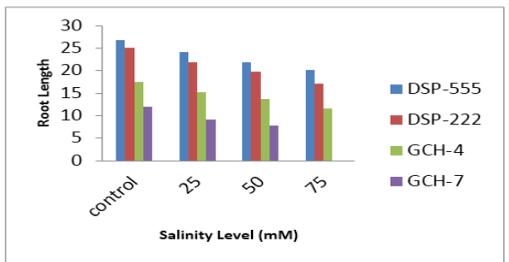


Fig. c. Effect of NaCl on Root Length

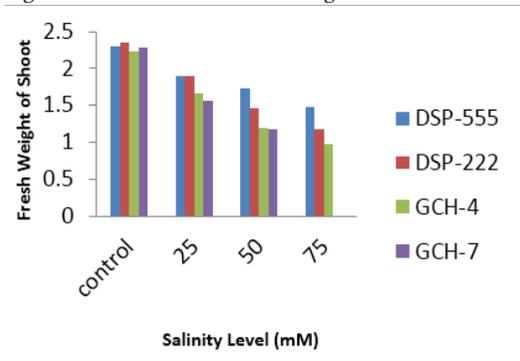


Fig. d. Effect of NaCl on Fresh Weight of Shoot

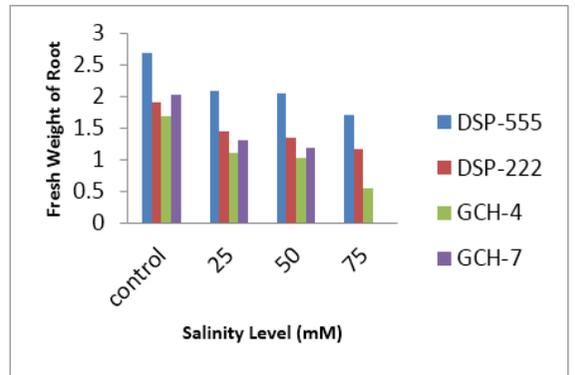


Fig. e. Effect of NaCl on Fresh Weight of Root

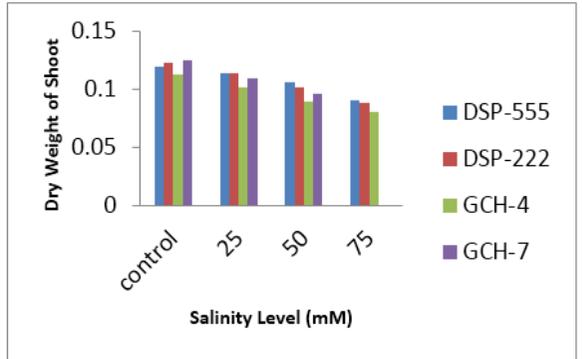


Fig. f. Effect of NaCl on Dry Weight of Shoot

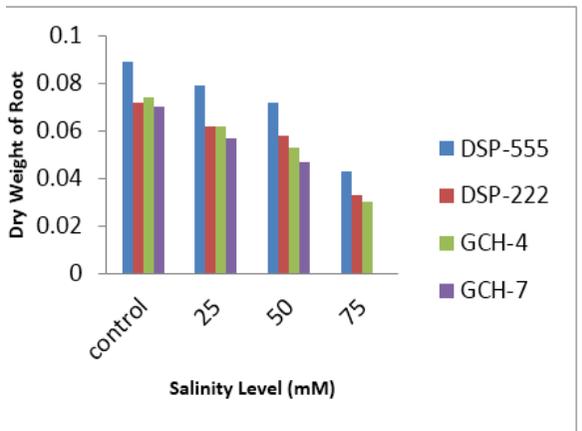


Fig. g. Effect of NaCl on Dry Weight of Root

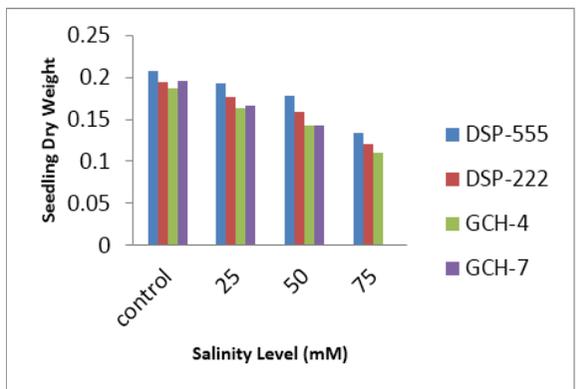


Fig. h. Effect of NaCl on Seedling Dry weight

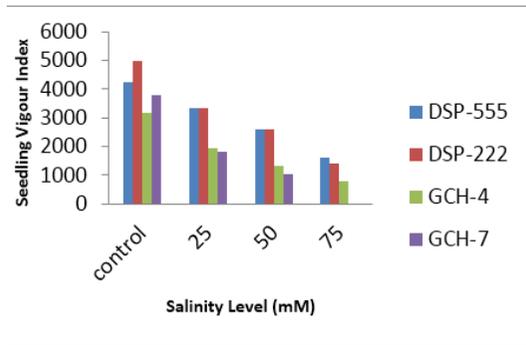


Fig. i. Effect of NaCl on Seedling Vigour Index

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