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Effect of salinity stress on seed germination and seedling vigour index in chickpea genotypes

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Abstract
Chickpea is grown under a wide range of climatic conditions and is known to be highly sensitive to salinity stress. Germination of seed depends on the imbibitions of water and which is affected by salt ion concentration of external medium in which seeds are sown. Germination process under salt stress condition was studied to identify the varieties tolerant to salinity as well as to understand the tolerance mechanism. Ten genotypes were tested in order to identify relatively tolerant genotypes at germination and early seedling growth in petriplates with graded salinity levels (0 dSm^{-1}, 3 dSm^{-1} and 6 dSm^{-1}) in the Department of Crop Physiology at College of Agriculture, Vijayapur. The maximum germination percentage was recorded at 0 dSm^{-1} (control) salt concentration level and increment of salinity levels caused significant reduction in germination percentage and vigour index at 3 dSm^{-1} and 6 dSm^{-1}, respectively. Based on these observations JG11, BGD103 and ICC1431 were found to be salt tolerant genotypes.

Keywords: Chickpea, germination, salt stress, genotypes and vigour index

Introduction
Soil salinity is known as a major inevitable problem, especially in arid and semi-arid regions of the world, where these regions are the main cultivation areas of chickpea (Flowers, *et al.*, 2010). Legumes, especially cool-season food legumes like chickpea, lentil and faba bean are relatively sensitive to soil salinity (Sheldon *et al*., 2004) [6].
From agriculture point of view soils with high salt concentration that adversely affect plant growth and crop productivity are called salt affected soils. Salt-affected soil is a broader term which includes both saline soil (soils having chlorides and sulphates of sodium) and alkaline soil (soils having carbonates and bicarbonates of sodium). Saline soil is formed when chlorides and sulphates of sodium, calcium, magnesium and potassium are abundant in the soil and the process is known as salinization. Apart from EC, pH and ESP (exchangeable sodium percentage) are also some of the quantitative parameters to distinguish saline soils. It has electrical conductivity (EC) > 4 dSm^{-1}, pH value < 8.5, exchangeable sodium percentage (ESP) < 15 and sodium absorption ratio (SAR) < 10. When the carbonates and bicarbonates of above said cations increase in the soil, then the formation of alkaline soil takes place and the process is known as alkalization. It has EC <4, pH >8.5, ESP >15 and SAR >10.
In recent decades considerable improvements in salinity tolerance have been made in crop species with respect to morphological and physiological characters and traits related to salinity tolerance but there is a limited literature in chickpea on salinity studies. Comparison of genotypic responses could be useful in identifying differences related to the relative ability of each cultivar to cope with salinity. Siddig *et al.* (2014) [7] reported that the number of germinated seeds, germination percentage, plumule length, radical length and heaviest wet and dry seedling weights were higher in the control treatment. High salinity level (1.5%) significantly ($p \leq 0.05$) reduced the germination percentage. Maximum germination percentage (98%) was observed in the control treatment and the minimum (58%) was found with 1.5 per cent NaCl concentration. It was observed that seedling length decreased with increasing NaCl concentration. The present study was conducted to investigate the effects of salinity on the chickpea seed germination and early seedling growth.

Materials and Methods
The present investigation was carried out in the laboratory of Department of Crop Physiology, College of Agriculture, Vijayapur. Germination studies were carried out using petriplate technique.

***In vitro* seed sterilization and seed germination:**
Germination studies were carried out using petriplate technique. Petridishes of 15 centimeter diameter were used to conducted germination studies. They were washed well, first with water and then with alcohol, oven dried and autoclaved. Germination paper was cut to the required diameter and autoclaved. The lower side of the germination paper was provided with a thin uniform layer of absorbent cotton and then placed in the petridishes. Seeds of uniform size were selected and surface sterilized before using for the experiment. They were first washed with tap water for 10 min followed by 0.5 per cent $HgCl_2$ treatment for 5 minutes and then washed with sterile distilled water. The air-dried seeds were then placed equidistantly (5 seeds / petridish) in each petridish. 10 ml solution of the three different concentrations of NaCI were poured into the respective petridishes. Petridishes were placed at room temperature of about 25.0 ± 2°C in the laboratory. A control was maintained for each set by using distilled water.

Germination percentage
Seed germination was started after 72 h (seeds were considered to be germinated with the emergence of the radical). Then the germinating seeds were counted at regular intervals. Furthermore the lengths of root and shoot of the germinated seeds which were more than 2 mm in length were measured and recorded after 14 days of sowing. In all treatments a continuous increase in the number of germinating seeds as well as in the lengths of roots and shoots was observed during the subsequent days of germination.

$$\text{Germination (\%)} = \frac{\text{Number of normal seedlings}}{\text{Number of seeds put for germination}} \times 100$$

Root length (cm)
Five normal seedlings were selected randomly in each treatment from all the replications on 14thday from germination test. The root length was measured from the tip of the primary root to base of hypocotyl with the help of a scale and mean root length was expressed in centimeters (cm).

Shoot length (cm)
Five normal seedlings were used for shoot length measurement. The shoot length was measured form the tip of the primary leaf to the base of the hypocotyl and mean shoot length was expressed in centimeter (cm).

Seedling dry weight (g)
Five normal seedlings attached with cotyledon used for root and shoot length measurements were put in a butter paper pocket and kept in hot air oven at 70 ± 1ºC for 24 hours. The dry weight of the seedlings was recorded and expressed in grams (g).

Seedling vigour indices
The seedling vigour index I was calculated by adopting the method as suggested by Abdul Baki and Anderson (1973) and expressed in number by using below formula.

Seedling vigour index (I) = Germination (%) x Seedling length (cm)

The seedling vigour index II was computed by multiplying germination percentage with seedlings dry weight and expressed as whole number.

Seedling vigour index (II) = Germination (%) x Seedling dry weight (g)

This laboratory experiment was to identify genotypic variations for salt tolerance with regards to seed germination and seedling establishment.

Results
Germination percentage
The germination percentage differed significantly with respect to genotypes, salinity levels and their interaction (Table 1). Among the salinity levels significantly maximum germination percentage (100%) was recorded under 0 dSm^{-1}, followed by 3 dSm^{-1} (85.33%) and under 6dSm^{-1} least germination percentage was recorded (80%). Among the chickpea genotypes, significantly maximum germination percentage was recorded by the genotypes MNK1, JG11 and BGD103 which were found on par with each other (100%,each), followed by ICC1431, ICC5003 and Annigeri 1 (97.78, 93.33 and 88.89, respectively) However, minimum germination percentage was observed in the genotype ICCV96029 (66.67%).

Seedling length
The seedling length differed significantly with respect to genotypes, salinity levels and their interactions (Table 1). Among the salinity levels significantly maximum seedling length was observed in 0 dSm^{-1} (31.98 cm) followed by 3 dSm^{-1} (16.25 cm) and significantly minimum seedling length was observed under 6 dSm^{-1} (2.30 cm). Similarly, the chickpea genotypes varied significantly for seedling length. The genotype JG11 recorded maximum seedling length (23.55 cm) followed by the genotype ICCV1431 and JAKI9218 (23.02 and 21.21 cm, respectively). Whereas, the genotype ICCV96029 recorded minimum seedling length (7.67 cm) compared to other genotypes.

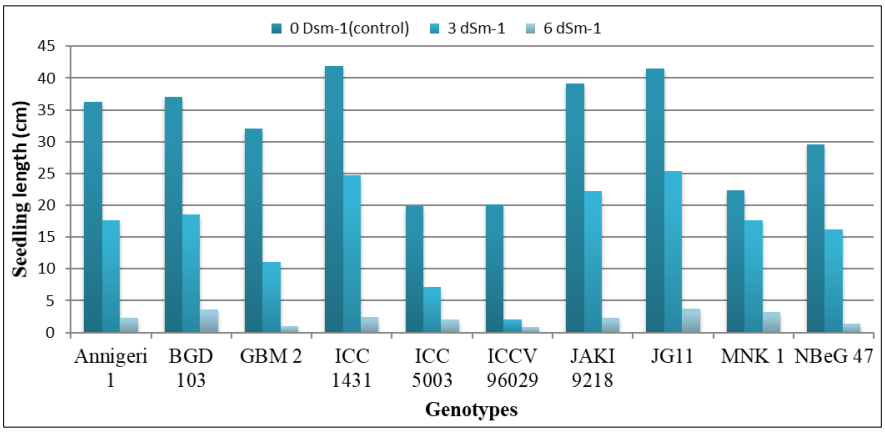

Fig 1: Effect of salinity stress on seedling length of chickpea genotypes

Seedling dry weight

The data on seedling dry weight influenced by genotype, salinity levels and their interaction is presented in Table 1. Among the genotypes, JG11 recorded significantly higher seedling dry weight (0.091 g) followed by the genotypesICCV1431, MNK1 and BGD103 (0.086 g, 0.08 g and 0.075 g, respectively). Whereas, significantly lower seedling dry weight (0.022 g) was recorded by the genotype ICCV96029.

Vigour Index I

The vigour index I differed significantly among the genotypes, salinity levels and their interactions (Table 2). The vigour index decreased with the increase in salinity levels. Among the genotypes, higher vigour index I was recorded in the genotype JG11 (2355.17) followed by ICC1431, BGD103, JAKI 9218 and Annigeri 1 (2296.41, 1975.08, 1953.76 and 1781.56 respectively). However, significantly lower vigour index was recorded by the genotype ICCV96029(717.70).

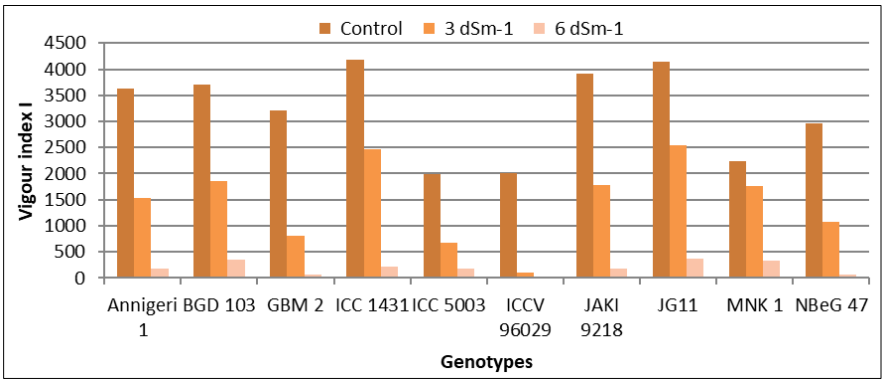

Fig 2: Effect of salinity stress on vigour index I of chickpea genotypes

Vigour index II

The vigour index II is influenced by genotypes, salinity concentration and their interactions (Table 2). Significantly higher value of vigour index II (10.42) was recorded under 0 dSm^{-1}, followed by 3 dSm^{-1} (5.20). Significantly least value of vigour index II (1.85) was observed in 6 dSm^{-1}.

Table 1: Effect of salinity stress on Germination percentage, seedling length and seedling dry weight in chickpea genotypes

Genotypes	Germination percentage (%)				Seedling length (cm)				Seedling dry weight (mg)			
	0 dSm^{-1} (Control)	3 dSm^{-1}	6 dSm^{-1}	Mean	0 dSm^{-1} (Control)	3 dSm^{-1}	6 dSm^{-1}	Mean	0 dSm^{-1} (Control)	3 dSm^{-1}	6 dSm^{-1}	Mean
Annigeri 1	89.96	72.26	68.04	76.76	36.28	17.65	2.28	18.74	99.70	57.25	19.00	58.65
JAKI 9218	89.96	68.04	59.19	72.40	39.10	22.18	2.35	21.21	121.58	66.28	19.93	69.27
BGD 103	89.96	89.96	89.96	89.96	37.05	18.62	3.58	19.75	109.87	79.40	35.30	74.86
MNK 1	89.96	89.96	89.96	89.96	22.35	17.59	3.23	14.39	131.83	78.00	28.83	79.56
JG11	89.96	89.96	89.96	89.96	41.50	25.36	3.80	23.55	147.60	87.83	37.17	90.87
GBM 2	89.96	59.19	54.97	68.04	32.10	11.08	1.05	14.74	83.85	37.50	6.67	42.67
NBeG 47	89.96	54.97	46.90	63.95	29.55	16.14	1.34	15.68	90.63	53.50	8.72	50.95
ICC 1431	89.96	89.96	81.11	87.01	41.89	24.73	2.44	23.02	155.33	74.08	27.40	85.61
ICC 5003	89.96	81.11	72.26	81.11	19.89	7.16	2.05	9.70	56.01	26.91	17.91	33.61
ICCV 96029	89.96	46.90	43.06	59.98	20.13	1.97	0.90	7.67	46.00	15.75	5.69	22.48
Mean	89.96	74.23	69.54		31.98	16.25	2.30		104.24	57.65	20.66	
	S.Em±		LSD @5%		S.Em±		LSD @5%		S.Em±		LSD @5%	
EC	1.31		3.49		0.05		0.12		1.57		4.18	
Genotypes	0.39		1.05		0.15		0.41		0.47		1.25	
Interaction (E*G)	3.93		10.46		0.46		1.23		4.71		12.54	

Table 2: Effect of salinity stress Vigour index I and Vigour index II in chickpea genotypes

Genotypes	Vigour index I				Vigour index II			
	0 dSm^{-1}(Control)	**3 dSm^{-1}**	**6 dSm^{-1}**	**Mean**	**0 dSm^{-1}(Control)**	**3 dSm^{-1}**	**6 dSm^{-1}**	**Mean**
Annigeri 1	3628.00	1535.33	181.33	1781.56	9.97	4.97	1.53	5.49
JAKI 9218	3910.00	1778.93	172.33	1953.76	12.16	5.32	1.47	6.32
BGD 103	3705.00	1862.00	358.25	1975.08	10.99	7.94	3.53	7.49
MNK 1	2235.00	1758.75	323.25	1439.00	13.18	7.80	2.88	7.96
JG11	4150.00	2535.50	380.00	2355.17	14.76	8.78	3.72	9.09
GBM 2	3210.00	812.53	66.22	1362.92	8.39	2.80	0.44	3.87
NBeG 47	2955.00	1081.60	71.68	1369.43	9.06	3.59	0.47	4.37
ICC 1431	4188.50	2473.00	227.73	2296.41	15.53	7.41	2.60	8.51
ICC 5003	1988.50	668.59	177.67	944.92	5.60	2.54	1.57	3.24
ICCV 96029	2013.00	104.11	36.00	717.70	4.60	0.84	0.27	1.90
Mean	3198.30	1461.04	199.45		10.42	5.20	1.85	
	S.Em±		LSD @5%		S.Em±		LSD @5%	
EC	6.94		18.45		0.05		0.13	
Genotypes	23.12		61.50		0.17		0.44	
Interaction (E*G)	69.36		184.50		0.50		1.33	

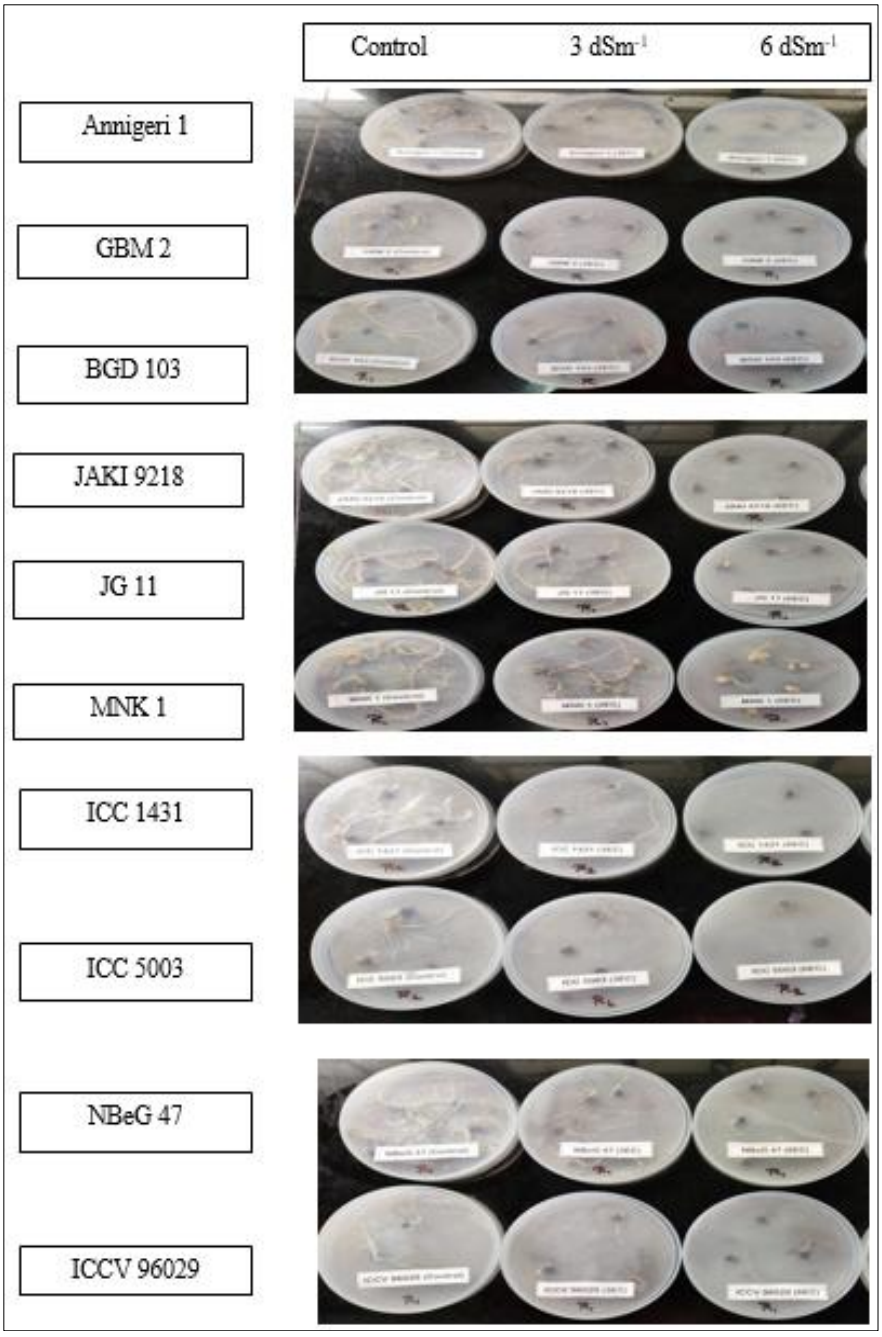

Fig 3: Effect of salinity stress on germination of chickpea genotypes in petriplates

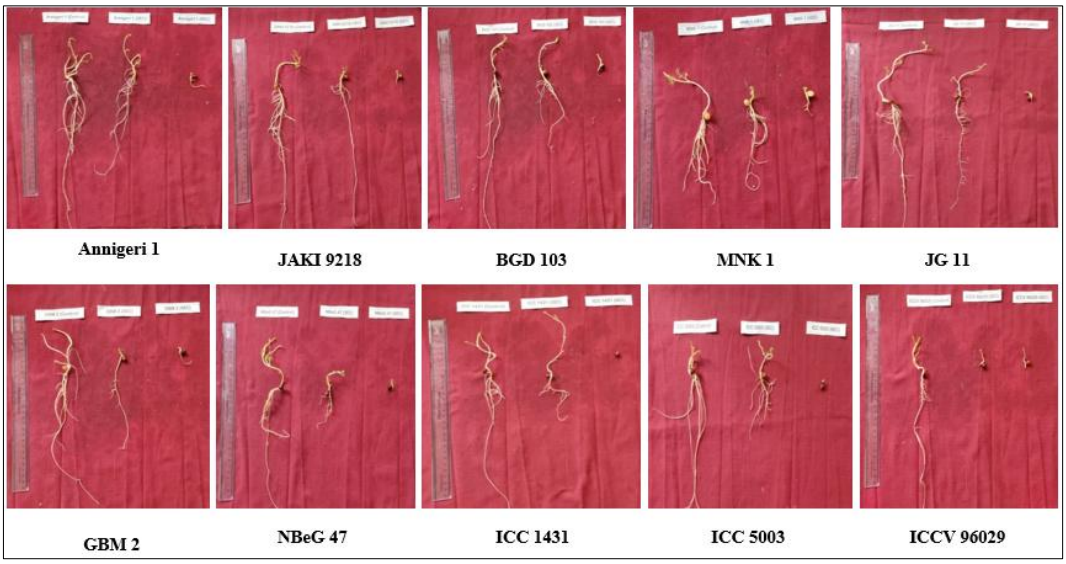

Fig 4: Effect of salinity stress on germination of chickpea genotypes

Discussion

Germination of seed depends on the imbibitions of water and which is affected by salt ion concentration of external medium in which seeds are sown. Germination process under salt stress condition was studied to identify the varieties tolerant to salinity as well as to understand the tolerance mechanism. Among the genotypes, the significant variation was observed at germination stage. Based on the results, genotypes categorized into three groups *viz*, salt tolerant (JG11, BGD103, MNK1 and ICC1431), moderately tolerant (ICC5003, JAKI9218 and Annigeri 1) and sensitive (ICCV96029, NBeG47 and GBM2). Reduction in rate of germination is due to toxic effects of various salts that diminish the water potential in the medium which avoids water absorption by germinating seeds (Jamil *et al*., 2006). Vigour index was low in salt sensitive genotypes whereas, tolerant genotypes did not show much difference in their germination ability at different salt stress levels. The germination percentage and germination rate decreased with reduction of water movement into the chickpea seeds during imbibition. Thus, it was observed that salt stress can affect germination of seed through osmotic effects which was also observed by Tsegazeabe *et al*. (2012) [8]. Krishnamurthy *et al*. (2007) [3] showed that the cultivars which attained higher germination rate under salt stress conditions produced higher yield and biomass. Similarly, Cicek and Cakirlar (2002) [2] reported that increased salt level in soil reduces germination rate.

The results of present investigation, it was confirmed that the maximum seedling length was observed in 0 dSm^{-1} (31.98 cm) followed by 3 dSm^{-1} (16.25 cm) and significantly reduced seedling length under 6 dSm^{-1} (2.30 cm). The presence of salt in growth medium leads to reduction of osmotic potential due to reduction in the absorption of water. Similar observations where length of radicle and plumule got negatively affected by salinity were observed by Zawude and Shanko which was attributed to ionic toxicity, disturbance in water absorption, reduction in biosynthesis of enzymes and plant hormones necessary for growth (2017). Kandil *et al*. (2012) reported gradually decreased gradually decreased average germination percentage, seedling vigour index, shoot and root fresh weight, shoot and root length and seedling dry weight due to salinity.

Conclusion

Initial screening of chickpea genotypes was carried out through germination studies in petriplates with salt (NaCl) stress and observation were recorded on 14th day. The data on germination percentage, seedling length, seedling dry weight and vigour index showed significant difference across genotypes, salinity levels and their interaction. As expected, the maximum germination percentage, seedling length, vigour index were observed under control (0 dSm^{-1}) as compare to 3 dSm^{-1} and 6 dSm^{-1}. Among genotypes MNK 1, JG 11 and BGD 103 showed maximum germination percentage.

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