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Influence of Seed Priming with Proline on Seed Quality Parameters of Chickpea (*Cicer arietinum*) under Salinity Stress

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ABSTRACT

An experiment was conducted to study the effect of seed priming with water, $CaCl_2$ at 2%, 6 ppm, 8 ppm, 10 ppm and 12 ppm of proline on germination and seedling vigour of chickpea (*Cicer arietinum*) exposed to four level of salinity *i.e.,* 2 dS/m, 4 dS/m, 6 dS/m and 8 dS/m NaCl and water. The experimental design was based on factorial completely randomized design with four replications and performed at the laboratory of crop physiology, college of agriculture, university of agricultural sciences, Raichur. The chickpea seeds were primed with water, $CaCl_2$ at 2% and different concentration of proline for six hours and kept for germination. The exposure of chickpea seeds to increasing concentration of NaCl had drastically reduced germination (%), seedling vigour index, seedling dry weight and seedling length. It is evident from the result that seed priming with 6 ppm of proline significantly increased the germination (%), seedling vigour index, seedling dry weight, seedling length of chickpea under normal as well as salinity condition.

Keywords: Seed priming; Proline; $CaCl_2$; Salinity

INTRODUCTION

Bengal gram or chickpea is an annual legume that belongs to the family Fabaceae. It is said to be one of the oldest pulses known and cultivated in Asia and Europe. The centre of origin of chickpea is stated to be Eastern Mediterranean, but it's probable place of origin lies in Southwestern Asia. Globally, chickpea is grown in an area of 137 lakh hectares with a production of 142.4 lakh tonnes and productivity of 1038 kg/ha. India contributes 70 percent of total world chickpea production of 116.2 lakh tonnes cultivated under 112 lakh hectares with productivity of 1036 kg/hectare in 2020-21. India is the largest producer of world gram production followed by Australia, Myanmar and Ethiopia. Salinity is one of the most important abiotic stresses that negatively affect plant growth and development around the world. It has been reported that approximately 19.5 percent of all irrigated land and 2.1 percent of dry land is affected by salt stress. Saline areas continue to increase in size because of mishandled irrigation. In addition, in arid and semi-arid regions the salinization process occurs because of high evaporation and inadequate amounts of precipitation for considerable leaching. Salinity inhibits the crop growth and development, through complex traits that include osmotic stress, ion toxicity, mineral deficits, and physiological and biochemical defects [1]. Soil salinity inhibits seed germination due to high

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osmotic potential created around the seed, which prevents water uptake [2].

Proline is only proteinogenic amino acid which has a unique rigidity by conformation and is necessary for primary metabolism. Proline accumulates in plants during condition of drought and salinity [3]. It has been reported that proline accumulation provides resistance to salinity stress [4]. In the view of above facts the experiment was conducted to standardize the proline concentration for seed priming in chickpea under salinity stress.

MATERIALS AND METHODS

The experiment was conducted during 2021-22 in the laboratory of department of crop physiology, college of agriculture, university of agricultural sciences, Raichur. Chickpea variety BGD 103 was used for the experiment. The experiment was laid out in factorial completely randomised design with two factors (Factor I: Salinity stress, Factor II: Seed priming) and four replications. The first factor salinity stress consists of five levels 0 dS/m, 2 dS/m, 4 dS/m, 6 dS/m, 8 dS/m of NaCl and the second factor seed priming consist of seven levels of control, water priming, $CaCl_2$ at 2%, 6 ppm, 8 ppm, 10 ppm and 12 ppm of proline. The chickpea seeds were first surface sterilized with 5 percent sodium hypochlorite and primed with water, $CaCl_2$ at 2%, and different concentration of proline. The primed seeds were placed in germination paper and the observations took from first day to 8^{th} day.

The germination test was conducted by following Between Paper (BP) towel method as per the procedure prescribed by ISTA rules. The germination count was taken daily till the 8^{th} day and the normal seedlings were considered for calculating the germination percentage. The seedling vigour index was calculated according to Abdul-Baki and Anderson by the following formula:

Seedling vigour index-I=Germination percentage × Total seedling length in cm

The speed of germination was calculated by using the formula suggested by Maguire.

$$\text{Speed of germination} = \frac{G1}{D1} + \frac{G2}{D2} + \frac{G3}{D3} + \ldots\ldots + \frac{Gn}{Dn}$$

Where,

G1, G2, ...Gn are the number of seeds germinated on D1, D2, ...Dn day

Germination index, co-efficient of velocity germination and mean daily germination was calculated by the formula suggested by Ranal and Santana. The data collected from the experiments were analysed statistically by following the procedure suggested by Sundarajan et al.

RESULTS AND DISCUSSION

Germination Percentage

Among different salinity stress levels, the higher salinity stress 8 dS/m recorded lower germination percent of 78 as compared to control. The reduction in the germination percent may be due to ion toxicity and hyperosmotic stress created by the sodium and chloride ions in the germinating seeds which delay or inhibit germination [3]. Salinity also affects the α-amylase activity by reduced bioactive gibberellin content. Seed priming with 6 ppm of proline showed higher germination of 96.3% (Table 1). Seed priming with 6 ppm of proline performed better when compared to all other priming treatments. Malik et al. also reported that seed priming with proline enhanced the germination percentage under salinity stress condition.

Table 1: Effect of seed priming on seed germination percentage of chickpea under salinity stress.

	Seed germination (%)							
Treatments	**P_1 control**	**P_2 water priming**	**P_3 $CaCl_2$ at 2%**	**P_4 proline 6 ppm**	**P_5 proline 8 ppm**	**P_6 proline 10 ppm**	**P_7 proline 12 ppm**	**Mean**
S_1 Control	98	88	96.4	98.7	96.6	90	94.5	94.6
S_2 2 dS/m	96.5	84.8	94.5	98.5	94	88.5	94	92.9
S_3 4 dS/m	84	78	90	96.5	92	84.5	88	87.5
S_4 6 dS/m	80	74.5	84.5	94.5	90	82	84.3	84.2
S_5 8 dS/m	64.5	68	70.5	94	86.5	80.7	82	78
Mean	84.7	78.6	87.1	96.3	91.8	85.1	88.5	

Factors	**SEm ±**	**CD at 1%**
Stress (S)	1	3
Priming (P)	1.2	3.6
S × P	2.8	8

Seedling Length

The seedling length of the chickpea significantly influenced by the salinity stress and seed priming treatments. The interaction effect also found significant (**Table 2**). The seedling length was decreased at the rate of 41 percent in stress condition S_5 (8 dS/m) when compared with control (S_1) based on mean values. Reduction in seedling growth at high NaCl levels may be the result from the interaction between negative effects of NaCl on cell membranes, ion toxicity [4]. From all seed priming treatments, based on mean values, the higher seedling length was recorded from treatment proline 6 ppm (33.2 cm) but lower seedling length was recorded in control (16.8 cm).

It was showed that, the seedling length was signi icantly increased in the order of 49 percent in treatment proline 6 ppm as compared with control. Here, the interaction effect was signi icant in S_1 P_4 with seedling length of 39.7 cm. Our studies agree with earlier studies, exogenous application of proline increased plant growth and yield characteristics in zea mays [5]. Heuer reported that external supplementation of proline helps in improving salt tolerance in crops.

Table 2: Effect of seed priming on seedling length of chickpea under salinity stress.

	Seedling length (cm)							
Treatments	P_1 control	P_2 water priming	P_3 $CaCl_2$ at 2%	P_4 proline 6 ppm	P_5 proline 8 ppm	P_6 proline 10 ppm	P_7 proline 12 ppm	Mean
S_1 Control	29.8	22.6	28.7	39.7	34.1	33.3	32.5	31.5
S_2 2 dS/m	18.6	21.8	27.8	37	31.5	31.9	29.4	28.3
S_3 4 dS/m	15.2	18.9	22.7	32.9	27.5	28.4	27.5	24.7
S_4 6 dS/m	11.9	15.7	22.2	29.1	26.4	27	26.5	22.7
S_5 8 dS/m	8.4	10.6	13.6	27.4	24.1	23.2	22.1	18.5
Mean	16.8	17.9	23	33.2	28.7	28.7	27.6	
Factors			**SEm ±**			**CD at 1%**		
Stress (S)			0.3			0.9		
Priming (P)			0.3			1		
S × P			0.8			2.4		

Seedling Dry Weight

The seedling dry weight of chickpea was significantly influenced by the salinity stress and seed priming treatments (**Table 3**). From the data, the seedling dry weight observed to be declined at the rate of 69.16 percent in S_5 (8 dS/m) stress condition over control. Moreover, the reduction in seedling dry weight may be due to the toxic effect of Na^+ on photosynthesis rate at higher concentrations [6]. Specifically, it was demonstrated previously that salinity reduces intercellular CO_2 concentration and then photosynthesis rate by stomatal closure [7]. In addition, under high salt levels, Na^+ can cause lower transport rate of essential ions such as NO_3 that reduce the N-containing compounds and ultimately inhibit plant growth and biomass accumulation.

Among the seed priming treatments, it was showed that treatment proline 6 ppm recorded more dry weight as 872 mg while the control (no stress) recorded less dry weight as 512 mg. It was clearly showed that the dry weight of chickpea in proline 6 ppm has perceptible increase of 41.28 percent when compared with control (no stress). It is well documented that certain concentrations of exogenous proline regulate different aspects of plant growth and development under salt stress including rises in biomass and productivity [8].

Table 3: Effect of seed priming on seedling dry weight of chickpea under salinity stress.

	Seedling dry weight (mg)							
Treatments	**P_1 control**	**P_2 water priming**	**P_3 $CaCl_2$ at 2%**	**P_4 proline 6 ppm**	**P_5 proline 8 ppm**	**P_6 proline 10 ppm**	**P_7 proline 12 ppm**	**Mean**
S_1 Control	690	640	750	1080	1010	880	930	854
S_2 2 dS/m	620	630	670	980	870	770	840	768
S_3 4 dS/m	550	590	560	850	850	710	760	695
S_4 6 dS/m	400	490	460	810	800	580	660	600
S_5 8 dS/m	300	310	350	640	590	560	520	467
Mean	512	532	558	872	824	700	742	
Factors			**SEm ±**			**CD at 1%**		
Stress (S)			8			24		
Priming (P)			10			29		
S × P			23			65		

Seedling Vigour Index-I

The seedling vigour index-I was significantly influenced by the salinity stress and the seed priming treatments. The interaction between salinity stress and seed priming treatments found significant as mentioned in **Table 4**. It was clearly shown that the seedling vigour index-I value was 49 percent decreased in S_5 (8 dS/m) level when compared with S_1 (no stress-control) level. Javed and Khan stated that shoot and root length provides an important clue to the response of plants to salt stress. The results are in confirmatory with findings of Jamil and Rha, where the shoot and root length of seedlings grown in salt solutions showed decreasing trend, indicating that the salt stress not only affected germination but also the growth of seedlings, which ultimately affects the seedling vigour. Similar results were also reported by Djanaguiraman et al. and Hakim et al. in rice.

Among the various seed priming treatments under this study, it was observed that higher seedling vigour index I of 3209 in proline 6 ppm and lower seedling vigour index-I was 1444 in water priming. Therefore, the seedling vigour index-I were 53 percent increase in proline 6 ppm over control. It shows that proline seed priming treatment with 6 ppm concentration were performed well under all level of salinity stress condition (S_1 to S_5). Proline reduces the ion toxicity under salt stress and decrease both Na^+ and Cl^- contents, but increase the K^+ content and the K^+/Na^+ ratio [9]. Yaqoob et al., reported that seed pre-treatment with proline improved the seedling length and germination percent of the quiona under cold stress. Increased germination percent and seedling length leads to increase in seedling vigour.

Table 4: Effect of seed priming on seedling vigour index-I of chickpea under salinity stress.

	Seedling vigour index-I							
Treatments	**P_1 control**	**P_2 water priming**	**P_3 $CaCl_2$ at 2%**	**P_4 proline 6 ppm**	**P_5 proline 8 ppm**	**P_6 proline 10 ppm**	**P_7 proline 12 ppm**	**Mean**
S_1 Control	2947	1994	2770	3890	3301	3004	3071	2997
S_2 2 dS/m	1794	1853	2628	3644	2962	2823	2771	2639
S_3 4 dS/m	1281	1480	2044	3180	2531	2399	2423	2191
S_4 6 dS/m	955	1174	1877	2757	2377	2214	2240	1942
S_5 8 dS/m	543	720	964	2575	2086	1872	1815	1511
Mean	1504	1444	2057	3209	2652	2462	2464	
Factors			**SEm ±**			**CD at 1%**		
Stress (S)			30			90		
Priming (P)			35			106		

S × P	79	225

Speed of Germination

Speed of germination is the rate of germination in terms of the total number of seeds that germinate in a time interval. The speed of germination was observed to be marginally decreased at the rate of 30 percent in salinity stress level S_5 (8 dS/m) as compared to control S_1 (no stress) (**Table 5**). Salinity may adversely influence seed germination by decreasing the amounts of seed germination stimulants such as GAs, enhancing ABA amounts, and altering membrane permeability and water behaviour in the seed [10]. This disruption in hormone balance will reduce the speed of germination of seeds under salinity stress condition.

Among all seed priming treatments, on an average, the faster speed of germination was found to be 30.92 which was 26 percent increased rate in proline 6 ppm than control, as well as the slower speed of germination was recorded as 22.88 in control. The interaction effect was signi icant. Karim et al. reported that seed priming will increase the speed of germination of seeds on yard long bean. Proline protects the cellular structures by acting as an antioxidant and ROS scavenger [11]. So, under stress condition proline primed seeds have more speed of germination compared to control.

Table 5: Effect of seed priming on speed of germination (% day^{-1}) in chickpea under salinity stress.

	Speed of germination (% day^{-1})							
Treatments	**P_1 control**	**P_2 water priming**	**P_3 $CaCl_2$ at 2%**	**P_4 proline 6 ppm**	**P_5 proline 8 ppm**	**P_6 proline 10 ppm**	**P_7 proline 12 ppm**	**Mean**
S_1 Control	28.5	24.33	27	36.5	31.11	29.5	33	29.99
S_2 2 dS/m	26.16	21.66	26	33	30.75	28.28	29.83	27.95
S_3 4 dS/m	21.25	21.31	25.83	31.41	29.45	25.83	25.5	25.79
S_4 6 dS/m	19.75	19.16	22.66	30.48	22.4	24	25.41	23.4
S_5 8 dS/m	18.75	16.41	21.66	23.23	21.33	19.18	25.33	20.84
Mean	22.88	20.57	24.63	30.92	27	25.35	27.81	
Factors		**SEm ±**				**CD at 1%**		
Stress (S)		0.32				0.9		
Priming (P)		0.38				1.07		
S × P		0.85				2.4		

Coefficient of Velocity of Germination

The coefficient of velocity of germination represents the velocity of germination of seeds. The impact on coefficient of velocity of germination due to seed priming under salinity stress was recorded and tabulated in **Table 6**. The coefficient of velocity was recorded a decline at the rate of 19 percent in salinity stress level S_5 (8 dS/m) as compared with control S_1 (no stress). Under salinity stress condition the velocity of seed germination affected because of osmotic stress and ion toxicity in the seeds [12]. Among the seven proline treatments of seed priming, on an average, lower coefficient of velocity of germination was recorded as 42.93 in control and higher was recorded from proline 6 ppm as 57.17 which was 24.9 percent increase over control. Seed priming hydrates the seed to activate the pre-germinative metabolic and biochemical activities without radical protrusion during phase II of seed germination. The primed seeds facilitate uniform germination by enzyme activation, cell repairing mechanism, synthesis of proteins, and improved antioxidant defense mechanisms as compared to non-primed seeds [13-15]. The coefficient of velocity of germination was found to be higher in treatment proline 6 ppm.

Table 6: Effect of seed priming on coefficient of velocity of germination chickpea under salinity stress.

	Coefficient of velocity of germination							
Treatments	**P_1 control**	**P_2 water priming**	**P_3 $CaCl_2$ at 2%**	**P_4 proline 6 ppm**	**P_5 proline 8 ppm**	**P_6 proline 10 ppm**	**P_7 proline 12 ppm**	**Mean**
S_1 Control	50	53.84	52.74	60.24	54.02	65.57	62.66	57.01

S_2 2 dS/m	48.48	51.57	51.11	57.31	53.24	53.57	54.21	52.78
S_3 4 dS/m	43.47	45.26	51.06	56.16	51.64	50	53.84	50.2
S_4 6 dS/m	38.59	43.75	48.05	56.14	46.6	49.43	51.08	47.66
S_5 8 dS/m	34.09	43.59	44.76	56	45.37	48.83	50.72	46.19
Mean	42.92	47.6	49.54	57.17	50.17	53.48	54.5	

Factors	SEm ±	CD at 1%
Stress (S)	0.64	1.8
Priming (P)	0.75	2.14
S × P	1.69	4.78

Peak Value

Peak value is the accumulated number of seeds germinated at the point on the germination curve at which the rate of germination starts to decrease [16-18]. The peak value of chickpea due to seed priming under salinity stress was recorded and tabulated in **Table 7**. The peak value was reduced at the rate of 36 percent in salinity stress level 8 dS/m over control (no stress). Among all proline seed priming treatments, on an average, the higher peak value was found as 77 in proline 6 ppm while the lower peak value was found to be 38 in control. The peak value of chickpea was an appreciable increase at the order of 50 percent in proline 6 ppm over control. Higher interaction was found at S_5P_4. The seed priming with proline at 6 ppm concentration was showed better performance in all levels of salinity stress [19].

Table 7: Effect of seed priming on peak value of chickpea under salinity stress.

	Peak value							
Treatments	**P_1 control**	**P_2 water priming**	**P_3 $CaCl_2$ at 2%**	**P_4 proline 6 ppm**	**P_5 proline 8 ppm**	**P_6 proline 10 ppm**	**P_7 proline 12 ppm**	**Mean**
S_1 Control	42	52	60	86	76	62	68	63
S_2 2 dS/m	42	46	58	80	68	62	66	60
S_3 4 dS/m	40	42	58	80	66	56	58	57
S_4 6 dS/m	38	38	52	74	60	50	58	52
S_5 8 dS/m	32	32	50	68	60	42	54	40
Mean	38	42	55	77	55	54	60	

Factors	SEm ±	CD at 1%
Stress (S)	0.7	2
Priming (P)	0.8	2.4
S × P	1.9	5.4

Mean Daily Germination

The data pertaining to mean daily germination was recorded and tabulated in **Table 8**. Based on mean values, the mean daily germination was found to be marginally decreased at the rate of 17 percent in salinity stress condition S_5 (8 dS/m) when compared with control S_1 (no stress). On an average, among all the seed priming treatments studied, the higher mean daily germination was found as 12.03 in proline 6 ppm but lower mean daily germination was recorded as 9.8 in P_2. The appreciable increase in mean daily germination at the order of 18.67 percent in proline 6 ppm as compared with P_2 [20]. Jamil et al., reported that salinity reduces the rate of germination in the vegetables. As already discussed in germination percentage, proline improved the mean daily germination of chickpea seeds under salinity stress.

Table 8: Effect of seed priming on mean daily germination of chickpea under salinity stress.

	Mean daily germination							
Treatments	P_1 control	P_2 water priming	P_3 $CaCl_2$ at 2%	P_4 proline 6 ppm	P_5 proline 8 ppm	P_6 proline 10 ppm	P_7 proline 12 ppm	Mean
S_1 Control	12.33	11	12.05	12.25	12.07	11.25	11.81	11.82
S_2 2 dS/m	12.06	10.6	11.81	12.31	11.75	11.06	11.75	11.62
S_3 4 dS/m	10.5	9.75	11.25	12.06	11.5	10.56	11	10.94
S_4 6 dS/m	10	9.31	10.56	11.81	11.25	10.25	10.53	10.53
S_5 8 dS/m	8.06	8.5	8.81	11.75	10.81	10.08	10.25	9.75
Mean	10.59	9.83	10.89	12.03	11.47	10.64	11.07	
Factors		SEm ±				CD at 1%		
Stress (S)		0.13				0.38		
Priming (P)		0.15				0.45		
S × P		0.35				1		

Germination Index

The impact of seed priming on germination index under salinity stress was recorded and tabulated in **Table 9**. On an average, the germination index was recorded a drop by 17.98 percent in salinity stress level S_5 (8 dS/m) over control S_1 (no stress). Malik et al., reported that salinity reduced the germination rate of desert forage grass. Among the seven seed priming treatments, on an average, higher germination index was observed as 340 in treatment proline 6 ppm and lower germination index was 267 in P_2. The germination index of proline 6 ppm was 21.47 percent increase when compared with control.

Table 9: Effect of seed priming on germination index of chickpea under salinity stress.

	Germination index							
Treatments	P_1 control	P_2 water priming	P_3 $CaCl_2$ at 2%	P_4 proline 6 ppm	P_5 proline 8 ppm	P_6 proline 10 ppm	P_7 proline 12 ppm	Mean
S_1 Control	346	300	338	367	329	308	348	317
S_2 2 dS/m	333	282	336	341	324	303	341	307
S_3 4 dS/m	282	273	321	333	322	299	307	292
S_4 6 dS/m	268	256	292	332	318	292	300	281
S_5 8 dS/m	231	228	246	331	292	273	296	260
Mean	292	267	306	340	317	295	318	
Factors		SEm ±				CD at 1%		
Stress (S)		3				10		
Priming (P)		4				12		
S × P		9				28		

CONCLUSION

Through the investigation it can be concluded that seed priming with 6 ppm of proline is effective in mitigating salinity stress in chickpea. Proline priming improved the germination percentage, speed of germination, seedling vigour and rate of germination.

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