Alleviation of Salt Stress Employing Priming with Sodium Chloride in Chickpea (Cv. Mahamaya-1, *Cicer arietinum* L) for Improved Germinability, Membrane Permeability and Enzyme Activity

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Abstract: ***The present study was performed in order to assess the influence of priming treatments in different salt concentrations of sodium chloride (0.00mM, 25mM, 50mM, 75mM, 100mM) in Chickpea desi (Mahamaya 1) variety. Seeds were collected from Baharampur Pulse and Oil Seed Research Station, Baharampur, Murshidabad and the experiments were conducted in the Institute of Agricultural Science, University of Calcutta. The seeds were soaked in different concentration of sodium chloride solutions and then seeds were lightly dried in the artificial dryer for 24 h. Germination test were conducted after 24h in different concentrations of salt solutions (0mM to 100mM) with primed and non-primed seeds revealed that 25mM sodium chloride solution primed seeds have shown greater seedling length and reduced leakage of electrolytes and sugar with a higher dehydrogenase enzyme activity than non-primed as well as other primed seeds. On the basis of the results, seed priming with 25mM sodium chloride solutions may be suggested for improved germination and seedling growth of Chickpea under salt stress conditions.***

Keywords: Seed priming, salt stress, chickpea, membrane permeability, dehydrogenase activity

1. Introduction

Chickpea or gram is a native of southern Europe, now successfully grown in India. It is a cold loving (70-85ºC) annual crop and seeds are edible, cotyledons of seeds are used in form of 'dal', 'besan' etc., the vegetative parts are used as salad and fodder.

In India, average annual area for cultivation and production is about 7.37 million hectares (2011) and production of 5.89 million tonnes (48.28%) respectively. The major chickpea producing states are Madhya Pradesh, Rajasthan, U.P., Bihar, Punjab, Haryana and Maharashtra. The area of gram in West Bengal were 0.850 million hectare (at 4^{th} position) during the year 2014-15.

Soil degradation through salinization has seriously affected the crop productivity of large areas. Saline soils are found in all agro ecological regions and own their existence to a wide variety of causes. In a fraction of these soils, salt accumulation the soil profile can be attributed to natural process, but in a majority of cases it is brought about by human intervention due to introduction of irrigation, use of excessive chemical fertilizer, use of saline water or due to other developmental works leading ultimately to accumulation of salts. Therefore, development of crops with higher salt tolerance has increased manyfold within the last decade due to increased salinity problems.

In general, plants do not develop salt tolerance unless they are grown in saline conditions. Levitt (1980) suggested that the plant should be hardened to salt stress for the development of salt tolerance. Salt tolerance of plant could be increased by treatment of seed with sodium chloride (NaCl) solution prior to sowing.

Although priming is one of the physiological methods, which improves seed performance and provides faster and synchronized germination (Sivritepe and Dourado, 1995). It has been shown that Sodium Chloride (NaCl) priming could be used as an adaptation method to improve salt tolerance of seeds. Nevertheless, beneficial effects of NaCl priming for later growth and development stages of plants remain unclear. The literature revealed that from 1980 to 1995, over 300 papers a year were published on mechanisms of salt tolerance in higher plants, yet fewer than a dozen salt tolerant cultivars were released, offering only slight improvement over the parent lines (Flowers and Yeo, 1995). In fact it has been questioned whether any cultivars bred for salt tolerance have been commercially successful. Farmers are still better off planting yield selected lines in salty soils (Richards, 1992). Two leading biochemists who take a molecular approach to salt tolerance research called for a moratorium on further plant breeding until the molecular genetics are better understood (Bohnert and Jensen, 1996), a leading breeder who takes a physiological approach responded that molecular biologists project the optimism of blue skies researches advertising their wares (reply by Flowers in Bohnert and Jensen, 1996). Chickpea *(Cicer arietinum L.)* is the third most extensively planted grain legume (D amore *et al*, 1996). Besides being an important source of human and animal food, the crop also plays an important role in the maintenance of soil fertility, particularly in arid regions (Saxena, 1990). Therefore, development of a suitable easily practicable and low cost seed priming methodology for improved crop performances will be helpful to the small and marginal farmers under salt stress conditions.

2. Materials and Methods

Freshly harvested seeds of Mahamaya 1 were taken for the experiment. Seeds were soaked in different concentrations of NaCl solutions (0.00mM, 25mM, 50mM, 75mM, 100mM) for 2 hours. After drying for 24 hours in the artificial light chamber, seeds were placed for germination following the method of Punjabi and Basu (1982) with minor modification. Blotting paper prior to germination were moistened with the different concentrations of respective NaCl solutions. During germination, seeds were slurry dressed with fungicide (Di-ethane M-45) to control seed borne fungus.

Priming Techniques

Two priming media were used such as water and NaCl solutions at different concentrations, 0.00mM, 25mM, 50mM, 75mM, 100mM. Seeds were fully immersed in priming media at a temperature of 24°C for durations of 2 hours in small container. Seeds were removed from priming media at the same time, then rinsed thoroughly with distilled water and lightly dried using blotting paper. The treated seeds were kept in light chamber for 24 hours and dried back to their half moisture content. The data on germination percentage and seedling length were recorded after germination for 5 days at 20±1℃.

3. Results and Discussion

The results indicate that the germination percentage alongwith seedling length of Chickpea significantly reduced with the increase of salt concentrations (Table 1, 2 and 3). These results confirm the earlier findings of Almansori *et al.* (2001), who reported that moderate stress insensitive delayed germination and higher concentration of Sodium Chloride reduced germination percentage. In the present experiment, hydropriming (using water alone) significantly improved vigour of the seedling as measured by root and shoot length under salt stress conditions. But hydropriming with sodium chloride solution at a concentration of 25mM can alleviate salt stress (sodium chloride solutions as salt stress) upto 100mM as evidenced by higher seedling length (root and shoot length) and germination percentage over non-primed seeds and other concentrations of sodium chloride priming seeds (Table 1, 2 and 3). Physiological and Biochemical studies revealed that hydropriming with sodium chloride solution (25mM) showed lower leakage of electrolytes and sugar alongwith higher dehydrogenase enzyme activity than the non-primed seeds and other primed seeds (Table 4).

In many crops, pre-soaking or priming causes improvement in germination and seedling establishment (Harris *et al.*, 2001). Increase in seedling growth correlated with higher water uptake by primed seed resulted in higher seedling growth. Kaur *et al.* (2002) reported that hydropriming showed three to four fold more growth with respect to root and shoot length in comparison with seedlings obtained from non-primed seeds in drought condition.

Salt tolerance mechanisms are either low-complexity or high-complexity mechanisms. Low-complexity mechanisms appear to involve changes in many biochemical pathways. High-complexity mechanisms involve changes that protect major processes such as photosynthesis and respiration, e.g., water use efficiency, and those that preserve such important features as cytoskeleton, cell wall, or plasma membrane-cell wall interactions (Botella *et al.*, 1994) and chromosome and chromatin structure changes, i.e., DNA methylation, polyploidization, amplification of specific sequences, or DNA elimination (Walbot and Cullis, 1985). It is believed that for the protection of higher-order processes, low-complexity mechanisms are induced co-ordinately (Bohnert *et al.*, 1995).

The decrease in antioxidants with increase in stress level was notable in chickpea. Previously, a decrease in the level of antioxidants was observed with increase in stress intensity in wheat by Zhang and Kirkham (1994). Enzymes activities showed great variation with increasing level of osmotic stress.

4. Conclusion

On the basis of the results, it may be advocated that seed priming with 25mM Sodium chloride solutions are suggested for improved germination and seedling growth of chickpea under salt stress conditions and it ranges upto 100mM.

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Table 1: Alleviation of salt stress employing priming with different concentrations of Sodium Chloride solutions on germination percentage in chickpea seeds

Treatments	Sodium Chloride Concentrations (mM)					Mean
	0.00	**25**	**50**	**75**	**100**	
Non-primed (control)	62.24	66.42	71.57	59.54	54.33	62.82
Primed (Water soaking)	62.73	65.35	71.19	58.69	55.98	62.79
Sodium chloride concentrations (mM) 25	75.46	78.40	84.26	65.88	59.15	72.63
50	53.55	58.05	53.13	48.04	45.97	51.75
75	42.71	43.28	40.80	42.30	42.88	42.39
100	35.24	37.29	36.45	37.46	30.20	35.33
Mean	55.32	58.13	59.57	51.99	48.09	-

L.S.D values for mean effects	**Probability at 0.05P**
Treatments	3.82
Salt concentrations	3.57
Interaction	6.31

Table 2: Alleviation of salt stress employing priming with different concentrations of sodium chloride solutions on mean root length of Chickpea

Treatments	Sodium chloride concentrations (mM)					Mean
	0.00	25	50	75	100	
Non-primed (control)	106	99	105	91	70	94.33
Primed (Water Soaking)	107	103	108	89	78	97.13
Sodium chloride concentrations (mM) 25	118	125	110	98	91	108.60
50	112	120	106	96	78	102.40
75	105	108	95	84	67	92.00
100	89	99	101	77	40	83.40
Mean	106.27	109.05	104.27	89.33	72.44	-

L.S.D Values for mean effects	**Probability at 0.05P**
Treatments	6.38
Salt Concentration	5.82
Interaction	14.27

Table 3: Alleviation of Salt stress employing priming with different concentrations of sodium chloride solutions on mean shoot length of Chickpea

Treatments	Sodium chloride concentrations (mM)					Mean
	0.00	25	50	75	100	
Non-primed (control)	42	52	53	44	34	45.13
Primed (Water soaking)	50	54	59	46	32	48.33
Sodium chloride concentrations (mM) 25	60	72	64	52	39	57.20
50	52	61	55	44	35	49.33
75	43	56	43	30	28	39.93
100	34	42	36	22	16	30.00
Mean	47.06	56.17	51.44	39.72	30.56	-

L.S.D Values for Mean effects	**Probability at 0.05P**
Treatments	3.501
Salt Concentrations	3.190
Interaction	7.827

Table 4: Alleviation of salt stress employing priming with different concentrations of sodium chloride solutions on electrical conductivity, sugar and dehydrogenase activity in Chickpea

Treatments	Electrical conductivity (μscm^2)	Leaching of Sugar (O.D at 580nm)	Dehydrogenase activity (O.D at 470nm)
Non-primed (Control)	199.9	0.137	0.827
Primed (Water Soaking)	128.0	0.058	0.847
Sodium chloride concentrations (mM) 25	94.0	0.042	0.917
50	197.2	0.102	0.795
75	205.0	0.126	0.726
100	234.9	0.247	0.707
L.S.D (0.05%)	0.965	0.001	0.003