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INDUCTION OF SALT TOLERANCE IN CHICKPEA BY USING SIMPLE AND SAFE CHEMICALS

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Abstract

Salinity is a common problem in Pakistan, which causes poor development of crops. Studies were conducted to make possible use of waste saline land by treating the seeds with some simple chemicals. Experiments were conducted under controlled and field conditions. Chickpea seeds of variety CM88 were surface sterilized and soaked in water, mannitol (2% & 4%), K_2HPO_4 (0.5% & 0.8%) and KNO_3 (0.5%, 1.0%, 1.5% and 2.0%) for 24 hours. Treated seeds were sown in pots, where salinity was created with 75ml solution of NaCl, along with three different types of control experiments; non-treated seed in saline soil, non-treated seeds in non saline soil and water treated seed in non-saline soil. It was observed that root length and biomass of roots and shoots were better when treated with water and mannitol. As far as K_2HPO_4 and KNO_3 are concerned, lower concentrations of these chemicals (0.5% K_2HPO_4 and KNO_3) gave comparatively better results than higher concentrations. Chickpea seeds of variety CM98 treated with same chemicals were sown under field conditions, where EC of the soil was 2.99-9.4 at 0-15cm depth, 2.8-10.5 at 15-30cm depth and pH was 7.7-9.5. Priming with mannitol and water improved plant survival and growth up to maturity under saline stress conditions.

Introduction

Induced systemic resistance (ISR) is a phenomenon, which enhances plants natural defenses against any biotic or abiotic stress. There are many abiotic stresses including drought and salinity, which affect the growth and productivity of the crops. ISR can be achieved by priming, conditioning or sensitization of plants (Sticher *et al*., 1997). Priming is one of the mechanisms which induced systemic resistance in plants (Conrath *et al*., 2001).

Priming of chickpea seeds with manitol and water improved seedling growth under salt stressed conditions (Kaur *et al*., 2003). Seed treatment with water and mannitol is also useful under water deficit stress and primed chickpea seeds gave high yield as compared to non-primed seeds (Kaur *et al*., 2002 a). Musa *et al*., (1999) reported that over night priming of chickpea seeds gave better crop production in Bangladesh. Priming of seeds with water promoted seedling vigor, yield and crop establishment of chickpea, maize and rice in India (Harris *et.al.*, 1999). Priming of tomato (*Lycopersicon lycopersicum)* seeds with NaCl had been reported to improve seedling growth.

Salinity is a common problem in Pakistan, which causes poor crop development. It was hypothesized to overcome this problem; preliminary treatment of seeds (priming) could give some good results. In this study, effect of salinity is checked on the growth of chickpea by using 'priming' which induces salt tolerance. The objective of this work was to induce salt tolerance in chickpea by using simple and safe chemicals, to use waste saline land, increase crop productivity, quality and profitability.

Materials and Methods

Pot experiment: Experiment was conducted in small plastic pots under controlled condition (temp. 22 ± 2^0C, humidity 70-80%, 12 hours photoperiod with 24000-26000 Lux fluorescent + incandescent light) in growth room. Chickpea seeds of variety CM-88 were washed with water, surface sterilized with Sodium hypochlorite and then soaked in water, mannitol (2 and 4%) K_2HPO_4 (0.5 and 0.8%) and KNO_3 (0.5, 1.0, 1.5 and 2.0%) for 24 hours. Treated and non-treated (control) seeds were sown in small pots in triplicate, salt stress was created by adding 75 mM NaCl with irrigation. Three different types of control experiments were run along with this experiment; non-treated seed in saline soil, non treated seed in non saline (normal) soil and water treated seeds in non saline soil. The length and biomass of roots and shoots of each seedling were recorded at 30 days after sowing. Germination was also observed.

Field experiment: Effect of priming with same chemicals as used in pot experiment, was studied under field conditions at Pacca Anna Biosaline Research Station, Nuclear Institute for Agriculture and Biology, Faisalabad, Pakistan. Chickpea seeds of variety CM-98 were soaked in water, mannitol (2 and 4%), K_2HPO_4 (0.5 and 0.8%) and KNO_3 (0.5, 1.0, 1.5 and 2.0%) for 24 hours before sowing. Unsoaked seeds served as control. Salinity level and pH of the soil in the field was measured at different depths and locations of the field. Germination and survival up to maturity were recorded. At maturity seven plants were randomly selected from each treatment and number of pods and dry weight of the plants were measured.

Results and Discussion

Pot experiment: Early emergence was observed in water, mannitol and in seeds treated with lower concentration of K_2HPO_4 and KNO_3. The effect of different treatments on root length was non-significant. Shoot length was the highest in seeds treated with KNO_3, which was equal to the shoot length in seeds treated with water and sown in non-saline soil (normal soil). Shoot length was higher in seeds treated with water, mannitol and lower concentration of K_2HPO_4 and KNO_3 as compared to seedlings grown from non-treated seed sown in saline soil (Table 1). Osmo-conditioning of cucmber (*Cucmis sativus*) seed with mannitol had also been reported to alleviate the adverse effects of salt stress on germination and growth of seedlings (Passam & Kakouriotis, 1994).

The biomass of roots and shoots of seedlings grown from treated with water, mannitol and lower concentration of K_2HPO_4 and KNO_3 were higher as compared to the biomass of seedlings grown from non-treated seeds sown in saline soil (Table 2). These results are in agreement with earlier studies; in tomato seeds, priming with KNO_3 has been reported to increase seedling growth under water and salt stressed conditions (Kang *et al*., 1996), priming of chickpea seeds with mannitol and water improved seedling growth under salt stressed conditions (Kaur *et al*., 2003).

Field studies: Studies on induction of salt tolerance in chickpea by using simple and safe chemicals were conducted at Pacca Anna Biosaline Research Station. Salinity level and pH of the soil in the field was measured at different depths and locations of the field.

Table 1. Average length of roots and shoots of chickpea seedlings of variety CM-88 grown from primed seeds with different chemicals.

S. No.	Treatment	Length of root (Cm.) ± SD		Length of shoot (Cm.) ± SD	
1.	Water	18	2.0	9	3.0
2.	2% Mannitol	17	4.5	13	0.6
3.	4% Mannitol	19	2.0	14	3.0
4.	0.5% K_2HPO_4	14	3.5	13	0.6
5.	0.8% K_2HPO_4	11	3.5	7	6.5
6.	0.5% KNO_3	14	1.7	14	1.0
7.	1.0% KNO_3	21	1.7	17	0.6
8.	1.5% KNO_3	17	4.3	10	2.5
9.	2.0% KNO_3	16	0.6	11	1.0
10.	Control (N.P & S)	12	3.1	10	2.3
11.	Control (N.P & N.S)	15	1.5	14	0.6
12.	Control (P & N.S)	16	2.0	16	2.0

Data represent mean ± SD of 3 replications with 15 seedlings in each replication.
N.P is non-primed seedlings, P is primed seedlings, N.S is non-saline soil and S is saline soil.

Table 2. Average Biomass (g) of roots and shoots of chickpea seedlings of variety CM88 grown from primed seeds with different chemicals.

S. No.	Treatment	Fresh weight (g)	±SD	Dry weight (g)	±SD	Fresh weight (g)	±SD	Dry weight (g)	±SD
1.	Water	1.20	0.1	0.50	0.02	2.14	0.07	0.52	0.00
2.	2% Manitol	1.53	0.3	0.55	0.13	2.59	1.41	0.59	0.31
3.	4% Manitol	1.22	0.7	0.51	0.28	2.34	1.19	0.60	0.32
4.	0.5% K_2HPO_4	0.90	0.3	0.44	0.13	2.25	0.45	0.60	0.08
5.	0.8% K_2HPO_4	0.20	0.2	0.08	0.04	0.29	0.04	0.05	0.00
6.	0.5% KNO_3	1.20	0.1	0.55	0.00	3.02	0.14	0.75	0.03
7.	1.0% KNO_3	1.87	1.1	0.98	0.47	2.67	1.02	0.70	0.21
8.	1.5% KNO_3	0.31	0.2	0.18	0.09	0.58	0.03	0.19	0.12
9.	2.0% KNO_3	1.37	0.6	0.37	0.06	1.08	0.07	0.27	0.02
10.	Control (N.P & S)	0.42	0.3	0.29	0.20	0.79	0.61	0.20	0.16
11.	Control (N.P&N.S)	2.97	0.1	1.23	0.08	5.28	0.74	0.82	0.21
12.	Control (P & N.S)	1.62	0.9	0.55	0.29	3.29	1.28	0.67	0.16

Data represent mean ± SD of 3 replications with 15 seedlings in each replication.
N.P is non-primed seedlings, P is primed seedlings, N.S is non-saline soil and S is saline soil.

Salinity levels and pH were recorded as follows:

0-15 cm depth: 2.97 – 9.4 EC; pH = 7.9 – 9.5
15-30 cm depth: 2.8 – 10.5 EC; pH = 7.7 – 9.5

Table 3. Growth parameters of chickpea crop grown from primed seeds (variety CM-98), with different chemicals, under field conditions at Pacca Anna Biosaline Research Station.

S. No.	Treatment	Germination (%)	Survival upto maturity (%)	No. of pods/ 7 plants	Dry weight (g)/ 7 plants
1.	Water	90	30	12	56
2.	2% Mannitol	92	70	80	71
3.	4% Mannitol	85	40	34	44
4.	0.5% K_2HPO_4	70	5	8	32
5.	0.8% K_2HPO_4	50	3	16	46
6.	0.5% KNO_3	40	2	24	37
7.	1.0% KNO_3	20	0	0	10
8.	1.5% KNO_3	5	0	0	0
9.	2.0% KNO_3	3	0	0	0
10.	Control (N.P)	95	4	0	0

N.P is non-primed.

Table 3 shows the growth parameters of field studies at Pacca Anna Biosaline Research Station with chickpea seeds of variety CM-98. In general, treatment of seeds with water and mannitol showed early emergence of seedlings. Mannitol 2% was found the best among the treatments, where 92% germination and 70% plants survived upto maturity. Water and 4% mannitol showed 90 and 85% germination whereas 30 and 40% survival was recorded, respectively, while other treatments showed 3-70% germination and survival up to maturity was very poor (0 - 5%). Upto 80 pods and 71 g dry weight of 7 plants from 2% mannitol treated plants were recorded.

Thus it is concluded that the treatment of seeds with water, 2 and 4% mannitol increased the length and biomass of roots and shoots of chickpea seedlings as compared to non-primed controls under salt stressed conditions. Priming treatment with 0.5% K_2HPO_4 and 0.5% KNO_3 gave better results as compared to higher concentrations of these chemicals. The use of 0.8% K_2HPO_4 and 1.0, 1.5 and 2.0% KNO_3 is not effective and there is no need of using higher concentrations of these chemicals, since better results are obtained by using lower concentrations. Higher concentrations of these chemicals did not show any beneficial effect on seedling growth. These results are similar with earlier studies where seeds priming overnight with water promoted seedling vigor, yield and crop establishment of chickpea, maize and rice in India (Harris *et al*., 1999). Similar results have also been reported with chickpea crop raised from overnight water primed seeds in Bangladesh (Musa *et al*., 1999). Priming may increase the activities of enzymes involved in carbohydrate metabolisms as reported by Kaur *et al*., (2002 b) the activities of enzymes, like amylase, invertase (acid and alkaline), sucrose synthase and sucrose phosphate synthase in shoots, roots and in cotyledons increased in primed stressed seedling as compared to non- primed stressed seedling, which help in germination and crop establishment. Thus by using this simple and cheap method, chickpea growers may produce better yield of the crop in saline soil.

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