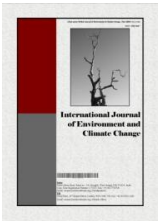

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Effect of Seed Hydropriming on Seedling Emergence and Growth of Chickpea (*Cicer arietinum* L.) under Adverse Climatic Condition of Drought and Salinity

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Aim: Assessment of influence of seed hydropriming in seed and seedling vigour enhancement for stress tolerance in chickpea which is important for successful crop production under erratic climate change causing drought and salinity stress severely affecting the seedling emergence and establishment especially in arid and semi arid regions of the world.
Study Design: A three factorial randomised block design was used in the study, involving seed hydropriming treatment, chickpea varieties and growth conditions.
Place and Duration of the Study: The experiment was conducted in Department of Seed Science and Technology, University of Agricultural Sciences, Dharwad, India, during the year 2018.
Methodology: Six months old chickpea seeds of variety JG-11 and Annigeri-1 were hydroprimed for 12 hours at 25°C in dark condition. The primed and unprimed control seeds are sown under

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normal, drought and salinity conditions imposed in pots under controlled conditions. The data recorded were analysed for Analysis of Variance (ANNOVA).

Results: The result of the experiment revealed that, upon seed hydropriming an average of 19 per cent increase in seedling emergence and 53.3 per cent increase in seedling vigour index was observed as compared to unprimed control seeds irrespective of variety and the abiotic stress conditions.

Keywords: Chickpea; hydropriming; seed vigour; abiotic stress; climate change.

1. INTRODUCTION

Chickpea is an important self-pollinated legume crop, cultivated across North Africa, West Asia and Indian subcontinent which are under severe consequence of climate change. Chickpea is also suitable for sustainable agriculture as it enriches the soil through biological nitrogen fixation [1]. In India chickpea is cultivated in winter season under irrigated as well as rainfed conditions in the states like Karnataka, Maharashtra, Andhra Pradesh, Madhya Pradesh, Rajasthan, Uttar Pradesh, Bihar and Haryana contributing about 95 per cent of total chickpea production of the country. An optimum temperature of 28-33°C with adequate soil moisture is required for field emergence of chickpea. In practise, generally chickpea sowing is taken in residual moisture after the *Kharif* crop is harvested *i.e.,* after first fortnight of October which coincides with the unfavourable environmental conditions and uneven distribution of rainfall for germination, seedling emergence and crop stand.

The abiotic stress drought is responsible for both inhibition and delayed seed germination resulting in poor seedling establishment in many areas. Similarly, salinity is also one of the major abiotic stresses especially in arid and semiarid regions, which severely impairs germination due to toxic effect of ions on embryo viability and decrease in water potential gradient between the seeds and external environment [2]. Germination may be seen under saline conditions after high precipitation where soil salinity is usually reduced due to leaching [3]. Drought and salinity stress also reduces nodule formation leading to reduced crop yield by altering the physiology of crop due to osmotic potential which prevents water up take. Therefore, seed germination and early seedling development are the vulnerable stages for the establishment of plant population [4] contributing for the final crop yield in any abiotic stress conditions.

One way to mitigate this problem is by developing seed technologies aimed at invigorating low-vigour seeds; for example, seed hydro-priming. Seed priming is a pre sowing seed treatment to hydrate partially the seed to a point where germination process is initiated but not completed. Most priming treatments involve imbibition of seed with restricted amount of water for sufficient hydration and advancement of metabolic processes, but preventing the actual protrusion of the radicle. Once after priming, seeds exhibits faster germination rate with uniform emergence, higher tolerance to prevailing environmental stresses and breakdown of dormancy in many species. The beneficial impacts of priming are ascribed with the antioxidant defence system activation, induction of the physiological and biochemical mechanisms of cell repair and initiation of enzymes responsible for catalysing the decomposition and reserve food mobilization [5]. Hence, the assessment of the extent of seed hydropriming and the varietal response in combating the abiotic stresses such as drought and salinity is undertaken based on physiological and biochemical parameters in the present experiment.

2. MATERIALS AND METHODS

2.1 Priming

Six months old chickpea seeds of variety JG-11 and Annigeri-1 were used for the priming. Seeds are first surface sterilized by soaking them for three minutes in sodium hypochlorite (1%) solution and then rinsed twice in distilled water to wash the sterilizing solution completely. Subsequently, the seeds were soaked in water for 12 hours at 25°C in dark condition. The seed weight to solution volume ratio during experiment was kept at 1:3 (g/ml) ratios. After priming the seeds were removed and rinsed with distilled water. Finally, the seeds are uniformly spread on blotting paper and were kept for air drying under shade to re-dry to their original moisture level as a method proposed by Afzal et al. [6].

2.2 Pot Experiment

The pots were internally covered with black plastics to maintain constant environmental temperature. The bottom of each pot was perforated to keep the soil well-drained and was filled with sterilized 2:6:2 sand to local soil (the growth medium) and organic manure, respectively [7]. Each treatment consisted of three replications and in each replication 10 seeds were sown. The pots are imposed with drought stress by withholding the water from 3rd day after sowing and soil moisture was monitored using theta probe to keep soil moisture below 35 percent to impose drought stress. Salinity stress was induced by saturating the pots to field capacity level with 150 mM salt solution. Control pots were irrigated regularly with normal water to maintain soil moisture to field capacity. The observation for seedling characters and sampling for proline and catalyse estimation was done on 8th day from sowing.

2.3 Seedling Emergence (%)

The seedling emergence was computed for 10 seeds sown per pot at each replication. The numbers of seeds germinated and emerged out of soil resembling the normal/ healthy seedling characters in each replication were counted on 8th day and the mean emergence was calculated and expressed in percentage.

Seedling emergence = (Number of normal seedling emerged/Number of seeds sown) × 100

2.4 Shoot Length (cm) and Root Length (cm)

From the emergence test, ten normal seedlings were randomly selected from each treatment on 8th day and the shoot length was measured from the tip of shoot to the hypocotyls point and root length was measured from tip of root to the hypocotyl point. The mean length was calculated and expressed in centimetres.

2.5 Seedling Dry Weight (mg)

The ten normal seedlings used for measuring root and shoot length were taken in butter paper and dried in a hot-air oven maintained at 70°C temperature for 24 h. Then, the seedlings were removed and allowed to cool in a desiccator for 20 minutes before weighing on an electronic balance. The average weight was calculated and expressed in milligram.

2.6 Proline Content

Total proline content in leaves was estimated according to Bates et al. [8] with slight modifications. Leaf sample (100 mg) was crushed in liquid nitrogen and homogenized in 2 ml of 3% aqueous sulphosalicylic acid. The homogenate was then centrifuged at 12000 rpm for 15 min and supernatant was collected. Two ml supernatant was mixed with 2 ml acid ninhydrin reagent and 2 ml of glacial acetic acid and incubated at 100ºC for 1hr. The reaction was stopped by keeping tubes in ice for 5 min. Four ml of toluene was added to the mixture and upper chromophore layer was taken for proline estimation. The absorbance was recorded at 570 nm. Proline concentration was determined using a calibration curve and expressed as µmol proline /protein gm.

3. RESULTS AND DISCUSSION

Seed priming has long been known to enable seeds to overcome biotic and abiotic stresses [9]. Among several prehydration treatments, seed hydropriming is currently employed to increase the speed and synchrony of seed germination which is crucial factor for seedling establishment under adverse climatic conditions. Seed priming aids in faster germination and uniform field emergence, which have practical agronomic implications, notably under unfavourable and unstable environmental conditions [10]. In accordance with the principle, our experimental results of seed hydropriming (Tables 1 and 2) significantly increased the average seedling emergence (83.8%), root and shoot length (11.7 and 8.9 cm, respectively), seedling dry weight (13.7 mg/seedling), seedling vigour index (1155) and Proline accumulation (1.17 µ moles/g fresh weight) as compared to unprimed control seeds (70.4%, 9.4 and 8.3 cm, 10.4 mg/seedling, 753 and 1.10 µ moles/g fresh weight, respectively). During priming sufficient imbibition of water takes place during phase I of the germination process to activate biochemical process inside seed (Phase II) and then dried back to prevent radicle protrusion (phase III). Once the primed seeds are sown and germinative conditions are appropriate, resumption of phase III leads to germination in much shorter time. Few processes ascribed to play a role during seed priming include cell cycle-related events [11], endosperm weakening by hydrolase activities [12] and mobilization of

Table 1. Influence of hydropriming on seed germination, root length and shoot length under differential stress condition in chickpea varieties JG-11 and Annigeri - 1

	Seedling emergence (%)					Root length (cm)					Shoot length (cm)			
	V_1		V_2			V_1		V_2			V_1		V_2	
	Control	HP	Control	HP		Control	HP	Control	HP		Control	HP	Control	HP
S_1:Normal	86.3	95.0	85.0	87.5	Normal	12.3	16.0	12.0	14.8	Normal	10.5	10.4	9.8	10.0
S_2:Drought	85.0	92.5	82.5	86.3	Drought	8.6	10.9	9.1	9.8	Drought	8.3	9.0	8.1	8.3
S_3:Salinity	47.5	76.3	36.3	65.0	Salinity	7.4	9.5	7.3	9.4	Salinity	6.5	8.1	6.5	7.9
Variables	**Means**	**Factors**	**S.Em±**	**C.D. at 1%**	**Variables**	**Means**	**Factors**	**S.Em±**	**C.D. at 1%**	**Variables**	**Means**	**Factors**	**S.Em±**	**C.D. at 1%**
JG 11	80.4	Variety	0.9	2.4	JG 11	10.8	Variety	0.1	0.3	JG 11	8.8	Variety	0.1	0.3
A1	73.8	Treatment	0.9	2.4	A1	10.4	Treatment	0.1	0.3	A1	8.4	Treatment	0.1	0.3
Control	70.4	V × T	1.2	NS	Control	9.4	V × T	0.2	0.4	Control	8.3	V × T	0.1	NS
Priming	83.8	Stress	1.0	2.9	Priming	11.7	Stress	0.1	0.4	Priming	8.9	Stress	0.1	0.3
Normal	88.4	V × S	1.5	4.2	Normal	13.8	V × S	0.2	NS	Normal	10.2	V × S	0.2	NS
drought	86.6	T × S	1.5	4.2	drought	9.6	T × S	0.2	0.5	drought	8.4	T × S	0.2	0.5
salinity	56.3	V × T × S	2.1	NS	salinity	8.4	V × T × S	0.3	NS	salinity	7.3	V × T × S	0.2	NS

Legend – V_1: JG-11; V_2: Annigeri-1; NS: Non-significant; HP: Hydro-priming S: Stress; T: seed hydropriming treatments, S.Em: Seedling Emergence

Table 2. Influence of hydropriming on seedling dry weight, seedling vigour index and proline content under differential stress condition in chickpea varieties JG - 11 and Annigeri - 1

	Seedling dry weight (mg/seedling)					Seedling vigour index					Proline (µ moles / g fresh weight)			
	V_1		V_2			V_1		V_2			V_1		V_2	
	Control	HP	Control	HP		Control	HP	Control	HP		Control	HP	Control	HP
Normal	12.6	15.7	11.8	15.8	Normal	1087	1488	996	1383	Normal	0.19	0.46	0.18	0.45
Drought	10.4	12.9	10.0	13.0	Drought	881	1190	806	1124	Drought	1.57	1.37	1.56	1.36
Salinity	9.9	12.3	7.5	12.4	Salinity	468	938	282	810	Salinity	1.55	1.69	1.54	1.68
Variables	**Means**	**Factors**	**S.Em±**	**C.D. at 1%**	**Variables**	**Means**	**Factors**	**S.Em±**	**C.D. at 1%**	**Variables**	**Means**	**Factors**	**S.Em±**	**C.D. at 1%**
JG 11	12.3	Variety	0.1	0.4	JG 11	1008	Variety	15	41	JG 11	1.14	Variety	0.001	0.002
A1	11.7	Treatment	0.1	0.4	A1	900	Treatment	15	41	A1	1.13	Treatment	0.001	0.002
Control	10.4	V × T	0.2	0.5	Control	753	V × T	21	NS	Control	1.10	V × T	0.001	0.003
Priming	13.7	Stress	0.2	0.4	Priming	1155	Stress	18	50	Priming	1.17	Stress	0.001	0.003
Normal	14.0	V × S	0.2	NS	Normal	1238	V × S	25	NS	Normal	0.32	V × S	0.001	NS
Drought	11.6	T × S	0.2	NS	Drought	1000	T × S	25	71	Drought	1.46	T × S	0.001	0.004
Salinity	10.5	V × T × S	0.3	NS	Salinity	624	V × T × S	36	NS	Salinity	1.62	V × T × S	0.002	NS

Legend – V_1: JG-11; V_2: Annigeri-1; NS: Non-significant; HP: Hydro-priming S: Stress; T: seed hydropriming treatments, S.Em: Seedling Emergence

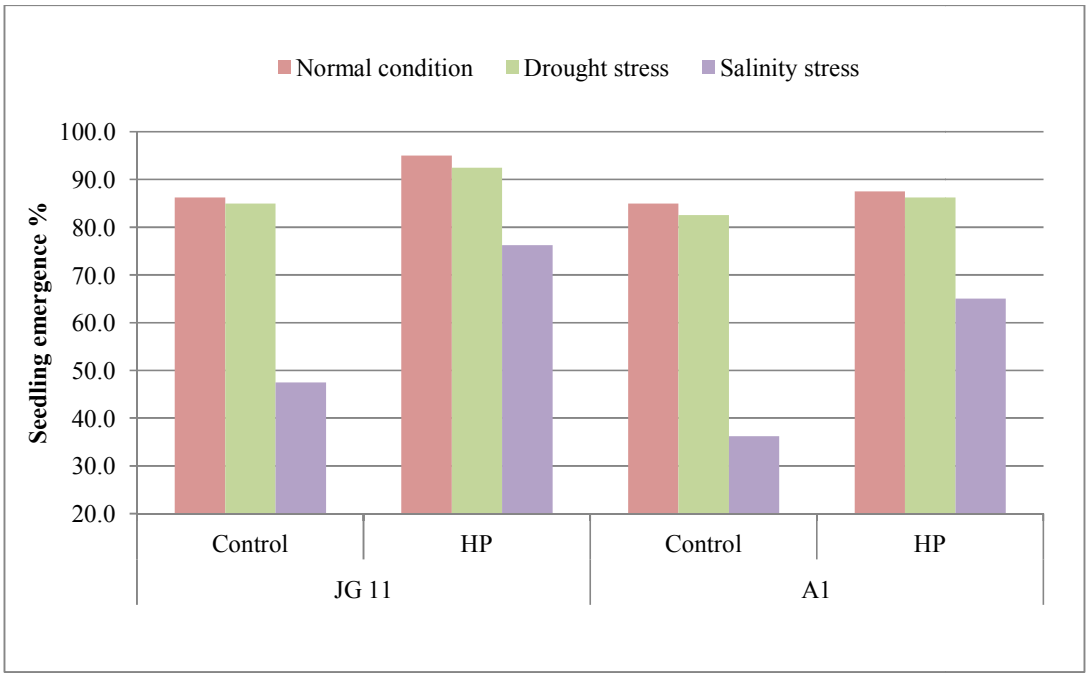

Fig. 1. Influence of seed hydro priming on seed emergence of chickpea in normal, drought and salinity stress

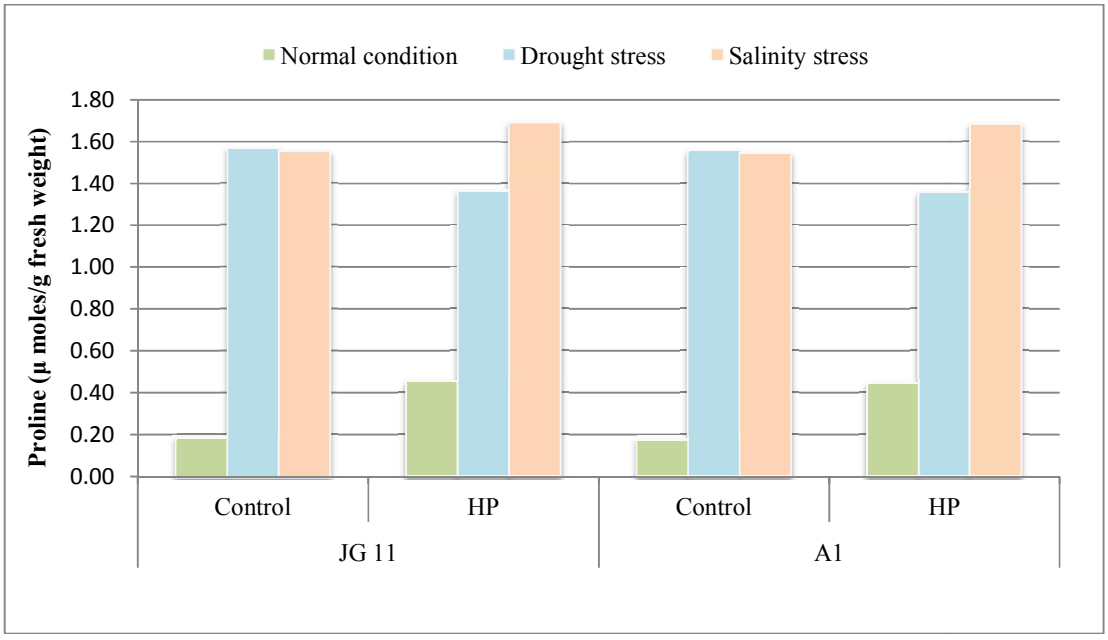

Fig. 2. Influence of seed hydro priming on proline accumulation in chickpea seedlings grown in normal, drought and salinity stress

storage proteins [13]. This helps the germinating seeds in complete hydrolysis of reserve food material for energy source and is made available to developing tissues apart from efficient water uptake mechanism for vigorous seedling emergence and establishment.

In between the varieties under study (Tables 1 and 2), JG-11 was significantly superior in its seedling emergence (80.4%), root and shoot length (10.8 & 8.8 cm, respectively), seedling dry weight (12.3 mg/seedling), seedling vigour index (1008) and proline content (1.14 moles/g fresh

weight) as compared to the variety Annigeri-1 (73.8%, 10.4 and 8.4 cm, 11.7 mg/seedling, 900 and 1.13 moles/g fresh weight, respectively). This variation may be due to the inherent genotypic difference for the tolerance to abiotic stresses and the amount of stored food reserve mobilized as reflected in test weight which ultimately contributed to higher growth of seedlings under stress conditions. Similar variations in crop performance among the varieties of chickpea was reported by Khoiwal et al. [14] and Tiwari et al. [15] under different moisture stress and Judith et al. [16], Diriba et al. [17] and Dharamvir et al. [18] under salinity conditions.

In the present experiment involving the abiotic stress, significantly higher seedling emergence (Fig. 1) was recorded in normal growth condition and drought (88.4 and 86.6%, respectively) as compared to salinity stress (56.3%). Salinity severely impairs germination due to toxic effect of ions on embryo viability and decrease in water potential gradient between the seeds and external environment [2]. The other physiological characters such as root and shoot length, seedling dry weight and seedling vigour index was also significantly lower in salinity stress (8.4 and 7.3 cm, 10.5 mg/seedling and 624, respectively) as compared to normal (13.8 and 10.2 cm, 14 mg/seedling and 1238, respectively) and drought (9.6 and 8.4 cm, 11.6 mg/seedling and 1000, respectively).

Proline accumulation was significantly higher in salinity stress condition (1.62 µ moles / g fresh weight) as compared to normal and drought stress (0.32 and 1.46 µ moles / g fresh weight, respectively) irrespective of the hydropriming treatment and varieties under the study (Fig. 2). Plants when exposed to frequent disturbance in climate and stressful conditions, they accumulate an array of metabolites, particularly amino acids. A large body of data suggests a positive correlation between proline accumulation and plant stress. In the present study, hydropriming enhanced proline synthesis in the seedlings subjected to normal, drought and salinity stress conditions as compared to unprimed control. Accumulation of proline is an adaptive response of plants to various abiotic stresses [19]. Proline could interfere with hydrophobic/hydrophilic amino acid side chain bonds and induce conformational changes in the enzyme protein and thus protects its cellular activity [20]. Tolerance to stress is imparted by maintaining cell turgor pressure or osmotic balance, stabilizing membranes, thereby preventing electrolyte leakage and bringing concentrations of reactive oxygen species (ROS) within normal ranges, thus preventing oxidative burst in plants.

Pre-soaking of seeds and drying back to original seed moisture content before sowing in soybean also permits early DNA replication, increased RNA production and protein synthesis, increased enzyme activity and greater ATP availability [21], faster embryo growth and efficient repair of deteriorated seed parts. All these activities might have initiated quicker radicle protrusion through seed coat and have accelerated the process of germination and other parameters by shortening the germination time [22] which in turn results in efficient utilization of available moisture and nutrients for quick growth and development and establishment into vigorous seedlings. The metabolic processes which are induced in seed by priming persist even after drying [23]. Thus, upon sowing, dried primed seed as soon as imbibes water and resumes the seed metabolism, resulting into faster and synchronous emergence. Kaur et al. [24] observed three to four folds more growth with respect to root and shoot length in chickpea of seven days old seedlings obtained from seeds primed with mannitol (4%) and water in comparison with seedlings obtained from non-primed seeds. Similar results were also reported by researchers like Jamadar and Deshpande [25] in pigeon pea.

4. CONCLUSION

Quality and vigorous seed use is important for successful farm practices where abiotic stress such as drought and salinity due to frequent climate change severely effects the chickpea seed germination and seedling establishment especially in arid and semi arid regions. Drought stress is responsible for both inhibition and delayed seed germination resulting in poor seedling establishment in many areas. Similarly, salinity stress impairs germination due to toxic effect of ions on embryo viability and decrease in water potential gradient between the seeds and external environment. To counter this obstacle, a simple technique of seed hydro priming for 12 hours helps in enhancing the vigour of seeds leading to vigorous seedling establishment even under unfavourable environmental conditions like drought and salinity stress during chickpea production.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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