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Research Article

IMPROVING FIELD PERFORMANCE OF AGED CHICKPEA SEEDS BY HYDRO-PRIMING UNDER WATER STRESS

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ABSTRACT: A sub-sample of chickpea (*Cicer arietinum* L. cv.ILC$_{482}$) seeds was kept as control (non-primed, P_1) and two other sub-samples were aged at 40°C for 10 and 12 days. Consequently, three seed lots with different levels of vigor were provided. These seed lots were soaked in distilled water at 15°C for 12 (P_2) and 18 (P_3) hours and then dried back to about 20% moisture content at a room temperature of 20-22°C. The lowest electrolyte leakage was recorded for high vigor and primed seed lots. Increasing seed vigor due to less deterioration and hydro-priming also resulted in increasing germination and seedling emergence rate and percentage and seedling dry weight. The highest improvement in germination and seedling vigor and emergence due to hydro-priming were observed in poor vigor seed lot. Early emergence and high density of plants from vigorous seeds increased competition of individual plants for water under limited irrigation conditions, reducing the superiority of these plants in yield and yield components under low watering. However, improvement of grains per plant and grain yield per plant due to hydro-priming was more evident under low water availability. Hydro-priming also enhanced grains per plant, grain yield per plant and per unit area more in plants from low vigor than high vigor seeds. This result clearly indicated that hydro-priming repaired deteriorated seeds and enhanced their performance in the field.

Keywords: Chickpea, Germination, Hydro-priming, Seedling emergence, Seed vigor

INTRODUCTION

Maximum seed vigor on the mother plant is achieved at the end of seed filling period [25, 42] or slightly after this phase [11, 9, 38, 21]. Seeds can then retain their high vigor for some time and thereafter begin to deteriorate on the mother plant or during storage, loosing germination capacity, vigor and viability [11, 31, 20]. Several biochemical and physiological changes have been observed in seeds during aging, resulting in a progressive decline in seed quality and performance [31]. Membrane disruption is one of the main reasons of seed deterioration. The major causes of membrane disruption are increase in free fatty acid level and free radicals productivity by lipid peroxidation [23]. Once these free radicals are initiated, they create profound damage to lipid bilayer especially of mitochondria membrane leading to reduce energy production [7] and enzymes, protein, DNA and ultimately cellular repair mechanism [44]. Free radicals attack membrane lipids, and cause major disruption of their viscosity and their permeability [43]. When deterioration is advanced, rate and uniformity of seed germination and seedling emergence and tolerance to environmental stresses decreases [36]. The slower rate of emergence frequently associated with low-vigor seeds resulting in smaller plants, compared with high- vigor seeds [12]. Different techniques could be used to enhance crop yields, particularly under unfavorable environmental conditions. One of the simple techniques which can improve seedling vigor and establishment and consequently crop performance in the field is seed priming [32, 15, 24]. Priming appears to reverse the detrimental effects of seed deterioration [32]. Reversal by priming of deteriorated seed generally occurs in the meristematic axis or the radicle tip [16]. Priming is also thought to increase free-radical scavenging enzymes activity and counteract the effects of lipid peroxidation and reduce leakage of metabolites [32]. The early improvements may increase the rate and uniformity of seed germination and seedling emergence [2, 13, 19], especially under stressful conditions.

Drought stress is undoubtedly one of the most important environmental problems limiting the productivity of crop plants around the world [6]. Chickpea is a major crop which is mainly grown in the arid and semi-arid zones and about 90% of world's chickpea is grown under rain fed conditions [30].Although, chickpea is an important drought tolerant food legume, but water deficit can limit growth and productivity of this crop [40]. Some of the deleterious effects of low-vigor seed lots and environmental stresses such as water limitation on crop performance may be also overcome by seed priming [5, 10, 22], via improving seedling vigor [17] and stand establishment [15].The beneficial effects of priming have been demonstrated for many field crops such as barley [1], maize [33], lentil [18], chickpea [22], sugar beet [37] and sunflower [39]. However, responses of deteriorated and non-deteriorated seed lots of chickpea to priming under limited and well irrigated conditions were not evaluated. Thus, the objective of this research is to investigate the effects of hydro-priming duration on seed invigoration and field performance of differentially deteriorated seed lots of chickpea under different irrigation treatments.

MATERIALS AND METHODS

Seeds of chickpea (*Cicer arietinum* L. cv.ILC_{482}) were obtained from Dry-land Agricultural Research Institute of Maragheh, Iran. These seeds were divided into three sub-samples. A sub-sample was kept as control with 100% germination (V_1). The other sub-samples with about 15% moisture content were artificially aged at 40°C for 10 and 12 days, reducing seed germination to 84 and 74% ($V_2$and V_3, respectively). Consequently, three seed lots with different levels of vigor were provided. Then, each seed lot were divided into three sub- samples, one of which was kept as control (non-primed, P_1) and two other samples were soaked in distilled water at 15°C for 12 (P_2) and 18 (P_3) hours and then dried back to about 20% moisture content at a room temperature of 20-22°C. Laboratory tests were carried out as factorial based on RCB design at the Seed Technology Laboratory of Tabriz University, Iran. Four replicates of 25 seeds were placed between moist filter papers and germinated in an incubator adjusted on 20°C for 14 days. At the end, percentage of normal seedlings and seedling dry weight were determined.

Unprimed seeds were thoroughly washed and then four replications of 50 pre-weighed seeds of each of primed and unprimed seed lots were soaked in 250 ml de-ionized water in plastic containers covered with caps to prevent evaporation loss and entry of foreign matter. A container of de-ionized water without seeds was prepared as the control. All the containers were then incubated at 20°C for 24 hours. Conductivity was measured with an electrical conductivity meter (WTW LF90). The results were expressed on initial seed weight basis (µS/cm/g).

The field experiment was conducted at the Research Farm of the University of Tabriz (Latitude 38˚05' N, Longitude 46˚17' E, Altitude 1360 m above sea level) in 2010. All the seeds were treated with benomyl at a rate of 2 g kg^{-1}before sowing. Seeds were hand sown in about 4 cm depth with a density of 60 seeds m^{-2}.Each plot consisted of 6 rows with 4 m length, spaced 25 cm apart. The experiment was arranged as split plot factorial, based on RCB design with three replications. All plots were irrigated immediately after sowing and subsequent irrigations were carried out after 70 (I_1), 120 (I_2) and 170 (I_3) mm evaporation from class A pan. Weeds were controlled by hand during crop growth and development. Plants were protected from heliothis caterpillar attack by spraying Diazinon at a rate of 2 ml l^{-1}before flowering.

Seedling emergence was recorded in daily intervals up to final establishment in each plot. Subsequently, seedling emergence rate and percentage were calculated. Finally, plants of 1 m^2in the middle part of each plot were harvested and grains per plant and per unit area,1000 grain weight and grain yield per plant and per unit area were recorded. Analysis of variance of the data appropriate to the experimental design and comparison of means at $p \leq 0.05$ were carried out, using MSTATC software.

RESULTS

The analysis of variance of the laboratory data showed significant effects of seed vigor and hydro-priming duration on electrical conductivity of seed leachates, germination rate, germination percentage and seedling dry weight ($p \leq 0.01$).The interaction of vigor× hydro- priming duration was also significant for these traits.

The lowest electrolyte leakage was achieved with high vigor seed lot and it was increased with decreasing seed lot vigor. Seeds of V_2 and V_3 germinated later than those of V_1. Decreasing seed vigor also resulted in reductions of germination percentage and seedling dry weight (Table 1). The electrical conductivity of non-primed seeds (P_1) was significantly higher than that of hydro-primed seeds (P_2 and P_3). However, hydro-priming duration had no significant effect on the electrical conductivity of seed leachates (Table 1). The lowest germination rate and the highest germination percentage and seedling dry weight were achieved with P_3 (hydro-priming for 18 h), which was not significantly different from P_2 (hydro-priming for 18 h).

Table1. Means of electrolyte leakage, germination rate, germination percentage and seedling dry weight of chickpea affected by seed vigor and hydro-priming duration

Treatments	Electrical conductivity (µs/cm/g)	Germination rate (per day)	Germination (%)	Seedling dry weight (g)
Vigor				
V_1	11.46 c	0.3491 a	100 a	0.9158 a
V_2	27.67 b	0.2753 b	84.67 b	0.8233 b
V_3	32.68 a	0.2622 b	74.33 c	0.7667 b
Hydro-priming				
P_1	33.48 a	0.2361 b	84 b	0.6367 b
P_2	19.77 b	0.3148 a	86.67 a	0.9250 a
P_3	18.56 b	0.3357 a	88.33 a	0.9442 a

Different letters at each column indicate significant difference at $p \leq 0.05$
V_1, $V_2$and V_3: Seed lots with 100, 85 and 74% viability, respectively
P_1,P_2 and P_3: non-primed, primed for 12 and 18 hours, respectively

Solute leakage of chickpea seeds with different levels of vigor reduced with hydro-priming, although this reduction in low-vigor seed lots (V_2 and V_3) was higher than that in high vigor seed lot (Table 2).The highest improvement in germination rate and percentage and seedling dry weight due to hydro-priming was also observed in poor vigor seed lot (V_3) (Table 2).

Table2. Means of electrolyte leakage, germination rate, germination percentage and seedling dry weight for different unprimed and primed seed lots

Traits	Treatment	P_1	P_2	P_3
Electrical conductivity (µs/cm/g)	V_1	16.95^{de}	10.08^{ef}	7.344^{f}
	V_2	37.70^{b}	23.46^{cd}	21.85^{cd}
	V_3	45.78^{a}	25.78^{c}	26.48^{c}
Germination rate (per day)	V_1	0.2894^{bc}	0.3751^{a}	0.3827^{a}
	V_2	0.2217^{de}	0.2568^{cd}	0.3081^{b}
	V_3	0.1971^{e}	0.3126^{b}	0.3162^{b}
Germination percentage (%)	V_1	100^{a}	100^{a}	100^{a}
	V_2	85^{b}	84^{b}	85^{b}
	V_3	67^{e}	76^{d}	80^{c}
Seedling dry weight (g)	V_1	0.8225^{b}	0.9500^{a}	0.9750^{a}
	V_2	0.6475^{c}	0.9125^{ab}	0.9100^{ab}
	V_3	0.4400^{d}	0.9125^{ab}	0.9475^{a}

Different letters at each column indicate significant difference at $p \leq 0.05$
V_1, $V_2$and V_3: Seed lots with 100, 85 and 74% viability, respectively
P_1,P_2 and P_3: non-primed, primed for 12 and 18 hours, respectively

Seedling emergence rate and percentage significantly ($P \leq 0.01$) affected by seed vigor and hydro-priming duration. The interaction of seed vigor × hydro-priming for these traits was also significant ($p \leq 0.01$). Although there was no significant difference between V_2 and V_3 in field emergence rate ($p > 0.05$), emergence rate decreased with decreasing seed vigor (Table3). The highest percentage of seedling emergence was obtained for the high vigor seed lot and it was significantly decreased with decreasing seed lot vigor (Table 3). Seedlings of P_2 and P_3 emerged earlier than those of P_1. However, emergence rate for P_2 and P_3 was statistically similar (Table3).Seedling emergence percentage for P_3 (hydro-priming for 18 h) was significantly higher than that for P_1 (non-primed seeds) and P_2 (hydro-priming for 12 h).

Table3. Means of emergence rate and percentage of chickpea seedlings affected by seed vigor and hydro-priming duration

Treatments	Seedling emergence rate (per day)	Seedling emergence (%)
Vigor		
V_1	0.07595 a	83.48 a
V_2	0.05555 b	26.86 b
V_3	0.05277 b	21.57 c
Hydro-priming		
P_1	0.04816 b	37.18 c
P_2	0.06617 a	45.63 b
P_3	0.06994 a	49.09 a

Different letters at each column indicate significant difference at $p \leq 0.05$

V_1, $V_2$and V_3: Seed lots with 100, 85 and 74% viability, respectively

P_1,P_2 and P_3: non-primed, primed for 12 and 18 hours, respectively

Field emergence rate of all seed lots enhanced as a result of hydro-priming. This advantage of hydro-priming on emergence rate of poor vigor seed lot (V_3) was considerably higher than thaton V_2 and V_1 (Figure 1- A). Hydro-priming also increased field emergence percentage of chickpea seedlings from different levels of vigor (Figure 1-B).

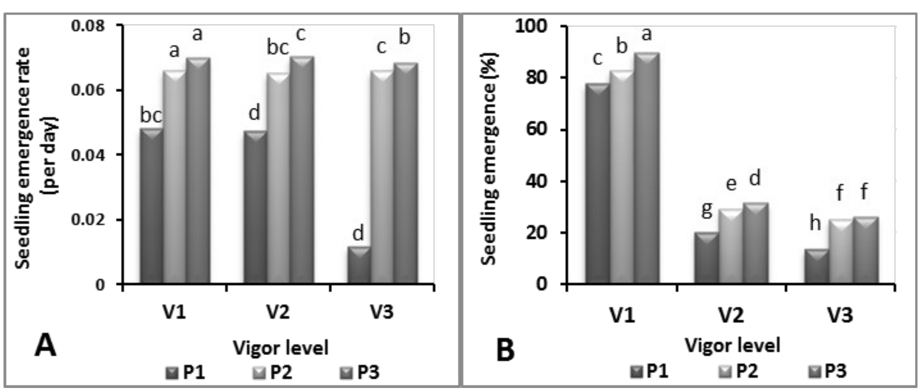

Figure1. Mean emergence rate and percentage of chickpea seedlings for unprimed and primed seed lots

The effects of irrigation on grains per plant, grains per unit area, 1000 grain weight, grain yield per plant and grain yield per unit area were significant ($p \leq 0.01$). Grains per plant, grains per unit area, 1000 grain weight, grain yield per plant and grain yield per unit area significantly decreased with decreasing water availability.

Seed vigor had significant effects on grains per plant, grains per unit area, 1000 grain weight and grain yield per unit area ($p \leq 0.01$), but not on grain yield per plant ($p>0.05$).Although the number of grains per plant, 1000 grain weight and grain yield per plant for plants from poor vigor seed lots (V_2 and V_3) were significantly higher than that from high vigor seed lot (V_1), the highest number of grains per unit area and grain yield per unit area were recorded for plants from vigorous seeds (Table 4).

Grain weight was not significantly affected by hydro-priming duration ($p>0.05$), but effects of hydro-priming duration on other traits were significant ($p \leq 0.01$). Grains per plant, grains per unit area, grain yield per plant and grain yield per unit area for plants from unprimed seeds (P_1) were considerably lower than those from primed seeds(P_2 and P_3) (Table 4).

Table4.Means of yield and yield components of chickpea affected by irrigation, seed vigor and hydro-priming duration

Treatments	Grains per plant	Grains per unit area	1000 grain weight (g)	Grain yield (g/plant)	Grain yield (g/m^2)
Irrigation					
I_1	9.369 ^{a}	180.4 ^{a}	309.6 ^{a}	2.943 ^{a}	60.43 ^{a}
I_2	7.905 ^{b}	151.3 ^{b}	307.5 ^{b}	2.214 ^{b}	42.98 ^{ab}
I_3	5.831 ^{c}	111.7 ^{c}	305.1 ^{c}	1.636 ^{c}	32.03 ^{b}
Vigor					
V_1	6.924 ^{b}	262.9 ^{a}	298.8 ^{c}	2.256 ^{a}	84.63 ^{a}
V_2	8.300 ^{a}	103 ^{b}	310.3 ^{b}	2.304 ^{a}	28.69 ^{b}
V_3	7.881 ^{a}	77.44 ^{c}	313.1 ^{a}	2.233 ^{a}	22.11 ^{b}
Hydro-priming					
P_1	6.766 ^{b}	102.3 ^{b}	309.4 ^{a}	2.053 ^{b}	35.13 ^{b}
P_2	8.167 ^{a}	165.3 ^{a}	308 ^{a}	2.414 ^{a}	50.02 ^{a}
P_3	8.173 ^{a}	175.8 ^{a}	304.9 ^{a}	2.327 ^{a}	50.29 ^{a}

Different letters at each column indicate significant difference at $p \leq 0.05$

I_1, I_2 and I_3: Irrigation after 70,120 and 170 mm evaporation from class A pan, respectively

V_1, $V_2$and V_3: Seed lots with 100, 85 and 74% viability, respectively

P_1, P_2 and P_3: non-primed, primed for 12 and 18 hours, respectively

Interaction of irrigation × seed vigor for grains per unit area, grain yield per plant and grain yield per unit area were significant ($p \leq 0.05$).Plants from high vigor seed lot (V_1) under different irrigation treatments had higher number of grains per unit area, compared with those from low vigor seed lots (Table 5). Grain yield per plant and grain yield per unit area among plants from various seed lots diminished with increasing water limitation (Table 5).

Interactions of irrigation × hydro-priming for grains per plant and grain yield per plant were statistically different ($p \leq 0.01$). Hydro-priming improved grains per plant and grain yield per plant under various irrigation treatments. This advantage for plants under sever water limitation was larger than that under other irrigation treatments (Table 6).

Interaction of seed vigor× hydro-priming for grains per plant, grains per unit area and grain yield per plant were significant ($p \leq 0.05$).Hydro-priming improved grains per plant, grains per unit area and grain yield of plants from different seed lots (Table 7).No significant interaction of irrigation ×vigor× hydro-priming on these traits were found ($p>0.05$).

Table5. Means of grains per unit area, grain yield per plant and grain yield per unit area for chickpea seed lots under different irrigation treatments

Traits	Treatment	V_1	V_2	V_3
Grains per unit area	I_1	324.6^{a}	120.6^{d}	96.22^{de}
	I_2	271.1^{b}	107.2^{de}	75.44^{ef}
	I_3	193.1^{c}	81.22^{ef}	60.67^{f}
Grain yield (g/plant)	I_1	3.158^{a}	2.794^{b}	2.878^{b}
	I_2	2.094^{d}	2.411^{c}	2.137^{d}
	I_3	1.515^{e}	1.708^{e}	1.685^{e}
Grain yield (g/m^2)	I_1	118.5^{a}	34.79^{d}	28^{de}
	I_2	77.99^{b}	29.49^{de}	21.44^{e}
	I_3	57.40^{c}	21.79^{e}	16.89^{f}

Different letters at each column indicate significant difference at $p \leq 0.05$

I_1, I_2 and I_3: Irrigation after 70,120 and 170 mm evaporation from class A pan, respectively

V_1, $V_2$and V_3: Seed lots with 100, 85 and 74% viability, respectively

Table 6. Means of grains per plant and grain yield per plant and grain yield per unit area for unprimed and primed seeds of chickpea under different irrigation treatments

Traits	Treatment	P_1	P_2	P_3
Grains per plant	I_1	8.978^{ab}	9.786^{a}	9.344^{a}
	I_2	7.330^{cd}	8.090^{bc}	8.296^{b}
	I_3	3.989^{e}	6.627^{d}	6.878^{d}
Grain yield (g/plant)	I_1	2.968^{a}	3.025^{a}	2.837^{a}
	I_2	1.966^{bc}	2.362^{b}	2.313^{bc}
	I_3	1.225^{d}	1.853^{c}	1.830^{c}

Different letters at each column indicate significant difference at $p \leq 0.05$

I_1, I_2 and I_3: Irrigation after 70,120 and 170 mm evaporation from class A pan, respectively

P_1, P_2 and P_3: non-primed, primed for 12 and 18 hours, respectively

Table7. Means of grains per plant, grains per unit area and grain yield per plant for different unprimed and primed seed lots

Traits	Treatment	P_1	P_2	P_3
Grains per plant	V_1	5.474^{d}	7.791^{bc}	7.507^{c}
	V_2	7.411^{c}	8.667^{ab}	8.822^{a}
	V_3	7.411^{c}	8.044^{abc}	8.189^{abc}
Grains per unit area	V_1	194.1^{b}	290.2^{a}	304.4^{a}
	V_2	68.11^{ef}	114.1^{cd}	126.8^{c}
	V_3	44.56^{f}	91.67^{de}	96.11^{cde}
Grain yield (g/plant)	V_1	2.149^{bc}	2.457^{a}	2.161^{bc}
	V_2	1.935^{c}	2.477^{a}	2.502^{a}
	V_3	2.075^{bc}	2.307^{ab}	2.318^{ab}

Different letters at each column indicate significant difference at $p \leq 0.05$

V_1, $V_2$and V_3: Seed lots with 100, 85 and 74% viability, respectively

P_1,P_2 and P_3: non-primed, primed for 12 and 18 hours, respectively

DISCUSSION

Increased membrane permeability associated with decreasing seed vigor [31] as indicated by high electrolyte leachates (Table1).The release of exudates such as sugars, inorganic ions and amino acids directly affects respiration and enzymatic activities and reduces macromolecular synthesis [4]. Reduction of electrolyte leakage in hydro- primed seeds (Table 1) could be attributed to repair mechanism of priming. Repair of DNA [41], RNA [29], protein [8], membrane [34] and enzymes [27] occurs during priming. This repair was more evident in low than in high vigor seed lots (Table 2). Priming increase germination metabolism in aged axes more than in those that are not aged [32].Decreasing seed vigor resulted in reductions of percentage and rate of germination and seedling dry weight (Table 1), which are improved by seed priming (Table 2).

Loss of seed vigor caused reduction in seedling emergence rate and establishment (Table 3), but hydro-priming improved these traits particularly in poor vigor seed lot (Figure 1).Since there is a strong relationship between plant density and yield [35], low plant density resulting from low vigor can potentially decrease crop yield per unit area. Because of poor stand establishment, plants from low vigor seed lots had the opportunity to use the environmental resources more efficiently, leading to the production of comparatively more and larger grains and grain yield per plant (Table 4). Early emergence of seedlings from primed seeds caused efficient and longer use of plants from light and soil resources during growth and development. Consequently, grains per plant and grain yield per plant were higher for plants from primed seeds compared with unprimed seeds. The superiority of plants from hydro-primed seeds in grain yield per unit area resulted from higher seedling establishment and production of more grains per unit area, compared with those from unprimed seeds (Table 4).Beneficial effects of hydro-priming on grain yield were also reported in wheat [28], safflower [3], sunflower [26], rice [14] and pinto bean [19].

The advantage of high vigor seeds in improving field performance and grain yield per unit area of chickpea directly related with rapid seedling emergence, optimal stand establishment and production of more grains per unit area under all irrigation treatments, particularly under well irrigation. Early emergence and high density of plants from vigorous seeds increased competition of individual plants for water and other resources under limited irrigation conditions. As a result, the superiority of plants from high vigor seeds decreased under low water supply (Table 5). However, improvement of grains per plant and grain yield per plant due to hydro-priming was more evident under low water availability (Table 6). Hydro-priming also enhanced grains per plant, grain yield per plant and per unit area more in plants from low vigor than high vigor seeds. This result clearly indicated that hydro-priming repaired deteriorated seeds [32] and enhanced their performance in the field (Table 7).

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