

DOI 10.2478/v10129-011-0066-2

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THE IMPACT OF SEED PRIMING AND AGING ON PHYSIOLOGICAL PERFORMANCE OF CHICKPEA UNDER DIFFERENT IRRIGATION TREATMENTS

ABSTRACT

A sub-sample of chickpea (*Cicer arietinum* L. cv. ILC₄₈₂) seeds was kept as control and two other sub-samples were aged at 40 °C for 3 and 5 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 and 18 hours and then dried back to initial moisture content at a room temperature of 20-22°C. Then seeds were sown in the field as split plot factorial based on RCB design. Hydro-priming improved leaf chlorophyll content index of plants from different seed lots. Hydro-priming also enhanced stomatal conductance of plants from all seed lots under all irrigation levels, but this advantage for plants from low vigor seed lots particularly under limited irrigations was higher than that for other treatments. Plants from high vigor seed lot under different irrigation treatments had higher relative water content, compared with those from low vigor seed lots. Hydro-priming improved relative water content, membrane stability and grain yield of chickpea plants from different seed lots under various irrigation treatments. It was concluded that hydro-priming to some extent can repair aged seeds and improve their performance under different irrigation treatments.

Key words: chickpea, hydro-priming, membrane stability, relative water content, stomatal conductance

INTRODUCTION

Chickpea (*Cicer arietinum* L.) is an important food legume crop which is grown in semi-arid regions (Labidi *et al.*, 2009). Drought is perhaps the major factor negatively affects plant growth and development and causes a sharp decrease of plants productivity (Pan *et al.*, 2002). Water deficit affects many physiological processes associated with plant growth and development (Toker and Cagirgan, 1998). In drought stress conditions, plants

close their stomata to avoid further water loss (Dulai *et al.*, 2006). This limits CO₂ assimilation which may promote an imbalance between photochemical activity at photosystem II (PSII) and electron requirement for photosynthesis, leading to a photoinhibitory damage of PSII reaction centers (Long *et al.*, 1994). Drought stress affects photosystem efficiency (Fv/Fm) and decreases quantum yield of photosystem II (Ahmed *et al.*, 2002). Investigations based on assessments of chlorophyll a fluorescence have shown that PSII is quite resistant to water deficits, being either unaffected (Shangguan *et al.*, 2000) or affected only under very severe drought conditions (Saccardy *et al.*, 1998). In fact, photo damage may be prevented through processes of thermal deactivation, down regulating PSII photochemistry, in a so-called dynamic photo inhibition (Osmond, 1994), that brings the electron transport capacity into balance with carbon metabolism (Epron *et al.*, 1992).

Another plant response to drought stress is change in photosynthetic pigment content. Leaf chlorophyll content play important role in harvesting light. The content of both chlorophylls a and b changes under drought stress (Farooq *et al.*, 2009). The effects of drought stress on membrane stability index (MSI) and relative water content (RWC) have also been decreased under water deficit (Bayoumi *et al.*, 2008). RWC is a reliable parameter for quantifying the plant drought stress response (Bayoumi *et al.*, 2008). The deleterious effects of water deficit on plants may be somewhat reduced by sowing high vigor seed lots or by priming of seeds before sowing (Ghassemi-Golezani *et al.*, 2012).

Maximum seed vigor is achieved at or slightly after mass maturity (end of seed filling period), which is previously termed physiological maturity (Shaw and Loomis, 1950). Thereafter seeds begin to deteriorate on the mother plant (Ghassemi-Golezani and Mazloomi-Oskooyi, 2008; Ghassemi-Golezani and Hossinzadeh-Mahootchy, 2009). When deterioration is advanced, rate and uniformity of seed germination and seedling emergence and tolerance to environmental stresses decreases (Khan *et al.*, 2003). The slower rate of emergence frequently associated with low-vigor seeds resulting in smaller plants, compared with high-vigor seeds (Ellis and Roberts, 1981; Ghassemi Golezani *et al.*, 2010b).

One of the simple and suitable methods which can improve seedling vigor and establishment and consequently crop performance in the field is seed priming (McDonald, 2000). Priming appears to reverse the detrimental effects of seed deterioration (McDonald, 2000). The early improvements may increase the rate and uniformity of seed germination and seedling emergence (Farooq *et al.*, 2005, Ghassemi-Golezani *et al.*, 2010a), especially under stressful conditions (Ghassemi-Golezani *et al.*, 2012). 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 (Demir *et al.*, 2006), via improving seedling vigor (Ghassemi-

Golezani, 1992) and stand establishment (Finch-Savage, 2000). Thus, this research was carried out to investigate the effects of seed vigor on some physiological characteristics and yield of chickpea under different irrigation treatments.

MATERIALS AND METHODS

Seeds of chickpea (*Cicer arietinum* L. cv. ILC₄₈₂) 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 20% moisture content were artificially aged at 40°C for 3 and 5 days, reducing seed germination to %98 and 89% (V_2 and V_3 , respectively). Consequently, three seed lots with different levels of vigor were provided. Then, each seed lot was divided into three sub-samples, one of which was kept as control (unprimed, 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 initial moisture content at a room temperature of 20-22°C for 24 hours.

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 2011. All the seeds were treated with benomyl at a rate of $2 \text{ g} \times \text{kg}^{-1}$ before sowing. Seeds were hand sown in about 4 cm depth with a density of $60 \text{ seeds} \times \text{m}^{-2}$. Each plot consisted of 8 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 $2 \text{ ml} \times \text{l}^{-1}$ before flowering.

Photochemical efficiency of photosystem II (F_v/F_m) was measured using a portable chlorophyll fluorometer. Measurements were made after 20 min dark adaptation (Maxwell *et al.*, 2000) from 3 plants. Chlorophyll content index of leaves was measured every week by a chlorophyll meter (CCM-200). Relative water content was determined according to Barr and Weatherley (1962). Fresh weight of the youngest fully expanded leaf was recorded within 24 h after excision. Turgid weight was obtained after soaking the leaf for 24 h in distilled water. After that, the leaves were quickly and carefully dried with tissue paper prior to determination of turgid weight. Leaf dry weight was obtained after drying the sample for 48 h at 75°C. Relative water content was calculated from the following equation:

$$RWC = \frac{(\text{fresh weight} - \text{dry weight})}{(\text{turgid weight} - \text{dry weight})} \times 100$$

Leaf samples (0.1 g) were taken in 10 ml double-distilled water in glass vials and kept at 40°C for 10 min. Initial conductivity (C_1) was recorded with a conductivity meter after transferring the sample to 25°C. The samples were kept at 100°C for 30 min and cooled at 25°C. Final conductivity (C_2) was measured according to Sairam (1994). The membrane stability index (MSI) was calculated as:

$$MSI = \left(1 - \frac{C_1}{C_2}\right) \times 100$$

Stomatal conductance of leaves was determined using a portable prometer (Delta-T AP4, Cambridge, UK). The measurements were taken on the surface of the leaf at the flowering stage. Finally, plants of 1 m² in the middle part of each plot were harvested and grain yield was 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

Chlorophyll Content Index (CCI)

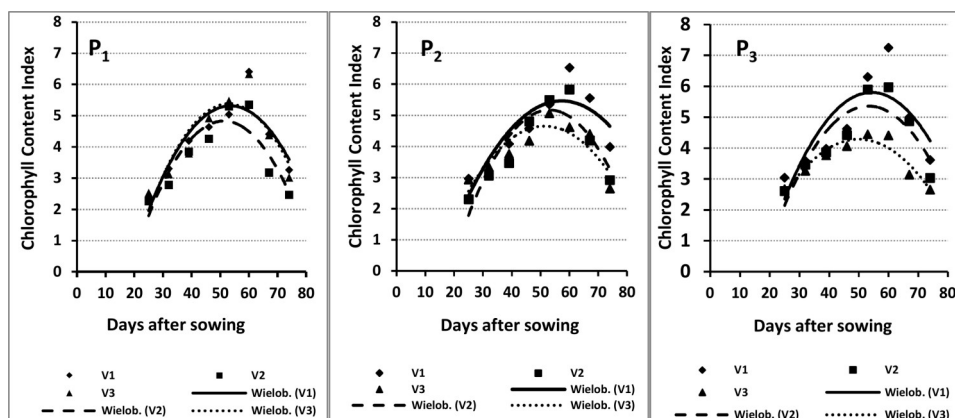


Fig.1. Changes in leaf chlorophyll content index (CCI) of chickpea at different stages of growth and development affected by seed vigor

Chlorophyll content index of chickpea leaves diminished with progressing plant development (Fig. 1). At the most stages of development, leaf chloro-

phyll content index of plants from low vigor seed lots (V_2 and V_3) was lower than that for plants from high vigor seed lot (V_1). CCI of plants from all seed lots decreased with increasing plant senescence at later stages of development. Reduction in CCI of V_3 plants was started earlier than that of plants from other seed lots. The rate of reduction was also much higher for V_3 than for V_2 and V_1 plants (Fig. 1).

The analysis of variance of data showed significant effects of irrigation level and seed vigor on maximum chlorophyll content index. Interaction of seed vigor \times hydro-priming duration for this trait was also significant (Table 1). Maximum chlorophyll content of chickpea was decreased with increasing water limitation (Table 2). Hydro-priming improved leaf CCI of plants from different seed lots (Table 2), but this beneficial effect of hydro-priming for plants from high vigor seed lot was higher than that for plants from other seed lots.

Table 1
Analysis of variance of the data of chickpea plants from different seed lots under different irrigation treatments

S.O.V	D.F.	CCI	Fv/Fm	MS			
				Stomatal con- ductance	RWC	MSI	Grain yield
Replication	2	0.219	0.080	703.494	33.309	11.751	853.758
Irrigation level	2	11.392*	0.207ns	216294.827**	880.805*	467.195*	285003.114**
Error	4	0.666	0.066	212.846	48.925	32.534	788.110
Vigor	2	8.217**	0.002ns	6189.123**	107.531*	10.814ns	117851.113**
I \times V	4	0.629ns	0.001ns	2081.420	60.095ns	23.202ns	20394.316**
Priming Duration	2	0.790ns	0.009ns	4688.346**	97.379*	26.933ns	50978.050**
I \times P	4	1.210ns	0.055ns	2802.531*	14.784ns	8.746ns	1100.462ns
V \times P	4	2.119*	0.032ns	3455.605**	74.978*	33.598*	604.905ns
I \times V \times P	8	0.991ns	0.023ns	3288.040**	67.162*	15.210ns	1321.139ns
Error	48	0.836	0.034	856.284	27.251	11.254	2051.446
C.V [%]		14.47	27.61	9.04	8.44	3.98	14.72

ns, *, **: No significant and significant at $p \leq 0.05$ and $p \leq 0.01$, respectively

Table 2
Means of maximum chlorophyll content index, stomatal conductance, relative water content, membrane stability and grain yield for different irrigation treatments

Treatments	CCI	Fv/Fm	Stomatal Conductance [mmol × m ⁻² × s ⁻¹]	RWC [%]	MSI [%]	Grain yield [g × m ⁻²]
Irrigation						
I ₁	6.975a	0.7624a	410.9a	67.73a	89.02a	418.387a
I ₂	6.307b	0.6350a	327.9b	61.53b	82.55b	289.290b
I ₃	5.676c	0.5946a	232.0c	56.32b	81.25b	215.392c

Different letters at each column indicate significant difference at $p \leq 0.05$
I₁, I₂ and I₃: Irrigation after 70, 120 and 170 mm evaporation from class A pan, respectively

Table 3
Means of maximum chlorophyll content index and membrane stability for different unprimed and primed seed lots

Traits	Treatment	P ₁	P ₂	P ₃
CCI	v ₁	6.448bc	6.804ab	7.570a
	v ₂	5.864bc	6.134bc	6.394bc
	v ₃	6.340bc	5.734c	5.587c
MSI (%)	v ₁	81.90b	87.46a	86.78a
	v ₂	83.22b	85.42ab	85.14ab
	v ₃	82.06b	85.05ab	85.44ab

Different letters at each column indicate significant difference at $p \leq 0.05$
V₁, V₂ and V₃: Seed lots with 100, 85 and 74% viability, respectively
P₁, P₂ and P₃: non-primed, primed for 12 and 18 hours, respectively

Fv/Fm

Efficiency of photosystem II (Fv/Fm) was not significantly affected by water limitation, seed vigor and hydro-priming duration.

Stomatal conductance

Irrigation treatments, seed vigor and hydro-priming duration had significant effects on stomatal conductance (SC) of chickpea leaves. Interactions of irrigation × hydro-priming, seed vigor × hydro-priming and irrigation × vigor × hydro-priming for SC were also statistically significant (Table 1). Stomatal conductance of plants from all seed lots significantly decreased with decreasing water availability (Table 2). Hydro-priming improved stomatal conductance of plants from all seed lots un-

der all irrigation levels, but this advantage for plants from low vigor seed lots particularly under limited irrigations was higher than that for other treatments (Fig. 2).

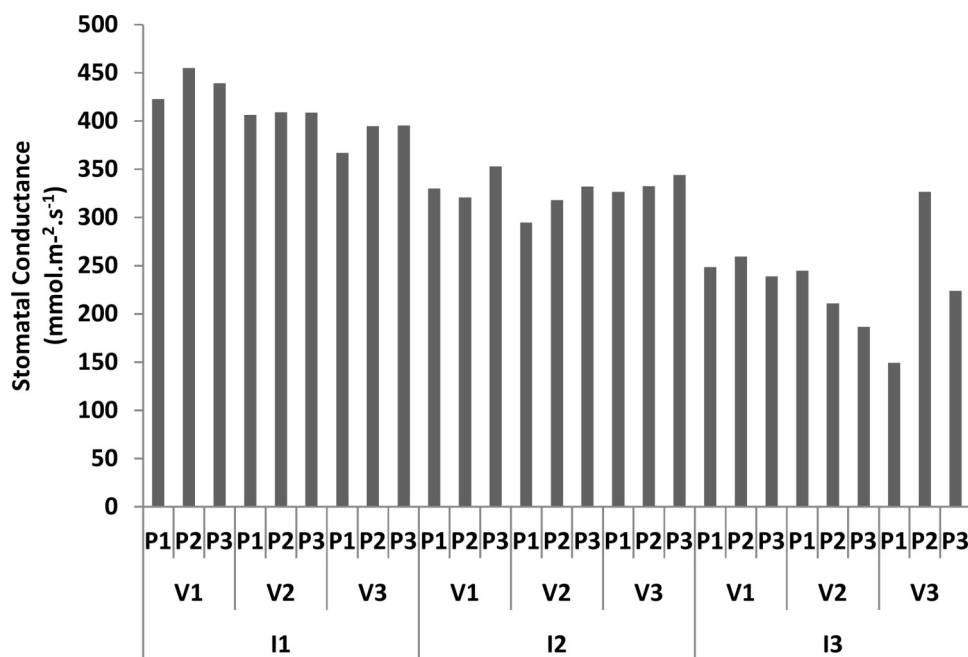


Fig. 2. Stomatal conductance of chickpea plants from various seed lots under different irrigation treatments I₁, I₂ and I₃: Irrigation after 70,120 and 170 mm evaporation from class A pan, respectively V₁, V₂ and V₃: Seed lots with 100, 85 and 74% viability, respectively P₁, P₂ and P₃: non-primed, primed for 12 and 18 hours, respectively

Relative Water Content (RWC)

Irrigation treatments, seed vigor and hydro-priming duration had significant effects on relative water content (RWC) of chickpea leaves (Table 1). Interactions of seed vigor × hydro-priming and irrigation × vigor × hydro-priming for RWC were also statistically different (Table 1). Leaf relative water content was decreased as water deficit increased. However, there was no significant difference between plants under I₂ and I₃ (Table 2). Plants from high vigor seed lot (V₁) under different irrigation treatments had higher relative water content, compared with those from low vigor seed lots. Hydro-priming improved relative water content of chickpea plants from different seed lots under various irrigation treatments. This improvement for plants from low vigor seed lot under severe water limitation was greater than that under other irrigation treatments (Fig.3).

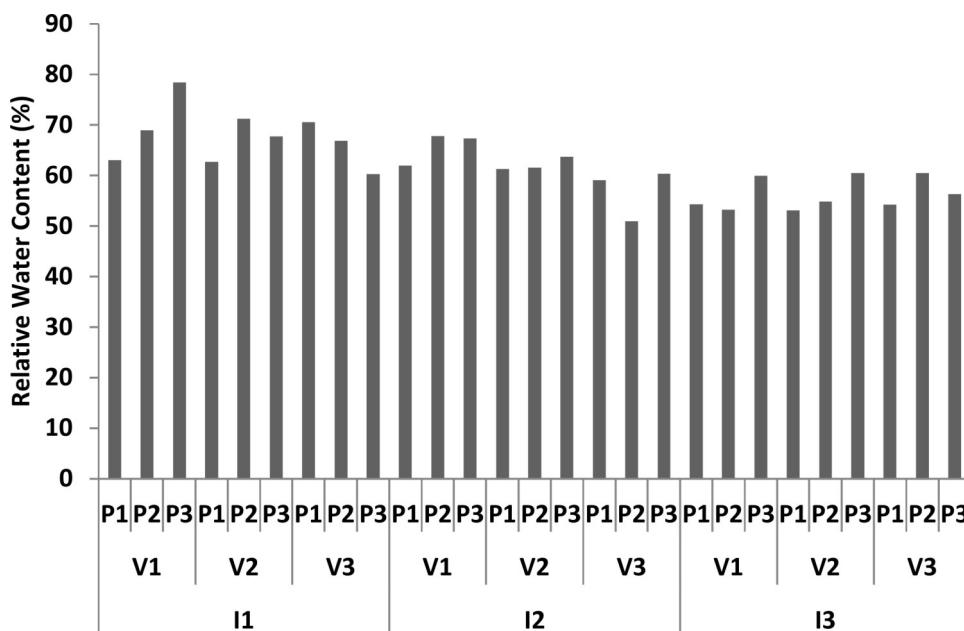


Fig. 3. Relative water content of chickpea plants from various seed lots under different irrigation treatments I₁, I₂ and I₃; Irrigation after 70, 120 and 170 mm evaporation from class A pan, respectively
V₁, V₂ and V₃: Seed lots with 100, 85 and 74% viability, respectively
P₁, P₂ and P₃: non-primed, primed for 12 and 18 hours, respectively

Membrane Stability Index (MSI)

Membrane stability index of chickpea leaves was significantly influenced by irrigation levels. However, no significant effects of seed vigor and hydro-priming duration on this trait were found (Table 1). Interaction of seed vigor × hydro-priming for MSI was also significant. Membrane stability of chickpea leaves was decreased with increasing water severity (Table 2). Hydro-priming enhanced membrane stability of chickpea plants from all seed lots (Table 3).

Grain yield

Grain yield was significantly influenced by irrigation treatments, seed vigor and hydro-priming duration (Table 1). Interaction of irrigation × seed vigor for grain yield was also significant (Table 1). Grain yield among plants from various seed lots diminished with increasing water limitation (Figure 4, Table 2). Plants from high vigor seed lot (V₁) under different irrigation treatments had higher grain yield, compared with those from low vigor seed lots (Fig. 4). Hydro-priming significantly enhanced grain yield from 258.19 g/m² to 339.55 g/m².

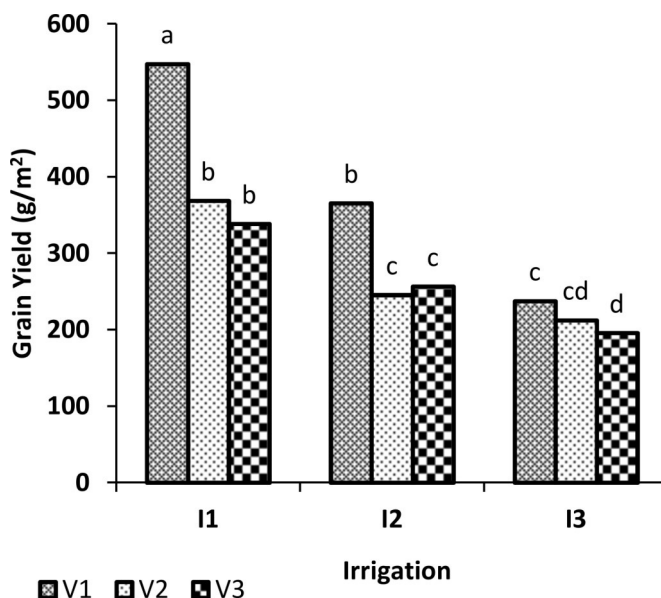


Fig. 4. Mean grain yield of chickpea for different seed lots under different irrigation treatments
 Different letters at each column indicate significant difference at $p \leq 0.05$ I_1 , I_2 and I_3
 I_1 : Irrigation after 70, I_2 : 120 and I_3 : 170 mm evaporation from class A pan, respectively
 V_1 , V_2 and V_3 : Seed lots with 100, 85 and 74% viability, respectively

Relative water content, membrane stability index, chlorophyll content index and stomatal conductance had significant and positive correlations with each other and also with grain yield. RWC and stomatal conductance showed the highest positive correlations with grain yield (Table 4).

Table 4
 Correlation coefficients among some physiological parameters of chickpea

Traits	1	2	3	4	5	6
1. Relative water content	1					
2. Membrane stability index	0.650**	1				
3. Fv/Fm	0.363 ^{ns}	0.550**	1			
4. Chlorophyll content index	0.680**	0.528**	0.348 ^{ns}	1		
5. Stomatal conductance	0.790**	0.771**	0.505**	0.670**	1	
6. Grain yield	0.809**	0.696**	0.355 ^{ns}	0.747**	0.808**	1

ns, **: no significant and significant at and $p \leq 0.01$

DISCUSSION

Greater beneficial effect of hydro-priming on chlorophyll content of plants from V_1 and V_2 seed lots (Fig.1) could be attributed to early improvements in rate and uniformity of seed germination and seedling emergence (Farooq *et al.*, 2005, Ghassemi-Golezani *et al.*, 2010a). Rapid emergence of seedlings could lead to the production of vigorous plants (Ghassemi-Golezani *et al.*, 2008a) with high chlorophyll content in their leaves (Ghassemi-Golezani *et al.*, 2008b) as it was shown for plants from high vigor seed lots of chickpea (Table 2). Delayed emergence of seedlings from poor vigor seed lots resulted in inefficient use of environmental resources. Early decline in leaf chlorophyll content of these plants at later stages of growth (Fig. 1) closely related with poor resistance to stressful condition. The reduction of chlorophyll content was probably related to the enhanced activity of the enzyme chlorophyllase (Reddy and Vora, 1986) and inducing the destruction of chloroplast structure and the instability of pigment protein complex (Singh and Dubey, 1995).

No significant effect of water stress on photosystem II efficiency (Fv/Fm) (Table 1) was a result of higher resistance of photosynthetic machinery to water limitation (Chaves *et al.*, 2002). Photo damage may be prevented through processes of thermal deactivation, down regulating PSII photochemistry, in a so-called dynamic photo inhibition (Osmond, 1994), that brings the electron transport capacity into balance with carbon metabolism (Epron *et al.*, 1992).

Decreasing water availability decreased stomatal conductance of chickpea plants (Fig. 2), due to the closure stomata which decrease transpiration rate and loss of water. During drought, leaves are subjected to both heat and water deficiency stress (Clarke *et al.*, 1993). As a consequence of the reduction in transpiration rates of leaves, leaf temperature increases (Kusvuran, 2012). Improved stomatal conductance of plants from primed seeds under all irrigation treatments (Fig. 2) was due to rapid emergence of plants and higher resistance of vigorous plants to unfavorable conditions (Ghassemi-Golezani *et al.*, 2012).

The decrease in leaf RWC (Fig. 3) could be related with low water availability under stress conditions (Shalhevet, 1993), or to poor root system, which is not able to compensate for water loss by transpiration (Gadallah, 2000). Higher RWC of plants from high vigor seed lot (V_1) and the efficiency of seed hydro-priming for better RWC under stressful condition were the result of rapid and uniform seedling emergence (Ghassemi-Golezani *et al.*, 2012). This can lead to the production of vigorous plants with a potential to use environmental resources efficiently. The resulting plants better tolerate drought stress, reduce pest damage and increase crop yield (Harris *et al.*, 1999).

Membrane lipids peroxidation, membrane damage and ion leakage under water stress (Katsuhara *et al.*, 2005) led to reduction in membrane stability index (MSI) of chickpea leaves (Table 2). Beneficial effects of hydro-priming on membrane stability of chickpea plants (Table 3) could be attributed to maintenance of positive leaf turgor, efficient and longer use of plants from soil resources under water stress by early establishment of seedlings.

Water limitation considerably reduced grain yield of chickpea, due to reductions in leaf, stomatal conductance, chlorophyll content, relative water content and membrane stability (Table 2). The earliest response to the leaf water deficit is stomata closure, which limits CO₂ diffusion to chloroplasts and limits photosynthesis (Cronic and Masacci, 1996). Inhibition of chlorophyll synthesis due to water stress (Fig. 1, Sayed, 2003) and decrease of relative water content affected by low water potential (Fig. 2, Krouma, 2010) can influence plant growth (Ohashi *et al.*, 2000) and field performance (Fig. 4).

Lower grain yield of plants from low vigor seed lots (Fig. 4) related to slow emergence of seedlings from aged seeds, poor stand establishment and delayed flowering of plants (Ghassemi-Golezani *et al.*, 2010b). The advantage of high vigor seeds in improving field performance and enhancement of grain yield of chickpea due to hydro-priming (Fig. 4) directly related with rapid seedling emergence, optimal stand establishment, efficient and longer use of plants from light and soil resources during growth and development and production of larger grains under all irrigation treatments (Ghassemi-Golezani *et al.*, 2012). Decreasing the superiority of plants from high vigor seeds under low water supply (Figure 4) is the result of early emergence and high density of plants from these seeds which increased competition of individual plants for water and other resources under limited irrigation conditions (Ghassemi-Golezani *et al.*, 2012). High positive correlations of stomatal conductance and relative water content with grain yield (Table 3) indicate that these parameters can be used to estimate potential field performance of chickpea under different irrigation conditions.

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