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Effects of water stress on some physiological traits and grain yield of chickpea (*Cicer arietinum* L.) cultivars

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Abstract

A field experiment was conducted in 2012 to determine the effect of different irrigation levels (I_1, I_2, I_3 and I_4: irrigation after 70, 100,130 and 160 mm evaporation from class A pan, respectively) on leaf-air temperature difference, chlorophyll fluorescence, ground cover and their consequences to crop yield of three chickpea cultivars (Azad, Arman and Jam from kabuli type). The experiment was arranged as split-plot based on randomized complete block design in three replications, with the irrigation treatments in main plots and chickpea cultivars in sub-plots. Results showed that with increasing water stress, leaf-air temperature difference and chlorophyll fluorescence of the PSII (Fv/Fm) decreased. Consequently, percentage and duration of ground cover and grain yield per unit area decreased. Azad was a superior cultivar in grain yield under all irrigation treatments.

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Introduction

Water availability is a key environmental factor limiting plant photosynthesis and growth (Flexas *et al*., 2002). Photosynthesis and cell growth are the primary processes which are affected by stress (Munns *et al*., 2006). Plant response depends on the nature of the water shortage, induction of physiological responses to short term changes (Ruiz-Sanchez *et al*., 1993), acclimation to a certain level of water availability and adaptation to drought (Alscher and Cumming, 1990).

According to Smirnoff (1993) low water availability is often associated with increased levels of reactive oxygen species, such as superoxide anion, hydrogen peroxide, hydroxyl radical and singlet oxygen. Low internal CO_2 concentration results in a reduction of oxidized $NADP^+$ pool, as an electron acceptor. Therefore, the light energy absorbed is not fully used by photosynthesis, photorespiration or heat generation and is diverted to molecular oxygen, which is abundant in the chloroplast (Chaves, *et al*., 2003). Knowledge of drought resistance mechanisms makes it easier to plant using deficit irrigation strategies designed to save water, while minimizing the negative impacts on yield (Domingo *et al*., 1996).

Drought causes huge decrease in crop yield by inhibiting plant growth and photosynthesis (Chaves and Oliveira, 2004). Under drought stress, plant leaves are dehydrated, and photosynthesis is decreased. The decrease in photosynthesis of dehydrated leaves is usually caused by stomatal limitation under moderate drought conditions and/or non-stomatal limitations under severe drought conditions (Cornic, 1994; Limousin *et al*., 2010). As water becomes limiting, stomatal conductance and transpiration decrease and leaf temperature increases. A temperature measurement on individual leaves is a good indicator of water potential (Ehrler *et al*., 1978) and plant stress (Reginato, 1983).

Under drought stress, disturbances of photosynthesis at the molecular level are connected with the restricted electron transport through PSII and/or with structural injuries to PSII (Flexas *et al*., 2004; Hura *et al*., 2007). Fluorescence of chlorophyll reflected the photochemical activities of PSII (Ganivea *et al*., 1998), with optimal values of around 0.832 measured from most plant species (Johnson *et al*., 1993). Environmental stresses that affect PSII efficiency leads to a characteristic decrease in the Fv/Fm ratio (Krause and Weis, 1991; Mamnouie *et al*., 2006).

Water stress during vegetative stages has the greatest impact on plant height and biomass (Ghassemi-Golezani *et al*., 2008a). Ghassemi-Golezani and Lotfi (2012) showed that percentage and duration of ground cover in soybean were sharply reduced due to water stress at later stages of plant development. There is a linear relationship between ground cover and light interception (Ghassemi-Golezani *et al*., 2008b). Reduction of this growth index can reduce photosynthesis, plant biomass, yield components and consequently grain yield. Similar results were reported for maize (Bismillah-Khan *et al*., 2001), faba bean (Nasrullahzadeh *et al*., 2007) and pinto bean (Ghassemi-Golezani *et al*., 2010).

In Iran, chickpea is mainly grown on reserve moisture which is progressively depleted with crop growth. The crop experiences drought stress from late vegetative stages until maturity. The intensity of drought stress varies from year to year, depending on the amount and distribution of rainfall and on spring and early summer temperatures. Therefore, this research was carried out to investigate changes in Leaf-air temperature difference, chlorophyll fluorescence and ground cover of three chickpea cultivars in response to water stress during growth and development and their consequences to crop yield.

Materials and methods

A split plot experiment (using RCB design) with three replications was conducted in 2012 at the Research Farm of the Faculty of Agriculture, University of Tabriz, Tabriz, Iran (Latitude 38° 05´N, Longitude 46° 17´E, Altitude 1360 m above sea level) to evaluate the effects of different irrigation levels on leaf-air temperature difference, chlorophyll fluorescence, ground cover and their consequences to crop yield of three chickpea (*C. arietinum* L.) cultivars. The climate is characterized by mean annual precipitation of 245.75 mm per year and mean annual temperature of 10°C. Irrigation treatments (I_1, I_2, I_3 and I_4: irrigation after 70, 100, 130 and 160 mm evaporation from class A pan, respectively) were located in main plots and cultivars (Azad, Arman and Jam) were allocated to sub plots.

Each plot had 6 rows of 5 m length, spaced 25 cm apart. Seeds were treated with Benomyl at a rate of 2 g/kg before sowing. The seeds were then sown by hand on 14 May 2012 in 4 cm depth of a sandy loam soil. All plots were irrigated immediately after sowing, but subsequent irrigations were carried out according to the treatments. Weeds were controlled by hand during crop growth and development as required.
Ground cover percentage (PGC) was measured in weekly intervals by viewing the canopy through a wooden frame (50 cm × 50 cm), divided into 100 equal sections. The sections were counted when more than half filled with crop green area. Changes in mean PGC was shown by regression fits on mean data.

After seedling establishment, at flowering stage of plants development a plant was marked in each pot and Leaf temperature (°C) of upper, middle and lower leaves were measured. Leaf temperature was recorded by an infrared thermometer (TES-1327) before irrigation of each plot in flowering stage. Before and after measurement of leaf temperature, air temperature was also recorded. Leaf-air temperature difference was calculated by subtracting average temperature (°C) of upper, middle and lower leaves from average air temperature.

The chlorophyll fluorescence induction parameters were measured in leaves by a chlorophyll fluorometer (OS-30, OPTISCIENCES, USA) at flowering stage of plants development before irrigation of each plot. Dark-adapted leaves (15 min.) were initially exposed to the weak modulate measuring beam, followed by exposure to saturated white light to estimate the initial (F0) and maximum (Fm) fluorescence values, respectively. Variable fluorescence (Fv) was calculated by subtracting F0 from Fm. The quantum yield (Fv/Fm) measures the efficiency of excitation energy capture by open PSII reaction centers, representing the maximum capacity of light-dependent charge separation in PSII (Rizza *et al.*, 2001; Basu *et al.*, 2004). At maturity, grain yield per unit area was determined.

Analysis of variance appropriate to the experimental design was conducted, using MSTATC software. Means of each trait were compared according to Duncan multiple range test at $p \leq 0.05$. Excel software was used to draw figures.

Results

Percentage ground cover (PGC) for all irrigations treatments and cultivars increased up to the points where maximum values were achieved and thereafter decreased with further plant development. Percentage ground cover was sharply reduced due to water stress. Maximum PGC under well-irrigation (I_1) was observed at 65 days after sowing, while under I_2 , I_3 and I_4 it was achieved at 55-60 days after sowing (Figure 1a). At the most stages of growth and development PGC of Azad was higher than that of other cultivars, but changes in PGC of Arman and Jam were almost similar. Maximum PGC for Azad was obtained at 60 days after sowing, while for Arman and Jam, it was attained at about 66 days after sowing (Figure 1b).

Analysis of the data (Table 1) showed that water stress had significant effects on leaf-air temperature difference, chlorophyll fluorescence, maximum ground cover and grain yield. Grain yield was also significantly affected by cultivars. The interaction of irrigation × cultivar was only significant for grain yield (Table 1).

Percentage ground cover, leaf-air temperature difference, chlorophyll fluorescence of the PSII (Fv/Fm) and grain yield decreased as water deficit increased. Maximum loss in grain yield was observed under severe water stress (I_4) (Table 2).

Azad was a superior cultivar in ground cover and grain yield, although differences in ground cover among cultivars were not significant (Table 2). Grain yield of Azad was higher than that of other cultivars under all irrigation treatments, but this superiority slightly decreased with decreasing water supply. No significant differences were observed in grain yield of Arman and Jam under all irrigation treatments (Figure 2).

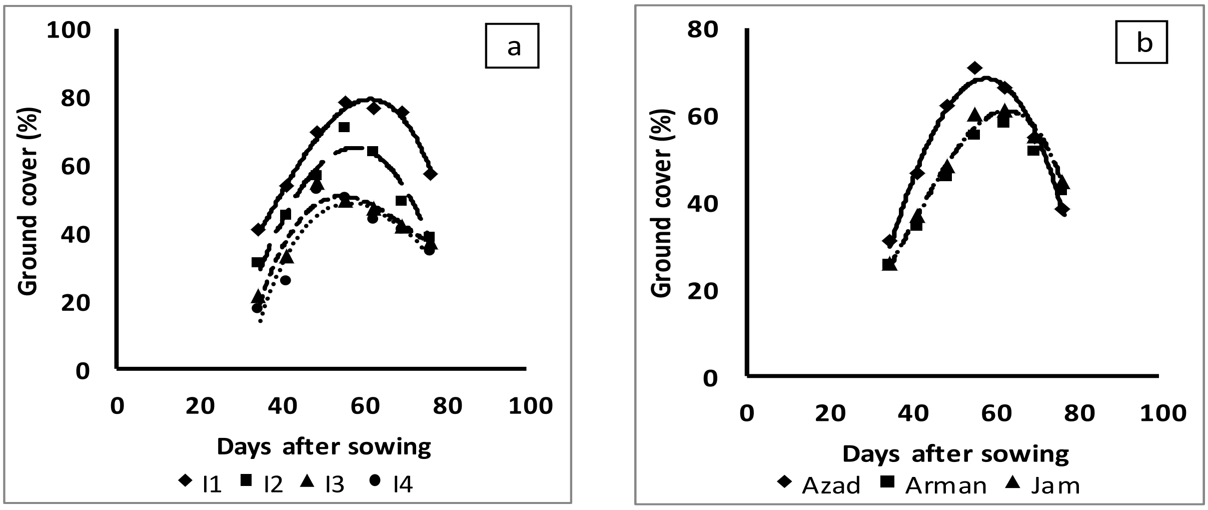

Fig. 1. Changes in ground cover (PGC) of chickpea for different irrigation treatments and cultivars
I_1, I_2, I_3 and I_4: irrigation after 70, 100, 130 and 160 mm evaporation from class A pan, respectively

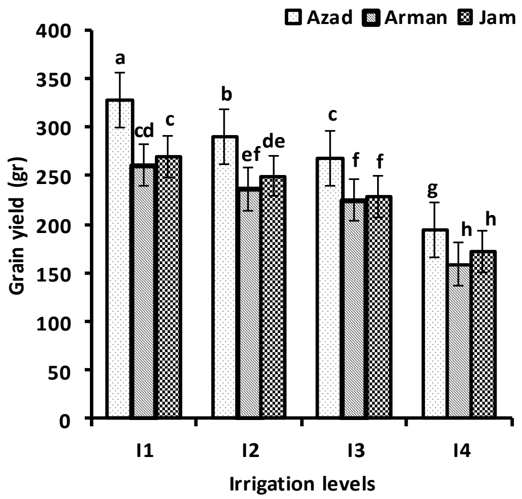

Fig. 2. Mean grain yield of chickpea cultivars under different irrigation treatments
Different letters indicate significant difference at P≤0.05.

Correlation coefficients of different traits showed that PGC and Fv/Fm positively correlated with grain yield, but the correlation between leaf-air temperature difference and grain yield was negative. The efficiency of photosystem II had the highest positive correlation with grain yield per unit area (Table 3).

Discussion

Reductions in percentage and duration of ground green cover due to water stress (Figure 1) can strongly reduce the absorption of incident PAR, either by drought-induced limitation of leaf area expansion or by temporary leaf wilting and early leaf senescence (Hugh and Richard, 2003). This can potentially reduce photosynthesis and consequently grain yield of crops (Ghassemi-Golezani and Lotfi, 2012). Since there is a linear relationship between percentage ground green cover and light interception (Burstall and Harris, 1983; Ghassemi-Golezani *et al.*, 2008b), it can be used as a reliable index to estimate yield potential of the crops under a wide range of environmental conditions (Ghassemi-Golezani and Mardfar, 2008). Reduction in percentage ground green cover due to water stress (Table 2) can be attributed to competition of plants for water and nutrients (Ghassemi-Golezani *et al.*, 2010).

Water deficit reduces leaf water potential and stomatal conductance, inhibits photosynthetic metabolism and eventually reduces plant productivity

(Baker and Rosenqvist 2004; Hassan 2006; Rouhi *et al.*, 2007; Degl'Innocenti *et al.*, 2008). Increasing leaf temperature due to water stress (Table 2) is possibly related to decreasing stomatal conductance and transpiration (Ehrler *et al.*, 1978; Reginato, 1983; Siddiaue *et al.*, 2000). 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.

Table 1. Analysis of variance of the the effects of water stress on some physiological characters and grain yield of chickpea cultivars under different irrigation treatments.

Source	df	Percentage ground cover	Leaf-air temperature difference	Fv/Fm	Grain yield
Replication	2	420.111	33.731	0.005	1026.493
Irrigation(I)	3	1640.889 *	90.881**	0.118 **	19961.108 **
Ea	6	225.333	5.909	0.004	116.758
Cultivar (C)	2	428.861 ns	0.308 ns	0.018 ns	8428.530 **
I*C	6	184.417 ns	1.768 ns	0.013 ns	215.456 *
Eb	16	190.194	2.582	0.008	76.914
Cv%		20.83	-54.45	19.07	3.65

*,** Significant at $p \leq 0.05$ and $p \leq 0.01$, respectively

Table 2. Means of the ground cover, leaf-air temperature difference, chlorophyll fluorescence and grain yield of chickpea for irrigation treatments and cultivars.

Treatment	Percentage ground cover	Leaf-air temperature difference	Fv/Fm	Grain yield
Irrigation				
I_1	82.44 a	-6.967 b	0.6588 a	71.54 a
I_2	72.22 ab	-4.389 b	0.4461 b	64.68 b
I_3	55.78 b	-0.983 a	0.4251 b	60.13 c
I_4	54.44 b	-0.066 a	0.3202 c	43.85 d
cultivar				
Azad	72.917 a	-2.917 a	0.475 a	67.57 a
Arman	61.417 a	-3.188 a	0.419 a	55.06 c
Jam	64.333 a	-3.200 a	0.494 a	57.52 b

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

I_1, I_2, I_3, I_4: Irrigation after 70, 100, 130 and 160 mm evaporation from class A pan, respectively

Table 3. Correlation coefficients of some physiological characters and grain yield of chickpea cultivars

	Percentage ground cover	Leaf-air temperature difference	Fv/Fm	Grain yield
Percentage ground cover	1			
Leaf-air temperature difference	-.715**	1		
Fv/Fm	.697*	-.813**	1	
Grain yield	.642*	-.631*	.852**	1

*,** Significant at $p \leq 0.05$ and $p \leq 0.01$, respectively

This may inhibits photosynthesis by limiting the availability of CO_2 within the leaf (Boyer, 1976; Chaves, 1991) and predispose leaves to photo-inhibition (Bjorkman and Powles, 1994) and decrease photosynthetic efficiency by stimulating photorespiration (Brooks and Farquhar, 1985). The rate of photosynthetic CO_2 assimilation is generally reduced by drought stress. This reduction is partly due to a reduced stomatal conductance and consequent restriction of the availability of CO_2 for carboxylation (Brugnoli and Lauteri, 1991).

It has been well documented that the photosynthetic system is very sensitive to many environmental stresses and that chlorophyll fluorescence analysis is a good index for measuring rapidly the change in photosynthetic metabolism of plants to such environmental stresses as drought (Conroy *et al.*, 1986; Genty *et al.*, 1987). Chlorophyll fluorescence analysis is a sensitive indicator of the tolerance of the photosynthetic apparatus to environmental stress (Maxwell and Johnson, 2000). Reduction in chlorophyll fluorescence of the PSII (Fv/Fm) under water stress (Table 2) indicates that occurrence of chronic photo-inhibition due to photo-inactivation of PSII probably associated with the degradation of D1 protein (He *et al.*, 1995; Giardi *et al.*, 1996). Some other researchers also showed that Fv/Fm reduced as a result of water stress (Xu *et al.*, 1999; Flexas and Medrano, 2002).

Water limitation considerably reduced grain yield in chickpea cultivars (Table 2) probably due to pod and flower abortion and reduction in mean grain weight (Ghassemi-Golezani *et al.*, 2012). The superiority of Azad in grain yield could be attributed to higher ground cover (Figures 1b) and lower leaf temperature (Table 2) of this cultivar, compared with other cultivars. Significant correlations of ground cover, Leaf-air temperature difference and Fv/Fm with grain yield per unit area (Table 3) clearly indicate that these physiological characters strongly influence grain yield of chickpea cultivars under different water availability.

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