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*Full Length Research Paper*

# Effects of water deficit stress on field performance of chickpea cultivars

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**Water deficit is one of the important factors limiting crop production in arid and semi-arid regions. This research was conducted in 2007 and 2008 to investigate field performance of three chickpea cultivars (Hashem and Arman from kabuli and Pirooz from desi type) under well watering ($I_1$: 70 mm evaporation from class A pan), gradual water deficit ($I_2$ and $I_3$: 70…90…110…130 and 70…100…130 mm evaporation, respectively) and severe water stress ($I_4$: 130 mm evaporation). Results showed that with increasing irrigation intervals, leaf proline content increased, while LAI and grain yield were decreased. These reductions were only significant under severe water deficit ($I_4$) as compared with well watering ($I_1$). No significant differences in chlorophyll content and quantum yield ($F_v/F_m$) were recorded among irrigation treatments. LAI, chlorophyll content and grain yield of kabuli type cultivars were more than those of desi type cultivars. It was concluded that gradually increasing irrigation intervals can prevent significant reductions in LAI and grain yield, due to drought hardening of chickpea plants.**

**Key words:** Chickpea, gradual water deficit, grain yield, LAI, proline, quantum yield.

## INTRODUCTION

Chickpea (*Cicer arietinum* L.) is an important cool-season food legume (pulse) in arid and semi-arid regions of West Asia and North Africa (Saxena et al., 1996). This crop with 17 - 24% protein, 41 - 51% carbohydrates and high percentage of other mineral nutrients and unsaturated linoleic and oleic acids is an important crop for human consumption (Farshadfar and Farshadfar, 2008). Due to low production cost, wide climate adaptation, use in crop rotation and atmospheric nitrogen fixation ability, chickpea is one of the most important legume plants in sustainable agricultural system (Anonymous, 2002; Farshadfar and Farshadfar, 2008). Because of short period growth of chickpea this crop is cultivated in Iran in spring with minimum energy consumption.

In west of Iran, chickpea is sown in early March and water deficit during late vegetative and reproductive stages is one of the most important limiting factors for production of this crop in the region (Soltani et al., 2001). The severity of water stress is varied from year to year, depending on the amount and distribution of rainfall. Chickpea yield in Iran is less than half of the world average yield (Sabaghpour et al., 2006).

Water is essential to plant growth because it provides the medium within which most cellular functions take place (Condon et al., 2002). Increasing crop tolerance to water limitation would be the most economical approach to enhance productivity and reduce agricultural use of fresh water resources. To survive against the stress, plants have involved a number of morphological properties and physiological and biochemical responses (Xiong et al., 2006; Gao et al., 2008).

Proline is one of osmolytes, which increase faster than other amino acids in plants under water stress and help the plants to maintain the cell turgor (Valentovic et al., 2006). Therefore increasing proline concentration can be used as an evaluating parameter for irrigation scheduling and for screening drought resistant varieties (Bates et

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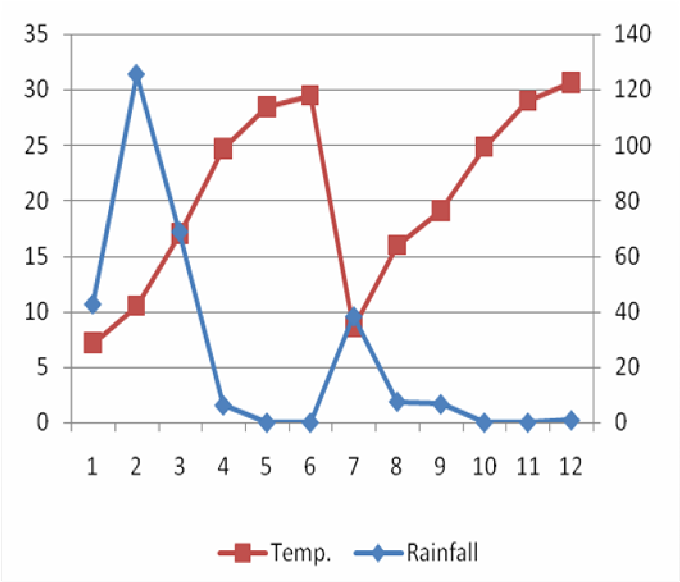

**Figure 1.** Pattern of monthly rainfall amounts and mean temperatures recorded during the crop season in 2007 and 2008.

al., 1973; Gunes et al., 2008).

The use of chlorophyll fluorescence from intact and attached leaves proved to be a reliable, non-destructive method for monitoring photosynthetic events and for judging the physiological status of the plant (Riza et al., 2001). Fluorescence induction patterns and derived indices have been used as empirical diagnostic tools in stress physiology (Strasser et al., 2000). This phenolmenon is a criterion for thylacoide membrane integrity and electron transfer efficiency from photosystem II (PSII) to photosystem I (PSI) (Ma et al., 1995). Thus, PSII fluorescence can be regarded as a biosensing device for stress detection in plants. The photochemical efficiency of PSII is determined by the $F_v/F_m$ ratio, which is reduced during periods of drought stress. The $F_v/F_m$ ratio represents the maximum quantum yields of the primary photochemical reaction of PSII. Environmental stresses that affect PSII efficiency leads to a characteristic decrease in the $F_v/F_m$ ratio (Krause and Weis, 1991; Mamnouie et al., 2006).

Water limitation in the West and North-West of Iran gradually increase during plant growth and development, particularly under rain-fed conditions. Therefore, this research was carried out for the first time to investigate the effects of gradual water deficit on some physiological traits and grain yield of desi and kabuli type chickpea cultivars.

## MATERIALS AND METHODS

Two field experiments were carried out for two years (2007 and 2008) at the Research Farm of Kermanshah Islamic Azad University (latitude 34°20' N, longitude 46º20' E, altitude 1351.6 m above sea level). Kermanshah is located in west of Iran and has a mean annual temperature of 13.8°C and annual rainfall of 478 mm. The soil texture of the research area was sandy-loam.

The experiments were arranged as split-plot, based on randomized complete block design in three replications, with the irrigation treatments ($I_1$, $I_2$, $I_3$, $I_4$: 70; 70…90…110…130; 70…100…130 and 130 mm evaporation from class A pan, respectively) in main plots and cultivars (Hashem and Arman from kabuli type and Pirooz from desi type cultivars) in sub plots. All plots were irrigated twice after sowing and subsequent irrigations were applied according to the treatments by furrow method. The plots under $I_1$ irrigation treatment received adequate water, and the water deficit increased progressively with the increasing irrigation intervals based on evaporation amount from the pan. In gradual water deficit treatments ($I_2$ and $I_3$), the plants were irrigated after 70 mm evaporation from the pan. The second, third and forth irrigations in $I_2$ were applied after 90, 110 and 130 mm evaporation, respectively. Irrigations intervals were increased in $I_3$ so that second and third irrigations were applied after 100 and 130 mm evaporation from the pan, respectively. Fertilizers were applied prior to sowing at the recommended rates of 20 and 30 kg/ha for N as urea and P as TSP, respectively. Seeds were pretreated with Mancozeb to minimize the probability of seed- and soil-borne diseases. The seeds were sown in six rows of 6 m length, spaced 25 cm apart (64 seeds per $m^2$) in the two years in early March. The size of main plots and sub plots were 36 and 12 $m^2$, respectively. The monthly rainfall amounts and mean temperature during the crop season in 2007 and 2008 were given in Figure 1. The experimental area was hand weeded.

After seedlings establishment, three plants were harvested from each sub plot every week, up to crop maturity and maximum leaf area index (LAI) was determined. LAI was estimated by weighting leaf disks of known area as described by Burstall and Harris (1983). Chlorophyll content was directly measured on five leaves of a plant at each plot, using a portable chlorophyll meter (SPAD 502-Minolta Co. Japan) at post-anthesis stage.

Chlorophyll fluorescence or photochemical efficiency (quantum yield, $F_v/F_m$) of the leaves was measured by using a Pulse Amplified Modulated Fluorometer (FMS 2 Hansatech, Inc. Co. UK) according to Basu et al. (2004). The fluorescence transients were measured within 1 s. The recordings were performed on the first fully developed leaf after dark adaptation period of 60 min. The quantum yield ($F_v/F_m$) measures the efficiency of excitation energy capture by open PSII reaction centers representing the maximum capacity of light dependent charge separation (Basu et al., 2004).

Proline content was determined according to Bates et al. (1973). Leaf tissues were rinsed with distilled water and oven-dried at 75°C for three days. Each dried leaf was crushed in a mortar with a pestle. 10 ml sulfosalicylic acid was added to each tube containing 0.1 g of the dried leaf. After 48 h, water extract, ninhydrin and glacial acetic were incubated in a water bath (100°C) for an hour. 0.2 ml toluene was added to each tube and the absorbance of top red aqueous layer was recorded at 520 nm in a spectrophotometer. The concentration of proline was calculated from a standard curve plotted with known concentrations of L-proline as standard.

Desi and kabuli type cultivars matured in early July and early August, respectively. At maturity, plants in 1 $m^2$ of middle part of each sub plot were hand harvested and brought back to the laboratory. The pods were then removed, threshed and grains detached from the pods and subsequently grain yield per unit area for each treatment at each replicate was determined.

Combined analysis of variance appropriate to the split plot design was carried out using SAS (version 9.1) General Linear Method (GLM) procedure. Years were considered as random effects, while irrigation treatments and varieties were fixed in the model. Duncan test was used to compare the differences between means of irrigation levels, varieties and interactions of year × cultivars at $P < 0.05$ probability.

**Table 1.** Combined analysis of variance of the effects of irrigation levels on various traits of three chickpea varieties.

| Source | Df | LAI | Chlorophyll content | Fv/Fm | Proline (µmol/g) | Grain yield (g/m$^2$) |
|---|---|---|---|---|---|---|
| Year (Y) | 1 | 7.75 | 3168.8 | 0.03 | 5176.5 | 57478.5 |
| Rep/Y | 4 | 11.4 | 33.01 | 0.01 | 14656.7 | 1266.3 |
| Irrigation (I) | 3 | 24.5* | 256.61 | 0.001 | 167973* | 56755* |
| Y×I | 3 | 2.51 | 220.68 | 0.01 | 26501* | 6002.4 |
| Ea | 12 | 3.76 | 79.66 | 0.006 | 6810.2 | 7124.9 |
| Cultivar (C) | 2 | 800.97* | 4773.5** | 0.005 | 2344.2 | 94483.3* |
| I×C | 6 | 1.04 | 32.51 | 0.006 | 3340.5 | 6062.1 |
| Y×C | 2 | 24.02** | 63.85 | 0.005 | 2510.8 | 94351** |
| Y×I×C | 6 | 1.86 | 65.15 | 0.005 | 3262.9 | 5838.1 |
| Eb | 32 | 3.03 | 48.5 | 0.003 | 3471.9 | 6190.7 |
| CV (%) | | 23.4 | 15.08 | 9.59 | 24.3 | 36.48 |

*, ** significant at $P < 0.05$ and $P < 0.01$, respectively.

**Table 2.** Mean values of analyzed traits for three chickpea varieties under four irrigation levels.

| Treatment | LAI | Chlorophyll content | Fv/Fm | Proline (µmol/g) | Grain yield (g/m$^2$) |
|---|---|---|---|---|---|
| **Irrigation** | | | | | |
| $I_1$ | 5.08a | 51.32a | 0.580a | 145.21c | 291.24a |
| $I_2$ | 4.60ab | 45.83a | 0.584a | 176.3bc | 200.3ab |
| $I_3$ | 4.51ab | 45.41a | 0.590a | 296.8ab | 215.78ab |
| $I_4$ | 3.94b | 42.22a | 0.570a | 348.34a | 156.77b |
| | | | | | |
| **Cultivar** | | | | | |
| $C_1$ | 4.88a | 57.76a | 0.595a | 250.41a | 205.26b |
| $C_2$ | 5.23a | 50.35a | 0.590a | 230.95a | 282.99a |
| $C_3$ | 1.89b | 30.48b | 0.565a | 243.69a | 158.81b |
| Year | | | | | |
| 2007 | 3.72a | 39.6a | 0.563a | 233.20a | 243.94a |
| 2008 | 4.28a | 52.8a | 0.604a | 250.6a | 187.44a |

Different letters in each column for each factor indicating significant difference at $P < 0.05$. $I_1$: 70…70; $I_2$: 70…90…110…130; $I_3$: 70…100…130; $I_4$: 130…130. $C_1$: Hashem; $C_2$: Arman; $C_3$: Pirooz.

## RESULTS

Combined analysis of variance of the data (Table 1) showed that the effects of year on all the measured traits were not significant. Leaf area index (LAI), proline content and grain yield were significantly affected by irrigation treatments ($P < 0.05$). However, chlorophyll content and photochemical efficiency (quantum yield, $F_v/F_m$) were not significantly different among the irrigation treatments. Cultivar had significant effects on LAI, chlorophyll content and grain yield, while quantum yield ($F_v/F_m$) and proline content were not significantly influenced by cultivar. Interactions of year × irrigation for proline content and year × cultivar for LAI and grain yield were also significant (Table 1).

Maximum LAI decreased, as water limitation increased. This reduction was only significant under severe water deficit ($I_4$), compared with control ($I_1$). In contrast, no significant differences in chlorophyll content and $F_v/F_m$ ratio were recorded among irrigation treatments (Table 2). The quantum yield ($F_v/F_m$) was not different among cultivars, but LAI and chlorophyll content of $C_3$ (desi type) were significantly less than those of kabuli type cultivars (Table 2). Although $C_2$ had the highest LAI in both years, the differences among all cultivars in the first year and between $C_1$ and $C_2$ in the second year were not statistically significant. The lowest LAI in both years was recorded for $C_3$ (Table 3).

Mean proline content increased with increasing irrigation intervals (Figure 2). Thus, proline accumulation was

**Table 3.** Mean values of LAI and grain yield of chickpea cultivars in two years.

| Traits | $Y_1$ | | | $Y_2$ | | |
|---|---|---|---|---|---|---|
| | $C_1$ | $C_2$ | $C_3$ | $C_1$ | $C_2$ | $C_3$ |
| LAI | 4.08b | 4.22b | 2.85b | 5.68a | 6.24a | 0.93c |
| Grain yield | 197.2b | 275.2ab | 259.5ab | 213.3ab | 280.8a | 58.2c |

Different letters in each row for each trait indicating significant difference at $P < 0.05$; $Y_1$: 2007; $Y_2$: 2008.

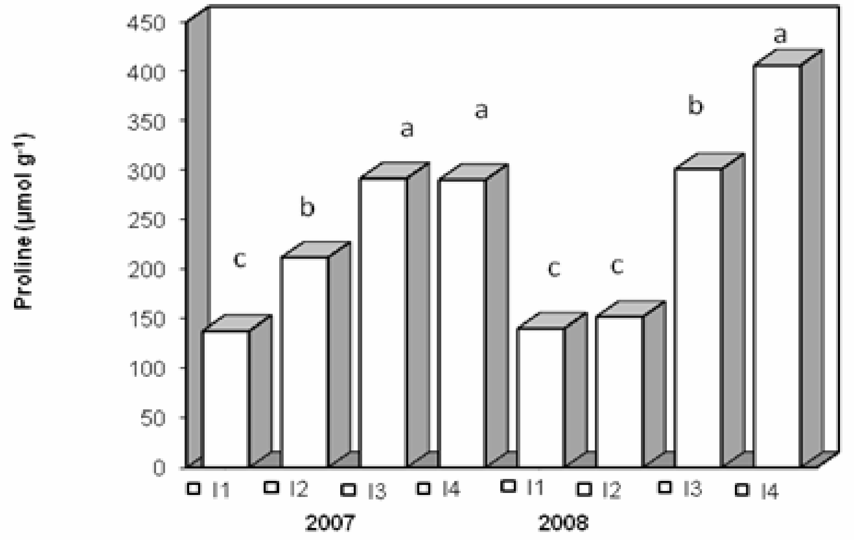

**Figure 2.** Proline content under four irrigation treatments in two years.

higher under $I_4$ compared with other irrigation treatments, although there was no significant difference between $I_3$ and $I_4$. Proline content of chickpea cultivars was statistically similar, but increased with increasing the severity of water stress (Table 2).

Mean grain yield under well-watering ($I_1$) and gradual water deficit ($I_2$ and $I_3$) was not statistically significant. However, grain yield per unit area significantly reduced as a result of severe water stress. Mean grain yield per unit area for $C_2$ was 37 and 78% higher than that for $C_1$ and $C_3$, respectively (Table 2). Grain yield of $C_1$ in the first year was slightly, but not significantly, lower than that of other cultivars. In contrast, grain yield of $C_3$ in the second year was significantly lower than that of $C_1$ and $C_2$. $C_2$ had the highest grain yield per unit area in both years (Table 3).

## DISCUSSION

Gradually increasing irrigation intervals improved chickpea resistance to water stress as indicated by non-significant differences in LAI and grain yield per unit area under $I_1$, $I_2$ and $I_3$ (Table 2). Significant reduction of these traits under $I_4$ suggests that chickpea plants cannot adapt to water stress, when it is severe and non-gradual. Increasing crop adaptation to water deficit conditions can be the most economic approach to reduce the use of fresh water resources and to improve crop productivity (Xiong et al., 2006). The adaptation of a crop variety is the ability of that variety to perform and produce to its maximum in a particular environment. Acclimation to water stress may also lead to a decrease in efficacy of the other processes like photosynthesis and growth.

The unaffected $F_v/F_m$ means that there is no loss in the yield of PSII photochemistry and confirms the resistance of the photosynthetic machinery to water deficit stress (Chaves et al., 2002; Cornic and Fresneau, 2002). This stability of photosynthetic components can be attributed to maintenance of positive leaf turgor under water stress as a result of osmotic adjustment (Basu et al., 2004).

Increasing leaf proline content with decreasing water supply (Table 2) means that an efficient mechanism for osmotic regulation, stabilizing sub-cellular structures and cellular adaptation to water stress was provided (Valentovic et al., 2006; Gunes et al., 2008). The stress adaptation effectors like protective proteins or osmolytes like proline usually undergo metabolic turnover and therefore, are not present once and for all (Beck et al., 2007). Higher leaf proline content under $I_4$ (severe water deficit) in the second year (Figure 2) was due to lower rainfall in this year, compared with that in the first year. A greater deal of effort has been made to develop plants that can withstand drought or production system that avoid water stress (Howell et al., 1998; Edmeades et al., 1999; Norwood, 2001; Ghassemi-Golezani et al., 2008). Resistance to water deficit stress can also be achievable by matching crop phenology with prevailing rainfall pattern (Edwards et al., 2005).

The superiority of $C_2$ in producing comparatively greater grain yield could be attributed to higher LAI of this cultivar in both years. Similar relationship was found for $C_3$ in the second year, which had the lowest LAI and grain yield (Table 3). In general, the impact of climatic conditions on chickpea development and productivity was not statistically different in the 2 years (Table 2). We found a highly significant positive correlation between LAI and grain yield (r = 0.8***). Therefore LAI can be used as a reliable criterion in selection of water stress tolerant chickpea cultivars.

## Conclusion

Progressively increasing irrigation intervals can help the chickpea plants to adopt water stress and prevent significant reductions in LAI and grain yield per unit area. Leaf area index (LAI) is closely related with grain yield and could be a reliable index for selecting high yielding chickpea cultivars. Increasing proline accumulation in chickpea leaves is a mechanism for osmotic adjustment under water stress. $C_2$ is a superior cultivar under both well watering and limited irrigation conditions.

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