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Full Length Research Paper

Effect of progressive water deficit stress on proline accumulation and protein profiles of leaves in chickpea

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Water deficit stress is one of the important factors limiting chickpea production in arid and semi-arid regions of West Asia and North Africa. When water deficit stress is imposed, different molecular and biochemical responses take place. This study was carried out to investigate proline accumulation and protein profiles of leaves in three chickpea cultivars under normal watering (I_1: irrigation based on 70 mm evaporation from class A pan), progressive 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). The experiment was split-plot, based on randomized complete block design (RCBD) in three replications. By increasing irrigation intervals, leaf proline content increased. Probable stress responsive proteins in relation to imposed water deficit stress was carried out by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) method. Water deficit stress increased concentration of soluble proteins in the leaves up to 43% in comparison with normal watering treatment (I_1: 70…70), but did not significantly affect electrophoretic pattern of protein profiles. It seems that chickpea can be adapted to progressive water deficit stress conditions.

Keywords: Chickpea, proline, protein profiles, water stress.

INTRODUCTION

Chickpea (*Cicer arietinum* L.) is the third important legume crop grown mainly in arid and semi-arid regions of West Asia and North Africa (Saxena et al., 1996). This crop is planted on 700,000 hectares in Iran and Iran ranks fourth in the world after India, Turkey and Pakistan. Most of the farmers grow chickpea in marginal areas in spring. Chickpea productivity in Iran is about half the world average yield. Due to water deficit during flowering, podding and seed filling, terminal drought stress is a major abiotic stress for reducing chickpea yield in Iran (Sabaghpour et al., 2006).

Water deficit stress is the most adverse environmental condition that can seriously reduce crop yield. To survive the stress, numerous morphological, physiological and biochemical changes occur in various plants species (Xiong et al., 2006). The alternation of protein synthesis or degradation is one of the fundamental metabolic processes that may influence water stress tolerance (Ouvrard et al., 1996; Jiang and Huang, 2002). Both quantitative and qualitative changes of proteins have been detected during the stress (Riccardi et al., 1998; Ahire et al., 2005; Kottapalli et al., 2009).

The accumulation of osmolytes may ensure the maintenance of the structural integrity of membranes (Conroy et al., 1988). There are some evidences that plants are more tolerant to water deficit when water is withheld under conditions that favour osmotic adjustment (Turner and Jones, 1980; Conroy et al., 1986; Moinuddin and Chopra, 2004). Osmotic adjustment is a part of drought avoidance mechanisms. Proline is one of the osmolytes, which increase faster than other amino acids in plants

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Table 1. Analysis of variance (ANOVA) for proline and total protein.

Source	df	Proline	Total protein
Replication	2	4925.3	0.016
Irrigation (I)	3	48797.39**	0.27*
Error (Ea)	6	2548.39	0.04
Cultivar (C)	2	1067.23	0.025
I×C	6	2071.4	0.028
Error (Eb)	16	1705.9	0.014
CV (%)		17.71	6.69

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

under water deficit stress and help the plants to maintain cell turgor (Valentovic et al., 2006). Thus, proline accumulation can be used as a criterion for drought resistance assessment of varieties (Bates et al., 1973; Gunes et al., 2008).

Alternations of proteins under water stress conditions have been studied widely in many plant species. Although, there is not enough information for chickpea in response to progressive water deficit stress in field conditions. In this study, our objectives were to investigate the changes in protein profiles and proline content in chickpea leaves under progressive water deficit stress, and to compare the biochemical responses of the chickpea cultivars to the water stress.

MATERIALS AND METHODS

Field trial

The field experiment was conducted in 2007 at the Research Farm of Razi University, Kermanshah (altitude 1351.6 m above sea level, longtitude 46°20' E, latitude 34°20' N). Kermanshah is located in west of Iran and has a mean annual rainfall of 478 mm and mean annual temperature of 13.8°C. Total amount of rainfall and mean temperature during the growth season (March to August) was 243 mm and 19.5°C, respectively. The soil texture of the research farm was sandy-loam.

The experiment was carried out as split-plot based on randomized complete block design (RCBD) with three replications. 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) were in main plots while cultivars (Pirooz from desi type and Hashem and Arman from kabuli type cultivars) were arranged in sub plots. Seeds were pretreated with benomil to minimize soil-borne diseases. The seeds were sown in six rows of 6 m length, spaced 25 cm apart and 6.5 cm between plants, in early March, 2007. All plots were irrigated twice after sowing to establish the seedlings and the next irrigations were exerted according to the treatments. The chickpea plants under I_1 irrigation treatment received sufficient water, while the water deficit increased progressively with the increasing irrigation intervals based on evaporation amount from the pan in I_2 and I_3. At post- anthesis stage, three leaves of five random plants at each plot were harvested for extraction of proline and protein.

Proline determination

Proline content was measured as described by Bates et al. (1973). Leaf tissues were rinsed twice with distilled water and oven-dried at 75°C for three days. Each dried leaf sample was crushed in a mortar with a pestle. 10 ml sulfosalicylic acid solution was added to each tube containing 0.1 g of the dried leaf. After two days, 1 ml of the extract was reacted with 1 ml glacial acetic acid and 1 ml ninhydrin (1.25 g ninhydrin warmed in 30 ml glacial acetic acid and 20 ml 6 M phosphoric acid until resolved) in a water bath (100°C) for an hour. The reaction was terminated in an ice bath to stabilize the purple color of the extract. 0.2 ml toluene was added to each tube and the absorbance of top purple aqueous layer was measured at 520 nm in a spectrophotometer. The concentration of proline samples was determined according to the standard curve plotted with known concentrations of L-proline.

Protein extraction and estimation

Leaf material (1 g fresh weight) was immediately frozen in liquid nitrogen and stored at -80°C. The leaf sample was crushed in a cold mortar with a pestle. Total proteins were extracted and estimated according to method of Bradford (1976) using bovine serum albumin as the standard. The absorbance of protein samples was measured at 595 nm using a spectrophotometer.

Electrophoresis

The proteins in the leaf samples were resolved by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE). Each sample at a concentration of 1 mg protein/ml was mixed with an equal volume of 2x sample buffer and the mixture was heated for 5 min in a water bath (100°C). The samples were loaded on a 12.5 and 4% polyacrylamide separating and stacking gel, respectively. Separation of proteins was carried out at constant voltage (50 V for 30 min and 150 V for 90 min). After electrophoresis, the gel was fixed in 20% trichloroacetic acid and stained in 0.1% Coomassie brilliant blue R-250, and then destained in 20% methanol and 7.5% (v/v) acetic acid.

Statistical data analysis

Statistical analyses of the data for the split plot design was performed using the Statistical Analysis System (SAS) (version 9.1) and analysis of variance (ANOVA) procedure. Least significant difference test (LSD) was used to test for the significance of the differences among means of irrigation levels and cultivars at $P < 0.05$ according to Gomez and Gomez (1984).

RESULTS AND DISCUSSION

The results of analysis of variance of data (Table 1) showed that proline and protein content of leaves were affected significantly by irrigation treatments but cultivar had no significant effect on these two traits. Interactions of irrigation × cultivar for proline and protein content were not significant (Table 1).

Progressive water deficit induced significant increase of proline content, so that proline accumulation in leaves at post-anthesis stage increased by more than twofold in severe stressed plants compared to well-watered plants. Proline content was higher under I_3 and I_4 when compared with other irrigation treatments (I_1 and I_2), although there was no significant difference between I_3 and I_4. Proline

Table 2. Mean values along with standard error (± SE) of proline and protein for three chickpea cultivars under four progressive irrigation treatments.

Treatment	Proline (µmol/gdw)	Total protein (mg/gfw)
Irrigation		
I_1	138 ± 4.8	4.23± 0.05
I_2	212.16 ± 6.86	4.67 ± 0.12
I_3	292.16 ± 24.52	6.07 ± 0.07
I_4	290.5± 11.11	5.73 ± 0.15
LSD 0.05	58.23	0.23
Cultivar		
C_1	241.5 ± 42.81	5.1 ± 0.39
C_2	224.4 ± 36.63	5.23 ± 0.45
C_3	232 ± 36.71	5.2± 0.43
LSD 0.05	35.75	0.1

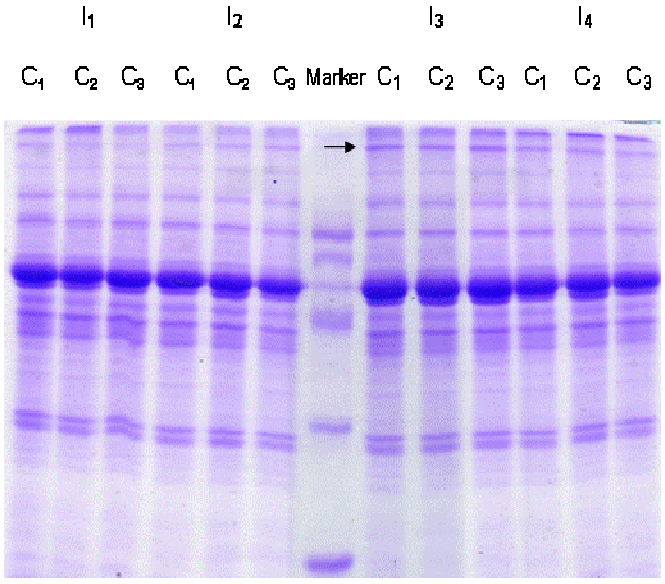

Figure 1. Reducing SDS-PAGE profiles of leaf soluble proteins under four irrigation treatments for three chickpea cultivars. The arrow indicates the increased band intensity in response to the water stress treatment.

content of chickpea cultivars was not statistically different, but increased by increasing the severity of water deficit, indicating that the response of desi and kabuli type cultivars were similar (Table 2). Increasing proline content of leaves with decreasing available water 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).

Water deficit stress increased total soluble protein content of leaves up to 43% in comparison with I_1. Leaf soluble proteins increased under I_2 as compared with I_1. Protein content of chickpea leaves under I_3 was slightly higher than that of I_4. All the cultivars had similar amount of soluble proteins (Table 2). High positive significant correlation was found between proline and protein content of the leaves ($r = 0.9$, $P < 0.001$).

Osmotic adjustment involves an active accumulation of cellular solutes such as proline and soluble proteins within the plant in response to lowering of the soil water potential and reducing the harmful effects of water deficit (Morgan, 1984). As a consequence of solutes accumulation, the osmotic potential of the cell is lowered, which in turn, attracts water into the cell and leads to maintaining its turgor (Moinuddin and Chopra, 2004). Osmotic adjustment has been reported also in chickpea under water deficit conditions (Leport et al., 1999; Gunes et al., 2008).

The SDS-PAGE analysis of soluble proteins from leaves revealed that progressive water deficit stress did not significantly change proteins profile of chickpea cultivars, with the exception that the band intensity of a polypeptide with molecular mass near 150 KDa was increased partly in all the cultivars under water stress. The level of this polypeptide was higher in I_3 and I_4 rather than I_1 and I_2 (Figure 1). Jiang and Huang (2002) reported that two polypeptides were intensified in dought-stressed tall fescue plants than well watered conditions. 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). 2D electrophoresis and proteome analysis is proposed for further analysis.

Conclusion

Progressive water deficit stress increased concentration of proline and soluble proteins in chickpea leaves. The accumulation of the osmolytes can help the chickpea plants to maintain the cell turgor and the structural integrity of membranes. SDS-PAGE analysis of the

proteins did not detect significant qualitative changes in protein synthesis in stressed plants than control. Kabuli and desi type chickpea cultivars did not differ in their biochemical responses to the water stress. It seems that chickpea can adapt to the progressive water deficit conditions.

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