Effect of drought stress on yield, proline and chlorophyll contents in three chickpea cultivars

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Abstract

Drought stress is one of the major abiotic stresses in agriculture worldwide. This study was carried out to investigate the effect of drought stress on proline content, chlorophyll content, photosynthesis and transpiration, stomatal conductance and yield characteristics in three varieties of chickpea (drought tolerant Bivaniej and ILC482 and drought sensitive Pirouz). A field experiment with four irrigation regimes was carried out in a randomized complete block design with three replications. Treatments included control (no drought), drought stress imposed during the vegetative phase, drought stress imposed during anthesis and drought stress during the vegetative phase and during anthesis. All physiological parameters were affected by drought stress. Drought stress imposed during vegetative growth or anthesis significantly decreased chlorophyll *a*, chlorophyll *b* and total chlorophyll content. Proline accumulation was higher in 'ILC482' than in 'Pirouz' both under control and drought stress conditions. Photosynthesis, transpiration, stomatal conductance and yield were higher but sub-stomatal CO_2 concentration was lower under drought stress conditions than under control conditions. The results showed that mesophyll resistance is the basic determinate of rate of phototosynthesis under drought stress conditions. Under drought conditions the drought tolerant variety 'Bivaniej' gave the highest yield whereas the drought sensitive variety 'Pirouz' gave the lowest yield. Drought stress at anthesis phase reduced seed yield more severe than that on vegetative stage.

Keywords: chickpea; *Cicer arietinum;* chlorophyll; Drought stress; photosynthesis; proline; stomatal conductance

Introduction

Drought is undoubtedly one of the most important environmental stresses limiting the productivity of crop plants around the world (Bohnert et al 1995) Drought is also a significant yield-limiting factor in chickpea (*Cicer arietinum* L.) production as the major chickpea growing areas are in the arid and semi-arid zones and about 90% of world's chickpea is grown under rain fed conditions (Kumar and Abbo, 2001) chickpea shows mechanisms for overcoming this condition. In this crop, yield losses might be the result of intermittent drought during the vegetative phase, due to drought during reproductive development or due to terminal drought at the end of the crop cycle (Serraj *et al*., 2004). Drought stress decreases the rate of photosynthesis (e.g., Kawamitsu *et al*., 2000). Plants grown under drought condition have a lower stomatal conductance in order to conserve water. Consequently, CO_2 fixation is reduced and photosynthetic rate decreases, resulting in less assimilate production for growth and yield of plants. Diffusive resistance of the stomata to CO_2 entry probably is the main factor limiting photosynthesis under drought (Boyer, 1970). Certainly under mild or moderate drought stress stomatal closure (causing reduced leaf internal CO_2 concentration (C_i)) is the major reason for reduced rates of leaf photosynthetic (Chaves, 1991; Cornic, 2000; Flexas *et al*., 2004). Severe drought stress also inhibits the photosynthesis of plants by causing changes in chlorophyll content, by affecting cholorophyll components and by damaging the photosynthetic apparatus (IturbeOrmaetxe et

al., 1998). Ommen *et al.* (1999) reported that leaf chlorophyll content decreases as a result of drought stress. Drought stress caused a large decline in the chlorophyll *a* content, the chlorophyll *b* content, and the total chlorophyll content in all sunflower varieties investigated (Manivannan et al., 2007). The decrease in chlorophyll under drought stress is mainly the result of damage to chloroplasts caused by active oxygen species (Smirnoff 1995). Plants can partly protect themselves against mild drought stress by accumulating osmolytes. Proline is one of the most common compatible osmolytes in drought stressed plants. For example, the proline content increased under drought stress in pea (Sanchez et al., 1998; Alexieva et al., 2001). Proline accumulation can also be observed with other stresses such as high temperature and under starvation (Sairam et al., 2002). Proline metabolism in plants, however, has mainly been studied in response to osmotic stress (Verbruggen and Hermans 2008). Proline does not interfere with normal biochemical reactions but allows the plants to survive under stress (Stewart, 1981). The accumulation of proline in plant tissues is also a clear marker for environmental stress, particularly in plants under drought stress (Routley, 1966). Proline accumulation may also be part of the stress signal influencing adaptive responses (Maggio et al. 2002). The purpose of the present study was to contribute to a better understanding of the physiology responses of chickpea plants to drought stress. We investigated the influence of four types of

drought stress on the chlorophyll (a, b, a/b) content, proline content, photosynthesis, transpiration and stomatal conductance in chickpea varieties differing in drought tolerance.

Material and methods

The research was carried out with three chickpea (*Cicer arietinum* L.) varieties contrasting in crop cycle duration, type (desi or kabuli), growth habit, and response to drought: Bivaniej (kabuli), ILC482 (kabuli) and Pirouz (desi). The first two are considered relatively drought tolerant, the latter is drought sensitive. Seeds of these varieties were obtained from the International Centre for Agricultural Research in the Kurdistan of Iran. The experiment was carried out in 2008 in a field of the Kurdistan University (47°1' N and 35°16' E, 1375 m above sea level) in Iran. The soil type was a sandy loam (pH until a depth of 30 cm was 7.6). The experiment was of a split-plot block design with three replications. The factors were variety (see above) as sub plot and drought treatment as main plot. To realize the drought treatments, plants were subjected to one of the following four irrigation regimes: Control; a well irrigated treatment (no drought stress), Drought stress imposed during the vegetative stage by withholding irrigation and re-watering at and after flowering, Drought stress imposed during anthesis by withholding irrigation, Drought stress imposed at both the vegetative and the anthesis stage by withholding irrigation. Individual plots were 6 rows (with a row distance of 0.30 m) of 6 m long. Plant distance within a row was 0.13 m. Plots were irrigated once immediately after sowing to ensure uniform emergence. Thereafter, plants were watered with tap water about once a week depending on treatment at -2 bar soil water potential. The plots were kept weed free by hand weeding.

Surface application and incorporation of 18 kg N ha^{-1} and 20 kg P ha^{-1} was carried out in experiment. Seeds were inoculated with fungicide protection before sowing.

Yield

At the end of the crop cycle, the effects of the drought treatments on seed yield were assessed. Samples were collected from a 1.0 m^2 area avoiding border effects. Also, 5 plants were selected randomly to assess plant height and number of pods per plant.

Proline content

Assessments of proline content were performed twice during the experimental period, at 40 days (vegetative stage) and 60 days (flowering) after the onset of the experiment. Proline was extracted from a sample of 0.5 g fresh leaf material samples in 3% (w/v) aqueous sulphosalycilic acid and estimated using the ninhydrin reagent according to the method of Bates et al. (1973). The absorbance of fraction with toluene aspired from liquid phase was read at a wave length of 520 nm. Proline concentration was determined using a calibration curve and expressed as µ mol proline g^{-1} FW.

Chlorophyll content

Assessments of chlorophyll content were performed twice during the experimental period, at 40 days (vegetative stage) and 60 days (flowering) after the onset of the experiment. Chlorophyll content was determined in 80% acetone extract.
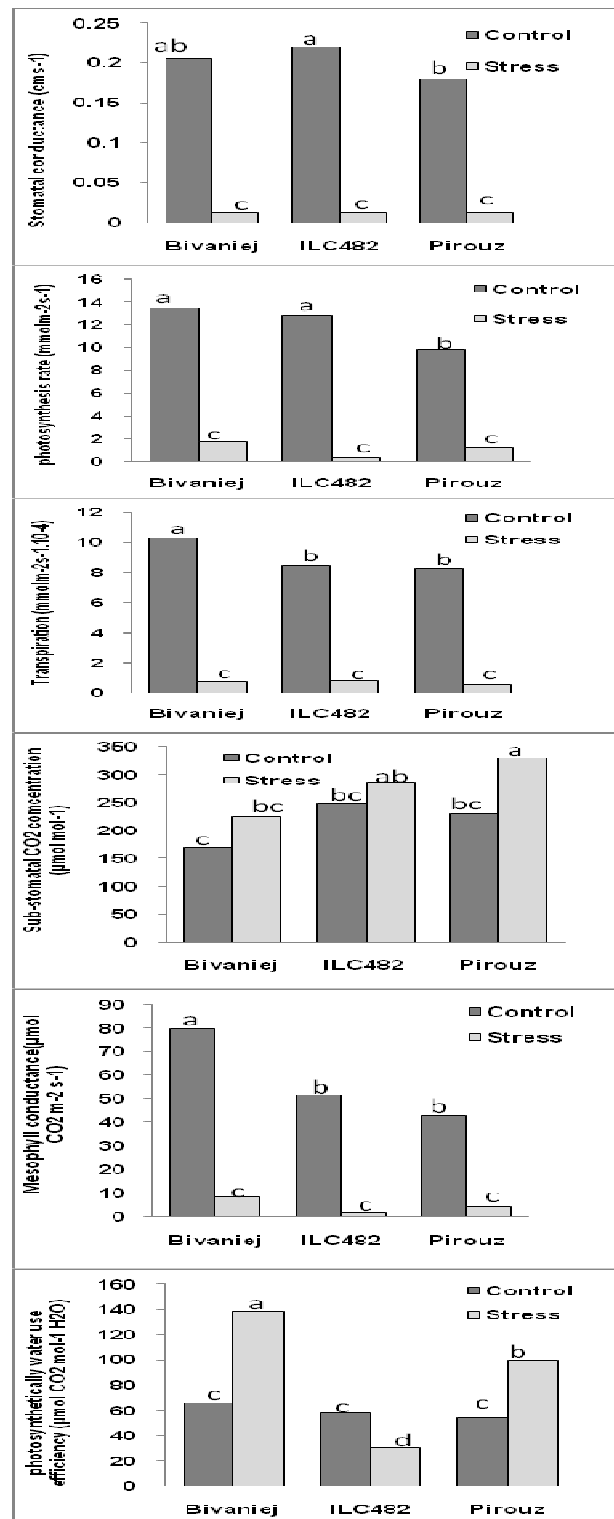

Fig 1. Transpiration, stomatal conductance, photosynthesis rate, sub-stomatal CO_2 concentration, mesophyll conductance and photosynthetically water use efficiency of three chickpea cultivars grown under control and drought stressed conditions. Values with different letters are significantly different at P=0.05.

After centrifugation (20 000 g, 20 min) the absorbance was read spectrophotometrically at 663 and 645 nm. Total chlorophyll as well as chlorophyll a and b concentrations were calculated according to Arnon (1949).

Gas exchange

Stomatal conductance (g_s), net photosynthesis (A), transpiration (E) and sub-stomatal CO_2 concentration (C_i) were determined at flowering using a portable gas exchange measuring system (Li 6400, Li-Cor, USA). Mesophyll conductance (MC) was calculated by dividing A by C_i (fischer et al 1998), photosynthetic water use efficiency (PWUE) was calculated by dividing A by g_s (Ahmadi and Siosemardeh 2005). Measurements were done at two levels of drought: the control (abundant water available) and a drought stress imposed at both the vegetative and the anthesis stage treatment. Measurements were doing between 10:00 and 12:00 h. under atmospheric Co2 and full sunlight.

Statistical analysis

Data were subjected to analysis of variance (ANOVA), and means were compared using Duncan's range test at P = 0.05. All calculations were performed with the help of the SAS software, version 9.1.

Result and discussion

Effects of drought on transpiration, stomatal and mesophyll conductance, photosynthesis, sub-stomatal CO_2 concentration and photosynthetically water use efficiency

Transpiration and stomatal conductance decreased in all three varieties when they were imposed to drought stress (Fig. 1) as one of the first responses of plants to drought is stomatal closure, restricting gas exchange between the atmosphere and the inside of the leaf. 'Pirouz' showed lowest stomatal conductance and seed yield under normal condition. A decreased as a result of the drought stress in all three varieties (Fig. 1). The internal CO_2 concentration increased in response to drought (Fig. 1). Varieties significantly differed in photosynthetic activities, but these differences could only be expressed under the control conditions. In many experiments it has been shown that A decreases when g_s decreases (e.g., Tenhunen et al., 1987; Nilsen and Orcutt, 1996). Chaves and Oliviera (2004) concluded that g_s only affects A at severe drought stress. The decrease in photosynthesis in drought stressed plants can be attributed both to stomatal (stomatal closure) and non-stomatal (impairments of metabolic processes) factors. Under control treatment, the yield of cultivars followed the same trend of A, under this condition 'Bivaniej' showed highest A and seed yield. At present most researchers agree that the stomatal closure and the resulting CO_2 deficit in the chloroplasts is the main cause of decreased photosynthesis under mild and moderate stresses (Flexas and Medrano, 2002). However, some authors claim that impaired ATP is a likely explanation for decreased photosynthesis under water stress (Lawlor, 2002; Tang et al., 2002). The effect of drought stress on transpiration was very similar to that on photosynthesis (Fig. 1). Better water supply resulted in significantly higher stomatal conductance (g_s; Fig. 1), net-photosynthesis (A) and transpiration rate (E) (see also de Souza et al., 2005). The varieties studied showed differences in sub-stomatatal CO_2 concentration and photosynthetically water use efficiency, but in this cases, the genetic differences were only statistically significant under drought conditions (Fig. 1), with 'Pirouz' showing the highest values of -stomatatal CO_2 concentration. Moreover, the effect of drought on sub-stomatal CO_2 concentration was only significant for the drought-sensitive variety Pirouz. No significant differences were observed in 'Bivaniej' and 'ILC482'. A decline in the photosynthetic rate under drought stress conditions could be attributed either to a decrease in stomatal conductance and/or to non-stomatal limitations (Cornic and Massacci, 1996). The increase in C_i in drought stressed Pirouz indicates the predominance of non-stomatal limitations (mesophyll resistance) to photosynthesis. Stomatal and non-stomatal limitation restricted crop yield under different conditions. Results of this research showed that under normal condition 'Bivaniej' had highest yield and mesophyll conductance and 'pirouz' showed lowest yield and mesophyll conductance (Fig. 1 and Table 1). Therefore, it can be concluded that under normal condition yield is primarily limited by non-stomatal rather than stomatal factors. Under water stress regime 'ILC482' showed lowest A and mesophyll conductance. Therefore, it can be concluded that under drought stress condition photosynthesis is limited by stomatal factors (Basu et al 2004).

Chlorophyll

Drought stress imposed at the vegetative stage, significantly decreased chlorophyll a content, chlorophyll b content and total chlorophyll content both at the vegetative and flowering stages, whereas drought stress imposed at anthesis also influenced these contents at flowering. The restricted water supply during the entire vegetative and anthesis stage had a mild effect on these contents. The lack of effects on the chlorophyll a/b ratio indicates that chlorophyll b is not more sensitive to drought than chlorophyll a (Table 1). At the vegetative stage variety ILC482 showed a higher chlorophyll a content than the other varieties (Table 1). At flowering stage, variety Pirouz showed the lowest chlorophyll a content in all four stress treatments. The interactions between variety and drought treatment were not significant. Differences between varieties in chlorophyll b and total chlorophyll content at flowering were not significant. The results are agreement with Nyachiro et al. (2001), who described a significant decrease of chlorophyll a and b caused by water deficit in six Triticum aestivum cultivars. Decreased or unchanged chlorophyll level during drought stress has been reported in other species, depending on the duration and severity of drought (Kpyoarissis et al., 1995). A decrease of total chlorophyll with drought stress implies a lowered capacity for light harvesting. Since the production of reactive oxygen species is mainly driven by excess energy absorption in the photosynthetic apparatus, this might be avoided by degrading the absorbing pigments (Herbinger et al., 2002).

Proline

Variety differences in proline content or interactions between variety and drought treatment were absent. The proline content of the leaf, however, increased at both growth stages in all varieties of chickpea in response to drought (Table 1).

The increase in proline content due to drought stress was more severe at flowering stage than at the vegetative stage. The proline content depends on plant age, leaf age, leaf position or

Table 1. Drought stress induced changes in chlorophyll contents (mg g^{-1} fresh weight) and proline (μ mol g^{-1} fresh weight) of three varieties of chickpea

Treatment	Variety	Chlorophyll a (mg g^{-1}fw)		Chlorophyll b (mg g^{-1}fw)		Total Chlorophyll (mg g^{-1}fw)		Chlorophyll a/b at flowering	Proline (μ mol g^{-1}fw)	
		vegetative	flowering	vegetative	flowering	vegetative	flowering		vegetative	flowering
Control	Bivaniej	1.76 a	1.51 a	0.84 a	0.75 ab	2.61 a	1.98 a	2.05 abc	0.32 b	0.67 c
	ILC482	1.82 a	1.47 ab	0.81 ab	0.77 a	2.53 a	1.96 ab	1.90 bc	0.22 b	1.26 c
	Pirouz	1.76 a	1.45 ab	0.92 a	0.80 a	2.69 a	1.91 ab	1.81 c	0.25 b	0.42 c
Drought during vegetative stage	Bivaniej	1.39 b	1.12 cd	0.55 c	0.45 d	1.94 d	1.57 c	2.49 ab	1.64 a	8.28 ab
	ILC482	1.52 b	0.91 d	0.71 bc	0.45 d	2.32 b	1.79 bc	2.15 abc	1.52 a	9.45 a
	Pirouz	1.48 b	0.91 d	0.64 c	0.49 cd	2.15 c	1.65 c	1.85 c	1.62 a	8.4 ab
Drought during anthesis	Bivaniej	-	1.25 c	-	0.51 cd	-	1.79 bc	2.49 ab	-	7.36 b
	ILC482	-	1.22 c	-	0.53 cd	-	1.64 c	2.32 abc	-	8.29 ab
	Pirouz	-	1.17 c	-	0.54 cd	-	1.86 ab	2.16 abc	-	7.30 b
Drought during vegetative and anthesis phase	Bivaniej	-	1.35 bc	-	0.53 cd	-	1.97 ab	2.55 a	-	1.00 c
	ILC482	-	1.36 abc	-	0.67 ab	-	1.92 ab	2.08 abc	-	1.20 c
	Pirouz	-	1.33 bc	-	0.62 bc	-	1.89 ab	2.38 abc	-	0.59 c

Data represent the mean values of three replicates. Within a column, mean values followed by different letters are statistically different based on Duncan's range test at P = 0.05.

Table 2. Drought stress induced changes in yield (kg/ha), number of pods (# per plant) and shoot height (cm) of three varieties of chickpea

Treatment	Variety	Yield (kg/ha)	Number of pods (# per plant)	Shoot height (cm)
Control	Bivaniej	2099 a	38.6 b	18.1 b
	ILC482	1452 b	34.1 b	22.7 a
	Pirouz	1047 c	45.1 a	15.4 cd
Drought during vegetative stage	Bivaniej	1507 b	13.4 ef	14.0 c
	ILC482	1149 c	16.1 de	15.8 bc
	Pirouz	707 de	20.1 c	11.4 e
Drought during anthesis	Bivaniej	1343 b	12.0 f	17.1 b
	ILC482	1062 c	11.7 f	20.1 ab
	Pirouz	627 e	18.1 cd	15.5 c
Drought during vegetative phase and during anthesis	Bivaniej	812 d	7.2 g	13.4 d
	ILC482	799 d	7.1 g	13.8 cd
	Pirouz	357 f	10.4 fg	11.5 e

Data represent mean values of three replicates. Within columns, mean values followed by different letters are statistically significantly different based on Duncan's range test at P = 0.05.

leaf part (Chiang and Dandekar, 1995). Under vegetative stage, drought stress increased proline content about tenfold, this increasing roles as an osmotic compatible and adjust osmotic potential which resulted in drought stress avoidance in chickpea. Prolin accumulation is believed to play adaptive roles in plant stress tolerance (Verbruggen and Hermans 2008). Accumulation of proline has been advocated as a parameter of selection for stress tolerance (Yancy et al., 1982. Jaleel et al., 2007).

Yield

The yield response to drought stress of chickpea is given in Table 2. The yield of all three varieties of pea was affected by drought stress. Plants stressed at the vegetative stage , but not stressed subsequently, gave a significantly higher yield than plants stressed during anthesis or during the vegetative stage and anthesis. The highest yield (under optimal and drought stress conditions) was obtained from 'Bivaniej'. The losses in yield in response to stress treatment were: 61% for 'Bivaniej', 45% for 'ILC482', and 66% for 'Pirouz'. However, interactions between cultivars and drought treatment were significant. Seed yield under drought stress at anthesis stage showed 10% less than that under drought treatment at vegetative stage.

Pod number and plant height

Drought had a significant effect on the number of pods and on plant height. Plants were usually tallest and had the highest number of pods when they were grown without drought stress. The effects of the drought during the vegetative phase and during the anthesis stage on the number of pods were more or less additive, but this was not true for the effects on the shot height (Table 2). Averaged across treatments 'Pirouz' showed the highest pod number and the shortest plants (Table 2). Although Pirouz had the highest pod numbers, it had the lowest yield (Table 2), probably due to decrease in percentage of filled pod and 1000 grain weight. The decrease in yield of grain legumes grown under drought conditions is largely due to the reduction in the number of pods per plant (Lopez *et al*., 1996; Pilbeam *et al*., 1992).

Conclusion

All physiological parameters responses of drought adapted (Bivaniej and ILC482) and drought sensitive (Pirouz) varieties chickpea to limited Water supply showed similar patterns: decreased chlorophyll a, b , a/b concentrations , transpiration, stomatal conductance and yield were associated with increased proline. Differences between varieties were mainly found in water Relation parameters, which indicates adaptations in physiology (stomata) or osmotic adjustments. Proline (Pro) accumulation is a common physiological response in many plants in response to drought stress. Photosynthesis is limited by drought stress due to stomatal (stomatal closure) and non-stomatal (impairments of metabolic processes) factors. The drought stress imposed in this study affected the vegetative growth of both, yield and pod of the pea plants, however yield was the most affected, limiting significantly the number of pod.

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