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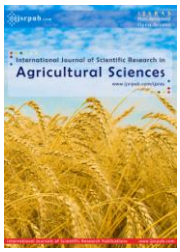

Full Length Research Paper

Physiological Studies on Moisture Stress Tolerance in Chickpea (*Cicer Arietinum* L.) Genotypes

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Abstract. Drought is a major constraint that limits seed yield in chickpea (*Cicer arietinum* L.). It is important to establish the relative importance many of these drought-related traits for prioritizing their consideration in breeding for drought tolerance improvements. The objective of this study was to categorize the drought tolerant and susceptible chickpea genotypes on the basis of physiological parameters. An experiment was conducted with five chickpea genotypes in field under irrigated and rain fed conditions. Observations were recorded on relative water content (RWC), and the contents of chlorophyll (Chl), carotenoid (Car), proline (Pro) and protein in the five chickpea genotypes. RWC and contents of Chl, Car and protein decreased under moisture stress, whereas Pro content increased with the increase in moisture stress. Pusa-1108, Pusa-362, Pusa-1103 were able to maintain relatively higher RWC, Chl, Car and protein content and greater Pro accumulation, while Flip 90-166 and SBD-377 showed comparatively greater decline in the RWC, Chl, Car and Protein and less accumulation of Pro under moisture stress. The data suggest that chickpea genotypes can be categorized into drought tolerant and susceptible types based on the variations in RWC, Chl, Car, Pro and protein content.

Keywords: Carotenoid, chickpea, chlorophyll, moisture stress, proline, protein, relative water content

1. INTRODUCTION

Chickpea (*Cicer arietinum* L.) is world's second most cultivated grain legume grown over 10.2 million hectare. Chickpea is grown as a winter crop in the Indian subcontinent, which accounts for nearly 85% of the chickpea area sown worldwide. It is also an important crop in West Asia and Mediterranean region. In India chickpea is generally grown using stored soil moisture after rainy season. Moisture stress is the most prevalent environmental factor limiting plant growth, survival and productivity in chickpea (Bohnert and Jenson, 1995). Moisture deficit affects seed germination and seedling establishment in the field, however, genotypes vary in their capacity to tolerate moisture stress. Chickpea is believed to be tolerant to drought condition, but there is little published evidence to support this contention (Saxena, 1984).

Relative water content (RWC) is one of the important parameter to measure water status of the tissue (Barrs and Weatherley, 1962). Gradual decrease in RWC with increase in stress and greater reduction afterwards under severe stress has been reported in chickpea (Deshmukh et al., 2000). High RWC under moisture stress denotes ability of plants to tolerate moisture stress (Uprety and Sirohi, 1987, Ritchi et al., 1990).Under moisture stress conditions tolerant types show less reduction in RWC as compared with susceptible ones (Sairam et al., 1997). Baisak et al. (1994) reported the decline in chlorophyll content with water stress. Reduction in chlorophyll content upon exposure to oxidative stress and a comparatively higher chlorophyll content in tolerant wheat and maize genotypes than susceptible ones has also been reported (Kraus et al., 1995). Sairam (1994) reported that under moisture stress chlorophyll content and chlorophyll stability index were higher in tolerant wheat genotypes in comparison to the susceptible

genotypes. Rahangdale et al. (1995) observed genotypic differences in chickpea for chlorophyll content under water deficit conditions. However, tolerant genotypes maintained relatively higher chlorophyll content.

Carotenoid provides protection against oxidative damage (Schmitz and Noga, 1997). Jiang et al. (1991) reported that carotenoids decreased markedly in rice plants on induction of water stress. They further reported that under osmotic stress the activities of endogenous protective enzyme systems and the contents of ascorbic acid and carotenoids were negatively correlated with membrane lipid peroxidation. Osmolytes are involved in signalling /regulating plant response to multiple stresses, including reduced growth, which may be part of plant's adaptation against stress. Among all osmolytes, proline is probably most widely distributed, and its accumulation seems to be involved in the process of adaptation to osmotic stress (Yoshiba et al., 1997). It has been shown to play an important role in ameliorating drought, salinity and heavy metal stresses (Andrade et al., 1995). The present study was, therefore, conducted to study the variability in RWC, Chl, Car, Pro and protein content in tolerant and susceptible chickpea genotypes.

2. MATERIALS AND METHODS

2.1. Sample preparation

An experiment was conducted with five chickpea genotypes *viz.* Pusa-362, Pusa-1103, SBD-377 (*desi*) and Pusa-1108, Flip 90-166 (*kabuli*)grown under irrigated (control) and rain fed (moisture stressed) environments for two seasons following the recommended package of practices. Pre-sowing irrigation was provided to ensure proper germination. In control plots irrigation was provided as and when required to avoid any stress to the crop, while under rain fed plantings no irrigation was given during entire crop season. The various parameters were studied at two growth stages of the crop *viz.* flowering and pod formation stage. Experiment was laid down in split plot design with 3 replications and the pooled data of two years was analysed by factorial randomised block design.

2.2. Laboratory determinations

Relative water content (RWC) was determined in flag leaf as per the method of Barrs and Weatherley (1962). Hundred mg leaf samples was taken and kept in distilled water in a petridish for four hours to make the leaf tissue turgid. The turgid weights of the leaf material were taken after carefully soaking the tissues between two filter papers, subsequently the leaf material was kept in a butter paper bag and dried in oven at 65 ^{0}C for 24 h and their dry weights were recorded. The RWC was calculated by using the formula given below:

$$\text{RWC (\%)} = \frac{\text{Fresh weight - Dry weight}}{\text{Turgid weight - Dry weight}} \times 100$$

Pigment extraction was done by the method of Hiscox and Israelstam (1979). Fifty mg of leaf tissue from fully emerged leaf was incubated in 10 ml of dimethyl sulphoxide (DMSO) for 4 h at 60 ^{0}C in an incubator. The absorbances (A) values were recorded at 645, 663 and 470 nm in a digital spectrophotometer (model: Specord 200). The amount of chlorophyll 'a', chlorophyll 'b' and total chlorophyll were calculated as per the formula given by Arnon et al. (1949) and the amount of carotenoid were calculated using the formula given by Lichtenthaler and Wellburn (1983).

Chlorophyll 'a' = [12.7 (A663) - 2.69(A645)] x V/(W x 1000)
Chlorophyll 'b' = [22.9 (A645) - 4.68(A663)] x V/(W x 1000)
Total Chlorophyll = [22.2 (A645) + 8.02(A663)] x V/(W x 1000)
where, A663 and A645 are the absorbance values at 663 and 645 nm respectively; W = weight of the sample in mg; V = volume of the solvent used (ml)
Carotenoid= [1000 x A_{470} – (3.27 x Chl a + 104 x Chl b)]
where, $A_{470}$is the absorbance value at 470 nm; Chl 'a' = Chlorophyll 'a'; Chl 'b' = Chlorophyll 'b'

Proline content was estimated in leaf tissues as per the protocol given by Bates et al. (1973). Fresh leaf sample was taken and homogenized with 10 ml sulpho-salicylic acid in chilled mortar and pestle. The homogenate was centrifuged at 10,000 rpm for 10 min. 0.2 ml of supernatant was taken in test tube to

which 2 ml of acid ninhydrin and 2 ml of glacial acetic acid were added. The resultant mixture was boiled at 100^0C in a water bath. The reaction was stopped by keeping the test tubes in an ice bath. Then, 4 ml of toluene was added to each tube and mixed vigorously on a vortex for 10-15 sec in order to facilitate quick diffusion/movement of chromophores from the aqueous phase to non-aqueous phase. The toluene layer (upper) was separated from the mixture and absorbance was read at 520 nm on a spectrophotometer using toluene as blank.

Soluble protein was estimated as per the method given by Lowry et al. (1951). Fresh leaf material (1 g) was homogenized in 10 ml extraction buffer (0.1 M phosphate buffer, pH 7.5, containing 0.5 mM EDTA) with the help of pre chilled mortar and pestle. The brie was passed through a 4 layers of cheese cloth, and the filtrate was centrifuged for 20 minutes at 15000 g (4^oC) and supernatant was used0.1 ml supernatant was mixed with 3.8 ml DDW, 1.0 ml reagent 'D' then shaken immediately and kept for 20 minutes. Added 0.1 ml 1:1 diluted (with DDW) Folin-ceo-catleu reagent and shaken immediately vigorously and leave for 20 minutes. Finally read OD at 500 nm.

Table 1: Effect of moisture stress on relative water content (%) in chickpea genotypes during flowering and pod formation stages (pooled data of 2006-07 & 2007-08).

Genotypes	Flowering stage			Pod formation stage		
	Irrigated	Rainfed	% Decrease	Irrigated	Rainfed	% Decrease
Pusa-1108	84.41±2.60	80.25±2.21	4.92	78.85±2.05	73.36±1.88	6.96
Pusa-362	90.22±2.58	86.33±2.35	4.31	86.33±2.24	80.51±2.20	6.74
Pusa-1103	85.96±2.51	80.11±2.18	6.81	87.49±2.20	81.07±2.12	7.34
Flip 90-166	80.95±2.31	65.23±1.98	19.42	68.09±2.02	52.64±1.68	22.69
SBD-377	83.99±2.49	63.81±1.96	24.03	74.64±2.08	55.36±1.62	25.83
Mean	**85.11**	**75.15**	**11.90**	**79.08**	**68.59**	**13.88**

CD at 5%: Stages (S)=0.886; Irrigation (I) = 0.886; S x I = 1.252; Genotype (G) = 1.401; G x S = 1.981; G x I =1.981; G x S x I = 2.807

Table 2: Effect of moisture stress on total chlorophyll content (mg g-1 dry wt.) in chickpea genotypes during flowering and pod formation stages (pooled data of 2006-07 & 2007-08).

Genotypes	Flowering stage			Pod formation stage		
	Irrigated	Rainfed	% Decrease	Irrigated	Rainfed	% Decrease
Pusa-1108	10.47±0.35	9.70±0.33	7.35	9.03±0.30	8.19±0.28	9.30
Pusa-362	12.25±0.41	11.47±0.39	6.37	10.68±0.28	9.82±0.33	8.05
Pusa-1103	11.27±0.38	9.60±0.32	14.82	10.88±0.27	9.84±0.33	9.56
Flip 90-166	10.54±0.28	7.94±0.27	24.67	10.25±0.27	7.45±0.20	27.32
SBD-377	12.71±0.43	9.11±0.24	28.32	11.83±0.31	7.59±0.26	35.84
Mean	**11.45**	**9.56**	**16.31**	**10.53**	**8.58**	**18.01**

CD at 5%: Stages (S) = 0.220; Irrigation (I) = 0.220; S x I = 0.311; Genotype (G) = 0.348; G x S = 0.492; G x I = 0.492; G x S x I = 0.697

3. RESULTS AND DISCUSSIONS

3.1. Relative water content

Chickpea crop responds to water deficit in the form of changes in various physiological and biochemical processes. Under mild stress the changes at physiological and biochemical level may not manifest morphologically, while under severe stress the physiological changes may lead to various morphological changes. Some of the biochemical and physiological changes observed under moisture stress are consequence of deleterious effects of water deficit on important metabolic processes.

Results on relative water content (RWC) under irrigated and moisture stress (rain fed) conditions are reported in table 1. Significant reduction was observed in RWC under moisture stress in all the cultivars. Under moisture stress condition the highest RWC at flowering stage was observed in Pusa-362 (86.33%) and at pod formation stage in Pusa-1103 (81.07%), while the lowest RWC was observed in SBD-377 (63.81%) and Flip 90-166 (52.64) at flowering and pod formation stages, respectively. The results are in agreement with the findings of Ritchi et al. (1990), who reported that drought resistant genotypes of wheat maintain high RWC under moisture stress.

Significant differences in RWC / water potential in tolerant and susceptible genotypes of maize (Pastori and Trippi, 1992) and wheat (Kraus et al., 1995; Sairam et al., 1997; Sairam and Srivastava, 2001) have also been reported.

3.2. Total chlorophyll content

Data on total chlorophyll content are presented in table 2. There was significant decrease in total chlorophyll content under moisture stress in all the genotypes. The moisture stress induced decrease in the chlorophyll content in gerbera was also reported by Qi-Xian et al. (2007).The percent reduction in chlorophyll was maximum in SBD-377 at both the stages (28.32% and 35.84%at the flowering pod formation stages, respectively), followed by Flip 90-166 (24.67% and 27.32% at flowering and pod formation stages, respectively), Pusa-1103 (14.81% and 9.56% at flowering and pod formation stages, respectively), Pusa-1108 (7.35% and 9.30% at flowering and pod formation stages, respectively) and the least reduction was observed in Pusa-362 i.e. 6.37% and 8.05% at flowering and pod formation stages, respectively. Higher chlorophyll content and lower percent decline under moisture stress in comparatively tolerant genotypes of wheat (Kraus et al., 1995, Sairam et al., 1997, 1998) and maize (Pastori and Trippi, 1992) have also been reported.

Table 3: Effect of moisture stress on carotenoid content (mg g-1 dry wt.) in chickpea genotypes during flowering and pod formation stages (pooled data of 2006-07 & 2007-08).

Genotypes	Flowering stage			Pod formation stage		
	Irrigated	Rainfed	% Decrease	Irrigated	Rainfed	% Decrease
Pusa-1108	2.90±0.10	2.49±0.09	14.14	2.57±0.09	2.20±0.08	14.40
Pusa-362	3.18±0.11	2.74±0.10	13.84	2.77±0.10	2.40±0.08	13.36
Pusa-1103	3.05±0.11	2.56±0.09	16.07	2.72±0.09	2.60±0.09	4.41
Flip 90-166	2.82±0.10	2.08±0.07	26.24	2.71±0.10	1.96±0.07	27.68
SBD-377	3.31±0.11	2.42±0.08	26.89	3.05±0.11	2.10±0.07	31.15
Mean	**3.05**	**2.46**	**19.43**	**2.76**	**2.25**	**18.20**

CD at 5%: Stages (S) = 0.046: Irrigation (I) = 0.046; S x I = 0.065; Genotype (G) = 0.073; G x S = 0.104; G x I = 0.104; G x S x I = 0.147

Table 4: Effect of moisture stress on proline accumulation (mg g-1 dry wt.) in chickpea genotypes during flowering and pod formation stages (pooled data of 2006-07 & 2007-08).

Genotypes	Flowering stage			Pod formation stage		
	Irrigated	Rainfed	% Increase	Irrigated	Rainfed	% Increase
Pusa-1108	15.22±0.43	49.10±1.38	222.60	34.40±0.96	85.60±2.40	148.84
Pusa-362	20.67±0.62	55.04±1.54	166.28	33.80±1.02	85.00±2.38	151.48
Pusa-1103	18.70±0.52	49.26±1.48	163.42	35.19±0.99	78.20±2.35	122.22
Flip 90-166	19.83±0.60	23.80±0.67	20.02	38.00±1.14	47.50±1.43	25.00
SBD-377	23.88±0.67	25.30±0.71	5.95	43.40±1.30	52.90±1.48	21.89
Mean	**19.66**	**40.50**	**115.65**	**36.95**	**69.84**	**93.89**

CD at 5%: Stages (S) = 0.806; Irrigation (I) = 0.806; S x I = 1.140; Genotype (G) = 1.275; G x S = 1.803; G x I =1.803; G x S x I = 2.551

Table 5: Effect of moisture stress on protein content (mg g-1fresh wt.) in chickpea genotypes during flowering and pod formation stages (pooled data of 2006-07 & 2007-08).

Genotypes	Flowering stage			Pod formation stage		
	Irrigated	Rainfed	% Decrease	Irrigated	Rainfed	% Decrease
Pusa-1108	17.50±0.59	14.70±0.50	16.00	10.21±0.34	8.20±0.28	19.69
Pusa-362	17.16±0.61	13.60±0.46	20.75	10.21±0.36	7.76±0.26	23.99
Pusa-1103	16.70±0.56	13.60±0.48	18.56	9.48±0.32	7.37±0.26	22.26
Flip 90-166	15.40±0.54	11.40±0.38	25.97	9.08±0.32	5.80±0.21	36.12
SBD-377	14.70±0.50	10.50±0.35	28.57	8.80±0.31	5.97±0.20	32.16
Mean	**16.29**	**12.76**	**21.97**	**9.55**	**7.02**	**26.84**

CD at 5%: Stages (S) = 0.205; Irrigation (I) = 0.205; S x I = 0.290 = Genotype (G) = 0.324; G x S = 0.458; G x I = 0.458; G x S x I = NS

3.3. Carotenoid content

Results on carotenoid content recorded at flowering and pod formation stages under irrigated and moisture stress conditions are presented in table 3.Moisture stress resulted in decrease in carotenoid content in all the genotypes. The results are in agreement with the findings of Sgherri et al. (1996) in sunflower, Sairam and Saxena (2000) in wheat and Qi-Xian et al. (2007) in gerbera. Reduction in carotenoid content under moisture stress was maximum in SBD-377 at both flowering and pod formation stages (26.89 and 31.15%) followed by Flip 90-166 (26.24 and 27.68%), Pusa-1103 (16.07 and 14.41%) and Pusa-1108 (14.14

and 14.40%). The minimum reduction in carotenoid content was observed in Pusa-362 at both the stages i.e. 13.84 and 13.36% at flowering and pod formation stages, respectively. Pigment bleaching under stress is caused by singlet oxygen. Carotenoids are responsible for the scavenging of singlet oxygen (Knox and Dodge, 1985) hence their comparatively less reduction in genotype will determine its relative tolerance.

3.4. Proline content

Significant genotypic variations were observed in proline content under irrigated (control) and rain fed conditions (Table 4). Proline content increased with age as well as under waster stress in all the genotypes. Increase in proline content in bermuda grass (Baynett and Naylor, 1966), barley (Singh et al., 1972), maize (Moussa and Abdel-Aziz, 2008) and mulberry (Ramanjulu and Sudhakar, 2000) has also been reported.

The increase in proline content under moisture stress over irrigated control at flowering stage was highest in Pusa-1108 (3.23 times), while at pod formation stage the highest increase was recorded in Pusa-362 (2.5 times). Pusa-1103 and Flip 90-166 showed 2.63 and 1.2 times increase at flowering stage, and 2.22 and 1.25 times increase at pod formation stage, while SBD-377 showed minimum increase at flowering and pod formation stages (1.06 and 1.22 times).An overall assessment of proline accumulation pattern under moisture stress condition showed that Pusa-1108 was superior at flowering stage, while Pusa-362 was superior at pod formation stage. Pusa-1103 was at third position at both the stages followed by Flip 90-166, while SBD-377, which showed lowest accumulation at both the stages. Ramanjulu and Sudhakar (2000) in mulberry reported that drought tolerant genotypes accumulated higher proline than susceptible types. Higher accumulation of proline seems to be involved in the process of adaptation to osmotic stress in many glycophytic plant species (Yoshiba et al., 1997). Direct evidence for a function of proline under osmotic stress has been provided by over expression of pyrroline-5-carboxylate synthetase in transgenic tobacco that contained elevated levels of proline and exhibited increased tolerance to osmotic stress (Kavikishor et al., 1995). Proline accumulation helps to maintain turgor and promotes continued growth under moisture stress condition (Mullet and Whitstitt, 1996).

3.5. Protein content

There was significant reduction in protein content in all the genotypes under moisture stress condition (Table 5). The decrease in protein content under rain fed condition was 16 and 19.67; 20.75 and 23.99; 18.56 and 22.26; 25.97 and 36.12; 28.57 and 32.16% at flowering and pod formation stages in Pusa-1108, Pusa-362, Pusa-1103, Flip 90-166 and SBD-377, respectively. The results are in conformity with the earlier findings in groundnut (Hui Fang and Xiao Ping, 2004) and maize (Mohammadkhani and Heidari, 2008). The decrease in protein content under moisture stress have also been reported in *Tortula* (Dhindsa, 1991), *Brassica napus* (Good and Zaplachinski, 1994), gerbera (Qi-Xian et al. 2007) and sesame (Fazeli et al., 2007).The reduction in protein content in all the cultivars could be due to decline in nitrate assimilation resulting in decline in reduced-N and consequently decrease in protein synthesis (Chandrashekhar et al., 2000). It could also have resulted from a ROS induced protein denaturation (Schwanz et al., 1996). The reduction in protein synthesis is also related to a decrease in the number of polysomes (Creelman et al., 1990). Drought induced decline in photosynthesis (Havuax et al., 1987) could also result in a reduction in carbon skeleton for amino acid resulting in a decrease in protein synthesis (Mohammadkhani and Heidari, 2008).

4. CONCLUSION

From the results it is evident that there were distinct variations among the genotypes in terms of decline in RWC and contents of chlorophyll, carotenoid, protein and increase in proline content under moisture stress. Considering the least decline in RWC, chlorophyll, carotenoids and protein contents and highest accumulation of proline, the genotypes Pusa-1108, Pusa-362 and Pusa-1103 could be considered as drought tolerant, while Flip 90-166 and SBD-377, which showed highest decline in RWC, contents of chlorophyll, carotenoids and protein, and lowest accumulation of proline as drought susceptible.

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