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MORPHO-PHYSIOLOGICAL RESPONSES OF CHICKPEA (*CICER ARIETINUM* L.) GENOTYPES UNDER DROUGHT STRESS

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Abstract: The physiological and morphological traits related to drought tolerance and to fix the criteria for reliable screening of drought tolerant chickpea genotypes, thirteen chickpea genotypes with seven checks were grown under control and drought stress in rainout shelter. The effect of drought stress on total chlorophyll content, stomatal conductance, transpiration rate, photosynthesis rate and number of pods at pre and post flowering stages was evaluated. A close relationship between plant growth, leaf water status and chlorophyll content was observed. In this study we recorded less percent reduction in total chlorophyll content, stomatal conductance, transpiration rate and photosynthesis rate in some wilt resistant genotypes JG 552476, JG 2001-4, JG 24 which was comparable to drought tolerant check ICC 4958. The entire drought related physiological parameters recorded slightly more decrease at postflowering stage of growth as compared to preflowering stage.
Keywords: Chickpea, Genotypes, Drought, Stress, Postflowering stage, Control.

Introduction

Chickpea (*Cicer arietinum* L.) is grown in many parts of the world and yields a total of about 9.8 M t from an area of 11.1 M ha (FAO STAT 2009). Despite the high yield potential of chickpea of over 6000 kg ha^{-1} (Singh 1990), the actual yields are significantly lower considered to be due to a combination of biotic and abiotic stresses (Singh 1993). Among the major chickpea producer countries, India, Pakistan, Turkey and Iran, most growing areas are classified as arid or semi-arid (Anonymous 2011). In these regions, chickpea is generally grown under rainfed conditions either on stored soil moisture in subtropical environments with summer-dominant rainfall or on current rainfall in winter-dominant Mediterranean type environments. In both environments, nonirrigated chickpea plantations suffer yield losses from terminal drought (Yadav *et al*. 2006; Toker *et al*. 2007). Kumar and Abbo (2001) reported that about 90% of the world's chickpea is grown under rainfed conditions where terminal drought is the major stress, accompanying with high temperature stress. Although chickpea is more drought-resistant than other cool-season food

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legumes, drought is the most important yield reducer in this crop (Johansen *et al*., 1994b).Deleterious responses to drought can include reduction of growth, decrease in chlorophyll, increase in hydrogen peroxide, which causes lipid peroxidation and consequently membrane injury (Mukherjee and Choudhuri 1983).

It is recognized that resistant plants under water stress conditions developed various physiological and biochemical responses of adaptive nature. These include changes of water use efficiency, pigment content, osmotic adjustment and photosynthetic activity (Dhanda *et al*. 2004; Serraj *et al*. 2004, Praba *et al*. 2009). These mechanisms play a key role in preventing membrane disintegration and provide tolerance against drought and cellular dehydration (Hanson and Hitz 1982, Bohnert and Jensen 1996; Mahajan and Tuteja 2005). Photosynthetic pigments play an important role in light harvesting and dissipation of excess energy. It is known that the content of both chlorophyll a and b changes under drought stress (Farooq *et al*. 2009). As a major crop, wheat has gained special attention with respect to morphological and physiological characters and traits affecting drought tolerance, but there is not enough information for chickpea about the relevant parameters and their relationships with drought susceptibility index (DSI) among chickpea cultivars.

As mechanisms of responses to drought stress varies with genotypes and growth stages of individual plants (Ashraf and Harris 2004). Knowledge on interrelationships among various physiological responses to dehydration can offer insight for developing useful strategies to improve drought stress tolerance in chickpea. The identification of suitable plant characters for screening large numbers of genotypes, in a short time at critical stages of crop growth, with the aim of selecting drought tolerant cultivars, remains a major challenge to the plant breeder. The present study was undertaken to assess the selection criteria for identifying drought tolerance in chickpea genotypes based on physiological attributes, so that suitable genotypes can be recommended for cultivation in the drought prone area.

Material and Methods

Seeds of thirteen genotypes with seven checks of chickpea were obtained from All India Co-ordinated Pulses Improvement Project, MPKV, Rahuri. The seeds were surface sterilized with 0.1% (v/v) sodium hypochlorite solution for 15 min, washed thoroughly with sterile distilled water and sown under rainout shelter in completely randomized block design. Pre and post flowering stress was created by withholding one irrigation at 40 DAS for pre flowering stress and 60 DAS at post flowering stage. After withholding irrigation seven days stress cycle was given, after seven days stress cycle leaf samples of control and stressed plants were evaluated for total chlorophyll content, transpiration rate, stomatal conductance photosynthesis rate and number of pods per plant.

Total leaf chlorophyll content of chickpea leaves was determined by the method described by Arnon (1949). The leaf samples of chickpea were cut into small pieces, known weight (0.2g) of fresh leaf sample was macerated in a mortar and pestle and extracted with 10 ml of 80 per cent acetone. The contents were centrifuged at 5000xg for 10 min and the supernatant was collected. The above steps were repeated until the residue became colourless. The final volume of extract was made to 50 ml. The extinction of chlorophyll extract was recorded at 645 and 663 nm on a spectrophotometer and a blank was run with 80 per cent acetone. The amount of total chlorophyll content was calculated by using the following formula and expressed in mg g^{-1} FW.

Total chlorophyll, mg g^{-1}FW = 20.2 (A645) + 8.02 (A 663) x V/ (1000 x W) Where, A= absorbance at specific wavelength

V= Final volume of chlorophyll extract in 80 % acetone

W= Fresh weight of tissue extracted in g.

The net photosynthetic rate (µmol CO_2 m^{-2} s^{-1}), transpiration rate (mmol H_2O m^{-2} s^{-1}) and stomatal conductance (mol H_2O m^{-2} s^{-1}) were measured using Infra-Red Gas Analyzer (IRGA; Model Portable Photosynthesis System LI 6400, LI-COR® Inc, Lincoln, Nebraska, USA). The transpiration rate and stomatal conductance were measured continuously monitoring H_2O of the air entering and existing in the IRGA headspace chamber and the measurements were made at mid day, between 11:30 and 12:00 eastern day time (1400–1800mmolm^{-2}s^{-1}PPFD), on top fully expanded leaf blades. The flow-rate of air in the sample line was adjusted to 500 µmol s^{-1}. The numbers of pods were counted from three randomly selected plants for each treatment and then the average was calculated. All the physiological parameters were analyzed in three replications. The data obtained for biochemical constituents and enzymes determination were subjected to simple completely randomized block design for significance of various data (Panse and Sukhatme, 1985).

Results and Discussion

Several attributes are related to drought tolerance, though also physiological attributes studied in this experiment are important for evaluation of genotypes under drought stress at different growth stages. Water deficit significantly decreased total chlorophyll content, net photosynthesis, stomatal conductance and transpiration at both the growth stages.

The mean total chlorophyll content of the stressed leaf samples decreased from 2.51 to 1.76 mg g^{-1}FW at preflowering stage, while it decreased from 2.08 to 1.38 mg g^{-1}FW at post flowering stage. Amongst the chickpea genotypes evaluated for chlorophyll content wilt susceptible genotype GL29285 recorded maximum reduction in chlorophyll content of 44.06 percent while wilt resistant genotype JG 552476 recorded minimum chlorophyll reduction of (19.56%) Wilt susceptible genotype PBG 5 recorded less chlorophyll reduction (25.87%) at preflowering stage. In genotype JG 552476 chlorophyll content decreased from 2.66 to 2.14 mg g^{-1}FW at preflowering stage under stressed condition (Table 1). Leaf samples of Vijay and ICC 4958 drought checks also recorded minimum reduction in total chlorophyll content at both the plant growth stages under stressed condition. Water stress resulted in the significant reduction of total chlorophyll content in chickpea genotypes but the magnitude of reduction was relatively higher at pod formation stage (Kataria and Singh, 2013). Ghiabi *et al*., 2013 reported that total chlorophyll content decreased in Kabuli chickpea accessions grown under rainfed conditions against irrigated conditions.

The mean stomatal conductance was decreased from 0.328 to 0.243 mol H_2O $m^{-2}s^{-1}$ at preflowering drought stage from control to stressed plants while it was decreased from 0.156 to 0.109 mol H_2O m^{-2} s^{-1} at post-flowering stage under drought stress. Wilt tolerant genotype JG552476 and JG2001-4 showed minimum decrease in stomatal conductance under drought situation at both the growth stages (Table 2). Maximum reduction in stomatal conductance was recorded in JG 62, Vikas and GL29285 under moisture stress at pre and postflowering stage. Hirich *et al*. (2014) revealed that chickpea genotypes exposed to increasing irrigation water salinity, exhibited a significant decrease in stomatal conductance both during growth period and during the day. Stomatal conductance significantly decreased in all three chickpea cultivars when they were imposed to drought stress (Mafakheri *et al*., 2010).

Genotype JG 24 recorded minimum reduction in transpiration rate from 5.35 to 4.79 mmol H_2O $m^{-2}s^{-1}$ (10.46 % reduction) under stressed conditions at preflowering stage. Wilt susceptible checks JG 62, Vikas with wilt susceptible genotypes GL29285 and GL 27014 recorded maximum reduction in transpiration rate. Wilt resistant genotypes JG 2000-7, JG 2-14-110 and GJG 0904 recorded maximum reduction in transpiration rate at pre and post-flowering stage (Table 3). The effect of drought stress on transpiration was very similar to that on photosynthesis. Better water supply resulted in significantly higher stomatal conductance, net photosynthesis and transpiration rate. Mafakheri *et al*. (2010) reported that transpiration rate was significantly decreased under drought stress as compared to control conditions in three chickpea cultivars *viz.*, Bivaniej, ILC 482 and Pirouz.

The mean photosynthesis rate of wilt resistant and susceptible genotypes with checks decreased from 16.89 µmolCO_2 $m^{-2}s^{-1}$ (control) to 13.42 µmolCO_2 $m^{-2}s^{-1}$ (water stress) at preflowering stage. Maximum reduction in photosynthesis rate was observed in GL 29285 from 16.17 to 10.08 µmolCO_2 $m^{-2}s^{-1}$ at preflowering stage while minimum reduction in photosynthesis rate was recorded in JG 552476 from 17.05 to 15.10 µmol CO_2 $m^{-2}s^{-1}$ under drought condition at preflowering stage (Table 4). Photosynthesis rate was decreased from control to stressed plants but maximum reduction was recorded at post flowering stage of crop growth. Decreased photosynthesis as a result of the drought stress has been reported in three varieties of chickpea (Mafakheri *et al*., 2010).Khan *et al*. (2010) observed that the linseed plants exposed to NaCl stress exhibited reduction in net photosynthesis. Amdoun genotype of chickpea was least affected by drought, followed by Kesseb and Chetoui. Net photosynthesis decreased by 33%, 48% and 51%, respectively, in Amdoun, Kesseb, and Chetoui (Krouma, 2010).

The mean number of pods per plant decreased from 69.22 to 55.08 at preflowering drought stage under stress condition, while number of pods decreased from 69.22 to 62.77 at post-flowering drought stress. Minimum number of pods was decreased in JG 2001-4 from 78 to 68.67 and from 78 to 75.20 respectively at pre- and post flowering stress. Amongst the chickpea genotypes JG 2001-4, JG552476 and JG 24 showed minimum reduction in number of pods per plant at preflowering and post-flowering drought conditions (Table 5). Jeena *et al.* (2005) studied the variability and correlation coefficients in 80 genetically diverse chickpea genotypes. High amount of genetic variability was expressed by pods per plant, l00 seed weight, biological yield per plant and seed yield per plant.

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Table 1. Effect of water stress on total chlorophyll content in the leaves of chickpea genotypes at pre and post-flowering stages under rainout shelter

Sr. No.	Genotype	Pre flowering Stage		% Decrease	Post flowering stage		% Decrease
		Control	Stress		Control	Stress	
	Total chlorophyll (mg g^{-1} FW)						
	Wilt resistant						
1	GJG 0919	2.74	2.12	22.62	2.62	1.88	28.24
2	JG 552476	2.66	2.14	19.56	2.18	1.54	29.49
3	GJG0922	2.59	2.03	21.83	2.20	1.54	29.85
4	IPC1048	2.39	1.32	22.88	2.14	1.48	30.84
5	GJG0904	2.12	1.22	23.51	1.87	1.24	33.86
6	JG 2-14-110	2.34	1.26	24.79	1.90	1.20	36.84
7	BCP2010-1	2.28	1.54	28.07	1.78	1.22	31.46
8	JG 2001-4	3.22	2.54	21.12	2.94	2.02	31.29
9	JG 24	3.02	2.35	22.18	2.42	1.62	33.06
10	JG 2000-7	2.30	1.40	39.12	1.64	1.17	28.66
	Wilt Susceptible						
11	GL 29285	1.88	1.05	44.06	1.52	0.70	53.95
12	PBG 5	2.58	1.91	25.87	1.91	1.34	30.18
13	GL 27014	2.44	1.45	40.47	1.82	0.84	53.85
	Checks						
14	Vijay	2.98	2.37	20.50	2.54	1.96	22.93
15	Digvijay	3.01	2.39	20.66	2.54	1.90	25.12
16	ICC 4958	3.15	2.50	20.63	2.75	2.17	20.97
17	SAKI9516	2.59	1.93	25.24	2.28	1.67	26.67
18	WR 315	1.91	1.30	32.09	1.58	0.89	43.67
19	Vikas	1.88	1.09	41.99	1.48	0.66	55.41
20	JG62	2.10	1.21	42.17	1.58	0.64	59.49
	Mean	**2.51**	**1.76**		**2.08**	**1.38**	
	Comparison	SE±	CD at 5%		SE±	CD at 5%	
1.	Genotype	0.019	0.054		0.030	0.090	
2.	Stage	0.015	0.041		0.025	0.070	
3.	Genotype x stage	0.066	0.184		0.050	0.148	

Table 2. Effect of water stress on stomatal conductance in the leaves of chickpea genotypes at pre and postflowering stages under rainout shelter

Sr. No.	Genotype	Pre flowering Stage		% Decrease	Post flowering stage		% Decrease
		Control	Stress		Control	Stress	
	Stomatal Conductance (mol $H_2Om^{-2}s^{-1}$)						
	Wilt resistant						
1	GJG 0919	0.298	0.236	20.60	0.154	0.122	20.78

2	JG 552476	0.317	0.261	17.68	0.162	0.129	20.16
3	GJG0922	0.311	0.250	19.61	0.148	0.106	28.38
4	IPC1048	0.353	0.268	24.15	0.155	0.110	29.18
5	GJG0904	0.341	0.240	29.62	0.143	0.102	28.84
6	JG 2-14-110	0.364	0.282	22.53	0.167	0.116	30.54
7	BCP2010-1	0.313	0.246	21.32	0.181	0.130	28.04
8	JG 2001-4	0.311	0.256	17.68	0.177	0.142	19.62
9	JG 24	0.341	0.273	19.84	0.190	0.154	18.95
10	JG 2000-7	0.313	0.228	27.08	0.154	0.104	32.47
	Wilt Susceptible						
11	GL 29285	0.328	0.209	36.45	0.147	0.068	53.64
12	PBG 5	0.291	0.221	24.08	0.183	0.139	24.09
13	GL 27014	0.357	0.226	36.64	0.125	0.057	54.26
	Checks						
14	Vijay	0.326	0.270	17.09	0.135	0.101	24.75
15	Digvijay	0.323	0.265	17.77	0.139	0.109	21.15
16	ICC 4958	0.349	0.292	16.32	0.143	0.121	15.81
17	SAKI9516	0.311	0.261	16.06	0.163	0.124	24.08
18	WR 315	0.351	0.234	33.27	0.135	0.086	36.30
19	Vikas	0.329	0.168	48.99	0.157	0.075	52.54
20	JG62	0.331	0.170	48.59	0.161	0.077	51.97
	Mean	**0.328**	**0.243**		**0.156**	**0.109**	
	Comparison	SE±	CD at 5%		SE±	CD at 5%	
1.	Genotype	0.002	0.005		0.001	0.003	
2.	Stage	0.002	0.006		0.002	0.006	
3.	Genotype x stage	0.008	0.024		0.004	0.012	

Table 3. Effect of water stress on transpiration rate in the leaves of chickpea genotypes at pre and postflowering stages under rainout shelter

Sr. No.	Genotype	Pre flowering Stage		% Decrease	Post flowering stage		% Decrease
		Control	Stress		Control	Stress	
	Transpiration rate (m mol $H_2Om^{-2}s^{-1}$)						
	Wilt resistant						
1	GJG 0919	4.22	3.58	15.16	4.07	3.21	21.1
2	JG 552476	5.13	4.50	12.28	4.86	3.72	23.4
3	GJG0922	4.65	4.15	10.89	4.24	3.07	27.6
4	IPC1048	5.22	4.34	16.86	3.83	2.68	29.9
5	GJG0904	4.87	3.98	18.22	3.57	2.48	30.6
6	JG 2-14-110	4.63	3.78	18.42	3.27	2.30	29.5
7	BCP2010-1	4.57	3.76	17.78	3.52	2.48	29.4
8	JG 2001-4	5.35	4.68	12.52	4.61	3.55	23.0
9	JG 24	5.35	4.79	10.46	4.49	3.29	26.7
10	JG 2000-7	4.36	3.56	18.35	3.29	2.38	27.5

	Wilt Susceptible						
11	GL 29285	4.23	3.24	23.40	3.82	1.97	48.5
12	PBG 5	4.51	3.89	13.76	4.16	2.90	30.2
13	GL 27014	4.15	3.02	27.17	4.05	2.22	45.2
	Checks						
14	Vijay	5.81	5.18	10.79	5.29	4.18	20.9
15	Digvijay	5.82	5.20	10.65	5.41	4.27	21.1
20	ICC 4958	5.94	5.32	10.44	5.44	4.26	21.7
17	SAKI9516	4.67	4.08	12.57	4.13	2.94	28.8
16	WR 315	4.19	3.30	21.30	3.68	2.34	36.4
18	Vikas	4.30	3.06	28.84	3.15	1.83	41.9
19	JG62	4.09	2.90	29.15	3.06	1.75	42.8
	Mean	**4.80**	**4.03**		**4.10**	**2.89**	
	Comparison	SE±	CD at 5%		SE±	CD at 5%	
1.	Genotype	0.049	0.138		0.044	0.124	
2.	Stage	0.038	0.107		0.034	0.097	
3.	Genotype x stage	0.170	0.512		0.153	0.452	

Table 4. Effect of water stress on photosynthesis rate in the leaves of chickpea genotypes at pre and postflowering stages under rainout shelter

Sr. No.	Genotype	Pre flowering Stage		% Decrease	Post flowering stage		% Decrease
		Control	Stress		Control	Stress	
	Photosynthesis rate (µmoles$CO_2m^{-2}s^{-1}$)						
	Wilt resistant						
1	GJG 0919	16.47	14.15	14.07	13.23	10.84	18.0
2	JG 552476	17.05	15.10	11.42	12.30	9.84	20.0
3	GJG0922	16.23	13.96	13.98	13.16	10.28	21.8
4	IPC1048	15.43	12.65	18.00	10.22	8.02	21.5
5	GJG0904	17.26	13.94	19.22	10.63	8.10	23.8
6	JG 2-14-110	16.64	13.34	19.83	11.11	8.62	22.4
7	BCP2010-1	17.78	14.20	20.13	10.29	7.69	25.2
8	JG 2001-4	15.82	13.53	14.50	12.33	9.64	21.8
9	JG 24	16.66	14.32	14.04	12.17	9.60	21.1
10	JG 2000-7	18.26	14.44	20.91	10.17	7.64	24.9
	Wilt Susceptible						
11	GL 29285	16.17	10.08	37.65	10.09	6.02	40.3
12	PBG 5	17.55	14.38	18.08	12.30	8.80	28.4
13	GL 27014	15.83	10.33	34.75	10.27	6.10	40.6
	Checks						
14	Vijay	18.83	16.12	14.41	12.84	10.32	19.6
15	Digvijay	18.15	15.64	13.81	12.59	10.02	20.4
20	ICC 4958	18.38	15.94	13.28	13.17	11.04	16.2
17	SAKI9516	17.35	13.98	19.41	11.83	9.34	21.0
16	WR 315	15.68	11.58	26.16	10.07	7.58	24.7

18	Vikas	16.13	10.85	32.70	9.63	6.24	35.2
19	JG62	16.21	9.95	38.61	9.59	5.54	42.1
	Mean	**16.89**	**13.42**		**11.40**	**8.56**	
	Comparison	SE±	CD at 5%		SE±	CD at 5%	
1.	Genotype	0.12	0.34		0.10	0.29	
2.	Stage	0.10	0.30		0.09	0.23	
3.	Genotype x stage	0.42	1.19		0.35	1.05	

Table 5. Effect of water stress on number of pods per plant of chickpea genotypes at pre and postflowering stages under rainout shelter

Sr.No.	**Genotype**	**Pre flowering Stage**		**% decrease**	**Post flowering stage**	**% decrease**
		Control	**Stress**		**Stress**	
	Number of pods/plant					
	Wilt resistant					
1	GJG 0919	79.33	66.55	16.11	75.30	5.08
2	JG 552476	70.67	58.00	17.92	67.67	4.25
3	GJG0922	78.33	67.00	14.47	74.32	5.12
4	IPC1048	68.33	56.33	25.37	61.33	10.24
5	GJG0904	78.00	62.67	25.38	66.67	14.53
6	JG 2-14-110	63.33	51.00	27.49	53.00	16.32
7	BCP2010-1	68.67	55.33	26.89	59.00	14.08
8	JG 2001-4	78.00	68.67	11.97	75.20	3.59
9	JG 24	72.67	61.24	15.72	68.00	6.42
10	JG 2000-7	68.00	55.67	25.99	61.67	9.31
	Wilt Susceptible					
11	GL 29285	60.67	46.33	20.55	54.67	9.89
12	PBG 5	74.67	60.67	18.75	68.00	8.93
13	GL 27014	57.33	42.33	26.17	48.00	16.28
	Checks					
14	Vijay	76.00	66.58	12.39	72.20	5.00
15	Digvijay	67.33	56.32	16.36	63.25	6.06
16	ICC 4958	54.67	44.30	18.97	51.84	5.18
17	SAKI9516	72.00	61.00	15.28	68.00	5.56
18	WR 315	66.00	51.67	26.26	55.33	16.16
19	Vikas	65.67	48.33	26.40	55.33	15.74
20	JG62	64.67	48.00	25.77	56.67	12.37
	Mean	**69.22**	**55.08**		**62.77**	
	Comparison	SE±	CD at 5%		SE±	CD at 5%
1.	Genotype	0.64	1.80		0.34	1.02
2.	Stage	0.50	1.39		0.38	1.20
3.	Genotype x stage	1.15	3.02		1.06	3.18