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Plant water status, canopy temperature and chlorophyll fluorescence in relation to yield improvement in chickpea (*Cicer arietinum L.*) under soil moisture stress environments

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ABSTRACT

The chickpea genotypes alongwith F_4 progeny lines differing in their behavior towards drought stress were evaluated for seed yield, yield attributes and physiological traits related to drought tolerance. Parental genotypes, HC 1 and ICC 4958 were grown under both irrigated and drought conditions and progenies were grown under drought conditions only. Plant water status, relative stress injury (RSI), canopy temperature depression (CTD) and chlorophyll fluorescence (F_v/F_m) were measured at 50% flowering stage under drought conditions. Drought stress decreased the leaf water potential (LWP), leaf osmotic potential (LOP) and relative water content (RWC) in the plants. The water potential, osmotic potential, relative water content and relative stress injury in HC 1 were -1.20 MPa, -1.31 MPa, 59.2% and 31.28% respectively and in ICC 4958 were -1.00 MPa, -1.74 MPa, 66.58% and 20.93% respectively, under drought condition. Increase in CTD was 0.9 ^{0}C in ICC 4958 as compared to 2.06 ^{0}C in HC 1. The ICC 4958 maintained higher F_v/F_m ratio than HC 1 in both irrigated and drought conditions. There is a significant positive correlation between water potential, osmotic potential and RWC with seed yield, whereas, RSI and seed yield were negatively correlated. CTD has a significant negative correlation with WP, OP, RWC and seed yield. The seed yield of HC 1 and ICC 4958 were decreased under drought condition but decrease in yield of genotype ICC 4958 (24.96%) was less than genotype HC 1 (37.32%).

Key words: Leaf water status, chlorophyll fluorescence, drought, yield, chickpea.

Chickpea (*Cicer arietinum L.*) is the third most important food legume which has a total global production of 11.6 m tons from 13.2 m ha (FAO 2012). Chickpea is an important self-pollinated grain legume crop, grown mainly in West Asia, North Africa and the Indian subcontinent. About 90% of world's chickpea is grown under rainfed conditions, where the crop grows and matures on a progressively depleting soil moisture profile (Kashiwagi, *et al*., 2013) and generally experiences terminal drought stress. Terminal drought stress is one of the major constraints limiting chickpea productivity and yield stability. It reduces the productivity by alteration in morpho- physiological metabolism in plant and quality of seed. Moisture deficit affects plant establishment in the field, photosynthetic ability and osmotic behavior of cells. However, species and genotypes vary in their capacity to tolerate water stress (Ulemale *et al*., 2013). There is a need to develop drought tolerant genotypes for an enhanced yield and its stability under terminal drought stress environments. In chickpea, some drought tolerant genotypes, including ICC 4958, have been identified by screening more than 1500 germplasm accessions directly for yields under drought conditions over a period of time (Saxena, 2003). The advantage of conservative water use during the vegetative growth stage with a low canopy conductance was proposed as an important trait (Zaman-Allah *et al*., 2011).

Plants adopt various defense mechanisms in response to terminal drought which are accomplished by regulating internal plant water status. Plant water status that includes leaf water potential, osmotic potential and relative water content represents an easy measure of water deficit and provides best sensor for stress. Therefore, crosses were made using parents HC 1 and ICC 4958 to obtain improved chickpea progeny lines exhibited better plant water status and high yield in drought prone areas.

Table 1 : Phenology (days after sowing) of parents and progeny lines of chickpea in 2013-14

Genotype / Progeny lines	50% flowering	50% poding	Physiological maturity
HC 1 (Irrigated)	96	112	140
HC 1 (Drought)	83	100	124
ICC 4958 (Irrigated)	94	116	152
ICC 4958 (Drought)	80	102	143
F_4 Progeny range HC 1× ICC 4958 (Drought)	73-95	96-110	118-150

MATERIALS AND METHODS

The experiment was conducted during the post rainy *(rabi)* seasons in drought plots with rainout shelters at Crop Physiology Field Laboratory, Department of Agronomy, CCS Haryana Agricultural University, Hisar ($29^0$10'N latitude, $75^0$48'E longitude, 215 m altitude), Haryana. The cross of progeny HC 1 and ICC 4958 was made in 2009-10 and the progeny rows were grown to get F_4 generation. The F_4 progeny lines along with parents (HC 1: drought sensitive and ICC 4958: drought tolerant) were planted in specially constructed facilities of concrete microplots (6 m long, 1 m wide and 1.5 m deep connected with iron gates and washing tanks) filled with sandy soil and irrigated up to field capacity. Both the parents were sown in six rows of 1 m length with inter row spacing of 30 cm and plant spacing of 10 cm under two environments, namely irrigated (I: two irrigations of 6 cm depth each at pre flowering and pod filling) and Drought (D: one irrigation of 30 mm equal to long-term average seasonal rainfall). The experiment was conducted in a randomized block design (RBD) with three replications. The plots were fertilized with 15 kg N ha^{-1} and 40 kg P_2O_5 ha^{-1} as basal dose before sowing. The F_4 progenies (140) of cross HC 1× ICC 4958 with three replications of each progeny in one row and spacing same as in case of parents were sown in drought conditions. Out of 140 progenies of cross, the data on 20 best performing progenies (G 06, G 13, G 17, G 19, G 20, G 21,G 30, G 33, G 40, G 41, G 42, G 45, G 48, G 54, G 55, G 61, G 81, G 83, G 93, G 95) are presented in results. The soil moisture content at the time of sowing was 12.3% upto 15 cm depth. The soil moisture content in the depth range of 45-135 cm in irrigated plots was 9.04% and 6.42% under drought conditions at the time of observations (80-92 days). The plant water relation traits and other characters were recorded on third fully expanded leaf from top between 1000-1200 hours at 50% flowering stage *i.e.* 94-96 days after sowing in irrigated and 80-83 days after sowing under drought conditions. The leaf water potential was measured using Pressure Chamber (PMS Instrument Co., Oregon, USA) on a clear sunny day. Osmotic potential was measured by Vapor Pressure Osmometer (Model 5100-B, Wescor, Logan, USA). Leaf relative water content was calculated using Kumar and Elaston (1992) method. Relative stress injury (RSI %) was determined by the method of Sullivan and Ross (1979) using conductivity meter. Transpirational cooling, i.e. canopy temperature depression (CTD) was measured using infra-red thermometer (Model AG-42 Tele-temp Corp, California, USA). Photochemical efficiency/ quantum yield of photosystem II was determined in intact plants in the field with an OS-30P chlorophyll flurometer (Opti-Science, Inc., Hudson, NY, USA). Initial (F_0) and maximum (F_m) fluorescence were recorded and variable fluorescence (F_v) were derived by subtracting F_0 from F_m. Photochemical efficiency/quantam yield , which is F_v / F_m ratio, was then calculated. The phenological observations such as days to 50% flowering, pod formation and physiological maturity were also recorded under both the environments. At maturity yield attributes of both parents under irrigated and drought conditions and all F_4 progeny lines under drought conditions were determined. Grouping of 140 F_4 progeny lines on the basis of seed yield and physiological traits into tolerant, moderate and susceptible lines to drought was made.

RESULTS AND DISCUSSION

Phenological observations

The chickpea parental genotypes matured earlier under drought than irrigated conditions. In the genotype ICC 4958, 50% flowering, poding and physiological maturity commenced in 80, 102 and 143 days, respectively under drought as compared to irrigated condition in 94, 116 and 152 days, respectively. Similarly in genotype HC 1, 50% flowering, poding and physiological maturity commenced under rainfed conditions in 83, 100 and 124 days, respectively as compared to irrigated condition in 96, 112 and 140 days, respectively. The range for 50% flowering, poding and physiological maturity in 140 F_4 progenies varied from 73-98 days, 96-120 days and 118-160 days, respectively under drought (Table 1). Early maturity is an important trait to avoid drought stress. Early flowering and early poding are two main components of drought escape in chickpea to avoid higher yield losses from drought. The differential

Table 2: Grouping on the basis of drought tolerance in 140 F_4 progeny lines of chickpea in 2013-14

	Seed yield plant^{-1}(g)	Water potential (-MPa)	Osmotic potential (-MPa)	Relative water content (%)	Relative stress injury (%)	CTD (^{0}C)
Tolerant(42 lines)	17.87-10.13	0.60-1.05	1.02-1.27	72.8-65.4	17.8-32.8	(-1.97)-(-0.70)
Moderate(59 lines)	10.09-7.03	1.06-0.95	1.27-1.83	65.8-60.7	32.9-31.0	(-0.70)-0.63
Susceptible(39 lines)	7.03-3.47	0.95-1.17	1.83-1.96	60.4-52	31.0-35.1	0.63-2.13

Table 3 : Plant water status, relative stress injury and chlorophyll fluorescence of parents and progeny lines of chickpea in 2013-14

Genotype / Progeny lines	Water potential (-MPa)	Osmotic potential (-MPa)	Relative water content(%)	Relative stress injury(%)	Canopy temperature depression(^{0}C)	Photochemical efficiency (F_v/F_m)
HC1 (Irrigated)	0.80	1.15	78.7	16.4	-0.4	0.624
HC1 (Drought)	1.20	1.31	59.2	31.3	2.06	0.429
ICC 4958 (Irrigated)	0.67	1.42	81.8	16.3	-1.1	0.798
ICC 4958 (Drought)	1.00	1.74	66.6	20.9	0.9	0.563
LSD (0.05%)	0.28	0.14	4.34	2.68	0.84	0.13
F_4 Progeny range (HC 1× ICC 4958) (Drought)						
G06	0.68	1.12	72.0	21.8	-1.7	0.759
G13	0.95	1.79	64.0	30.8	0.5	0.774
G17	0.83	1.20	70.0	26.0	-0.9	0.614
G19	0.60	1.02	72.8	17.8	-1.9	0.654
G20	0.68	1.08	72.7	18.8	-1.8	0.751
G21	0.96	1.74	63.0	31.7	0.5	0.770
G30	0.90	1.27	68.0	29.8	-0.8	0.680
G33	0.90	1.54	64.0	30.9	0.2	0.672
G40	0.76	1.10	70.6	24.2	-1.4	0.708
G41	0.92	1.54	64.7	30.9	-0.6	0.672
G42	0.67	1.00	72.1	20.1	-1.7	0.766
G45	0.67	1.08	72.6	18.8	-1.8	0.721
G48	0.85	1.22	68.8	27.2	-0.9	0.563
G54	0.88	1.20	69.6	28.3	-0.9	0.753
G55	0.69	1.15	71.9	20.4	-1.6	0.683
G61	0.70	1.16	70.8	24.3	-1.4	0.740
G81	0.93	1.60	64.0	31.2	0.3	0.717
G83	0.96	1.79	64.0	32.1	0.5	0.663
G93	0.77	1.17	71.4	26.0	-1.0	0.655
G95	0.90	1.53	65.8	30.4	-0.7	0.668
L LSD (0.05%)	0.21	0.24	4.54	2.92	0.7	0.17

genotypic response to drought stress, as a result of variation in physiological parameters has also been reported by Gunes *et al.*, (2008).

Leaf water status

The observed physiological changes could be the result of deleterious effect of water deficit on important metabolic processes as well as responses of various defense mechanisms by the plant under drought stress environments (Talebi *et al.*, 2013). The plant water status would be evaluated as drought tolerance selection criteria. The results showed that under drought stress there is decrease in water

Table 4 : Yield and yield attributes of parents and 20 best yielding progeny lines in chickpea in 2013-14

	# Branches plant^{-1}	# Pods plant^{-1}	100 Seed weight(g)	Biomass yield plant^{-1} (g)	Seed yield Plant^{-1} (g)
HC 1 (Irrigated)	4.4	49.8	14.72	39.6	11.1
HC 1 (Drought)	3.6	40.4	11.47	24.3	6.9
ICC 4985 (Irrigated)	6.0	35.2	27.26	44.5	13.5
ICC 4958 (Drought)	4.9	30.0	24.15	34.7	10.1
LSD (0.05%)	0.74	3.42	2.46	8.62	2.42
F_4 Progeny range (HC 1× ICC 4958) (Drought)					
G 06	7.0	61.0	14.20	49.4	14.6
G 13	6.6	48.3	14.67	42.5	11.8
G 17	5.3	57.6	15.37	44.3	13.2
G 19	6.6	76.6	16.37	56.3	17.8
G 20	5.3	70.0	15.47	52.9	16.5
G 21	5.6	47.6	15.93	37.6	11.5
G 30	5.0	55.6	19.23	39.8	12.5
G 33	6.0	52.0	13.13	36.3	12.2
G 40	6.6	60.8	14.66	49.2	13.6
G 41	4.6	53.3	18.81	42.8	12.3
G 42	6.0	63.0	14.57	50.3	14.9
G 45	7.0	68.3	14.23	56.5	16.1
G 48	5.6	56.6	13.81	41.6	12.7
G 54	6.6	56.3	14.2	47.0	12.5
G 55	6.0	60.6	14.23	45.3	14.3
G 61	7.3	60.4	14.77	40.0	13.5
G 81	5.0	50.3	25.9	46.9	11.9
G 83	5.0	47.3	11.67	38.9	11.4
G 93	6.0	60.3	14.68	50.3	13.3
G 95	5.0	55.6	13.45	44.2	12.3
LSD (0.05%)	0.63	4.72	2.81	8.73	2.65

Table 5 : Correlation matrix of physiological traits and yield attributes of 20 best progeny lines of chickpea in 2013-14

	LWP	LOP	RWC	RSI	CTD	#Pods	BY	SY
LWP	1.00							
LOP	0.88	1.00						
RWC	0.93	0.96	1.00					
RSI	-0.98	-0.86	-0.93	1.00				
CTD	-0.92	-0.95	-0.95	0.91	1.00			
#Pods	0.92	0.86	0.89	-0.93	-0.91	1.00		
BY	0.75	0.68	0.74	-0.79	-0.72	0.82	1.00	
SY	0.92	0.79	0.84	-0.95	-0.85	0.97	0.83	1.00

Where, LWP- leaf water potential; LOP- leaf osmotic potential; RWC- relative water content; RSI-relative stress injury; CTD-canopy temperature depression; #Pods- number of pods per plant; BY-biomass yield per plant; SY-seed yield per plant potential, osmotic potential and relative water content (Table 3). The leaf water potential of HC 1 decreased from -0.8 MPa under irrigated condition to -1.20 MPa under drought condition, whereas, in genotype ICC 4958 from -0.6 MPa under irrigated condition to -1.0 MPa under drought. Osmotic potential decreased from -1.15 MPa (Irrigated) to -1.31 MPa (Drought) in HC1 and in ICC 4958 from -1.42 MPa (Irrigated) to -1.74 MPa (Drought). The decrease in osmotic potential of parent ICC4958 was recorded high since it may accumulate higher amount of solutes under drought as compared to HC 1. The osmo-regulatory activities helped the plant to cope up with moisture stress (Ulemale *et al*., 2013). The RWC was recorded relatively low under drought stress as compared to non stress condition in both parental genotypes. The parent ICC 4958 maintained less decrease in RWC (15.2%) than in HC 1 (19.6%) at 50% flowering. Variation in RWC is achieved through differences in plant ability to absorb water from soil by developing a high water potential gradient from soil to plant, extending rooting depth or ability to control water loss through stomata (Omae *et al*., 2005). A decrease in the relative water content (RWC) in response to drought stress has been recorded in wide variety of plants as reported by Nayyar and Gupta (2006). In F_4 progeny lines, LWP, LOP and RWC ranges from -0.67 to -1.13 MPa, -1.02 to -1.94 MPa and 54.9% to 76.9% respectively. Among the F_4 progeny lines G 19 recorded highest plant water status (LWP -0.68 MPa, LOP -1.12 MPa and RWC 72%) followed by G 20 and G 45. Whereas, lowest plant water status was recorded in progenies G 21 followed by G 13 and G 81. Out of the total 140 F_4 progeny lines, 42 lines had leaf water status near the tolerant parent and were considered as drought tolerant, 59 lines were moderate and 39 lines which had leaf water status near susceptible parent were considered as drought susceptible lines (Table 2).

Relative stress injury, CTD and photochemical efficiency

In drought sensitive parent HC 1 relative stress injury was recorded 16.4% under irrigated whereas 31.3% under drought condition. Whereas, in drought tolerant ICC 4958, RSI was recorded 16.3% under irrigated and 20.9% under drought conditions (Table 3). In 140 progeny lines RSI ranges from 17% to 34.6% under drought situation. Out of 20 highest yielding progeny lines the lowest stress injury was recorded in progeny G19 (21.8%). It had been reported that tolerant and intermediate genotypes were superior to susceptible ones in maintaining membrane stability and lower membrane injury under drought stress condition (Pouresmael, *et al*., 2013). Canopy Temperature Depression is expressed as canopy temperature minus ambient temperature and this value is higher and positive in drought stress. In tolerant parent ICC 4958, there is increase in CTD from -1.1 under irrigated condition to 0.9 under drought conditions is less as compared to HC 1 from -0.4 in irrigated condition to 2.6 in drought conditions (Table 3). In best yielding progeny lines, CTD ranges from -1.97 to 0.50 under drought condition. The photosynthetic efficiency was significantly reduced by drought in both the parent genotypes. Parent ICC 4958 maintained higher F_v/F_m ratio than HC 1 in both irrigated and drought situation. In HC 1, F_v/F_m was recorded 0.624 under irrigated and 0.429 under drought conditions whereas in ICC 4958 it was observed to be 0.798 under irrigated and 0.563 under drought. The photochemical efficiency was recorded in the range of 0.654 to 0.770 for highest yielding 20 progeny lines. Increase in CTD might have occurred due to decreased transpiration resulting from stomatal closure. The photosynthetic efficiency, transpiration and the values of relative stress injury declined in chickpea under drought conditions (Kumar *et al*., 2012).

Yield and yield attributes

Drought stress reduces the seed yield of both parental genotypes and 140 F_4 progeny lines (Table 4). The yield of HC 1 and ICC 4958 were decreased in drought stress condition but decrease in yield of ICC 4958 (24.96%) was less as compared to HC 1 (37.32%). Drought stress decreases number of pods in both the parents under drought as well as irrigated condition. In HC 1, number of pods plant^{-1} were 49.9 in irrigated and 40.4 in drought condition whereas, ICC 4958 had 35.2 number of pods in irrigated and 30.0 in drought. Number of pods was less in ICC 4958 than HC 1 however, 100 seeds weight of ICC 4958 was more over HC 1 in irrigated as well as drought conditions. Seed yield was more in ICC 4958 than HC 1 under both irrigated and drought condition. Under irrigated condition, seed yield of HC 1 was recorded 11.12 g plant^{-1} and ICC 4958 was 13.5 g plant^{-1} whereas, under drought conditions seed yield of 6.97 and 10.13 g plant^{-1} was recorded in HC 1 and ICC 4958, respectively. Reduction in number of branches and biological yield was less in ICC 4958 as compared to HC 1. Among the progeny lines the maximum seed yield was recorded in G 19 with highest number of branches, number of pods, 100 seed weight and biological yield. Out of 140 progeny lines, 42 lines had seed yield ranging from 10.13 to 17.87 g plant^{-1} and were considered as drought tolerant lines, 59 lines had seed yield ranging from 7.0 to 10.09 g plant^{-1} and were considered as moderately tolerant lines whereas, 39 lines had seed yield ranging from 3.47 to 7.03 g plant^{-1} and were considered as drought susceptible lines (Table 2). A significant pod abortion has been observed under severe moisture stress causes yield losses (Anyia and Herzog, 2004; Leport *et al*., 2006).

Leaf water potential, osmotic potential and RWC had a significant positive association with seed yield such as 0.92, 0.79 and 0.84, respectively. RSI and CTD were negatively correlated with seed yield as -0.95 and -0.85 respectively. The number of pods has a significant positive association with LWP, LOP, RWC and seed yield whereas, negative correlation with CTD and RSI (Table 5).

The results of this study showed that the plant water relation parameters had direct bearing on yield formation via yield attributes. Therefore, the measurement of RWC, LWP, CTD and chlorophyll fluorescence during midday hours which is simple and rapid could be exploited in chickpea progenies for crop improvement programmes of drought tolerance. The ICC 4958 was more promising with better plant water status, low membrane injury and cooler canopy temperature and higher seed yield. The results in the correlation matrix showed a positive correlation of LWP, LOP, RWC with seed yield.

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