

## Identification of traits related to drought tolerance in chickpea (*Cicer arietinum* L.) genotypes

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**Abstract:** Drought is the most common abiotic stress limiting chickpea production because chickpea is usually grown under the residual soil moisture. The experiment was carried out with 39 chickpea genotypes at International Crops Research Institute for the Semi-Arid Tropics (ICRISAT) during post-monsoon season of 2009-2010 based on alpha design with two replications under two set (rainfed and irrigated conditions) to observe information on yield under drought condition and potential yields and to investigate the relationship of physiological traits related to drought tolerance. Rainfed condition significantly reduced seed yield due to poor partitioning operated along with terminal drought stress. Nine genotypes resulted superior or similar to the seed yield of drought tolerant check genotype (ICC 4958) under rainfed condition. These genotypes were observed well performed under irrigated condition. The SPAD chlorophyll meter reading (SCMR) was increased but specific leaf area (SLA), and relative water content (RWC) were decreased under rainfed condition as compared to irrigated condition. This study also identified promising genotypes for high SCMR on ICCV 03110, ICCV 00108 and ICCV 04110, low SLA on ICCV 04303, ICCV 03302, ICCV 04301 and ICCV 01303 and high RWC on ICCV 00108, Yezin 6 and Yezin5. Results showed that the SCMR was significantly related with seed yield and SLA. The genotypes having high SCMR and low SLA seemed to be resistance to drought.

**Key words:** Chickpea, drought stress, drought tolerance traits, relationship, yield.

### Introduction

Chickpea (*Cicer arietinum* L.) is an important food legume crop because of its high quality protein for the human diet and its straw for valued animal feed. It is grown in over 50 countries in all continents of the world. The major chickpea growing countries fall in the arid and semi-arid regions where terminal drought is one of the major constraints which affect the yield (Turner *et al.*, 2001). This problem is more serious in Myanmar where chickpea is traditionally planted towards the end of the rainy season and generally grown on progressively declining residual soil-moisture. In some production areas, the rainfall is poorly distributed over the growing season and stops before growth of chickpea is completed even in case of early sowing. Consequently, terminal drought stress, which during the reproductive phase of the crop, is common and critical.

Yield losses due to terminal drought estimates range from 35 to 50% across the Semi-Arid Tropic (SAT) and West Asia and North Africa (WANA) (Sabaghpour *et al.*, 2003). A large portion of the losses can be prevented through crop improvement and better drought adapted genotypes would reduce the yield losses. Several physiological, morphological and phenological traits have been listed to play a significant role in crop adaptation to drought stress (Ludlow and Muchow, 1990). Thus, alternative breeding strategies using physiological traits as selection criteria have been proposed by some researchers. Rapid progress in drought resistance breeding has been achieved in groundnut based on characters such as harvest index (HI), water use efficiency (WUE), specific leaf area (SLA), and SPAD chlorophyll meter reading (SCMR) (Nagam *et al.*, 2005). Early studies have indicated differential responses for relative water content (RWC) in chickpea (Bahavar *et al.*, 2009) and it was positively correlated with chlorophyll content and grain yield in rice under drought conditions (Pirdashti *et al.*, 2009).

In addition, information on the heritability of these traits will be useful for planning the suitable breeding strategies for improving drought tolerance. Phenotypic correlations among these traits are also important when simultaneous selection of multiple traits is to be carried out for high

yield under drought stress conditions. Therefore, the objective of this study was to observe information on yield under drought conditions and potentials yields and to investigate the relationship of physiological traits related to drought tolerance.

### Materials and Methods

**Experimental site, design and plant materials:** The experiment was carried out at the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), Patancheru (17°30' N; 78° 16' E; altitude 549 m) in post-monsoon chickpea growing season (October-February) of 2009-2010. This study was evaluated in two sets (rainfed and irrigated conditions) of 13 x 3 alpha designs (39 genotypes) with two replications. Thirty-nine genotypes of chickpea which comprising 8 genotypes developed at Myanmar were evaluated. The plot size was 4 m length with a single row. Under irrigated treatment, furrow irrigation was applied at 40 days after sowing (DAS). Crop management for both trials was followed by ICRISAT's practices.

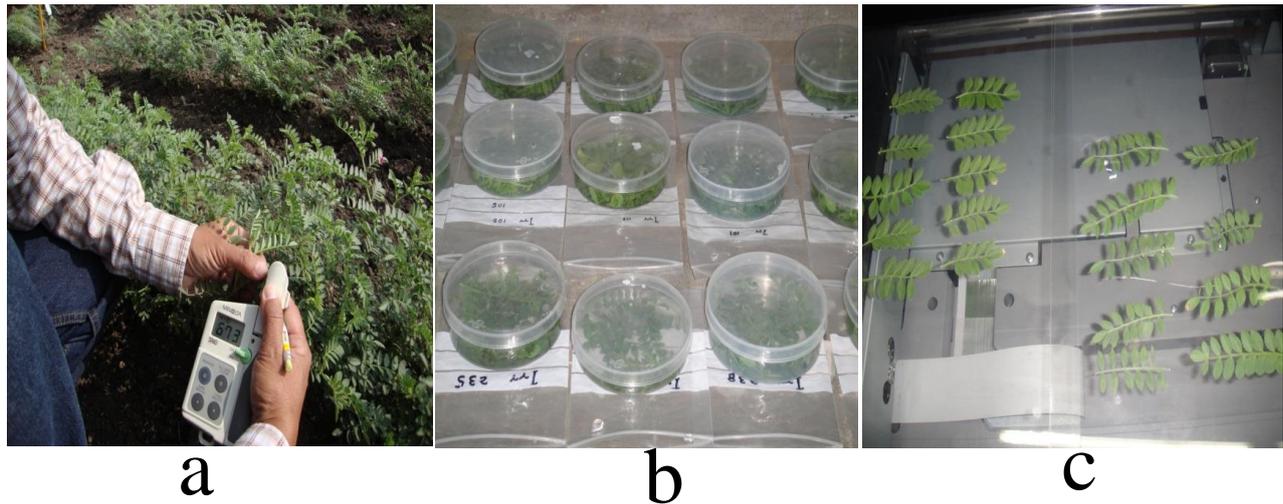
**Data collection:** Yield and yield attributes were recorded. Moreover, drought tolerance traits such as SPAD Chlorophyll Meter Reading (SCMR), Relative Water Content (RWC) and Specific Leaf Area (SLA) were measured at 75 DAS under both conditions (Fig. 1).

**Statistical analysis:** The data from each individual experiment were analyzed using the following linear additive mixed effects model:  $Y_{ijk} = \mu + r_i + b_{ij} + g_k + e_{ijk}$ , where,  $Y_{ijk}$  = the observation recorded on genotype  $k$  in incomplete block  $j$  of replicate  $i$ ,  $\mu$  = the general mean,  $r_i$  = the effect of replicate  $i$ ,  $b_{ij}$  = the effect of block  $j$  within replicate  $i$ ,  $g_k$  = the effect of genotype  $k$ ,  $e_{ijk}$  = the effect of the plot.

Using the above model, the statistical procedure of residual maximum likelihood (ReML) method with GenStat (version 12.1) statistical computing software was employed to obtain the unbiased estimates of the variance components  $\delta^2_b$ ,  $\delta^2_g$  and  $\delta^2_e$ , and the best linear unbiased predictions (BLUPs) of the performance of the genotypes. Heritability was estimated as  $h^2 = \delta^2_g / (\delta^2_g + \delta^2_e)$ . The significance of genetic variability among genotypes was

assessed from the standard error of the estimate of genetic variance  $\delta^2_g$ , assuming the ratio  $\delta^2_g/S.E.$  ( $\delta^2_g$ ) to follow normal distribution asymptotically. Moreover, the

correlations were calculated separately in both conditions for seed yield and drought tolerance traits.



**Fig. 1.** (a) SPAD Chlorophyll Meter Reading, (b) Relative Water Content determining, and (c) Specific Leaf Area (scanning)

### Results

**Seed yield:** The analysis showed significant genotypic differences for seed yield under rainfed and irrigated conditions (Table 1). The mean, range and heritability of seed yield were low under rainfed condition comparison with irrigated condition. Under rainfed conditions, the highest seed yield was found in PCHL 04-5 (2985 kg ha<sup>-1</sup>) followed by ICCV 03107 (2905 kg ha<sup>-1</sup>), Annigeri (2854 kg ha<sup>-1</sup>), ICCV 00108 (2715 kg ha<sup>-1</sup>) and the drought

tolerant genotype ICC 4958 (2675 kg ha<sup>-1</sup>), while the lowest in ICCV 03406 (1351 kg ha<sup>-1</sup>) (Table 2). This was due to significantly higher in their yield attributes *viz.*, biomass yield, HI and number of pods per plant of these genotypes (data not shown). Under irrigated condition, the highest seed yield was observed in ZCHL 05-2 (3701 kg ha<sup>-1</sup>) followed by Shwenilonegi (3605 kg ha<sup>-1</sup>), while the lowest yield was in Yezin 5 (1578 kg ha<sup>-1</sup>) (Table 2).

**Table 1.** Trial mean, range of best linear unbiased predicted means (BLUPs) and variance of seed yield and drought tolerance traits of chickpea genotypes under rainfed and irrigated conditions at ICRISAT during post-monsoon season, 2009-2010

Traits	Trial mean	Range of predicted means	$\delta^2_g$	S.E	Significance	Heritability ( $h^2$ )
Seed yield (kg ha <sup>-1</sup> )						
Rainfed	2236	1351-2985	88991	38792	*	0.37
Irrigated	2725	1578-3701	101854	48153	*	0.42
SCMR						
Rainfed	64.98	57.99-70.07	4.40	1.47	**	0.56
Irrigated	60.32	56.02-66.78	4.03	1.46	**	0.52
SLA						
Rainfed	208.8	150.8-291.5	913.3	290.2	**	0.59
Irrigated	232.2	185.3-306.3	650.2	225.9	**	0.53
RWC						
Rainfed	78.97	69.66-86.03	13.35	6.54	*	0.35
Irrigated	81.44	70.90-95.85	9.18	3.78	*	0.44

\*, \*\*, Significant at the  $p \leq 0.05$  and  $p \leq 0.01$ , respectively

### Physiological traits related to drought tolerance:

**SPAD Chlorophyll Meter Reading (SCMR):** Significant differences were also observed among the tested genotypes for SCMR under both rainfed and irrigated conditions (Table 1). The mean, range and heritability of SCMR were high under rainfed condition comparison with irrigated condition. Under rainfed condition, ICCV 03110 showed the highest SCMR of (70.07) followed by ICCV 00108 (69.05) and ICC 4958 (68.15). Under irrigated

conditions, these genotypes also showed high in SCMR readings (Table 3).

**Specific Leaf Area (SLA):** There was a significant reduction in SLA under rainfed compared to irrigated conditions. In this study, genotypic differences for SLA were found to be significant under rainfed and irrigated conditions (Table 1). This finding was supported by good heritability of SLA. Low SLA is preferable as it indicates higher drought tolerance. The lowest SLA was obtained in

ICCV 04303 (150.8 cm<sup>2</sup>g<sup>-1</sup>) followed by ICCV 03302 (157.0 cm<sup>2</sup>g<sup>-1</sup>) and ICCV 04301 (158.1 cm<sup>2</sup>g<sup>-1</sup>) (Table 3). The values of these genotypes were significantly lower than that of ICC 4958. Under irrigated conditions, there were no genotypes significantly better than ICC 4958. However, nine genotypes showed consistency results of lower SLA under both rainfed and irrigated conditions.

**Table 2.** Seed yield (kg ha<sup>-1</sup>) of chickpea genotypes under rainfed and irrigated conditions at ICRISAT during post-monsoon season, 2009-2010

Genotypes	Rainfed yield	Irrigated yield
Annigeri	2854	3464
ICCV 37	2082	3118
ICCV 00108	2715	2968
ICCV 00401	2031	2939
ICCV 01303	2500	2225
ICCV 03103	1735	3444
ICCV 03107	2905	2457
ICCV 03110	2609	2759
ICCV 03111	2368	2589
ICCV 03203	2158	3361
ICCV 03302	1881	2632
ICCV 03403	2264	2373
ICCV 03406	1351	2989
ICCV 03407	2584	2426
ICCV 04103	2658	3348
ICCV 04110	2119	2596
ICCV 04111	1917	2726
ICCV 04301	1472	2043
ICCV 04303	1726	2884
ICCV 04304	2158	2793
ICCV 04306	2542	3202
ICCV 95311	1787	2650
ICCV 97024	1951	2512
ICCV 97306	2484	2510
ICCV 97314	2193	2709
Karachi	2242	1894
PCHL 04-2	2391	2326
PCHL 04-32	2271	2668
PCHL 04-34	2600	2400
PCHL 04-5	2985	2611
Shwenilongi	2129	3605
Yezin 3	1918	2754
Yezin 4	2119	2731
Yezin 5	1631	1578
Yezin 6	2082	2966
ZCHL 05-2	2616	3701
ZCHL 05-20	2164	2807
ZCHL 05-73	2341	2003
ICC 4958 ©	2675	2526
Mean	2236	2725
LSD <sub>(0.05)</sub>	748	844

**Relative Water Content (RWC):** Significant differences for RWC were observed among chickpea genotypes under rainfed and irrigated conditions (Table 1). The heritability of RWC was good due to the genetic variation of RWC. Under rainfed condition, nine genotypes were observed as promising genotypes for high RWC. The highest RWC was observed in ICCV 00108 (86.03%) followed by Yezin 6 (85.84%), Yezin 5 (85.61%) (Table 3). However, no consistency results were obtained under irrigated condition due to an interaction of genotype x environment.

**Correlation between seed yield and drought tolerance traits:** Correlations between seed yield and drought tolerance traits provide information on expected responses in seed yield from selection for drought tolerance traits. In the present study, a significant positive relationship was observed between seed yield and SCMR ( $r = 32$  at  $p$

<0.05) under rainfed condition (Table 4). A negative correlation was found between SLA and SCMR ( $r = -0.16$  and  $-0.18$ ) and RWC ( $r = -0.08$  and  $-0.05$ ) under rainfed and irrigated conditions, but not significant.

Lower seed yield, higher SCMR, lower SLA and RWC indicated that rainfed condition suffered from more moisture stress to certain extent than irrigated conditions. In this study, the adverse effect of moisture stress on seed yield was clearly evident by its lowest value in the rainfed conditions with a reduction in terms of 18 per cent in comparison with irrigated conditions. The yield reduction can be ascribed to statistically retarded performance with respect to various yield attributes especially pods per plant and biomass yield (data not shown). However, the reductions in seed yield could not be observed in ICCV 03107, PCHL 04-5, Karachi, ZCHL 05-73, ICCV 01303, PCHL 04-34, ICCV 03407, ICC 4958, PCHL 04-2 and Yezin 5. It indicated that these genotypes may have inbuilt capacity to resist moisture stress effectively.

The present study has also shown that, ICCV 03110, ICCV 00108 and ICCV 04110 showed superior and more consistent SCMR values than the others. Besides, ICC 4958 is a well known drought resistant genotype had better SCMR. It was possibly due to its strong root systems (Kashiwagi *et al.*, 2006). The SCMR is an indicator of the photosynthetically active light transmittance characteristics of the leaf, which is dependent on the unit amount of chlorophyll per unit leaf area (Chlorophyll density) (Richardson *et al.*, 2002). Leaf photosynthesis is generally correlated with chlorophyll content per unit leaf area and hence the SPAD chlorophyll meter reading can provide a useful tool to screen for genotypic variation in potential photosynthetic capacity under drought conditions (Nageswara Rao *et al.*, 2001).

Although SLA was reduced by drought stress, SLA in certain genotypes under rainfed was dependent on that under residual moisture conditions. ICCV 04303, ICCV 03302, ICCV 04301 and ICCV 01303 showed consistently lower SLA than other genotypes in both rainfed and irrigated conditions. In addition, the high seed yielding genotype PCHL 04-5 showed lower SLA than ICC 4958 under the rainfed conditions. The variation and consistency of SLA make it useful for the application as a selection criterion in drought tolerance breeding program.

The low value of RWC was recorded under rainfed conditions, which might be due to the impact of lower soil moisture supply. According to Reddy *et al.* (2003), biochemical components in leaves of stressed plants were changed although the plants could maintain RWC as high as those for non-stressed plants and RWC in a range lower than 85% is considered severely stressed. In the present study, the mean value of rainfed condition for RWC was 78.97 %. Thus, the tested chickpea genotypes faced moisture stress as a terminal drought. Similar findings were reported by Arunyanark *et al.* (2008), who found significant differences for RWC between drought treatment and control treatment as early as 33-35 days after withholding water. According to this result, ICCV 00108, Yezin 6 and Yezin 5 had the highest RWC and may be assumed as promising genotypes for high RWC for drought tolerance.

**Table 3.** Drought tolerance trait of chickpea genotypes under rainfed(R) and irrigated (I) conditions at ICRISAT during post-monsoon season, 2009-2010

Genotypes	SCMR		SLA		RWC	
	R	I	R	I	R	I
Annigeri	65.49	58.92	291.5	306.3	75.40	93.19
ICCV 37	67.08	58.12	243.1	287.3	84.92	82.92
ICCV 00108	69.05	66.78	186.4	205.9	86.03	80.89
ICCV 00401	62.11	60.19	194.8	236.6	72.65	89.19
ICCV 01303	63.33	60.80	160.3	207.0	81.22	79.65
ICCV 03103	66.06	57.34	182.4	234.6	80.05	83.15
ICCV 03107	63.27	56.02	227.5	261.1	76.68	72.62
ICCV 03110	70.07	65.03	267.9	271.3	80.06	78.80
ICCV 03111	67.21	59.88	215.7	232.2	77.76	73.01
ICCV 03203	67.31	62.22	176.0	201.5	75.88	80.28
ICCV 03302	63.42	63.14	157.0	217.8	77.55	88.72
ICCV 03403	65.32	61.29	257.3	218.2	80.33	75.28
ICCV 03406	62.01	61.10	175.6	192.8	76.22	76.67
ICCV 03407	65.21	63.50	221.9	193.6	76.46	80.71
ICCV 04103	65.40	59.96	216.9	247.4	70.69	77.00
ICCV 04110	67.41	63.39	183.8	260.7	78.79	82.04
ICCV 04111	66.24	60.08	172.3	254.5	73.23	70.90
ICCV 04301	64.15	61.40	158.1	216.5	82.19	80.89
ICCV 04303	63.39	59.69	150.8	200.9	77.30	95.85
ICCV 04304	62.74	57.05	228.1	216.2	83.45	81.01
ICCV 04306	64.13	64.49	172.4	193.7	80.53	87.41
ICCV 95311	64.11	58.35	252.2	271.3	69.66	78.99
ICCV 97024	66.11	60.88	273.7	282.6	79.96	84.16
ICCV 97306	65.86	57.38	223.8	252.5	77.86	85.21
ICCV 97314	66.41	59.25	197.5	204.5	80.75	85.87
Karachi	65.64	57.70	228.3	236.8	80.49	81.00
PCHL 04-2	68.03	61.16	173.9	244.6	78.12	82.88
PCHL 04-32	62.60	61.55	194.0	188.8	82.42	79.51
PCHL 04-34	62.43	56.49	212.1	201.7	76.97	86.19
PCHL 04-5	66.08	60.26	185.1	267.7	79.86	78.88
Shwenilongi	67.30	60.05	214.3	217.0	81.97	76.01
Yezin 3	59.85	57.00	189.1	238.6	80.05	76.70
Yezin 4	63.01	59.93	259.3	221.5	75.99	82.92
Yezin 5	61.03	57.52	232.0	185.3	85.61	81.03
Yezin 6	66.56	60.92	184.9	258.7	85.84	82.00
ZCHL 05-2	66.42	60.72	249.7	267.7	78.93	78.08
ZCHL 05-20	66.16	62.20	226.5	218.1	81.36	80.23
ZCHL 05-73	57.99	58.23	196.2	211.3	81.99	86.83
ICC 4958 ©	68.15	62.28	209.7	229.9	74.47	79.51
Mean	64.98	60.32	208.8	232.2	78.97	81.44
LSD <sub>(0.05)</sub>	3.85	4.14	50.5	48.7	7.17	10.08

**Table 4.** Correlation coefficients between seed yield and drought tolerance traits of chickpea genotypes under rainfed and irrigated conditions at ICRISAT during post-monsoon season, 2009-2010

Traits	Seed Yield (kg ha <sup>-1</sup> )	SCMR	SLA	RWC
Seed Yield (kg ha <sup>-1</sup> )				
Rainfed	-			
Irrigated	-			
SCMR				
Rainfed	0.32*	-		
Irrigated	0.17	-		
SLA				
Rainfed	0.29	-0.16	-	
Irrigated	0.25	-0.18	-	
RWC				
Rainfed	-0.07	0.02	-0.08	-
Irrigated	0.05	0.00	-0.05	-

\*, Significant at the p≤0.05

Differential responses of genotypes for drought tolerance traits indicated that several drought resistance mechanisms might exist. Combining these characters in chickpea breeding programs should increase drought resistance in chickpea. In this study, SCMR was significantly correlated with seed yield under rainfed conditions (Table 4). Similar

positive correlation between SCMR and seed yield has earlier been reported in groundnut (Nageswara Rao *et al.*, 2001). Higher SCMR seems to be an indication of the genotype's capacity for higher carbon assimilation and in turn seed yields even under moisture-limited situations. Significant and positive correlation between SCMR and

chlorophyll content was observed and SCMR was also closely related with chlorophyll density (Nageswara Rao *et al.*, 2001). A positive correlation was found between seed yield and SLA under rainfed and irrigated conditions (Table 4). A negative correlation was found between SLA and SCMR under rainfed and irrigated conditions (Table 3). Similar relationship between SLA and SCMR has been reported in groundnut (Upadhyaya, 2005). Genotypes with lower SLA (thicker leaves) are known to have more of photosynthetic machinery, i.e. more chlorophyll content (Nageswara Rao and Wright, 1994). Moreover, negative correlations were also found between SLA and RWC under rainfed and irrigated conditions. This indicated that genotypes with thicker leaves may have more RWC under drought conditions.

Among drought tolerance traits (SCMR, SLA and RWC) SCMR had the highest correlation with seed yield and the measurement of SCMR was easy and simple. Moreover, these traits have lower G × E interaction than do SLA and RWC. It would be possible to improve yield by selecting high SCMR. Thus, the SPAD chlorophyll meter provides an easy opportunity to integrate a surrogate measure of WUE with seed yield, in the selection scheme of a drought tolerance breeding program in chickpea.

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