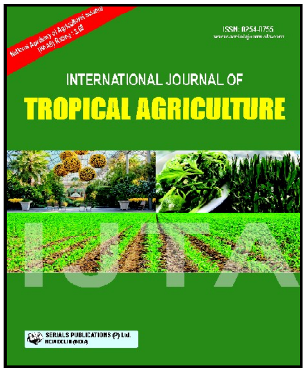

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Morpho-Physiological Grouping of Chickpea (*Cicerarietinum* L.) Genotypes on the Basis of their Response to Drought Stress

Supriya Sachdeva[1a*], C. Bharadwaj*[1b], Vinay Sharma[2], Neeraj Kumar[1a], Kv Bhat[3], B. S. Patil[1c], K. R. Soren[4a], S. K. Chaturvedi[4b], Manish Roorkiwal[5a], S. K. Chauhan[1d] and Rajeev Varshney[5b]

[1a] Research Scholar, Principal Scientist, [1c] Senior Scientist,[1d]ACTO, Genetics DivisionICAR-IARI, Pusa, New Delhi, India 110012;
[2] Professor, Head and Dean Faculty of Science, Banasthali University,
[3] Head, Division of Genomic Resources, ICAR-NBPGR, Pusa, New Delhi
[4a]Senior Scientist,[4b] Principal Scientist ICAR-IIPR Kanpur,
[5a] Programme Leader, [5b] Senior Scientist, Genomics Programme, ICRISAT, Patancheru
[*]Corresponding Author. E-mail: chbharadwaj@yahoo.co.in

Abstract: Drought is a major constraint that limits seed yield in chickpea (*Cicerarietinum* L.). To identify the most limiting trait under drought conditions is important so that one can breed for varieties with more resilience to drought. The objective of this study was to categorize the drought tolerant and susceptible chickpea genotypes based on the morpho-physiological genotypes so that the most divergent genotypes could be identified to be involved in the crossing programme. The set consisted of forty genotypes which included the lines form Training Population, released varieties and identified donors. A pot experiment was carried out as a randomized complete block design under water stressed conditions and control conditions and observations were recorded on plant height, 100 seed weight, biomass, plant yield, chlorophyll index(CI), membrane stability index (MSI), relative water content (RWC) and protein content in these chickpea genotypes. SAHN grouping was done using the traits relative water content and membrane stability index to cluster these genotypes into homogenous groups. Three distinct clusters could be identified with the most tolerant genotypes grouping into a distinct cluster. The genotypes from this cluster can be used to cross with the lines from the farthest cluster to generate trans-aggressive segregants for these traits so that greater selection gain can be obtained.

Key words: Morpho-physiological, drought stress, rain-fed, SAHN grouping.

INTRODUCTION

Chickpea is an important pulse crop of India with over 40% share in the country's total pulse production. In India chickpea is grown from 32°N in northern India with cooler long season environment to 10°N in southern India with warmer short season environment. Globally, an area of 8.25 Mha is under chickpea producing 7.33 Mt (PC report 2015-16). Despite being the largest chickpea producing country with a share of over 68% in the global chickpea production, India has to import large quantities of chickpea every year in order to meet the growing domestic demand. A major shift in chickpea area has taken place in the last two and half decades with major production area shifting to South and Central India from North India (PC report 2015-16). With the increase in irrigation potential in the northern parts there was replacement of chickpea area to mustard, wheat and rice. The chickpea area reduced from 3.2 m ha to 1.0 m ha in northern states (Punjab, Haryana and Uttar Pradesh).

On the contrary, there has been substantial increase in area from 2.6 Mha to 4.3 Mha in central and southern states (Madhya Pradesh, Maharashtra, Andhra Pradesh and Karnataka). This shift from productive Northern climatic conditions to hot, short duration less productive south Indian conditions that too limited to marginal and sub marginal tracts which are drought prone has greatly affected chickpea yields over the past few years. Further with the delayed onset of receding rains, there is delay in chickpea sowing time. The rice fallows where chickpea needs to be expanded as a niche crop also falls under late sown conditions (in Jharkhand, Chhattisgarh, Madhya Pradesh, Orissa and West Bengal) and is exposed to heat and drought stresses during reproductive stage. Terminal heat and drought stresses have become the most serious constraints to chickpea production in India due to the above reasons. Even in the traditional regions terminal drought has become a major constraint for production and varieties with resilience to terminal drought and heat stress have become essential for very much needed for realizing higher yields and stabilizing.Since, growth and development are the two major processes obliterated by water stress leading to major yield losses in chickpea. Yield losses upto 50% have been projected due to drought in chickpea all over the world (Tapan *et. al.,* 2015, Ahmad *et. al.*, 2005). The responses to abiotic stresses are complex and a basic understanding of morpho-physiological, biochemical and gene regulatory mechanisms involved is essential so that one can breed for varieties with more resilience to drought. The objective of thisstudy was to study the morpho-physiological responses of chickpea genotypes to drought stress, categorize the drought tolerant and susceptible genotypes and select the most divergent genotypes to be involved in crossing program.

MATERIALS AND METHODS

Selection of Experimental Material

The present study was conducted at the National Phytotron Facility, Indian Agricultural Research Institute, New Delhi which is located at 28°08′N 77°12′E under glasshouse conditions in 6 cm × 6 cm plastic pots. Theglasshouse temperature was maintained at 18°C and 15°C during day and night respectively. The experimental design was completely randomized design with three replications for each genotype and in two environments irrigated and stressed. Forty chickpea genotypes from genetic stock maintained at IARI were selected and used as planting material for the experiment.

Selection of Soil and Stress Treatment

The experimental soil with electric conductivity 0.4ds/m and pH 8.1 respectively, was taken from the IARI field. The plants were maintained well and watered regularly upto the pre-stress period and drought stress was imposed at the pre-flowering stage as per Mafakheri *et. al.*, 2010, after which morpho-physiological parameters were recorded. The drought stress was imposed at 35 days after sowing. After the stress was terminated, plants were watered regularly till harvesting. Though, chickpeais grown mostly on residual soil moisture in arid and semi-arid regions all over the world, however, a greater variability for yield performance of different chickpea genotypes under drought conditions has been reported by many workers. Our study focuses on physiological approaches to improve the chickpea productivity under adverse environmental conditions thus, observations were recorded on numerous growth parameters *viz.,* plant height, protein content, CI, MSI, RWC, biomass, 100 seed weight and plant yield and DSI.

Physiological Parameters

Membrane stability index (MSI) (Blum and Ebercon, 1981)

Membrane Stability Index (MSI) was calculated by taking 400 mg freshleaf sample in test tube and immersing it in 10ml of distilled water. This test tube was keptin water bath at 45°C for 30 minutes and then water conductivity of sample (C1) was measured using electrical conductivity meter. Again, the test tube was kept in water bath at 100°C for 10 minutes and thefinal conductivity meter reading of the sample (C2) was measured. The membrane stability index (MSI) was calculated using following formula.

$$\text{MSI} = 1 - (\text{C1}/\text{C2})*100$$

Relative water content (RWC)(Barrs and Weatherley, 1962)

$$\text{RWC} = \frac{[(\text{Fresh Weight} - \text{Dry Weight})] \times 100}{[(\text{Turgid Weight} - \text{Dry Weight})]}$$

Protein Content

Protein content in leaves was estimated as per the method of Bates *et. al.*, (1973).

Chlorophyll Index

Chlorophyll index was measured at around 12 noon using a chlorophyll meter SPAD 502 Plus.

Morphological Traits

Among various factors minimizing the crop yield, the drought stress affects growth parameters and reduce the crop yield to a greater extent, thus, the crop observations were recorded on plant height (PH), biomass, 100 seed weight and plant yield (PY).

Statistical Analysis

The data of individual characters was analyzed statistically and all statistical observation were carried out on the mean value of the three replications. Phenogram was generated using the morpho-physiological parameters *viz.*, MSI and RWC by sequential agglomerative hierarchical non-overlapping (*SAHN*) *grouping* method (Sokal and Sneath 1963) using the NTSYS-pc program Version 2.1 (Rohlf, 2000).

RESULTS AND DISCUSSION

Chickpea is the third most important pulse crop of India (45% total pulse production). India produces about 70% world production (FAOSTAT, 2012). Chickpea is highly susceptible to climate change, drought and heat both limit its production severely.The mean of the characters under study indicate presence of large amount of variability (Table 1) in the genotypes. Such diverse genotypes can be used to generate trans-aggressive segregants in crossing program and increase the selection gains. RWC and MSI of the forty genotypes wereevaluated. Under stress conditions, the mean RWC was 63.22 while it ranged from 40.67 (ICCV3103) to 80.45 (ICC4958) and MSI ranged from 39.87(ICC1882) to 77.12(ICCV97309) with a mean of 60.49. The genotypes ICC4958 and ICCV97309 have been identified to be drought tolerant based on their high RWC and MSI values (Table 1). The lower the

Table 1

		PH		*MSI*		*RWC*		*CHL. Index*		*Protein*		*Biomass*		*100sw*		*Yield*	
Sr. No.	*Genotype*	*N*	*S*	*N*	*S*	*N*	*S*	*N*	*S*	*N*	*S*	*N*	*S*	*N*	*S*	*N*	*S*
1.	*ICC1882*	27.33	23.67	47.44	39.87	61.99	49.18	51.05	50.6	27.77	23.43	708.74	656.37	16.54	16.22	222.00	165.4
2.	*ICC4958*	33.00	31.33	78.67	76.15	82.03	80.45	61.75	53.05	33.52	31.47	733.08	725.48	28.87	28.44	184.40	178.3
3.	*PUSA1103*	31.00	29.00	68.26	61.69	72.56	69.09	54.55	46.3	27.56	24.67	523.89	491.65	21.12	19.81	246.80	223.6
4.	*BGD72*	30.00	28.67	70.62	69.12	72.48	71.77	53.05	42.4	28.85	27.79	626.25	615.28	15.48	14.08	461.00	400
5.	*P-1003*	25.33	21.33	51.50	47.02	50.54	60.93	60.75	41.25	26.15	25.75	462.10	127.90	12.87	10.10	146.00	61.9
6.	*CSG8962*	33.00	29.33	71.25	70.07	80.45	70.63	54.05	56.2	34.38	32.10	727.83	687.92	10.38	10.1	245.30	208
7.	*C-235*	26.67	23.67	58.90	44.00	62.64	60.27	47	53.1	29.86	28.92	361.80	104.80	24.20	15.53	142.00	45.6
8.	*ICCV3310*	26.00	24.33	67.82	64.83	62.99	62.40	60.3	40.55	32.01	28.60	500	60	33.4	30.7	116.78	47.33
9.	*ICCV3311*	33.33	30.67	77.05	73.11	72.30	65.83	52.55	40.2	29.64	27.71	581.81	155	28.86	25.69	115.06	90.913
10.	*ICCV3403*	32.33	31.00	44.67	43.98	69.01	66.56	47.05	37.85	30.75	30.63	471.87	50	30.61	27.02	139.19	114.13
11.	*ICCV3404*	24.33	20.67	65.89	64.31	45.14	45.01	52.85	23.9	29.29	27.70	461.11	110	37.13	34.29	168.75	124.26
12.	*ICCV7301*	28.00	26.00	66.87	66.33	63.08	60.34	57.95	56.6	29.76	32.06	333.33	120	36.09	29.66	155.00	69.66
13.	*ICCV4303*	22.67	19.67	56.76	56.48	69.77	66.67	62.05	29.2	31.59	30.26	536.84	40	35.97	33.97	127.14	76.48
14.	*ICCV4310*	27.00	23.33	59.59	56.27	67.87	67.10	57.4	53.3	30.61	29.60	294.44	100	32.04	27.65	129.97	60.78
15.	*ICCV5312*	31.67	30.67	73.60	70.89	69.58	66.18	59.75	45.4	32.05	32.02	488.88	75	35.25	32.67	42.00	39.38
16.	*ICCV9312*	26.00	24.00	55.88	52.14	70.21	68.59	45.6	54.45	29.57	27.81	380	220	37.68	34.88	119.03	56.723
17.	*ICCV9313*	25.33	24.33	65.78	59.84	64.95	60.42	55.15	44.3	29.75	26.67	475	325	38.42	35.82	70.63	66.6
18.	*ICCV9314*	24.33	21.90	69.30	54.13	73.28	72.41	44.55	28.05	28.41	26.46	309.09	150	36.36	32.46	187.82	169.54
19.	*ICCV10313*	29.33	28.33	70.95	63.57	84.72	76.50	47.6	54	28.41	21.24	720	400	37.07	35.88	365.00	240.23
20.	*ICCV10*	32.33	24.67	72.59	70.59	82.73	77.62	52.6	52.2	31.09	31.27	364.70	235	18.84	16.36	154.70	136.5
21.	*ICCV2*	25.00	21.00	48.26	44.04	68.00	55.64	56.55	46.95	30.15	26.90	728.57	335	20.37	17.88	176.86	137.73
22.	*ICCV92337*	28.33	27.67	67.65	65.94	65.07	64.04	47.05	44.15	30.53	29.85	461.53	170	29.8	26.3	87.24	77.26
23.	*ICCV8310*	28.00	26.33	56.29	52.07	72.35	68.22	49.5	46.6	30.08	24.83	294.25	285	28.76	26.06	87.32	40.724
24.	*ICCV97309*	29.00	26.33	78.09	77.12	68.36	65.97	52.3	41.95	30.42	26.08	723.07	350	24.48	21.94	140.51	128.26
25.	*ICCV1309*	34.33	29.00	64.21	65.15	52.15	50.00	55.3	55.05	29.89	24.65	925	80	30.81	29.01	142.48	68.28

Contd. Table 1

		PH		*MSI*		*RWC*		*CHL. Index*		*Protein*		*Biomass*		*100sw*		*Yield*	
Sr. No.	*Genotype*	*N*	*S*	*N*	*S*	*N*	*S*	*N*	*S*	*N*	*S*	*N*	*S*	*N*	*S*	*N*	*S*
26.	*ICCV10304*	32.33	29.33	70.80	68.96	62.63	61.92	49.75	32.2	30.10	28.16	311.11	170	21.14	17.74	78.08	72.09
27.	*ICCV10307*	25.67	22.33	65.02	60.82	65.02	70.65	67.15	40.75	30.68	26.92	420	325	34.03	32.4	81.91	67.23
28.	*ICCV10306*	24.00	22.00	67.35	59.82	69.67	68.04	54.75	59.15	31.28	27.41	378.94	145	34.4	31.9	98.42	78.905
29.	*ICCV10316*	33.33	29.00	60.50	55.73	64.00	63.47	51.2	55.95	29.51	27.09	454.54	450	40.18	36.08	136.52	85.386
30.	*ICCV00109*	20.33	18.67	61.76	55.17	63.44	60.26	49.9	62	29.46	27.88	446.66	310	20.32	18.35	153.33	54.16
31.	*ICCV3103*	26.00	23.33	66.84	61.61	70.82	40.67	63.05	57.2	30.53	29.88	335	210	23.48	20.78	105.75	57.167
32.	*ICCV9307*	30.67	28.00	73.04	71.14	68.78	61.75	54.35	53.95	29.82	26.75	388.8	180	38.84	38.31	119.82	44.43
33.	*ICCV95423*	32.33	28.00	60.81	60.33	64.65	62.33	48.5	53.4	25.24	23.32	400	235	25.91	24.33	413.64	155.83
34.	*ICCV97404*	28.33	15.67	67.02	63.84	56.59	52.91	46.3	46.2	29.42	29.57	700	360	23.68	21.28	252.00	152.65
35.	*ICCV0301*	31.33	26.67	58.50	54.29	59.66	57.64	50.35	49.75	32.04	31.34	560	160	16.776	13.27	124.50	45.133
36.	*ICCV0302*	23.00	25.33	56.25	51.96	63.30	60.00	55.15	54.6	29.88	29.86	413.3	170	31.7	29.91	125.20	50.571
37.	*ICCV1301*	29.00	25.00	68.12	61.67	56.16	54.78	55.95	50	30.39	28.04	355.5	225	26.83	24.3	111.63	102.4
38.	*L-550*	26.67	18.67	61.76	55.78	64.46	62.61	45.95	46.7	29.66	28.18	713.0	683.9	17.9	16.84	167.00	73.86
39.	*ICCV5308*	28.33	17.00	63.04	54.55	68.00	64.81	52.1	29.4	33.02	25.35	132.3	70	35.3	33.13	288.06	242.95
40.	*ICCV5313*	30.33	26.67	77.13	75.31	71.49	65.28	52.3	50.75	31.28	26.84	420	95	32.96	31.47	194.54	111.32
	Mean	28.38	25.06	64.64	60.49	66.82	63.22	53.38	46.99	30.11	27.88	490.5	261.4	28.12	25.57	165.58	110.54
	Min	20.33	15.67	44.67	39.87	45.14	40.67	44.55	23.90	25.24	21.24	132.3	40.00	10.38	10.10	42.00	39.38
	Max	34.33	31.33	78.67	77.12	84.72	80.45	67.15	62.00	34.38	32.10	925.0	725.4	40.18	38.31	461.00	400.00

Where PH- plant height, MSI-membrane stability index,RWC-relative water content, Chl. Index-chlorophyll index, 100SW-100 seed weight, YLD- plant yield, N: Normal and S: Water stressed environments difference in the values under normal and stress conditions, the greater is thetolerance to stress and such lines can be used as a donor for thattrait.

The genotypes ICC4958 and ICCV97309 had lower variation in MSI and RWC values under normal and drought stress conditions and higher yield, thus, are very promising indicating their suitability to be used as donors. 100 seed weight ranged from 10.10 gm (P-1003) to 38.31gm (ICCV9307). Yield for single plant ranged from 39.38 gm (ICCV5312) to 400 gm (BGD-72) with the mean of 110.54gm. Biomass ranged from 40gm (ICCV4303) to 725.48gms (ICC4958) with a mean of 261.46gm. The mean CI was 46.99 while it ranged from 23.90(ICCV3404) to 62 (ICCV00109) and proteinranged from 21.24 µg/ml (ICCV10313) to 32.10µg/ml (CSG8962) with a mean of 27.88µg/ml. The plant height ranged from 15.67cm (ICCV97404) to 31.33cm (ICC4958) with a mean of 25.06cm.

The dendrogram grouped the forty genotypes into three major clusters (Figure 1). Out of 3 clusters, the largest was cluster II which comprised of 33 cultivars, whereas and cluster III emerged as smallest cluster with 2 cultivars (ICCV3404 and ICCV3103). Cluster I comprised of five cultivars. The cluster II had 33 cultivars which were further divided into two sub-clusters (IIa, IIb). In the IIb sub-cluster twointra clusters IIb(i) and IIb(ii) could be identified. The SAHN clustering further delineated IIb(ii) into two sub groups *viz.*, IIb(ii)a and IIb(ii)b (Figure 2 and Table 2). Bharadwaj *et. al.*, 2001 proposed that phenotypic or genotypic diversity *per se* should not be considered as a direct measure of genetic diversity. It is an inferential criterion andmay not be useful for discrimination among the genotypes for selecting them as parents for crossing programme which generally most breeders do. Numerous classificatory techniques have been used by different workers to

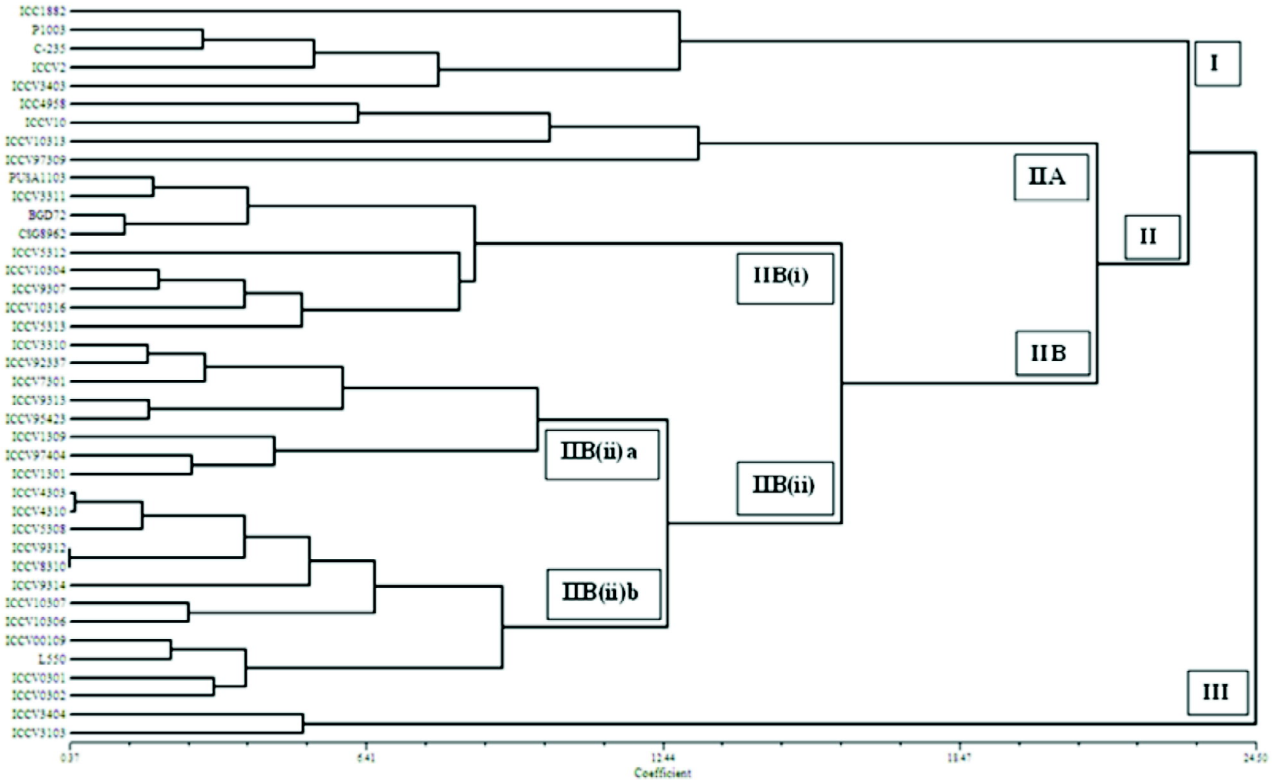

Figure 1: SAHN grouping based on morpho- physiological parameters showing genetic relatedness among the forty chickpea genotypes under stress environment

Table 2
Clustering based on SAHN grouping of the forty chickpea genotypes under stress environment

	Average values				
Major Cluster	*Subcluster*	*Minor cluster*	*Genotypes*	*RWC*	*MSI*
I (5)			ICC1882, P-1003, C-235, ICCV-2,ICCV-3403	58.51594	43.78124
II (33)	IIA (4)		ICC4958, ICCV10, ICCV10313, ICCV97309,	75.13591	71.85814
	II B (29)	II B (i) – (9)	P-1103, ICCV-3311, BGD-72, CSG-8962, ICCV-5312, ICCV-10304, ICCV-9307, ICCV-10316, ICCV-5313	66.21491	68.44663
		II B (ii)a –(8)	ICCV-3310, ICCV-92337, ICCV-7301, ICCV-9313, ICCV-95423, ICCV-1309,ICCV-97404, ICCV-1301	58.40224	63.49048
		II B (ii)b –(12)	ICCV-4303, ICCV-4310, ICCV-5308, ICCV-9312, ICCV-8310, ICCV-9314, ICCV-10307, ICCV-10306, ICCV-00109, L-550, ICCV-0301, ICCV-0302	65.58313	55.28988
III (2)			ICCV-3404, ICCV-3103	42.83877	62.95827

Figures in parenthesis indicate the number of genotypes in that cluster

quantify the geneticdivergence in a given set of genotypes based on the data collected (Bharadwaj *et. al.* 2011 in rice; Jeena and Arora, 2002 in chickpea, Bharadwaj *et. al.* 2011 in chickpea).

Cluster average values could clearly delineate the tolerant genotypes from the susceptible ones (Figure 4). Cluster IIa had the most tolerant genotypes *viz.*, ICC4958, ICCV10, ICCV10313 and ICCV97309 grouping together while the most susceptible ones were grouped in cluster I and cluster III. Cluster II, in general, comprised of the tolerant and the moderately tolerant genotypes. The average cluster value in cluster IIa having the most tolerant genotypes was 71.85 (MSI) and 75.13 (RWC) under stress conditions (Figure 2-3). Further these genotypes also had very low variation under normal versus stress conditions in their membrane stabilities. If the breeding program intends to create variability for these traits, ideal would be crossing the genotypes of cluster IIa with cluster III. Crosses among such diverse parents would produce a broad spectrum of variability from which selection could be done for the desirable phenotypes. The breeding lines which mostly constituted of training population lines developed at ICRISAT have grouped into different groups. The clustering pattern clearly suggested that there was considerable diversity in the material used. This probably would have occurred due to differential selection exercised by breeder for seed yield components and other traits which have been referred to as genetic drift due to selection (Murty

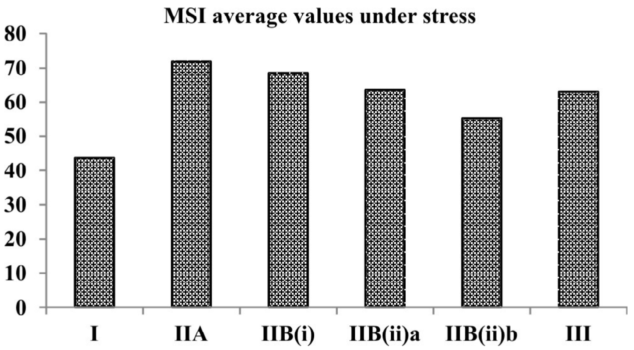

Figure 2: Clusters v/s average values of MSI under stress

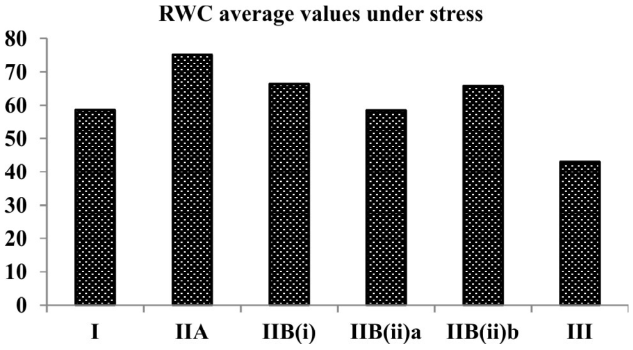

Figure 3: Clusters v/s average values of RWC under stress

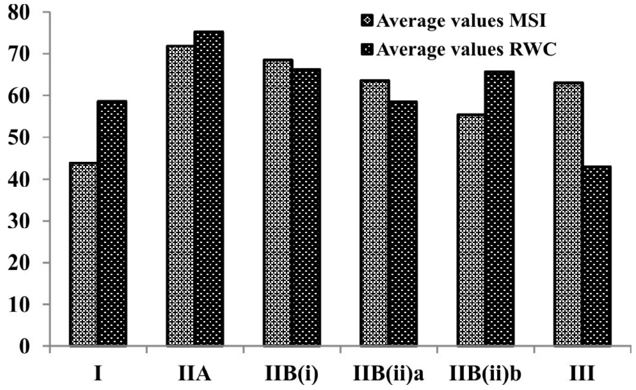

Figure 4: Clusters v/s Average values of MSI and RWC under stress

and Arunachalam, 1966). Thus, crosses between ICC4958, ICCV10 with that of ICCV3404 and ICCV3103 would result in a wider variability for selection to be exercised.

Madan Pal *et. al.*, 2013 studying the growth dynamics and temperature sensitivity of late planting chickpea genotypes to Delhi conditions inferred that low temperature stress during vegetative stage apart from high temperature stress at flowering and podding stages affects yield. In South Indian conditions, terminal heat and drought are the main yield deterrents. In such a scenario, it is pertinent to identify genotypes that have lower drought susceptibility index (DSI) but simultaneously also have higher yield and high biomass. Such lines can directly be deployed for yield improvement in niche areas like rice fallows. ICC4958 and ICCV97309 though having a very high drought tolerance due their MSI values. However, have lower yields and could be ideal donors. BDG72, Pusa-1103 on the other hand not only have higher MSI values but also greater yield under stress, lower DSI and higher biomass indicating their plasticity to produce higher yield even under vegetative and terminal drought stress conditions.

CONCLUSION

The occurrence of distinct groups of chickpea lines used in the study as identified through SAHN grouping would possibly draw the attention of breeders for planning efficient breeding program for yield resilience under drought stress conditions. The gains obtained through the use of the lines identified in the study would provide a large variability in which breeders can exercise their option. Root trait QTLs have already been identified in ICC4958 (Varshney *et. al.*, 2014) are already been deployed in marker-assisted backcrossing (MABC) in chickpea for improving drought. It is further proposed that greater gains can be obtained by crossing these lines (ICC4958 with Pusa-1103 and BGD72) to develop a high yielding drought tolerant line. This high × high stress tolerance interaction can also pave way for getting super aggressive trans-aggregants. Tracking the root QTLs for drought tolerance from ICC4958 with the already identified markers along with selection exercised based on MSI and RWC shall identify high yielding drought tolerant lines.

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