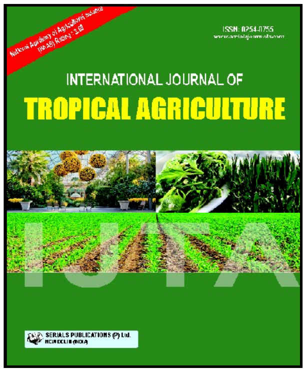

INTERNATIONAL JOURNAL OF TROPICAL AGRICULTURE

ISSN : 0254-8755

available at http: www.serialsjournal.com

Volume 35 • Number 1 • 2017

Selection Indices to Discern Response of Chickpea (*Cicer Arietinum* L.) Genotypes to Drought Stress

Supriya Sachdeva[1a], C. Bharadwaj[*1b] , Vinay Sharma[2], B.S. Patil[1c], K.R. Soren[3], Tapan Kumar[4] , Sanjay Kumar[6a], Sarvajeet Singh[5], S.K. Chauhan[1d] and KV Bhat[6]

[1a]Research Scholar, [1b]Principal Scientist, [1c] Senior Scientist, [1c]ACTO Genetics Division ICAR-IARI, Pusa, New Delhi, India 110012;
[2] Professor, Head and Dean Faculty of Science, Banasthali University,
[3]Senior Scientist, ICAR-IIPR Kanpur,
[4]ICARDA Amlah Centre Bhopal,
[5]PAU, Ludhiana, Punjab
[6A]Research Scholar, [6]Head, Division of Genomic Resources, ICAR-NBPGR, Pusa, New Delhi
[*]Corresponding Author. E-mail:chbharadwaj@yahoo.co.in

Abstract: Breeding for drought tolerance in chickpea is a challenging task. The present investigation was undertaken to identify the morpho-physiological responses of chickpea genotypes subjected to drought stress and identify tolerant genotypes. Further it also aimed at identifying the best selection indices that can be used for identification of tolerant genotypes under drought stress. A pot experiment was carried out under water stressed conditions (no irrigation) and rain-fed (control) conditions in three replications and observations were recorded on plant height, protein content, chlorophyll index, Membrane Stability Index (MSI), Relative Water Content (RWC), Drought Susceptibility Index (DSI), days to flowering, days to maturity, biomass, 100seed weight and plant yield in the ten chickpea genotypes. ICC4958, Pusa 1103, CSG 8962, ICCV10313, ICCV9314, ICCV10 maintained higher MSI and also had lower DSI indicating their tolerance to drought. IC1882, ICCV9312, ICCV9313, and ICCV2 showed higher DSI. The results suggest that growth and photosynthesis are completely or partially abolished by drought stress leading to major yield losses in chickpea and in order to minimize the yield losses, it is important to understand the morpho-physiological basis of yield variation occurring due to drought stress. DSI and MSI would serve as an important selection index for identifying the genotypes tolerant to drought stress.

Key words: Protein, Chlorophyll index, MSI, RWC, DSI, variability, chickpea.

INTRODUCTION

Chickpea (*Cicer arietinum* L.) is the second most important legume crop in Asia belonging to the family Fabaceae (Varshney *et al*, 2013). Chickpea grows in more than 50 countries and 90 per cent of its global area is in Asia (Gaur *et al*, 2012). Globally, an area of 8.25Mha is under chickpea producing 7.33Mt (PC report, 2015-16) and India produces 68% of total production of the world. Chickpea has been acknowledged as best composed legumes with good nutritional value containing 40% carbohydrate, 20-30% protein (Gill *et al*, 1996) and several minerals as well. Chickpea grows mostly in areas with depleting soil moisture and maintains the soil fertility by symbiotic nitrogen fixation (Roy *et al.*, 2010). The average productivity of chickpea is very low about 889kg.ha^{-1} (http://agricoop.nic.in/) and has remained about the same since many years partly because of numerous environmental stresses and insufficient genetic diversity in various traits due to the domestication process (Bharadwaj *et al* 2011; Gur and Zamir, 2004). Environmental stresses mainly drought, low temperature, terminal heat, high salt (Ryan, 1997), acidity, water logging and toxicity stresses (Siddique *et al*, 2000) and susceptibility to pathogens adversely reduce the chickpea yield and affecting growth and development in chickpea.

Yield losses upto 50% have been projected due to drought in chickpea all over the world (Ahmad *et al*, 2005). Breeding for drought tolerance and high temperature stresses in chickpea is constrained by the lack of sound selection indices that can be used for stress tolerance, thus, there is an urgent need to understand the physiological responses of chickpea plants to drought stress and identify the stress tolerant genotypes tolerant to drought stress. The objective of this study was to develop a better understanding of the morpho-physiological responses of chickpea genotypes subjected to drought stress, determine their tolerance and also identify the best selection indices that can be used for identification of high yielding and genotypes tolerant to drought stress for use in future crop improvement programs.

MATERIALS AND METHODS

The present research was carried out with ten chickpea cultivars at the National Phytotron Facility, Indian Agricultural Research Institute, New Delhi which is located at 28°08′N 77°12′E under glasshouse conditions during the year 2016-17. The glasshouse temperature was maintained at 18°C and 15°C during day and night respectively. The experiment was conducted under water stress conditions and control conditions as per Mafakheri *et al* (2010) protocol. The plants were maintained well and watered regularly upto the pre-stress period and drought stress was imposed at the pre-flowering stage.

INDUCTION OF DROUGHT STRESS IN POTS

The experimental soil with electric conductivity 0.4dsm^{-1} and pH 8.1 respectively, was taken from the IARI field. The experiment was laid down in in a Completely Randomized Designwith three replications in 6cm × 6cm plastic pots in both stress and normal condition as per the protocol by Mafakheri *et al* (2010). The drought stress (vegetative) was imposed at 35 days after sowing. After the stress was terminated, plants were watered regularly till harvesting. Though, chickpea grows well in drought prone conditions, there exists variability for yield performance of different chickpea genotypes grown under drought conditions. Attempts to measure the degree of tolerance with a single parameter have limited value, thus, observations were recorded on numerous morpho-physiological parameters *viz*, plant height, protein content, chlorophyll index, MSI, RWC, DTF, DTM, biomass, 100 seed weight and plant yield and DSI.

PHYSIOLOGICAL PARAMETERS

Relative Water Content (RWC) (Barrs and Weatherley, 1962)

Top three completely open healthy leaves were collected from three different plants randomly for calculating the relative water content. 400 mg of leaf sample was taken and immersed in distilled water in a petriplate for 4 hours at room temperature with sufficient sunlight and their turgid weights were recorded. Leaves were then oven dried at 60°C for 72 hours and weighed and the mass was recorded as the plant dry mass quickly to avoid retention of atmospheric moisture. RWC was calculated for all the ten genotypes as follows:

$$RWC = FW - DW/TW - DW \times 100$$

Where, FW-Fresh weight; DW-Dry weight; TW-Turgid weight

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 water conductivity (C1) was measured using electrical conductivity meter. Again, the test tube was kept in water bath at 100°C for 10 minutes and the final conductivity meter reading (C2) was measured. The MSI was calculated using following formula.

$$MSI = 1 - (C1/C2) \times 100$$

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.

Drought susceptibility index (DSI) was used as the measure of drought tolerance. Drought susceptibility index (DSI) was also calculated by the formula given by Fischer and Maurer (1978) (Figure 2).

$$DSI = (1 - Yd/Yp)/D$$

Where, Yd = Grain yield of the genotype under moisture stress condition.

Yp = Grain yield of the genotypes under irrigated condition

D = Mean yield of all strains under moisture stress condition

Mean yield of all strains under irrigated condition

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), Days to 50 per cent flowering (DTF), Days to maturity (DTM), biomass, 100 seed weight and plant yield (PY).

RESULTS AND DISCUSSION

The mean, range and coefficient of variation(CV) for plant height, protein content , chlorophyll index, MSI, RWC, days to flowering, days to maturity, biomass, 100seed weight and plant yield are given in Table 1. Breeding for complex traits like drought tolerance is difficult since drought is a quantitative trait controlled by many genes and highly influenced by environment, thus, the responses to drought stress are not well understood, making it difficult to develop drought-tolerant. The analysis of variance for normal and drought stress conditions revealed that the differences among the genotypes were significant. The mean sum of squares is highly significant for all the characters *viz.,* plant height, protein content, chlorophyll index, MSI, RWC, days to 50 per cent flowering, days to maturity, biomass, 100 seed weight and plant yield in both normal and drought conditions studied indicating presence of significant variability in the genotypes (Table 2).

Tapan *et al* (2015), Aslam *et al* (2008) also reported significant variation in morphological, physiological, phenological characters and yield, yield components in chickpea. Under normal conditions, mean plant height was found to be 65.97cm, with a minimum of 52cm and amaximum of 86.1cm at 40

Table 1
Mean, Range, CV of the characters under study among the chickpea genotypes

	DTF		*DTM*		*BIOMASS*		*100 SW*		*YLD*		*DSI (Under Stress)*
	N	*S*	*N*	*S*	*N*	*S*	*N*	*S*	*N*	*S*	
ICC1882	125	110	145	127	708.7	656.4	16.5	16.22	222	165.4	0.3729
ICC4958	110	109	145	138	733.1	725.5	28.9	28.44	184.4	178.3	0.0483
PUSA1103	110	108	146	130	523.9	491.7	21.1	19.81	246.8	223.6	0.1374
CSG8962	100	96	146	125	727.8	687.9	10.4	10.1	245.3	208	0.2224
ICCV9312	52	73	151	124	414.9	220	37.7	34.88	119	36.72	0.7656
ICCV9313	49	82	142	124	476.4	325	38.4	35.82	70.63	66.6	0.0833
ICCV9314	47.8	80	148	124	309.1	150	36.4	32.46	187.8	169.5	0.1423
ICCV10313	48	78	145	125	720	400	37.1	35.88	365	240.2	0.4999
ICCV10	104	90	148	128	364.7	235	18.8	16.36	154.7	136.5	0.1720
ICCV2	43	73	143	124	730.4	335	20.4	17.88	176.9	137.7	0.3236
Mean	78.8	90	145.9	165.7	570.9	422.6	26.6	24.79	197.3	134.9	0.2768
Min	43	73	142	124	309.1	150	10.4	10.1	70.63	36.72	0.0483
Max	125	110	151	138	733.1	725.5	38.4	35.88	365	240.2	0.7656
CV	0.42	0.2	0.018	0.027	0.3	0.492	0.39	0.392	0.407	0.482	0.7998

	PH		*Protein content*		*Chl. Index*		*MSI*		*RWC*	
Genotype	*N*	*S*	*N*	*S*	*N*	*S*	*N*	*S*	*N*	*S*
ICC1882	60.1	55.3	27.77	23.4	51.1	50.6	47.436	39.87	61.99	49.18
ICC4958	77.9	75.3	33.52	31.5	61.8	53.1	78.667	76.15	76.39	73.75
PUSA1103	86.1	81.8	27.56	24.7	54.6	51.3	68.255	61.69	72.56	69.09
CSG8962	70.7	66.2	34.38	32.1	54.1	56.2	71.255	70.07	80.45	70.63
ICCV9312	62	58	29.57	27.8	49.6	44.5	55.879	52.14	69.35	65.83
ICCV9313	52	50	29.75	26.7	55.2	44.3	65.777	59.84	64.95	60.42
ICCV9314	72	68	28.41	26.5	44.6	28.1	69.297	54.13	72.79	71.78
ICCV10313	60	56	28.41	21.2	57.5	54	70.947	63.57	84.72	79.2
ICCV10	62.9	62.8	31.09	31.3	52.6	52.2	72.589	70.59	75.64	71.1
ICCV2	56	54	26.3	24.1	56.6	47	48.256	44.04	68	58.8
Mean	65.97	62.74	29.68	26.9	53.7	48.1	64.836	59.21	72.68	67.71
Min	52	50	26.3	21.2	44.6	28.1	47.436	39.87	61.99	49.18
Max	86.1	81.8	34.38	32.1	61.8	56.2	78.667	76.15	84.72	79.2
CV	0.038	0.161	0.088	0.14	0.09	0.17	0.1642	0.198	0.096	0.128

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

Table 2
ANOVA for the 10 characters under study in stress environment in chickpea genotypes

Source	*Rep*	*Treatment Mean SS*	*Error Mean SS*
Df	2	9	18
PH	101.45	425.66*	66.14
Protein	8.22	47.53**	3.31
Chl. Index	20.64	146.59**	3.39
MSI	4.53	414.63**	13.07
RWC	23.63	211.65**	2.63
DTF	0.21	564.12**	9.1
DTM	11.03	89.32**	9.66
Biomass	104.22	134,772.28**	141.29
100 SW	8.92	221.40**	1.52
YLD	8.09	13,310.15**	22.52

**Significant at $P < 0.05$; *Significant at $P < 0.01$

PH-plant height, Chl. Index-chlorophyll index MSI-membrane stability index, RWC-relative water content DTF-days to flowering, DTM-days to maturity, 100SW-100 seed weight, YLD- plant yield

days of sowing whereas the plant height in drought stressed pot ranged from 50cm to 81.8 cm with an average of 62.74cm. There exists highly significant variation in biomass from 309.1g to 733.1g with an average value of 570.9g under normal condition while underdrought stress condition it varied from 150.0g to 725.5 g with an average of 422.64 g. The 100 seed weight varied from 10.4g to38.42 g with mean value of 26.6g under normal condition and 10.1g to 35.8g with mean value of 24.79g under stress condition which indicate that drought stress significantly affects yield parameters. Under drought stress condition there was significant decrease in the mean of most the characters under study. Drought stress reduced the plant height by (54.03%) followed by plant yield (31.62%), biomass reduced by (25.96%), chlorophyll index (10.45%) and protein content by (9.26%), membrane stability index by (8.68%), relative water content by (6.83%) and 100 seed weight showed reduction up to (6.70%) under drought stress conditions (Table 3).

Table 3
Percentage reduction of different traits under stress

Sr. No.	*Character*	*Avg of normal*	*Avg of stress*	*% Reduction*
1.	Plant Height	65.97	62.74	54.03
2.	Protein	29.68	26.92	9.26
3.	Chl. Index	53.74	48.11	10.45
4.	MSI	64.84	59.21	8.68
5.	RWC	72.68	67.71	6.83
6.	Biomass	570.90	422.64	25.96
7.	100 SW	26.57	24.79	6.70
8.	Plant Yield	197.25	134.86	31.62

A low heritability for plant height and plant yield indicating greater role of environment factors which was also reported by Rachna *et al* (2016) in chickpea. Leport *et al* (2006) reported that moisture stress at pre-flowering stage is the most damaging stage to yield and yield parameters, thus, artificial stress at this stage will lead to screening of genotypes resilient to drought and heat in chickpea. Drought tolerant genotypes with low mean yield have no commercial value however can be used as donors in breeding program. Drought tolerant genotypes with good economic yield under stress conditions could be of immense potential for further direct utilization in varietal development for stress environments.

In general, there was a reduction of all the traits studied under stress environment (Table 3) compared to that of normal. This shows irrespective of genotypes the normal grown seedlings had higher values for all the parameters under study while those grown under drought stress had reduced values for all the traits. However, the highest per cent reduction over all the genotypes was seen for the trait plant height (54.03%) followed by plant yield (31.62%) and biomass (25.96%) (Figure 1). 100 seed weight had relatively lower reduction of 6.7% indicating that character is lesser affected by any environment compared to above ones. A greater G × E interaction with more preponderance of non-additive gene action may be a cause for such variability. The star microplot (Figure 2) of yield under normal and stress conditions showed that ICC4958, Pusa-1103, CSG8962, ICC9314 and ICCV10 had lower yield penalty compared to the other genotypes. Drought susceptibility index (Figure 3) clearly brought about the resilience of above genotypes when grown under stress conditions.

Relationships between DSI and relative water content and membrane stability index was determined in order to find out whether physiological parameters could be used as genotypic selection criteria for drought tolerance (Figure 4-5). Studies into RWC and MSI for these genotypes were done to analyze how the resistant genotypes mitigated the drought stress. It was seen that in ICCV9314, higher

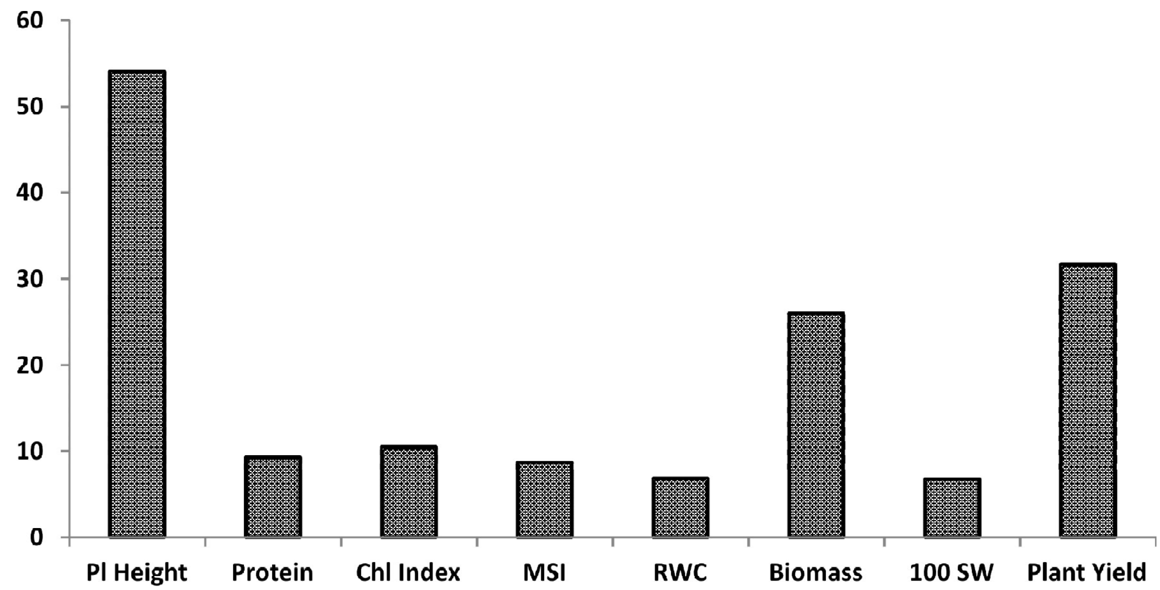

Figure 1: Percentage Reduction of different seedling growth parameters under drought stress conditions

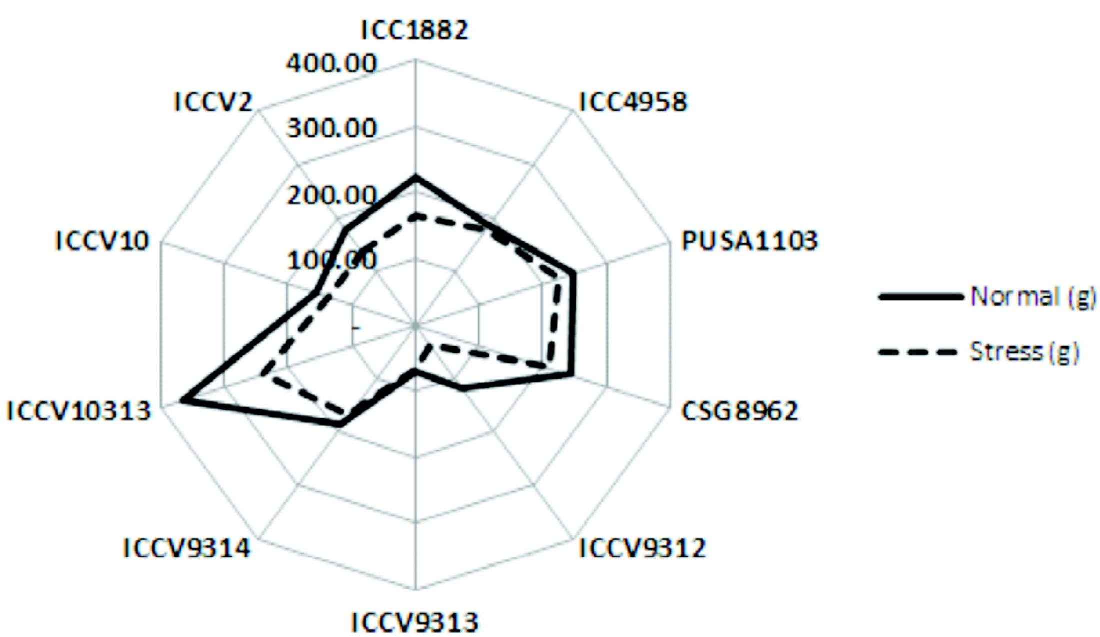

Figure 2: Star Micro plot of yield under normal and yield under stress

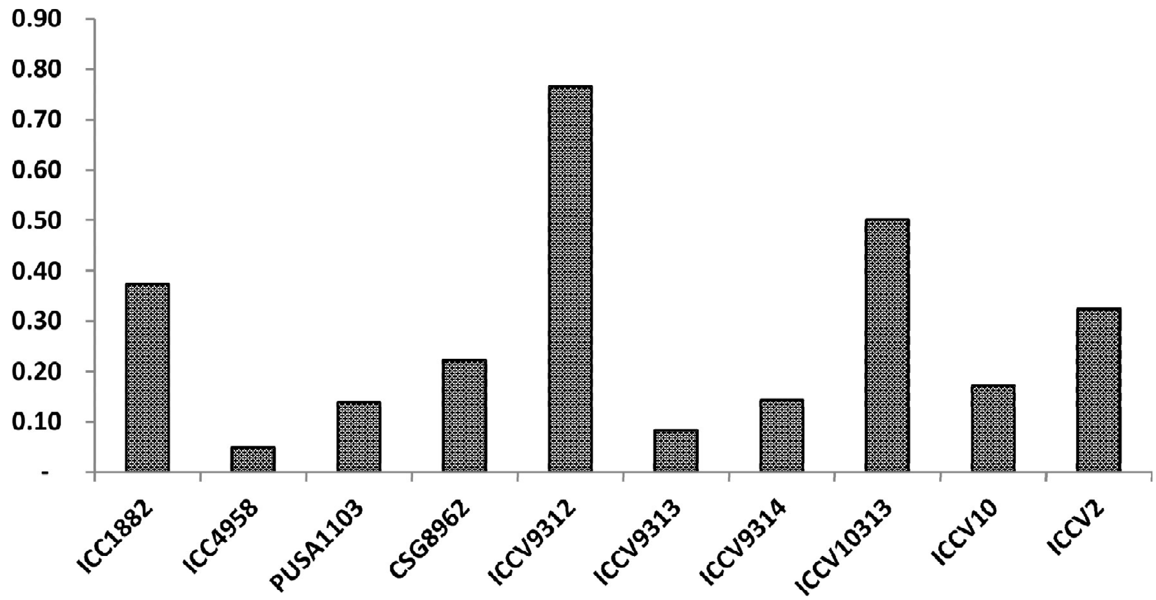

Figure 3: Drought Susceptibility Index (DSI) of the chickpea genotypes under study

relative water content could have played role in drought tolerance while in other tolerant genotypes a higher membrane stability index was the cause. Further, when the reduction in MSI and RWC of these genotypes under stress *viz-à-viz* normal conditions is compared the tolerant genotypes ICC4958, ICCV9313, ICCV9314 and Pusa-1103 had a lower reduction (Figure 4-5). It can be inferred that under drought stress conditions these genotypes could maintain integrity of their membrane even at lower water regimes and thus, could withstand stress. Similar findings were reported by Tapan *et al* (2015), Neeraj *et al*, 2016 who reported that those lines which had lower reduction in MSI and RWC values compared to normal were relatively able to withstand better the stress. In this study, we could find a stronger correlation for MSI with DSI.

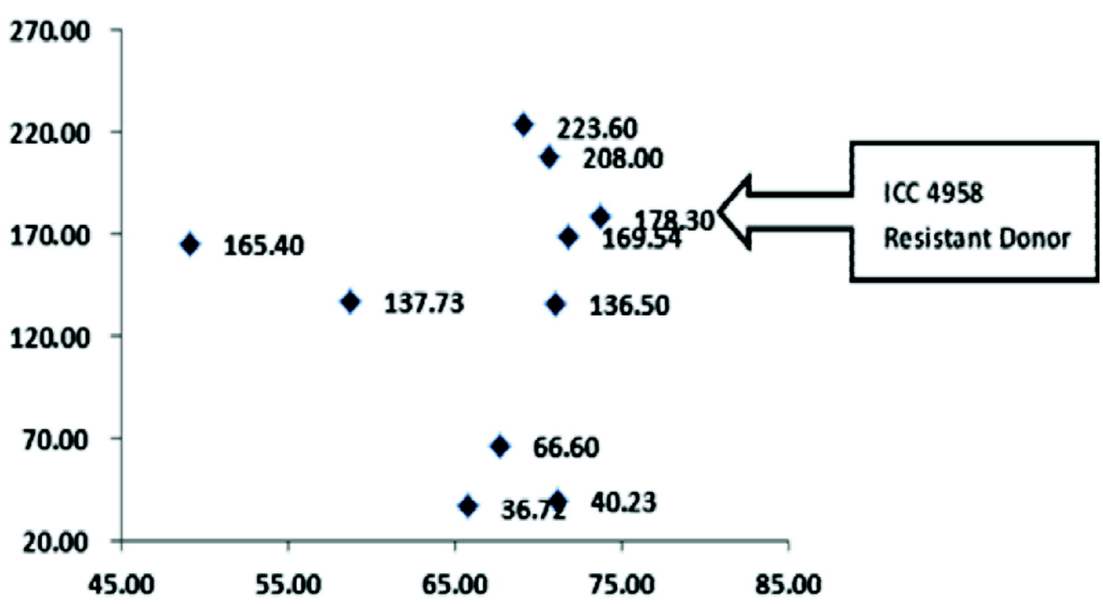

Figure 4: Maximax- Minimax plot of Yield (Stress) vs RWC of 10 genotypes

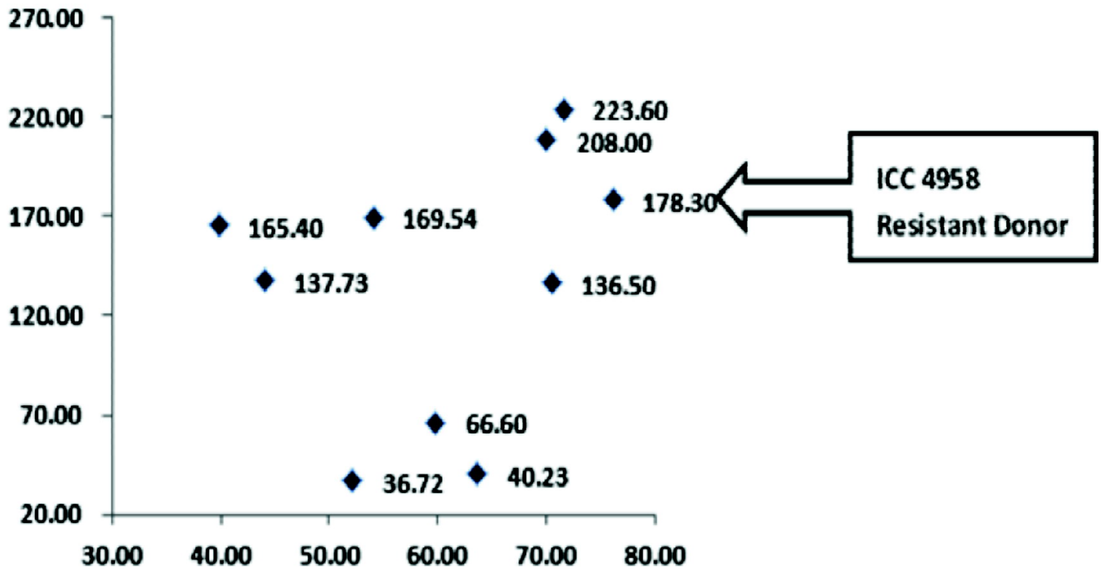

Figure 5: Maximax- Minimax plot of Yield (Stress) vs MSI of chickpea genotypes

Breeders are interested in identifying lines which give lesser reduction of yield under drought. DSI though is always a reliable estimate but evaluation for DSI is tedious, time consuming and requires rain-out shelters, so that the experiment does not fail due to unseasonal rains. MSI can easily be estimated and a large number of genotypes can be evaluated through paper cup protocol (Vadez *et al*, 2007) for seedling tolerance to drought. The dip in MSI values of these genotypes can be used to identify the most tolerant genotypes. Those genotypes which have a lower deviation from their non-stress values are the tolerant ones.

CONCLUSION

Drought is a major constraint minimizing chickpea yield to a greater extent.Chickpea responds to these stresses with an array of biochemical and physiological mechanisms which include reduced cell growth and decreased leaf area, biomass and yield. Drought stress tolerance becomes more complicated in cases where the plant response varies with the stage of stress and the environmental conditions in which it is grown. Since growth and photosynthesis are the two main processes abolished by drought stress, maximum reduction was seen in plant height, yield and biomass. From the results it is evident that there are distinct variations amongst the genotypes in terms of decline in plant height, chlorophyll index, relative water content, membrane stability index, protein content, days to flowering, days to maturity and yield parameters under drought stress. The per cent decrease in MSI would serve as an ideal selection index for identification of drought tolerant genotypes.Genotypes showing minimum reduction in growth parameters and low drought susceptibility index under stress conditions in this study were ICC4958, Pusa-1103, CSG-8962, ICCV-10313, and ICCV- 10. These genotypes can be used in breeding programme to develop drought tolerant lines.

ACKNOWLEDGEMENTS

The authors acknowledge ICAR-IARI for the financial support for the first author and to Incentivizing Research on Agriculture (Component IV: Chickpea) Project funded by ICAR, Government of India and Department of Biotechnology, AISRF.

REFERENCES

Ahmad, F., Gaur, P., Croser, J., (2005), Chickpea (*Cicer arietinum*L.) *In: Singh R, Jauhar P (eds)* Genetic resources, chromosome engineering and crop improvement-grain legumes. *CRC Press, Boca Raton, FL.* pp 185-214.

Aslam, M. M., M. R. Ismail, M. Ashrafuzzaman, K. M. Shamsuzzaman and M. M. Islam. (2008), Evaluation of chickpea lines/mutants for high growth and yield attributes. *Int. J. Agric. Biol.* **10**(5): 493-498.

Barr, H.D., and Weatherley, P.E. (1962), A re-examination of the relative turgidity technique for estimating water deficit in leaves. *Aust. J. Biol. Sci.* **15**: 413-428.

Bharadwaj C., Srivastava R., Chauhan S. K., Satyavathi C. T., Kumar J., Faruqui A., Yadav S., Rizvi A. H. and Kumar T. (2011), Molecular diversity and phylogeny in geographical collection of chickpea (*Cicer* sp.) accessions. *J. Genet.*(Springer) 90, e94–e100. http://www.ias.ac.in/jgenet/OnlineResources/90/e94.pdf

Blum A. and Ebercon A. (1981), Cell membrane stability as a measure of drought and heat tolerance in wheat. *Crop. Sci.* **21**: 43-47.

Bohnert, H., D.E. Nelson and R.G. Jensen, (1995), Adaptations to environment stresses. *Plant Sci.* **7**: 1099-1111.

Gaur, P. M., Jukanti, A. K., Varshney, R. K., (2012), Impact of genomic technologies on chickpea breeding strategies. *Agronomy.* **2**, 199-221.

Gil, J., Moreno, M.T., Nadal, S., Luna, D., De Haro, A., (1996), Variability of some physico-chemical characters in Desi and Kabuli chickpea types. *J. Sci. Food. Agric.* **71**: 179-184.

Gur, A., Zamir, D., (2004), Unused natural variation can lift yield barriers in plant breeding. *PLoS Biol.* **2**: e245.

Leport, L., N. C. Turner, S. L. Davies, and K. H. M. Siddique, (2006), Variation in pod production and abortion among chickpea cultivars under terminal drought. *Eur. J. Agron.* **24**, 236-246.

Mafakheri, A., Siosemardeh, A., Bahramnejad, B., Struik, P.C., Sohrabi, Y., (2010), Effect of drought stress on yield, proline and chlorophyll contents in three chickpea cultivars. *Aust. J. Crop Sci.* **4**, 580-585.

Neeraj kumar, C. Bharadwaj, C. Tara Satyavathi, Madan Pal, Tapan Kumar, Tripti Singhal, P.K. Jain, B. S. Patil and K. R. Soren. Yield Correlation of chickpea (*Cicer arietinum*L.)genotypes based on physiological and morphological traits for salt tolerance. *International Journal of Tropical Agriculture.* **34**(3): 693-699.

Project Coordinator's Report 2015-16. All India Co-ordinated Research Project on Chickpea, ICAR-Indian Institute of Pulses Research-208024, pp 1-47.

Rachna Srivastava, C. Bharadwaj, Sanjay Vyas, Tapan Kumar, Sabhyta Bhatia, Swarup Parida, C. Tara Satyavathi, B.S. Patil, S.K. Chauhan and Rajesh Yadav (2016), Genetic variability and Heritability in Recombinant Inbreed Lines Developed for Seed Traits in Chickpea (*Cicer arietinum L.*). *International Journal of Tropical Agriculture.* **34**(6): 1583-1586

Roy, A.F., Boye, J.I., and Simpson, B.K. (2010), A review bioactive proteins and peptides in pulse crops: Pea, chickpea and lentil. *Food Research International.* **43**: 432-442.

Ryan, J.G. (1997), A global perspective on pigeon pea and chickpea sustainable production systems: present status and future potential. *In: Asthana, A.N. and Ali, M. (eds) Recent Advantages in Pulses Research.* Indian Society of Pulse Research and Development, IIPR, Kanpur, India, pp. 1-31.

Siddique, K.H.M. (2000), Understanding growth stresses of cool season pulses. *In: O'Connell, L. (eds.) Shared Solutions Cropping Manual, Australian Grain*. Berekua, Brisbane, pp. 12-16.

Tapan Kumar, C. Bharadwaj, A. H. Rizvi, Ashutosh Sarker, Shailesh Tripathi, Afroz Alamand S. K. Chauhan. (2015), Chickpea landraces: a valuable and divergent source for drought tolerance. *International Journal of Tropical Agriculture.* **33**(2): 633-638.

Vadez, V., Krishnamurthy, L., Serraj, R., Gaur, P.M., Upadhyaya, H.D., Hoisington, D.A., Varshney, R.K., Turner, N.C. and Siddique, K.H.M. (2007), Large variation in salinity tolerance in chickpea is explained by differences in sensitivity at the reproductive stage. *Field Crops Res.* **104**: 123-129.

Varshney, R.K., Song, C., Saxena, R.K.,Azam, S., Yu, S., Sharpe, A.G., *et al.* (2013), Draft genome sequence of chickpea (*Cicer arietinum*) provides a resource for trait improvement. *Nature biotechnology.* **31**: 240-246.