Indian J. Genet., (2023); 83(1): 135-138
doi: 10.31742/ISGPB.83.1.17
ISSN: 0975-6906
www.ijgpb.org

SHORT RESEARCH ARTICLE

Harnessing genetic variability for shoot and root morphophysiological traits contributing to drought stress tolerance in pre-breeding lines chickpea (*Cicer arietinum* L.) under hydroponics condition

Rintu Jha[1], Hemant K. Yadav[1], Uday C. Jha[2], Rahul Raiya[1], Pronob J. Paul[3], Shailesh Tripathi[1] and Prashant Singh[*]

Abstract

Under the global climate change, drought stress is becoming a recurrent phenomenon influencing plant growth and yield negatively, thus jeopardizing global food security. Chickpea (*Cicer arietinum* L.) grown in South Asia as a post-rainy season crop under residual soil moisture, is more often exposed to terminal drought stress, especially during the pod filling stage ultimately results in significant yield reduction in chickpea. Crop wild relatives are natural reservoirs of novel genes, including drought tolerance, that are not often found in cultivated species. Aiming to identify novel drought tolerance sources, a total of 60 pre-breeding lines of chickpea derived from the wild progenitor *C. reticulatum* and cultivated *C. arietinum* cross were screened based on various morpho-physiological traits under controlled as well as water-stressed condition in a hydroponic system. Based on the results, 15 PBLs were found to be promising as compared with reported drought-tolerant cultivar ICC 4958 and susceptible genotype ICC17264 (both were used as check). Thus, these identified pre-breeding lines could be potentially used for developing high-yielding drought resilient chickpea genotypes.

Keywords: Chickpea, pre-breeding, hydroponic technique, drought, root length, shoot length.

Introduction

Chickpea is one of the important nutritionally rich grain legume mostly grown on residual moisture in south-Asia and India (Rani et al. 2020). Thus, chickpea frequently encounters terminal moisture stress during the reproductive stage, especially pod development and pod filling stage, leading to serious yield challenge (Jha et al. 2019). It is estimated that drought stress causes yield loss up to 50% in chickpea. Thus, exploring genetic variability for drought stress tolerance across the various chickpea gene pool is urgently needed to sustain chickpea yield under water stress environment. Chickpea wild relatives are a reservoir of novel gene(s) conferring various biotic and abiotic stress tolerance, including drought stress (Mohanty et al. 2022). To identify novel source of drought tolerance, a set of sixty chickpea PBLs developed from *C. reticulatum* and cultivated *C. arietinum* cross were screened based on various morpho-physiological traits under non stress and water stress conditions. Significant genetic variation for the evaluated traits was recorded. High and positive significant correlation between various traits related to shoot and root morpho-physiological and yield related traits viz., fresh shoot weight (FSW) and shoot length (SL), fresh root weight (FRW) and root length (RL), fresh shoot weight (FSW) and fresh root weight (FRW), fresh plant weight (FPW) and RL, dry root

---

Department of Botany, Institute of Science, Banaras Hindu University, Varanasi 221 005, Uttar Pradesh, India

[1]Present address [1]Division of Genetics, ICAR-Indian Agricultural Research Institute, New Delhi 110 012, India

[2]Crop Improvement Division, ICAR-Indian Institute of Pulses Research, Kanpur 208 024, Uttar Pradesh, India

[3]International Rice Research Institute, South-Asia Hub, Hyderabad 500 030, Andhra Pradesh, India

[*]Corresponding Author: Prashant Singh, Department of Botany, Institute of Science, Banaras Hindu University, Varanasi 221 005, Uttar Pradesh, India, E-Mail: p.singh@bhu.ac.in

How to cite this article: Jha R., Yadav H.K., Jha U.C., Raiya R., Paul P.J., Tripathi S. and Singh P. 2023. Harnessing genetic variability for shoot and root morphophysiological traits contributing to drought stress tolerance in pre-breeding lines of chickpea (*Cicer arietinum* L.) under hydroponics condition. Indian J. Genet. Plant Breed., **83**(1): 135-138.

Source of support: ICAR-IARI, Govt. of India

Conflict of interest: None.

Received: Nov. 2022 **Revised:** Jan. 2023 **Accepted:** Feb. 2023

weight (DRW) and RL, DRW and FSW, DRW and FRW, dry plant weight (DPW) and DSW, relative water content (RWC) and RL, RWC and DRW, number of pods/plant (PNO) and RL, PNO and FSW, PNO and FSW, PNO and FPW, PNO and DRW, and PNO and RWC was recorded.

To conduct the experiment, seeds of 60 pre-breeding lines (PBLs) of chickpea (Supplementary Table S1), derived from a cross (ICC 4958 × ICC 17264) × IG 69978 along with checks, ICC 4958 and ICC17264 were disinfected with 1% sodium hypochlorite for 2 minutes and rinsed thoroughly with double distilled water and then germinated on seed germination paper in incubator adjusting temperature 18°C and1 week old seedlings were transferred to the hydroponic nutrient solution (Singh et al. 2013). Drought stress was imposed on 3 weeks old seedling plants by removing them from the nutrient solution such that their roots were exposed to air for 5 hours daily up to the entire growth phase. Plants grown under controlled conditions were kept in the nutrient solution for the entire development period without any interruption. The experiments were carried under controlled and stressed conditions (Fig. 1) with two replications at the National Phytotron Facility, Indian Agricultural Research Institute, New Delhi. Growth conditions have fixed with the following parameter: air temperature was maintained at 22/18°C day/night; photoperiod was 10/14 h light/dark; and the relative humidity was approximately 45%.

SL and RL were measured by using a centimetre scale. FSW, FRW and FPW were taken in grams (g) using electronic weighing balance. To determine DSW, DRW and DPW plants have dried at 80°C for 72 hours. ELWL (%) have measured by taking fresh weight (immediately after sampling) of leaves then leaf samples were left for 6 hour for drying at 28°C with 50% RH and samples were re-dried for 72 hour in an oven for 24 hours at 70°C and %ELWL was calculated as (Fresh Weight–Weight after 6 h/Fresh Weight– Dry Weight)×100 as suggested by Clarke and Townley-Smith (1986) RWC (%). For this parameter fresh leaf material was weighed and kept in double distilled water in a petridish for 2 hours to make the leaf tissue turgid. The turgid weight and dry weight of the leaf materials was measured and RWC was calculated with the help of formula as suggested by Barrs and Weatherley (1962) (Fresh Weight of Sample–Dry Weight of Sample/ Turgid weight of Sample-Dry Weight of Sample)×100. PNO was counted in each plant manually.

For statistical analysis, PBLs were planted in an augmented design (with 6 block with each block contained 10 PBLs along with the ICC4958 and ICC 17264 checks. The checks were replicated in each block. The ANOVA was applied to analyse the data and estimates of heritability in broad-sense were calculated as $H^2 = Vg/(Vg + Ve/nr)$. All the traits mentioned above were analyzed using maximum residual likelihood (REML) in GenStat 15 (https:// www.vsni.co.uk/) in mixed model approach considering genotypes as random effect and environment as fixed effect. The significance of environments was tested using Wald's statistic. Variance components due to genotype (sg2) their standard errors (SE) were estimated. Best linear unbiased predictors (BLUPs) were obtained for the tested traits. Using the R package cluster, the euclidean dissimilarity matrix was constructed using all the tested traits and the accessions were clustered following Ward's method (Ward 1963).

Fig.1. (a-b) Appearance of chickpea plants under non stress and drought stress condition; (c) Exposure of plants in dried air condition and (d) Root length variability of same plant under non stress and stress condition

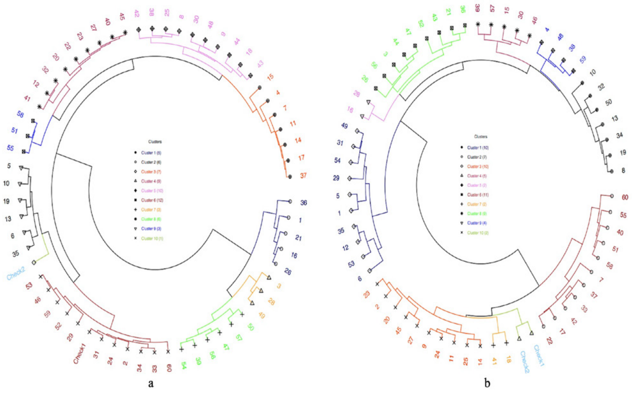

Fig. 2. (a-b) Cluster analysis of chickpea plants (PBLs) in response to both drought stress and non-stress respectively

The data analysis indicated that all the traits studied showed significant genetic variation both under non-stress and drought stress conditions, except the DRW under non-stress conditions (Supplementary Table S2). All the traits displayed wide variation among the PBLs for different traits. Under drought stress, the value for shoot length varied from 14.6 to 22.4 cm with 74% heritability value, while root length varied from 15.03 to 26.3 cm with high heritability (97.8%). Considering fresh shoot and root weights, a wide range of genetic variability ranging from 0.26 to 1.26 g with 93.4% heritability and 0.54 to 1.44 g with 95% heritability was noted under water stress. Likewise, wide range of genetic variability for fresh plant weight (1.19 g to 2.44 g) and dry shoot weight (0.15 to 0.66 g) was recorded under drought stress. Similarly, large range of genetic variability for shoot biomass, specific leaf area, leaf area index was reported in chickpea (Ramamoorthy et al. 2016) under drought stress, indicating the importance of these traits for selecting drought-tolerant genotype in chickpea. Considering dry root weight and dry plant weight, a sufficient amount of genetic variability was captured under water stress, ranging from 0.22 to 0.57 g with 95.8% heritability, and 0.64 to 1.15 g with 88.3% heritability condition. Likewise, root and root related traits are one of the critical parameters for screening and assessing drought tolerance in chickpea as drought tolerance has a positive relationship with deeper root and vigorous root system (Kashiwagi et al. 2013). Considerable works have been reported on root and root-related traits for selecting drought-tolerant genotypes in various crops, including chickpea. In the current experiment, sufficient amount of genetic variability for root and root related parameters such as root length (15.77–30.4 and 16.07–24.87 cm), fresh root biomass (1–3 and 0.54–1.44 g), and dry root weight (0.3–0.77 and 0.2–0.57 g) was noted under drought stress condition. Similarly, based on assessing various root parameters viz., root length density, root dry weight, root: shoot ratio allowed selecting drought tolerant genotypes at vegetative and reproductive in chickpea (Ramamoorthy et al. 2017).

Aiming at examining the other important physiological parameter, significant amount of genetic variability for excised leaf water loss (5.5–8.4%) with high heritability (90.4%) and relative water content (39.9–88.9%) with high heritability (97.8%) was recorded. Sufficient genetic variability for RWC (40.9–87.3%) and high heritability (93.5%) in the present study indicated that this trait can be used for selecting drought-tolerant lines in chickpea.

Emphasizing yield trait, a wide range of genetic variability for number of pods/plant (25.3–63.3) with high heritability (94%) was obtained under drought stress. Considering most important trait, number of pods per plant (PNO), the identified seventeen pre-breeding lines showed an average number of pods per plant (PNO) in the range of 62.55 -82.62, whereas the check cultivar ICC4958 had an average PNO of 55.08 under water stress conditions. Previously, relying on the high value of pods/plant and seed yield/plant some important chickpea lines were identified under water stress condition (Arif et al. 2021; Shah et al. 2020; Jha et al. 2019). RL's positive and highly significant association with FPW, DSW, DRW, DPW, RWC and PNO was recorded under non-stress conditions. Similarly, FPW exhibited positive and highly significant correlation with DSW, DRW, DPW, and RWC. High and significant positive correlation of DSW with DRW, DPW, RWC and PNO was noted. Considering PNO trait, it showed high and positive significant association with DPW and RWC traits under normal condition. Under drought stress, RL showed positive and highly significant association with all the traits except DSW, DPW and ELWL. Similarly, FSW exhibited positive and high significant association with all traits except DSW, DPW and ELWL. Considering FPW, it showed a positive and high significant correlation with DRW, RWC and PNO traits. Likewise, PNO showed a significantly positive association with all traits except SL, DSW, DPW and ELWL traits under drought stress.In common bean high canopy biomass enable plant to accumulate high grain yield under drought stress (Polania et al. 2017). Significant and positive association of root length with fresh shoot weight, fresh root weight, fresh plant weight, dry root weight and relative water content suggested deep root length allows in acquiring higher amount of water content for improving photo-assimilation under drought stress. A positive association of shoot trait with grain yield and positive association of root trait with grain yield has been reported in chickpeas under water stress (Ramamoorthy et al. 2016, 2017). Thus, selecting a genotype with high relative water content, high fresh shoot weight, root weight, high pods/plant and high fresh plant weight under drought stress could enable in selecting drought tolerant chickpea genotype.

Genetic variability for various shoot and root related morphological traits in the given pre-breeding lines assessed under non-stress and drought stress at vegetative stage indicated presence of wide range of genetic variability. Results of correlation analysis of various morpho-physiological traits related to root and shoot were found to be positive and significant. Cluster analysis based on the response of all the tested pre-breeding lines (PBLs), ICCP 171056, ICCP 171052, ICCP 171033, ICCP 171011, ICCP 171041,ICCP 171038, ICCP 171064, ICCP 171012, ICCP 171058, ICCP 171023, ICCP 171057, ICCP 171072, ICCP 171061, ICCP 171084, and ICCP 171071 indicated promising results advocating their use for developing climate resilient drought tolerant chickpea varieties (Fig.2). Further, based on morpho-physiological traits analysis in response to drought stress some of the PBLs, ICCP 171056, ICCP 171052, ICCP 171033, ICCP 171011, ICCP 171038) showed better-improved number of pods/plant, an important yield parameter under drought stress as well as normal condition. Hence, these selected drought tolerant PBLs could be further used in breeding programme to develop high-yielding drought tolerant chickpea cultivars.

Authors' contribution

Conceptualization of research (RJ); Designing of the experiments (RJ); Contribution of experimental materials (ST); Execution of field/lab experiments and data collection (RJ, HKY, RR); Analysis of data and interpretation (PJP, UCJ, RJ); Preparation of the manuscript (RJ, UCJ, PS).

Supplementary material

Supplementary Tables S1 and S2 are provided, which can be accessed online www.isgpb.org.

References

Arif A., Parveen N., Waheed M.Q., Atif R.M., Waqar I. and Shah, T.M. 2021. A comparative study for assessing the drought-tolerance of chickpea under varying natural growth environments. Front. Plant Sci., **11**: 607869.

Barrs 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**: 413–28.

Clarke J.M. and Townley-Smith T.F. 1986. Heritability and relationship to yield of excised leaf water retention in durum wheat. Crop Sci., **26**: 289–92.

Jha U.C., Bohra A. and Nayyar H.2019. Advances in "omics" approaches to tackle drought stress in grain legumes. Plant Breed., **00**: 1–26.

Kashiwagi J., Krishnamurthy L., Gaur P.M., Upadhyaya H.D., Varshney R.K. and Tobita S., 2013. Traits of relevance to improve yield under terminal drought stress in chickpea (C. arietinum L.). Field Crops Res., **145**: 88-95.

Mohanty J.K., Jha U.C., Dixit G.P. et al. 2022. Harnessing the hidden allelic diversity of wild *Cicer* to accelerate genomics-assisted chickpea crop improvement. Mol. Biol. Rep., doi: 10.1007/s11033-022-07613-9

Polania J., Rao I.M., Cajiao C., Grajales M., Rivera M., Velasquez F., Raatz B. and Beebe S.E. 2017. Shoot and Root Traits Contribute to Drought Resistance in Recombinant Inbred Lines of MD 23–24 × SEA 5 of Common Bean. Front. Plant Sci., **8**: 296.

Ramamoorthy P., Lakshmanan K., Upadhyaya H.D., Vadez V. and Varshney R.K. 2017. Root traits confer grain yield advantages under terminal drought in chickpea (*Cicer arietinum* L.). Field Crops Res., **201**: 146-161.

Ramamoorthy P., Lakshmanan K., Upadhyaya H.D., Vadez V. and Varshney R.K. 2016. Shoot traits and their relevance in terminal drought tolerance of chickpea (*Cicer arietinum* L.). Field Crops Res., **197**: 10-27.

Rani A. , Devi P., Jha U.C., Sharma K.D., Siddique K.H.M.and Nayyar H.S. 2020.Developing Climate-Resilient Chickpea Involving Physiological and Molecular Approaches With a Focus on Temperature and Drought Stresses. Front. Plant Sci., **10**: 1759.

Shah T. M., Imran M., Atta B. M., Ashraf M. Y., Hameed A., Waqar I., et al. 2020. Selection and screening of drought tolerant high yielding chickpea genotypes based on physio-biochemical indices and multi-environmental yield trials. BMC Plant Biol., **20**: 171. doi:10.1186/s12870-020-02381-9

Singh D., Dikshit H.K. and Singh R. 2013. A new phenotyping technique for screening for drought tolerance in lentil (L ens culinaris M edik.). Plant Breed., **132**(2): 185-190.

Supplementary Table S1. List of pre breeding lines (PBLs) derived from a cross [(ICC4958×ICC17264) ×IG69978] that were used in the study

Code No.	PBLs	Code No.	PBLs	Code No.	PBLs	Code No.	PBLs	Code No.	PBLs
1	ICCP 171003	13	ICCP 171017	25	ICCP 171033	37	ICCP 171048	49	ICCP 171065
2	ICCP 171004	14	ICCP 171018	26	ICCP 171034	38	ICCP 171052	50	ICCP 171066
3	ICCP 171006	15	ICCP 171020	27	ICCP 171035	39	ICCP 171053	51	ICCP 171068
4	ICCP 171007	16	ICCP 171021	28	ICCP 171036	40	ICCP 171054	52	ICCP 171071
5	ICCP 171008	17	ICCP 171022	29	ICCP 171037	41	ICCP 171055	53	ICCP 171072
6	ICCP 171009	18	ICCP 171023	30	ICCP 171038	42	ICCP 171056	54	ICCP 171074
7	ICCP 171010	19	ICCP 171025	31	ICCP 171039	43	ICCP 171057	55	ICCP 171079
8	ICCP 171011	20	ICCP 171027	32	ICCP 171040	44	ICCP 171058	56	ICCP 171081
9	ICCP 171012	21	ICCP 171028	33	ICCP 171041	45	ICCP 171060	57	ICCP 171082
10	ICCP 171013	22	ICCP 171029	34	ICCP 171043	46	ICCP 171061	58	ICCP 171083
11	ICCP 171014	23	ICCP 171031	35	ICCP 171046	47	ICCP 171062	59	ICCP 171084
12	ICCP 171015	24	ICCP 171032	36	ICCP 171047	48	ICCP 171064	60	ICCP 171087

Supplementary Table S2. Genetic variability for the traits studied under non-stress and drought stress conditions

Traits	Genotypic Variance	Mean	Range	Heritability (%)
Non-stress				
SL	8.0**	23.68	15.2-29.59	95
RL	8.4**	22.88	15.96- 31.2	96.3
FSW	0.09**	1.8	1.04-2.30	94.4
FRW	0.26**	1.84	1.12-2.9	96.1
FPW	0.29*	3.76	2.3-4.5	91.3
DSW	0.03**	0.7	0.4-1.12	74
DRW	0.01	0.56	0.33-0.76	86.6
DPW	0.05**	1.26	0.7-1.46	90.7
RWC	157.6**	74.3	41.9-98.5	98.5
ELWL	0.41**	7.79	6.5-9.0	86.6
PNO	37.5**	54	42.0-66.4	90.2
Traits	Genotypic Variance	Mean	Range	Heritability (%)
Drought stress				
SL	4.2**	18.5	14.64-22.4	74
RL	12.01*	19.7	15.03-26.29	97.8
FSW	0.09**	0.64	0.26-1.26	93.4
FRW	0.06**	0.9	0.54-1.44	95
FPW	0.07**	1.45	1.19-2.44	98.2
DSW	0.013**	0.44	0.15-0.66	96.1
DRW	0.012**	0.37	0.22-0.57	95.8
DPW	0.02**	0.94	0.64-1.15	88.3
RWC	125.6**	65.9	39.9-88.9	97.8
ELWL	0.5**	6.8	5.5-8.4	90.4
PNO	92.6*	41.2	25.3-63.2	94

*Significant at 5% and **Significant at1% level of significance