Indian J. Genet. Plant Breed., (2025); 85(4): 675-679

https://doi.org/10.31742/ISGPB.85.4.17

ISSN: 0975-6906

www.isgpb.org

SHORT RESEARCH ARTICLE

Dissecting gene action for heat stress-responsive traits in chickpea (*Cicer arietinum* L.) across heat stress environments

Mahaveer Prasad Ola*, Shailesh Kumar Jain[1], Rekha Choudhary[1], S. S. Punia[1] and C. Bharadwaj[2]

Abstract

Limited information is available in the literature on the dissection of non-additive gene action under contrasting sowing environments, leaving a gap in breeding strategies for heat-tolerant chickpea varieties. Therefore, an investigation was carried out on the gene interactions influencing physio-biochemical traits in chickpea (*Cicer arietinum L.*) under heat stress conditions. Three chickpea crosses, namely, RSG807 × RSG895, RSG895 × HC5, and RSG974 × Avrodhi, were evaluated under two sowing environments during rabi seasons of 2019-20 to 2021–22. Genetic analysis revealed the presence of duplicate epistasis for several traits in specific cross-environment combinations. Relative water content in RSG-974 × Avrodhi under late sowing (E2); membrane stability index in RSG-807 × RSG-895 under E2; total chlorophyll content in RSG-807 × RSG-895 under timely sowing (E1), RSG-974 × Avrodhi under E2, and RSG-895 × HC-5 under both E1 and E2; proline content in RSG-974 × Avrodhi under E1; protein content in RSG-807 × RSG-895 and RSG-974 × Avrodhi under E2; and seed yield per plant in RSG-895 × HC-5 under E1 were observed. Overall, the inheritance of most physio-biochemical traits across both sowing environments was predominantly governed by non-additive gene actions.

Keywords: Chickpea, gene interaction, duplicate epistasis and heat stress

Chickpea (*Cicer arietinum* L.) is the second most important pulse crop globally after the common bean, serving as a vital source of protein in semi-arid regions. However, its productivity is increasingly threatened by rising temperatures due to climate change. High temperature, especially above 35°C during the reproductive phase, can cause up to 39% yield loss by affecting key physiological and biochemical processes (Danakumara et al. 2024). Heat stress adversely impacts traits such as chlorophyll content, membrane stability, relative water content, and proline accumulation, critical for plant growth and productivity. In India, delayed sowing following crops like rice and maize often exposes chickpea to terminal heat stress, reducing grain yield. Physiological traits like RWC, MSI, and proline accumulation are reliable indicators of stress tolerance. Understanding their genetic control is crucial for developing heat-tolerant genotypes. Gene action studies help in identifying additive, dominance, and epistatic effects influencing these traits. Such insights guide breeders in selecting superior parents for hybridization. Comprehensive knowledge of these mechanisms supports breeding programs aimed at enhancing chickpea resilience to heat stress (Munns et al. 2021).

All plant materials used in the present investigation complied with the research regulations and standard protocols of S.K.N. Agriculture University, Jobner. The experimental materials were developed and maintained at the Rajasthan Agricultural Research Institute (RARI), Durgapura, Jaipur, Rajasthan, India. The study on chickpea was conducted over three consecutive *rabi* seasons (2019–20 to 2021–22). During 2019–20, three crosses, viz., RSG 807 × RSG 895, RSG 895 × HC 5, and RSG 974 × Avrodhi, were

Department of Agriculture, Vivekananda Global University, Jaipur 303 012, Rajasthan, India

[1]Division of Genetics and Plant Breeding, RARI, Sri Karan Narendra Agriculture University, Jobner, Durgapura 302 018, Jaipur, Rajasthan, India.

[2]ICAR-Indian Agricultural Research Institute, New Delhi 110 012, India

***Corresponding Author:** Mahaveer Prasad Ola, Department of Agriculture, Vivekananda Global University, Jaipur 303 012, Rajasthan, India, E-Mail: mahaveerprasadola37@gmail.com

How to cite this article: Ola M.P., Jain S.K., Choudhary R., Punia S.S. and Bharadwaj C. 2025. Dissecting gene action for heat stress-responsive traits in chickpea (*Cicer arietinum* L.) across heat stress environments. Indian J. Genet. Plant Breed., **85**(4): 675-679.

Source of support: Nil

Conflict of interest: None.

Received: Aug. 2025 **Revised:** Oct. 2025 **Accepted:** Nov. 2025

---

made using five diverse parents (HC 5, Avrodhi, RSG 974, RSG 807 and RSG 895). The F_1 plants were backcrossed during 2020–21 to produce B_1 and B_2 generations. In 2021–22, six generations (P_1, P_2, F_1, F_2, B_1, and B_2) of each cross were evaluated in a randomized block design with three replications under two sowing environments—timely sown (E1, 1st November) and late sown (E2, 1st December). Each block comprised twenty 3.0 m long rows with a spacing of 30 × 15 cm. The generations P_1, P_2, and F_1 were planted in single rows each, while F_2, B_1, and B_2 generations were planted in four rows each. Observations were recorded on five competitive plants of P_1, P_2, and F_1; ten of B_1 and B_2; and twenty of F_2 per replication under both environments. Data were collected for pollen viability (%), relative water content (Slavik 1974), membrane stability index (Sairam et al. 1997), total chlorophyll and carotenoid content (Talebi et al. 2013), proline content (Bates et al. 1973), and seed protein content (Lowry et al. 1951). Pooled analysis of variance was carried out as per Panse and Sukhatme (1985). The adequacy of the additive–dominance model was tested using the joint scaling test (Cavalli 1952). A non-significant chi-square indicated the adequacy of the three-parameter model (Jinks and Jones 1958), while the six-parameter model (Hayman 1958) was applied otherwise.

Analysis of variance

The pooled analysis of variance over timely (E1) and late-sown (E2) environments revealed highly significant differences among all crosses for each trait studied, confirming the presence of substantial genetic variability (Supplementary Table S1). Significant environmental effects indicated that the expression of most traits was influenced by sowing conditions. A significant generation × environment interaction for all crosses further demonstrated differential genetic responses across environments, a trend commonly reported in chickpea. Mean performance across environments showed that most traits recorded lower values under late sowing, except proline content, which increased under delayed planting (Supplementary Fig. S1). The greatest reductions under E2 were observed for carotenoid content and seed yield per plant, indicating their sensitivity to heat stress. Conversely, pollen viability exhibited the least reduction, suggesting better tolerance. These findings align with Praween et al. (2024), who also reported similar responses under heat stress in chickpea. F_1 means varied among crosses and traits, and in most cases exceeded parental means under both environments. The cross RSG-807 × RSG-895 recorded the highest F_1 seed yield per plant even under late sowing, indicating its potential utility for developing high-yielding cultivars suitable for heat-prone environments. In almost all crosses, F_2 means were lower than their corresponding F_1 means across traits and environments, indicating inbreeding depression and the predominant involvement of non-additive gene action. Backcrosses (B_1, B_2) generally showed intermediate performance and a tendency towards the recurrent parent, although no consistent pattern was observed.

Study on gene action

The six-parameter model was adequate for most cross–trait–environment combinations, indicating that both main gene effects (additive and dominance) and epistatic effects (additive × additive, additive × dominance and dominance × dominance) governed the inheritance of physio-biochemical traits. However, the additive–dominance model was sufficient for certain cases: pollen viability in RSG-807 × RSG-895 under E1 and RSG-974 × Avrodhi under both environments; relative water content in RSG-807 × RSG-895 under E2; membrane stability index in RSG-895 × HC-5 under E1; carotenoid content in RSG-807 × RSG-895 under E1, RSG-974 × Avrodhi under E2, and RSG-895 × HC-5 under both environments; and proline content in RSG-807 × RSG-895 under E1 (Table 1). The adequacy of the simple model in these combinations suggests that the respective traits are largely governed by additive and dominance components without significant interaction effects. For total chlorophyll content, protein content, and seed yield per plant, the additive–dominance model was inadequate across all crosses and environments, implying the essential role of epistasis in their inheritance. Similar inconsistencies in model adequacy across environments were reported by Nehra et al. (2020). Generation mean analysis showed that the magnitude and direction of the genetic components differed across environments and cross combinations. The 'm' component was significant for all traits across all crosses, indicating significant differences among the generations. Duplicate epistasis was detected for several traits, including relative water content (RSG-974 × Avrodhi, E2), membrane stability index (RSG-807 × RSG-895, E2), total chlorophyll content (RSG-807 × RSG-895, E1; RSG-974 × Avrodhi, E2; RSG-895 × HC-5, E1 and E2), proline content (RSG-974 × Avrodhi, E1), protein content (RSG-807 × RSG-895 and RSG-974 × Avrodhi, E2) and seed yield per plant (RSG-895 × HC-5, E1). Duplicate epistasis generally slows genetic progress and limits the efficiency of early-generation selection. Additive and additive × additive gene effects were predominant for seed yield per plant in RSG-974 × Avrodhi under late sowing (E2), in agreement with Choudhary et al. (2023), who reported similar patterns for physio-biochemical traits in chickpea. In several crosses, a mixture of additive (d, i) and non-additive effects (h, j, l) governed seed yield, indicating limited scope for rapid improvement through simple early-generation selection. To enhance the probability of identifying superior recombinants, recurrent selection or inter-crossing of selected segregants in early generations is recommended. Subsequent fixation through pedigree breeding may help develop homozygous,

Table 1. Estimates of chi-square value ($\chi 2$) and gene effects for pollen viability, relative water content, membrane stability index and total chlorophyll content under E1 and E2 environments

	RSG807 × RSG-895 (C1)		RSG895 × HC5 (C2)		RSG974 × Avrodhi (C3)	
	E1	E2	E1	E2	E1	E2
Pollen viability						
χ^2	3.07	17.59**	68.96**	45.51**	3.63	4.15
Gene effects (Three/six parameter) with epistasis type						
M	92.6** ±3.87	82.53**±1.28	87.63** ±0.63	82.2** ± 0.96	84.89** ± 3.80	88.63** ± 5.11
D	1.86* ± 0.89	0.56 ± 1.98	-0.06 ± 1.06	0.79 ± 1.24	-2.03* ± 0.84	-3.43** ± 0.72
H	-9.59 ± 9.89	3.46 ± 6.72	-6.19 ± 3.48	3.03 ± 4.80	17.03 ± 9.12	-5.69 ± 12.69
I	-	2.86 ± 6.50	-7.06* ± 3.30	0.93 ± 4.60	-	-
J	-	0.30 ± 2.40	1.13 ± 1.33	0.89 ± 1.62	-	-
L	-	14.66 ±10.08	30.93** ±5.43	21.13** ±6.87	-	-
	--	--	--	--	--	--
Relative water content						
χ^2	44.88**	0.93	32.40**	14.45**	62.37**	11.29*
Gene effects (Three/six parameter) with epistasis type						
M	79.53** ±0.34	70.21** ± 5.41	76.32** ± 1.19	67.84** ± 1.19	68.30** ± 0.21	64.32** ± 1.10
D	3.51** ± 1.01	-3.9** ± 1.37	4.78 ± 2.42	0.57 ± 2.35	-2.82 ± 1.61	-1.31 ± 1.31
H	0.80 ± 2.80	4.11 ± 13.51	3.43 ± 6.98	15.36* ± 6.97	11.73** ± 3.94	4.59 ± 5.54
I	-7.34** ±2.45	-	-2.10 ± 6.82	13.76* ± 6.70	13.63** ± 3.34	4.41 ± 5.13
J	7.65** ± 1.61	-	6.99* ± 2.65	1.36 ± 2.59	1.85 ± 2.04	-0.91 ± 1.76
L	0.06 ± 5.07	-	22.91* ± 11.23	-5.32 ± 11.23	-1.56 ± 7.73	8.87 ± 8.04
	--	--	--	--	--	--
Membrane stability index						
χ^2	12.34**	24.50**	3.20	18.29**	23.94**	61.53**
Gene effects(Three/six parameter) with epistasis type						
M	86.35** ±0.49	81.06** ±0.73	81.63** ±4.43	75.70** ± 0.7	78.68** ± 0.65	67.53** ± 0.84
D	1.44 ± 1.20	-4.23** ± 1.38	0.80 ± 1.04	-2.25 ± 2.03	-0.25 ± 1.13	2.51 ± 1.70
H	-5.37 ± 3.50	-15.35** ±4.26	6.40 ± 11.28	-4.55 ± 5.42	-4.41 ± 3.71	7.82 ± 5.04
I	-8.73** ±3.11	-14.96** ±4.05	-	-4.60 ± 5.15	-4.72 ± 3.45	2.93 ± 4.79
J	3.80* ± 1.68	-2.97 ± 1.69	-	-1.71 ± 2.25	2.27 ± 1.57	1.27 ± 2.13
L	12.60* ± 6.11	14.18* ± 6.83	-	23.86* ± 9.34	20.10** ± 5.88	25.18** ± 8.21
	--	Duplicate	-	--	--	--
Total chlorophyll content						
	33.44**	17.33**	64.20**	95.21**	28.25**	27.99**
Gene effects (Three/six parameter) with epistasis type						
M	34.15** ± 0.2	31.31** ± 0.35	30.46** ±0.26	26.07** ±0.40	33.03** ± 0.27	30.73** ± 0.44
D	0.68 ± 0.37	0.91 ± 0.66	-0.19 ± 0.40	-0.55 ± 0.71	0.57 ± 0.51	1.36 ± 0.85
H	8.13** ± 1.28	-1.56 ± 2.06	-7.23** ± 1.52	-4.14** ±1.29	0.28 ± 1.62	-7.41** ± 2.59
I	5.26** ± 1.10	-5.92** ± 1.94	-5.41** ± 1.34	-3.58 ± 2.17	0.75 ± 1.51	-9.57** ± 2.47
J	2.06** ± 0.58	1.47 ± 0.76	-1.15 ± 0.60	-0.69 ± 0.94	1.99** ± 0.69	0.86 ± 0.98
L	-7.29** ±2.15	11.72** ±3.32	17.63** ± 2.41	22.57** ± 3.61	4.61 ± 2.62	21.15** ± 4.18
	Duplicate	--	Duplicate	Duplicate	--	Duplicate

Table 1. Cont..

	RSG807 × RSG-95 (C1)		RSG895 × HC-5 (C2)		RSG974 × Avrodhi (C3)	
	E1	E2	E1	E2	E1	E2
Carotenoid content						
χ^2	4.21	7.67	3.29	2.53	15.88**	4.46
Gene effects(Three/six parameter) with epistasis type						
M	4.58** ± 0.71	5.15** ± 0.63	4.74** ± 0.92	4.10** ± 0.69	5.60** ± 0.17	4.26** ± 0.79
D	0.21 ± 0.16	0.05 ± 0.16	-0.49* ± 0.18	-0.18 ± 0.22	0.37 ± 0.26	-0.17 ± 0.13
H	-0.23 ± 1.88	-3.28 ± 1.67	1.25 ± 2.21	1.73 ± 1.8	2.31* ± 0.9	3.04 ± 1.94
I	-	-	-	-	1.49 ± 0.85	-
J	-	-	-	-	0.75* ± 0.31	-
L	-	-	-	-	-0.22 ± 1.38	-
	--	--	--	--	--	--
Proline content						
χ^2	5.79	10.50*	8.17*	19.54**	45.04**	18.48**
Gene effects (Three/six parameter) with epistasis type						
M	4.16** ± 0.69	5.92** ± 0.15	4.956** ±0.14	5.48** ± 0.13	5.54** ± 0.13	6.23** ± 0.14
D	-0.61* ± 0.22	0.15 ± 0.31	0.27 ± 0.26	-0.04 ± 0.24	0.26 ± 0.25	0.26 ± 0.29
H	2.68 ± 1.82	0.85 ± 0.91	0.14 ± 0.81	0.19 ± 0.75	-1.58* ± 0.78	-0.40 ± 0.88
I	-	0.3 ± 0.87	0.65 ± 0.78	0.62 ± 0.71	-1.84* ± 0.72	-0.51 ± 0.81
J	-	0.48 ± 0.36	0.20 ± 0.31	0.04 ± 0.31	0.20 ± 0.30	0.58 ± 0.34
L	-	1.65 ± 1.50	0.50 ± 1.28	1.64 ± 1.21	6.69** ± 1.27	3.67* ± 1.45
	--	--	--	--	Duplicate	--
Protein content						
χ^2	10.69*	27.47**	6.61	0.91	25.65**	90.69**
Gene effects (Three/six parameter) with epistasis type						
M	24.71** ±0.18	22.15** ± 0.17	22.89** ± 2.6	20.72** ± 1.44	24.16** ± 0.13	21.49** ± 0.14
D	-0.69 ± 0.38	-1.32** ± 0.43	-0.60* ± 0.22	-0.39 ± 0.23	0.13 ± 0.32	0.35 ± 0.30
H	3.01* ± 1.16	4.63** ± 1.19	3.22 ± 6.44	2.83 ± 3.97	-1.21 ± 1.06	1.67 ± 0.97
I	3.09** ± 1.07	4.19** ± 1.11	-	-	-0.91 ± 0.85	1.98* ± 0.84
J	0.21 ± 0.48	-0.54 ± 0.52	-	-	-0.96* ± 0.42	-0.93* ± 0.4
L	-4.72* ± 1.94	-2.92 ± 2.05	-	-	7.21** ± 1.89	5.99** ± 1.66
	Duplicate	--	--	--	--	--
Seed yield per plant						
χ^2	22.87**	19.13**	27.41**	7.90*	53.75**	31.55**
Gene effects (Three/six parameter) with epistasis type						
M	8.88** ±0.19	7.68** ±0.17	9.02** ± 0.17	7.26** ±0.17	7.60** ±0.18	6.50** ±0.17
D	-0.51 ± 0.32	0.42 ±0.32	-0.66 ± 0.42	0.67* ±0.28	1.16** ±0.29	1.51** ±0.33
H	-1.82 ±1.07	-1.84 ±1.03	-3.97** ±1.15	0.12 ±1.07	-0.29 ±0.98	1.52 ±1.01
I	-2.69** ±1.01	-2.78** ±0.94	-4.05** ±1.10	-0.60 ±0.89	-1.04 ±0.93	1.09 ±0.97
J	0.19 ±0.37	1.01* ±0.4	-1.32** ±0.44	0.10 ±0.34	0.35 ±0.33	0.24 ±0.38
L	6.98** ±1.67	6.8** ±1.69	7.41** ±1.94	3.85* ±1.79	6.67** ±1.51	3.05 ±1.63
	--	--	Duplicate	--	--	--

*, ** Significant at 5 % and 1 % level, respectively

heat-tolerant, high-yielding lines. Promising transgressive segregants identified under stress conditions may serve either as improved varieties or as potential parents in future breeding programmes.

Supplementary materials

Supplementary Tables S1 and Fig. S1 are provided, which can be accessed at www.isgpb.org

Author's contribution

Conceptualization of research (SKJ, MPO); Designing of the experiments (SKJ, MPO); Contribution of experimental materials (SKJ); Execution of field/lab experiments and data collection (MPO, SKJ, RC, SSP); Analysis of data and interpretation (MPO, SKJ, RC, SSP, CB); Preparation of the manuscript (MPO, SKJ, SSP, CB).

Acknowledgment

The first author gratefully acknowledges the research guide who helped in the research experiments and interpretations of findings. This research paper is a part of the Ph.D. thesis submitted to the SKNAU, Jobner, Jaipur, Rajasthan.

References

Bates L.S., Waldren R.P. and Teare I.D. 1973. Rapid determination of free proline for water-stress studies. Plant and Soil **39**: 205-207.

Cavalli L.L. 1952. An analysis of linkage in quantitative inheritance. In: Reeve ECR, Waddington CH (eds) Quantitative Inheritance. HMSC, London, pp 135-144.

Choudhary R., Jain S.K., Ola M.P. and Gupta D. 2023. Gene action and combining ability for yield and other quantitative traits under timely and late sown conditions in chickpea (*Cicer arietinum* L.). Indian J. Genet. Plant Breed., **83**(2): 273-277.

Danakumara T., Kumari J., Singh A. K., Sinha S. K., Pradhan A. K., Sharma S., Jha S.K., Bansal R., Sundeep K., Jha G.K., Yadav M.C. and Prasad P.V. 2021. Genetic dissection of seedling root system architectural traits in a diverse panel of hexaploid wheat through multi-locus genome-wide association mapping for improving drought tolerance. Int. J. Mol. Sci., **22**: 7188.

Hayman B.I. 1958. The separation of epistasis from additive and dominance variation in generation means. Heredity, **12**: 371-390.

Jinks J.L. and Jones R.M. 1958. Estimation of the components of heterosis. Genetics **43**: 223-224.

Lowry O.H., Rosebrough N.J., Farr A.L. and Randall R.J. 1951. Protein measurement with the Folin phenol reagent. J. Biol. Chem., **193**: 265-275.

Munns R., James R.A. and Sirault X.R.R. 2021. The physiology of heat stress in chickpea and other pulses: From biology to breeding. Field Crops Res., **258**: 107948.

Nehra M., Panwar R.K., Verma S.K., Arora A., Bhardwaj R. and Choudhary R. 2020. Generation mean analysis for seed yield and its contributing quantitative traits in chickpea (*Cicer arietinum* L.). Int. J. Chem. Stud., **8**(6): 172-175.

Panse V.G. and Sukhatme P.V. 1985. Statistical Methods for Agricultural Workers. Indian Council of Agricultural Research Publication, 87-89.

Praween N., Kumar Y., Sikarwar R.S., Syed M.Q., Basu P.S., Biswajit M. and Dixit G.P. 2024. Response of chickpea (*Cicer arietinum* L.) genotypes to high temperature under late sown condition. Indian J. Genet. Plant Breed., **84**(3): 415-424.

Sairam R.K., Shukla D.S. and Saxena D.C. 1997. Stress induced injury and antioxidant enzymes in relation to drought tolerance in wheat genotypes. Biol. Plant, **40**(3): 357-364.

Slavik B. 1974. Methods of studying plant water relations. Academia Publishing House, Prague and Springer-Verlag, Berlin, pp 449.

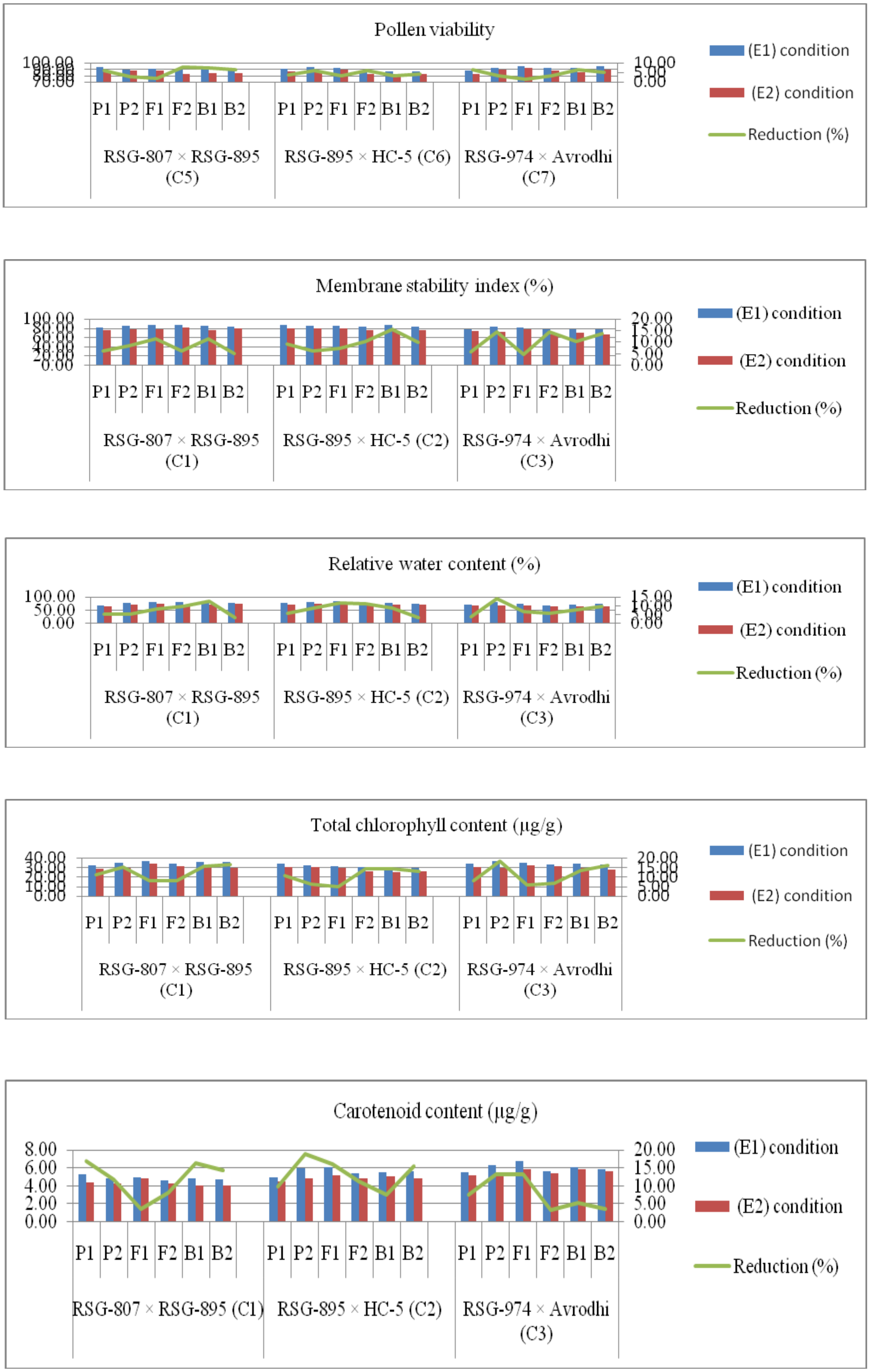
Talebi R., Ensafi M.H., Baghebani N. and Karami E. 2013. Physiological responses of chickpea (*Cicer arietinum* L.) genotypes to drought stress. Environ. Exp. Biol., **11**: 9-15.

Supplementary Table S1. Pooled analysis of variance (mean squares) for different physio-biochemical characters in three crosses of chickpea

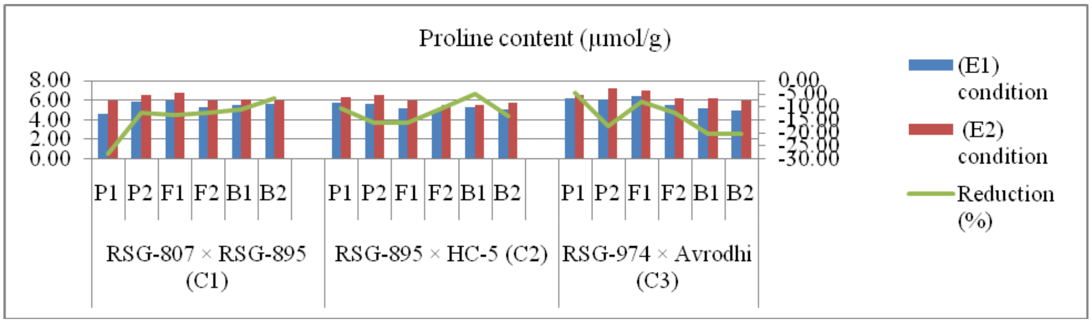
Crosses	Source of variations / (DF)				
	Environment (1)	Replication × Environment (2)	Generations (5)	Generation × Environment (5)	Error (20)
Pollen viability (%)					
RSG-807 × RSG-895 (C1)	218.279**	1.929	20.680	6.471**	1.292
RSG-895 × HC-5 (C2)	150.922**	0.538	52.730**	4.935*	1.556
RSG-974 × Avrodhi(C3)	146.541**	0.922	46.906	4.489**	0.709
Relative water content (%)					
RSG-807 × RSG-895 (C1)	284.993**	0.457	90.323	11.744**	0.590
RSG-895 × HC-5 (C2)	367.929**	0.693	44.236	10.669**	1.165
RSG-974 × Avrodhi (C3)	316.075**	0.265	52.474	13.596**	1.849
Membrane stability index (%)					
RSG-807 × RSG-895 (C1)	424.489**	1.483	19.076	9.3529**	0.873
RSG-895 × HC-5 (C2)	617.310**	0.840	12.896	11.699**	1.421
RSG-974 × Avrodhi (C3)	625.860**	1.315	50.951	18.855**	1.395
Total chlorophyll content (µg/g)					
RSG-807 × RSG-895 (C1)	168.011**	2.090	13.131	2.692**	0.627
RSG-895 × HC-5 (C2)	96.016**	2.010	27.964*	2.987*	0.777
RSG-974 × Avrodhi (C3)	136.917**	0.360	7.279	5.057**	0.431
Carotenoid content (µg/g)					
RSG-807 × RSG-895 (C1)	3.019**	0.024	0.270	0.106**	0.018
RSG-895 × HC-5 (C2)	5.010**	0.018	0.589	0.220**	0.043
RSG-974 × Avrodhi (C3)	2.016*	0.090	0.695	0.228*	0.068
Proline content (µmol/g)					
RSG-807 × RSG-895 (C1)	4.877*	0.014	0.981	0.434*	0.127
RSG-895 × HC-5 (C2)	3.627**	0.115	0.783	0.217**	0.066
RSG-974 × Avrodhi (C3)	5.311**	0.062	1.604*	0.315*	0.069
Protein content (%)					
RSG-807 × RSG-895 (C1)	19.951*	0.283	6.678*	1.264*	0.316
RSG-895 × HC-5 (C2)	35.370**	0.156	6.955*	0.893*	0.247
RSG-974 × Avrodhi (C3)	29.745*	0.483	3.968	1.921**	0.386
Seed yield per plant (g)					
RSG-807 × RSG-895 (C1)	14.279**	0.419	2.842**	0.845**	0.204
RSG-895 × HC-5 (C2)	10.934**	0.023	1.868	0.518*	0.151
RSG-974 × Avrodhi (C3)	5.973**	0.043	6.215**	0.633*	0.211

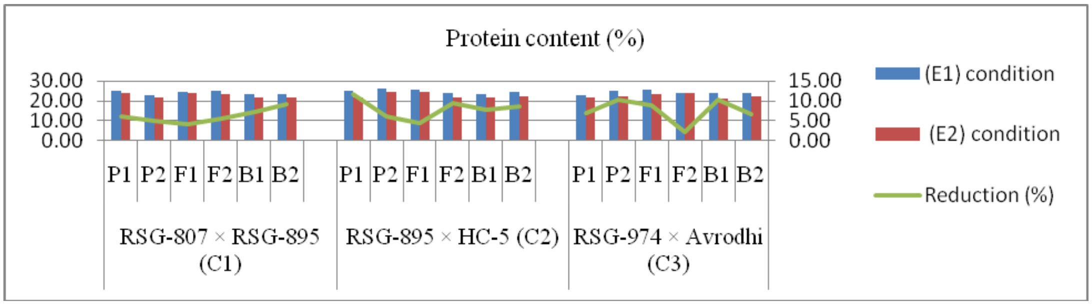
*, ** Significant at 5 % and 1 % level, respectively; DF = degrees of freedom

Supplementary Fig. S1. Mean performance of all six generations of three crosses



(iii)


Proline content (µmol/g)
8.00
6.00
4.00
2.00
0.00
0.00
-5.00
-10.00
-15.00
-20.00
-25.00
-30.00
P1 P2 F1 F2 B1 B2
P1 P2 F1 F2 B1 B2
P1 P2 F1 F2 B1 B2
RSG-807 × RSG-895 (C1)
RSG-895 × HC-5 (C2)
RSG-974 × Avrodhi (C3)
(E1) condition
(E2) condition
Reduction (%)


Protein content (%)
30.00
20.00
10.00
0.00
15.00
10.00
5.00
0.00
P1 P2 F1 F2 B1 B2
P1 P2 F1 F2 B1 B2
P1 P2 F1 F2 B1 B2
RSG-807 × RSG-895 (C1)
RSG-895 × HC-5 (C2)
RSG-974 × Avrodhi (C3)
(E1) condition
(E2) condition
Reduction (%)