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UNVEILING GENETIC VARIABILITY OF PHYSIOLOGICAL TRAITS DRIVING DROUGHT TOLERANCE IN CHICKPEA (*CICER ARIETINUM* L.)

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ABSTRACT

Chickpea (*Cicer arietinum* L.) is one of the important legumes widely grown for dietary proteins in semi-arid Mediterranean climatic conditions. Its crop yield is highly susceptible to abiotic stresses such as heat, frost and drought, especially during the reproductive phase, causing major yield losses and production instability worldwide. With climate change intensifying the frequency and severity of drought and heat stress, the development of stress-tolerant varieties is crucial for sustaining and enhancing chickpea production. Many researchers have emphasized the genetic improvement of yield-related traits under drought conditions as the primary avenue for developing high-yielding, drought-tolerant varieties. However, drought tolerance can also be achieved through the selection of physiological traits directly associated with stress adaptation. Therefore, an investigation was conducted at the Regional Agricultural Research Station, Vijayapur, during the post-rainy season to assess the genetic differences for physiological traits among chickpea genotypes. The experimental material consisted of 30 chickpea genotypes including six checks. Analysis of variance revealed significant genotypic differences for all the four physiological characters with wide range of variations. Genotypic and phenotypic co-efficient of variation were high for proline content under both stress and non-stress condition. Broad sense heritability was higher for all the four traits. High heritability coupled with high genetic advance over mean was exhibited by SPAD chlorophyll meter reading and Proline content at 45 days after sowing under both stress and non-stress conditions. Based on drought indices, DBGV211, DIBG205, DBGV219, DBGV210, ICCV191116 and ICCV191102 were identified as superior genotypes and can be used as parents to develop breeding populations to identify genomic regions linked to specific traits.

Keywords: Broad sense heritability, Chickpea, SPAD chlorophyll meter reading, Proline content

Introduction

Chickpea is the most important legume crop and a source of nutrition to millions of people globally due to its richness in protein, fibre and minerals. It also remediates the soil by its ability to fix nitrogen in a symbiotic relationship with rhizobacteria upon nodulation. It belongs to the family Fabaceae, subfamily Faboideae. The genus *Cicer* consists of 44 species including 35 perennial and 8 wild species, with *Cicer arietinum* L. as the domesticated species. The crop is self-pollinated, diploid ($2n=2x=16$) with a

relatively small genome (740 Mb). The cultivated chickpea is divided into two groups as microsperma and macrosperma on the basis of plant and seed characteristics (Varshney *et al.*, 2014).

Terminal drought is major limiting factor because in majority of the areas, chickpea is cultivated as post rainy season crop. The crop is generally planted after the main rainy season and grown on stored soil moisture, making terminal drought stress a primary constraint to productivity (Leport *et al.*, 1999 and Serraj *et al.*, 2004). The crop experiences drought

stress from late vegetative stages until maturity. Drought stress intensity varies from year to year and from place to place, depending on amount and distribution of rainfall, as well as spring and early summer temperatures. Limited irrigation to meet crop needs at critical times of growth and development may be vital for realizing chickpea cultivars' yield potential. However, the majority of chickpea production takes place in areas where irrigation is not available. Farmers with limited resources who grow chickpeas find it difficult to provide supplemental irrigation. Therefore, developing high yielding drought tolerant genotypes is the only way to address or overcome this constraint.

JG 11 and JAKI 9218 are widely cultivated chickpea varieties in North Karnataka; however, their performance is largely confined to the normal sowing window (September 25th–October 30th). When sown beyond this period, these varieties experience substantial yield losses, primarily due to exposure to terminal high-temperature stress during the reproductive phase, which adversely affects flowering, pod set and seed filling (Devasirvatham *et al.*, 2015). Availability of irrigation water in command areas also necessitates the need to identify suitable breeding lines for late sown cultivation. In this context, the identification of well-adapted, drought-tolerant genotypes is crucial for improving both the production and productivity of chickpea.

Understanding the variability in physiological traits is essential for improving drought tolerance in crops. Traits such as chlorophyll content, membrane stability, relative water content, and proline accumulation reflect how plants cope with water stress. When considerable genetic variability exists for these traits, it gives plant breeders a fortuitous to select genotypes that perform better under drought (Kashiwagi *et al.*, 2005). Practicing selection for these traits in breeding programs helps in developing crop varieties that are more resilient and stable in yield even during dry conditions. This ultimately supports sustainable crop production and food security in drought-prone areas. The present investigation was aimed to estimate the genetic variability, heritability and genetic advance for different physiological traits along with drought tolerance indices based on yield performance.

Materials and Methods

The experimental material comprised 30 chickpea genotypes, including six standard checks; A-1, SA-1, ICC4958, BGD111-1, JAKI9218 and JG11. The trial was conducted under both stress (Rainfed) and non-stress (Irrigated) conditions during the *Rabi* 2021-22

season at the rainout shelter, III Block, Regional Agricultural Research Station (RARS), College of Agriculture, Vijayapur. A Randomized Block Design (RBD) with two replications was employed to ensure reliable evaluation of genotypic performance. The spacing was maintained at 30 cm between rows and 10 cm between plants, with each plot measuring 36 m² (2 m × 18 m). The crop was raised with all standard agronomical package of practices *i.e.* fertilizer dose N, P and K applied as basal and all plant protection measures were adopted to raise healthy crop. The observations were recorded on SPAD chlorophyll meter reading (SCMR), membrane stability index (MSI), relative water content (RWC) and proline content (PC) at 45 and 75 days after sowing. The various genetic parameter *viz.* genotypic and phenotypic coefficients of variation, broad sense heritability, genetic advance over mean were formulated as suggested by Burton and Devane (1952).

Statistical analysis

The mean data of physiological parameters recorded for all the genotypes, were subjected to various standard statistical procedures to estimate mean, range, ANOVA and variability parameters. The data was analyzed using WINDOSTAT *ver.* 9.1 software programme. Genotypic and phenotypic coefficients of variability were computed as per the formula. Broad sense heritability was calculated as the ratio of genotypic variance to the total phenotypic variance, as suggested by Hanson *et al.* (1956) and expressed as percentage. Genetic advance and Genetic advance over mean were estimated by using the formula given by Johnson *et al.* (1955). In addition, five drought tolerance indices include Drought and Productivity Index (DPI), Drought tolerance efficiency (DTE), Mean productivity (MP), Drought susceptibility index (DSI) and Tolerance index (TOL) were estimated using the following formula.

- Drought and Productivity Index (DPI) = $(3Y_S + 1Y_{NS} - 2DC_S) / 2$
- Drought susceptibility index (DSI) = $1 - (Y_S / Y_{NS}) / D$ (Fischer and Maurer, 1978)
- Mean productivity (MP) = $(Y_{NS} + Y_S) / 2$ (Rosielle and Hamblin, 1981)
- Tolerance to Drought index (TDS) = $Y_{NS} - Y_S$
- Drought tolerance efficiency (DTE) = $(Y_S / Y_{NS}) \times 100$

Where

Y_S = Grain yield of the genotype under moisture stress condition

Y_{NS} = Grain yield of the genotype under non-stress condition

DC_S = Grain yield of drought tolerant check under stress condition

$$D = 1 - \frac{\text{Mean grain yield of all strain under moisture stress condition}}{\text{Mean grain yield of all strains under non-stress condition}}$$

Results and Discussion

The mean performance of genotypes showed significant variation for all four physiological traits under both stress and non-stress conditions, indicating adequate scope for effective selection in crop improvement (Fig. 1). The analysis of variance carried out on four physiological traits reveal that mean sum of squares due to genotypes was significant at both 45 and 75 days after sowing under stress and non-stress conditions, indicating the presence of substantial genetic variability for the traits studied among the genotypes (Table 1). Variability within a population is fundamental to crop improvement, as it provides the basis for identifying and selecting superior genotypes in a breeding programme. It can be quantified through several approaches, among which the phenotypic coefficient of variation (PCV) and genotypic coefficient of variation (GCV) are the most widely used indicators. Generally, higher PCV values compared to GCV suggest a positive influence of the environment on the expression of physiological traits. Estimates of PCV, GCV, heritability, and genetic advance over mean for physiological traits at both intervals under both stress and non-stress conditions are presented in Table 2.

The phenotypic and genotypic coefficient of variation were high for PC at 45 days after sowing under both stress and non-stress condition, indicates ample improvement in the trait when selection was practiced. However, the magnitude of difference between GCV and PCV was very low representing that they were majorly under genetic control and the influence of environment was negligible. These results were in conformity with earlier reports of Singh *et al.* (2013) and Singh *et al.* (2021). Moderate GCV and PCV were observed for PC at 75 days, SCMR at 45 days, MSI at both intervals and RWC at 75 days under both conditions, indicates the moderate variability in these traits. Higher estimates of PCV than that of the corresponding GCV indicates the positive influence of environmental factors in expression of these traits and were in line with the findings of Meena *et al.* (2006), Singh *et al.* (2013) and Manasa *et al.* (2020). Low PCV

and GCV for RWC at 45 days under both conditions indicated limited genetic variation, making selection less effective for this trait.

The proportion of variability due to genetic cause is projected as heritability. Heritability imprints on the transmission of traits from parents to offspring. It plays a key role in the selection process in plant breeding as it is estimated from fixable genetic variance. High heritability was recorded for SCMR at 45 days, MSI at 75 days, and PC at 45 days under both conditions, suggesting genetic improvement of these traits will be effective. Although high heritability indicates the reliability of phenotypic performance in selection, it does not always reflect the expected genetic gain.

High heritability accompanied with high genetic advance over mean were observed for SCMR and PC at 45 days under both stress and non-stress conditions, MSI at both intervals, and PC at 75 days under non-stress conditions, indicates that the majority of the variations in this character are attributable to additive gene effects and selection for these traits may be effective. High heritability coupled with moderate genetic advance were exhibited by SCMR and RWC at 75 days under both conditions, MSI at both intervals, RWC at 45 days, and PC at 75 days under stress condition indicate a combination of additive and non-additive gene action. High heritability with low genetic advance exhibited by RWC at 45 days, suggested the prevalence of non-additive gene action. These findings corroborate earlier reports by Singh *et al.* (2021), Sanjay *et al.* (2019), and Gautam *et al.* (2021).

Based on yield performance under stress and non-stress conditions, the genotypes were screened using drought tolerance indices like TDS, MP, DSI, TDE and DPI. The genotypes DBGV211, DIBG205, DBGV219, DBGV210, ICCV191116, and ICCV191102 performed well under both stress and non-stress conditions, indicating their potential for use in future breeding programmes without any yield penalty (Table 3).

Conclusion

Genetic variability study revealed high phenotypic and genotypic coefficient of variation, heritability and genetic advance over mean for traits like membrane stability index and proline content indicate the predominance of additive gene effect and therefore simple selection based on phenotypic performance is likely to yield beneficial results in improving these characters will help in development of drought tolerant high yielding superior genotypes. The genotypes performed superior under both stress and non-stress condition can be used as parents in crossing program to

develop breeding populations without compromising yield trait to identify genes/QTLs governing the specific trait and further can also be used in marker assisted selection for developing drought resilient genotypes.

Table 1 : Analysis of variance for physiological related traits in chickpea genotypes under stress and non-stress conditions

Source of variations	Situation	DF	SCMR @45	SCMR @75	RWC @45	RWC @75	MSI @45	MSI @75	PC @45	PC @75
Replications	NS	1	0.02	0.75	95.63	99.61	4.68	25.29	1.19	1.97
	S	1	4.89	0.75	59.28	79.61	61.49	15.29	12.48	4.53
Genotypes	NS	29	49.66**	32.47**	87.33**	116.40*	244.45**	112.96*	9.74**	3.91**
	S	29	39.02**	32.47**	73.96**	116.39*	140.10**	102.96**	18.33*	8.03**
Residual	NS	29	7.12	10.37	46.71	38.08	13.07	15.11	2.29	0.47
	S	29	3.22	10.37	20.71	38.08	47.97	15.11	4.61	2.23
CD @ 5%	NS	-	5.46	6.59	13.98	12.62	7.40	7.95	3.10	1.40
	S	-	3.67	5.29	9.31	10.62	14.17	5.95	4.39	3.06
CV%	NS	-	7.41	9.42	8.77	9.62	5.55	6.17	18.08	7.64
	S	-	5.17	8.32	5.94	7.62	10.14	4.17	14.68	10.62
S.E. d	NS	-	2.67	3.22	6.84	6.17	3.62	3.89	1.52	0.69
	S	-	1.79	1.22	4.55	4.17	6.92	3.88	2.14	1.49

*Significant at P = 0.05, ** P = 0.01.

SCMR-SPAD chlorophyll meter reading; RWC- relative water content; MSI-Membrane stability index; PC-Proline content

Table 2 : Estimation of genetic variability parameters for physiological related traits in chickpea genotypes under stress and non- stress condition

Particulars	Traits		SCMR @45	SCMR @75	RWC @45	RWC @75	MSI @45	MSI @75	PC @45	PC @75
GCV	NS		12.81	8.43	5.78	12.98	16.51	12.31	23.01	14.6
	S		12.18	9.72	6.74	9.76	9.94	11.1	20.21	12.08
PCV	NS		14.8	15.4	10.51	14.21	17.42	15.3	29.27	16.48
	S		13.23	13.53	8.98	13.7	14.2	12.7	23.15	16.08
MEAN	NS		36.01	34.21	77.92	64.13	65.15	74.5	8.38	8.98
	S		34.73	34.2	76.6	64.13	68.27	63.02	14.63	14.09
GA	NS		8.22	6.45	5.11	10.65	21	14.65	3.13	2.39
	S		8.02	4.92	7.97	9.18	9.79	12.59	4.17	2.64
h ² _{bs}	NS		74.89	51.57	62.39	65.70	89.84	76.40	61.83	78.5
	S		84.73	62.27	56.24	60.07	48.99	56.40	60.79	56.43
GAM (%)	NS		22.83	17.76	6.56	16.98	32.24	22.76	37.28	26.65
	S		23.09	14.38	10.41	14.31	14.33	19.98	28.51	18.7
RANGE	NS	Min	25.5	27.09	55.59	54.31	44.5	47.9	3.19	7.17
		Max	45.37	41.72	92.5	87.00	85.2	83.7	11.96	13.91
	S	Min	26.3	23.09	58.41	44.31	42.17	43.5	10.23	6.81
		Max	45.3	36.02	87.58	88.87	83.57	72.94	22.72	18.32

Standard range= (maximum – minimum) / mean

SCMR-SPAD chlorophyll meter reading; RWC- relative water content; MSI-Membrane stability index; PC-proline content

Table 3 : Top six potential genotypes based on drought tolerance indices

ENTRIES	PYIR	PYS	TDS	MP	DSI	DTE	DPI
DBGV211	118.931	106.75	21.655	117.58	-15.46	147.58	168.755
DIBG205	206.9262	148.5	97.425	158.215	-0.77	91.775	207.015
DBGV219	151.165	112.47	38.695	131.8175	-62.315	130.64	236.275
DBGV210	194.0117	138.255	55.755	166.1325	-1.985	104.505	319.895
ICCV191116	193.8485	139.1	94.75	141.475	-1.315	78.23	223.95
ICCV191102	244.8	191.81	67.01	203.305	-13.785	144.255	236.93

PYIR=Irrigated yield; PYRF=Rain fed yield; TDS=Tolerance to drought stress; MP=Mean productivity; DSI=Drought susceptibility index; DTE=Drought tolerance efficiency; DPI=Drought and productivity index

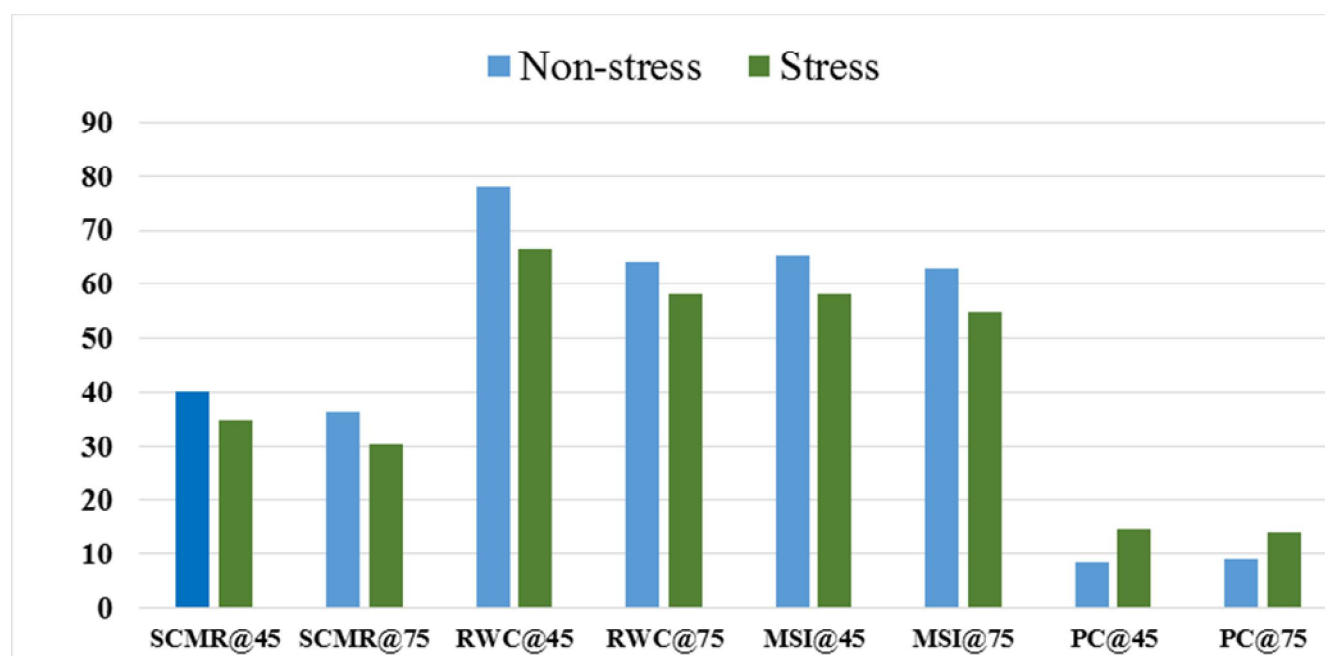


Fig. 1 : Overall mean performance of genotypes for different physiological traits at two intervals under both stress and non-stress conditions

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