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Genetic analysis of traits related to cold tolerance and yield in chickpea: Estimates of genetic variance from Diallel mating design

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Abstract
In the present investigation, eight parents *viz.* ICCV88503, ICCV88506, ICCV88510, PG 5, IPC 09-50, IPC 97-29, GNG 2171 and ICC 1205 that were crossed in half diallel fashion to obtain twenty-eight F_1 hybrids to estimate combining ability variances for five biochemical parameters related to cold tolerance and thirteen yield related traits. The genetic analysis findings indicated that the inheritance of all eighteen traits involves significant contributions from both additive and non-additive genetic components. However, the non-additive (sca) variance was notably higher than the additive (gca) variance for all traits, except for one biochemical trait (total soluble proteins), as well as four yield-related traits: days to 50% flowering, days to maturity, plant height, and biological yield per plant. This suggests that non-additive gene action predominates in these traits. Considering the parallel importance of both additive and non-additive genetic effects in determining the inheritance of different traits, it is recommended to simultaneously utilize both through the adoption of a biparental approach.

Keywords: Chickpea, GCA, SCA, Variance

1. Introduction
Chickpea, scientifically known as *Cicer arietinum* L., belongs to the Fabaceae family and has a chromosome count of 2n=2x=16. It has since spread to various countries, including India many parts of the world's form its origin Western Asia. Considered one of the earliest legumes cultivated by humans (Pokorny *et al.*, 2015) [18], chickpea seeds are valued for their affordability and high-quality dietary proteins. Additionally, this contains essential minerals as well as vitamins such as thiamine, riboflavin, and niacin along with unsaturated fatty acids, dietary fibers and carbohydrates (Jimenez-Lopez *et al.*, 2020) [13]. India holds a significant position in global chickpea production, contributing approximately 70% of the total output. In India it is grown as *rabi* season crop and it is important to note that chickpeas have a threshold temperature requirement of 21 °C and temperatures below this range can be stressful for the crop. Consequently, northern regions and foot hills around India are vulnerable to cold stress, leading to significant yield losses.

Chickpea, being native to the warm climates of the Mediterranean region, is particularly sensitive to low temperatures. The impact of low temperatures on chickpea depends on the growth stage and the specific cultivation region. In northern India, for example, the months of December, January, and February experience low temperatures ranging from 0-10°C, resulting in significant yield losses (15-20%) for the susceptible cultivars. Temperature plays a crucial role in determining the timing of the reproductive phase in chickpea. Chilling temperatures below 10°C have detrimental effects on yield due to the crop's sensitivity during the flowering stage. The influence of cold stress on chickpea is well-documented, with various adverse effects observed at different levels of plant organization. These effects include reduced vegetative and reproductive growth, delayed phenology (timing of growth stages), increased leaf chlorosis and necrosis, changes in leaf hydration status, abnormalities in flower structure, and damage to reproductive structures, ultimately impacting yield. Several studies, including those by Croser *et al.* (2003) [7], Thakur *et al.* (2010) [24], Rani *et al.* (2020) [19] and Kiran *et al.* (2021) [14], have highlighted the negative consequences of cold stress on chickpea at the physiological and agronomic levels.

To improve productivity, it is important to address the current low levels by making concerted efforts.

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Before starting the breeding program, it is crucial to carefully select the parent plants for the hybridization process. The analysis of combining ability allows the breeder to effectively choose the parents. It also provides essential information about the genetic mechanisms that influence the expression of specific traits, helping to determine future breeding strategies. Therefore, evaluating combining ability becomes necessary as it assesses the potential of parents and reveals the nature and extent of various gene actions involved. This information greatly assists plant breeders in selecting desirable genotypes for breeding programs and identifying cross combinations with commercial value. Thus, the current study aims to investigate the additive and non-additive genetic variances in chickpea for five biochemical traits related to cold tolerance and thirteen yield related traits using half diallel crosses.

2. Material and Methods
The current investigation was carried out during the *rabi* season of 2021-22. The experimental material for this study consisted of eight parents, namely ICCV88503, ICCV88506, ICCV88510, PG 5, IPC 09-50, IPC 97-29, GNG 2171, and ICC 1205. Crossing was performed in diallel fashion, resulting in a total of twenty-eight F_1 hybrids. Both the 28 F_1 hybrids and the eight parent plants were sown in randomized block design with two replications. Standard practices for chickpea cultivation were employed to ensure the growth of a healthy crop.

The observations were made on five biochemical parameters related to cold tolerance and thirteen yield related traits.

2.1 Estimation of biochemical parameters related to cold tolerance
All the biochemical parameters were estimated in vegetative stage from leaves taken when average temperature was equal to or less than 10 ºC.
1. Total Chlorophyll was estimated according to procedure given by Arnon (1949) [2] with slight modification.
2. Proline content was estimated according to procedure given by Bates (1973) [3] with slight modification.
3. Total soluble sugars were estimated according to procedure given by Dubois *et al.* (1956) [8] with slight modification.
4. Phenol content was estimated according to procedure given by Malik and Singh (1980) [15] with slight modification.
5. Total soluble proteins content was estimated according to procedure given by Bradford (1976) [4] with slight modification

2.2 Statistical and genetic analysis
The analysis of variance was conducted following the standard method outlined by Panse and Sukhatme in 1967 [17] for all the traits under investigation. To determine the general combining ability (GCA) and specific combining ability (SCA) effects, Griffing's method 2 model 1, proposed in 1956 [11], was employed. These calculations provide insights into the overall genetic potential of the parent plants (GCA) as well as the specific interactions between the parents in producing desirable traits (SCA).

Variance components of combining ability and other genetic components

GCA and SCA variances were estimated as follows
i. GCA components

$$\sigma^2 gca = \frac{1}{n+2}[M_g - M_s]$$

ii. SCA components

$$\sigma^2 sca = [M_s - M_e{}']$$

iii. Components due to error

$$\sigma^2 e = MS_e$$

Where
$\sigma^2 sca$ = SCA variance
$\sigma^2 gca$ = GCA variance
σ^2_e = Environmental variance
n = Total number of parents
M_g = MSS gca
M_s = MSS sca
MS_e = MSS error

3. Result and Discussion
Analysis of variance of combining ability
Mean sum of squares for both GCA and SCA were showing significant for all the biochemical and agronomic parameters under investigation (Table 1 and 2). Reddy (2012) [20] conducted combining ability studies on total chlorophyll and recorded significant differences for the traits. Similarly, Shivani (2019) [22] recorded significant GCA and SCA for chlorophyll content and proline content. Singh (2020) [23] recorded significant GCA and SCA for phenol content. Similar studies for agronomic traits were also conducted by Jha *et al.* (2019) [12], Gaur *et al.* (2020) [9] and Sasane *et al.* (2022) [21] recorded significant difference for traits under investigation.

Table 1: Combining ability analysis of variance cold tolerance related

	DF	Total Chlorophyll	Proline content	Total soluble sugar	Total phenols	Total soluble proteins
GCA	7	1293.40 ***	27028.11 ***	1354.943 ***	858.970 ***	160.498 ***
SCA	28	.456.40***	7 973.784 ***	484.657 ***	564.104 ***	16.176 ***
Error	35	15.573	23.857	8.466	2.764	2.183

Table 2: analysis of variance of combining ability for yield related traits

Source of Variation	GCA	SCA	Error
DF	7	28	35
Days to maturity	74.701 **	3.026	4.264
Days to 50% flowering	58.877 **	2.807 **	0.648
Plant height (cm)	216.902 **	23.872 **	4.478
Height of first pod bearing node (cm)	44.276 **	17.794 **	0.68
Primary branches	0.406 **	0.304 **	0.007
Secondary branches	2.227 **	0.835 **	0.057
Number of pod per plant	628.897 **	84.258 **	5.023
Pod length (cm)	1689.948 **	308.004 **	29.002
Number of seeds per pod	339.900 **	410.298 **	32.933
100 seed weight	12.020 **	3.768 **	0.156
Seed yield per plant (g)	52.281 **	6.756 **	0.182
Biological yield (g)	280.653 **	29.077 **	1.928
Harvest index (%)	110.120 **	58.282 **	5.283

Genetic components and genetic parameters

The estimation of genetic parameters from the combining ability analysis is an important aspect to understand genetic nature of the traits. The σ^2 A is twice the value of σ^2 GCA and σ^2 D is same as that of σ^2 SCA. The GCA variance attributes to additive gene action and SCA variance is attributed to non-additive effect, hence if GCA to SCA ratio is more the one it marks the preponderance of additive gene action and ratio less the one indicates involvement of non- additive action.

In case of biochemical parameter total soluble proteins, σ^2 GCA was found higher than σ^2 SCA (Table 3). In contrast, all other traits under study had higher σ^2 SCA than σ^2 GCA. Similarly, Only one parameter *viz.* total soluble proteins showed the ratio more than 1, whereas all other traits showed <1. Most of the traits exhibit the ratio in between 0 to 1 show that the existence of high SCA variance. The trait total protein had higher σ^2A than σ^2D. Remaining traits had higher σ^2D than the σ^2 A. Parameters like total chlorophyll, total phenols, proline content and total soluble sugars showed high significant SCA variance and having fewer additive gene actions out of total genotypic variance.

Observation recorded for yield related revealed that the estimates of σ^2 GCA were higher than σ^2 SCA in case of four traits. These included two phonological traits days to 50% flowering and days to maturity along with two agronomic traits plant height and biological yield per plant (Table 4).

Similarly, for these traits σ^2A was also greater than σ^2D and ratio of GCA to SCA was more than one. For all remaining nine traits under study higher σ^2 SCA than σ^2 GCA was recorded along with higher σ^2D and GCA to SCA ratio of less the one. This showed that majority of the traits have preponderance of SCA variance which is attributed to non-additive gene action. Seed yield per plant showed high significant SCA variance and having fewer additive gene actions out of total genotypic variance.

In the study additive gene actions along with non-additive gene actions were involved in both expression of biochemical traits and yield related traits. Non- additive gene actions were more prominently involved in the expression biochemical traits. Thus, the results revealed that non-additive gene action existed more prominently for total chlorophyll, total phenols, proline content and total soluble sugars except for total soluble proteins. The importance of biochemical like chlorophyll, prolines, total souble sugars, phenols and soluble proteins imparting cold tolerance in chickpea and other plant species has been well documented in past. In parity with the present findings, non- additive gene action for chlorophyll was also obtained by Reddy *et al*. (2012) [20]. Shivani (2020) [22] reported non- additive gene action for expression of total chlorophyll and proline content. Singh (2020) [23] reported involvement of non- additive gene action in expression of phenol content in chickpea.

Table 3: Components of genetic variance for cold tolerance related parameters

Parameter	σ² GCA	σ² SCA	σ²a	σ² D	σ² GCA/σ² SCA Ratio
Total Chlorophyll	1289.063	4520.929	2578.126	4520.929	0.285
Proline content	2700.326	4950.927	5400.652	4950.927	0.545
Total soluble sugars	134.648	476.191	269.295	476.191	0.283
Total phenols	85.621	561.339	171.241	561.339	0.153
Total soluble proteins	15.832	13.992	31.663	13.992	1.131

Table 4: Genetic variance components for yield related traits

Characters	σ²GCA	σ²SCA	σ²a	σ²D	σ²GCA/σ² SCA Ratio
Days to Maturity	7.044	-1.238	14.087	-1.238	-5.690
Days to 50% Flowering	5.823	2.159	11.646	2.159	2.697
Plant Height (cm)	21.242	19.394	42.485	19.394	1.095
Height of First Pod Bearing Node (cm)	4.360	17.114	8.719	17.114	0.255
Number of Primary Branches	0.040	0.298	0.080	0.298	0.134
Number of Secondary Branches	0.217	0.779	0.434	0.779	0.279
Number of Pod per Plant	62.387	79.235	124.775	79.235	0.787
Pod Length (cm)	166.095	279.002	332.189	279.002	0.595
Number of Seeds per Pod	30.697	377.365	61.394	377.365	0.081
100 Seed Weight	1.186	3.612	2.373	3.612	0.328
Seed Yield per Plant (g)	5.210	6.574	10.420	6.574	0.792
Biological Yield (g)	27.872	27.149	55.745	27.149	1.027
Harvest Index (%)	10.484	52.999	20.967	52.999	0.198

Additive gene action was crucial for four traits, out of which two were phonological triats days to 50% flowering and days to maturity along with two agronomic trait plant height and biological yield. Rest of the traits was more prominently controlled by non-additive gene action existed. Observed results agreed with the findings of Gowda *et al.* (1978) [10] they also observed significant SCA variance for seed yield and number of branches along with high GCA variance for flowering time and plant height. Naveed *et al.* (2012) [16], reported significant SCA effects for number of pods per plant, days to maturity, number of seeds per pod, 100 seed weight and seed yield per plant, where as significant GCA for biomass per plant. Ambadabade *et al.* (2014) [1], they also observed preponderance of non-additive gene action for plant height, number of primary branches, number of seeds per pods and grain yield per plant. Involvement of both additive and non-additive gene action for expression of yield related traits in chickpea has also reported by Chauhan *et al.* (2013) [5], Jha *et al.* (2019) [12] and Gaur *et al.* (2020) [9].

4. Conclusion
The findings of the study indicate that both the gene actions contribute to the expression of biochemical traits as well as yield-related traits. Non-additive gene actions were found to be more significant in the expression of the majority of the traits studied. Considering the parallel roles played by both additive and non-additive genetic effects in determining the inheritance of different traits, the study suggests the simultaneous utilization of these effects through the adoption of a biparental approach. This approach involves carefully selecting and crossing two parent plants to exploit the favorable additive and non-additive genetic components and enhance the expression of desirable traits for the development of high yielding varieties tolerant for cold stress.

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