Genetic Association Studies for indirect selection for important yield and yield attributing traits in grain cowpea (*Vigna unguiculata* L. Walp subsp *unguiculata*)

.

ABSTRACT:

Aims: The present study encompasses genetic association studies among F_4 and F_5 families generated from various crosses in grain cowpea (*Vigna unguiculata* L. Walp subsp *unguiculata*).
Study design: Augmented design-II for F_4 families and checks during *kharif* 2023 and Randomized block design with two replications for selected F_5 families and checks during *rabi-summer* 2023-24.
Place and Duration of Study: The current experiment was conducted at the Botany garden, Department of Genetics and Plant Breeding, University of Agricultural Sciences, Dharwad, Karnataka for the evaluation of 36 F_4 families and six checks during *kharif* 2023 and 27 selected F_5 families and five checks during *rabi-summer* 2023-24.
Methodology: The material under experiment included 36 F_4 families and six checks evaluated in augmented design-**II** during *kharif* 2023. Then during *rabi-summer* 2023-24, 27 selected F_5 families and five checks evaluated in randomized block design. Observations were recorded for nine yield and yield attributing traits *viz*., total yield (kg/ha), single plant yield (g), number of pods per plant, test weight (g), number of seeds per pod, pod length (cm), number of clusters per plant, days to fifty per cent flowering and days to maturity. Correlation and path coefficients were estimated using standard procedures.
Results: Correlation studies indicated that yield estimates were highly positively correlated to all yield attributing traits except to days to fifty per cent flowering and days to maturity. Characters like number of pods per plant (0.59, 0.64), pod length (0.65, 0.58) and test weight (0.39, 0.58) had highest correlations with yield in F_4 and F_5 generation respectively. Path coefficients further highlighted the character; number of pods per plant (0.363, 0.594) to have the highest direct effect on yield at both phenotypic and genotypic level respectively.

Keywords: Cowpea, Correlation, Path, Direct effect, Indirect effects

1. INTRODUCTION

Cowpea [*Vigna unguiculata* (L.) Walp subsp *unguiculata*] is one of the most ancient legumes majorly cultivated in tropical and sub-tropical regions of the world. Cowpea was introduced in India during the end of Neolithic period. Owing to this ancient introduction, India possesses exceptional diversity in the forms of both wild cowpea and cultivated cowpea. Therefore, India is regarded as the secondary centre of diversity for the crop (Timko *et al.*, 2007[1]; Deshpande *et al.* 2018[2]). Cowpea is commercially grown across India as a multipurpose legume. It is often identified as "Poor man's meat" due to protein rich nature (Sabale *et al.,* 2018[3]). On a global scale, productivity of cowpea is 643.00 kg ha^{-1} on an average, with highest in Ghana (1662.00 kg/ha) (Anonymous, 2022-23[4]). But in India, productivity records at 567.00 kg ha^{-1} (Anonymous, 2019-20[5]). The productivity in the country is relatively low due to several reasons, one of which is the tendency of cultivating local landraces and lack of adoption of high yielding improved varieties among the cowpea growers in the country. This necessitates development of high yielding cowpea varieties with farmers' interest in mind.

In field condition the realized yield often do not truly represent the underlying genetic potential in a plant. As a result; direct selection depending on yield is unreliable. So to select for higher yielding genotypes plant breeders rely on several yield component traits along with yield *per se*. The inter-relationships among yield and yield related parameters determine the nature of influence of a component character to yield. Hence, studying correlation is essential to understand the desirable direction of selection pressure on each component trait [Patel *et al.,* 2016[6]]. The component characters are not only related to yield but also are interconnected. So each character influences yield directly and also indirectly via other correlated component characters. Path coefficient analysis helps to partition the correlation coefficients into direct and indirect effect of a component trait on yield. Estimates of both correlation coefficient and path coefficient lead to a better understanding of the characters at genetic level and thus help in careful selection of desirable genotypes (Khan *et al.,* 2022[7]). Hence, the present experiment studied the correlation, direct and indirect effects among F_4 and F_5 families of various crosses in grain cowpea to help select for the desirable families.

2. MATERIAL AND METHODS

The present study entitled "Genetic Association Studies for indirect selection for important yield and yield attributing traits in grain cowpea (*Vigna unguiculata* L. Walp subsp *unguiculata*)" was carried out at the Botany garden, Department of Genetics and Plant Breeding, University of Agricultural Sciences, Dharwad, Karnataka. The material under experiment included 36 F_4 families [Pedigree enclosed in supplementary table 1] and six checks [Four released varieties- DC 15, DCS 47-1, RC 101, GC 3 and two advanced breeding line- DC 16 and DC 17] evaluated in augmented design-II during *kharif* 2023. Observations were recorded for nine yield and yield attributing traits *viz.*, total yield (kg/ha), single plant yield (g), number of pods per plant, test weight (g), number of seeds per pod, pod length (cm), number of clusters per plant, days to fifty per cent flowering and days to maturity. Correlation coefficients were estimated among these traits. Then during *rabi-summer* 2023-24, 27 F_5 families were selected based on yield, earliness and overall performance on field and evaluated along with five checks [Three released varieties- DC 15, DCS 47-1, RC 101 and two advanced breeding line- DC 16 and DC 17] in randomized block design. Observations on the same group of characters were taken. Correlation as well as path coefficients were estimated from the replicated data of F_5 families and checks.

2.1 Statistical Analysis

Correlation and path coefficients were estimated using standard procedures with the help of RStudio [version 2023.12.0]. Both genotypic and phenotypic coefficients of correlation between two characters were determined by using the variance and covariance components, as suggested by Al- Jibouri *et al.* (1958)[8]. For testing the significance of correlation coefficients, the estimated values were compared with the table value (Fisher and Yates, 1938[9]) at n-2 degrees of freedom (where n denotes the number of entries tested) at 5 % and 1 % levels of significance.

3. RESULTS AND DISCUSSION

Results

3.1 Correlation Studies: Phenotypic correlation coefficients were estimated for yield and yield attributing traits among 36 F_4 families of various crosses and checks (Table 1). Highest positive correlation was estimated between total yield (kg/ha) and single plant yield (g) (0.80). A high positive correlation between days to fifty per cent flowering and days to maturity (0.75) and between pod length (cm) and number of pods per plant (0.70) were also evident. The characters pod length (cm) and number of pods per plant showed significant positive correlation with total yield (kg/ha) (0.65 and 0.59 respectively) as well as with single plant yield (g) (0.60 and 0.62 respectively).

Phenotypic as well as genotypic correlation coefficients were estimated for yield and yield attributing traits using the replicated data of 27 F_5 families of various crosses and checks (Table 2 and 3). Highest significant correlation at phenotypic level was found between number of seeds per pod and pod length (0.79) followed by days to fifty per cent flowering and days to maturity (0.75). The characters single plant yield (g), number of pods per plant and test weight (g) exhibited significant positive phenotypic correlation with total yield (kg/ha) (0.71, 0.64 and 0.58 respectively). At the genotypic level, highest positive correlation was found between single plant yield (g) and test weight (g) (0.92) followed by number of pods per plant and total yield (kg/ha) (0.89). Characters like number of pods per plant, test weight (g) and single plant yield (g) exerted high positive correlation with total yield (kg/ha).

3.2 Path analysis: Path analysis was carried out by considering yield as the ultimate dependent character which is influenced by seven major component characters *viz.*, number of pods per plant, test weight, number of seeds per pod, pod length, number of clusters per plant, days to fifty per cent flowering and days to maturity. The correlation coefficients between yield and each of these characters were split between direct and indirect effects at both phenotypic and genotypic level. In this study, phenotypic as well as genotypic path coefficients were estimated for yield and yield attributing traits using the replicated data of 27 F_5 families of various crosses and checks (Table 4 and 5).

At the phenotypic level, the component character number of pods per plant (0.363) exhibited the highest positive direct effect on the dependent character total yield followed by characters like test weight (0.276) and number of clusters per plant (0.252). The character number of pods per plant displayed positive effect towards yield directly as well as indirectly through traits like test weight (0.166), number of clusters per plant (0.107) and pod length (0.021). The characters test weight, number of seeds per pod, pod length and number of clusters per plant displayed highest positive indirect effect via number of pods per plant (0.219, 0.195, 0.159 and 0.154 respectively).

At the genotypic level also, the component character number of pods per plant (0.594) exhibited the highest positive direct effect on the dependent character total yield followed by characters like number of clusters per plant (0.442) and test weight (0.299). The character number of pods per plant displayed positive effect towards yield directly as well as indirectly through traits like number of clusters per plant (0.228), test weight (0.215) and pod length (0.103). The characters test weight, number of seeds per pod, pod length and number of clusters per plant displayed highest positive indirect effect via number of pods per plant (0.427, 0.368, 0.300 and 0.307 respectively).

Table 1: Phenotypic correlation coefficients among F_4 families of diverse crosses and checks in grain cowpea during *kharif*-2023

	Total Yield (kg/ha)	Single Plant Yield (g)	Number of pods per plant	Test weight (g)	Number of seeds per pod	Pod Length (cm)	Number of clusters per plant	Days to fifty per cent flowering	Days to maturity
Total Yield (kg/ha)	**1.00**	0.80**	0.59**	0.39**	0.35*	0.65**	0.42**	0.06	0.11
Single Plant Yield (g)		**1.00**	0.62**	0.50**	0.34*	0.60**	0.39**	0.02	0.10
Number of pods per plant			**1.00**	0.60**	0.33*	0.70**	0.26*	-0.33*	-0.04
Test weight (g)				**1.00**	0.21	0.59**	0.05	-0.29*	-0.10
Number of seeds per pod					**1.00**	0.53**	0.19	-0.12	0.02
Pod Length (cm)						**1.00**	0.24	-0.08	0.19
Number of clusters per plant							**1.00**	0.37**	0.37**
Days to fifty per cent flowering								**1.00**	0.75**
Days to maturity									**1.00**

***Significant at 5% level of significance **Significant at 1% level of significance**

Table 2: Phenotypic correlation coefficients among F_5 families of diverse crosses and checks for yield and yield attributing traits in grain cowpea during *rabi-summer* 2023-24

	Total Yield (kg/ha)	**Single Plant Yield (g)**	**Number of pods per plant**	**Test weight (g)**	**Number of seeds per pod**	**Pod Length (cm)**	**Number of clusters per plant**	**Days to fifty per cent flowering**	**Days to maturity**
Total Yield (kg/ha)	**1.00**	0.71**	0.64**	0.58**	0.39**	0.37**	0.47**	0.001	0.11
Single Plant Yield (g)		**1.00**	0.59**	0.61**	0.41**	0.42**	0.50**	0.14	0.35**
Number of pods per plant			**1.00**	0.60**	0.54**	0.44**	0.42**	-0.12	0.11
Test weight (g)				**1.00**	0.34**	0.45**	0.29*	-0.04	0.10
Number of seeds per pod					**1.00**	0.79**	0.36**	0.10	0.28*
Pod Length (cm)						**1.00**	0.24	0.08	0.30*
Number of clusters per plant							**1.00**	0.37**	0.37**
Days to fifty per cent flowering								**1.00**	0.75**
Days to maturity									**1.00**

***Significant at 5% level of significance **Significant at 1% level of significance**

Table 3: Genotypic correlation coefficients among F_5 families of diverse crosses and checks for yield and yield attributing traits in grain cowpea during *rabi-summer* 2023-24

	Total Yield (kg/ha)	**Single Plant Yield (g)**	**Number of pods per plant**	**Test weight (g)**	**Number of seeds per pod**	**Pod Length (cm)**	**Number of clusters per plant**	**Days to fifty per cent flowering**	**Days to maturity**
Total Yield (kg/ha)	**1.00**	0.80**	0.89**	0.83**	0.49**	0.46**	0.69**	0.02	0.16
Single Plant Yield (g)		**1.00**	0.77**	0.92**	0.53**	0.56**	0.76**	0.26	0.49**
Number of pods per plant			**1.00**	0.72**	0.62**	0.51**	0.52**	-0.13	0.10
Test weight (g)				**1.00**	0.48**	0.60**	0.40*	-0.05	0.08
Number of seeds per pod					**1.00**	0.86**	0.50**	0.11	0.33
Pod Length (cm)						**1.00**	0.30	0.08	0.32
Number of clusters per plant							**1.00**	0.47**	0.44**
Days to fifty per cent flowering								**1.00**	0.75**
Days to maturity									**1.00**

***Significant at 5% level of significance **Significant at 1% level of significance**

Table 4: Phenotypic path coefficients estimated for yield and yield attributing traits among F_5 families of diverse crosses and checks for yield and yield attributing traits in grain cowpea during *rabi-summer* 2023-24

Characters	**Number**	**Test**	**Number**	**Pod**	**Number**	**Days to**	**Days to**	**Correlation**

	of pods per plant	**weight (g)**	**of seeds per pod**	**Length (cm)**	**of clusters per plant**	**fifty per cent flowering**	**maturity**	**Coefficients of yield attributing traits with Total yield per ha**
Number of pods per plant	**0.363**	0.166	-0.005	0.021	0.107	-0.001	-0.007	**0.64****
Test weight (g)	0.219	**0.276**	-0.003	0.022	0.074	0.000	-0.007	**0.58****
Number of seeds per pod	0.195	0.095	**-0.010**	0.038	0.091	0.001	-0.018	**0.39****
Pod Length (cm)	0.159	0.125	-0.008	**0.049**	0.060	0.001	-0.020	**0.37****
Number of clusters per plant	0.154	0.080	-0.004	0.012	**0.252**	0.003	-0.024	**0.47****
Days to fifty per cent flowering	-0.043	-0.011	-0.001	0.004	0.094	**0.007**	-0.049	**0.001**
Days to maturity	0.041	0.028	-0.003	0.014	0.093	0.005	**-0.066**	**0.11**

Residual effect: 0.480
***Significant at 5% level of significance **Significant at 1% level of significance**

Table 5: Genotypic path coefficients estimated for yield and yield attributing traits among F_5 families of diverse crosses and checks for yield and yield attributing traits in grain cowpea during *rabi-summer* 2023-24

Characters	**Number of pods per plant**	**Test weight (g)**	**Number of seeds per pod**	**Pod Length (cm)**	**Number of clusters per plant**	**Days to fifty per cent flowering**	**Days to maturity**	**Correlation Coefficients of yield attributing traits with Total Yield per ha**
Number of pods per	**0.594**	0.215	-0.259	0.103	0.228	0.011	0.003	**0.89****

plant								
Test weight (g)	0.427	**0.299**	-0.199	0.121	0.176	0.004	0.002	**0.83****
Number of seeds per pod	0.368	0.142	**-0.418**	0.174	0.223	-0.010	0.008	**0.49****
Pod Length (cm)	0.300	0.178	-0.357	**0.203**	0.135	-0.007	0.008	**0.46****
Number of clusters per plant	0.307	0.119	-0.210	0.062	**0.442**	-0.041	0.011	**0.69****
Days to fifty per cent flowering	-0.076	-0.015	-0.048	0.016	0.207	**-0.087**	0.018	**0.02**
Days to maturity	0.061	0.023	-0.138	0.064	0.195	-0.066	**0.024**	**0.16**

Residual effect: 0.023
***Significant at 5% level of significance **Significant at 1% level of significance**

Discussion

Yield is a complex trait affected by action and interaction of several important attributes in various different ways. The detailed study of these associations helps a breeder to make informed decision in day to day plant breeding activities especially in selection of desired genotype. In the present study, correlation coefficients and path coefficients are used to describe these important associations in segregating generations of grain cowpea.

From the correlation study over the generations, it can be concluded that all yield attributing traits except days to fifty per cent flowering and days to maturity under study exhibited significant positive correlation with total yield (kg/ha) at both genotypic and phenotypic level which suggests that these traits can be effectively used for indirect selection of yield. A similar observation was made by many other scientists for example, Sabale *et al.* (2019) [10] showed a significant positive correlation for number of pods per plant and number of clusters per plant with total yield; Snehal *et al.* (2021) [11] showed a significant positive correlation for test weight with total yield; Vijayakumar *et al.* (2020) [12] reported a significant positive correlation for number of seeds per pod and pod length with total yield. On the other hand, studies by Owusu *et al.* (2021) [13] and Elteib *et al.* (2021) [14] also reported non-significant correlation among maturity traits (days to fifty per cent flowering and days to maturity) and total yield. The positive significant correlation between single plant yield (g) and days to maturity among F_5 families indicates that for selection of early maturity, a reduction in grain yield per plant will occur. The high positive correlation between pairs of yield attributing traits like number of seeds per pod and pod length, days to fifty per cent flowering and days to maturity, number of pods per plant and pod length, test weight and number of pods per plant suggesting the possibility of simultaneous improvement in these characters.

From the path analysis, Genotypic path coefficient analysis revealed that the highest positive direct effect was exerted by number of pods per plant followed by other traits like number of clusters per plant, test weight and pod length. So a positive selection pressure on these traits will increase total yield substantially. Similar conclusions for these traits were drawn by Singh *et al.* (2018) [15] and Singh *et al.* (2022) [16]. The character number of seeds per pod exhibits a negative direct effect on yield. But this trait positively effects yield through other traits like number of pods per plant, number of clusters per plant, test weight and pod length. The magnitude of these positive indirect effects overcomes the negative effect. So the ultimate correlation between number of seeds per pod and total yield remains positive and significant. A similar observation for this trait was evident from many previous studies [Walle *et al.* (2018) [17], Snehal *et al.* (2021) [11], Aliyu *et al.* (2022) [18], Aishwarya *et al.* (2023) [19] and Parmar *et al.* (2024) [20]].

4. CONCLUSION

The underlying genetic background and influence of environment on each trait vary from trait to trait and from material to material. A classical polygenic character like yield is highly influenced by environment and thus does not provide a reliable estimate under field condition. This necessitates the utilization of several yield attributing traits as indirect selection criteria for high yield. An association study among the yield and yield attributing traits helps to comprehend the actual genetic relation present between two traits. From the correlation study, it was evident that yield estimates were highly positively correlated to all yield attributing traits except to days to fifty per cent flowering and days to maturity. Notably, the characters like number of pods per plant, pod length and test weight had highest correlation with yield in F_4 and F_5 generation. So these traits can be confidently used for indirect selection of yield. Path coefficients further highlighted the character number of pods per plant to have the highest direct effect on yield. So, for the material under study this character should be prioritized for selection of high yielding genotypes besides yield *per se*.

Disclaimer (Artificial intelligence)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc.) and text-to-image generators have been used during the writing or editing of this manuscript.

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SUPPLEMENTARY TABLE 1: Details of the pedigree of F_4 families of various crosses

Sl No.	Pedigree	Source
1	EC 724160 × EC 724157	Department of Genetics and Plant Breeding, UAS, Dharwad
2	EC 724160× EC 738126	
3	EC 724160 × DC 15	
4	EC 724160 × DC 16	
5	EC 724160 × DC 17	
6	EC 724160 × GC 3 (F_4-I)$^{\#}$	
7	EC 724160 × GC 3 (F_4-II)$^{\#}$	
8	EC 724157 × EC 738126	
9	EC 724157× GC 3	
10	EC 724157× DCS 47-1	
11	EC 724157× DC 16	
12	EC 724157× DC 17	
13	EC 724157× RC 101	
14	EC 724157× EC 724153	
15	EC 724153× DC 16	
16	EC 724153× DC 17	
17	EC 724153× DCS 47-1	
18	EC 724153× DC 15	
19	EC 724153× GC 3	
20	EC 724153× RC 101	
21	EC 738126 × DC 15	
22	EC 738126× DC 16	
23	EC 738126× DC 17	
24	EC 738126× GC 3	
25	EC 738126× DCS 47-1	
26	EC 738126× RC 101	

27	DC 15 × DCS 47-1	
28	DC 15 × RC 101	
29	DC 16× RC 101	
30	DC 16 × Phule CP05040	
31	DC 16 × DC 17	
32	DC 16 × GC 3	
33	DC 16 × Phule CP05040× RC 101	
34	DCS 47-1× RC 101	
35	DCS 47-1× EC 724160	
36	DCS 47-1× DC 17	

[#] Sister families derived from common pedigree