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Breeding Cowpea (*Vigna unguiculata* L.) for High Seed Yield, Protein Content and Harvest Index: Genetic Parameters to Identify Useful Parents

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ABSTRACT

Background: Cowpea is a highly adaptable, versatile and nutrient-dense grain legume hampered by low productivity across the globe. The selection of parents for getting high frequency of heterotic hybrids is a topic of recurrent contention among plant breeders. So with regard to obtaining high seed yield, harvest index and protein content, the goal of the current study is to identify parents which upon hybridization will generate superior heterotic hybrids.

Methods: Three testers and seven lines were used to develop 21 crosses, totalling to 31 genotypes in the experimental material evaluated at Pulses Research Station, SDAU, Sardarkrushinagar, Gujarat, India. The overall heterotic status (high or low) and gca status (high or low) of each parent and hybrid were determined based on three characters *viz.,* harvest index, protein content and seed yield. Furthermore, the hybrids were classified into various classes according to the parents gca status.

Result: Examining the heterotic effect of the crosses along with the individual performance showed that the GC 7 × GC 4 and EC 724035 × PGCP 12 crosses were the best for seed yield per plant, harvest index and protein content. Three of the 7 lines *viz*., JLS 60, EC 72 3909 and PL 4 and two of the three testers *i.e.* PGCP 12, PL 7 displayed high overall gca status. Among the hybrids, eleven (52.38%) and twelve (57.14%) were categorised as having high (H) overall sca and heterotic status, respectively, while the remaining crosses were categorised as having low (L) overall sca and heterotic status. As non-additive gene action for these traits predominated in current study, heterotic hybrids for these traits were generated by parents with all combinations *i.e.* high, contrasting, or even low gca effects. Therefore, when selecting parents based on gca effects, careful consideration should be given to the sort of gene action governing that character.

Key words: Combining ability, Cowpea, Heterotic status, Hybrid.

INTRODUCTION

Cowpea [*Vigna unguiculata* (L.) Walp.] is a popular grain legume that is versatile, drought-tolerant and nutritious. Indigenous terms like "lobia," "chowlee" and many more have been applied to cowpea in India (Singh, 2016; Sharma *et al.,* 2022). Cowpea is cultivated and utilised in a variety of ways and it differ from other crops in that this crop has a wide range of plant type, pod and seed forms as well as a varied growth habit and maturation period. According to Singh (2016), the cowpea grain has a dry weight containing roughly 55-60% carbohydrates, 1% fat and 20-30% protein. Cowpea has the natural capacity to fix nitrogen in even the poorest soils and are resistant to heat and drought. It is a valuable commercial crop and is considered a vital source of protein for both urban and impoverished rural populations. Cowpea leaves and grains are edible by products that are abundant and reasonably priced sources of high-quality protein. All growth phases of the fruits *i.e.* green pods, fresh or dried seeds, *etc*., are eaten and young leaves are frequently added to soups (Singh, 2016). Cowpea grains have a similar nutritional profile to regular beans, however they contain less anti-nutritional and flatulence-causing substances and more folic acid. Global cowpea production was 8.9 million tonnes, occupying 14.9 million hectares (FAOSTAT, 2022). This crop was grown on 55,800 hectares in India, yielding 35,600 tonnes of yield at a productivity of 638.6 kg/ha (INDIASTAT, 2022). It is mostly grown in the Indian states of Tamil Nadu, Andhra Pradesh, Gujarat, Rajasthan, Maharashtra and Madhya Pradesh.

Shull (1914), provided a lucid explanation of the concept of heterosis. The term "heterosis" describes whether an F_1 hybrid is superior or inferior to its parents in one or more characteristics. Taking use of heterosis is a crucial

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perspective for crop genetic advancement. As growing genotypes across a range of environmental conditions will significantly reduce their potential output, high yielding stable genotypes is the need of the hour (Patel *et al.,* 2021a; Sharma *et al.,* 2025 and Sharma *et al.,* 2024). In self-pollinated crops, hybridization with various mating techniques and segregation in subsequent generations are key components of varietal development. The yield and related traits of the ensuing advanced generations are then evaluated in order to determine their suitability as breeding materials (Sharma and Sridevi, 2016; Patel *et al.,* 2021b and Parmar *et al.,* 2024). The most appropriate method for maximising a crop species' potential for productivity is heterosis breeding, provided that producing F_1 hybrids is both technically and financially possible (Sharma and Shadakshari, 2021 and Gandhi *et al.,* 2024). The probability of a suitable heterotic combination in F_1 hybrids is extremely low. Only a few number of favourable heterotic combinations remain after screening many F_1 crosses (Sharma and Shadakshari, 2021; Sharma *et al.,* 2022b and Gandhi *et al.,* 2025). Plant breeders frequently argue over the choice of parents for the high frequency of heterotic hybrids that are developed. One crucial factor that was and is utilised to select the parents in order to produce a higher frequency of heterotic hybrids is combining ability (CA). The ability to forecast hybrids performance is where CA is most useful when implemented (Griffing, 1956). Therefore present study was undertaken for identifiying parents which will produce heterotic hybrids for harvest index, protein content and seed yield in cowpea.

MATERIALS AND METHODS

Location and genotypes

In Summer 2023, at Pulses Research Station, Sardarkrushinagar Dantiwada Agricultural University, Sardarkrushinagar, twenty-one crosses were made utilising the line × tester mating design (Kempthorne, 1957) with seven lines and three testers. Therefore, 31 genotypes total-21 crosses, 10 parents including 7 lines and 3 testers were used as the experimental material. These genotypes were asse-ssed using a randomised block design with three replications during the *Kharif* season of 2023-24. Every entry was planted in a single, 4-meter-long row, with 0.45 metres separating rows and 0.10 metres separating plants within a row. Plant protection measures and agronomic practices were implemented in accordance with guidelines and recommendations.

Observations and statistical analysis

Observation were recorded on five random plants, at different growth stages of the crop and average values for the traits worked out for 21 F_1s and 10 parents and in each replication on 3 productivity *per* se traits *viz.*, seed yield per plant, harvest index and protein content. By using Near Infrared (NIR) mass spectroscopy, the protein content was calculated and reported as a percentage. Harvest index was calculated as ratio of seed yield by biological yield and expressed as a percentage. Statistics were applied to the replication-wise mean values of each genotype for a range of characteristics. Using the methods proposed by Panse and Sukhatme (1978), analysis of variance was used to examine the differences between the genotypes for all the features under consideration. The formula for heterobeltiosis was derived using Fonseca and Patterson's (1968) methodology. It was quantified as the percentage of the F_1 value's departure from the superior parent value. The variations resulting from the general combining ability (gca) impacts of three testers and seven lines, as well as the specific combining ability (sca) effects of twenty-one F_1 hybrids, were estimated (Kempthorne, 1957). As a result, overall status regarding the gca effects of the parents and overall status regarding the sca effects of the hybrids and BPH throughout three features were ascertained. Crosses were categorised as HH (both parents in a cross with high overall gca status), HL/LH (one parent with high and the other parent with low overall gca status) and LL (both parents with low overall gca status) based on the overall gca status of the parents.

RESULTS AND DISCUSSION

Analysis of variance for combining ability

Analysis of variance for combining ability presented in Table 1 revealed that, the mean square due to lines were significant for protein content; whereas, mean square due to testers was found non-significant for all traits. Significant mean sum of squares due to lines × testers for all the traits suggested that experimental material possessed considerable variability and there were possibilities to improvement of various traits under study through heterosis breeding. A perusal of variance ratio (σ^2 gca/σ^2 sca) less than unity suggested the preponderance of non-additive genetic variance for all the characters which further complicates the selection of parents but increases the probability of identifying transgressive segregants. Non-additive gene action for these traits were also obtained by Thangaraj (2018) and Debbarma *et al.* (2022).

Trait-wise parental gca effects and hybrid sca and heterosis

Variations in the frequency of genes that are passed down to the progeny with the additive effects account for the variations in gca effects between lines and testers. Line PL 4 and tester PL 7 were found to be good general combiner for all the characters having high gca effect. Tester PGCP 12 was good general combiner for harvest index. Harvest index is found to have direct and positive effect of on seed yield per plant. Therefore, these parents could be utilized in future breeding programmes to generate desirable segregates for seed yield and harvest index. Since gca effects are attributed to additive and additive × additive gene effects, the above mentioned parents for gca effects have good potential for respective characters and may be used in a multiple crossing programme to synthesize a dynamic population with most of the favourable genes accumulated.

As is true with respect to lines and testers for gca effects (Table 2), the hybrids differed significantly for their sca effects (Table 3). Hybrid GC 7 × GC 4 was desirable specific combiner for seed yield per plant and harvest index. Hybrids *viz.,* GC 3 × GC 4 and GC 7 × PGCP 12 were desirable specific combiner for harvest index and protein content. Hence, these crosses were identified as potential for getting good transgressive segregants for these traits. This result was in agreement with Debbarma *et al.* (2022) and Joshi *et al.* (2022).

Hybrid *viz.,* EC 724035 × PGCP 12 (Fig 1) and JLS 60 × PL 7 had high better parent heterosis for all the characters except protein content. Cross, GC 3 × GC 4 showed high better parent heterosis for protein content. Therefore, these crosses should be included for further evaluation in the generation advancement for getting good transgressive segregants. However, it was evidenct that characters like protein content may adversely contribute towards the heterosis for seed yield per plant. This may be due to the

Table 1: Analysis of variance for combining ability and estimates of component of variance for different characters in cowpea.

Source of variation	d.f.	Seed yield per plant	Harvest index	Protein content
Replications	2	14.95	0.61	0.30
Crosses	20	385.93**	105.29**	2.09**
Lines	6	447.57	112.35	3.97*
Testers	2	583.06	48.49	2.05
Lines × Testers	12	322.25**	111.24**	1.15*
Error	40	19.96	1.17	0.54
		Estimates of variance component		
σ^2 gca		1.65*	-0.15	0.02**
σ^2 sca		100.76**	36.68**	0.20*
σ^2 gca/ σ^2 sca		0.02	0.01	0.1

*, **, significant at 5% and 1% levels, respectively, d.f = Degree of freedom.

Table 2: Desirable general combiners for productivity *per se* traits in cowpea.

Traits	Parents (L/T)	gca effect
Seed yield per plant	JLS 60 (L)	7.04**
	PL 4 (L)	5.63**
	PL 7 (T)	4.03**
Harvest index	JLS 60 (L)	2.92**
	EC 72 3909 (L)	2.65**
	EC 724035 (L)	2.53**
	PL 4 (L)	1.63**
	PGCP 12 (T)	0.99**
	PL 7 (T)	0.76**
Protein content	GC 7 (L)	0.58*
	PGCP 6 (L)	0.91**

*, **, Significant at 5% and 1% level, respectively. L- Line; T- Tester.

Table 3: Desirable specific combinations based on sca effect and better parent heterosis (BPH) for productivity *per se* traits in cowpea.

Traits	Crosses	*sca* effect	Crosses	Estimates of BPH
Seed yield per plant	GC 7 × GC 4	19.32**	PL 4 × PL 7	35.13**
	JLS 60 × GC 4	6.76*	EC 724035 × PGCP 12	27.05**
	EC 724035 × PGCP 12	12.14**	JLS 60 × PL 7	24.76**
			PL 4 × PGCP 12	22.49**
Harvest index	GC 7 × GC 4	6.87**	EC 724035 × PGCP 12	21.10**
	JLS 60 × PL 7	1.85**	JLS 60 × PL 7	13.89*
Protein content	GC 3 × GC 4	0.89*	GC 3 × GC 4	8.21**
	GC 7 × PGCP 12	1.04*	GC 7 × PGCP 12	7.24**

*, **, Significant at 5% and 1% levels, respectively.

condition which favoured the development of one component could be adversely affected the other one. Therefore, to obtain good yield with high protein content, desired level of each component should be identified to make an effective selection programme. The results were confirming with those of Ratnakumari *et al.* (2023) in cowpea.

Parental overall gca status and hybrids overall sca and heterotic status

Three out of 7 lines *viz.,* JLS 60, EC 72 3909 and PL 4 and two of the three testers *viz.,* PGCP 12, PL 7 displayed high overall gca status and the remaining exhibited low overall gca status (Table 4). Similarly among hybrids, eleven (52.38%) and twelve (57.14%) crosses were classified as having high (H) overall sca and heterotic status, respectively and remaining were classified as having low (L) overall sca and heterotic status (Table 5 and 6). Similar results were found in the studies of Bandyopadhyay and Arunachalam (1980); Ramesh *et al.* (2000) and Keerthi *et al.* (2016).

Relationship of overall parental gca status with hybrids overall sca and heterotic status

Requirement of parents with contrasting gca effects to realise higher frequency of hybrids with high overall sca and heterotic hybrids have been observed in many previous studies (Ramesh *et al.,* 2000 and Keerthi *et al.,* 2016). Results obtained in the present study contrastingly indicated that in Cowpea for the concerned traits high

Table 4: Overall general combining ability status of parents across productivity *per se* traits in cowpea.

Parents	Total rank	Overall status
Lines		
GC 3	13	L
GC 7	16	L
PGCP 6	13	L
JLS 60	7	H
EC 72 3909	8	H
EC 724035	15	L
PL 4	12	H
Final norm	12.0	
Testers		
PGCP 12	6	H
PL 7	5	H
GC 4	7	L
Final norm	6.0	

H = High overall gca status; L = Low overall gca status.

Table 5: Overall sca status of crosses across productivity *per se* traits in cowpea.

Lines / Testers	PGCP 12 (H)		PL 7 (H)		GC 4 (L)	
	Total score	Status	Total score	Status	Total score	Status
GC 3 (L)	41	L	33	L	25	H
GC 7 (L)	30	H	23	H	16	H
PGCP 6 (L)	32	H	42	L	33	L
JLS 60 (H)	32	H	33	L	45	L
EC 723909 (H)	55	L	16	H	21	H
EC 724035 (L)	23	H	47	L	22	H
PL 4 (H)	43	L	19	H	54	L
Final norm	32.61					

H = High overall sca status; L = Low overall sca status.

(H) = High overall gca status of parents; (L) = Low overall gca status of parents.

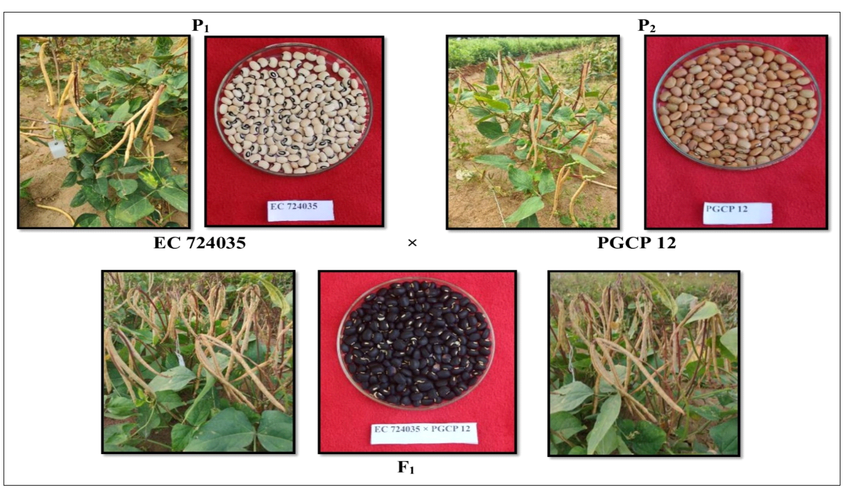

Fig 1: Parents and their heterotic hybrid.

Table 6: Overall heterotic status of crosses across productivity *per se* traits in cowpea.

Lines / Testers	PGCP 12 (H)		PL 7 (H)		GC 4 (L)	
	Total score	Status	Total score	Status	Total score	Status
GC 3 (L)	38	L	25	H	23	H
GC 7 (L)	26	H	37	L	23	H
PGCP 6 (L)	33	H	29	H	28	H
JLS 60 (H)	41	L	13	H	56	L
EC 723909 (H)	53	L	18	H	15	H
EC 724035 (L)	44	L	44	L	18	H
PL 4 (H)	42	L	29	H	48	L
Final norm	33					

H = High overall sca status; L = Low overall sca status.

(H) = High overall gca status of parents; (L) = Low overall gca status of parents.

Table 7: Distribution of crosses with high overall sca and heterotic status in relation to overall parental gca status across productivity *per se* traits in cowpea.

	Number of crosses		
Parental gca status of crosses	Under the category	With high (H) overall sca status	With high (H) heterotic status
H × H	6	3	5
H × L / L × H	11	5	5
L × L	4	3	2
Total		11 (52.38%)	12 (57.14%)

HH: Both parents are high in their overall general combining ability.

HL / LH: One parent is high and other one is low in their overall general combining ability.

LL: Both parents are low in their overall general combining ability.

magnitude of heterosis can be produced by either of three combination of parents *viz*., H × H or L × L or H × L / L × H (Table 7). Such results can be attributed to non-additivite gene action found for these traits in our study. So it may not always be possible to identify parents based on gca effects to realise hybrids with high heterosis and before doing so one must consider the gene action governing the trait of interest.

CONCLUSION

The ability to forecast parental gca impacts on hybrid heterosis, which would save a significant amount of time and resources, is essential to the effectiveness of any breeding strategy using hybridization. The line PL 4 and tester PL 7 registered as good general combiner for seed yield per plant and harvest index. The crosses GC 7 × GC 4 recorded high mean seed yield heterotic effect along with positive significant sca effect for seed yield per plant and harvest index. The perusal of per se performance as well as heterotic effect of crosses revealed that, the crosses GC 7 × PGCP 12 and GC 3 × GC 4 were proved to be best for protein content. Hence, these crosses were identified as potential for getting good transgressive segregants for these traits and suggested for further evaluation in advance generations. Further findings of this study contrast the requirement of parents with contrasting gca effects to realise higher frequency of heterotic hybrids as the present study showed that any combination of parents with regards to gca can lead to heterotic hybrids depending upon the type of gene action governing that trait.

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Conflict of interest

Authors declare there is no conflict of interest to disclose.

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