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Decoding the Gene Action for Pod Length, Seed Weight and Yield in Intra-Specific Crosses of Mungbean [*Vigna radiata* (L.) Wilczek]

Nivethitha T.[1*], Babu C.[2] and Jayamani P.[1]
1. Department of Genetics and Plant Breeding, Centre for Plant Breeding and Genetics, Tamil Nadu Agricultural University, Coimbatore, INDIA
2. Directorate of Research, Tamil Nadu Agricultural University, Coimbatore, INDIA
*nivethitha120897@gmail.com

Abstract
Comprehensive knowledge of gene action and interactions involved in the inheritance of pod length, seed weight and single plant yield is essential for formulating breeding strategies to develop a consumer-preferable, bold-seeded, high-yielding mungbean variety. Therefore, in the present study, a generation mean analysis (five-parameter model) was carried out in six different inter-varietal populations developed from contrasting parents to evaluate the gene action for the above traits. The experiment was conducted during the summer, 2023 at the Department of Pulses, Centre for Plant Breeding and Genetics, Tamil Nadu Agricultural University, Coimbatore. Significant variation in the mean performance of traits was observed across different generations of all crosses. The scaling test figured out the presence of inter-allelic interaction (epistasis) for pod length, seed weight and single plant yield in the populations evaluated.

Collectively, the results revealed that pod length, seed weight and single plant yield were under the influence of fixable gene effects viz. additive and additive × additive interaction effects. However, the magnitude of additive × additive gene effect was relatively higher, necessitating the selection at later generations. The selected early-maturing segregants, characterized by high-yield, bold seeds and resistance to MYMV from the populations of COGG 13-39 x VGG 16-058 and COGG 13-39 x VGG 18-002 can be integrated into the breeding program to develop a commercially valuable mungbean variety.

Keywords: Epistasis, Gene action, Generation mean analysis, Mungbean, Scaling test.

Introduction

Mungbean [*Vigna radiata* (L.) Wilczek] often referred to as ‘poor man’s meat’, has gained significance in the aftermath of the COVID outbreak, owing to its nutritional value, affordability, sustainability and role in ensuring nutritional security. The nutritional value is exceptionally high and sprouts from bold seeds are preferred for their size and increased nutrition[17]. The nutritional value of sprouts is equivalent to asparagus and mushrooms. During sprouting, there is an increase in thiamine, niacin and ascorbic acid contents[13]. Bold seeds also signify higher germination rates and vigour. Overall, it emphasizes the need of high yielding varieties with bold seeds to meet consumer demands.

Earlier findings in mungbean pointed out a positive correlation between seed weight and pod length. Moreover, seed weight and pod length also had a direct positive effect on yield[14]. The seed weight, pod length and yield are crucial factors in determining productivity and quality. Therefore, identifying the genetic factors responsible for traits such as bold seed (test weight >5 g), pod length and yield are also important to improve the trait value. These traits are governed by complex gene actions *viz.* intra-allelic and inter-allelic interactions (epistasis) and are strongly influenced by the environment.

However, there is limited research focusing exclusively on genetic studies related to seed weight and pod length. Hence, the genetic information on the inheritance of genes associated with yield and its closely related components *viz.* hundred seed weight and pod length, is essential for devising an efficient breeding program.

A simple additive or additive-dominance model, wherein epistasis is rarely considered, might lead to misconstrued results. Alternatively, models based on generation mean analysis can accurately reflect the nature of the gene effect and epistasis involved[16]. In this context, it is crucial to emphasize that the scale and nature of epistasis can significantly affect both the accuracy of predictions and the formulation of breeding strategies. Therefore, the present investigation aims to estimate the additive, dominance and epistatic effects of the pod length, seed weight and single plant yield in selected segregating populations derived from parents with contrasting characteristics under study.

Material and Methods

Plant genetic material: Seven mungbean genotypes *viz.* IPM 409-4, ADT 3, COGG 13-39, VGG 16-058, GAM 5, VGG 18-002 and WGG 42 were used to generate six segregating populations *viz.* IPM 409-4 × VGG 18-002, IPM 409-4 × WGG 42, ADT 3 × VGG 16-058, ADT 3 × GAM 5, COGG 13-39 x VGG 16-058 and COGG 13-39 x VGG 18-002. Details of the parental source and characteristics are presented in table 1. Among the six crosses, based on the contrasting nature of the parents, crosses *viz.*, IPM 409-4 × VGG 18-002 and IPM 409-4 × WGG 42 were selected to explore gene action for pod length ADT 3 × VGG 16-058 and ADT 3 × GAM 5 for seed weight and COGG 13-39 x

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VGG 16-058 and COGG 13-39 x VGG 18-002 for single plant yield. The experiment was carried out at the field facility of the Department of Pulses, Centre for Plant Breeding and Genetics, Tamil Nadu Agricultural University, Coimbatore (11°N and 77°E, 411.98 m elevation).

Population development: The crossing program involving emasculation and pollination was carried out during summer, 2022. During *kharif,* 2022, F_1s resulting from six inter-varietal crosses were raised and the true hybrids were morphologically confirmed based on contrasting traits between parents. Each plant identified as a true hybrid was harvested individually and the F_2 populations were raised during *rabi,* 2022 to procure the seeds for F_3 generation.

Evaluation of genetic materials: The five generations that included P_1, P_2, F_1, F_2 and F_3 of the six populations were raised during the summer, 2023 in a row length of 4 m with 30 x 10 cm spacing. Standard cultivation practices were precisely followed to maintain a healthy crop. The population size of all the generations of six crosses is presented in table 2. Observations were recorded for pod length (cm) in IPM 409-4 × VGG 18-002 and IPM 409-4 × WGG 42; 100-seed weight (g) in ADT 3 × VGG 16-058 and ADT 3 × GAM 5 and seed yield per plant (g) in COGG 13-39 x VGG 16-058 and COGG 13-39 x VGG 18-002.

Statistical analysis: The scaling test as suggested by Mather[9] was used to test the adequacy of a simple additive-dominance model. The significance of scales, C and D was tested using a t-test. The model was considered inadequate when either of the scales *viz.* C or D was significant. Considering the presence of epistasis, generation mean analysis with a five-parameter model as described by Hayman[2] was performed in the present study to estimate additive (d), dominance (h) and non-allelic interaction components i.e. additive x additive (i) and dominance x dominance (l) for pod length, seed weight and single plant yield. The five-parameter model does not offer insights into the additive × dominance (j) type of interaction. The data analysis was carried out using the TNAUSTAT statistical package[8].

Results and Discussion

Prediction accuracy and formulation of breeding strategies are mainly influenced by gene action governing the trait expression.

Table 1
Parent materials involved in the study with their characteristic features

S.N.	Genotypes	Source	Characteristics (special features)
Female parents			
1	IPM 409-4	ICAR-Indian Institute of Pulses Research, Kanpur	Variety: Shiny seeds, short pod (pod length < 8 cm)
2	ADT 3	Tamil Nadu Rice Research Institute, Aduthurai	Variety: Dull seeds, small seed size (test weight, 2.0 -2.5 g/100 seeds)
3	COGG 13- 39	Department of Pulses, Tamil Nadu Agricultural University, Coimbatore	Breeding line: High yielding
Male parents			
4	VGG 16-058	National Pulses Research Centre, Vamban	Breeding line: Shiny, bold seeds (test weight > 5g/100 seeds)
5	VGG 18-002		Breeding line: Shiny, bold seeds (test weight > 5g/100 seeds), long pod (pod length > 10 cm)
6	WGG 42	Professor Jayashankar Telangana State Agricultural University, Hyderabad	Variety: Shiny seeds, long pod (pod length > 10 cm)
7	GAM 5	Anand Agricultural University, Gujarat	Variety: Shiny, bold seeds (test weight > 5g/100 seeds)

Table 2
Details of six inter-varietal crosses and population size of five generations (P_1, P_2, F_1, F_2 and F_3) of mungbean

Trait	Crosses	Population size				
		P_1	P_2	F_1	F_2	F_3
Pod length	IPM 409-4 × VGG 18-002	10	10	10	48	89
	IPM 409-4 × WGG 42	10	10	10	99	253
Seed weight	ADT 3 × VGG 16-058	10	10	10	133	199
	ADT 3 × GAM 5	10	10	10	193	122
Single plant yield	COGG 13- 39 × VGG 16-058	10	10	10	84	210
	COGG 13- 39 × VGG 18-002	10	10	10	152	179

Therefore, a profound knowledge of the gene action that regulates trait expression facilitates selecting the most effective breeding and selection procedure. To assess the gene action of a trait, contrasting parents must be selected for unbiased estimation of additive-dominance variation with equal precision[4]. Thereby, in the present study, different populations developed from contrasting parents were used to evaluate the gene action of seed weight, pod length and single plant yield.

The mean values along with their respective standard errors, for the analyzed traits in various populations across five generations (P_1, P_2, F_1, F_2, F_3) are presented in table 3. In all the generations (F_1, F_2, F_3), the observed traits exhibited intermittent values within the range of parental values. On dissection of the generation mean into five different genetic components, the effect of the mean was highly significant indicating variation across the generations in all crosses. Among the two crosses evaluated for each trait, the significance and relative strength of additive, dominance and epistatic components were different (Table 3). However, mungbean, being a highly self-pollinated crop, only the fixable gene effects *viz.* additive (main effect) and additive × additive (interaction effect) could be exploited for the trait improvement.

Scaling test: In all six inter-varietal crosses evaluated for three different traits *viz.* pod length, seed weight and single plant yield, at least one of the two scales (C and D) consistently exhibited statistical significance (Table 3). The significant deviation of scales from zero indicated the presence of epistasis (non-allelic interaction). Consequently, the additive-dominance model was not adequate for studying pod length, seed weight and single plant yield in the evaluated populations. In instances where epistasis is overlooked, there is a likelihood of increasing dominance variance and decreasing additive variance, thereby ultimately reducing the efficiency of the breeding program. The presence of epistatic interaction for pod length, seed weight and single plant yield was in accordance with inter-varietal crosses of mungbean[5, 7].

Gene action

Pod length: Two crosses, *viz.*, IPM 409-4 × VGG 18-002 and IPM 409-4 × WGG 42 were evaluated for pod length. The genotype IPM 409-4 exhibited a shorter pod length (<8 cm) in comparison to VGG 18-002 and WGG 42, which had longer pods (>10 cm). A notable contrast in pod length was observed between female and male parents.

Table 3
Mean ± standard error, direct and interaction gene effects for pod length, seed weight and yield in six different crosses in various generations

Trait	Crosses	Mean ± Standard error					Scales		Parameters					
		P_1	P_2	F_1	F_2	F_3	C	D	m	d	h	i	l
Pod length (cm)	IPM 409-4 × VGG 18-002	7.05 ± 0.11	10.15 ± 0.13	7.88 ± 0.11	7.65 ± 0.12	7.95 ± 0.08	-2.348**	-0.698	7.653**	-1.552**	-0.645	-3.030**	2.200*
	IPM 409-4 × WGG 42	7.05 ± 0.11	9.56 ± 0.14	7.18 ± 0.16	7.75 ± 0.07	6.8 ± 0.03	0.020	-4.885**	7.747**	-1.258**	2.133**	0.744**	-6.540**
Seed weight (g)	ADT 3 × VGG 16-058	2.26 ± 0.1	5.03 ± 0.03	2.9 ± 0.14	3.49 ± 0.04	3.7 ± 0.04	0.862*	0.526*	3.486**	-1.384*	-0.958*	-2.975*	-0.449
	ADT 3 × GAM 5	2.26 ± 0.1	5.06 ± 0.13	3.15 ± 0.05	3.23 ± 0.04	3.19 ± 0.04	-0.39	-0.705**	3.228**	-1.398**	0.05	-2.234**	-0.42
Single plant yield (g)	COGG 13- 39 × VGG 16-058	23.09 ± 0.9	14.24 ± 0.35	15.47 ± 1.22	15.79 ± 0.38	14.32 ± 0.28	-5.122	-11.602**	15.786**	4.426**	3.691**	15.732**	-8.640
	COGG 13- 39 × VGG 18-002	23.09 ± 0.9	15.97 ± 0.48	20.97 ± 0.77	16.35 ± 0.53	16.5 ± 0.39	-15.593**	-5.764**	16.351**	3.56**	2.688	8.364**	13.105*

* and **, significant gene effect at 0.05 and 0.01 probability levels.
m, mean; d, additive; h, dominance; i, additive × additive; l, dominance × dominance

In the cross, IPM 409-4 × VGG 18-002, the mean pod length observed was in ascending order of $P_1 < F_2 < F_1 < F_3 < P_2$ (Fig.1). Significant additive (d), additive × additive (i) and dominance × dominance (l) components were recorded. However, a high magnitude of additive × additive (i) component was observed. (Table 3). Conversely, in the cross, IPM 409-4 × WGG 42, the mean pod length was in ascending order of $P_1 < F_3 < F_1 < F_2 < P_2$ and four genetic parameters *viz.* d (additive), h (dominance), i(additive × additive) and l (dominance × dominance) were found to be significant.

Similarly, epistatic studies in four inter-varietal populations of mungbean also revealed the involvement of dominance (h) and dominance × dominance (l) in governing pod length[6]. The mean pod length of different generations recorded intermittent values within the parental range in both crosses. The significance and magnitude of genetic components varied between the two crosses. It is possibly attributed to differences in gene frequencies and the opposing or synergistic effects of the pollen parent. However, in mungbean, the dominance type of gene action cannot be exploited. Significant additive and additive × additive gene actions were recorded for both crosses.

Since, the scale of the interaction component, additive × additive was higher, the selection of the trait should be postponed to later generations. In accordance, researchers[7] also evaluated six intraspecific populations of mungbean and highlighted the significance of additive × additive gene actions for pod length in two of the populations studied.

Seed weight: The two crosses *viz.* ADT 3 × VGG 16-058 and ADT 3 × GAM 5 were evaluated for seed weight. The genotype ADT 3 was small-seeded (2.26 g/100 seeds) when compared to VGG 16-058 (5.03 g/100 seeds) and GAM 5 (5.06 g/100 seeds). In ADT 3 × VGG 16-058, the mean value of seed weight was in the ascending order of $P_1 < F_1 < F_2 < F_3 < P_2$. In ADT 3 x GAM 5, the mean value was also in the ascending order of $P_1 < F_1 < F_3 < F_2 < P_2$ (Fig. 2). On comparing both crosses, F_1 means were within the range of corresponding parental values. The additive (d) and additive × additive (i) types of gene action were found to be highly significant in both the crosses.

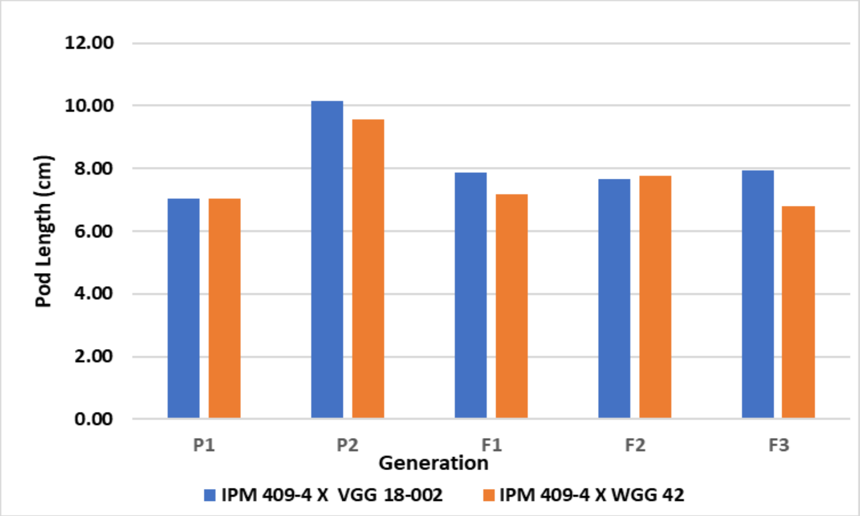

Figure 1: Mean pod length among various generations in IPM 409-4 × VGG 18-002 and IPM 409-4 × WGG 42

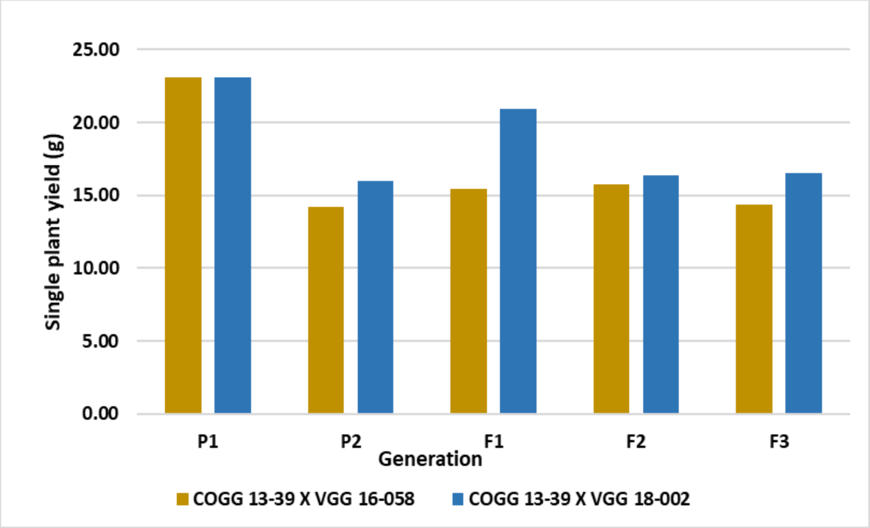

Figure 2: Mean seed weight among various generations in ADT 3 × VGG 16-058 and ADT 3 × GAM 5

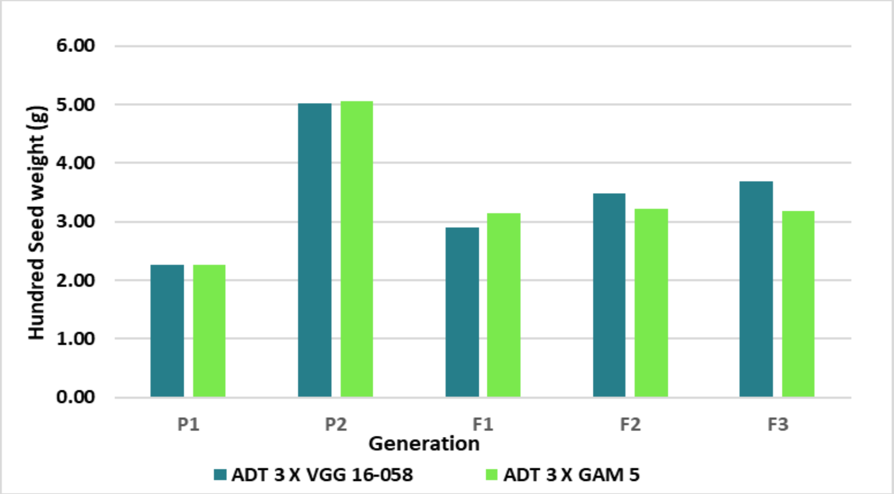

Figure 3: Mean single plant yield among various generations in COGG 13- 39 × VGG 16-058 and COGG 13- 39 × VGG 18-002

Though the dominance(h) was significant in one of the crosses, ADT 3 × VGG 16-058, the magnitude was comparatively very low. In addition, the relative strength of additive × additive (i) gene action was higher compared with other genetic parameters in both the crosses (Table 3). Therefore, selection for seed weight must be deferred to later generations for effective improvement. Similarly, the impact of additive and additive × additive gene action on seed weight in inter-varietal crosses of mungbean was documented earlier[1,7,15].

Single plant yield: The two crosses *viz.* COGG 13-39 x VGG 16-058 and COGG 13-39 x VGG 18-002 were evaluated for single plant yield. The genotype, COGG 13-39 was a comparatively high yielding breeding line. The male parents (VGG 16-058 and VGG 18-002) were early maturing with bold seeds compared to the female parent. The genotype, VGG 16-058 was Mungbean Yellow mosaic virus (MYMV) disease resistant. In the cross, COGG 13-39 x VGG 16-058, the mean seed yield observed was in ascending order of $P_2 < F_1 < F_3 < F_2 < P_1$. Significant additive (d), dominance (h) and additive × additive (i) components were recorded. The relative strength of the additive × additive (i) component was higher (Table 3). Conversely, in cross COGG 13-39 x VGG 18-002, the mean yield was in ascending order of $P_2 < F_2 < F_3 < F_1 < P_1$ (Fig. 3).

The genetic parameters *viz.* additive (d), additive × additive (i) and dominance × dominance (l) components were significant. In the present study, the contrary results obtained for these two populations might be due to the differences in the single plant yield and gene frequencies of the male parents involved. However, it is not feasible to harness dominance × dominance (l) in mungbean. The predominance of dominance × dominance (l) component for single plant yield in two inter-varietal populations of mungbean was also observed in early studies[3]. In the presence of additive × additive gene action in both crosses, selection for single plant yield at early generation is not likely to be rewarding. The findings of previous researchers similarly suggested adopting a selection procedure i.e. delayed selection, that fixes the additive × additive (i) genetic effect in later generations[3,10-12]. The two populations developed by utilizing early-maturing and bold seeded pollen parent enabling the selection of high yielding seggregants coupled with early maturity and bold seeds in later generations. Furthermore, the MYMV screening accompanied by selection in the population of COGG 13-39 x VGG 16-058 will be helpful in the development of MYMV resistant high-yielding mungbean variety.

Collectively, it is evident that additive component and epistatic interactions played a crucial role in the inheritance of pod length, seed weight and single plant yield. As these traits were under the influence of additive and additive × additive (fixable) gene effects, improvement through selection in later generations is feasible.

Conclusion

Additive gene action and epistatic interactions were prevalent for the traits *viz.* pod length, seed weight and single plant yield in the present study. These traits collectively exhibited fixable gene effects *viz.* additive and additive × additive with the predominance of additive × additive gene action. Therefore, selection should be carried out in later generations, specifically through pedigree or modified pedigree methods. Moreover, within the population evaluated for single plant yield, COGG 13-39 x VGG 16-058 and COGG 13-39 x VGG 18-002, early-maturing, high-yielding segregants with bold seeds can be selected for subsequent incorporation into breeding programs.

In addition, due to the involvement of MYMV resistant male parent (VGG 16-058), the selection strategy coupled with MYMV screening will result in the development of early-maturing, high yielding MYMV resistant varieties with bold seeds. The development of such consumer preferrable bold seeded varieties boosts the farmers' income in terms of higher marketability and also ensures nutritional security.

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