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RESEARCH ARTICLE

Unveiling a novel gene inheritance model for peduncle length in moth bean (*Vigna aconitifolia* L.)

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Abstract

Understanding the inheritance patterns of specific traits related to plant architecture is crucial for selecting appropriate breeding methods. Crosses were conducted between RMO-257 (short peduncle, female parent) and CZMO-18-4 (long peduncle, male parent), as well as between CZM-45 (short peduncle, female parent) and CZMO-20-2-2 (long peduncle, male parent) for peduncle length, an important trait for plant architecture. The results revealed that peduncle length follows a ratio of 2.82:1 and 3.28:1 in the respective crosses, indicating control by a major gene, with minor genes or environmental factors contributing to its continuous variation. Although peduncles are categorized as either long or short, the long peduncle phenotype ranges from 5 cm to 15 cm, suggesting the involvement of multiple factors influencing the dominant gene. The short peduncle trait was found to be recessive. Non-significant $\chi 2$ values further confirmed that the observed results adhered to Mendelian inheritance patterns. This pioneering study on moth bean provides novel insights into the genetic control of peduncle length, offering a foundation for the development of improved breeding approaches in this underutilized crop. The above elucidation is a first report on the genetic inheritance of peduncle length or any trait in the orphan legume moth bean.

Keywords: Moth bean, Inheritance, Peduncle, Genetic control, Plant architecture.

Introduction

Moth bean [*Vigna aconitifolia* (Jacq.) Maréchal; 2n = 22], a member of the Fabaceae family, is uniquely adapted to tropical arid climates, exhibiting remarkable tolerance to drought and heat stress compared to other species in the subgenus Ceratotropis (Somta et al. 2018; Choudhary et al. 2024). Its agronomic resilience, characterized by a deep root system, short life cycle, broad canopy, and trailing growth habit, makes it a critical crop for low-input agricultural systems in the arid regions of western India, particularly on sand dunes, degraded soils, and marginal lands. Despite its potential, the area under moth bean cultivation has been decreasing in recent years (Choudhary 2022). This trend is compounded by limited breeding efforts and the crop's sensitive floral morphology, which complicates artificial hybridization (Sharma et al. 2020).

Although previous improvement programs have focused primarily on mutation breeding (Choudhary et al. 2022), these have often narrowed the genetic base and constrained variability in desirable traits. The small, fragile flowers of moth bean have posed practical barriers to recombination breeding, hindering the development of hybrids. Nevertheless, recent progress at ICAR-Central Arid Zone Research Institute (CAZRI) has resulted in stable genotypes with contrasting phenotypes, particularly in peduncle length and plant stature, traits that can be exploited for effective hybrid identification and trait introgression (Mahla and Sharma 2022).

A comprehensive understanding of the inheritance of such morphological traits is imperative for informed selection and the formulation of effective breeding strategies. Contrasting traits not only facilitate phenotypic selection but also enable the precise mapping of genes and the identification of associated biochemical pathways. Segregating populations plays a crucial role in advancing both classical and molecular breeding programs. The

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present study was therefore undertaken to find out the genetic control of the peduncle trait in moth bean.

Materials and methods

The experiment was conducted at the ICAR-Central Arid Zone Research Institute (CAZRI), Jodhpur, Rajasthan, during the *kharif* seasons of 2021 and 2022. The site is located in the hot arid zone, characterized by extreme temperatures and erratic rainfall patterns.

Development of crosses

During *kharif* 2021, four stable genotypes chosen based on the contrasting traits for peduncle length were grown in crossing blocks. The material consists of two short peduncle genotypes, RMO-257 and CZM-45, two long peduncle genotypes, CZMO-18-4 and CZMO-20-2-2, for synthesizing F_1 hybrids. Crossing was done by following the method reported by Mahla and Sharma (2022). Two F_1 crosses were synthesized, and the crossed seeds were collected combination-wise to obtain the F_1 hybrid seeds. Experimental materials with details of the parental lines, their pedigree, important traits and special characteristics used for genetic inheritance studies of two different crosses were described in Table 1

Scoring for segregation of peduncle length

During *kharif* 2022, the F_1 seeds of crosses were grown along with their parents. The seeds born on F_1 plants were considered as F_2 seeds. The F_2 seeds from all the F_1 plants were maintained separately, and then they were grouped into two separate groups: long peduncle *vs* short peduncle based on the visual field observation of peduncle length. Moreover, observations were also taken for each plant of F_2population in both crosses.

Statistical analysis

Observations on parents and F_1 as well as F_2 plants were subjected to data analysis. F_2 plants of these cross combinations were used to study the mode of inheritance. The large number of F_2plants was grouped into two separate group reference to the presence of long peduncle *vs* short peduncles. The segregation patterns were fitted to standard Mendelian ratios and the goodness of fit was tested by applying the chi-square (χ2) test.

The chi-square values were computed using the following formula:

$$\text{Chi-square value} = \Sigma \frac{(O-E)^2}{E}$$

Where O = observed number of seeds and E = expected number of seeds.

The significance of chi-square values was tested against the table of χ2 values with n-1 degrees of freedom, where n is the total number of segregating classes (McHugh, 2013).

Density distribution analyses were done in the extension XLSTAT 2019 from Microsoft Excel (Addinsoft 2019).

Results

Difference in peduncle length of various generations

Analysis of variance revealed significant differences in peduncle length across parental, F_1, and F_2 generations in both crosses. F_1 plants exhibited enhanced peduncle length relative to both parents, suggesting the presence of hybrid vigor. Boxplots (Fig. 1) visually confirmed these generational differences. The mean peduncle lengths and standard errors (Table 2) highlighted significant variation. LSD analysis showed significant differences between P_1 and P_2, as well as between parents and F_1 plants in both crosses. However, F_2 populations exhibited variable and often overlapping means, indicating continuous variation. Egbadzor et al. (2013) also found similar kinds of results in the genetic studies for seed size in cowpea.

Genetic control of peduncle length

Peduncle length was found to be a visually and metrically distinguishable trait in field conditions. F_2 populations from both crosses (n = 222 in RMO-257 × CZMO-18-4; n = 137 in CZM-45 × CZMO-20-2-2) showed clear segregation into short and long peduncle classes. Chi-square analyses revealed non-significant deviations from expected Mendelian ratios (Table 4), supporting the hypothesis of single major gene control.

Kernel density estimation of peduncle length in both F_2 populations revealed a bimodal distribution with modes near ~1–3 cm (short) and ~5–7 cm (long) and a local minimum within ~3–5 cm (Fig. 4). Using thresholds defined at this trough (<3.0 cm = short; 3.0–5.0 cm =

Table 1. Details of experimental materials used for genetic inheritance studies

S. No.	Parental Line	Pedigree	Trait details	Contrasting trait	Crosses
1	RMO-257 (P1)	Mutant from the Jadia moth	Semi-erect growth, Broad leaves and shallow lobed with 2–3 pod clusters/leaf axil, bears 3-6 branches/plant	Short Peduncle	RMO-257 x CZMO-18-4
2	CZMO-18-4 (P2)	Stable mutant of GMO-2	Upright with longer peduncles	Long Peduncle	
3	CZM-45 (P1)	Jadia x RMO-40	Semi-erect growth habit	Short Peduncle	CZM-45 x CZMO-20-2-2
4	CZMO-20-2-2 (P2)	Mutant of CZM-45	Semi Spreading	Long Peduncle	

Table 2. Average of peduncle length (cm) and standard errors for various moth bean generations with a matrix showing LSDs (5%) with significant pairs indicated with asterisks(*)

Cross	Generation	Mean ± se (cm)	Lsd			
Rmo-257 x czmo-18-4	P1 (rmo-257)	$1.38^a \pm 0.15$	-			
	P2 (czmo-18-4)	$9.32^a \pm 0.32$	7.94*	-		
	F_1	$11.03^b \pm 0.32$	9.65*	1.71	-	
	F_2	$6.18^c \pm 0.22$	4.80	3.14	4.85	-
Czm-45 x czmo-20-2-2	P1 (czm-45)	$1.10^c \pm 0.96$	-	-		
	P2 (czmo-20-2-2)	$9.15^a \pm 0.96$	8.05*	-		
	F_1	$11.15^a \pm 0.68$	10.05*	1.2	-	
	F_2	$6.29^b \pm 0.26$	5.19	2.86	4.86	-

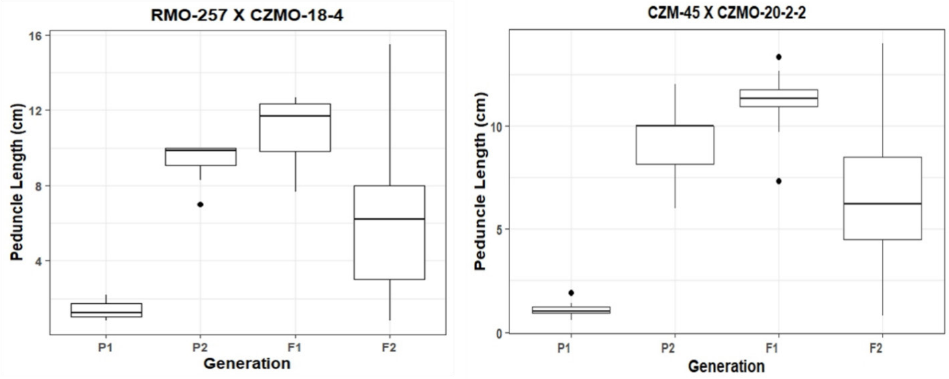
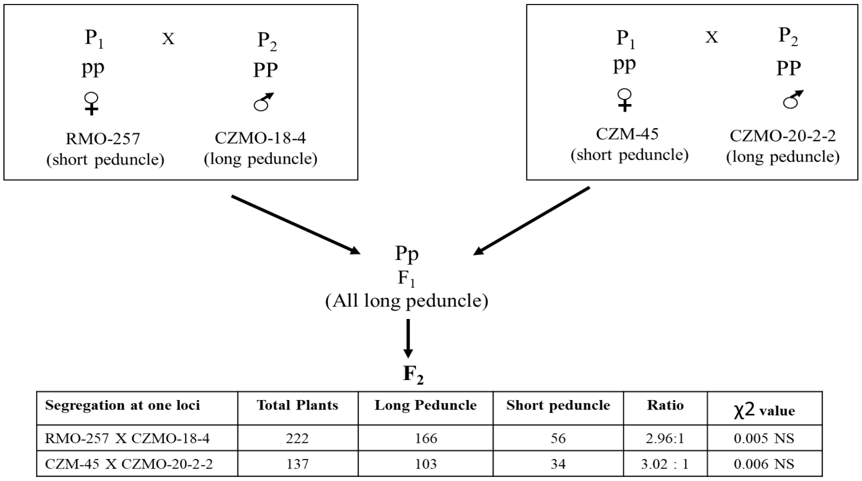


Fig. 1. Boxplot of peduncle length (cm) of different generations of moth bean in both the crosses



Segregation at one loci	Total Plants	Long Peduncle	Short peduncle	Ratio	χ2 value
RMO-257 X CZMO-18-4	222	166	56	2.96:1	0.005 NS
CZM-45 X CZMO-20-2-2	137	103	34	3.02 : 1	0.006 NS

Fig. 2. Chi-square values for peduncle length in both the crosses

intermediate; >5.0 cm = long), 72% of plants (RMO-257 × CZMO-18-4) and 67% (CZM-45 × CZMO-20-2-2) were classified as long (>5.0 cm) (Table 3). These results show predominance of the long-peduncle phenotype in the F_2 populations, while the continuous variation inside the long and intermediate classes suggests polygenic modification and/or environmental effects. Though the clear distribution among long peduncles was not visible, no prejudiced ratio was observed in both crosses, clearly depicting that this trait is governed by major genes, but these genes are also affected by several influencers or minor genes, which may contribute to producing different long-sized peduncles or a normally distributed long-sized peduncle, or a normally distributed short-sized peduncle.

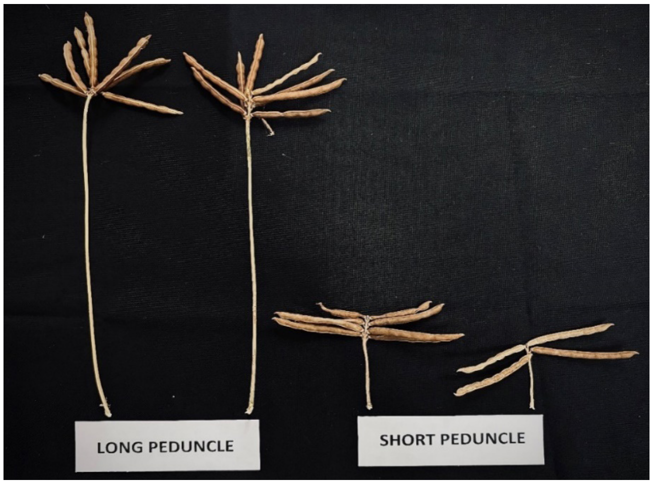


Fig. 3. Long Peduncle and Short Peduncle observed in F_2 population of crosses

Kernel density plots (Fig. 4) revealed bimodal distributions in both F_2 populations, corresponding to short and long peduncle classes, with normally distributed peaks. This pattern supports a model where a major gene controls peduncle length, but minor genes or polygenic factors modulate the trait's expression.

CZM-45 X CZMO-20-2-2 w

The analysis of peduncle length data from the F_2 population of the cross between CZM-45 (short peduncle, female parent) and CZMO-20-2-2 (long peduncle, male parent) reveals insightful patterns regarding the inheritance of this trait. The data, characterized by a mean of 6.29 and a standard deviation of 3.35, exhibit a wide range of values from 0.80 to 14.00, indicating considerable variability in peduncle lengths among the offspring. Statistical tests suggest that while the data approximate a normal distribution, there is slight skewness (0.077) and platykurtosis (-0.508), implying

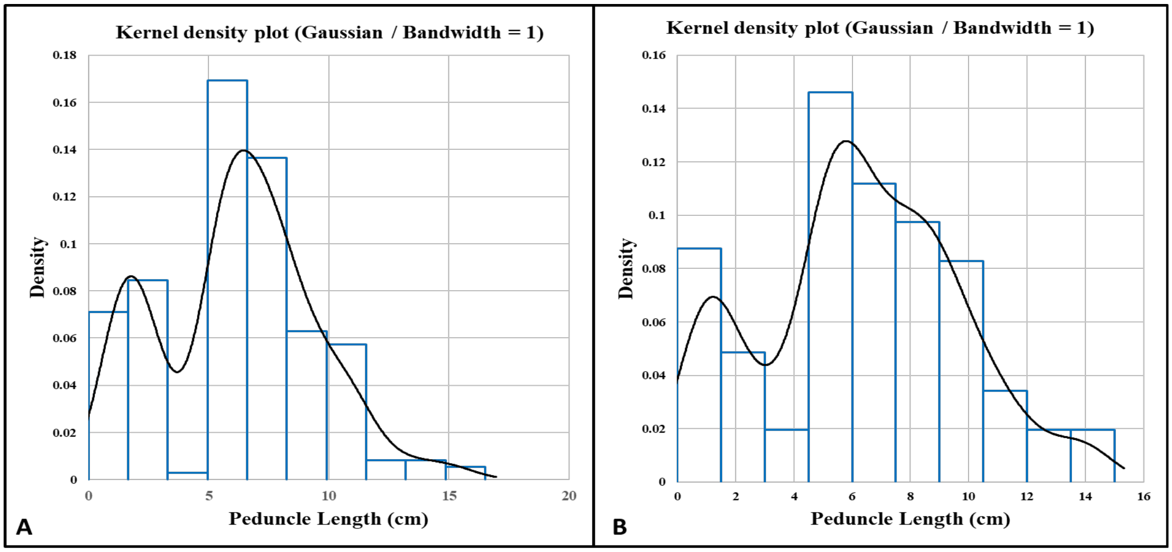


Fig. 4. Kernel density distribution of peduncle length (cm) in F_2 population of **A:** RMO-257 X CZMO-18-4 and B: CZM-45 X CZMO-20-2-2

Table 3. Range, frequency and density for peduncle length (cm) in F_2 generation

RMO-257 X CZMO-18-4			CZM-45 X CZMO-20-2-2		
Peduncle length Range (cm)	Frequency	Density	Peduncle length Range (cm)	Frequency	Density
0.00–1.65	26	0.071	0.00–1.5	18	0.088
1.65–3.30	31	0.085	1.5–3.0	10	0.049
3.30–4.95	1	0.003	3.0–4.5	4	0.019
4.95–6.60	62	0.169	4.5–6.0	30	0.146
6.60–8.25	50	0.137	6.0–7.5	23	0.112
8.25–9.90	23	0.063	7.5–9.0	20	0.097
9.90–11.55	21	0.057	9.0–10.5	17	0.083
11.55–13.20	3	0.008	10.5–12.0	7	0.034
13.20–14.85	3	0.008	12.0–13.5	4	0.019
14.85–16.50	2	0.005	13.5–15.0	4	0.019

Table 4. Descriptive statistics for peduncle length in both the crosses

Variable	Cross	No. of F_2 Plants	Mean (cm)	STD (cm)	Shapiro-Wilk test		
					W	*p-value* (Two-tailed)	alpha
Peduncle Length	RMO-257 X CZMO-18-4	222	6.19	3.23	0.961	<0.0001	0.05
	CZM-45 X CZMO-20-2-2	137	6.29	3.35	0.964	0.001	0.05

a trait influenced by multiple genetic factors rather than a simple Mendelian pattern. The higher frequencies observed in intermediate peduncle length intervals (4.50 to 7.50) suggest polygenic inheritance, where multiple genes with additive effects contribute to the observed phenotypic variation. For checking the normality of the peduncle length data, the Shapiro-Wilk Test was analyzed. It suggested that the *p-value* (0.001) is less than the significance level (0.05), indicating that the peduncle length distribution in the F_2 generation does not follow a perfectly normal distribution. These findings underscore the complex genetic architecture underlying peduncle length in this cross, warranting further investigation through genetic mapping to elucidate the specific loci and pathways involved in controlling this trait.

RMO-257 X CZMO-18-4

The mean peduncle length (6.187) falls within the range observed in the F_2population. The distribution shows a skewness close to zero (0.168), indicating a near-normal distribution, though the kurtosis is slightly negative (-0.351), suggesting a platykurtic distribution (less peaked than a normal distribution). The skewness and kurtosis values indicate that while the trait may not strictly follow a normal distribution, it shows characteristics of quantitative trait variation, typical of polygenic inheritance. To check the normality of the peduncle length data we performed the Shapiro-Wilk Test. It suggested that the *p-value* (<0.0001) is less than the significance level (0.05), indicating that the peduncle length distribution in the F_2 generation does not follow a perfectly normal distribution. Moreover, Interval Analysis indicates that, peduncle length frequencies across intervals show variability, with higher frequencies in the middle ranges (4.950 to 8.250), suggesting possible polygenic inheritance or influence of multiple genes on this trait. The continuous distribution and the observed frequencies across intervals suggest that peduncle length in the F_2population is likely influenced by multiple genetic factors rather than a simple Mendelian inheritance pattern. The consistent findings across the Shapiro-Wilk test in both crosses strongly indicate that the distribution of peduncle lengths in the F_2 generation does not conform to a normal distribution. This departure from normality suggests that peduncle length in this cross is likely influenced by non-Mendelian genetic factors or environmental influences that create a skewed or non-symmetrical distribution of phenotypic outcomes. Such patterns are common in traits governed by multiple genes with varying degrees of dominance, epistasis, or environmental interactions. Further genetic analysis, possibly using quantitative trait locus (QTL) mapping or other genetic mapping techniques, could help identify specific genes or genomic regions responsible for the variation in peduncle length observed in the F_2 population. In conclusion, these results highlight the complex genetic architecture underlying peduncle length in the RMO-257 X CZMO-18-4, emphasizing the need for further genetic analysis to uncover the specific genetic loci and mechanisms responsible for the observed phenotypic variation.

Discussion

The complexity of conducting hybridization in minor legumes such as moth bean, owing to its small, delicate flowers prone to shedding, has long hindered genetic studies (Kanishka et al., 2023). Consequently, no prior inheritance studies have been reported for morphological traits in moth bean. This study represents the first report on the genetic control of peduncle length in this crop, an important trait influencing plant architecture and agronomic adaptability.

Peduncle length is a key morphological trait in moth bean, typically short in most released cultivars. Long peduncles have traditionally been associated with primitive, spreading plant types. However, recent breeding efforts have led to the stabilization of long peduncle traits in upright and high-yielding genotypes. The present investigation suggests that peduncle length is primarily governed by a major gene, as evidenced by clear phenotypic segregation in the F_2 population. Nonetheless, continuous variation within each phenotypic class, especially among plants with long pedunclesindicates the influence of minor genes or environmental factors.

Visual phenotyping effectively distinguished long and short peduncle types, with density distribution analyses further supporting the presence of both major and polygenic control. Kernel density plots revealed bimodal distributions corresponding to short and long phenotypes with normal-like variation within each class. This suggests that although a major gene governs peduncle length, its expression is modified by multiple minor genes, possibly operating in an additive or epistatic manner.

In both crosses, the F2 populations exhibited a wide phenotypic spectrum of peduncle lengths, extending beyond the range of the parental genotypes. In the RMO-257 × CZMO-18-4 cross, although the F_2 mean peduncle length (6.18 cm) was intermediate, individual plants displayed transgressive phenotypes, with lengths shorter than the short parent and longer than the long parent. A similar pattern was observed in the CZM-45 × CZMO-20-2-2 cross, where F2 individuals exceeded both parental extremes. This transgressive segregation suggests that, in addition to the major gene governing peduncle length, multiple minor genes with additive effects contribute to the continuous variation observed in the F_2 generation.

Chi-square (χ^2) analysis of the segregating classes for short and long peduncle phenotypes in F2 populations was non-significant, confirming the inheritance of a major gene with a dominant-recessive relationship. The observed continuous variation in the long peduncle phenotype, ranging from 5 cm to 15 cm, further indicates that the expression of the dominant allele is modulated by polygenic additive effects and possibly environmental influences, resulting in quantitative variation around the major gene effect.

The findings of the present study align with those in related legumes. For example, Kumar et al. (2009) reported complete dominance for long peduncle length in lentil, with F_2 segregation ratios fitting a 3:1 pattern. In wheat, dominant gene action influencing peduncle length has also been observed (Joshi et al. 2024; Farooq et al. 2018). These studies collectively support the hypothesis that peduncle length is under simple genetic control with potential quantitative modifiers.

The absence of prior inheritance studies in moth bean for this or related traits underlines the significance of the present work. While genetic control of plant type, leaf shape, flower color, pod morphology, and seed characteristics has been extensively studied in crops like mungbean (Singh et al. 2016), moth bean has remained underexplored. This study fills a critical gap by providing empirical evidence of the inheritance of peduncle length, thereby contributing to a better understanding of plant architectural traits in this underutilized legume.

Hence, conclusively, understanding the genetic basis of morphological traits such as peduncle length, which primarily contributes towards plant architecture, is essential for moth bean breeders seeking to exploit genetic variability to create novel hybrids and varieties. Our inheritance study has revealed significant insights into this trait. Peduncle length may be primarily controlled by a major gene, with several minor genes or environmental factors contributing to its continuous variation, or may be controlled by more than one gene. Visually, peduncles are either long or short, but the long peduncle phenotype shows a range of 5 cm to 15 cm in density distribution. This variation suggests that the major dominant gene is influenced by several factors, whereas the short peduncle trait is recessive. Future studies in the F_3population are needed to confirm the segregation ratios observed in the F_2population. The population developed in this study will be instrumental in the genetic mapping of genes associated with peduncle length. Continued selection within these crosses will help identify lines with optimal grain yields, paving the way for the commercialization of these unique traits. This study provided the first documented analysis of the inheritance of peduncle length in moth bean. The findings contribute foundational knowledge to guide selection strategies, develop improved genotypes, and enhance the adaptability and yield of this valuable arid legume.

Authors' contribution

Conceptualization of research (HRM, KBC, RS); Designing of the experiments (KBC); Contribution of experimental materials (HRM, RS); Execution of field/lab experiments and data collection (HRM, KBC); Analysis of data and interpretation (KBC, RS); Preparation of the manuscript (HRM, KBC, RS).

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