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Genetics of growth habit in horse gram (*Macrotyloma uniflorum* (Lam.)Verdc.)

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Short Report

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Abstract

Growth habit is a plant architectural trait in grain legumes with no exception of horse gram. Determinacy and indeterminacy are the two types of growth habits reported in horse gram. Relative advantages of the two types of growth habit depend on the production systems to which cultivars are targeted. Dependable information on genetics of growth habit provide clues for adopting the most appropriate selection strategy to breed high yielding horse gram varieties with desired growth habit. Taking cues from the past studies, we hypothesize that growth habit in horse gram is controlled by two genes displaying inhibitory epistasis and indeterminacy is dominant over determinacy. To test this hypothesis, we monitored the inheritance of growth habit in F_1, F_2 and F_3 generations derived from two crosses involving parents differing for growth habit. Contrary to our hypothesis, determinate growth habit of F_1s of both the crosses suggested dominance of determinacy over indeterminacy. A good fit of observed segregation of F_2 plants to that of the hypothesized segregation in the ratio of 13 determinate: 3 indeterminate plants, besides confirming dominance of determinacy, suggested classical digenic inhibitory epistatic control of growth habit. These results were further confirmed in F_3 generation based on goodness of fit between observed numbers of plants segregating for determinacy and indeterminacy and those expected in the ratio of 49 determinate: 15 indeterminate plants. To the best of our knowledge, this is the first report on the inheritance of growth habit in horse gram.

Introduction

Horse gram is one of the ancient, indigenous and underutilized grain legume crops in India (Verdcourt 1982). It is the fifth most important grain legume crop grown in India (Fuller and Murphy 2018). Its ability to grow in nutrient-poor soils and tolerate moisture stress makes it a crop of choice for resource-poor farmers in drought prone areas. Besides this, it serves as a valuable crop component to address present and future environmental challenges to agricultural production (Kahane et al. 2013). Despite its importance, horse gram has received disproportionately little research (Fuller and Murphy 2018).

Determinate and indeterminate are the two types of growth habits reported in horse gram. Unlike in other legumes such as common bean (Campos et al. 2010), chickpea (Sandhu et al. 2010) and dolichos bean (Keerthi et al. 2016) where the main meristem terminates in inflorescence in determinate genotypes, the main stem stops growing but will not terminates in inflorescence even when flowering starts in the lateral branches in horse gram (Ashwini et al. 2021). Almost all the currently used horse gram cultivars for commercial production display indeterminate growth habit. Asynchronous flowering, and pod development and maturity driven by long overlapping vegetative and reproductive phases followed by shattering of first formed pods are attributed as important causes (among others) for poor grain productivity of cultivars with indeterminate growth habit (Huyghe 1998). On the other hand, greater synchronous flowering, pod development and maturity driven by shorter overlapping vegetative and reproductive phases are important features of genotypes with determinate growth habit (Kwak et al. 2012; Kato et al. 2019). Determinacy is a domestication-driven plant architectural modification in grain legumes (Huyghe 1998). The compact growth habit of determinate genotypes facilitates high density planting that help maximize productivity (Kim et al. 1992). Synchronous pod maturity minimizes grain yield losses attributable to pod shattering in determinate genotypes. These features of determinate genotypes along with short crop growth and maturity period make them most ideal cultivar types suitable for mechanical harvesting and for multiple and intercropping production systems.

Wide acceptance and popularity of determinate cultivars in other legumes such as common bean (Mekbib 2003), chickpea (Sandhu et al. 2010; Hegde 2011), dolichos bean (Ramesh and Byregowda 2016) and soybean (Kato et al. 2019) suggests breeding and deployment of determinate cultivars should receive high priority in horse gram as well. However, the productivity of determinate cultivars is low due to their fewer branching and pod bearing abilities. Nevertheless, their compact growth habit enable high density planting which adequately compensates for fewer branches and pods and hence offers possibility to maximize their productivity (Ashwini et al. 2021). A precise knowledge on genetic basis of growth habit would provide useful clues (among others) for adopting the most appropriate selection and breeding strategies for developing determinate cultivars in horse gram. Taking cues from reported studies in related legumes such as pigeonpea

(Waldia and Singh 1987) and chickpea (Sandhu et al. 2010; Hegde 2011), we hypothesize that the growth habit in horse gram may be controlled by two genes and indeterminacy is dominant over determinacy. The present study is aimed to test this hypothesis in horse gram.

Material And Methods

The basic material consisted of two indeterminate cultivars namely Palem 1 and CRIDA-18-R and two determinate genotypes, namely HPKM-320 and IC 361290 (Table 1).

Development and characterization of experimental material for growth habit

CRIDA-18-R and Palem 1 were used as male parents and HPKM-320 and IC 361290 as female parents to synthesize crosses. The tiny flowers were emasculated in female parent, the evening of the day before pollination on next day morning. The two crosses, namely HPKM-320 × CRIDA-18-Rand IC 361290 × Palem 1 were synthesized during 2018 post rainy season at the experimental plots of the Department of Genetics and Plant breeding (GPB), University of Agricultural Sciences, (UAS), Bangalore, India. A total of only eight and five well-filled F_1 seeds could be obtained from HPKM-320 × CRIDA-18-R and IC 361290 × Palem 1, respectively. The seeds of four parents, F_1 seeds were planted in 2019 summer season. The seeds of the four parents and all the F_1 seeds germinated and survived to maturity in 2019 rainy season. A total of 10 plants each of the four parents were maintained. The selfed pods from F_1s of the two crosses were harvested, hand-threshed and sun-dried to obtain F_2 seeds. F_2 plants from these two crosses were raised in 2019 post rainy season. A total of 259 and 245 F_2 plants from HPKM-320 × CRIDA-18-R and IC 361290 × Palem 1, respectively survived to maturity. Selfed pods from each F_2 plants were manually harvested, hand-threshed and seeds were sun-dried for use in raising F_3 population during 2020 rainy season.

As a result of mortality of several plants at seedling stage due to natural infection by yellow mosaic virus, fewer than expected numbers of F_3 families survived to maturity. However, total number of plants that survived to maturity in F_3 generations of both the crosses (389 in HPKM-320 × CRIDA-18-R and 211 in IC 361290 × Palem 1) was still sufficiently large enough to get reliable data on growth habit. The recommended production package was practiced to raise parents, F_1, F_2 and F_3 generations.

Data recording and statistical analysis

Each plant of the parents, F_2 and F_3 plants of the two crosses were inspected and their growth habit was recorded at maximum pod formation stage. Two distinct growth habits could be observed in F_2 and F_3 generations of both crosses. All the plants bearing elongated flowering branches that terminated in vegetative bud similar to those in indeterminate parents were classified as indeterminate (ID); and plants bearing short primary and secondary branches with their apical buds developing into a flower bud or fully opened flower similar to those in determinate parents as determinate (D) (Hegde 2011). Based on hypothesized inheritance pattern (digenic inhibitory epistasis), goodness of fit between observed and expected segregation ratio of 13 determinate: 3 indeterminate F_2 plants and 49 determinate: 15 indeterminate F_3 plants were examined using χ^2 test. Non-significance of χ^2 test was considered as an evidence for digenic inhibitory epistatic genetic control of growth habit and dominance of determinacy over indeterminacy.

Results And Discussion

Contrary to our hypothesis, the F_1's of both the crosses displayed determinate growth habit (Fig. 1). Hence, we suspected that the F_1's are not true crosses. However, appearance of both determinate and indeterminate plants in F_2 generation not only confirmed that the F_1's were true crosses but also dominance of determinacy contrary to our hypothesis. Further, a greater frequency of determinate plants and a good fit of observed numbers of determinate and indeterminate plants to those expected based on hypothetical segregation ratio of 13 determinate to 3 indeterminate plants in F_2 generation (Table 2) suggested that growth habit in horse gram is controlled by two genes that display classical inhibitory epistasis. These results further confirmed dominance of determinacy. The dominance of determinacy over indeterminacy and digenic inhibitory epistatic control of growth habit was confirmed in F_3 generation based on goodness of fit between observed and expected segregation ratio of 49 determinate: 15 indeterminate F_3 plants (Table 3). Thus, our results based on F_2 and F_3 segregation indicated that the growth habit in horse gram is controlled by two genes that exhibit inhibitory epistasis and determinacy is dominant over indeterminacy. The dominance of determinacy over indeterminacy has also been reported in spaghetti squash (*Cucurbita pepo*) (Edelstein et al. 1989) and common bean (*Phaseolus vulgaris*) (Campos et al. 2010).

Waldia and Singh (1987) in pigeonpea, and van Rheenen et al. (1994) and Sandhu et al. (2010) in chickpea have also suggested digenic inhibitory epistatic control of growth habit. However, they reported dominance of indeterminacy over determinacy. We believe that dominance of determinacy in horse gram is not surprising considering that dominance of any trait's alternate alleles (Kearsey and Pooni 1996) depends on the level of organization of phenotypes controlled by them (Mike 2008), and that the architecture of determinate phenotype in horse gram is different from that reported in common bean (Koinange et al. 1996; Campos et al. 2010), chickpea (van Rheenen et al. 1994; Sandhu et al. 2010; Hegde 2011) and in dolichos bean (Keerthi et al. 2014; Keerthi et al. 2016). It is likely that different source genotypes' may harbor different genes controlling determinacy (Edelstein et al. 1989). It is also possible that dominance of alternate alleles at different genes may vary with source genotype. These possibilities suggest that determinacy in horse gram might also inherit as a dominant phenotype if sources different from those used in the present investigation will be used to decipher inheritance of determinacy. The reported results on dominance of both indeterminacy (Koinange et al. 1996) and determinacy (Campos et al. 2010) using different sources of determinacy in common bean lends adequate support to our results and arguments thereof.

We designate the two genes controlling growth habit in horse gram as '*D*' and '*ID*'; while gene '*D*' controls determinacy, '*ID*' controls indeterminacy. The gene '*D*' either in dominant or recessive state in the absence of dominant allele '*ID*' produces determinate growth habit. The gene '*ID'* in dominant state inhibits '*D*' only in its recessive state. Based on this mode of action of '*D*' and '*ID*' genes controlling the inheritance of growth habit, we have proposed most probable genotypes of parental, F_1 and F_2 populations (Table 4). To the best of our knowledge, this is the first report on the inheritance of growth habit in horse gram.

Implications in breeding horse gram

If the objective is to develop determinate cultivars in horse gram, determinacy should be fixed in early segregating generations followed by selection for high grain yield potential. Dominance of determinacy and digenic inhibitory epistatic control of its inheritance results in high frequency (81.25 %) of determinate genotypes in F_2 generation. Further, considering that 44% plants do not segregate from F_2 generation onwards, it is possible to fix determinacy in F_2 generation itself. High probability of recovering determinate genotypes enable enhanced pace of developing determinate cultivars in high yielding genetic background in horse gram.

Declarations

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Compliance with ethical standards

Conflict of interest: Authors of this manuscript declare that we have no conflict of interests.

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Tables

Table 1 Growth habit and pedigree/source of parents used to derive crosses in horse gram

Parents	Growth habit	Source	Pedigree
Palem1	Indeterminate	Agriculture Research station, Palem, Andhra Pradesh	Unknown
CRIDA-18-R	Indeterminate	CRIDA, Hyderabad	Mutant of K-42
HPKM-320	Determinate	CSK HPKV, Palampur	Unknown
IC 361290	Determinate	NBPGR, Bhowali	Unknown

CRIDA: Central Research Institute for Dryland Agriculture, NBPGR: National Bureau of Plant Genetic Resources,

CSK HPKV: Chaudhary Sarwan Kumar Himachal Pradesh Krishi Vishvavidyalaya

Table 2 The observed pattern of segregation for growth habit and its goodness of fit to that expected in F_2 generations derived from two crosses in horse gram

Crosses	Observed number of F_2 plants with two types of growth habit		Hypothesized ratio of D and ID plants	Expected number of F_2 plants based on hypothesized ratio of 13 D: 3 ID		Chi square statistic	Probability
	Determinate (D)	Indeterminate (ID)		Determinate (D)	Indeterminate (ID)		
HPKM-320 (D) × CRIDA-18-R (ID)	208	51	13 D: 3 ID	210	49	0.15	0.69
IC 361290 (D) × Palem 1 (ID)	195	50	13D: 3 ID	199	46	0.44	0.50

Table 3 The observed pattern of segregation for growth habit and its goodness of fit to that expected in F_3 generations derived from two crosses in horse gram

Crosses	Observed number of F_3 plants with two types of growth habit		Hypothesized ratio of D and ID plants	Expected number of F_3 plants with two types of growth habit		Chi square statistic	Probability
	Determinate (D)	Indeterminate (ID)		Determinate (D)	Indeterminate (ID)		
HPKM-320 (D) × CRIDA-18-R (ID)	398	110	49 D:15 ID	389	119	0.90	0.34
IC 361290 (D) × Palem 1 (ID)	211	58	49 D:15 ID	206	63	0.52	0.47

Table 4 The proposed genotypes of parents, and F_1 and F_2 generations in horse gram

Generation	Genotypic frequency	Growth habit
Parents		
DDidid	–	Determinate
ddIDID	–	Indeterminate
F_1		
DdIDid	–	Determinate
F_2		
Segregating		
DdIDid	4/16	Determinate
DDIDid	2/16	Determinate
DdIDID	2/16	Determinate
Ddidid	2/16	Determinate
ddIDid	2/16	Indeterminate
Non-segregating		
DDIDID	1/16	Determinate
DDidid	1/16	Determinate
ddidid	1/16	Determinate
ddIDID	1/16	Indeterminate

Figures

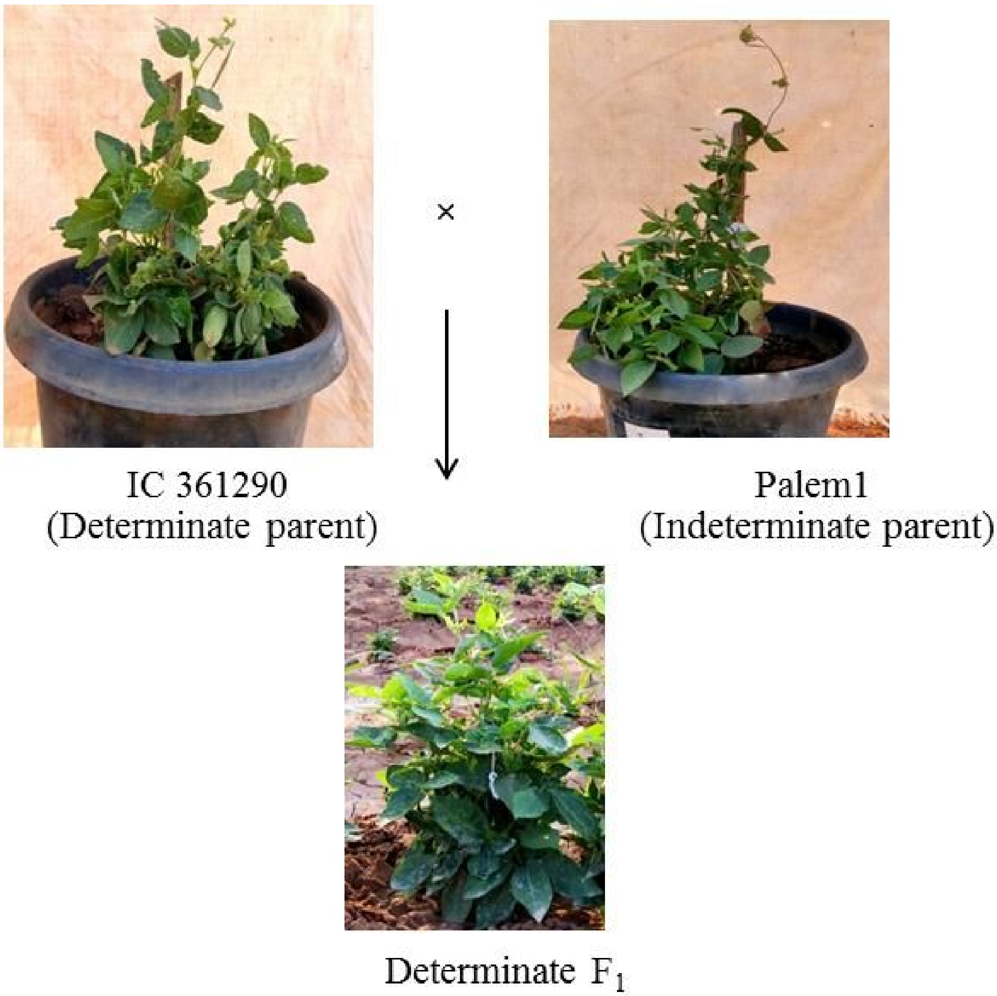

Figure 1

The photographs showing determinate growth habit of the F1 derived from crossing determinate genotypes as females and indeterminate genotypes as males