

RESEARCH NOTE

Genetics of growth habit and photoperiodic response to flowering time in dolichos bean (*Lablab purpureus* (L.) Sweet)

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Introduction

Development of photoperiod insensitive and determinate type cultivars is one of the major objective of Dolichos bean breeding. For this, an understanding of the genetics of photoperiodic sensitivity to flowering time and growth habit is a prerequisite. Genetics of photoperiod sensitivity and growth habit was investigated in two crosses, HA 4 × GL 103 and HA 4 × GL 37 derived from parents contrasting for photoperiod sensitivity and growth habit. Results revealed monogenic control of photoperiodic response to flowering time; photoperiod sensitivity being dominant to insensitivity. Growth habit is controlled by three genes, *GH*₁, *GH*₂ and *GH*₃ of which, one (*GH*₁) is independent and the other two (*GH*₂ and *GH*₃) are complementary; indeterminacy being dominant to determinant growth habit. The genes controlling growth habit and photoperiodic response to flowering time were linked in coupling phase in these lines.

Dolichos bean (*Lablab purpureus* L. Sweet) is commonly known as field bean, hyacinth bean, Indian bean, sem, butter bean, Egyptian kidney bean and lubia bean etc., belongs to the family Fabaceae and is one of the most ancient crops among the cultivated plants. In India, dolichos bean is primarily cultivated in Karnataka and adjoining districts of Tamil Nadu, Andhra Pradesh and Maharashtra (Mahadevu and Byregowda 2005) and used as vegetable (immature green soft pods and immature grains) and forage (NRC 2006). It is cultivated either as a pure crop or intercropped with finger millet, groundnut, castor, corn, pearl millet or sorghum. In Karnataka, dolichos bean is grown in an area of 85,000 hectares with a production of 30,000 tones and

contributes nearly 90% of both area and production in India (www.vijayakarnataka.com).

Photoperiod sensitivity for flowering time and indeterminate type of most of dolichos germplasm accessions (Shivashankar and Kulkarni 1989) poses difficulty in evaluating them across seasons and in prediction and synchrony of flowering time. Synchrony in flowering time is essential to synthesize planned crosses to generate variability to combine and augment frequency of favourable alleles controlling targets traits. Also, photoperiod sensitivity for flowering time restricts the cultivation of dolichos bean cultivars to specific seasons and hence cultivars cannot be fit into different cropping systems. Determinate type cultivars have relatively more synchronous flowering and hence have more uniform pod maturity facilitating cost-effective harvesting particularly in the face of severe manpower shortage. Thus determinate cultivars are gaining increased popularity among the farmers. An understanding of the genetics and linkage relationship of genes controlling growth habit and photoperiodic response to flowering time is a prerequisite for breeding photoperiod insensitive with determinate type dolichos bean cultivars.

Materials and methods

Materials consisted of HA 4 (HA 3 × Magadi local), a released photoperiod insensitive and determinate type cultivar and photoperiod sensitive, indeterminate type germplasm accessions, GL 37 and GL 103. The genotypes are being maintained at Zonal Agricultural Research Station (ZARS), UAS, Gandhi Krishi Vignana Kendra (GKVK), Bangalore. Emasculated flowers of variety HA 4 were pollinated by pollen grains collected from GL 37 and GL 103 during 2010 post-rainy season. Twenty F₁ seeds were collected from each

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of the crosses. The seeds of the two F_1 's HA 4 \times GL 103 and HA 4 \times GL 37 were planted during 2011 rainy season at experimental plots of ZARS and GKVK. The F_2 populations of two crosses, HA 4 \times GL 103 and HA 4 \times GL 37 were grown in a block of 18 and 28 rows of 3 m length at ZARS, GKVK (during August 2012 rainy season). The parents (HA 4, GL 37 and GL 103), and their F_1 's were each grown in a single row of 6 m length.

The data on days to flowering and growth habit were recorded on parents, their F_1 's and 176 individual F_2 plants derived from HA 4 \times GL 103 and on 273 individual F_2 plants derived from HA 4 \times GL 37. The F_2 plants which flowered in less than 52 days from planting date were classified as photoperiod insensitive (PIS) and those which took more than 52 days for flowering as photoperiod sensitive (PS) plants. As all PS accessions required a maximum of 50 ± 2 days to flowering comparable to PIS accessions when they were planted in December (data not shown) that matched with short days, 52 days to flowering was considered as critical for classification of accessions into PS and PIS. Similarly, those F_2 plants whose main axis terminated in inflorescence were classified as determinate (D) and those whose main axis continued to produce vegetative buds as indeterminate (ID).

Statistical analysis

The goodness of fit of observed ratio of PIS and PS plants and of D and ID plants with expected ratios of 3 PS : 1 PIS (Hanumantha Rao 1987; Prasanthi 2005) and 57 ID : 7 D (Girish and Byregowda 2009), respectively were tested using χ^2 test statistic. The linkage between genes controlling photoperiodic response to flowering time and growth habit was detected using χ^2 test. After confirming linkage between genes, the frequency of recombination and hence distances between them were estimated using maximum-likelihood method (Fisher 1921) and Haldane mapping function (Haldane 1919), respectively.

Results and discussion

Genetics of photoperiod sensitivity

The F_1 's of both crosses were photoperiod sensitive suggesting dominance of sensitivity. Among 176 F_2 plants of HA 4 \times GL 103, 141 were found PS and the remaining 35 plants were PIS with a χ^2 value of 2.45 ($P = 0.117$) when tested against expected ratio of 3 PS : 1 PIS. Among 273 F_2 plants of other cross, HA 4 \times GL 37, 192 F_2 plants were found PS and 81 plants were PIS with a χ^2 value of 3.18 ($P = 0.074$) when tested against expected ratio of 3 PS : 1 PIS (table 1). The results indicated that photoperiodic response to flowering time is controlled by a monogenic biallelic locus; photoperiod sensitivity being dominant to insensitivity.

Genetics of growth habit

The F_1 's of both crosses exhibited indeterminate growth habit suggesting dominance of indeterminacy. Among 176 F_2 plants derived from HA 4 \times GL 103, 160 F_2 plants were found ID and the remaining 16 were D with a χ^2 value of 0.62 ($P = 0.43$) when tested against the expected ratio of 57 ID : 7 D. Among 273 F_2 plants of HA 4 \times GL 37, 237 F_2 plants were found ID and 36 plants were D with a χ^2 value of 1.42 ($P = 0.23$) upon testing against expected ratio of 57 ID : 7 D (table 1). These results indicated that the growth habit is controlled by three genes GH_1 , GH_2 and GH_3 of which, one (GH_1) is independent and other two (GH_2 and GH_3) are complementary and dominance of indeterminate growth habit.

Thus, the present study validated the previously reported mode of action of genes controlling photoperiodic response to flowering time (Hanumantha Rao 1987; Prasanthi 2005) and growth habit (Girish and Byregowda 2009) in different genetic backgrounds of dolichos bean. However, joint segregation and linkage relationship of genes controlling photoperiod sensitivity and growth habit have not yet been reported.

Joint segregation of photoperiod sensitivity and growth habit

Analysis of joint segregation of genes controlling photoperiodic response to flowering time and growth habit was performed to detect linkage between genes controlling the two traits. Among 176 F_2 plants of HA 4 \times GL 103, 141 were found PS and ID, 21 plants PIS and D, five plants PS and ID and nine plants were PIS and D with a χ^2 value of 22.93 ($P < 0.0001$) when tested against the expected joint segregation of 171 PS and ID : 21 PS and D : 57 PIS and ID : 7 PIS and D. Among 273 F_2 plants derived from HA 4 \times GL 37, 187 F_2 plants were found PS and ID, 50 were PIS and D, six were PS and ID and 30 were PIS and D with a χ^2 value of 82.13 ($P < 0.0001$) upon testing against the expected joint segregation of 171 PS and ID : 21 PS and D : 57 PIS and ID : 7 PIS and D (table 2). These results provided the sufficient evidence for the linkage between genes controlling photoperiodic response to flowering time and growth habit in both the crosses. Occurrence of plants with a combination of PS and D, and PIS and ID, rules out the possibility of pleiotrophic genetic control of the two traits.

Estimates of recombination fraction

A rather lower magnitude of recombination of 0.212 and 0.258 which translate into distances of 27.58 and 36.28 cM between genes controlling photoperiodic response to flowering time and growth habit in the two crosses, HA 4 \times GL 103 and HA 4 \times GL 37, respectively (table 2) suggested close linkage between them. The results also indicated that the genes are linked in coupling phase.

Table 1. Test of significance of deviation of observed segregation ratio of genes controlling photoperiodic response to flowering time and growth habit from expected ratio in dolichos bean.

Traits	Crosses	Phenotypic class	Observed number of plants in F ₂	Predicted number of plants in F ₂	Expected ratio	χ^2 statistic	Pr > χ^2																												
Photoperiodic response to flowering	HA 4 × GL 103	PS	141.00	132.00	3 PS:1 PIS	2.45	0.117																												
		PIS	35.00	44.00					HA 4 × GL 37	PS	192.00	204.75	3 PS:1 PIS	3.18	0.074	PIS	81.00	68.25	Growth habit	HA 4 × GL 103	ID	160.00	156.75	57 ID:7 D	0.62	1.420	D	16.00	19.25		HA 4 × GL 37	ID	237.00	243.14	57 ID:7 D
	HA 4 × GL 37	PS	192.00	204.75	3 PS:1 PIS	3.18	0.074																												
		PIS	81.00	68.25				Growth habit	HA 4 × GL 103	ID	160.00	156.75	57 ID:7 D	0.62	1.420	D	16.00	19.25		HA 4 × GL 37	ID	237.00	243.14	57 ID:7 D	0.43	0.230	D	36.00	29.86						
Growth habit	HA 4 × GL 103	ID	160.00	156.75	57 ID:7 D	0.62	1.420																												
		D	16.00	19.25					HA 4 × GL 37	ID	237.00	243.14	57 ID:7 D	0.43	0.230	D	36.00	29.86																	
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		D	36.00	29.86																															

Table 2. Detection of linkage, phase and estimates of recombination fraction (\hat{r}) and distance (in cM) between genes controlling photoperiodic response to flowering time and growth habit in dolichos bean.

Crosses	Phenotypic class	Observed number of plants in F ₂	Predicted number of plants in F ₂	Expected ratio	χ^2 statistic	Probability > χ^2	Estimated recombination fraction	Distance between genes (cM)	Phase of linkage
HA 4 × GL 103	PS & ID	141.00	117.60	171:57:21:07	22.93	<.0001	0.212 ± 0.036	27.58	Coupling
	PIS & ID	21.00	39.19						
	PS & D	5.00	14.14						
	PIS & D	9.00	4.81						
HA 4 × GL 37	PS & ID	187.00	182.40	171:57:21:07	82.13	<.0001	0.258 ± 0.039	36.28	Coupling
	PIS & ID	50.00	60.79						
	PS & D	6.00	22.39						
	PIS & D	30.00	7.46						

Implications for dolichos bean breeding

To our knowledge, detection of linkage and its phase, and estimation of recombination fraction and distance between genes controlling photoperiodic response to flowering time and growth habit reported in the present study is first of its kind in dolichos bean. The strong linkage in coupling phase suggests effectiveness of selection for PIS plants in breeding populations using growth habit as a surrogate, as the latter is easily observable/assayable under field conditions. Also, relatively small-sized F₂ populations will suffice to recover and select PIS plants with determinate growth habit. Cultivars with PIS and D growth habit are desirable from farmers' point of view, as they facilitate high density planting and hence help maximize productivity of dolichos bean (Vishwanath *et al.* 1971). Further, PS accessions with ID growth habit could be converted into PIS accessions with D growth habit by repeated backcrossing using simple visual assay. Photoperiod insensitiveness to flowering time helps enhance the use of germplasm in dolichos breeding. Large-scale conversion of PS sorghum germplasm accessions into their PIS counterparts which enabled enhanced use of exotic germplasm, broaden genetic diversity and providing new sources of desirable traits (Rai *et al.* 1999) stands

testimony to the utility of PIS accessions in breeding crop plants and dolichos bean is no exception to this. As both photoperiodic response to flowering time and growth habit are easily assayable under field conditions and have monogenic/oligogenic control, they serve as diagnostic descriptors of germplasm accessions and hence useful to avoid mistakes in labelling, aid identification and minimize duplication in the germplasm database (Smith and Smith 1992). They also serve as morphological markers to identify true F₁'s in dolichos bean.

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