

RESEARCH ARTICLE

Further evidence for the genetic basis of qualitative traits and their linkage relationships in dolichos bean (*Lablab purpureus* L.)

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Abstract

An investigation on inheritance of qualitative traits in dolichos bean revealed biallelic monogenic control of photoperiod-induced sensitivity to flowering time and flower colour in F₂ and F₃ generations. While, growth habit and pod curvature are each controlled by two genes that exhibit classical complementary epistasis, raceme emergence was controlled by two genes that displayed classical inhibitory epistasis. The dominant alleles, at two different unlinked pairs of genes are necessary for plants to exhibit indeterminate growth habit and bear straight pods. Any other combination of alleles at the two pairs of genes result in plants displaying determinate growth habit and bearing curved pods. While, the genes controlling growth habit, PSFT and raceme emergence are linked. Those controlling flower colour and pod curvature are segregated independent of each other. These results are discussed in relation to strategies for breeding dolichos bean.

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Introduction

In our recently published article (Keerthi *et al.* 2014a), we reported inheritance of genes controlling growth habit (GH) and PSFT (PSFT) and phase of linkage and recombination frequency between them in dolichos bean using F₂ populations derived from two crosses involving parents contrasting for GH and PSFT. Dolichos bean is one of the important food grain legume extensively grown as a rainfed intercrop / sole crop in the southern states of India (Keerthi *et al.* 2014a). Evidences point to its origin in Indian subcontinent (Nene 2006). It is grown for fresh grains for use as a vegetable and for whole dry grains and split dhal, used in various food preparations and snacks (Shivashankar and Kulkarni 1989). It is predominantly a self-pollinated crop with $2n = 22$ chromosomes (Goldblatt 1981). It is also known for its fodder potential (Magoon *et al.* 1974). Considering its multipurpose utility and ability to tolerate drought stress (Ewansiha and Singh 2006), dolichos bean is regarded as an underexploited crop in terms of area planted, consumption level and efforts for its genetic improvement. The crop has evolved

as highly responsive to photoperiod and requires short-days for switching over from vegetative to reproductive phase and exhibit indeterminate GH (Shivashankar and Kulkarni 1989). Most cultivars grown by farmers are photoperiod sensitive (PS) and display indeterminate GH (Keerthi *et al.* 2014b). Photoperiod insensitive (PIS) determinate growth type cultivars have been developed at University of Agricultural Sciences (UAS), Bengaluru, India (Vishwanath *et al.* 1971; Girish and Gowda 2009). In the germplasm maintained at UAS, Bengaluru, India, variation in PSFT, GH, flower colour (FC), raceme emergence (RE) and pod curvature (PC) have been documented (Vaijayanthi *et al.* 2014). Human selection for specific variants of PSFT over several years has permitted significant expansion and improvement in adaptation of several legumes from prehistoric times to the present day (Weller and Ortega 2015) and dolichos bean is no exception to this. PS varieties have narrow adaptation and could be grown only during short-day seasons. On the other hand, PIS varieties have wide adaptation and could be grown in all seasons irrespective of day length, a necessity driven by demand for dolichos bean round the year. Raceme emergence and pod curvature are unique to dolichos bean. While, raceme emergence is associated with higher

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economic product yield, curved pods are highly preferred by farmers and consumers. Though flower colour is not associated with economic product productivity, it serves as visual marker to identify the germplasm accessions during their maintenance and identifying true hybrids while effecting crosses to generate variability. Based on these considerations, these five traits were chosen for genetic investigation. A thorough understanding of the genetic basis of these traits would guide in formulating suitable selection strategies to combine these traits in pureline varieties for commercial dolichos bean production. Further, from strategic and basic plant breeding research point of view, an understanding of their genetic basis help to develop genetic stocks such as near isogenic lines (NILs) to assess their linked/pleiotropic effects on economically important traits and unravel metabolic pathways regulating these traits. However, comprehensive genetic analyses of production and productivity-related traits in poorly researched crops like dolichos bean have not been attempted. Under these premises, we provide further evidence for the genetic basis of GH and PSFT using F_2 and F_3 populations derived from three different crosses (in terms of geographical origin) from those used in our published article (Keerthi *et al.* 2014a). We also report genetic basis of raceme emergence, flower colour and pod curvature, and linkage relationship, recombination frequency and distance among the genes controlling PSFT, GH, raceme emergence, flower colour and pod curvature in dolichos bean.

Materials and methods

Experimental material

The basic genetic material for the study consisted of germplasm accessions, GL 424, GL 127 and GL 16 and a released variety HA 4 being maintained at Zonal Agricultural Research Station (ZARS), UAS, Bengaluru, India. The four genotypes were selfed for two generations to ensure non-segregations for the GH, PSFT, raceme emergence, flower colour and pod curvature. Using three germplasm accessions as male parents and HA 4 as female parent, three crosses (i) HA 4 \times GL 424, (ii) HA 4 \times GL 127 and (iii) HA 4 \times GL 16 were effected. The first cross involved parents

contrasting for five qualitative traits, namely GH (determinate and indeterminate), PSFT (PS and PIS), raceme emergence (raceme emerge out from foliage and racemes remain within foliage), flower colour (purple and white) and pod curvature (straight and curved). The second cross involved parents with contrasting GH, PSFT and raceme emergence while, the third cross involved parents with contrasting GH and PSFT (table 1). The three crosses were affected by pollinating pollen from male parents (GL 424, GL 127 and GL 16) to stigma of emasculated flowers of female parent (HA 4) between 6 a.m. and 8 a.m. at the experimental plots of ZARS during 2011 rainy season. The true F_1 's were identified using phenotypes (except RE) of male parents as markers, as they were dominant.

The seeds of the three F_1 s (HA 4 \times GL 424, HA 4 \times GL 127 and HA 4 \times GL 16) were planted during 2012 rainy season. A total of 15 F_1 plants of each of the three crosses were maintained. The phenotypes of the three F_1 's for each of the five traits were noted and selfed. The seeds from each of the three F_1 's were separately bulked, and were planted to raise F_2 populations in 2013 rainy season. The final crop stand consisted of 135 F_2 plants of HA 4 \times GL 424, 179 F_2 plants of HA 4 \times GL 127 and 161 F_2 plants of HA 4 \times GL 16. To confirm the inheritance of qualitative traits, 80 F_3 families of HA 4 \times GL 424 and 58 F_3 families of HA 4 \times GL 127 each consisting of 20 plants were grown in a single row of 3-m length during 2014 rainy season. Standard crop production and protection practices were followed to raise healthy plants.

Collection of data

The data on days to flowering, GH, raceme emergence, flower colour and pod curvature were recorded on 135 F_2 plants derived from HA 4 \times GL 424; data on days to flowering, GH and raceme emergence were recorded on 179 F_2 plants derived from HA 4 \times GL 127 and data on days to flowering and GH were recorded on 161 F_2 plants derived from HA 4 \times GL 16. The plants planted during June experience long-days under Bengaluru conditions. Those F_2 plants which flowered within 52 days from planting date were classified as PIS and those which took more than 52 days were classified as PS plants (Keerthi *et al.* 2014a). Similarly, F_2

Table 1. Phenotypes of the four parents used for effecting three crosses in dolichos bean.

Parent	Pedigree/place of collection	Phenotypes of five traits				
		GH	PSFT	Raceme emergence	Flower colour	Pod curvature
HA 4	HA 3 \times Magadi Local	Determinate	PIS	Racemes emerge from foliage	White	Curved
GL 424	Kenya	Indeterminate	PS	Racemes remain within foliage	Purple	Straight
GL 127	Karnataka	Indeterminate	PS	Racemes remain within foliage	White	Curved
GL 16	Andhra Pradesh	Indeterminate	PS	Racemes emerge from the foliage	White	Curved

plants whose main axis terminated in raceme were classified as determinate (D) and those whose main axis continued to produce vegetative buds as indeterminate (ID). Based on flower colour, F_2 plants were classified into those producing purple (P) and white (W) flowers. Based on raceme emergence, F_2 plants were classified as those plants with racemes emerging from the foliage (REF) and those where racemes remain within the foliage (RWF). Based on PC, F_2 plants were classified as those bearing straight pods (SP) and curved pods (CP). Number of F_3 families true breeding and segregating for PS, GH, raceme emergence, flower colour and pod curvature were recorded.

Statistical analysis

Test for traits segregation in expected ratio: The goodness of fit of observed ratio of PIS and PS plants and of determinate and indeterminate plants, and plants bearing purple and white flowers, plants with racemes emerging from the foliage and those where racemes remain within the foliage and straight pods and curved pods in F_2 population with expected ratios of 3:1 (Hanumantha Rao 1987; Prasanthi 2005; Keerthi *et al.* 2014a) and 9:7 (Hanumantha Rao 1987), 3:1, 13:3 and 9:7, respectively were tested using chi-square (χ^2) statistic. The observed number of F_3 families true breeding for PS, segregating for PS and PIS, and true breeding for PIS based on PSFT were counted. Similarly, the observed number of F_3 families true breeding for purple flowers, segregating for purple and white flowers and true breeding for white flowers based on flower colour were counted. The goodness of fit of these observed number of F_3 families with those expected (based on 1:2:1 ratio) for PSFT and flower colour (table 2) was tested using χ^2 statistic. The observed number of F_3 families true breeding for indeterminacy, segregating for indeterminacy and determinacy and true breeding for determinacy based on GH were counted. Similarly, the observed number of F_3 families true breeding for straight pods, segregating for straight pods and curved pods and true breeding for curved pods based on pod curvature were counted. The goodness of fit of these observed number of F_3 families with those expected (based on 1:8:7 ratio) for GH and pod curvature (table 2) was tested using χ^2 statistic. Similarly, the observed number of F_3 families true breeding for racemes emerge from the foliage, segregating for racemes emerge from the foliage and racemes remain within the foliage and true breeding for racemes remain within the foliage based on raceme emergence were counted. The goodness of fit of these observed number of F_3 families with those expected (based on 7:6:3 ratio) for raceme emergence (table 2) was tested using χ^2 test. A test for heterogeneity of goodness of fit between observed and expected number of F_2 plants derived from different crosses was performed using χ^2 statistic to determine whether it was appropriate to pool data from all F_2 plants.

Detection of linkage and estimation of recombination frequency: The linkage if any between genes controlling PSFT, GH, raceme emergence, flower colour and pod curvature was detected using χ^2 test (Mather 1951). After confirming linkage between genes, the frequency of recombination between them was estimated following maximum likelihood method in $F_2 \times$ population (Fisher and Balmukandh 1928; Mather 1951). The averaged recombination frequency across F_2 generations of three crosses was used to estimate distance between the linked genes using Haldane mapping function (Haldane 1919).

Results and discussion

Genetics of PSFT, GH, raceme emergence, flower colour and pod curvature

The F_1 s derived from the cross HA 4 \times GL 424 were indeterminate, PS and bore purple flowers on racemes which emerged from the foliage and produced straight pods (table 2). On the other hand, the F_1 s of the cross HA 4 \times GL 127 were indeterminate, PS and bore racemes which emerged from the foliage and the F_1 s of cross HA 4 \times GL 16 were PS and indeterminate (table 2). The expected dominance of indeterminate over determinate; PS over PIS; purple (P) over white (W) and straight over curved pods confirmed trueness of F_1 s.

Biallelic monogenic inheritance of PSFT and flower colour was evident from segregation of F_2 plants derived from all the three crosses in three PS: one PIS ratio for PSFT (table 3) and of those derived from HA 4 \times GL 424 in 3 P : 1 W ratio for flower colour (table 4), respectively. The results indicated dominance of PS over PIS and of purple flowers over white flowers. The test for heterogeneity χ^2 test for expected segregation of F_2 plants derived from the three crosses indicated consistent expected monogenic phenotypic 3 PS : 1 PIS ratio across three crosses. Analysis of F_3 progenies segregation confirmed these results. F_3 families derived from PS F_2 plants segregated in the ratio of 1 family homozygous for PS : 2 families segregating for PS and PIS : 1 family homozygous for PIS in HA 4 \times GL 424 and HA 4 \times GL 127 crosses (table 3). The F_3 families derived from purple flower bearing F_2 plants segregated in the ratio of 1 family homozygous purple flowers : two families segregating for purple and white flowers : one family homozygous for white flowers in HA 4 \times GL 424 cross (table 4). Hanumantha Rao (1987), Prasanthi (2005) and Keerthi *et al.* (2014a) have also reported monogenic control of PSFT in dolichos bean in different genetic backgrounds. The majority of loci controlling PSFT have been detected as quantitative traits loci (QTL), such as *Hdl* in rice (Yano *et al.* 2000), *CONSTANS* (*CO*) in *Arabidopsis* (Putterill *et al.* 1995) and *Ppd* in common bean (Wallace *et al.* 1993). Of these, *Hdl* locus is a homologue of *CO*, which codes for a zinc-finger-containing protein, which interacts

Table 2. Proposed phenotypes (PT), genotypes (GT) and expected ratio (ER) used to test the goodness of fit of observed ratio and expected ratio in F₂ and F₃ generations of five qualitative traits in dolichos bean.

Trait	P ₁		P ₂		F ₁		F ₂			F ₃		
	PT	GT	PT	GT	PT	GT	PT	GT	ER	PT	GT	ER
PSFT	PS	SS	PIS	ss	PS	Ss	PS	1 SS	3	PS	1 SS	1
							PS	2 Ss		Segregate	2 Ss	
							PIS	1 ss		1	PIS	
FC	Pr	RR	W	rr	Pr	Rr	P	1 RR	3	Pr	1 RR	1
							P	2 Rr		Segregate	2 Rr	
							W	1 rr		1	W	
GH	ID	GGHH	D	gghh	ID	GgHh	ID	1 GGHH	9	Segregate	1 GGHH	1
							ID	2 GGHh		Segregate	2 GgHH	
							ID	2 GgHH		D	4 GgHh	
							D	1 GGhh		D	2 Gghh	
							D	2 Gghh		D	1 ggHH	
							D	1 ggHH		D	2 ggHh	
PC	SP	PPCC	CP	ppcc	SP	PpCc	SP	1 PPCC	9	Segregate	1 PPCC	1
							SP	2 PPCC		Segregate	2 PpCC	
							SP	2 PpCC		CP	4 PpCc	
							CP	1 PPcc		CP	2 Ppcc	
							CP	2 Ppcc		CP	1 ppCC	
							CP	1 ppCC		CP	2 ppCc	
RE	REF	RRee	RWF	rrEE	REF	RrEe	REF	1 RREE	13	REF	1 RREE	7
							REF	2 RREe		REF	2 RREe	
							REF	1 RRee		Segregate	1 RRee	
							REF	2 RrEE		Segregate	2 Rree	
							REF	4 RrEe		Segregate	4 RrEe	
							RWF	1 rree		Segregate	1 rree	
RE	REF	RRee	RWF	rrEE	REF	RrEe	RWF	1 rrEE	3	RWF	1 rrEE	3
							RWF	2 rrEe		RWF	2 rrEe	
							RWF	2 rrEe		RWF	rrEe	

PSFT, photoperiod-induced sensitivity to flowering time; PS, photoperiod sensitive; PIS, photoperiod insensitive; FC, flower colour; Pr, purple flower; W, white flower; GH, growth habit; ID, indeterminate; D, determinate; PC, pod curvature; SP, straight pods; CP, curved pods; RE, raceme emergence; REF, racemes that emerge from the foliage; RWF, racemes that remain within the foliage.

between circadian rhythms and the flowering signalling pathway (Hayama and Coupland 2004). *Ppd* is a Mendelized locus mapped on linkage group 1 where recessive alleles

confer reduced photoperiod response and early flowering under long days (Koinange *et al.* 1996). The *Ppd* locus is also syntenic with region containing *E3/PHYA3* gene

Table 3. Test of significance of segregation for genes controlling PSFT in F₂ and F₃ generations in dolichos bean.

Cross	Parent/ generation	Observed number of F ₂ plants / F ₃ families				Expected ratio	Expected number of F ₂ plants / F ₃ families			χ^2 statistic	Pr > χ^2
		Total	PS	SEG	PIS		PS	SEG	PIS		
HA 4 × GL 424	HA 4	10	0	–	10	–	0	–	10	–	–
	GL 424	10	10	–	0	–	10	–	0	–	–
	F ₁	15	15	–	–	–	15	–	–	–	–
	F ₂	135	92	–	43	3 : 1	101.25	–	33.75	3.38	0.07
	F ₃ (PIS)	17	0	0	17	–	0	0	17	–	–
	F ₃ (PS)	63	17	37	9	1 : 2 : 1	15.75	31.50	15.75	3.95	0.139
HA 4 × GL 127	HA 4	10	0	–	10	–	0	–	10	–	–
	GL 127	08	08	–	0	–	08	–	0	–	–
	F ₁	15	15	–	–	–	15	–	–	–	–
	F ₂	179	126	–	53	3 : 1	134.25	–	44.75	2.03	0.154
	F ₃ (PIS)	15	0	0	15	–	0	0	15	–	–
	F ₃ (PS)	43	14	24	5	1 : 2 : 1	10.75	21.50	10.75	4.35	0.114
HA 4 × GL 16	HA 4	09	0	–	09	–	0	–	09	–	–
	GL 16	11	11	–	0	–	11	–	0	–	–
	F ₁	15	15	–	–	–	15	–	–	–	–
	F ₂	161	114	–	47	3 : 1	120.75	–	40.25	1.51	0.219
Heterogeneity χ^2	F ₂	–	–	–	–	–	–	–	–	0.28	0.87

PS, photoperiod sensitive; PIS, photoperiod insensitive; SEG, segregated; ‘0’ zero number of individuals; ‘–’ absence of characters.

in soybean (McClellan *et al.* 2010). In chickpea, classical genetic analyses have distinguished four Mendelian loci, designated as *early flowering 1* (*Efl1*) to *Efl4* (Gaur *et al.* 2015). Recessive alleles at these loci confer early flowering and at least two are likely to be widespread within chickpea germplasm and have major impact on flowering time adaptation. Kumar *et al.* (2000) in chickpea, Sreevalli *et al.* (2002) in periwinkle and Gettys and Wofford (2007) in pickereel weed, have also revealed monogenic inheritance of flower colour. Stephens and Nickell (1992) reported the *wp* locus, which conditions a change in flower colour from purple to pink in soybean. Moreau *et al.* (2012) reported the single major gene *Clariroseus* (B) controlling flower pigmentation in pea which controls hydroxylation at the 59 position of the B ring of flavonoid precursors of the anthocyanins.

The F₂ plants derived from all the three crosses, HA 4 × GL 424, HA 4 × GL 127 and HA 4 × GL 16 segregated in

the ratio of 9 indeterminate : 7 determinate for GH (table 5). The nonsignificant heterogeneity χ^2 statistic suggested consistent segregation of F₂ plants derived from all the three crosses in 9 indeterminate : 7 determinate ratio. These results were further confirmed through expected segregation of F₃ families. The F₃ families derived from F₂ plants displaying determinate GH bred true for determinacy in all the three crosses. The F₃ families derived from F₂ plants displaying indeterminate GH segregated in the ratio of 1 family homozygous/nonsegregating for indeterminate : 8 families segregating for indeterminate : 7 families homozygous/nonsegregating for determinate in all the three crosses (table 5). The consistent segregation of F₂ plants and F₃ families in expected ratio for GH in HA 4 × GL 424 and HA 4 × GL 127 crosses suggested similar genetic constitutions of the three indeterminate parents GL 424, GL 127 and GL 16. As was true with respect to GH, F₂ plants derived from HA 4 × GL 424 segregated in the ratio of 9 straight pods :

Table 4. Test of significance of segregation for genes controlling flower colour in F₂ and F₃ generations in dolichos bean.

Cross	Parent/ generation	Observed number of F ₂ plants / F ₃ families				Expected ratio	Expected number of F ₂ plants / F ₃ families			χ^2 statistic	Pr > χ^2
		Total	Pr	SEG	W		Pr	SEG	W		
HA 4 × GL 424	HA 4	10	10	–	0	–	10	–	0	–	–
	GL 424	10	0	–	10	–	0	–	10	–	–
	F ₁	15	15	–	–	–	15	–	–	–	–
	F ₂	135	97	–	38	3 : 1	101.25	–	33.75	0.71	0.398
	F ₃ (W)	19	0	0	19	–	0	0	19	–	–
	F ₃ (Pr)	61	17	34	10	1 : 2 : 1	15.25	30.50	15.25	2.41	0.300

Pr, purple; W, white; SEG, segregated; ‘0’ zero number of individuals; ‘–’ absence of characters.

Table 5. Test of significance of segregation for genes controlling GH in F₂ and F₃ generations in dolichos bean.

Cross	Parent/ generation	Observed number of F ₂ plants / F ₃ families				Expected ratio	Expected number of F ₂ plants / F ₃ families			χ^2 statistic	Pr > χ^2
		Total	ID	SEG	D		ID	SEG	D		
HA 4 × GL 424	HA 4	10	0	–	10	–	0	–	10	–	–
	GL 424	10	10	–	0	–	10	–	0	–	–
	F ₁	15	15	–	–	–	15	–	–	–	–
	F ₂	135	87	–	48	9 : 7	75.94	–	59.06	3.68	0.06
	F ₃ (D)	16	0	0	16	–	0	0	16	–	–
	F ₃ (ID)	64	7	36	21	1 : 8 : 7	4	32	28	4.50	0.105
HA 4 × GL 127	HA 4	10	0	–	10	–	0	–	10	–	–
	GL 127	8	8	–	0	–	8	–	0	–	–
	F ₁	15	15	–	–	–	15	–	–	–	–
	F ₂	179	105	–	74	9 : 7	100.69	–	78.31	0.42	0.516
	F ₃ (D)	19	0	2	17	–	0	0	19	–	–
	F ₃ (ID)	39	5	23	11	1 : 8 : 7	2.44	19.50	17.06	5.48	0.07
HA 4 × GL 16	HA 4	9	0	–	9	–	0	–	9	–	–
	GL 16	11	11	–	0	–	11	–	0	–	–
	F ₁	15	15	–	–	–	15	–	–	–	–
	F ₂	161	89	–	72	9 : 7	90.56	–	70.44	0.06	0.804
Heterogeneity χ^2	F ₂	–	–	–	–	–	–	–	–	2.58	0.27

ID, indeterminate GH; D, determinate GH; SEG, segregated; '0' zero number of individuals; '–' absence of characters.

7 curved pods for pod curvature. Segregation of F₂ plants for pod curvature in 9 : 7 ratio was further confirmed through nonsegregation of curved poded F₂ plants-derived F₃ families for curved pods and segregation of straight poded F₂ plants-derived F₃ families into 1 family homozygous straight pods : 8 families segregating for straight pods and curved pods : 7 families homozygous for curved pods (table 6).

The results indicated that GH and pod curvature are each controlled by two genes with complementary epistasis; indeterminate GH being dominant to determinate GH and straight pods are dominant to curved pods. Hanumantha Rao (1987) in dolichos bean, Bernard (1972) in soybean and Van Rhenen *et al.* (1994) in chickpea have also documented digenic control of GH. Elkind *et al.* (1991) in tomato have also documented the digenic (*sp* and *sdt*) control of GH. The dominant alleles at two loci are necessary for plants to exhibit indeterminate GH. Any other combinations of alleles at two genes result in plants displaying determinate GH (table 2). Similarly, the dominant alleles at different pairs of genes are necessary for plants to bear straight pods and any other

combination of alleles at two genes result in plants bearing curved pods (table 2).

Segregation of F₂ plants derived from crosses HA 4 × GL 424 and HA 4 × GL 127 in 13 racemes that emerge from the foliage : 3 racemes that remain within the foliage ratio (table 7), indicated that raceme emergence is also controlled by two genes but exhibit inhibitory epistasis; racemes emergence from the foliage being dominant to lack of raceme emergence from the foliage. The plants carrying one of the two genes, either in dominant or recessive condition irrespective of dominant/recessive condition, at the second locus bear racemes which emerge out from the foliage. The plants carrying second locus in dominant state with the former in recessive state bear racemes that remain within the foliage (table 2). The segregation of raceme emergence in the ratio of 13 : 3 was further confirmed through segregation of F₃ families. F₃ families belonging to F₂ plants with racemes that emerge from the foliage group segregated in the ratio of 7 families homozygous for racemes that emerge from the foliage : 6 families segregating for racemes that emerge from

Table 6. Test of significance of segregation for genes controlling pod curvature in F₂ and F₃ generations in dolichos bean.

Cross	Parent/ generation	Observed number of F ₂ plants / F ₃ families				Expected ratio	Expected number of F ₂ plants / F ₃ families			χ^2 statistic	Pr > χ^2
		Total	SP	SEG	CP		SP	SEG	CP		
HA 4 × GL 424	HA 4	10	10	–	0	–	10	–	0	–	–
	GL 424	10	0	–	10	–	0	–	10	–	–
	F ₁	15	15	–	–	–	15	–	–	–	–
	F ₂	135	81	–	54	9 : 7	75.94	–	59.06	0.77	0.380
	F ₃ (CP)	33	0	5	28	–	0	0	33	–	–
	F ₃ (SP)	47	06	26	15	1 : 8 : 7	2.94	23.50	20.56	4.96	0.084

SP, straight pods; CP, curved pods; SEG, segregated; '0' zero number of individuals; '–' absence of characters.

Table 7. Test of significance of segregation for genes controlling raceme emergence in F₂ and F₃ generations in dolichos bean.

Cross	Parent/ generation	Observed number of F ₂ plants / F ₃ families				Expected ratio	Expected number of F ₂ plants / F ₃ families			χ^2 statistic	Pr > χ^2
		Total	REF	SEG	RWF		REF	SEG	RWF		
HA 4 × GL 424	HA 4	10	10	–	0	–	10	–	0	–	–
	GL 424	10	0	–	10	–	0	–	10	–	–
	F ₁	15	15	–	–	–	15	–	–	–	–
	F ₂	135	106	–	29	13 : 3	109.68	–	25.31	0.66	0.416
	F ₃ (RWF)	17	0	4	13	–	0	0	17	–	–
	F ₃ (REF)	63	31	26	6	7 : 6 : 3	27.56	23.63	11.81	3.53	0.171
HA 4 × GL 127	HA 4	10	10	–	0	–	10	–	0	–	–
	GL 127	08	0	–	8	–	0	–	8	–	–
	F ₁	15	15	–	–	–	15	–	–	–	–
	F ₂	179	141	–	38	13 : 3	145.44	–	33.56	0.72	0.395
	F ₃ (RWF)	19	0	3	16	–	0	0	19	–	–
	F ₃ (REF)	39	19	11	9	7 : 6 : 3	17.06	14.63	7.31	1.51	0.471
Heterogeneity χ^2	F ₂	–	–	–	–	–	–	–	–	0.003	0.96

REF, racemes that emerge from the foliage; RWF, racemes that remain within the foliage; SEG, segregated; ‘0’ zero number of individuals; ‘–’ absence of characters.

the foliage and racemes that remain within the foliage : 3 families homozygous for racemes that remain within the foliage in both the crosses (table 7).

Linkage and recombination frequency between the genes controlling the five qualitative traits

Joint segregation of PSFT with GH, raceme emergence, flower colour and pod curvature: Analysis of joint segregation of genes controlling PSFT and GH indicated significant departure of observed segregation ratio from that of expected ratio of 27 PS and indeterminate : 21 PS and determinate:

9 PIS and indeterminate : 3 PIS and determinate in all the three crosses. These results provided sufficient evidence for the coupling phase linkage between genes controlling these traits in all the three crosses with recombination fraction of 0.29, 0.24 and 0.20, respectively (table 8). In a different genetic background, Keerthi *et al.* (2014a) have also documented linkage with recombination frequency of 0.235 between genes controlling PSFT and GH in dolichos bean. Kwak *et al.* (2008) found similar map positions of *PvZTL* in common bean, the flowering locus and GH locus. They observed positive correlation between earliness and GH, and attributed this to cosegregation of early flowering and determinate GH as is true in dolichos bean (Keerthi *et al.* 2014b).

Table 8. Detection of linkage, estimates of recombination fraction (r) and phase of linkage between genes controlling different qualitative traits in dolichos bean.

Cross	Linkage status	χ^2 probability	Recombination fraction $r \pm SE$	Distance (cM)	Phase of linkage	
HA 4 × GL 424	PSFT & GH	Linked	< 0.05	0.29 ± 0.07	42.83	Coupling
	PSFT & FC	Unlinked	> 0.05	–	–	–
	PSFT & PC	Unlinked	> 0.05	–	–	–
	PSFT & RE	Linked	< 0.05	0.38 ± 0.08	69.57	Repulsion
	GH & FC	Unlinked	> 0.05	–	–	–
	GH & PC	Unlinked	> 0.05	–	–	–
	GH & RE	Linked	< 0.05	0.32 ± 0.09	50.62	Repulsion
	FC & RE	Unlinked	> 0.05	–	–	–
	FC & PC	Unlinked	> 0.05	–	–	–
	PC & RE	Unlinked	> 0.05	–	–	–
HA 4 × GL 127	PSFT & GH	Linked	< 0.05	0.24 ± 0.06	32.52	Coupling
	PSFT & RE	Linked	< 0.05	0.33 ± 0.07	53.23	Repulsion
	GH & RE	Linked	< 0.05	0.31 ± 0.07	48.25	Repulsion
HA 4 × GL 16	PSFT & GH	Linked	< 0.05	0.20 ± 0.06	25.48	Coupling

PSFT, photoperiod-induced sensitivity to flowering time; GH, growth habit; FC, flower colour; RE, raceme emergence; PC, pod curvature.

Further, Kwak *et al.* (2008) hypothesised that *PvZTL* locus could be a candidate gene for GH locus in common bean.

Significant departure of the observed joint segregation ratio of PSFT and raceme emergence from that of expected ratio (39 PS and raceme emerge from the foliage : 9 PS and raceme remain within the foliage : 13 PIS and raceme emerge from the foliage : 3 PIS and racemes remain within the foliage) indicated repulsion phase of linkage between them in two crosses, HA 4 × GL 424 and HA 4 × GL 127, with the recombination fraction of 0.38 and 0.33, respectively (table 8). On the other hand, the comparable observed and expected joint segregation ratio of PSFT with flower colour (9 PS and purple : 3 PIS and purple : 3 PS and white : 1 PIS and white) and pod curvature (27 PS and straight pods : 9 PIS and straight pods : 21 PS and curved pods : 3 PIS and curved pods) suggested independent segregation of genes controlling them in the cross HA 4 × GL 424.

Joint segregation of GH with raceme emergence, flower colour and pod curvature: The significant departure of the observed joint segregation ratio from that of expected ratio of 91 determinate and raceme emerge from the foliage : 21 indeterminate and raceme emerge from the foliage : 117 indeterminate and raceme emerge from the foliage : 21 indeterminate and raceme remain within the foliage indicated repulsion phase of linkage between genes controlling GH and raceme emergence in two crosses, HA 4 × GL 424 and HA 4 × GL 127, with the recombination fraction of 0.32 and 0.31, respectively (table 8). However, comparable observed and expected joint segregation of GH with flower colour (27 indeterminate and purple : 21 determinate and purple : 9 indeterminate and white : 3 determinate and white) and pod curvature (81 indeterminate and straight pods : 63 determinate and straight pods : 63 indeterminate and curved pods : 49 determinate and curved pods) indicated independent segregation of genes controlling them in the cross HA 4 × GL 424 (table 8).

Joint segregation of raceme emergence with flower colour and pod curvature and of flower colour with pod curvature: The observed and expected joint segregation of raceme emergence with flower colour (39 racemes that emerge from the foliage and purple : 9 racemes that remain within the foliage and purple : 13 racemes that emerge from the foliage and white : 3 racemes that remain within the foliage and white) and pod curvature (117 racemes that emerge from the foliage and straight pods : 27 racemes that remain within the foliage and straight pods : 91 racemes that emerge from the foliage and curved pods : 21 racemes that remain within the foliage and curved pods), and of flower colour with pod curvature (27 straight pods and purple : 21 curved pods and purple : 9 straight pods and white : 3 curved pods and white) were comparable indicating independent segregation of genes controlling them in the cross HA 4 × GL 424 (table 8).

The results clearly indicate that the genes controlling GH, PSFT and raceme emergence are linked and

hence map to one linkage group, while those controlling flower colour and pod curvature segregate independently and hence map to two different linkage groups. Thus, the genes controlling the five qualitative traits considered in the present study are located on three different linkage groups. Involvement of only one/two gene systems in the inheritance of five traits investigated in the present study could be due to the use of limited number of crosses involving a few diverse parents. Studies using crosses involving large number of diverse parents could possibly reveal the role of genes other than those reported in the inheritance of five traits.

Implications in strategic and applied dolichos bean breeding

As GH, raceme emergence, flower colour and pod curvature are easily assayable/scorable with stable expression and monogenically/digenically inherited, they serve as diagnostic descriptors for characterization of germplasm accessions which help to classify and distinguish each accession from other similar accessions and for identification of true F_1 's. They serve as markers for identification and minimize duplications in the germplasm accessions and hence help to avoid mistakes in labelling and easy retrieval of desired accessions from the database (Smith and Smith 1992). They are also useful for finger printing varieties for meeting the criteria of distinctiveness, uniformity and stability (DUS) test, a mandatory requirement to protect them under protection of plant varieties and farmers rights act of India.

PS indeterminacy and PIS-determinacy are the two combinations of twin-traits required in cultivars suitable for traditional single season (rainy season) production-subsistence consumption and all-season production-large scale consumption situations, respectively. PS-indeterminate cultivars (compared to PIS-determinate cultivars) have greater ability/flexibility to match their growth and development cycle to prevailing rainfall duration in production regions, where sowing dates are highly variable (Keerthi *et al.* 2014b). PS-indeterminate cultivars also have the ability to compensate for large variation in plant stand density (a common feature in rainfed production system) and produce additional vegetative and reproductive organs and acceptable economic product yield (Huyghe 1998). Besides these favourable attributes, PS indeterminacy result in staggered flowering, pod formation and pod maturity leading to the availability of dolichos bean pods over a longer period of time, an essential requirement for subsistence consumption. Despite these advantages, PS indeterminacy is associated with low or unstable harvest index and consequently low or unstable seed yield due to competition between vegetative and reproductive phases for photosynthate partitioning (Huyghe 1998). Also, PS cultivars could be grown only during seasons with short-day lengths. These features restrict the suitability of PS-indeterminate cultivars to only rainy season-subsistence production and consumption system. However, market-led economy has necessitated production of dolichos bean throughout the year to ensure

continuous supply of pods on a commercial scale. The changed production–consumption scenario has triggered requirement for PIS determinate cultivars.

Determinacy is a plant architectural modification in grain legumes (Huyghe 1998). Determinacy is associated with more compact GH, reduced branching, shorter internodes, reduced above ground biomass and accelerated, and synchronized flowering (Kwak *et al.* 2012) leading to more uniform pod formation and maturity, and hence stable harvest index and seed yield (Jeuffroy and Ney 1997). Further, determinate cultivars enable high density planting to harness complete productivity potential of cultivars (Vishwanath *et al.* 1971; Shivashankar and Kulkarni 1989), the highly preferred feature for commercial production of dolichos bean. Determinate cultivars exhibit greater performance stability than their indeterminate counterparts in dolichos bean (Keerthi *et al.* 2014b). PIS facilitates farmers to produce dolichos bean across a range of production environments throughout the year irrespective of prevailing photoperiods. Also, farmers can control the date of flowering of PIS cultivars either by varying sowing time or choosing a cultivar with a different heat unit requirement in dolichos bean (Keerthi *et al.* 2014b). These attributes render suitability of PIS-determinate cultivars to all-season commercial production–consumption system. Due to these advantages, PIS determinate type cultivars are gaining popularity among the farmers. Hence, development of PIS determinate cultivars is one of the major objectives of dolichos bean breeding. Single/digenically-controlled linked farmers-preferred traits such as photoperiod insensitiveness, determinate GH and complete emergence of racemes suggest effectiveness of simple visual selection for these traits even from smaller early segregating generations. Further, independent (of PSFT, GH and raceme emergence) segregation of curved podedness, yet another farmer preferred trait indicated the possibility of combining all the four traits in pure-line varieties. Simple genetic control of the investigating traits facilitates development of NILs differing in each of these traits to uncover the possible biochemical/physiological pathways controlling the traits.

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