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Molecular tagging of photoperiod responsive flowering in Indian bean [*Lablab purpureus* (L.) Sweet]

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Abstract

Photoperiod responsive flowering and growth habit might have played a key role in domestication of lablab bean (*Lablab purpureus*) and currently shifting its cultivation from intercropping to monoculture. Most of the landraces of lablab bean exhibit photoperiod sensitivity in flowering and indeterminate growth habit. A cross was made between GNIB21 and GP189 which are phenotypic extremes for photoperiod responsive flowering. The F_1 hybrid exhibited dominant traits like indeterminate growth habit and photo-sensitive flowering endowed from male parent. Segregation pattern of 3:1 in F_2 generation indicated monogenic recessive nature of photoperiod insensitive flowering. Bulk segregant analysis in F_2 population revealed association of *PvTFLy1,* a locus governing determinate growth habit in lablab bean, with photoperiod responsive flowering where an amplicon of 300 bp was observed in photo sensitive GP189 while it was absent in photo insensitive variety GNIB21. Significant $\div^2$ test indicated coupling phase of linkage between *PvTFLY1* and photoperiod responsive flowering. Linkage analysis placed *PvTFLY1* at the distance of 19.23 cM from the locus governing photoperiod responsive flowering. The linkage between growth habit and photoperiod responsive flowering in common bean, soybean and Indian bean suggest that these traits may be governed by mutation or deletion of *E3* (or *GmPhyA3*) and *Dt1* homologs in Indian bean. Information available on characterized genes for photoperiod responsive flowering and determinate growth habit from common bean, soybean and other related legumes may be utilized for isolation, characterization, mapping and molecular dissection of genes involved in regulation of photoperiod responsive flowering in Indian bean.

Key words: Molecular tagging, photoperiod responsive flowering, bulk segregant analysis, terminal flowering locus, linkage analysis

Introduction

Indian bean [*Lablab purpureus* L. (Sweet)] is a predominantly self-fertilizing species with chromosome number 2n = 22, belongs to the family Fabaceae sub family Faboidene, currently regarded as monospecific. The common names for Indian bean include hyacinth bean, lablab bean, bonavist bean/pea, dolichos bean, seim bean, sem, Egyptian kidney bean, bataw and Australian pea. Genome size of the *Lablab purpureus* subsp. *purpureus* is 367 Mb (Iwata et al. 2013). Though, considerable variability exists in India, it is native to Africa and cultivated throughout the tropics for food and forages. It is a multipurpose crop grown for pulse or vegetable for human consumption or as forage for animals (Murphy and Colucci 1999). Indian bean is often grown as a weed suppressor, as a cover crop to avoid soil erosion or as a green manure crop. Looking to its ancient cultivation, immense genetic diversity and adaptability, it has potential to become model pulse crop in the era of genomics. Unfortunately, it has not attained the level of agricultural significance evident from lack of information on molecular mapping and genome sequence. No single marker has yet been reported linked with economically important trait. The reason could be severe competition by major pulses with better economics and non-realization of its importance due to scattered cultivation in tribal area as well as urban home gardens.

Human selection for different versions of growth habit and photoperiod responsive flowering during the

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process of domestication have changed the flowering time and crop duration of several legumes. These two traits are prime factors related to yield potential of legume crop like Indian bean. Growth habit and photoperiod responsive flowering might have played crucial role in domestication and evolution of Indian bean. Immense genetic resources, being a reservoir of valuable traits, offer scope for selection of agro-morphological traits in this crop. Most land races / cultivars of this crop are photoperiod sensitive (PS) and display indeterminate growth habit, whereas, improved varieties are photoperiod insensitive (PIS) as well as determinate types making possible its cultivation across the year either as pure or mixed crop. Photoperiod sensitive flowering restricts cultivation to specific crop season, hence it does not fit into different cropping pattern. Photoperiod insensitive flowering enables the transfer of valuable adaptive traits from land races to high yielding varieties by making hybridization feasible across the year.

Most of the landraces of lablab bean exhibit photoperiod sensitivity in flowering and indeterminate growth habit. Previous results revealed monogenic control of photoperiod responsive flowering. The genes controlling growth habit and photoperiod responsive flowering were linked in coupling phase (Keerthi et al. 2014b, 2016). Recessive *fin* (determinacy) and the dominant *Ppd* (photoperiod sensitivity) loci have been located on the B1 linkage group in common bean (Kwak et al. 2008). Ten loci *viz*., *E1-E9* and *J* controlling flowering time and maturity have been characterized at phenotypic and genetic levels in soybean (Cao et al. 2015). Of these, *E3* locus causes flowering delays under long days (Cober and Voldeng 1996). The recessive *e3* allele is associated with the control of long day insensitivity (Buzzell 1971). Watanabe et al. (2009) characterized and fine mapped *Dt1* linked *E3* (or *GmPhyA3*) locus in soybean located on chromosome number 19. The linkage between growth habit and photoperiod responsive flowering in common bean, soybean and Indian bean suggest that photoperiod insensitivity in Indian bean may be governed by mutation or deletion of *E3* homologue. Kong et al. (2010) detected two *FT* homologues *GmFT2a* and *GmFT5a* involved in the control of photoperiodic flowering in soybean. They concluded that loss-of-function alleles of the two *PHYA* genes (*e3e4*) and *FT* homologs coordinately control flowering and enable the adaptation of soybean to a wide range of photoperiodic environments. Fan et al. (2014) suggested the possibility of involvement of complicated *CO-FT* regulons in the photoperiod regulation of flowering time in soybean. Cao et al. (2015) pointed out that *GmCOL1a/b* may serve as suppressors of photoperiodic flowering in soybean under long day conditions by suppressing the florigens *GmFt2a/ GmFT5a* in cordination with *E1*, *E2*, *E3* and *E4*. Such *CO-FT* regulons might also exist in Indian bean. Recently, Cai et al. (2018) demonstrated CRISPR/ Cas9 mediated targeted mutagenesis of *GmFT2a*, which delayed flowering time in soya bean.

Development of photoperiod insensitive and determinate type cultivars is one of the major objectives for genetic improvement of Indian bean. Bulked Segregant Analysis (BSA) (Michelmore et al. 1991) is a rapid method for identification of molecular markers linked to qualitative trait. Identification of molecular marker linked to locus responsible for photoperiod responsive flowering may enable efficient selection as well as germplasm conversion to combine desirable yield attributes from photosensitive to photo-insensitive genotypes which are otherwise difficult to combine. It may eventually help in discovering possible biochemical pathways controlling this trait. The comparison of sequenced regions of common bean and soybean reveals high degree of conservation between them (Choi et al. 2004). Phylogenetic analysis and sequence information available in evolutionary related pulse crops may be helpful for identification and characterization of homologs governing photoperiod responsive flowering and determinate growth habit in Indian bean. Such molecular dissection may eventually aid to manipulation of flowering and growth habit in this crop. The present study was, therefore, conducted to determine the genetic nature of photoperiod responsive flowering and growth habit in lablab bean. Linkage analysis and molecular profiling was also done through bulk segregant analysis using SSR markers.

Materials and methods

The experimental material consisted of released variety GNIB21 and germplasm line GP189 obtained from Pulses and Castor Research Station, Navsari Agricultural University, Navsari, Gujarat, India. Variety GNIB21 is of determinate growth habit and photo insensitive nature, which flowers across the year irrespective of photoperiod. GP189 is indeterminate type and photosensitive, flowers only under short days of winter. The cross was made between keeping GNIB21 as female. True F_1s were distinguished from selfers on the basis of male dominant traits like

indeterminate growth habit, photo-sensitive flowering and purple flower. True hybrids identified in such a manner were selfed to generate F_2 population comprising of 386 plants. Parents, F_1s and F_2 population were screened for photoperiod responsive flowering. F_2 individuals which flowered within 45 days were considered as photo insensitive (PIS) and those flowered later than that under short days were considered as photo sensitive (PS) following the approach adopted by Prasanthi (2005). Segregation for photoperiod responsive flowering was confirmed using χ^2 test.

Bulk Segregant Analysis was followed for molecular tagging of photoperiod responsive flowering using parents, F_1 and F_2 population with various gene specific/SSR (Simple Sequence Repeats) primers from related pulse crops like common bean and soybean, as till now, there has been no molecular study conducted in Indian bean in relation to photoperiod responsive flowering. Genomic DNA was extracted from juvenile and healthy trifoliate leaves by CTAB method (Zidani et al. 2005) with some modifications. Quantification of DNA was done with the help of spectrophotometry using Nanodrop (Thermo, USA) at absorbance ratio of 260/280 nm. Quality of genomic DNA was analyzed by 0.8 % agarose gel electrophoresis using 1x Tris EDTA (TE) buffer and ethidium bromide (5 µl/100 ml of Agarose) as staining agent. The forward and reverse primer sequences 5'CTTCTTGTGATGTAAGTGTTTG3' and 5'GATGTT CCWGGWCCTAGTGAYCC3' (Kwak et al. 2008), associated with growth habit and photoperiod responsive flowering in common bean were used for genotyping of parents, F_1 and F_2 individuals as only this pair showed polymorphism between parents in dominant fashion. Equal quantity of DNA was bulked from 15 photoperiod insensitive and 15 photoperiod sensitive F_2 plants. The amplification was carried out in Eppendorf Thermal Cycler. PCR conditions for amplification of gene specific primer *PVTFL1y* followed was initial denaturation at 94°C for 5 min, denaturation 94°C for 1 min, annealing 57°C for 45 sec, extension 72°C for 1 min, final extension 72°C for 10 min, halt 4°C. PCR amplified products were fractionated on 1.5 % (w/v) agarose gel electrophoresis.

Linkage analysis of marker and locus governing photoperiod responsive flowering was done manually. The recombinant fraction calculated by maximum likelihood method (Immer, 1930) was converted into map distance and expressed as centi Morgan (cM) using the Kosambi map function (Kosambi, 1944). The standard error for linkage distance was calculated using the method suggested by Adams and Joly (1980).

Results and discussion

Two phenotypic extremes *viz*., GNIB-21 and GP-189 were selected to identify marker linked with the locus governing photoperiod responsive flowering. GNIB-21 is early flowering and early maturing determinate variety while GP-189 is late flowering/maturing indeterminate variety with continuously growing vegetative shoot apex (Fig. 1). True F_1 hybrid obtained

GNIB-21 GP-189 GNIB-21 GP-1890

Fig. 1. Parents and F_1 derived from the cross GNIB 21 × GP-189

from these parents exhibited dominant traits like indeterminate growth habit, photo-sensitive flowering and purple flower colour endowed from male parent. The parents were also contrasting for flower colour, pod curvature, pod size, pod colour, pod shape, pod length, pod beak etc which resulted into tremendous variation in F_2 population at field level.

Out of total 386 F_2 individuals, 304 plants manifested photo sensitive flowering, while 82 plants exhibited photo insensitive flowering. χ^2 test (0.200, > 0.01) was found non-significant with expected ratio of 3 : 1 (PS : PIS, Table 1), revealing monogenic recessive nature of photoperiod insensitive flowering. The results of present investigation revealed that photoperiod insensitive flowering is governed by single recessive gene under biallelic monogenic control, photosensitive flowering being dominant over photo-insensitive flowering. Similar findings were reported by Prasanthi (2005) and Keerthi et al. (2014a). However, flowering and maturity traits are under control of several loci *viz*., *E1* to *E9* and *J* in soybean (Xia *et al*., 2012). Flowering and maturity in Indian bean may also be controlled by multiple loci and quantitative in nature. However, loss of function mutation at one locus, probably gene governing phytochrome, may result into monogenic inheritance of photo-insensitive flowering

Table 1. Segregation of photoperiod responsive flowering in F_2 population of a cross GNIB-21 x GP-189

Classes	Observed frequencies in F_2	Expected frequencies in F_2	Expected ratio	χ^2 probability
Phenotypic segregation				
PS	304	289	3 : 1	0.200
PIS	82	97		
Marker segregation				
Presence of band	113	135	3 : 1	0.012
Absence of band	67	45		
Marker trait co-segregation				
90 PS (+)*	85	101	9:3:3:1	0.00001
90 PS (-)	05	34		
90 PIS (+)	28	34		
90 PIS (-)	62	11		

* : + and - indicates presence and absence of bands, respectively

Out of total 36 primer pairs tested, 8 pairs manifested monomorphic bands between the parents, while 11 and 16 primer pairs resulted into multiple bands and absence of polymorphism, respectively. Surprisingly, one of the primer pairs showing multiple bands belonged to *E3* locus. Only one primer pair exhibited polymorphism between the parents (Table 2). Among all gene specific primers, only primer pair

Table 2. Amplification success for the 36 gene specific or SSR primers of related pulse crops in *Lablab purpureus*

S.No.	Description	No. of primer pairs
1	Monomorphic bands	08
2	Polymorphic bands	01
3	Double/ Multiple bands	11
4	No amplification	16
5	Total number of primers tested	36

specific to *PvTFL1y* (terminal flowering locus) of common bean showed polymorphism (Kwak et al. 2012). The primer pair amplified band of approximately 300 bp in photo sensitive parent GP-189 while it was absent in photo insensitive female parent GNIB-21. However, as expected the band was observed in F_1 hybrid (Fig. 1). BSA method given by Michelmore et al. (1991) was followed for tagging of locus responsible for photoperiod responsive flowering utilizing gene specific primer pair belonging to *PvTFLy*. Equal quantity of DNA was pooled from 15 photo insensitive and 15 photo sensitive F_2 individuals to create PIS bulk (PISB) and PS bulk (PSB), respectively. DNA samples from parents, F_1, PISB and PSB were subjected to PCR amplification utilizing polymorphic primer pair. A band of 300 bp was amplified in photosensitive parent GP-189, F_1 and PSB while it was absent in GNIB-21 and PISB. These results indicated association of *PvTFLy* homologue with locus responsible for photoperiod responsive flowering in Indian bean (Fig. 2).

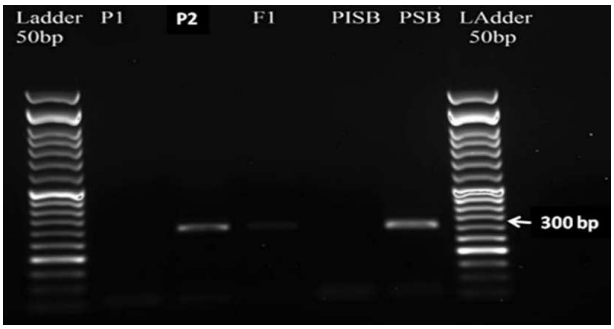

Fig. 2. DNA polymorphism between parents [P1 = GNIB21 (Photoperiod insensitive), P2 = GP189 (Photoperiod sensitive)], F_1 (GNIB-21 × GP-189), PISB = Photoperiod insensitive bulk and PSB = Photoperiod sensitive bulk

Molecular profiling of 180 individuals from F_2 indicated that among 90 photoperiod insensitive individuals, the band was absent in 62 individuals same as parents GNIB-21 while in 28 individuals, band was present indicating their recombinant nature (Table 1). Among 90 photoperiod sensitive individuals, the band was present in 85 individual same as photo sensitive parent GP-189. However, this band was absent in 5 photosensitive individuals suggesting their recombinant nature. χ^2 test (0.012, > 0.01) indicated independent segregation of marker alleles. The significant χ^2 test for independent assortment between marker and locus responsible for photoperiod responsive flowering indicated presence of linkage in coupling phase (χ^2 = 0.0001, <0.01). Linkage map distance was manually calculated from recombination fraction following maximum likelihood method and kosambi mapping function. The marker *PvTFLy* is situated at the distance of 19.23 + 0.03 cM from the locus governing photoperiod responsive flowering.

The linkage between determinate growth habit and photoperiod responsive flowering was observed by Keerthi et al. (2014a, 2016) at phenotypic level in Indian bean. Cober and Voldeng (1996) also reported a linkage relationship between the *E3* and *Dt1* loci which is related to a growth habit in soybean. *PvTFLy* locus in common bean is related with growth habit and cosegregated with determinacy locus "*fin*" (Kwak et al. 2008), *PvTFLy* governing indeterminate growth habit while its recessive counterpart *Pvtfly* is responsible for determinate growth habit. Watanabe et al. (2009) characterized and fine mapped *Dt1* linked *FT3* (26 cM) locus corresponding to *E3* or *GmPHYA3* located on chromosome number 19 utilizing RHL (Residual Heterozygous Line) in soybean. The findings of present study clearly indicated that photoperiod insensitive flowering and determinate growth habit in Indian bean are governed by recessive alleles of *GmPHYA3* and *Dt* homologs.

Similar to other pulses, determinate growth habit and photoperiod insensitivity might have played very important role in evolution and domestication of Indian bean, however, the actual genes responsible for these traits have not been identified nor even the markers linked to it. Even reports on inheritance pattern of these traits in Indian bean are very scanty. Phylogenetic analysis indicated a close evolutionary relationship between Indian bean, common bean and soybean (McClean et al. 2010). The linkage between growth habit and photoperiod responsive flowering in common bean, soybean and Indian bean suggest that these traits may be governed by mutation or deletion of *GmPHYA3* and *Dt1* homologs. This study is first report of molecular tagging of photoperiod responsive flowering in the world as well as probability of involvement of *GmPHYA3* and *Dt1* homologs in governing photoperiod responsive flowering and growth habit in Indian bean. The information available on characterized genes for photoperiod responsive flowering and determinate growth habit as well as markers linked to them from common bean, soybean and other related legumes may be utilized for isolation, characterization and mapping of genes involved in regulation of flowering and growth habit in Indian bean. Utilization of next generation sequencing based genomics as well as transcriptomics tools and mapping population like recombinant inbred lines (RILs), near isogenic lines (NILs) and RHL may further accelerate this process of molecular dissection and manipulation of photoperiod responsive flowering in Indian bean.

Authors' contribution

Conceptualization of research (KGM); Designing of the experiments (K G M, V P); Contribution of experimental materials (BHK, KGM); Execution of field/lab experiments and data collection (VR, KGM, GV, BHK, VPAB); Analysis of data and interpretation (VR, KGM, VP); Preparation of the manuscript (KGM, RKP, VP).

Declaration

The authors declare no conflict of interest.

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