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RESEARCH ARTICLE

Heterogenous inbred families-derived Near Isogenic Lines for growth habit in Dolichos bean [*Lablab purpureus* (L.) Sweet]

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Abstract

Identification of markers linked to loci controlling economically important traits, including growth habits, helps the selection of genotypes with desired growth habits at the seedling stage itself in crops, with no exception of the dolichos bean. Near isogenic lines (NILs) differing for a target locus are the most appropriate genetic resources for the identification of dependable genomic resources for use in a marker-assisted selection of genes controlling growth habit. In the present study, we found that 30 $F_{2:5}$ progenies derived from a cross between determinate and indeterminate parents segregated for a few morphological traits, including growth habit. A total of 30 $F_{2:5}$ progenies were regarded as heterogeneous inbred families (HIFs). Among these 30 $F_{2:5}$ HIF, seven were segregated only for growth habits. From these seven HIFs, seven pairs of plants differing in growth habit were selected and were regarded as candidate NILs for growth habit. Of these, only six pairs were segregated for growth habit as inferred by polymorphism for alleles at SSR marker (LPD 19) linked to growth habit. Of these, only four pairs of NILs showed monomorphism (>80%) at most of the 94 background SSR markers and hence were regarded as the most putative NILs. These NILs serve as ideal genetic resources to assess the effect of growth habit genes on non-target traits in the dolichos bean.

Keywords: Dolichos bean**,** NILs, HIFs, SSR, Growth habit

Introduction

Dolichos bean or hyacinth bean or Indian bean [*Lablab purpureus* (L.) Sweet] is one of the oldest legume crops grown in dry and semi-arid regions of Asia, Africa and America (Ramesh and Byregowda 2016). In Asia, India and Bangladesh are the major countries where dolichos bean is grown. It is grown in both rainfed and irrigated ecosystems for fresh beans for use as a vegetable. While fresh pods are harvestable and marketable economic products, fresh beans are consumable economic products in dolichos beans. In dolichos bean, determinate and indeterminate types of growth habits have been reported. While the main stem continues to grow and only primary and secondary branches produce racemes in indeterminate genotypes, the main stem terminates in racemes with primary and secondary branches also producing racemes in determinate genotypes (Ramesh and Byregowda, 2016). As is true in other grain legumes (Krylova et al. 2020), growth habit is one of the economically important domestication-driven adaptive traits. Traditionally, most former cultivars exhibit indeterminate growth habits. Of late, increasing trends towards practicing mechanized agriculture driven by an

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acute shortage of labor have enhanced demand for cultivars with determinate growth habits. Determinate cultivars, owing to their short crop cycle, synchronous flowering, pod development and maturity, are most suitable for mechanical harvesting. These considerations have prompted breeders to develop high-yielding cultivars in determinate growth habit backgrounds. Though growth habit is an easily observable and monitorable trait under field conditions, researchers need to wait at least 40-45 days after seed planting for the selection of genotypes with desired growth habits. Identification of DNA markers closely linked to loci controlling growth habit would facilitate the identification of determinate genotypes either in germplasm and/or in breeding populations early in the seedling stage. Early identification of determinates of the genotypes helps enhance the pace and efficiency of breeding *Dolichos* bean cultivars. Near isogenic lines (NILs) differing for growth habit is the most appropriate genetic material to identify dependable DNA markers linked to loci controlling growth habit. Our objective is to report phenotypic and DNA markers-assisted identification of four pairs of growth habit-differing NILs derived from heterogeneous inbred families (HIFs) (Tuinstra et al. 1997) developed from a cross between highly related determinate and indeterminate genotypes.

Materials and methods

Development of pairs of NILs differing for growth habit

The basic material for our study consisted of determinate (HA 4) and indeterminate (HA 5) genotypes(Table 1). HA 5 is highly related to HA 4 as it is developed using pedigree selection from segregating population derived from HA 4 × GL 153 (Ramesh et al. 2018). HA 5 was crossed as a male parent to HA 4 as a female parent using hand emasculation and pollination in the experimental plots of the Department of Genetics and Plant Breeding (GPB), College of Agriculture (CoA), University of Agricultural Sciences (UAS), Bangalore, India during 2020 summer season. Seeds collected from HA 4 were planted. Selfedseeds from 10 true F_1s (identified based on the dominance of indeterminacy over determinacy) were planted during the 2020 rainy season to raise F_2 generation. Survived 144 F_2 plants were advanced to F_5 generation by single seed descent method. Seeds collected from 144 $F_{2:5}$ plants were planted in plant-to-row progeny families during the 2022 summer season.

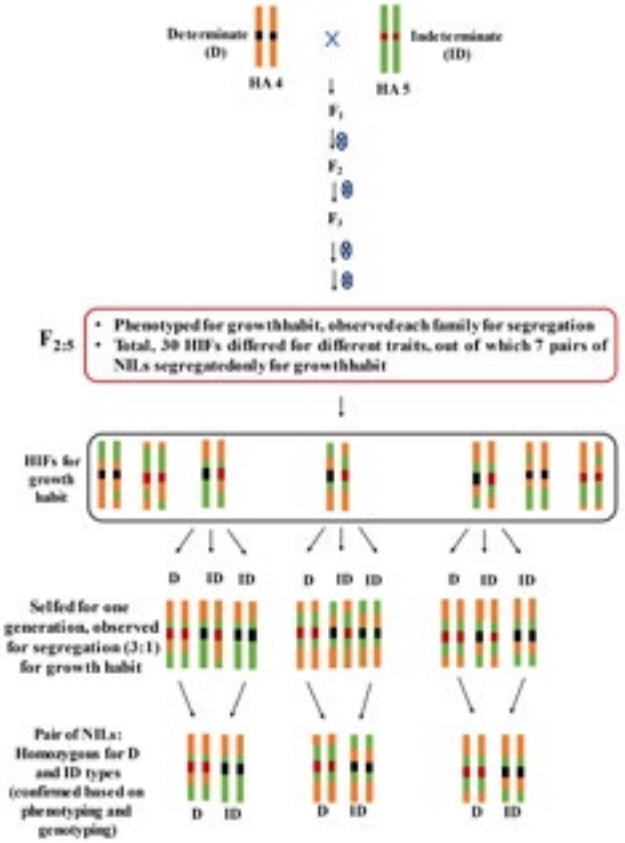


Fig. 1. Schematic representation of development of pairs of near isogenic lines derived from heterogenous inbred families (HIFs) differing for locus controlling growth habit

Identification of candidate NILs

Given that parents are highly related and based on empirical results (Tuinstra et al. 1997), we hypothesized that 144 $F_{2:5}$ progenies would have attained homozygosity at most loci and, therefore, most $F_{2:5}$ progenies would become homogeneous. However, upon visual inspection of each of the 144 $F_{2:5}$ progenies, we found that 30 $F_{2:5}$ progenies were segregated for a few morphological traits, including growth habit. We regarded these 30 $F_{2:5}$ progenies as HIFs. From among these 30 $F_{2:5}$ HIF, seven were segregated only for growth habit and not for any other trait for which the two parents differed. From these seven HIFs, seven pairs of plants differing in growth habit were selected and were regarded as candidate NILs for growth habit (Fig. 1).

Table 1. Growth habit, photoperiod sensitivity and pedigree/source of parents used to derive crosses in dolichos bean

Parents	Growth habit	Photoperiod sensitivity	Source	Pedigree/Source	References
HA 4	Determinate	Insensitive	Karnataka, India	HA 3 × Magadi local	Ramesh and Byregowda (2016)
HA 5	Indeterminate	Insensitive	Karnataka, India	HA 4 × GL 153	Ramesh et al. (2018)

Table 2. SSR marker-based background genome similarity between the pairs of NILs identified from heterogeneous inbred families derived from HA 4 × HA 5 cross

NILs	Pair of NILs	No. of Markers	Monomorphic markers	%Background genome similarity
HA4/HA5-2020-F_5-	1-D and 1-ID	94	62	67.39
	6-D and 6-ID	94	77	83.69
	19-D and 19-ID	94	67	72.82
	22-D and 22-ID	94	81	88.04
	67-D and 67-ID	94	80	86.96
	125-D and 125-ID	94	79	85.86

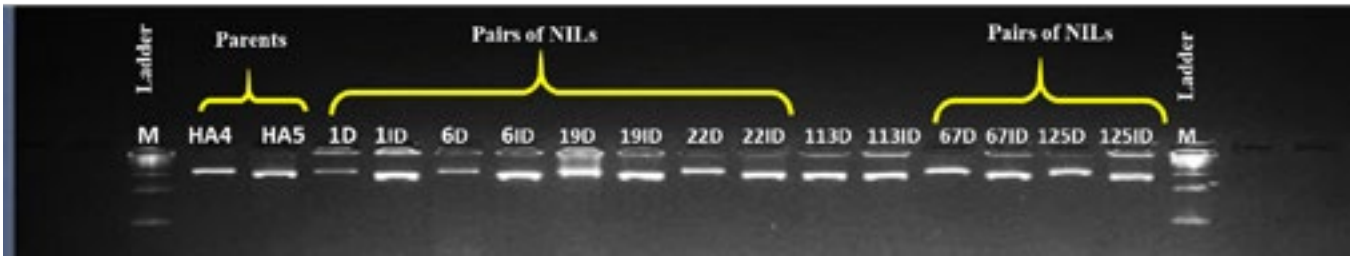


Fig. 2. SSR marker-based confirmation of NILs (identified from $F_{2:5}$ HIFs derived from HA 4 ×HA 5) for growth habit using linked SSR marker (LPD 19)

Phenotypic and DNA marker-assisted confirmation of candidate NILs

The seeds from seven pairs of candidate NILs were collected and selfed. The selfed seeds were planted during the 2022 rainy season. The surviving plants from candidate NILs were observed for segregation, if any, for growth habit. The determinate and indeterminate component lines of all the seven pairs of NILs bred true indicating that NILswere fixed for growth habit.

To confirm that NILs differ only for growth habit, we genotyped the seven pairs of NILs using the reported growth habit-linked SSR marker (LPD 19) (Basanagouda 2022). The PCR products were resolved on 4% meta-agarose gel electrophoresis. The NILs were examined for marker alleles specific to determinate (HA 4) and indeterminate (HA 5) parents. The presence of linked SSR marker allele-specific to HA 4 and HA 5 in determinate and indeterminate components of NIL pairs indicates that NILs truly differ in growth habit. All the seven pairs of candidate NILs except one (113-D and 113-ID) differed for alleles at SSR marker (LPD 19) linked to loci controlling growth habit (Fig. 2). We, therefore, considered only these six candidates as true NILsdiffering for growth habit (Table 2).To confirm that NILs share similar genomic regions, we genotyped NILs using 94 background SSR markers for which parental genotypes (HA 4 and HA 5) differed. The PCR products were visualized on 3.5% agarose gel electrophoresis. The % background genome similarity between pairs of NILs was estimated as (number of monomorphic markers/total number of background markers) × 100. Monomorphism for most background SSR markers indicates similar genomic regions of NILs except for regions controlling growth habit genes. The % of parental allele contribution to each determinate and indeterminate NIL was estimated as (number of HA 4 and HA5 type alleles/total number of alleles) × 100 at all the 94 background markers. As NILs were selected from $F_{2:5}$ RIL population, the determinate and indeterminate components of NILs were expected to display a mosaic of genomic regions derived from their parents. To visualize this, graphical genotyping was performed by randomly assigning the positions of markers on 11 different linkage groups (LGs) (as 2n = 22 chromosomes in dolichos bean) using GGT version 2.0 software (Van Berlo 2008).

Results and discussion

Development of NILs from HIFs is a cost-effective approach (Wang et al. 2019). The special feature of HIFs is that their genomic composition, although homozygous, is a mosaic of genomic regions of the two parents. This feature enables the selection of more than one HIF with different genetic backgrounds. The selection of pairs of genotypes from these HIFs differing only for target traits (such as growth habit in our study) results in NILs with different genetic backgrounds. This means that HIFs are useful for developing multiple sets of NILs with different genetic backgrounds from the RIL population derived from a single cross (Loudet et al. 2005; Wang et al. 2019). Considering these advantages of HIFs, we identified six four pairs of NILs differing in growth habits from HIFs derived from crosses involving highly

Table 3. Representation of the parental alleles (HA 4 & HA 5) among the six pairs of NILs identified from the heterogeneous inbred families of HA 4 × HA 5 cross

NILs	NIL-D		NIL-ID	
	HA 4	HA 5	HA 4	HA 5
HA4/HA5-2020-F_5-6	59 (64.13)	33 (35.87)	45 (48.91)	47 (51.09)
HA4/HA5-2020-F_5-22	46 (50.00)	46 (50.00)	43 (46.74)	49 (53.26)
HA4/HA5-2020-F_5-67	54 (58.70)	38 (41.30)	46 (50.00)	46 (50.00)
HA4/HA5-2020-F_5-125	39 (42.39)	53 (57.61)	36 (39.13)	56 (60.87)
Average parental alleles (%)	53.81	46.19	46.19	53.81

Values in the parentheses indicate the % parental allele

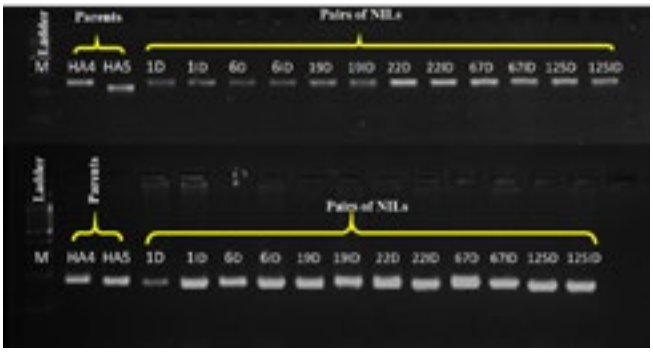


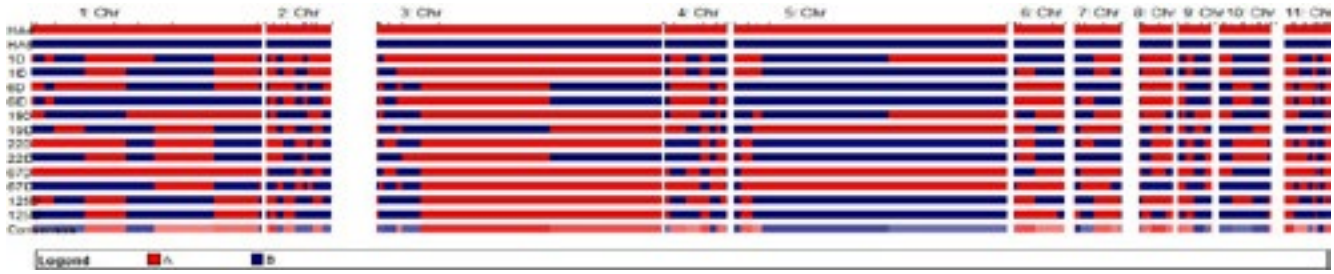
Fig. 3. Agarose gel image showing monomorphism of NILs (differing for growth habit) for background SSR marker

related determinate and indeterminate parents (Table 2). All six pairs of NILs differed for alleles at the SSR marker (LPD 19) linked to genes controlling growth habit. Determinate and indeterminate component lines of all the six pairs of NILs produced alleles specific to determinate (HA 4) and indeterminate (HA 5)parents, respectively (Fig. 2). The extent of similarity between six pairs of NILs (as indicated by %monomorphic markers) ranged from 67.39 to 88.04% (Table 2, Fig. 3). Out of six pairs of NILs, four pairs showed genome similarity of more than 80%. We, therefore, finally considered these four pairs (6-D/6ID, 22-D/22-ID, 67-D/67-ID and 125-D/125-ID) as NILs differing for growth habit.

The relative representation of the two parental genomes among the four pairs of NILs using the 94 background SSR markers was also examined in the present study. Representation of parental genomes varied widely among four pairs of NILs, while HA 4 specific alleles ranged from 39.13 to 64.13%, those of HA 5 type alleles ranged from 35.87 to 60.87% among four pairs of NILs (Table 3). The average representation of determinate parental (HA 4) alleles was marginally greater than those of indeterminate parental (HA 5) alleles within determinate NILs. Reverse was true within indeterminate NILs (Table 3). Further, these NILs differed in the composition of parental genomes, as could be inferred from wide variation with respect to the representation of parental alleles. The differences in the composition of parental genomes among NILs are a result of the transmission of recombined parental chromosomal segments driven by meiotic cross-over events right from F_1 to F_5 generations (Tuinstra et al. 1997). The distribution of parental origin and genome composition across entire genomes of NILs is evident from graphical genotypes (Fig. 4) mapped using graphical genotyping (GGT). The concept of GGT was proposed by Young and Tanskley (1989) to display the mosaic of parental genomes in progeny derived from biparental crosses. This is the first report on developing NILs differing in growth habits from HIFs in *Dolichos* beans. To date, HIF-derived NILs have been reported in other legume crops such as groundnut (Yeri et al. 2014) and soybean (Kato et al. 2018; Kato et al. 2019).

Implications in Dolichos bean breeding

The NILs reported in the present study could be used for fine mapping and functional characterization of loci controlling



*LG – Linkage groups

Fig. 4. Graphical genotypes of HIF-derived four pairs of NILs in dolichos bean

growth habit. Fine-mapped and functionally characterized genes facilitate the design of markers for use in a selection of genotypes with desired growth habits without linkage drag. The unique advantage of HIF-derived NILs is that by virtue of their independent genetic background (Kooke et al. 2012), they offer the possibility of assessing the effect of growth habit locus that exist in different genetic backgrounds on non-target economically important traits such as fresh pod yield, the harvestable and marketable economic product in dolichos bean.

Authors' contribution

Conceptualization of research (MPK, SR); Designing of the experiments (MPK, SR); Contribution of experimental materials (MPK, SR, KM); Execution of field/lab experiments and data collection (MPK,CBS, GB, HS, NK, DS, MK, RK, BRC, JVG); Analysis of data and interpretation (MPK); Preparation of manuscript (MPK, SR).

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