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Identification and characterization of contrasting genotypes for productivity traits from a core set of dolichos bean germplasm

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Abstract
A study was undertaken to identify contrasting accessions for pod productivity traits from a core set of dolichos bean germplasm. Among different combinations of phenotypically contrasting accessions for pods plant^{-1}, highest numbers of SSR markers were polymorphic between GL 577 and KA followed by GL 577 and GL 142. Similarly, between GL 201 and GL 527, which are contrasting for fresh pod yield plant^{-1}, eleven SSR markers, were found polymorphic. Nine SSR markers differentiated two pairs of genotypes GL 201 and KA and GL 355 and GL 576 which were contrasting for fresh seed yield plant^{-1}. These quantitative traits and SSR marker alleles-based diverse genotypes could be used as putative parents for developing mapping population to identify QTLs controlling yield associated traits and/or could be used to effect crosses to derive superior pure lines for use as varieties for commercial cultivation of dolichos bean.

Keywords: Core set, Diversity, Germplasm, Lab lab, SSR

Introduction
Dolichos bean (*Lablab purpureus* L. Sweet) with 2n=22 chromosomes (She and Jiang, 2015) [20] is commonly known as field bean, hyacinth bean, Indian bean, sem, bonavista bean, lubia bean, butter bean and Egyptian kidney bean. It belongs to the family Fabaceae, sub-family Faboideae, tribe Phaseoleae and sub-tribe Phaseolineae. It is one of the oldest legume crops grown in Asia, Africa, and Australia (Ayyangar and Nambiar, 1935) [2] and known for its food (Vishwanath, *et al*.,1971) [29] and fodder value (Magoon, *et al*., 1974) [14].The dolichos bean is believed to have originated in India (Nene, 2006) [16] as it is documented by archaeo-botanical finds in India from 2000 to 1700 BC at Hallur, the earliest Iron Age site in Karnataka, to 1200-300 BC at the Veerapuram excavation site in Andhra Pradesh (Fuller, 2003) [9]. From India, it is believed to have been introduced into China, Western Asia and Egypt (Ayyangar and Nambiar, 1935) [2].

Dolichos bean is highly popular in South Asia, where it is grown in rainfed ecosystems (Rahman, *et al.,* 2002; Haque, *et al.,* 2003) [17& 10]. It is the third most important vegetable in central and south-western parts of Bangladesh (Rashid, *et al.,* 2007) [18]. In India, dolichos bean var. lignosus is primarily grown as a rainfed crop in Karnataka and adjoining districts of Tamil Nadu, Andhra Pradesh and Maharashtra both as an inter-crop and pure crop (Mahadevu and Byregowda, 2005) [15]. Despite its importance as a vegetable, pulse, forage, cover and green manure crop (Adebisi and Bosch, 2004) [1], dolichos bean has remained as an 'underutilized crop' as evidenced from limited area planted to this crop and efforts towards its genetic improvement (Shivashankar and Kulkarni, 1989) [20].

Enhancing the pace and efficiency of dolichos bean breeding for pod yield and its component traits require adoption of a well-conceived strategy that hinges on increased use of available plant genetic resources (PGR), identification of trait based genotypes, identification and introgression of key genes/ Quantitative Trait Loci (QTLs) etc,. The core collections have been suggested as the most efficient and reliable sources of PGR for initial search for trait-specific accessions (Upadhyaya, 2015) [26]. Following the concept of Frankel and Brown (1984) [8], a core set (n=64; 10% of the whole collection) representing ≥ 90% diversity of entire collections was developed in dolichos bean (Vaijayanthi, *et al*., 2015a) [27]. Due to its small size, the core set provided an easy means for evaluation across multilocations/ years to identify promising

germplasm accessions as new and potential donors for various qualitative and quantitative economically important traits. Under this premise, the present investigation was undertaken to identify and characterize contrasting germplasm accessions for productivity traits. This will enable their utilization as putative parents for developing mapping population to identify key genes/QTLs controlling productivity traits in dolichos bean.

Material and Methods

Experimental material: The material consisted of 64 core set germplasm accessions of dolichos bean and two check entries [HA-4 and kadalavare (KA)] maintained at All India Coordinated Research Project (AICRP) on pigeon pea, University of Agricultural Sciences (UAS), Bengaluru. Half of the cores set accessions are of Indian origin (mainly from Karnataka, Andhra Pradesh, Maharashtra, Gujarat, Tamil Nadu and Kerala) and some are of exotic origin, while others are of unknown origin (Vaijayanthi, *et al*., 2015b) [28].

Phenotyping core germplasm accessions: The core germplasm accessions, along with two check entries, were sown in an augmented design (Federe, 1956) [7] in seven compact blocks during 2014 and 2015 late rainy seasons at the experimental plot of ZARS, UAS, Bengaluru. Each block consisted of 13 germplasm accessions, two checks (replicated twice) and two border entries. The seeds of each entry were sown in a single row of 2.5 m length, with a row spacing of 0.45 m. Ten days after sowing; seedlings were thinned by maintaining a spacing of 0.2 m between plants within a row. A basal dose of 25:50:25 Kg ha^{-1} of NPK was applied to the experimental plots. Recommended management practices were followed during the crop-growing period to raise a healthy crop.

Data were recorded on five randomly tagged plants on three farmer and consumer preferred qualitative traits, *i.e.,* growth habit, pod fragrance and pod constriction. The data were also recorded on three important pod productivity traits, i.e., pods plant^{-1}, fresh pod yield plant^{-1} and fresh seed yield plant^{-1} following the descriptors (Byre Gowda, *et al*., 2015) [5].

Genotyping core germplasm accessions: Young leaves from 21 days old seedlings were collected from each germplasm accessions and genomic DNA was extracted following cetyl Trimethyl Ammonium Bromide (cTAB) extraction method (Doyle and Doyle, 1987) [6] with minor modifications. To assess the quality of DNA, samples were run on 0.8 per cent agarose gel. The quantity of DNA was assessed by comparing the band intensity with that of λ DNA (50bp). The core germplasm accessions were genotyped with a total of 234 SSR markers which included 198 in-house developed SSR markers and 36 transferable cross legume species/genera SSR markers. PCR was carried out in a total reaction volume of 10 µl mixture. PCR products were visualized on 4 % agarose gel. The genotype profiles produced by SSR markers were scored manually. The amplicons of SSR priming regions of genomic DNA at defined product size range (the amplicons in the same row) were scored as '1' for presence of amplicon and '0' for absence of amplicon for each SSR marker locus. The variation in amplicon intensity was not taken into consideration to avoid confusion in scoring.

Statistical analysis

The quantitative trait means of each germplasm accession and each check were used for analysis of variance (ANOVA). Quantitative trait means of each of the 64 core set of germplasm accessions were adjusted for block effect. The adjusted quantitative trait means were used for identifying contrasting accessions (those represent extreme trait values). Further, the number of polymorphic SSR markers between phenotypically most contrasting pairs of accessions (for pods plant^{-1}, fresh pod yield plant^{-1} and fresh seed yield plant^{-1}) was assessed manually.

Results and Discussion

Variability for qualitative traits: Availability of trait-specific germplasm and its judicious use is critical for success of any breeding programme (Upadhyaya, *et al*., 2013) [25]. In the present study, the core set of 64 germplasm accessions were characterized for three consumer/end-user preferred qualitative traits such as growth habit, pod fragrance and pod constriction. High level of polymorphism was detected for all the qualitative traits. Indeterminate type of accessions dominated the core set (57.81%) followed by semi-determinate (28.13%) and determinate (14.06%) types (Table 1). Highly frequent indeterminate/ semi-determinate types of accessions are expected as farmers preferred cultivars with indeterminate/semi-determinate types owing to their staggered production of pods over a period of time (Keerthi, *et al*., 2014) [11]. The accessions with high pod fragrance were abundant (42.18%) than those with moderate (34.37%), low (17.18%) and absence of fragrance (6.25%) (Table 1). Pod fragrance has been attributed to oily exudates which have been reportedly composed of a mixture of fatty acids of which trans-2-dodecenoic acid and tetra-dodecenoic acids are predominant (Uday Kumar, *et al*., 2016) [23]. The pods with slight constriction were present in 53.12% of the accessions, while those with constriction and absence of constriction were present in 17.19% and 29.69% of the accessions, respectively (Table 1).

Table 1: Variability for growth habit, pod fragrance and pod constriction and their frequency in a core set of dolichos bean germplasm accessions

Sl. No.	Traits	Score	Classified as	Frequency	Per cent
1	Growth habit	1	Determinate	09	14.06
		2	Semi-determinate	18	28.13
		3	Indeterminate	37	57.81
2	Pod fragrance	0	Absent	04	6.25
		1	Low	11	17.18
		2	Medium	22	34.37
		3	High	27	42.18
3	Pod constriction	0	No constriction	19	29.69
		3	Slightly constricted	34	53.12
		5	Constricted	11	17.19

As these qualitative traits are easily scorable/assayable, show simple inheritance (single/oligogenic) and stable expression, and selectively neutral (Smith and Smith, 1992) [22], they serve as diagnostic markers of germplasm accessions for maintaining their identity and purity. They also aid in identification and minimizing duplication and avoid mistakes in labeling the germplasm. They are also useful in conduct of Distinctness (D), Uniformity (U) and Stability (S) test, a mandatory requirement for protecting crop varieties under Protection of Plant Varieties and Farmers Rights (PPV&FR) Act in India.

Variability for quantitative traits: The pooled ANOVA revealed highly significant mean squares attributable to

'germplasm accessions' for all traits. Mean squares attributable to 'check varieties' and 'accessions *vs* check varieties' was highly significant for all traits. These results suggested significant differences among the accessions and they differed from the checks.

Identification of contrasting accessions: The two year-pooled phenotypic data and data on alleles at 95 polymorphic SSR marker loci were used to identify contrasting germplasm accessions for important productivity traits. The accessions such as KA, GL 142, GL 633, FPB 35 and GL 110 showed highest number of pods per plant. While the accessions, GL 204, GL 237, GL 577, KNR and FPB 20 showed least number of pods. GL 576, GL 527, GL 142, FPB35 and KA (high pod yield) and GL 661, GL 228, GL 201, GL 204 and GL 205 (less pod yield) were the contrasting accessions for fresh pod yield plant^{-1}. The accessions such as KA, FPB 35, GL 576, GL 142 and GL 12 (higher seed yield) and accessions such as GL 385, GL 228, GL 355, GL 201 and GL 661(less seed yield) were the contrasting accessions for fresh seed yield plant^{-1}. These contrasts are also exceptionally desirable for pod fragrance and constriction, the two most farmer/consumer preferred qualitative traits.

Several DNA-based markers are available for genetic diversity analysis. The SSR markers are now the markers of choice in various applications of plant breeding research as they are codominant, multi-allelic, highly polymorphic even between closely related lines, require low quantity of DNA, can be easily automated for high throughput genotyping, can be exchanged between laboratories and are highly transferable between populations. SSR marker assay help understand genetic relationship among germplasm accessions/ breeding lines, selection of parents for hybridization, organization of variation in germplasm accessions and identification of cultivars (Benabdelmouna, *et al*., 2001) [3]. The SSR markers were used to study the genetic diversity in core collections of chickpea (Upadhyaya, *et al*., 2008) [24], genus *Arachis* (Koppolu, *et al*., 2010) [12], common beans (Blair, *et al*., 2009) [4], peanut (Kottapalli, *et al*., 2007) [13], etc.

Of the 234 SSR markers used in the study, 187 markers were amplified. Among the 187 amplified markers, 95 (50.80%) were found polymorphic and the number of alleles per SSR locus varied from 1 to 3 with an average of 2.21 alleles. As many as 20 markers were tri-allelic (most informative); 75 markers were bi-allelic and 92 markers were mono-allelic (least informative). The 20 tri-allelic SSR markers exhibited greater ability to discriminate the germplasm accessions as reflected by higher estimates of polymorphic PIC and Nei's gene diversity. Saravanan *et al*. (2013) [19] also reported higher discriminating power of 10 SSR loci and a fairly higher polymorphism (81 %) among 39 dolichos bean genotypes.

Among different combinations of phenotypically contrasting accessions for fresh pods plant^{-1}, highest numbers of SSR markers were polymorphic between GL 577 and KA followed by GL 577 and GL 142 (Table 2). Similarly, between GL 201 and GL 527, which are contrasting for fresh pod yield plant^{-1}, eleven SSR markers, were found polymorphic (Table 3). Nine SSR markers differentiated two pairs of genotypes GL 201 and KA and GL 355 and GL 576 which were contrasting for fresh seed yield plant^{-1} (Table 4).

Table 2: SSR marker-based polymorphism between pairs of core set of dolichos bean germplasm accessions contrasting for fresh pods plant^{-1}

High / Low	Fresh pods plant^{-1}				
	KA(37.5)	**GL 142(34)**	**GL 633(33)**	**FPB 35(31.5)**	**GL 110(37.5)**
GL 204(10)	5	5	3	3	2
GL 237(10)	6	5	5	4	5
GL 577(12.5)	10	9	8	7	8
KNR(14)	7	7	5	5	4
FPB-20(15)	8	7	7	6	6

*Figures in parenthesis indicate mean number of fresh pods plant^{-1}

Table 3: SSR marker-based polymorphism between pairs of core set of dolichos bean germplasm accessions contrasting for fresh pod yield plant^{-1}

High / Low	Fresh pod yield plant^{-1}(g)				
	GL 576 (222.28)	**GL 527 (203.60)**	**GL 142 (194.25)**	**FPB35 (189.23)**	**KA (187.57)**
GL 661(73.06)	7	9	6	4	6
GL 228(76.19)	5	9	5	3	6
GL 201(80.16)	8	11	7	4	9
GL 204(83.15)	4	8	5	3	5
GL 205(86.34)	6	9	5	3	4

*Figures in parenthesis indicate mean fresh pod yield plant^{-1}

Table 4: SSR marker-based polymorphism between pairs of core set of dolichos bean germplasm accessions contrasting for fresh seed yield plant^{-1}

High / Low	Fresh seed yield plant^{-1}(g)				
	KA (116.64)	**FPB35 (116.37)**	**GL 576 (110.94)**	**GL 142 (107.48)**	**GL 12 (105.17)**
GL 385(38.56)	7	3	7	6	5
GL 228(38.76)	6	3	5	5	4
GL 355(39.43)	7	5	9	8	7
GL 201(43.71)	9	4	8	7	7
GL 661(44.88)	6	4	7	6	5

*Figures in parenthesis indicate mean fresh pod yield plant^{-1}

The identified contrasting germplasm accessions could be used preferentially in dolichos bean breeding programmes. These genotypes may be recombined with elite advanced breeding genotypes and selection for economically important traits may be practiced in F_2 or in F_3 generations. Likewise limited backcrossing and selection in BC2 or BC3 generations will help to combine desirable favouarable alleles in defined proportions to derive transgressive segregants for yield and yield related traits. These genotypes are suggested for preferential use in multiple crossing programmes to generate variability for recovering farmer acceptable varieties with consumer/end-user preferred traits.

Conclusion

The contrasting genotypes identified based on phenotypic and SSR marker alleles-based analysis could be used as putative parents for developing mapping population to identify SSR markers linked to genomic regions controlling yield associated traits, the harvestable economic product and/or could be used to effect crosses to derive superior pure lines for use as varieties for commercial cultivation of dolichos bean.

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