

Genetic diversity among and within accessions of a lablab (*Lablab purpureus*) collection maintained in the ILRI forage genebank

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Abstract

Lablab (*Lablab purpureus* L.) is an important annual multi-purpose legume used as a vegetable for human consumption, as forage for livestock, and as green manure and a cover crop to improve soil fertility. It has a high feed value with good digestibility and high crude protein content. The International Livestock Research Institute (ILRI) forage genebank holds a diverse set of about 340 lablab accessions collected from different regions of the world. A total of 1,843 plants from 142 lablab accessions (1 to 29 plants per accession genotyped individually) were genotyped by the genotyping-by-sequencing (GBS) method of the DArTseq platform. The genotyping produced a total of 38,824 and 64,793 genome-wide single nucleotide polymorphism (SNP) and SilicoDArT high-density markers, respectively, on 1,768 plants from 139 accessions. The short sequence reads corresponding to the markers were mapped on the mungbean (*Vigna radiata*) reference genome, with approximately 37% of the SNPs and 26 % of the SilicoDArTs able to be mapped. A subset of 1,000 robust markers was filtered by different criteria and used for the diversity analysis. Plants within accession were classified into “true-to-type”, “progeny”, or “contaminant” based on a pairwise IBD (Identity-By-Descent) analysis and 1440 true-to-type plants from 120 accessions were filtered after removing contaminants. Clustering analysis using the discriminant analysis of principal components (DAPC) detected four major groups. Analysis of molecular variance (AMOVA) showed a highly significant ($P < 0.001$) variation, explaining more than 95 % of the variance among the accessions. However, the variation among plants within accessions was not significant and explained only 5 % of the variation. The results of this study provide a useful guide for the management and rationalization of activities of the lablab germplasm collection at the ILRI genebank. The substantial genetic diversity observed in the collection reveals the potential of the population for further genetic studies.

Introduction

Lablab (*Lablab purpureus* L.) is an important annual multi-purpose legume used as food for human consumption (Duke et al. 1981; Smartt 1985), as forage in commercial and smallholder agriculture (Pengelly and Maass 2001), and as a green manure and cover crop to improve soil fertility (Nyawade et al, 2019). It has a high feed value with good digestibility and high content of crude protein (<https://feedsdatabase.ilri.org/>). Lablab is one of the tropical forage legumes that are highly demanded for research and agricultural production in Africa and other regions in the world. In the years leading to 2017, over 2,300 samples of *Lablab purpureus* were distributed by the ILRI genebank to germplasm requesters both internationally and nationally, showing the high demand for this species. The ILRI Genebank holds around 340 accessions of this species, with some information on the genetic diversity among the accessions. Previously, about 200 accessions of the collection were characterized using morphological and Amplified fragment length polymorphism (AFLP) markers, which revealed a significant amount of genetic diversity in the collection and enabled the development of a core collection and the identification of best bet accessions for dryland and sub-humid environments (Whitbread et al., 2011; Pengelly and Maass 2001; Wiedow 2001). Those marker types, however, have limitations associated with reproducibility and distribution across the genome. In addition, the analyses were based only on variability among accessions, but there is no information on the genetic diversity of the within accessions. This study aimed to assess genetic diversity within and among *Lablab purpureus* accessions held at the ILRI genebank using genome-wide DArTseq markers.

Materials and methods

Seedlings were raised from seeds of 142 *Lablab purpureus* accessions and genomic DNA was extracted from leaves using a DNeasy[®] Plant Mini Kit (Qiagen Inc., Valencia, CA). The DNA samples were genotyped by the DArTseq genotyping platform at Diversity Arrays Technology, Canberra, Australia. A pairwise IBD (Identity-By-Descent) analysis was conducted using the PLINK software (Purcell, et al., 2007). Genetic diversity analysis was done in R statistical software (<https://www.r-project.org/>).

Results and discussion

A total of 1,843 plants from 142 accessions, with 1 to 29 plants per accession, were genotyped by the genotyping-by-sequencing (GBS) method of the DArTseq platform. The remaining 75 samples from three accessions were failed to produce analyzable marker data. The genotyping generated a total of 38,824 and 64,793 genome wide SNP and SilicoDArT markers respectively. The short sequence reads corresponding to the markers were mapped onto the mungbean (*Vigna radiata*) reference genome (Kang et al. 2014), with approximately 37% of the SNPs and 26 % of the SilicoDArTs mapped across the eleven chromosomes (Figure 1).

In both SNP and SilicoDArT markers, the polymorphic information content (PIC) and heterozygosity (He) values ranged from 0 to 0.38 and 0 to 0.50 with an average value of 0.05, respectively. The number of SNP markers with PIC and He values above 0.2 were only 2,685 (7%) and 2,805 (7%), respectively. Similarly, for the SilicoDArT markers, only 4,771 (7%) and 4,884 (8%) markers had PIC and He values above 0.2, respectively. This low level of polymorphism in the marker sets might be attributed to the low sequence diversity of the species.

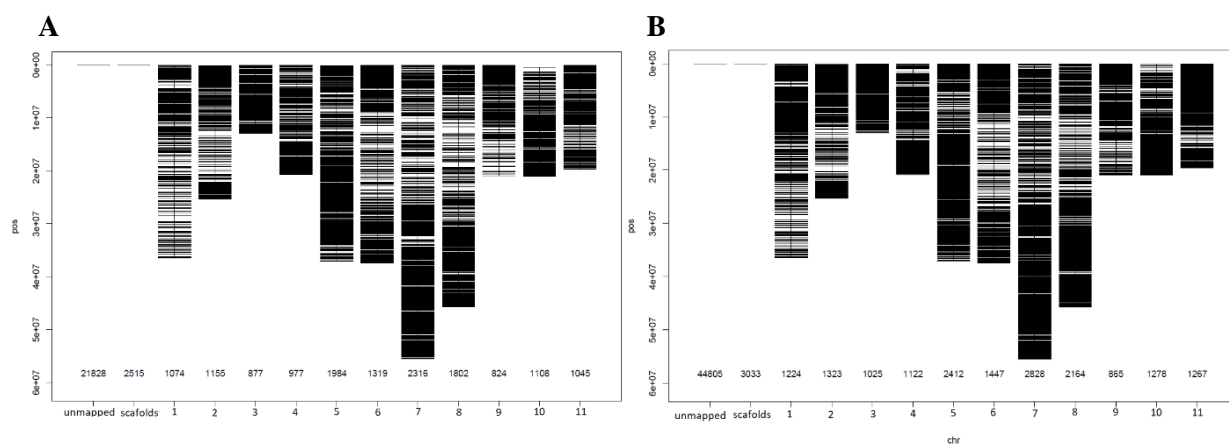


Figure 1. Genome-wide distribution of SilicoDArT (A) and SNP (B) markers across the eleven chromosomes of the mungbean (*Vigna radiata*) reference genome. The markers that were not mapped are shown as “unmapped”, and those markers that were mapped onto different scaffolds as “scaffolds”.

To analyze the genetic diversity, 1,000 robust SNP markers were selected based on the marker’s minor allele frequency ($MAF \geq 5\%$), missing values (less than 10%), independence from each other (Linkage disequilibrium- $LD \leq 0.5$), and their distribution across the genome. The PIC and He values of the markers ranged from 0.13 to 0.37 and 0.14 to 0.50 with an average value of 0.30 and 0.38, respectively. The MAF of the markers was above 5%, while the missing values were less than 10%.

Before the genetic diversity analysis, the genetic integrity of plants within accessions was tested based on a pairwise IBD (Identity-By-Descent) analysis, which classified the plants into “true-to-type”, “progeny”, or “contaminant” based on a PI_HAT (Purcell, *et al.*, 2007) value. A total of 1440 true-to-type plants from 120 accessions were filtered after removing the plants detected as “contaminants” by the IBD analysis.

Clustering analysis using discriminant analysis of principal components (DAPC) detected four major groups (Figure 2). All plants per an accession were clustered in the same group, except for accessions ILRI_14466-4, ILRI_11617-5, and ILRI_18636-3. Few plants from these three accessions were grouped differently from the majority. Group 1 was the largest containing 46 accessions, followed by group 4 with 36 accessions and group 3 with 27 accessions (Table 1). Group 2 was the smallest having 13 accessions, including accession #147, the cultivar Highworth (<https://doi.org/10.18730/FT38T>) that was used for genome sequencing (Chang et al. 2018). Analysis of molecular variance (AMOVA) showed the presence of significant genetic variance among accessions and the variation among accessions was greater (95%) than the within (5%) (Table 2), which is expected as lablab is predominantly a self-pollinating species. The genetic variation within accessions may be as a result of segregation occurring in those accessions, or due to cross pollination during regeneration in the field. The generated information provides an improved understanding of the genetic diversity held in the collection and is useful in guiding the management and rationalization of activities of the lablab germplasm collection at the ILRI genebank. The information also help to enhance the conservation and utilization of the genetic resources particularly by the plant breeding community.

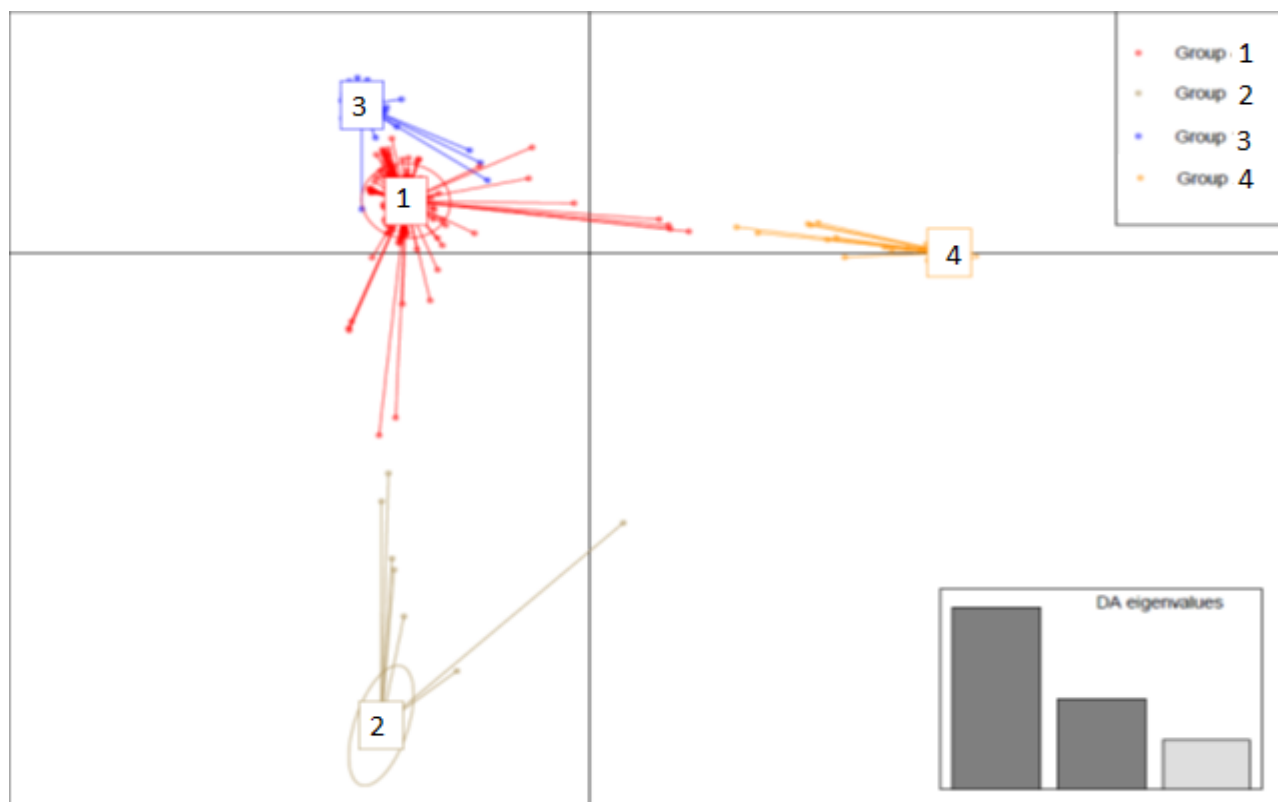


Figure 2. A DAPC plot showing the four major groups in the collections and plants within accessions.

Table 1. Lablab accessions and plants within accessions in each of the four major groups detected by DAPC

Group 1	No. of plants/acc.	Group 2	No. of plants/acc.	Group 3	No. of plants/acc.	Group 4	No. of plants/acc.
ILRI_14907-4	9	ILRI_14468-4	13	LRI_14410-2	5	ILRI_14452-3	14
ILRI_14905-4	21	ILRI_14415-3	9	ILRI_14413-3	16	ILRI_14438-2	13
ILRI_18604-5	18	ILRI_16803-4	15	ILRI_14417-2	11	ILRI_14465-3	18
ILRI_18597-5	14	ILRI_14486-2	13	ILRI_14420-2	6	ILRI_14454-3	16
ILRI_18599-5	4	ILRI_14436-8	5	ILRI_14424-3	16	ILRI_14445-3	14
ILRI_18601-5	18	ILRI_147-23	25	ILRI_14427-3	8	ILRI_14451-3	18
ILRI_21082-4	9	ILRI_14414-6	18	ILRI_14428-2	10	ILRI_18632-4	16
ILRI_14480-3	12	ILRI_14466-4	16	ILRI_14434-2	4	ILRI_14459-3	16
ILRI_18609-4	13	ILRI_14487-2	9	ILRI_14435-3	14	ILRI_14449-3	18
ILRI_18635-8	13	ILRI_18627-5	4	ILRI_14457-3	14	ILRI_14430-2	16
ILRI_21062-4	15	ILRI_18636-3	2	ILRI_14463-2	11	ILRI_14490-2	25
ILRI_21059-3	17	ILRI_14461-2	4	ILRI_14471-6	11	ILRI_14481-4	15
ILRI_21055-3	15	<u>ILRI_7379-6</u>	<u>13</u>	ILRI_14474-6	20	ILRI_14426-2	16
ILRI_11640-11	19			ILRI_14475-7	11	ILRI_14441-2	15
ILRI_21081-3	4			ILRI_14476-4	15	ILRI_14470-3	14
ILRI_14901-4	12			ILRI_14477-8	11	ILRI_14439-3	4
ILRI_11631-3	6			ILRI_14478-5	13	ILRI_11630-3	14
ILRI_6533-15	14			ILRI_14493-2	7	ILRI_14422-4	15
ILRI_18592-6	4			ILRI_15436-7	13	ILRI_14485-2	16
ILRI_13693-11	15			ILRI_11612-4	3	ILRI_14418-2	20
ILRI_18607-7	3			ILRI_11617-5	1	ILRI_14448-3	13
ILRI_11614-6	21			ILRI_18593-7	10	ILRI_14453-3	15
ILRI_14896-15	4			ILRI_18605-5	11	ILRI_14469-2	3
ILRI_11617-5	5			ILRI_18618-3	5	ILRI_14482-2	14
ILRI_18636-3	6			ILRI_21065-3	10	ILRI_6528-2	17
ILRI_18600-12	13			ILRI_24777-3	14	ILRI_14419-3	14
ILRI_18617-1	13			<u>ILRI_13701-3</u>	<u>8</u>	ILRI_14458-2	14
ILRI_21071-5	10					ILRI_14479-2	10
ILRI_13689-6	14					ILRI_14489-2	12
ILRI_11620-2	11					ILRI_6536-1	15
ILRI_11629-4	15					ILRI_6529-25	15
ILRI_14902-4	13					ILRI_14425-2	19
ILRI_18622-12	14					ILRI_14431-2	17
ILRI_18611-8	7					ILRI_14488-2	11
ILRI_18619-6	5					ILRI_14492-4	15
ILRI_18625-6	5					<u>ILRI_14466-4</u>	<u>1</u>

Group 1	No. of plants/acc.	Group 2	No. of plants/acc.	Group 3	No. of plants/acc.	Group 4	No. of plants/acc.
ILRI_21060-4	6						
ILRI_21072-3	11						
ILRI_21085-3	6						
ILRI_21056-4	4						
ILRI_10953-6	3						
ILRI_11619-3	5						
ILRI_14898-6	15						
ILRI_14914-3	10						
ILRI_11641-5	4						
ILRI_14906-4	10						

The number after the minus (-) in each accession number indicate lot number, which is the location of the accession in the ILRI forage genebank

Table 2. AMOVA showing the genetic variance among and within accessions

Accession/ Groups	Source of variation	Degrees of freedom (df)	Sum of squares	Mean sum of squares	Percentage of variation	P-value
Among and within accessions	Among accessions	119	976163.31	8203.05	95.11	0.001
	Within accession	1320	46223.32	35.02	4.89	0.540

Acknowledgements

Lablab purpureus seeds of more than 142 accessions were obtained from the ILRI forage genebank. The genotyping was supported by the A15 genotyping project of the Genebank platform. All data is freely available under a 'creative commons' license.

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