Genetic Diversity among and within Accessions of a Lablab (*Lablab purpureus*) Collection Maintained in the ILRI Forage Genebank

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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 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. 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. Clustering analysis using the discriminant analysis of principal components (DAPC) detected five major groups, each with further subgroups. Analysis of molecular variance (AMOVA) showed a highly significant ($P < 0.00001$) variation, explaining more than 73 % of the variance among the accessions. A significant variation ($P < 0.005$) was also observed among plants within accessions, which explained about 27 % 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 purpureus 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, but very little information is available on the genetic diversity among and within the accessions. Previously, about 100 accessions of the collection were characterized using morphological and Amplified fragment length polymorphism (AFLP) markers, which revealed a significant amount of genetic diversity within the collection and enabled the development of a core collection and the identification of best bet accessions for dryland and sub-humid environments (Pengelly and Maass 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. 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 of 15 days old seedlings 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. All data analysis was done in 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 used to analyse genetic diversity among and within the accessions. For the within accession analysis, 102 accessions of 10 and more plants per accession, were used. Genotyping by the DArTseq platform 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.

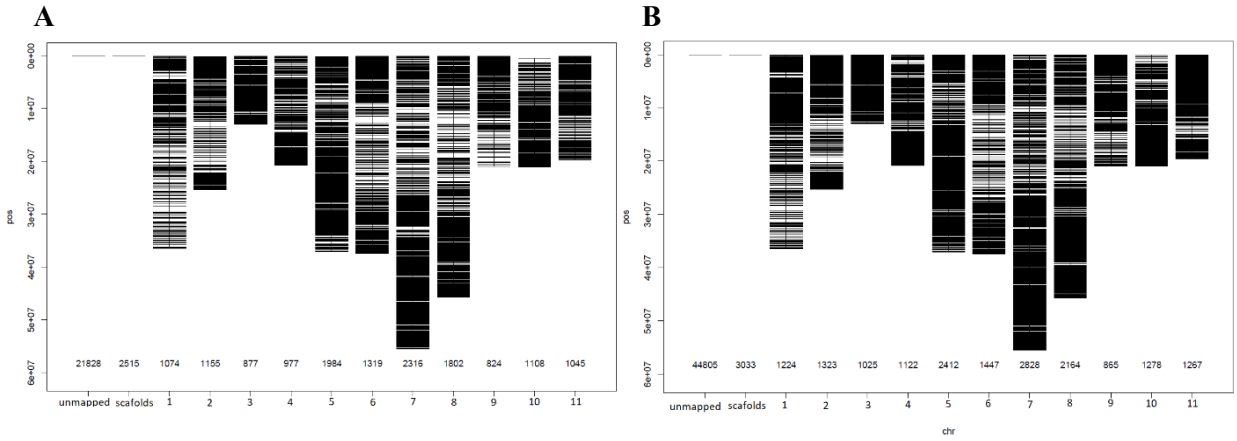

Figure 1. Genome-wide distribution of SilicoDArT (A) and SNP (B) markers across the eleven chromosomes of the mungbean (*Vigna radiata*) reference genome. The markers that were not mapped are shown as "unmapped", and those markers that were mapped onto different scaffolds as "scaffolds".

To analyze the genetic diversity, 1,000 robust SNP markers were selected based on the marker's minor allele frequency (MAF ≥ 5 %), missing values (less than 10 %), independence from each other (Linkage disequilibrium-LD ≤ 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 %.

Clustering analysis using discriminant analysis of principal components (DAPC) detected five major groups (Figure 2). Group 1 contains 18 accessions, including accession #147, the cultivar Highworth (https://doi.org/10.18730/FT38T) that was used for genome sequencing (Chang et al. 2018). Groups 2, 4, and 5 were the largest containing 52, 54, and 55 accessions, respectively (Table 1). In Group 1, all progeny plants from accessions Acc_14468.4, Acc_16603.4, Acc_147.23, Acc_14414.6, and Acc_14487.2 clustered within the group, while plants from the other 10 accessions in the group also clustered with plants in other groups. This may suggest the presence of admixtures in those 10 accessions, and they may require further checking for genetic purity. Similar trends were also observed in the other four groups. Analysis of molecular variance (AMOVA) showed the presence of significant genetic variance among accessions as well as among plants within an accession. However, the genetic variation among accessions was greater (73%) than the within (27%) (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 that the accessions have been mixed up with seeds from other accessions. 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.

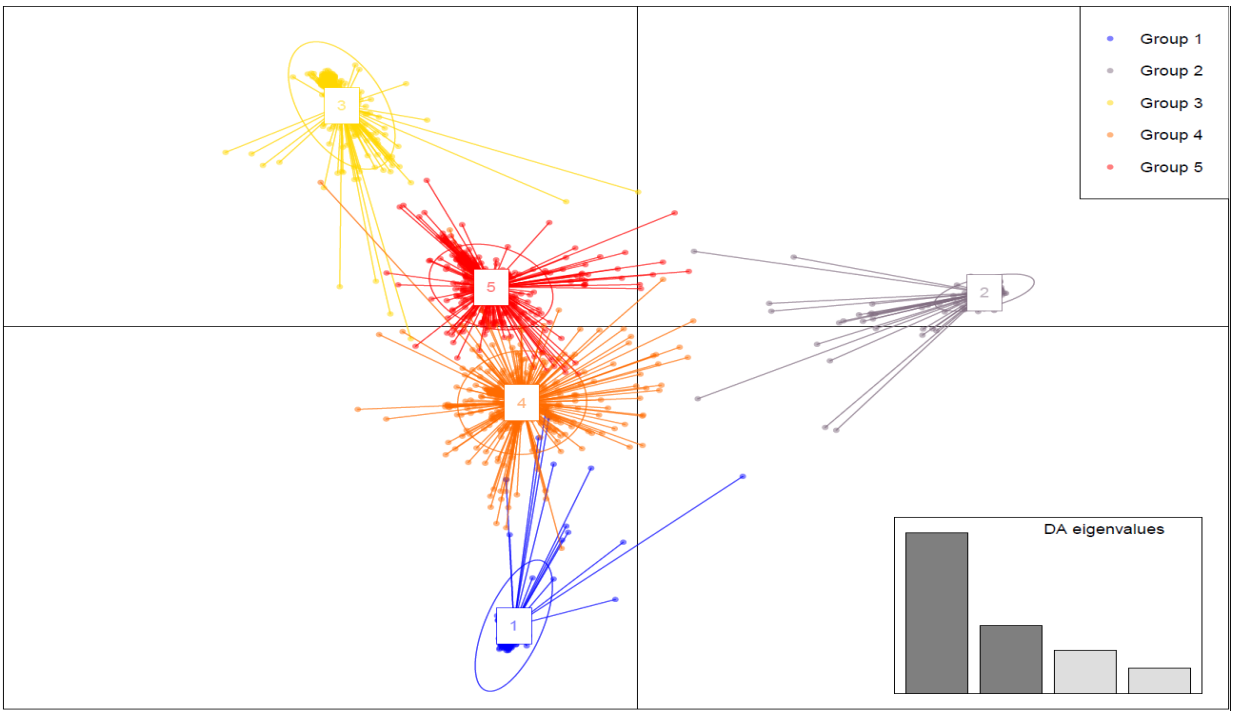

Figure 2. A DAPC plot showing the five major groups in the collections and plants within accessions.

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

Group 1	No. of progeny	Group 2	No. of progeny	Group 3	No. of progeny	Group 4	No. of progeny	Group 5	No. of progeny
Acc_14468.4	14	Acc_14438.2	13	Acc_13701.3	8	Acc_10953.6	2	Acc_10953.6	1
Acc_14415.3	7	Acc_14465.3	18	Acc_14410.2	2	Acc_10979.10	1	Acc_11641.5	4
Acc_16603.4	15	Acc_14454.3	16	Acc_14413.3	16	Acc_11631.3	6	Acc_11642.4	13
Acc_14486.2	13	Acc_14445.3	14	Acc_14417.2	6	Acc_11640.11	20	Acc_14410.2	14
Acc_14436.8	8	Acc_14451.3	18	Acc_14424.3	16	Acc_13685.11	14	Acc_14417.2	7
Acc_14453.3	1	Acc_14452.3	14	Acc_14427.3	4	Acc_13689.6	14	Acc_14419.3	2
Acc_14419.3	1	Acc_14466.4	4	Acc_14428.2	11	Acc_13693.11	17	Acc_14420.2	12
Acc_147.23	25	Acc_18632.4	16	Acc_14434.2	6	Acc_13697.4	2	Acc_14425.2	1
Acc_14414.6	18	Acc_14459.3	16	Acc_14435.3	15	Acc_14415.3	12	Acc_14427.3	12
Acc_14466.4	16	Acc_14449.3	20	Acc_14445.3	1	Acc_14428.2	3	Acc_14428.2	2
Acc_14487.2	9	Acc_14430.2	16	Acc_14448.3	7	Acc_14436.8	3	Acc_14434.2	14
Acc_21085.3	1	Acc_14490.2	29	Acc_14457.3	15	Acc_14479.2	2	Acc_14452.3	2
Acc_18627.5	4	Acc_14481.4	15	Acc_14463.2	16	Acc_14480.3	12	Acc_14456.3	1
Acc_18636.3	1	Acc_14426.2	16	Acc_14470.3	1	Acc_14493.2	4	Acc_14457.3	1
Acc_14489.2	1	Acc_14441.2	15	Acc_14471.6	13	Acc_14896.15	8	Acc_14466.4	1
Acc_14461.2	4	Acc_14901.4	4	Acc_14474.6	20	Acc_14898.6	10	Acc_14470.3	1
Acc_7379.6	13	Acc_21042.4	1	Acc_14476.4	15	Acc_14901.4	12	Acc_14471.6	3
Acc_14452.3	1	Acc_14470.3	14	Acc_14483.3	1	Acc_14902.4	13	Acc_14475.7	11
		Acc_14439.3	4	Acc_14493.2	4	Acc_14904.4	1	Acc_14477.8	16
		Acc_18637.5	2	Acc_15436.7	13	Acc_14905.4	1	Acc_14478.5	14
		Acc_21059.3	1	Acc_18593.7	10	Acc_14906.4	12	Acc_14483.3	4
		Acc_14422.4	16	Acc_18605.5	11	Acc_14914.3	1	Acc_14493.2	6
		Acc_14485.2	17	Acc_18618.3	5	Acc_11613.4	2	Acc_14896.15	1
		Acc_14418.2	21	Acc_6529.25	1	Acc_11614.6	21	Acc_14898.6	5
		Acc_14448.3	13			Acc_11619.3	5	Acc_14904.4	5
		Acc_14453.3	15			Acc_11629.4	15	Acc_14905.4	1
		Acc_14469.2	3			Acc_11630.3	2	Acc_14906.4	6
		Acc_14482.2	14			Acc_18592.6	6	Acc_14907.4	16
		Acc_6528.2	17			Acc_18593.7	5	Acc_14914.3	11
		Acc_14419.3	14			Acc_18595.3	11	Acc_11612.4	3
		Acc_14436.8	2			Acc_18596.3	2	Acc_11617.5	15
		Acc_14458.2	14			Acc_18597.5	14	Acc_11620.2	11
		Acc_14479.2	10			Acc_18600.12	15	Acc_11630.3	1
		Acc_14489.2	12			Acc_18601.5	18	Acc_18592.6	1
		Acc_6536.10	15			Acc_18607.7	1	Acc_18593.7	4
		Acc_6529.25	15			Acc_18611.8	14	Acc_18596.3	2
		Acc_7278.4	1			Acc_18617.10	13	Acc_18599.5	7

Acc_1629.4	1	Acc_18619.6	5	Acc_18604.5	18
Acc_1630.3	12	Acc_18622.12	14	Acc_18607.7	3
Acc_14425.2	19	Acc_18623.7	17	Acc_18609.4	13
Acc_14431.2	17	Acc_18625.6	7	Acc_18611.8	1
Acc_14433.8	1	Acc_18628.13	8	Acc_18636.3	6
Acc_14488.2	11	Acc_18635.8	15	Acc_21055.3	1
Acc_14902.4	1	Acc_18636.3	4	Acc_21059.3	17
Acc_14492.4	15	Acc_21042.4	2	Acc_21062.4	15
Acc_14486.2	2	Acc_21055.3	15	Acc_21065.3	10
Acc_6930.10	2	Acc_21056.4	4	Acc_21071.5	10
Acc_14483.3	1	Acc_21060.4	6	Acc_21072.3	11
Acc_7403.3	1	Acc_21066.5	2	Acc_21082.4	2
Acc_14904.4	1	Acc_21081.3	8	Acc_21085.3	6
Acc_15436.7	1	Acc_21082.4	15	Acc_24777.3	14
Acc_13685.11	1	Acc_6533.15	16	Acc_6529.25	1
		Acc_6535.5	1	Acc_6533.15	1
		Acc_6930.10	1	Acc_6930.10	4
				Acc_7379.6	1

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

Source of variation	**Degrees of freedom (df)**	**Sum of squares**	**Mean sum of squares**	**Percentage of variation**	***P*-value**
Among accessions	102	457447.419	4484.779	73%	0.00001
Within accession	3121	161112.801	51.622	27%	0.005

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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