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Microsatellite (SSR) Analysis of Dolichos Lablab (*Lablab purpureus* (L.) Sweet) Germplasm in Namibia

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Abstract— Dolichos lablab (Lablab purpureus (L.) Sweet)) is a multipurpose drought tolerant protein-rich legume crop native to Africa and grown in warm temperate to tropical climates for its edible seeds and manure. Literature informs that Lablab purpureus holds significant benefits to subsistence farmers and offers a great promise for sustainable crop productivity, especially in marginalised areas. Its uses range from human consumption as a vegetable to improving soil fertility, and as forage. Not with standing Lablab purpureus crucial potential functions in Namibia, there is currently limited information regarding the plant's genetic diversity. This study followed a descriptive quantitative research approach where the data collected was used to describe the samples collected. The study aimed at evaluating the genetic diversity among 26 accessions of Lablab purpureus. The plant's genetic diversity was determined by PCR amplification of three SSR markers namely VM38, AGB8 and GATS911. The SSR markers had PIC values of 0.452663 for marker VM38, 0.473373 for marker AGB8 and 0.260355 for marker GATS911. The Shannon Index of diversity gave a value of 1.25 meaning low levels of genetic diversity among the accessions. The study was the first report of Lablab purpureus's genetic diversity in Namibia.

Keywords— Genetic diversity, SSR, microsatellite markers, Lablab purpureus, Dolichos lablab, Namibia

I. INTRODUCTION

Dolichos lablab (*Lablab purpureus* (L) Sweet) also known as Dolichos bean, Indian bean or Hyacinth bean is a perennial herb from the family *Fabaceae* endowed with high protein content and nutritional value [1]. This drought tolerant under-utilised crop has a global social recognition that stretches from human food (vegetable and pulse) to soil fertility improvement to high quality animal fodder [2]– [4]. Some pictures of *Lablab purpureus* are shown below (Fig.1). *Lablab purpureus*, indigenous to India and Africa, is primarily produced by small farmers in the semi-arid region, dry poor soils of America, Southeast Asia, and Africa [5]. It has been classified as a neglected legume that is one of the lost crops of Africa [4].

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Fig.1 Pictures of *Lablab purpureus*

Lablab purpureus has been described as having several benefits; young, green pods and seeds are consumed as a delicious vegetable low in lipids and carbohydrates, but extraordinarily rich source of dietary fibre and once matured, the harvested dry seeds are kept and consumed as a pulse throughout the year [1]. Nutritionally, it is particularly important as a good source of protein, minerals (Phosphorus, Iron, Sulphur, Magnesium, Sodium, and Calcium), vitamins (Riboflavin, C and A), and amino acids (lysine) [1]. According to [1] the seeds and leaves of *Lablab purpureus* contain 20-28% protein. This makes *Lablab purpureus* nutritionally important for healthier food habits as low fat and low-calorie vegetarian foods are gaining popularity with changing lifestyle patterns [1]. It is planted to be used as either a pulse or for the young pods which are used for stock feed by some farmers or as a fresh vegetable. The field type is utilised for cover crops, green manure, forage, and seed production. whereas garden type is used mostly as a green vegetable.

In the south Indian diet, it serves as the main protein source where it grows and is also popularly grown in northeast and eastern parts of the country. It is either intercropped with groundnut and other cereals like sorghum, corn, pearl millet and finger millet or grown in pure stand [1]. The green pods, fresh leaves, mature grains, and immature grains are also used for some medicinal purposes [6].

In animal feeding, it is used as fresh forage, grain, straw, grazing, hay, browsing or forage meal [1]. The great crude protein of *Lablab purpureus*' green leaves adds to the nutritional content and palatability of green fodder, it is also used when making better quality silage with improved protein content. The better-quality silage is obtained by mixing it with other cereals such as sorghum in a 2:1 ratio [1].

Analysing the untapped population genetics of a crop species through the application of highly informative DNA molecular markers is necessary to expand and diversify the genetic foundation of crop cultivars [6].

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Breeding populations with a wide genetic base could be created by analysing germplasm with unique DNA profiles [7]. In Namibia, *Lablab purpureus* has not attained the expected level of agricultural significance.

By initially starting out to identify the genetic similarity of these varieties through microsatellite analysis, it will serve as a starting point in the establishment of breeding programs for the species in Namibia. The aim of this study was to evaluate the degree of diversity of *Lablab purpureus* in Namibia using microsatellite (SSR) markers.

II. METHODS

A. *Sample collection*

For the microsatellite analysis of the germplasm, seeds from the 26 *Lablab purpureus* varieties were germinated (Fig. 2). Fresh young leaves were then collected and used for analysis.

Fig. 2 *Lablab purpureus* seed varieties

B. *DNA Extraction and Purification*

With a few minor modifications, the cetyl-tri-methyl-ammonium bromide (CTAB) method was used to extract the genomic DNA from the young, fresh leaves [8]. The DNA's quality and concentration were assessed using a Nanodrop spectrophotometer (NANADROP 2000 spectrophotometer from Thermo Scientific) (Table II). Further quality check done on a 0.8% agarose gel (Fig.3). For each of the samples extracted, DNA dilutions were carried out for the normalization and homogenization of DNA to a concentration 50ng/µl and then stored at 4˚C for use in PCR analysis.

C. *Primers used*

The primers were purchased in lyophilized form from Inqaba Biotech (South Africa) and reconstituted in accordance with the manufacturer's instructions. From a stock solution of 100 µM, a working solution (10 µM) was made for each primer. They were kept until use at -20˚C. The table below shows the primer sequences that were used for SSR analysis (Table I).

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TABLE I
PRIMER SEQUENCES USED FOR MICROSATELLITE ANALYSIS

Primer		Sequence 5ˊ-3ˊ	Source
VM38	F	AATGGGAAAAGAAAGGG AAGC	[9]
	R	TCGTGGCATGCAGTGTCAG	
AGB8	F	CACCGGGAGTGGCTGACA	[9]
	R	GTTTGGGGCGGAGTTCGA	
GATS911	F	GAGTGCGGAAGCGAGTGAG	[9]
	R	TCCGTGTTCCTCTGTCTGTG	
KTD249	F	ACTACCCTATAGTCTCTCTGTGCT	[10]
	R	AGAAGATGATCTCAGATTCCAC	
LABRRT2	F	GCCATGTTCTGAAAGATGTAACAGTG	[11]
	R	GGCAAGCAGTCATATCCAGAAACT	

D. *PCR for amplification*

Qualitative PCR was performed following the samples' DNA extraction. A 25 µl total reaction consisting of 12.5 µl of OneTaq Quick- Load 2X Master mix with standard buffer (VWR life sciences), 0.5 µl of forward primer, 0.5 µl of reverse primer, 8.5 µl of water and 3 µl of template DNA from samples was done. Additionally, a negative control was included, consisting of all the components but with 3µl of nuclease-free water in place of the template DNA. This was carried out with each PCR reaction.

Thermo Scientific's ARKTIK Thermo Cycler was used for the PCR. The PCR parameters used were denaturation at 95˚C for 4minutes, final denaturation at 95˚C for 35 seconds; Annealing at 54˚C for 45 seconds (for primers AGB8, VM38 and KTD249) an annealing at 56˚C for 45 seconds (for primers GATS911 and LABRRT2); extension at 72˚C for 1 minute; final extension at 72˚C for 10 minutes for 35 cycles and final hold at 4˚C.

E. *Data analysis*

Analysis was considered for SSR primers that displayed scoreable DNA bands (Fig. 4, Fig.5). Scoring was based on whether the generated DNA bands were present (score of 1) or not (score of 0). Python software (3.12.0) was used to analyse the sample size, allele counts per polymorphic locus, Shannon diversity index, and Polymorphism Information Content (PIC) statistics. The Unweighted Pair Group Method with Arithmetic Mean (UPGMA) was applied to the similarity matrix.

III.RESULTS

TABLE II
NANODROP RESULTS OF EXTRACTED DNA

Sample	*Lablab purpureus* Variety	Nucleic Acid (ng/µl)	A260/A280	A260/A230
1	IC-0623025	1352.0	2.01	2.18
2	IC-0623029	3382.3	2.12	2.33
3	IC-0623043	885.1	2.05	2.39
4	HA 4	1934.2	2.09	2.29
5	IC-0623022	2051.6	2.16	2.40
6	IC-0623020	2140.5	2.10	2.32
7	IC-0623011	1303.3	2.13	2.30
8	IC-0623093	1380.3	2.11	2.28
9	IC-0623005	884.1	2.15	2.38
10	IC-0623069	2423.8	2.13	2.29
11	IC-0623075	2255.3	2.11	2.28
12	IC-0623063	1749.4	2.05	2.07
13	Whk 1	512.5	2.12	2.08

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14	IC-0623096	764.7	2.08	2.23
15	IC-0623019	1344.1	2.10	2.25
16	IC-0623072	843.8	2.09	2.25
17	IC-0623028	317.9	2.05	1.90
18	IC-0623095	621.6	2.12	2.27
19	IC-0623038	936.5	2.12	2.09
20	Whk 2	1550.4	2.19	2.27
21	IC-0623099	1796.2	2.16	2.45
22	IC-0623008	4282.3	2.17	2.38
23	IC-0623088	3591.2	2.14	2.31
24	IC-06230100	2910.5	2.11	2.32
25	IC-0623094	3545.0	2.15	2.11
26	IC-0623001	3346.7	2.10	2.05

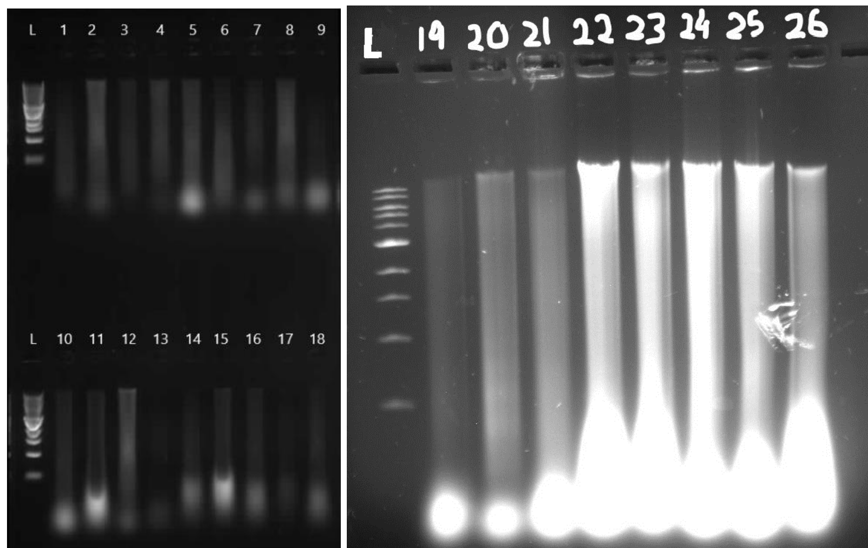

Fig.3 Agarose gel of extracted gDNA from 26 samples: L= 1kb ladder, 1= IC-0623025, 2= IC-0623029, 3= IC-0623043, 4= HA 4, 5= IC-0623022, 6= IC-0623020, 7= IC-0623011, 8= IC-0623093, 9= IC-0623005, 10= IC-0623069, 11= IC-0623075, 12= IC-0623063, 13= Whk 1, 14= IC-0623096, 15= IC-0623019, 16= IC-0623072, 17= IC-0623028, 18= IC-0623095, 19= IC-0623038, 20= Whk 2, 21= IC-0623099, 22= IC-0623008, 23= IC-0623088, 24= IC-06230100, 25= IC-0623094 and 26= IC-0623001

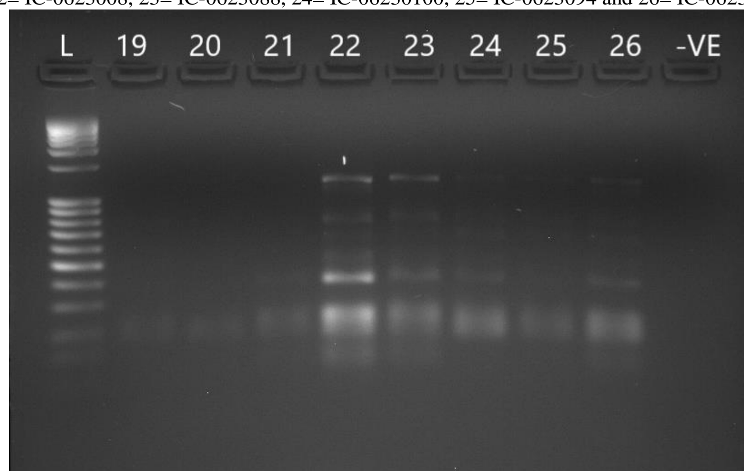

Fig.4 Agarose gel showing some polymorphic bands from some samples PCR using Primer VM38: L= 100bp DNA ladder, 19= IC-0623038, 20= Whk 2, 21= IC-0623099, 22= IC-0623008, 23= IC-0623088, 24= IC-06230100, 25= IC-0623094, 26= IC-0623001 and -VE = negative control

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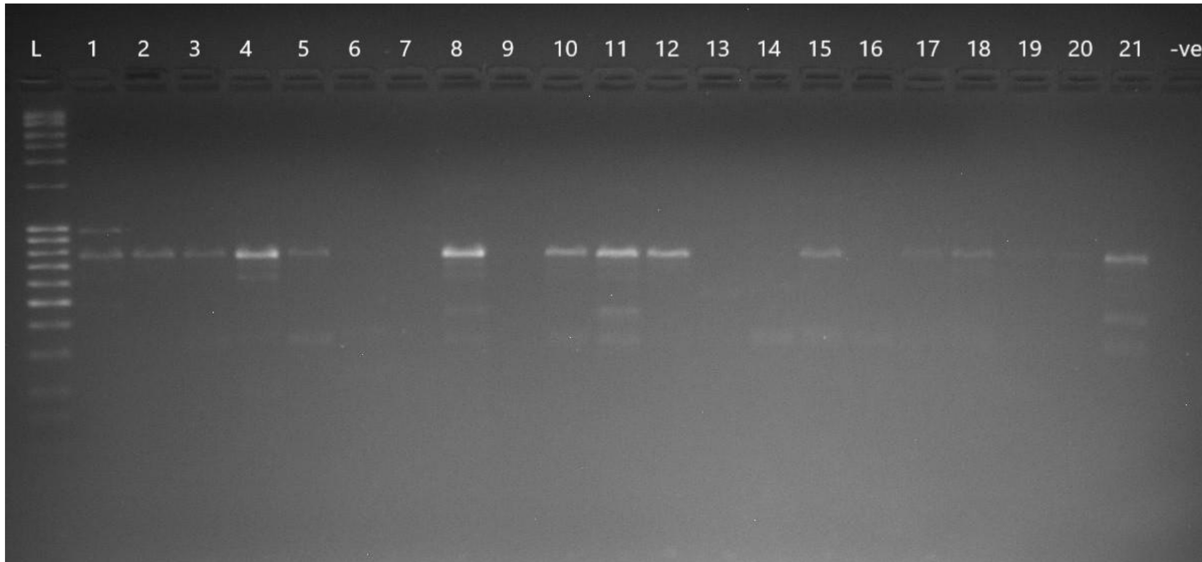

Fig.5 Agarose gel showing some polymorphic bands from PCR using Primer AGB8. L= 100bp DNA ladder, 1= IC-0623025, 2= IC-0623029, 3= IC-0623043, 4= HA 4, 5= IC-0623022, 6= IC-0623020, 7= IC-0623011, 8= IC-0623093, 9= IC-0623005, 10= IC-0623069, 11= IC-0623075, 12= IC-0623063, 13= Whk 1, 14= IC-0623096, 15= IC-0623019, 16= IC-0623072, 17= IC-0623028, 18= IC-0623095, 19= IC-0623038, 20= Whk 2, 21= IC-0623099 and -ve= negative control

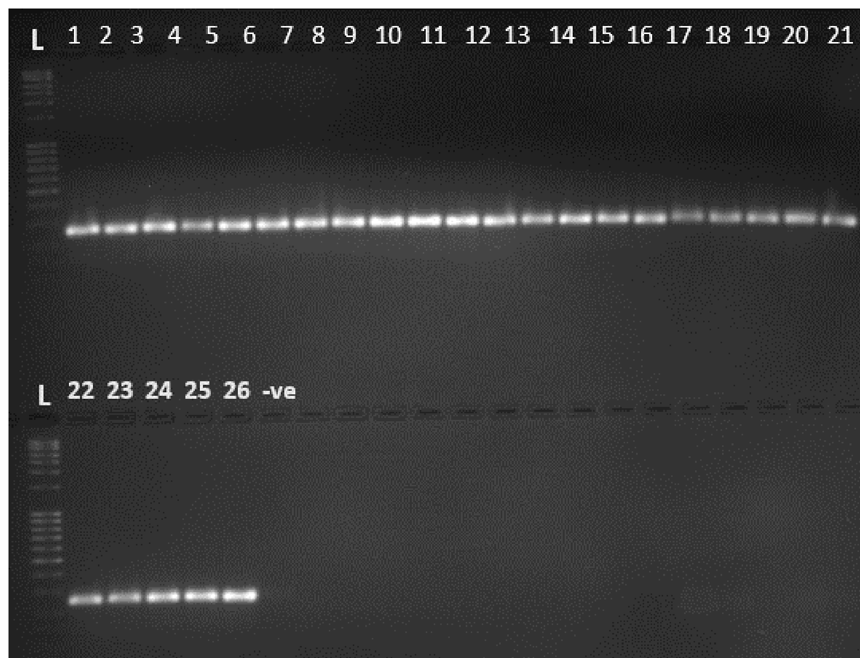

Fig.6 Agarose gel showing monomorphic bands from PCR using Primer KTD249: L= 100bp DNA ladder, 1= IC-0623025, 2= IC-0623029, 3= IC-0623043, 4= HA 4, 5= IC-0623022, 6= IC-0623020, 7= IC-0623011, 8= IC-0623093, 9= IC-0623005, 10= IC-0623069, 11= IC-0623075, 12= IC-0623063, 13= Whk 1, 14= IC-0623096, 15= IC-0623019, 16= IC-0623072, 17= IC-0623028, 18= IC-0623095, 19= IC-0623038, 20= Whk 2, 21= IC-0623099, 22= IC-0623008, 23= IC-0623088, 24= IC-06230100, 25= IC-0623094, 26= IC-0623001 and -ve = negative control

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A. Microsatellite analysis

Three out of the five primers gave polymorphic bands, the other 2 primers had monomorphic bands and were not considered for further analysis (Fig. 6). Based on these 3 polymorphic SSR primers: AGB8, VM38 and GATS911, the genetic diversity of the 26 accessions of *Lablab purpureus* were calculated using the Shannon Diversity Index. The Shannon Diversity Index calculated value for the dataset was discovered to be 1.25 which indicates that the genetic diversity among the accessions is relatively low. Most ecological studies indicate that index values are rarely greater than 4 and are usually between 1.5 and 3.5 [12].

Table III
Allele frequency and Polymorphic Information Content (PIC) values from primers that produced polymorphic bands

Primer	Alleles per locus	PIC
Primer AGB8	16	0.473373
Primer VM38	17	0.452663
Primer GATS911	4	0.260355

B. Cluster analysis

By using the unweighted pair group method with arithmetic mean averages (UPGMA) cluster analysis on the similarity matrix data, a dendrogram was created (Fig.7). According to the findings, the 26 accessions could be divided into 2 major clusters. According to the dendrogram one cluster had 7 accessions whilst the other had 19. One cluster had accessions Whk 2, IC-0623038, IC-0623095, IC-0623072, IC-0623005, IC-0623020, and IC-0623011. The other cluster contained 2 subclusters: One subcluster had accessions Whk1, IC-0623096, and IC-0623028 the other subcluster had the accessions IC-0623043, IC-0623029, HA4, IC-0623022, IC-0623069, IC-0623075, IC-0623063, IC-0623019, IC-0623099, IC-0623094, IC-0623088, IC-0623008, IC-06230100, IC-0623001, IC-0623093, and IC-0623025.

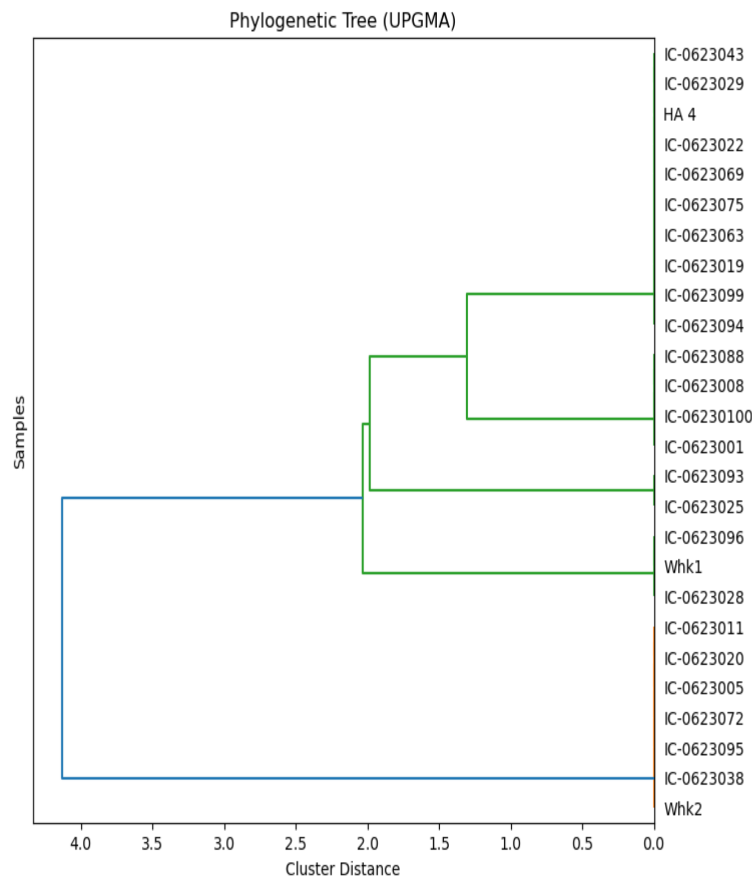

Fig. 7 Dendrogram (UPGMA) showing the genetic relationship among 26 populations of *Lablab purpureus*

IV. DISCUSSION

A. *Microsatellite analysis*

Planning a plant breeding program and preserving germplasm depend heavily on knowledge of the structure and diversity of a crop's gene pool. Characterising the genetic material is essential to breeding programs for efficient and effective management of plant genetic resources. A common DNA marker system used in plant diversity analysis is the SSR marker, which has several benefits including being multi-allelic, co-dominant, easy-to-score and PCR-based [13]. In this research, analysis of the genetic diversity of 26 *Lablab purpureus* accessions was done.

Three out of the five SSR primers gave polymorphic bands, the other 2 primers had monomorphic bands and were not considered for further analysis. Absence of allele amplification in certain genotypes might be the creation of a PCR product in an amount that is too small to detect or the outcome of a mismatch in the microsatellite's flanking sequences, which results in a null allele [6], [13].

Polymorphic information content (PIC), which considers both the number of expressed alleles and the relative frequency of alleles, provides an estimate of a locus's discriminatory power [14]. The higher a marker's PIC, the more alleles it has discovered. PIC values range from zero, which indicates monomorphism, to one, which indicates an extremely high level of discrimination between many alleles present at the same frequency. The SSR marker's utility and informativeness increase with increasing PIC value [14]. Consequently, the polymorphic information content (PIC) value was computed to describe each primer's ability to detect polymorphic loci. The values calculated were Primer AGB8 with PIC of 0.473373, Primer VM38 with PIC of 0.452663 and Primer GATS911 with PIC of 0.260355 (Table III). PIC values between 0.25 and 0.50 are said to be reasonably informative, whereas those with PIC below 0.25 are not recommended [10]. Thus, these three primers are reasonably informative as they fall within this range ($0.5 > PIC > 0.25$).

The SSR marker LABRRT2, used in this study was non informative as it produced monomorphic bands after PCR. However, according to [6] the same primer LABRRT2 which was used in their study was highly informative with PIC of 0.668. The same authors concluded to recommend using it in upcoming *Lablab purpureus* diversity studies. The same was seen with SSR marker KTD249 which had a PIC value of 0.68 and was informative in the [10] study and recommended but this same primer was non informative (monomorphic) in this study.

B. *Cluster analysis (UPGMA)*

Individuals are mathematically grouped into clusters based on how similar their depictions are using cluster analysis to group objects or people based on characteristics they share [14]. By categorising or putting similar data items in one group, clustering reduces the amount of data.

The 26 accessions in this study were grouped into 2 main clusters. One cluster had 7 accessions: Whk 2, IC-0623038, IC-0623095, IC-0623072, IC-0623005, IC-0623020, and IC-0623011 whilst the other had 19 .The other cluster with 19 accessions contained 3 subclusters: One subcluster had the accessions Whk1, IC-0623096, and IC-0623028 the second subcluster had the accessions IC-0623043, IC-0623029, HA4, IC-0623022, IC-0623069, IC-0623075, IC-0623063, IC-0623019, IC-0623099, IC-0623094, IC-0623088, IC-0623008, IC-06230100 and IC-0623001 whilst the third subcluster had the accessions IC-0623093, and IC-0623025. The closest genotypes, according to the genetic distance, were IC-0623099 and IC-06230100, while IC-0623099 & IC-06230100 and Whk 2 had the greatest distance (Figure 7).

V. CONCLUSION

The findings of this study demonstrate that SSR markers successfully assessed the genetic variability among genotypes of *Lablab purpureus*. It indicated they are of a narrow genetic diversity. These results are expected to benefit in the conservation of *Lablab purpureus* germplasm in Namibia and aid in their breeding efforts as well. The findings of this study can be shared with local agricultural communities involved in *Lablab purpureus* cultivation. It can serve as a valuable resource to educate farmers, improving the overall management and utilisation of this crop.

Declaration of Interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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