Research Article **GJMB (2021) 3:8**

Global Journal of Molecular Biology
(ISSN:2637-5141)

DIVERSITY AND POPULATION STRUCTURE OF LOCAL AND EXOTIC *LABLAB PURPUREUS* ACCESSIONS IN KENYA AS REVEALED BY MICROSATELLITE MARKERS

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ABSTRACT

Lablab purpureus is an important pulse crop in some parts of sub-Saharan Africa and Asia but has largely remained underutilized. Understanding the genetic diversity is prerequisite for genetic improvement and utilization of this leguminous crop. The relationships of the local lablab genotypes and those collected from other diverse geographic origins including the wild accessions remain unknown in Kenya. The study was undertaken to determine genetic diversity and population structure of germplasm accessions collected from Kenya and other global regions. Eight simple sequence repeat primer pairs were used to genotype the 189 lablab accessions. A total of 39 alleles were revealed by eight SSR with an average of 4.88 alleles per polymorphic loci. The average PIC was 0.42. The gene diversity among the accessions ranged from 0.26 to 0.52 with an average of 0.38, indicating moderate genetic diversity. Germplasm collected from Kenya showed a moderate genetic diversity of 0.36. Higher genetic diversity (He<0.5) was detected within the Ethiopian and South Africa populations. Analysis of molecular variance (AMOVA) revealed that 94% of the allele diversity was attributed to individuals within populations while only 6% was distributed among the populations. The Bayesian model-based Structure method and Principal coordinate analysis (PCoA) scatter plot clustered the accessions into three groups with germplasms collected from Kenya showing distribution among all the three groups. The wild accessions clustered mainly with those from Southern and Eastern Africa confirming earlier suggestions that lablab is of African origin. The results of this study are discussed in light of the crop improvement of this crop.

Keywords: Lablab purpureus; Population structure; SSR; Genetic diversity; Improved varieties

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How to cite this article:
Eliezah M. Kamau, Miriam G. Kinyua, Charles N. Waturu, Oliver Kiplagat, Bramwel W. Wanjala, Robert K. Kariba and David R. Karanja. DIVERSITY AND POPULATION STRUCTURE OF LOCAL AND EXOTIC *LABLAB PURPUREUS* ACCESSIONS IN KENYA AS REVEALED BY MICROSATELLITE MARKERS. Global Journal of Molecular Biology, 2021; 3:8.

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Website: http://escipub.com/

Introduction

Lablab purpureus L. Sweet (2n = 22) is a leguminous crop that is currently grown in Africa, India and parts of Southeast Asia [1]–[3]. The crop is cultivated in diverse climatic conditions worldwide and displays wide genetic diversity. The production of lablab in India, a major global lablab producer, is about 0.03 million tonnes, grown on approximately 0.085 million hectares [4]. In Kenya, it remains a minor crop where it is grown in less than 0.01 million hectares, with an approximate yield of less than 0.5 tons ha^{-1} [5].

Lablab is an important pulse crop in some developing countries of Asia and Africa where it is mainly consumed as dry seeds and fresh green pods [1]–[3]. As food, it serves as a vital source of protein (20-28%), minerals such as Zinc (34mg/kg) and iron (57 mg/kg) [6]. The immature pods are commonly used as vegetable while the crop residue after harvest are utilized for making manure. It is also important as livestock forage and feed especially in Australia [7] while the spreading varieties are used in soil conservation and in weed management. Lablab has also been utilized in the management of *diabetes mellitus*, inflammations, coronary heart diseases and anaemia [8]. The ability of lablab to extract soil water at deep depth even at heavy-textured soil makes it a crop of choice for farmers in semi- arid areas [2],[9]. In Kenya, in addition to subsistence use, the excess grains are sold in the local market where it usually fetches higher prices than most of the other common legumes [10].

In Africa, lablab has remained neglected and underutilized despite the crop being well adapted to arid and semiarid areas which are characterized by food insecurity [7]. Like other underutilized crops in Africa, less research attention has been given towards the improvement of lablab in Kenya. This is evident by: limited literature on the crop, poor crop productivity and low grain yield on farmers' fields. The genetic potential of this leguminous crop is enormous and can be fully realised through research and crop improvement. Understanding the genetic diversity of the existing germplasm will be critical for crop improvement [2]. In Kenya, minimal effort has been made to study the diversity of genes present in the local lablab genetic resource [2], [5], [11]. However, most of the germplasm collections used in earlier studies were small compared to the available local collections, had no reference accessions or varieties included so that no complete picture of diversity could be derived from these studies. In addition, these studies did not include collections from outside the country to understand the diversity of the local materials in relation to the worldwide collections.

Various techniques have been used in discrimination of plant genotypes. This include the use of morphological markers, biochemical evaluation and DNA marker analysis [12]–[14]. Molecular or DNA markers are increasingly being used to complement morphological markers in germplasm characterization because of being independent of environmental influence [13]. Various types of molecular markers have been used to assess the level of genetic variations of plant populations. Among them is simple sequence repeats (SSRs) or microsatellites marker. SSRs are short tandem repeats motifs usually with varying number of repeats at a given locus. This variation in the number of repeats is as a result of the high mutation rate at SSRs loci caused by DNA slippage during the DNA replication process [15] have been used widely in genetic analysis of crops due to their wide distribution in genome, can reveal multiple alleles, are co-dominant and are highly reproducible [16] [17] [12] [18] [19]. Limited studies have been reported on genetic diversity analysis of lablab using SSRs in Kenya. For instance, [11] employed SSRs developed from common beans (*Phaseolus vulgaris*) to study genetic diversity of 13 lablab accessions while [20] used SSRs designed from lablab in genetic diversity analysis of 96 accessions grown by farmers in Kenya. The genetic diversity of Kenyan lablab accessions and their phylogenic relationships with lablab collected from other parts of the globe is still not well understood. This study set to assess the genetic diversity in Kenyan and other introduced lablab accessions based on SSR markers.

Materials and methods

Plant materials

A total, 189 accessions were evaluated in this study and described in Supplementary Table 1. These materials included accessions previously collected from farmers' fields and markets (40 accessions) in various lablab growing regions of Kenya, some advanced determinate early maturing lines from Kenya Agriculture and Livestock Research Organization (KALRO) lablab breeding program (8 lines), released variety (1 variety) and accessions preserved at the GBK (43 accessions). The advanced breeding lines were pedigrees of crosses between accession KDD 2, Njoro and GBK 028663. Also included was a collection from the International Livestock Research Institute Forage Germplasm (ILRI-FG) which comprised of cultivars from other parts of Africa, USA, Asia, Australia and accessions of unknown origin. The ILRI-FG was established and has been maintained since 1982.

Genomic DNA isolation

The activity was undertaken at Kenya Agricultural and Livestock Research Organization (KALRO) Biotechnology Research Centre, Nairobi. Seeds of the 189 accessions were planted in a greenhouse. At two leaves stage, approximately one gramme of leaf tissue of each genotype was harvested, placed on labelled zip lock papers and immediately placed on ice box. DNA was isolated using modified cetriamonium bromide method (CTAB) [21]. Approximately 0.3g of the leaf tissues were placed in self-standing tubes each containing a ceramic bead. Nine hundred micro-liters (900µl) of extraction buffer was added to the leaf tissue containing the ceramic bead. The samples were crushed by genogrinder machine (Benchtop homogenizer) set at revolution speed of 4 meter per second for one minute. The samples were then incubated in a water bath with constant shaking at 65^0C for 15 minutes. The tubes were then centrifuged at 14000 rpm for five minutes. Six hundred microliters (600µl) of the supernatant were transferred into a fresh eppendorf tube and an equal volume of chloroform: isoamyl alcohol (24:1) added. The above step was repeated. Four hundred microliters (400µl) of the aqueous phase was transferred to a fresh tube and an equal volume of ice-cold isopropanol added and mixed by inverting several times and incubated at -20^{o}C for 2 hours to precipitate the DNA. The tubes were centrifuged at 14000rpm for 5 minutes. The supernatant was decanted leaving the DNA pellet at the bottom of the tube. The pellets were washed using 500µl of 70% ethanol and centrifuged for one minute at 14000rpm before they were air dried for one hour. The dried pellets were re-suspended in 50µl of sterile distilled water. RNA was removed by adding two microliters (2µl) of pancreatic ribonuclease A (RNase A) (10mg/ml) and incubating the samples for 30 minutes at 37^{o}C. The samples were stored at -20^{o}C.

The concentration and purity of the sample DNA was determined using a Nanodrop spectrophotometer (Thermo Scientific NanoDrop 2000C). The samples were then diluted with molecular water to a final concentration of 20 ng/ µl for use in the PCR.

Polymerase chain reaction and Polyacrylamide gel electrophoresis

Eight SSR markers were used to discriminate the 189 lablab accessions. These markers were pre-selected based on their ability to reveal many clear polymorphic bands on diverse lablab accessions. The eight markers are listed in Table 2 below.

The following were used in the 25ul PCR; pureTaq Ready-to-Go PCR beads (Illustra, UK) containing (dNTPs, $MgCl_2$, Tris-HCl (pH 9.0), KCl and Taq), 19µL of sterile distilled water, 2µL of 0.1 pmoles/ul of forward and reverse primer and 2µL of template of 20ng/ul DNA. Amplification was carried out in a Thermocyler machine (Applied Biosystems Veriti systems) programmed with the following regime 94°C for 3 minutes, 35 cycles of 94°C for 1-minute, annealing temperature of 57^{o}C for 1 minute, 72°C for 1 minute and final extension at 72°C for 10 minutes and final hold at 4^0C.

Data collection

The PCR products were size separated using 6% PolyAcrylamide gel (PAGE). The 6% gel was prepared as follows; 53.8 ml of distilled water

was put in a clean 500 ml glass beaker, 18.8 ml of 40% acrylamide/ bis-acrylamide 19:1 was added to the beaker, 2.5ml of 50x TAE (Tris acetate EDTA) was then added and mixed by stirring, one tablet containing ammonium persulfate (APS) and TEMED was dissolved in 50ml distilled water in a 50 ml falcon tube, the APS/TEMED solution was then added to the glass beaker and gently stirred without introducing any air bubble, the solution was immediately poured into the gel tray with in-built combs and was covered with glass plate treated with silane solution and allowed to gel for 1 hour. The glass plate with the attached PAGE was then transferred to the horizontal PAGE (hPAGE) (Cleaver Scientific) electrophoresis tank containing 1X TAE solution. Two micro liters (2uL) of samples were loaded into the wells alongside 3.0µl 1kb DNA ladder and run at 140V for 3hrs. The glass plate was removed from the gel tank and placed in another glass tray for post staining of the gel. To stain the PAGE, 6uL of ethidium bromide at a concentration of 10mg/ml was dissolved in 200 ml of water and poured into the glass tray to cover the glass plate containing the PAGE. The tray was kept in a dark room for 1 hour. The staining solution was then drained off and the gel de-stained using clean water for 30-45 minutes.

The amplified products were then viewed under UV light in a gel box (G: Box, Syngene). The images were captured using a camera connected to the gel box and saved in the computer. To score the bands, the images were pasted in MS power point program. On the gel image, a straight line was drawn across the bands that had migrated the longest distance. The accessions that had bands at that position were scored as 1 while those that didn't have were scored 0. The straight line was moved to another allele and scoring was done. The process continued until all the bands were scored.

Data analysis

The number of alleles per locus, major allele frequency per marker, gene diversity, heterozygosity and the polymorphic information content (PIC) for each locus was computed using PowerMarker version 3.25. GenAlEx version 6.501 [22] was used to estimate, the number of different alleles per locus, number of effective alleles per locus, expected heterozygosity, Shannons Information Index, percentage of polymorphic loci and analysis of molecular variance (AMOVA). Dissimilarity matrix was generated using simple matching coefficient of DARwin software version 6.0.17. The dissimilarity matrix was used to construct Principal Coordinate Analysis (PCoA) scatter plot. The Bayesian based STRUCTURE software version 2.3.3 was used to investigate population structure of the 189 lab-lab accessions. The number of clusters (K) in STRUCTURE were set from 2 to 8. Each run consisted of a burn-in period of 5000 steps followed by 50000 Monte Carlo Markov Chain (MCMC) replicates. We assumed an admixture model and uncorrelated allele frequencies. To determine the most likely number of clusters we used delta value (ΔK) method proposed by [23] using the STRUCTURE Harvester available online at http://taylor0.biology.ucla.edu/struct_harvest/. To assign accessions to various groups, the accessions with estimated memberships above 0.8 were allocated to the matching group while those with below 0.8 were assigned to a mixed group.

Results

SSR Polymorphism

A total of 39 alleles were revealed by the eight SSRs with an average of 4.88 alleles per polymorphic loci (Table 3). The number of alleles per locus ranged from 3 for locus Lab RRT77 to 7 for Locus Lab T12. The PIC ranged from 0.149 (Lab RRT77) to 0.772 (Lab T12) with an average of 0.420. Half of the markers were highly polymorphic with PIC values of over 0.5. The gene diversity varied among the loci with an average of 0.45 recorded. All the markers had lower observed heterozygosity (Ho) compared to expected heterozygosity (He). The average observed heterozygosity was low with an average of 0.02. The locus Lab RRT53 did not display any observed heterozygosity. The inbreeding coefficient (***f***) of the SSR loci was high with an average of 0.94.

Genetic diversity within and among 189 lablab accessions

The summary of genetic diversity indices of the nine lablab populations are shown in Table 4. The average number of different alleles (Na) were highest for Kenyan population (4.375), South Africa population (3.875) and Ethiopia population (3.750) but lowest for Uganda population at 1.625. The number of effective loci (Ne) ranged from 2.452 to 1.570 with an overall mean of 1.930. High gene diversity of > 0.5 was recorded only in Ethiopia and South Africa populations. However, moderate gene diversity of 0.3-0.4 was observed for advanced lines, Australia, India, Kenya and West Africa populations of lablab. The average expected heterozygosity was moderate at 0.38. The highest Shannon's Index (I) was observed on Ethiopia (0.964) and South Africa (0.929) populations while the least was in Uganda population (0.661). The observed heterozygosity (Ho) values were very low for all the populations studied indicating the inbreeding nature of the lablab crop. However, the highest observed heterozygosity of 0.09 was recorded on advanced lines suggesting that these lines could still be segregating at some loci.

Population differentiation

Analysis of molecular variance (AMOVA) revealed that 94% of the allele diversity was attributed to individuals within populations (94%) while only 6% was distributed among the populations (Table 5). A low value of ΦPT of 0.061 was observed confirming that there was only a small differentiation among the populations. However, the p-value (0.01) indicated that there was significant differences among the populations.

Population structure analysis using Bayesian-model Structure

We explored population structure in the 189 *Lablab purpureus* germplasm using the Bayesian model-based Structure method. According to [23], the real number of population is detected at the modal value or the upper most value of Delta K. In this study, the highest value of delta K was observed at K=3 (Figure 1) suggesting that the 1 89 lablab accessions are assigned to three subgroups.

The 3 subpopulations detected using STRUCTURE are shown in Figure 2. Each lablab accession is represented by a vertical bar. The bars are segmented into different coloured fragments which represented the estimated membership of a certain subpopulation. The STRUCTURE cluster 1 comprised of 68 accessions while clusters 2 and 3 had 77 and 22 accessions respectively. The STRUCTURE cluster 1 contained accessions from Kenya, Ethiopia, SA, Australia, India, USA and West Africa. The accessions from Kenya included those collected from gene bank of Kenya (GBK 12219, 012215, 011723, 011719, 013096), from central Kenya (Kagio, Muranga), coastal Kenya (Lamu, Kibwezi), western Kenya (Kakamega, Kitale) and those preserved at ILRI (ILRI 14901, ILRI 14445). The eleven accessions from SA included in STRUCTURE cluster 1 were mainly from sub species purpureus. Materials from Ethiopia allocated to cluster 1 included ILRI 13686, ILRI 13688, ILRI 13700, ILRI 13701, ILRI 6528, ILRI 6533, ILRI 6537 and ILRI 6930, those from Australia included ILRI 21071, ILRI 21059, ILRI 14414, ILRI 21087 and ILRI 11617. All accessions from India with exception of one bengalensis (ILRI 21032) were also grouped in STRUCTURE cluster 1. Similarly, included in cluster 1 were all the accessions from USA and five accessions from West Africa which consisted of two accessions (ILRI 11615 and ILRI 24810) belonging to sub species bengalensis.

STRUCTURE cluster 2 was assigned accessions from Kenya, Ethiopia, SA, Australia, West Africa and India. The Kenyan materials in this cluster originated from all major lablab growing areas and markets such as central region (Kahuro), Rift valley region (Nakuru, Njoro, Bahati, Namanga, Eldoret), western region (Kisumu and Bungoma) and eastern region (Kitui and Meru). Majority (79%) of the Kenyan accessions from the gene bank of Kenya were included in this cluster. Cluster 2 also included 11 accessions from Ethiopia such as ILRI 6535, ILRI 6536, ILRI 7278, ILRI 13685, ILRI 13688, ILRI 13687, five from South Africa (ILRI 14437, ILRI 14435, ILRI

14419, ILRI 24777, CPI 666243), from Australia (ILRI 21061, ILRI 21076, ILRI 11617), from West Africa (ILRI 11630, ILRI 10953) and India (ILRI 21032).

All the accessions allocated to cluster 3 were of African (South Africa, Ethiopia, Kenya, Uganda) origin. The accessions from South Africa included the two seeded wild accessions (ILRI 21045, ILRI 24800 and ILRI 21083), four seeded wild accessions (ILRI 21048, ILRI 24749) and cultivated sub species purpureus (ILRI 14437, ILRI 21084, ILRI 21085). The accessions from Kenya consisted of a two seeded wild germplasm (ILRI 14440) and five local collection. The accessions included in cluster 3 from Ethiopia consisted of a bengalensis (ILRI 13692) and other three sub species purpureus (ILRI 13694, ILRI 13695, and ILRI 13704). This group also included accession ILRI 21081 and a four seeded wild germplasm (ILRI 24756) from Uganda. A total of 22 accessions showed some mixed ancestry (the membership value of less than 80% of any sub population). The 15 accessions in [24] core collection included in this study were distributed in all the three STRUCTURE clusters. For instance, accessions ILRI 13700, ILRI 13701, ILRI 6533, ILRI 11615, ILRI 13702, ILRI 6930 and ILRI 20134 were grouped in STRUCTURE cluster 1. Included in cluster 2 were ILRI 11630, ILRI 13687, ILRI 14411 and ILRI 14437. The third cluster consisted of accessions ILRI 14440, ILRI 13692, ILRI 13694 and ILRI 13695. All the accessions from sub species unicinatus with exception of accession ILRI 24778 were grouped in the STRUCTURE cluster 3 which was dominated by accessions from Africa. The accessions belonging to sub species bengalensis were split into all the three STRUCTURE clusters. All the seven advanced lines (AL lines) from a breeding program in Kenya were grouped in clusters 1 and 2 together with their parental lines (Njoro, GBK 028663B, KDD).

Principal coordinate analysis (PCoA) analysis was done using DARwin 6.0.17 software to further understand the population structure of the 189 lablab accessions. The PCoA analysis separated the accessions into three clusters thereby confirming the results obtained with the STRUCTURE software. The membership of each Structure cluster was similar to those of PCoA clusters. The degree of distribution of the accessions in the PCoA scatter plot varied across the clusters (Figure 3). For instance, accessions in cluster 1 and cluster 3 were widely dispersed in their distribution, while those in cluster 2 were concentrated on the upper left-hand quadrant of the plot. This suggest that the accessions within cluster 1 and 3 are more diverse than those in cluster 2.

Discussion

The understanding of genetic diversity within a population and among the germplasm populations is important for both plant conservation and breeding program [25]. This knowledge enables the breeders to identify suitable parents for making genetic crosses to create recombination for selection of improved crops [26]. Genetic markers are preferred to morphological markers in germplasm characterization because they provide variability at genetic level thereby providing better estimate of genetic diversity [11]. In the present study, 8 SSR markers were used for genetic diversity study in 189 lablab germplasm accessions. The microsatellite marker analysis found moderate level of polymorphism in the worldwide lablab collection. The number of alleles per locus detected by the microsatellites were moderate ranging from two to seven with mean of 4.88 respectively. The number of alleles suggests the richness of the population [27]. The higher the allelic richness at a locus, the higher the degree of diversity. The intensity of the alleles per locus identified in this study compares well with those reported in lablab by [11], [20], [28]. However, allelic richness in this study is lower than 7.4 alleles per locus reported in lablab by [29]. The lower number of alleles per locus observed here could have been attributed to the difference in genetic population tested. In addition, the DNA fragments analysis method PAGE used in this study has moderate resolving power while [29] used automatic capillary sequencing which has a higher resolving power.

Polymorphic information content (PIC) is a good estimator of the informativeness of molecular marker loci because it takes into account both the number alleles and the relative frequencies of the alleles. The higher the PIC value, the more informative is the SSR marker [20], [30]. This study revealed polymorphisms of moderate informativeness (mean PIC value 0.419) suggesting their suitability for diversity analysis. The average PIC value in this study compares with 0.492 by [29] in lablab and 0.56 in [16] but slightly lower than 0.63 by [20] in lablab. Microsatellites are more informative than other markers due to their high mutations that evade correction during DNA mismatch repair system thereby allowing formation of new alleles in those loci [19].

The total gene diversity (GD) or average expected heterozygosity (H), is a good descriptor of the diversity of the alleles revealed in germplasm by the molecular markers [13]. The GD value ranges from 0-1 with values close to one indicating very high allelic diversity. In this study, majority of the markers recorded moderate to high expected heterozygosity of more than 0.4 suggesting that the markers were able to reveal fairly high number of equally frequent alleles. The SSR markers used revealed high level of polymorphism and therefore supported their application in genetic studies such as genetic diversity and genetic linkage map construction.

Previous genetic studies of Kenyan accessions reported a low to moderate genetic diversity and suggested the need to diversify the genetic base of the local accessions by introducing the wild cultivars and exotic germplasm [2], [11]. The diversity of Kenyan germplasm in relation to those from the region and other continents remain unknown. This study considered diversity of a wide range of accessions collected from Kenya, other parts of Africa and beyond the continent. The present study showed that the heterozygosity expected under Hardy Weinberg Equilibrium (He) was moderate (mean He=0.38) for the 189 lablab accessions studied across eight SSR loci. In addition, the low number of different allele (Na=2.8) and average effective locus (Ne=1.93) further reflected the low to moderate genetic diversity of these accessions. The expected heterozygosity, number of different allele and effective number of locus are important indicators of population diversity and its potential for adaptability [31]. The higher the value of these parameters the higher the degree of diversity present in the germplasm under study.

The highest gene diversity of > 0.5 was detected for Ethiopia and South Africa populations. This is not unexpected because the origin of lablab has been suggested to be Southern and Eastern Africa while Ethiopia is considered as a Centre of diversity [32]. Regions of origin and diversification are expected to contain larger genetic diversity [33]. Another reason for high genetic diversity in the South Africa population could be due to the high number of wild accessions included in the population. Among the 27 accessions originating from South Africa, nine accessions belonged to sub species unicinatus. [24], reported that the wild accessions from Eastern and Southern Africa represented a far wider diversity than the cultivated accessions. [1], further classified the wild accessions belonging to sub species unicinatus into two type's namely 4-seeded pod and 2-seeded pod and noted that the 2-seeded pod types were distinct from the other crop germplasm. In this study, out of the nine accessions of sub species unicinatus included in the South Africa population, four belonged to the 2-seeded pod type further explaining the high gene diversity in this Southern Africa population. The moderate gene diversity (He=0.363) for Kenya population observed in this study compares well with that of [20] in lablab. Whereas there is substantial amount of genetic diversity in Kenyan lablab accessions, the country breeding programs can benefit more from introducing germplasm from other countries especially those from South Africa and Ethiopia because they may contain valuable genes of interest like drought tolerance, diseases and insect pest resistance.

In the present study, there was low genetic differentiation among the populations as indicated by the AMOVA results which showed that variation within populations accounted for the majority

(94%) of the total variation. Low inter-population variability could be as a result of the populations not been separated long enough to accumulate detectable genetic differences or may arise from high levels of gene flow [34]. There is a long history of exchange of lablab germplasm within Africa and other regions of the world as reviewed by [32]. For instance, before 2000 B.C lablab was transferred from Africa to India through Sabaean lane. During the slave trade lablab was transferred from West Africa to Brazil and Caribbean region where it was cultivated. After the abolishment of slave trade, the Indians carried lablab from the Caribbean region back to India subcontinent. In the present era, gene flow between populations could also be attributed to sharing of germplasm conserved in the gene banks. The cross boarder grain trade between countries could also have contributed to the high gene flow between populations. For instance, in 2014, Kenya imported lablab grain worth 124,000 Mt from the region [10]. Some of these grains could have been used as seeds by small holder farmers who purchase grain in the market for planting [5].

To further understand the population structure in the 189 world wide lablab accessions, STRUCTURE and Principal Coordinate Analysis (PCoA) were used to identify genetically similar individuals based on the SSR allele frequencies. The Bayesian model-based STRUCTURE analysis method clustered the accessions into three groups not related to their location of origin. The overlay in clustering between individuals from different regions and countries observed in this study suggest widespread genetic exchange among the regions [35] . The results were consistent with that of [1], [36]. The study of [1] showed that using AFLP markers, 103 lablab accessions from Africa and Asia were clustered into 12 groups that represented plant types and not the geographical origin. The study of [36] used 22 SSRs to screen 24 lablab accessions from China and Africa and showed that some materials from the two regions were clustered together in one group. Notably, STRUCTURE analysis clusters, only accessions from African origin were present in all the groupings suggesting huge genetic variation from this continent.

The proportion of the accessions allocated to the STRUCTURE admixture cluster in this study was small (22 accessions out of 189). This suggest that even though some evidence of widespread genetic exchange in the region exist, there is low intraspecific hybridization occurring among the populations. This would support the argument by [7] that lablab as an underutilized species has undergone limited crop improvement and that the few available improvement programs are based on limited genetic diversity. Lablab is predominantly self-fertilizing crop [5] and therefore any population admixture can significantly occur through the organized crossing of parents in breeding programs.

The present study showed that in all the two population structure analysis approaches (STRUCTURE analysis and PCoA) the accessions belonging to sub species unicinatus were clustered together and shared the clusters only with individuals originating from Africa. The accessions which clustered closely to sub species unicinatus were all from Southern and Eastern Africa (Ethiopia, Uganda and Kenya) regions suggesting the origin of lablab to these areas. Previous studies have indicated Africa as origin for lablab [1], [29]. These accessions belonging to sub species unicinatus require to be integrated in the lablab breeding program because they carry valuable gene resources [1].

The accessions collected from gene bank of Kenya and those obtained from ILRI gene bank were split across all the three clusters of STRUCTURE indicating their wide diversity. This suggests that these gene banks are important reservoirs of lablab allelic diversity that is much needed for the genetic improvement of this underutilized legume. Gene banks are centres of *ex-situ* conservation of wide range of plant genetic diversity necessary for improvement of crops. Lablab improvement programs should therefore introduce and integrate into their breeding programs the unique germplasm collection held at various gene banks [7].

The Kenyan accessions evaluated in this study were distributed in all clusters of both PCoA scatter plot and STRUCTURE but some clusters had more membership than others. The overlap of the Kenyan genotypes with other accessions from the rest of Africa and beyond the continent in the population structure indicate that the local germplasm is a significant representation of the genetic diversity available globally. An important consideration in plant breeding is in the selection of very divergent parents for use in artificial crosses to generate superior recombinant genotypes [37].

Conclusion

In this study, SSR allelic data was used to explore genetic diversity and population structure among a worldwide lablab collection. The study revealed moderate genetic diversity (He= 0.36) within the Kenyan lablab accessions. Higher gene diversity (He<0.5) was detected within the Ethiopian and South Africa populations. This is probably because the two are considered as regions of origin and domestications. The low genetic differentiation detected between populations as indicated by AMOVA could be due to gene flow across the regions. The Kenyan accessions were distributed in all clusters of both PCoA scatter plot and STRUCTURE but some clusters had more membership than others suggesting that the local germplasm is a fair representation of the genetic diversity available globally. Majority of the wild accessions grouped far away from cultivated materials revealing the dissimilarity of wild and cultivated lablab germplasm. To broaden the genetic base of Kenyan lablab accessions, breeders must target the new alleles from the wild accessions and those from other distantly related accessions especially from Ethiopia and South Africa.

Conflict of interest

The authors have no conflict of interest to declare.

Acknowledgment

We thank Kirkhouse Trust for their financial support and laboratory reagents and consumables used in this study. We also thank Forage diversity project, International Livestock Research Institute (ILRI), Ethiopia for providing some Lablab purpureus accessions used in this study.

Supplementary Table 1: List of 189 lablab accessions genotyped using 8 SSR markers

Code	Accession	Name of population	Region of collection	Sub species	Code	Accession	Name of population	Region of collection	*Sub species*
1	NH13	Advan. Line	KALRO	*Purpureus*	34	ILRI 13694	Ethiopia	ILRI	*Purpureus*
2	NH17	Advan. Line	KALRO	*Purpureus*	35	ILRI 13686	Ethiopia	ILRI	*Purpureus*
3	NH7	Advan. Line	KALRO	*Purpureus*	36	ILRI 6930 A	Ethiopia	ILRI	*Bengalensis*
4	OH12	Advan. Line	KALRO	*Purpureus*	37	ILRI 13695	Ethiopia	ILRI	*Purpureus*
5	NH8	Advan. Line	KALRO	*Purpureus*	38	ILRI 6533	Ethiopia	ILRI	*Purpureus*
6	NH4	Advan. Line	KALRO	*Purpureus*	39	ILRI 6537	Ethiopia	ILRI	*Purpureus*
7	OH2	Advan. Line	KALRO	*Purpureus*	40	ILRI 6528	Ethiopia	ILRI	*Purpureus*
8	NH1	Advan. Line	KALRO	*Purpureus*	41	ILRI 6535	Ethiopia	ILRI	*Purpureus*
9	ILRI 21061	Aus	ILRI	*Purpureus*	42	ILRI 7278	Ethiopia	ILRI	*Purpureus*
10	ILRI 21076	Aus	ILRI	*Purpureus*	43	ILRI 18618	Ethiopia	ILRI	*Purpureus*
11	ILRI 11617	Aus	ILRI	*Purpureus*	44	ILRI 18619	India	ILRI	*Purpureus*
12	ILRI 21087	Aus	ILRI	*Purpureus*	45	INDIA 534	India	ILRI	*Purpureus*
13	ILRI 11612	Aus	ILRI	*Purpureus*	46	DOL 414-2	India	ILRI	*Purpureus*
14	ILRI 21071	Aus	ILRI	*Purpureus*	47	DOL 414-3	India	ILRI	*Purpureus*
15	ILRI 21059	Aus	ILRI	*Purpureus*	48	ILRI 21032	India	ILRI	*Bengalensis*
16	ILRI 14414	Aus	ILRI	*Purpureus*	49	ILRI 21034	India	ILRI	*Purpureus*
17	ILRI 13692 B	Eth	ILRI	*Bengalensis*	50	ILRI 147	India	ILRI	*Purpureus*

18	ILRI 6930	Eth	ILRI	*Bengalensis*	51	ILRI 21034	India	ILRI	*Purpureus*
19	ILRI 6536	Eth	ILRI	*Purpureus*	52	ILRI 21070	India	ILRI	*Purpureus*
20	ILRI 13692	Eth	ILRI	*Bengalensis*	53	ILRI 21033	India	ILRI	*Purpureus*
21	ILRI 13702	Eth	ILRI	*Purpureus*	54	ILRI 21088	India	ILRI	*Bengalensis*
22	ILRI 13701	Eth	ILRI	*Purpureus*	55	KDD-1	Kenya	Kenya	*Purpureus*
23	ILRI 13688	Eth	ILRI	*Purpureus*	56	KDD-2	Kenya	Kenya	*Purpureus*
24	ILRI 13687	Eth	ILRI	*Purpureus*	57	ILRI 14901B	Kenya	ILRI	*Purpureus*
25	ILRI 13688 B	Eth	ILRI	*Purpureus*	58	ILRI 14445	Kenya	ILRI	*Purpureus*
26	ILRI 13685	Eth	ILRI	*Purpureus*	59	ILRI 14443	Kenya	ILRI	*Purpureus*
27	CPI 195851	Eth	ILRI	*Purpureus*	60	ILRI 14490	Kenya	ILRI	*Purpureus*
28	ILRI 13700	Eth	ILRI	*Purpureus*	61	ILRI 14478	Kenya	ILRI	*Purpureus*
29	ILRI 14442	Eth	ILRI	*Purpureus*	62	ILRI 14481	Kenya	ILRI	*Purpureus*
30	ILRI 13697	Eth	ILRI	*Purpureus*	63	ILRI 14901	Kenya	ILRI	*Purpureus*
31	CPI 347628	Eth	ILRI	*Purpureus*	64	ILRI 14488	Kenya	ILRI	*Purpureus*

Table 1 continued……

Code	Accession	Name of population	Region of collection	Sub species	Code	Accession	Name of population	Region of collection	Sub species
65	GBK 005380	Kenya	Eastern	*Purpureus*	96	GBK 010494	Kenya	Unknown	Purpureus
66	GBK 010409	Kenya	Unknown	*Purpureus*	97	GBK 010707 B	Kenya	Eastern	Purpureus
67	GBK 010436	Kenya	Eastern	*Purpureus*	98	GBK 012026	Kenya	Coast	Purpureus
68	GBK 010439	Kenya	Eastern	*Purpureus*	99	GBK 010708	Kenya	Coast	Purpureus
69	GBK 010494 A	Kenya	Unknown	*Purpureus*	100	GBK 012038	Kenya	Coast	Purpureus
70	GBK 010707	Kenya	Eastern	*Purpureus*	101	GBK 010392	Kenya	Unknown	Purpureus
71	GBK 010708	Kenya	Coast	*Purpureus*	102	GBK 011733	Kenya	Eastern	Purpureus
72	GBK 010822	Kenya	Coast	*Purpureus*	103	GBK 013083 B	Kenya	Eastern	Purpureus
73	GBK 010824 A	Kenya	Rift	*Purpureus*	104	Kakamega mkt.	Kenya	West	Purpureus
74	GBK 010837	Kenya	Rift	*Purpureus*	105	Namanga	Kenya	Rift	Purpureus
75	GBK 010843	Kenya	Rift	*Purpureus*	106	Nakuru 2	Kenya	Rift	Purpureus
76	GBK 012230	Kenya	Unknown	*Purpureus*	107	Bungoma 1	Kenya	West	Purpureus
77	GBK 10708	Kenya	Coast	*Purpureus*	108	Kagio mkt.	Kenya	Central	Purpureus
78	GBK 012221	Kenya	Unknown	*Purpureus*	109	Kahuro A	Kenya	Central	Purpureus
79	GBK 012219	Kenya	Unknown	*Purpureus*	110	Muranga mkt.	Kenya	Central	Purpureus
80	GBK 012215	Kenya	Rift	*Purpureus*	111	Kisumu	Kenya	West	Purpureus
81	GBK 012187	Kenya	Coast	*Purpureus*	112	Lamu	Kenya	Coast	Purpureus
82	GBK 012038 B	Kenya	Coast	*Purpureus*	113	Kagio B	Kenya	Central	Purpureus
83	GBK 012033	Kenya	Unknown	*Purpureus*	114	Bahati	Kenya	Rift	Purpureus
84	GBK 012032	Kenya	Unknown	*Purpureus*	115	Kahuro B	Kenya	Eastern	Purpureus
85	GBK 012026 C	Kenya	Coast	*Purpureus*	116	Meru	Kenya	Eastern	Purpureus
86	GBK 012000	Kenya	Eastern	*Purpureus*	117	Mukinduri	Kenya	Eastern	Purpureus
87	GBK 011803	Kenya	Unknown	*Purpureus*	118	Kakamega mkt.14	Kenya	West	Purpureus
88	GBK 011733 B	Kenya	Eastern	*Purpureus*	119	Kitale mkt15	Kenya	Rift	Purpureus
89	GBK 011723	Kenya	Nairobi	*Purpureus*	120	Kitui 20 A	Kenya	Eastern	Purpureus
90	GBK 011719	Kenya	Eastern	*Purpureus*	121	Kitui 20 C	Kenya	Eastern	Purpureus

91	GBK 013083	Kenya	Eastern	*Purpureus*	122	Kitui 17	Kenya	Eastern	Purpureus
92	GBK 013096	Kenya	Eastern	*Purpureus*	123	Kitale mkt.16	Kenya	Rift	Purpureus
93	GBK 028663 B	Kenya	Eastern	*Purpureus*	124	Makindu 18	Kenya	Eastern	Purpureus
94	GBK 045372	Kenya	Eastern	*Purpureus*	125	Matiliku mkt.11	Kenya	Eastern	Purpureus
95	GBK 010824	Kenya	Rift	*Purpureus*	126	Machakos	Kenya	Eastern	Purpureus

Table 1 continued……

Code	Accession	Name of population	Region of collection	Sub species	Code	Accession	Name of population	Region of collection	Sub species
127	Kitale Wamuini 2	Kenya	Rift	*Purpureus*	159	ILRI 14415	SA	ILRI	Purpureus
128	Kitale Kala 1	Kenya	Rift	*Purpureus*	160	ILRI 14412	SA	ILRI	Purpureus
129	Kibwezi 5	Kenya	Eastern	*Purpureus*	161	ILRI 24800 B	SA	ILRI	Unicin 2
130	Bungoma 3	Kenya	West	*Purpureus*	162	ILRI 24749	SA	ILRI	Unicin 4
131	Kibwezi	Kenya	Eastern	*Purpureus*	163	ILRI 24781	SA	ILRI	Purpureus
132	Matiliku mkt.12	Kenya	Eastern	*Purpureus*	164	ILRI 24796	SA	ILRI	Unicin 4
133	Kitale Mkt.16 B	Kenya	Rift	*Purpureus*	165	ILRI 21085	SA	ILRI	Purpureus
134	Machakos 13	Kenya	Eastern	*Purpureus*	166	CPI 666245	SA	ILRI	Purpureus
135	Nakuru 1	Kenya	Rift	*Purpureus*	167	ILRI 14435	SA	ILRI	Purpureus
136	DL 1002	Kenya	Eastern	*Purpureus*	168	ILRI 24778	SA	ILRI	Unicin 4
137	GBK 028663	Kenya	Eastern	*Purpureus*	169	ILRI 21049	SA	ILRI	Purpureus
138	Kahuro	Kenya	Central	*Purpureus*	170	ILRI 24780	SA	ILRI	Purpureus
139	Eldoret	Kenya	Rift	*Purpureus*	171	CPI 666243	SA	ILRI	Purpureus
140	Njoro	Kenya	Rift	*Purpureus*	172	ILRI 14419	SA	ILRI	Purpureus
141	Kakamega	Kenya	West	*Purpureus*	173	ILRI 24777 B	SA	ILRI	Purpureus
142	Lamu B	Kenya	Coast	*Purpureus*	174	ILRI 24799	SA	ILRI	Purpureus
143	Kagio	Kenya	Central	*Purpureus*	175	ILRI 21083	SA	ILRI	Unicin 2
144	Bungoma 2	Kenya	West	*Purpureus*	176	ILRI 21084 B	SA	ILRI	Purpureus
145	Meru B	Kenya	Eastern	*Purpureus*	177	ILRI 21081	Uganda	ILRI	Purpureus
146	ILRI 14440	Kenya	ILRI	*Unicin 2 seeded*	178	ILRI 21081 B	Uganda	ILRI	Purpureus
147	ILRI 14460	Kenya	ILRI	*Purpureus*	179	ILRI 24756	Uganda	ILRI	Unicin 4
148	ILRI 14458	Kenya	ILRI	*Purpureus*	180	ILRI 10979	USA	ILRI	Purpureus
149	ILRI 14411	Kenya	ILRI	*Purpureus*	181	ILRI 18611 G	USA	ILRI	Purpureus
150	ILRI 21046	SA	ILRI	*Purpureus*	182	ILRI 18611 P	USA	ILRI	Purpureus
151	ILRI 14437	SA	ILRI	*Purpureus*	183	ILRI 10953 B	WA	ILRI	Purpureus
152	ILRI 21084	SA	ILRI	*Purpureus*	184	ILRI 14441	WA	ILRI	Purpureus
153	ILRI 21045	SA	ILRI	*Unicin 2*	185	ILRI 11615	WA	ILRI	Bengalensis
154	ILRI 14437 B	SA	ILRI	*Purpureus*	186	ILRI 10953	WA	ILRI	Purpureus
155	ILRI 24800	SA	ILRI	*Unicin 2*	187	ILRI 24810	WA	ILRI	Bengalensis
156	ILRI 21048	SA	ILRI	*Unicin 4*	188	ILRI 11630	WA	ILRI	Purpureus
157	ILRI 21048 B	SA	ILRI	*Unicin 4*	189	ILRI 11614	WA	ILRI	Purpureus
158	ILRI 24777	SA	ILRI	*Purpureus*					

Table 2: List of 8 lablab specific SSR markers used to evaluate 189 accessions

Primer name	Forward primer (5´-3´)	Reverse primer (5´-3´)	Expected Product size in bp	Primer melting temp. (Tm) in ^{0}C
Lab T2	GTGCGCGTCACTTATTAG-TTCTTA	CAATATCTTCACGTAACCAC-GGTA	224	59.3 & 61.0
Lab RRT23	GGGAGTGTGAAATAGA-GAATCAGTT	CAGCACTATCCACACCTGCAA-TAC	136	59.0 & 62.7
Lab RRT28	AATCGAACAAAGTGAAGTGC CTTG	AAA-TAGCCTCCAACTTCTCCCACT	96	59.3 & 61.0
Lab RRT44	AAGCTTCGTT-GTTTCTGCGATTAG	CGAGCTTTAAACCAATCAG-GACAC	92	59.3 & 61.0
Lab RRT90	ATAACTCTGGCTCGCTCTGTG G	GTGCATTTGATTTGGTGGGAAA	234	56.5 & 62.1
Lab RRT53	ACACCACATCACACAC-TTATTC	CTTGCTGACTGTTCTCCATT	176	58.4 & 59.0
Lab RRT77	CTTTCTCCTTCTCTTTCTCACT C	GAAGACGGGTAGTTCCTAG-TTAT	181	59.3 & 61.3
Lab T12	CACCACCTCCAACTTCTAC-GGTTA	TGACCTCCATTATGGGAT-TCAGAT	193	59.3 & 62.7

Table 3: Summary of PowerMarker data analysis of 189 lablab accessions with 8 SSR markers

Marker	MAF	SS	No.	AN	Avai.	GD	Obs.Heter	PIC	f
Lab T12	0.2971	189	170	7	0.8995	0.8008	0.0176	0.7722	0.9781
Lab T2	0.5772	189	162	6	0.8571	0.6146	0.0062	0.5783	0.9900
Lab RRT23	0.6872	189	187	4	0.9894	0.4712	0.0588	0.4174	0.8758
Lab RRT 44	0.9181	189	177	5	0.9365	0.1550	0.0621	0.1514	0.6009
Lab RRT53	0.6044	189	182	5	0.9630	0.5843	0.0000	0.5461	1.0000
Lab RRT77	0.9144	189	187	3	0.9894	0.1581	0.0107	0.1485	0.9327
Lab RRT28	0.5027	189	186	5	0.9841	0.6360	0.0054	0.5763	0.9916
Lab RRT90	0.9091	189	187	4	0.9894	0.1696	0.0267	0.1630	0.8431
	0.6763	189	179	4.88	0.9511	0.4487	0.0234	0.4191	0.9480

MAF: Major allele frequency; SS: Sample size; No.: Number of observations; AN: Allele number; Avai.: Availability; GD: gene diversity; PIC: Polymorphic information content; f: Inbreeding coefficient.

Table 4. Mean number of different loci (Na), number of effective loci (Ne) Shannon index (I), observed heterozygosity (Ho) and Expected heterozygosity (He) among the 189 lablab accessions studied.

Pop		Na	Ne	I	Ho	He
Adv line	Mean	2.875	1.950	0.666	0.096	0.362
	SE	0.549	0.381	0.191	0.021	0.099
Australia	Mean	2.000	1.731	0.521	0.000	0.328
	SE	0.327	0.241	0.162	0.000	0.100
Ethiopia	Mean	3.750	2.452	0.964	0.031	0.518
	SE	0.491	0.392	0.151	0.021	0.071
India	Mean	2.750	1.880	0.685	0.022	0.396
	SE	0.313	0.245	0.143	0.015	0.084
Kenya	Mean	4.375	1.940	0.735	0.018	0.363
	SE	0.324	0.421	0.178	0.008	0.090
South Afr	Mean	3.875	2.372	0.929	0.015	0.508
	SE	0.350	0.322	0.139	0.007	0.076
Uganda	Mean	1.625	1.575	0.383	0.000	0.257
	SE	0.263	0.255	0.154	0.000	0.100
USA	Mean	1.750	1.570	0.429	0.083	0.285
	SE	0.250	0.203	0.137	0.055	0.089
West Afri	Mean	2.250	1.897	0.639	0.036	0.402
	SE	0.313	0.224	0.147	0.036	0.090
Total	Mean	2.806	1.930	0.661	0.033	0.380
	SE	0.160	0.103	0.054	0.009	0.030

Table 5: Analysis of molecular variance (AMOVA) for 9 populations of *Lablab purpureus* and partitioning of the total diversity into population components

Source	Df	SS	MS	Est. Var.	% molecular variation	P value	PhiPT
Among Pops	8	121.280	15.160	0.477	6	0.01	0.061
Within Pops	180	1313.339	7.296	7.296	94		
Total	188	1434.619		7.773	100		

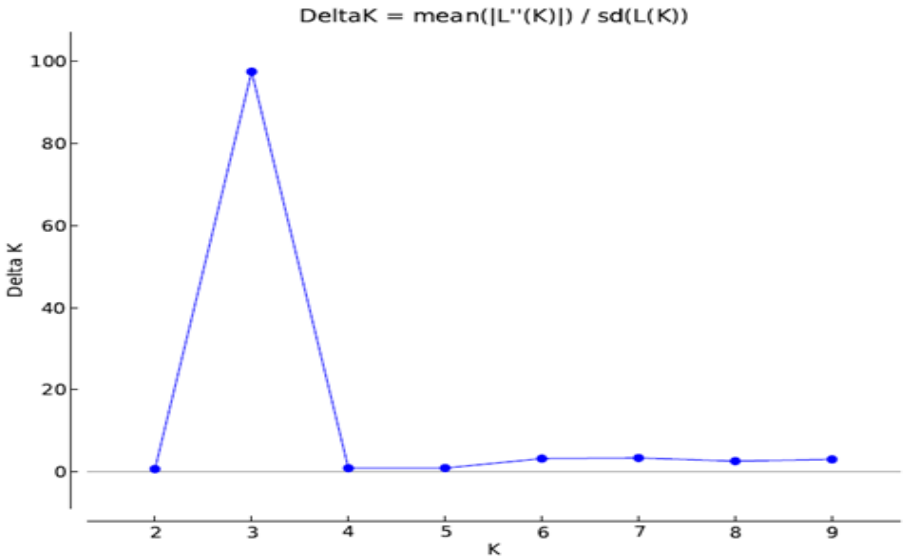

Figure 1: Plot of Delta K against the likely sub populations (K) generated according to Evanno *et al*. (2005)

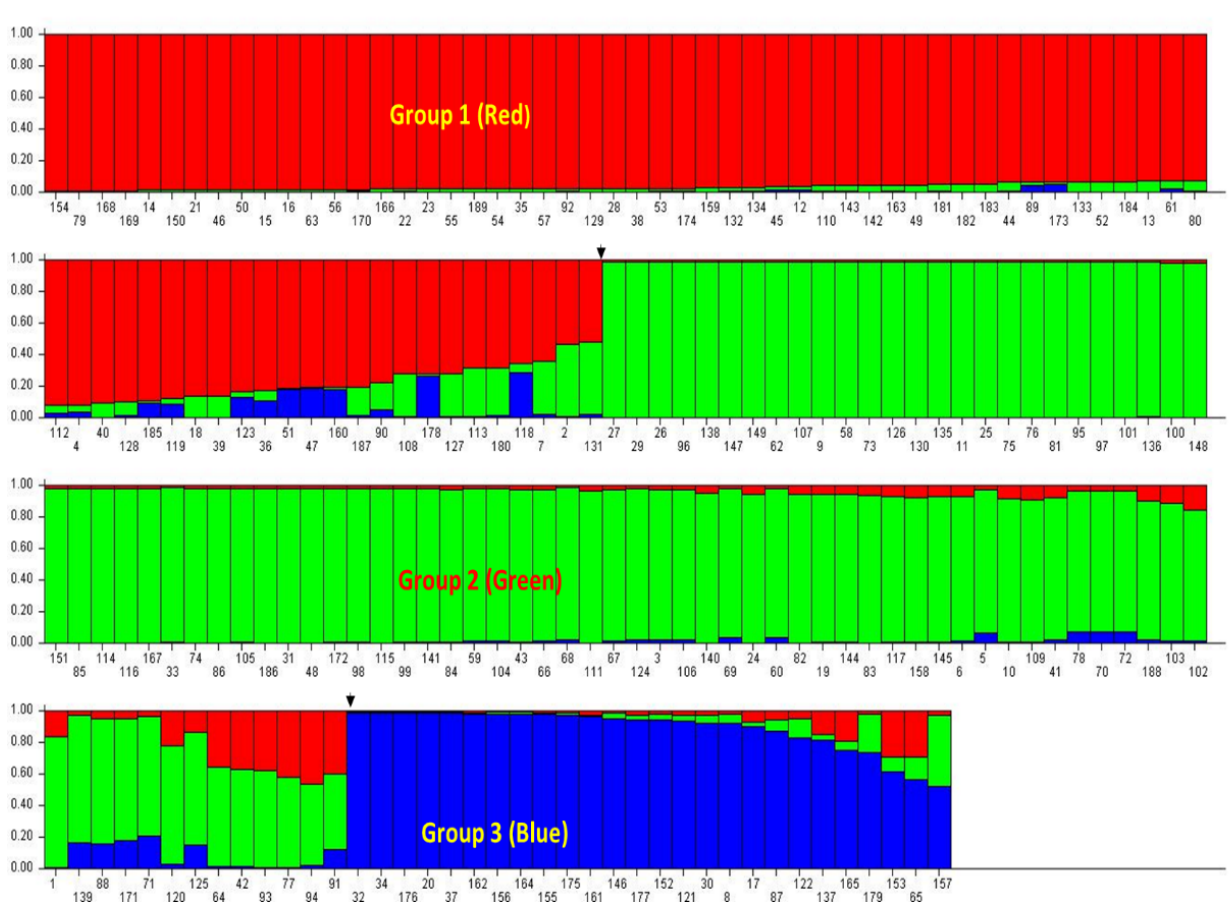

Figure 2: Population structure of 189 accessions of *Lablab purpureus* as determined by STRUCTURE analysis based on SSR allelic data at 8 loci. The subpopulations 1, 2 & 3 are denoted by red, green and blue colors respectively. The values on the x axis represents the accessions code, y axis value represent the estimated group membership of accessions and the small black arrow separates the groups.

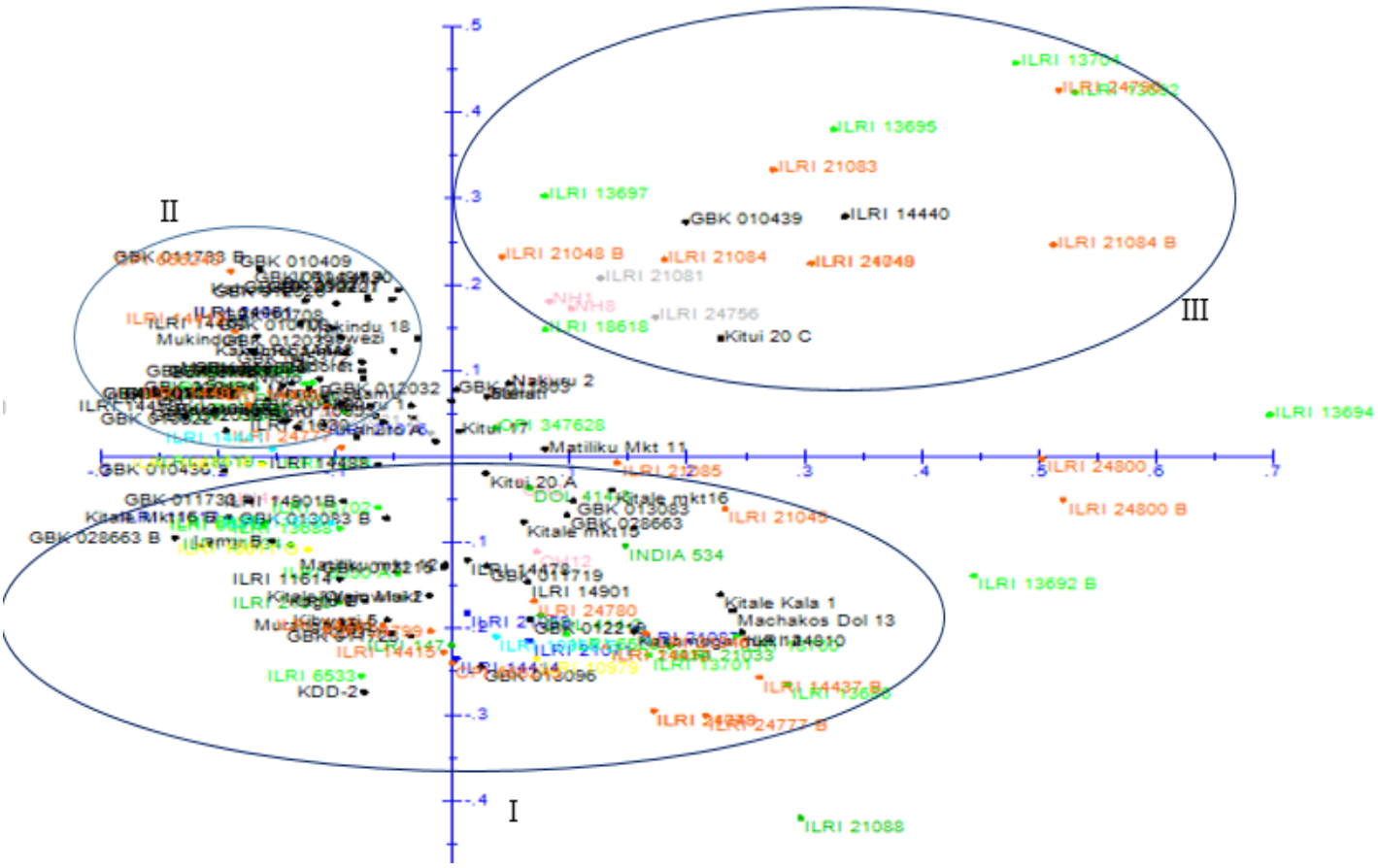

Fig. 3: Principal coordinates analysis (PCoA) of 189 lablab accessions using 8 selected SSR. The clusters identified are I, II and III. Different colors have been used to differentiate the sources of the accessions. Black (Kenya), dark red (South Africa), Light green (Ethiopia), dark green (India), light blue (West Africa), Blue (Australia), grey (Uganda), light red (advanced lines) and yellow (USA).

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