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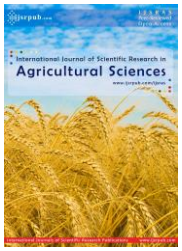

Full Length Research Paper

Agronomic and Molecular Evaluation of Six Lablab Bean (*Lablab Purpureus* L.) Cultivars

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Abstract. The experiment was done to assess the genetic diversity of six lablab varieties and determine their growth and productivity during the season 2014/2015. Field experiment was arranged in Randomized Complete Block Design with four replications. Seven different parameters for each variety and yield quality were taken (plant height,/cm, stem diameter/mm , number of leaves, yield of green fodder, ton/ha), analysis of crude protein and crude fiber were taken for each variety. Soil analysis for N, K, pH, EC was taken before and after cultivation. Molecular Analysis using four ISSR markers was done for the six varieties. Results showed that Cultivar 11640T was the tallest (91.87Cm) while cultivars (11609T) and (147T) were the lowest at late stages (60day). Number of leaves at 60 days showed that cultivar 11609T had the highest (127.41). Cultivar (11609T) had the highest fresh matter yield, whereas, the local cultivar gave the lowest yield. Cultivar (11640T) showed the highest crude fiber and cultivar (11609T) the lowest crude fiber. The result of protein revealed that cultivar (147I) contained the highest crude protein and cultivar (11640I) contained the least. Molecular diversity using inter simple sequence repeats (ISSR) analysis generated polymorphic alleles. The clustering of genotypes was done using the UPGMA method, which gave 3 clusters. ISSR was suitable for discriminations of the studied lablab bean.

Keywords: Lablab bean, Cultivars, Productivity, Crude protein, ISSR markers

1. INTRODUCTION

The ablab (*Lablab purpureus* L.); Family: Fabaceae (alt. Leguminosae); subfamily: Faboideae; tribe: Phaseoleae subtribe: Phaseolinae, also placed in: Papilionac. The biosystematics of hyacinth bean and its relatives were reviewed and revised. Formerly, Lablab was included in the genus Dolichos following Linnaeus, but is now assigned to the monotypic genus Lablab. Three subspecies are recognized in *L. purpureus*; ssp. uncinatus: the wild ancestral form distributed mainly in East Africa with small, scimitar-shaped pods of about 40 mm x15 mm; ssp. purpureus, cultivated as a pulse crop, has larger, scimitar-shaped pods, 100 mm x 40 mm; includes commercial varieties; and ssp. *bengalensis*, Asiatic origin, has linear-oblong shaped pods, longer than other subspecies, up to 140 mm x 10-25 mm. Although pod shape is a significant morphological difference, it is widely believed that ssp. *bengalensis* and ssp. *purpureus* are genetically very similar. Although most domesticated material is either ssp. *purpureus* or ssp. *bengalensis*, ssp. *uncinatus* has been domesticated in Ethiopia. Studies in lablab have shown that the perennial types have considerable genetic and morphological diversity. Hybrids between perennial and forage types have been produced at CSIRO, Australia and have resulted in new cultivars being released.

The lablab bean (lubia) is an old established irrigated crop in the Sudan. Commercial crop *Lablab purpurues* is grown as a pulse crop (crop harvested for dry seed) in Africa, Asia, and the Caribbean. It is also consumed as a green vegetable (green bean, pod, leaf). Maass et al. (2010) observe that *L. purpurues* may suffer from low yields when grown as a main cash crop, and suggest that it is more popular in home gardens and mixed-cropping schemes. Protein isolate from the bean can be used as a food additive for improving cake quality (Maass et al., 2010).

L. purpureus is used as forage, hay, and silage. As forage, it is often sown with sorghum or millet. The leaf is very palatable but the stem is not. The seeds are moderately palatable. Overall, it is one of the most palatable legumes for animals (Valenzuela and Smith, 2002).

The leaf has crude protein of 21 to 38% and the seed contains 20 to 28% crude protein. The seeds contain large amounts of various vitamins and minerals, but contain tannins and trypsin inhibitors so must be soaked or cooked before human consumption. The leaves make excellent hay for cattle and goats, but the stem is difficult to dry, and must be mechanically conditioned through crushing (FAO, 2012). Silage made from a mix of *L. purpureus* and *Sorghum* sp. raised the protein content of sorghum by roughly 11% with a 2:1 mixture of lablab: sorghum (FAO, 2012). *L. purpureus* is used as a nitrogen-fixing green manure to improve soil quality. It often produces more dry matter than cowpea (*Vigna unguiculata*), especially during drought, and can produce roughly 1,750 lb of leaf matter or 2.5 tons of total biomass per acre (Valenzuela and Smith, 2002). Each ton of biomass produced 50 lb of nitrogen (Valenzuela and Smith, 2002). It not only produces nitrogen through fixation, but returns nitrogen through leaf decay (FAO, 2012). Initially growth is slow, but once established, it competes well with weeds. It has an extensive root system that improves the physical soil properties.

Molecular markers are segments of chromosomes which don't necessarily encode any traits and are not affected by the environment but which are inherited in a Mendelian fashion. Some segments of the chromosome change faster than others (i.e. coding vs. non coding DNA). As a result it is recommended to use fast changing markers for closely related individuals and slow changing markers for less related individuals (different species). Different marker types therefore have different usefulness in fingerprinting individuals and populations. Moreover; a good marker for fingerprinting studies will be cheap to run, or gives a lot of information per run; very repeatable between assays; experience very low error rate and easy, unambiguous to score; and contain many alleles (high information content). The following techniques are those most used in genetic diversity studies and listed in chronological order: RFLP (restriction fragment length polymorphism), (Rallo et al., 2000). Genetic diversity in microorganisms 96 polymorphism) (Botstein et al., 1980), SSR (simple sequence repeats or just microsatellites) (Tautz, 1989), RAPD (randomly amplified polymorphic DNA) (Williams et al., 1990) or AP-PCR (arbitrarily primed PCR) (Welsh and McClelland 1990), ISSR (inter-simple sequence repeats) (Zietkiewicz et al., 1994), AFLP (amplified fragment length polymorphism) (Vos et al., 1995), SNPs (single nucleotide polymorphisms) (Chen and Sullivan, 2003) and, more recently, DarT (diversity array technology) (Kilian et al., 2005) and other high throughput platforms. A review summarizes various tools of DNA markers technology for application in molecular diversity analysis with special emphasis on wildlife conservation was presented by Arif et al. (2011). However, authors reviewed only mitochondrial DNA based markers including ribosomal DNA (12S and 16S rDNA). As a laboratory methodology, fingerprinting and diversity studies require the following steps: a) isolation of DNA, b) digestion, hybridization, and/or amplification of DNA into specific fragments, c) sizing /or separation of DNA fragment combinations or patterns into a set of individual DNA fingerprints, d) comparison of DNA fingerprints from different individuals) calculation of similarity (or dissimilarity) coefficients for all pairs of entries in the genetic study, f) creation of a dendrogram or graph to visualize the differences. Therefore the Objectives of the study were to:

(1) Evaluate the growth and yield of six lablab cultivars. (2) Measure the quality of the cultivars fresh leaves by determining the protein and fiber content. (3) Document the DNA fingerprints of the cultivars by using the ISSR technique. (4) Study the interaction effects of *lablab purpureus* on the soil by analyzing the pH, N, P,K , salinity.

2. MATERIALS AND METHODS

2.1. Experimental Site and Seed material

This study was conducted at the Experimental Farm in Shambat, Khartoum North, Sudan (15◦-40◦N) (32◦-35◦E) elevation (Adam, 2005). The soil of the experimental site is described as loam clay and characterized by a deep cracking moderately alkaline with low permeability, low nitrogen content and PH of 7.5-8 and a higher exchangeable sodium percentage (ESP) in sub soil (Abdel Hafiz 2001). The climate of the locality is semi arid with low relative humidity; the temperature varies between 45◦C and 21◦C in summer and 15◦C in winter (Adam, 2002).

2.2. Field layout and Seed material

This experiment comprised of six treatments (six forage legumes) viz., lablab- 147T, lablab-11609T, lablab-7403T, lablab-11640T, lablab (local) and lablab baladi obtained from the market. The treatments were arranged in a randomized complete block design replicated four times. The experiment was planted on 12 of August 2013, seeds were planted in holes 20cm apart at a rate of two seeds per hole. One adjacent ridge, next to one of the margin ridge was allocated to yield (yield area) while one ridge was allocated to groin yield. For forage yield, harvesting was done when plants reached 50% flowering .The fresh matter was immediately weighted in the field

and a sub-sample of .5 kg was over dried at 80^{o} C to 48hrs for dry matters determination.

2.3. Analysis methods

Data were statistically analyzed according to randomized complete block design by using *MSTAT* package. Means were separated by least significant difference (LSD).

Electrical conductivity (EC) was determined by portable EC meter model 410. Micro-kjeldahl method was used pre-moistened soil treated with concentrated sulphuric acid for digestion. Saturated solution of NaOH was used for distillation and liberated ammonia was received in 2% boric solution acid and titrated with hydrochloric acid. The pH reading of soil extracts, were determined using pH meter model 3510. Potassium was determined by flame photometer model C 410. Available Phosphors was determined by Olsen sodium bicarbonate extract method and color intensity measured by spectrophotometer model 6305. Crude fiber was determined according to AOAC (1990). Protein was determined by kjeldhal method according to the AOAC (1990).

2.4. DNA Extraction and PCR Reactions

Genomic DNA was extracted from fresh leaf tissues for each gene type using the modified CTAB method. Then the Agarose Gel for Electrophoresis was performed. DNA quantification was done by the comparison of the extracted DNA and compared it with several aliquots of lambda DNA, at known concentration using gel electrophoresis. 12 ISSR primers were used. Each reaction contained 25 u1 total volume of reaction in the table. For each of the primers, a master mix was done separately for the six samples plus a control sample. 24 u1 of the master mix were placed into each PCR tube and 1 u1 of DNA (20 ng) was added at last. Each 25 u1master mix contained 2.5 ul 10 X Tag Buffer, 1.5 ul Mg Cl2 (50 mM), 2.5 ul DNTPs (2mM), 2ul Primer (10 Pmol), 0.5 Taq DNA polymerase (5U\ul), 15 ul DDW (Double distilled water). The control sample contained no DNA, substituted with 1 ml DDH 2O.

The PCR amplification protocol was programmed for 5 min at 94∘ C for initial denaturation, followed by 40 cycles of 1min at 94∘C, 1 min at 34– 36 ∘C and 1 min at 72∘ C final extension was programmed for 7 min at 72∘ C, followed by hold time at 4∘ C until samples were collected. The PCR amplification of the ISSR markers was repeated twice and gels showing consistent banding pattern and polymorphism were used for scoring.

2.4. Data Scoring

The bands were scored visually for their presence or absence with the ISSR primers. The scores were obtained in the form of matrix which Indicate the presence and absence of a band for each accession. The scoring data in the form of binary values was used for the construction of dendrogram. The genetic association between accessions was evaluated by calculating the Jacquards' similarity coefficient for pair-wise comparisons based on the proportions of shared bands produced by the primer.

3. RESULTS AND DISCUSSIONS

The results presented in table (1) showed the highly significant difference in plant height at 60 days. However there was no significant difference between cultivars at 30 days (fig 1). There was slight increase in height at the local cultivar which was significant from the rest at 60 days. Cultivar 11640T had the tallest height (91.87Cm) while cultivars 11609T and 147T were the lowest. The difference in height between cultivars at late stages might be due to genetic difference between cultivars. The number of leaves per plant was presented in table (1) and Fig.2 and showed the significant difference in number of leaves at 60 days. However, there were significant differences between cultivars at 30 days as shown in (fig 2). There were significant differences between cultivars at 45 days and the highest number was for the local (57.67) Balady (62.32) 11690T (57.41). At 60 days cultivar (11609T) had the highest number of leaves (127.41). The difference in number of leaves between cultivars was due to the genetic differences between cultivars. The results presented in table (1) showed no significant difference between the treatments on the stem diameter at 60 days after planting according to LSD. Table (1) also showed significant difference between the treatments on the Fresh matter yield at 60 day after planting according to LSD. Cultivar (11609T) gave the highest fresh matter yield, whereas the local cultivar gave the lowest yield. As far as the dry matter there was no significant difference between the treatments at 60 days after planting according to LSD.

The results presented in table (1) showed significant difference between the treatments on percent crude protein. Cultivar (11640T) showed the highest crude fiber and cultivar (11609T) the lowest. The results presented in figure (3) showed significant difference between the treatments. Cultivar (147I) contained the highest crude protein, and cultivar (11640I) contained the least crude protein. There were obvious differences in variation and distribution of crude protein among the lablab lines. Obvious

differences in variation and distribution of crude protein among the lablab cultivars were reported. This implied that the genotypic variations provided opportunities to select materials with high contents of crude protein.

Table 1: Summary of ANOVA for lablab (*lablab purpureus* L.) cultivars

Source	D.F	F value					
		Plant Height (cm)	Number of Leaves	Stem Diameter (mm)	Fresh Weight	Dry Weight	Crude Fiber
Replication	3	754.623	2012.687	2.083	126.000	8.428	1.682
Cultivar	5	5389.765**	2810.010*	1.493*	75.627NS	1.507NS	14.615**
Error	15	-	-	-	-	-	-
Total	23	-	-	-	-	-	-
EMS	-	98.486	124.842	0.397	21.247	6.975	0.770
C.V	-	8.88	10.67	5.91	23.33	21.45	5.98
S.E	-	4.0515	4.5613	0.2573	1.8818	1.0782	0.3581
LSD	-	14.52	16.34	0.9494	3.863	6.743	1.512

NS=Not signification; *=Significant (5%); **=Highly significant (1%)

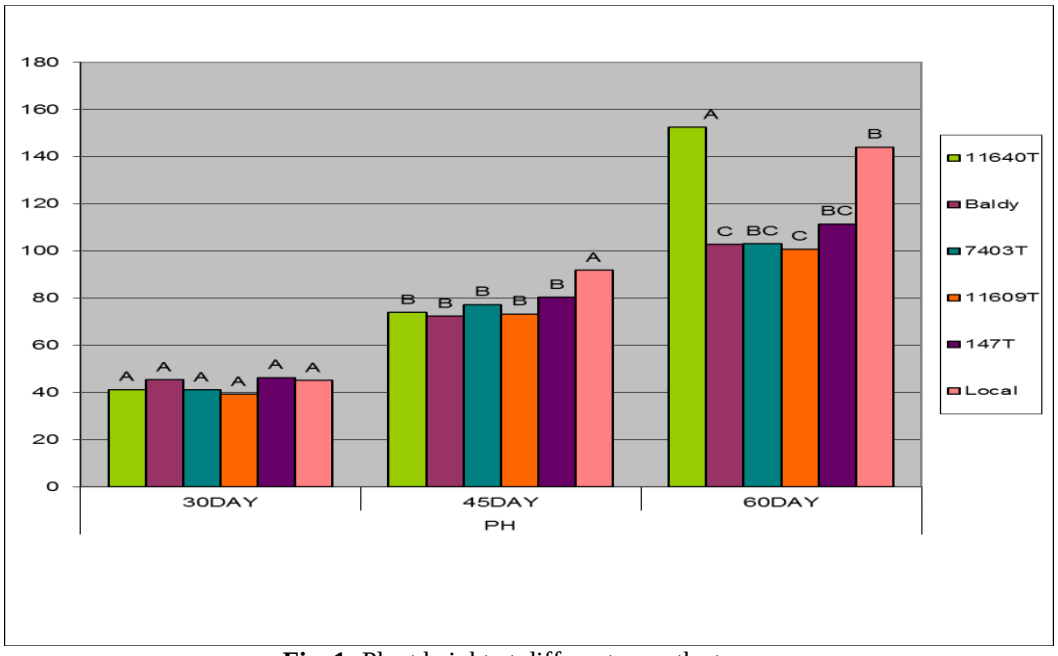

Fig. 1: Plant height at different growth stages.
Mean followed by the same letter were not significantly at 5% (LSD)

The present study also aimed to understand the genetic diversity of lablab bean using ISSR markers. The six lablab cultivars were studied for their genetic diversity at molecular level. As shown in table (2), primer 872 gave the highest number of polymorphic bands, with percentage of polymorphism of 71%, followed by primer 848 which gave 4 polymorphic bands out of 10 bands with a percentage of 40%. Primer 814 gave the lowest number of polymorphic bands (2) and the least percentage of polymorphism (22%). Figure (4) show the different banding pattern of the genotypes with the ISSR primers 872 used in the study. Kimani et al. (2012) studied 50 lablab accessions from Kenya using AFLP technique found low level of species diversity, which is due to the self-pollination of the *lablab purpureus*. However, some lablab accessions in their study were out crossing, they assumed also that the gene flow as a result of exchange of germplasm between farmers can be another reason causing the low genetic diversity. To increase the genetic diversity of lablab, they recommended that there is a need to diversify the genetic base of the cultivars by including wild cultivars from other regions. Saravanan et al. (2013) found larger amount of genetic diversity by using 3 types of DNA markers (ISSR, SSR, RAPD).

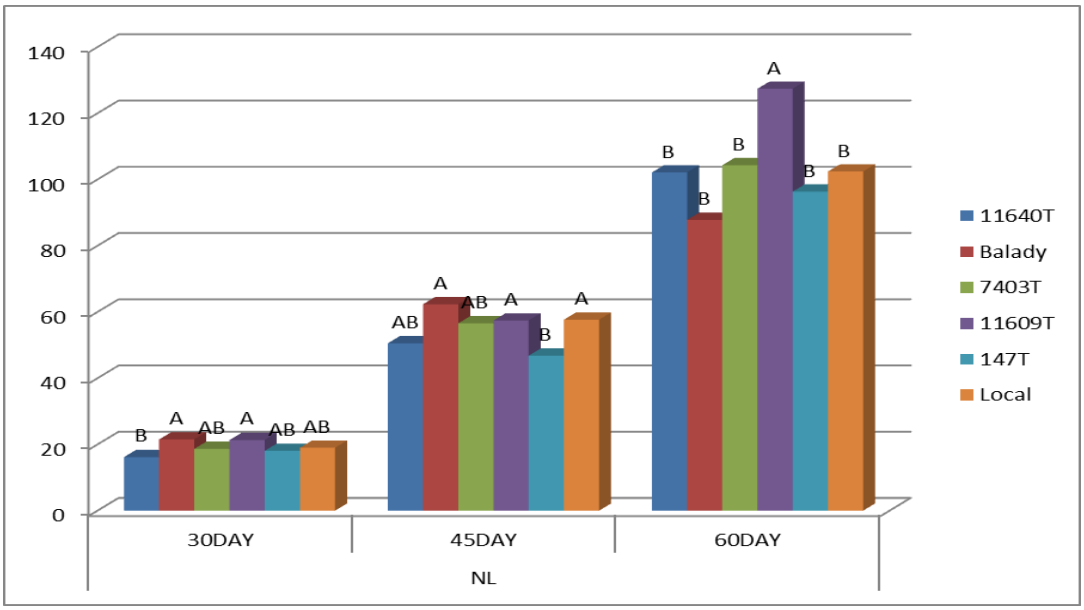

Fig. 2: Number of leaves at different growth stages after 30, 45, and 60 days
Mean followed by the same letter were not significant at 5% (LSD)

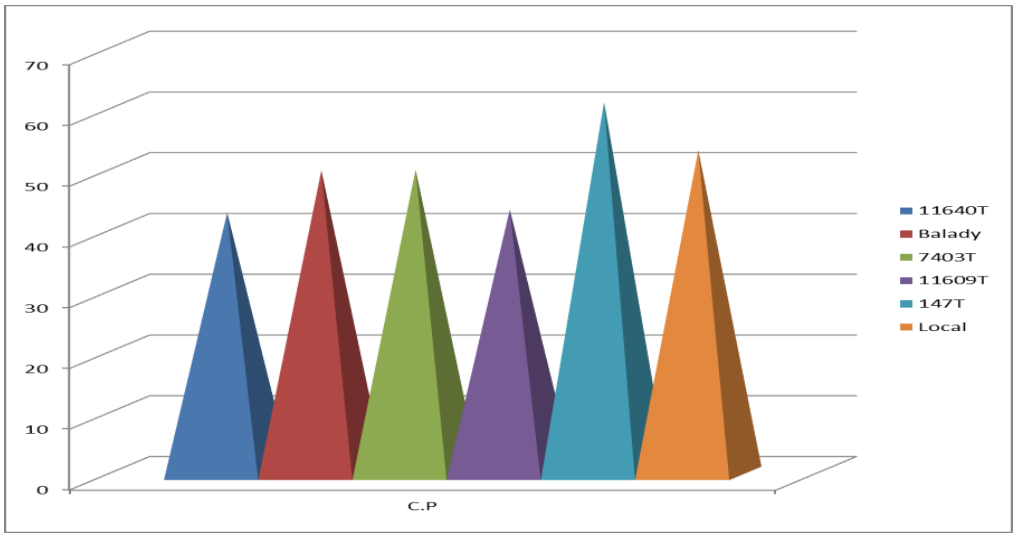

Fig. 3: The level of protein for the six lablab cultivars

They attributed this high diversity to the wide diversity among the cultivars and their relatives. The three types of the DNA marker systems were able to differentiate the accessions. They found that ISSR markers were more efficient than RAPD to study lablab, based on all parameters, as ISSR gave more markers with fewer primers than RAPD. The study results were in agreement with Saravanan et al. (2013), as by using four polymorphic ISSR primers to study the variation between six lablab cultivars, the ISSR proved to be very useful and could differentiate between the six cultivars. Also, wide genetic distance range was found from 2 to 3.87 as shown in table (3). The UPGMA figure also gave very clear relationships among the cultivars based on their genetic distances (Figure 5). Ajibade et al. (2000) also concluded that ISSR is better technique than RAPD for phylogenetic studies.

Table 2: Results of the polymorphism of the six lablab cultivars using four ISSR primers

	primer	Sequence (5'-3')	Total Number of bands	Poly-morphic bands	Mono-morphic bands	Percentage of polymorphic bands
1	807	(AG)8 T	4	2	2	50%
2	814	(CT)8 A	9	2	7	22%
3	848	(CA)8 RG	10	4	6	40%
4	872	(GATA) 4	7	5	2	71.4%

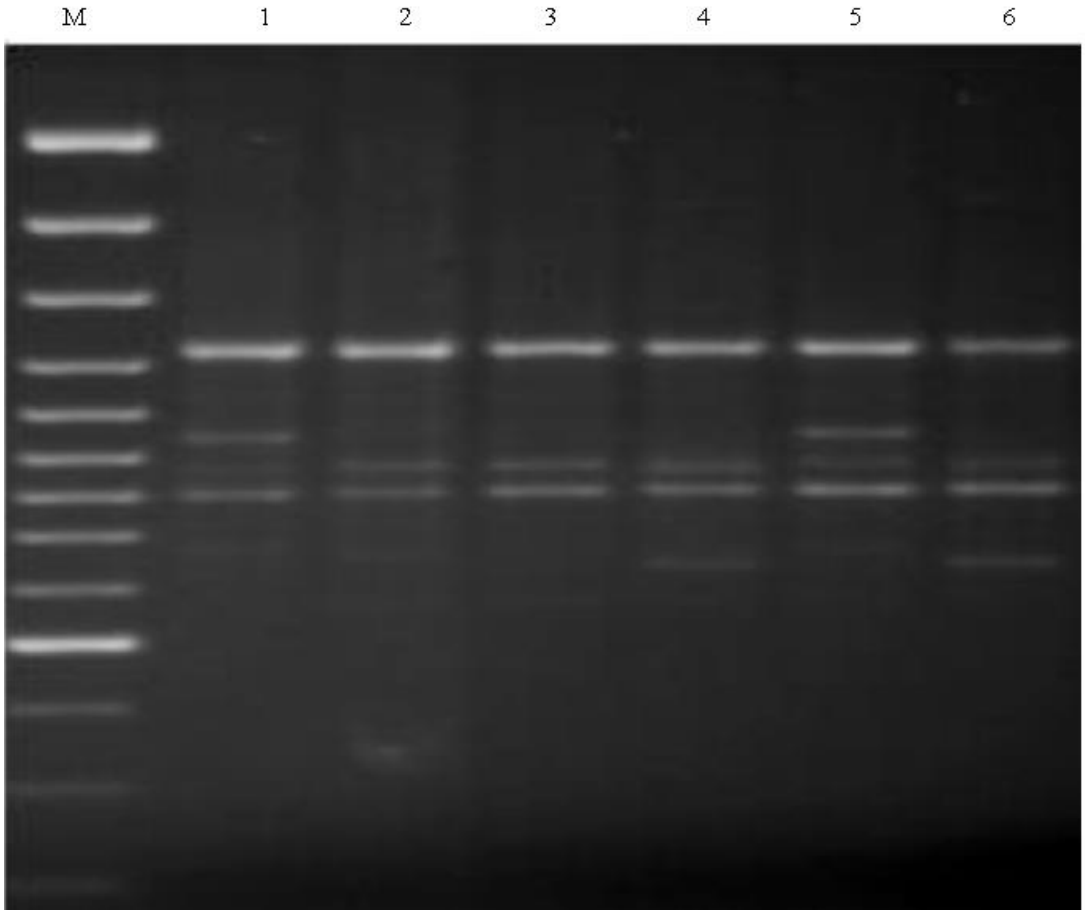

Fig. 4: ISSR markers profile of six lablab genotypes using the primer 872
Whereas: lablab cultivars: 1=7403T, 2=Balady, 3=local, 4=147T, 5=11640T, 6=11609T

Table 3: Genetic distance matrix for the six lablab cultivars

	1	**2**	**3**	**4**	**5**	**6**
1	0.00					
2	3.61	0.00				
3	3.74	2.24	0.00			
4	3.16	3.00	3.46	0.00		
5	3.61	3.16	2.24	3.87	0.00	
6	3.32	2.00	2.24	2.65	2.83	0.00

As presented in table (3) the genetic distance between the six lablab cultivars, the highest genetic distance was found between cultivar 147T, and cultivar, 11640T, the lowest genetic distance was found between cultivar, Balady, and cultivar 11609T. This illustrates that the cultivar, 7403T, and cultivar, 147T, are very different from each other.

The tree based on genetic distances showed that cultivar 1 (7403T), is genetically different from the other 5 lablab cultivars (2=Balady, 3=local, 4=147T, 5=11640T, 6=11609T). From these five cultivars, cultivar Balady, and cultivar 11609T are genetically close cultivars whereas, cultivar local and cultivar 11640T are closer to each other. Cultivar 147T appeared as out group and is genetically far from all other four cultivars (Balady, 11609T, local, 11640T).

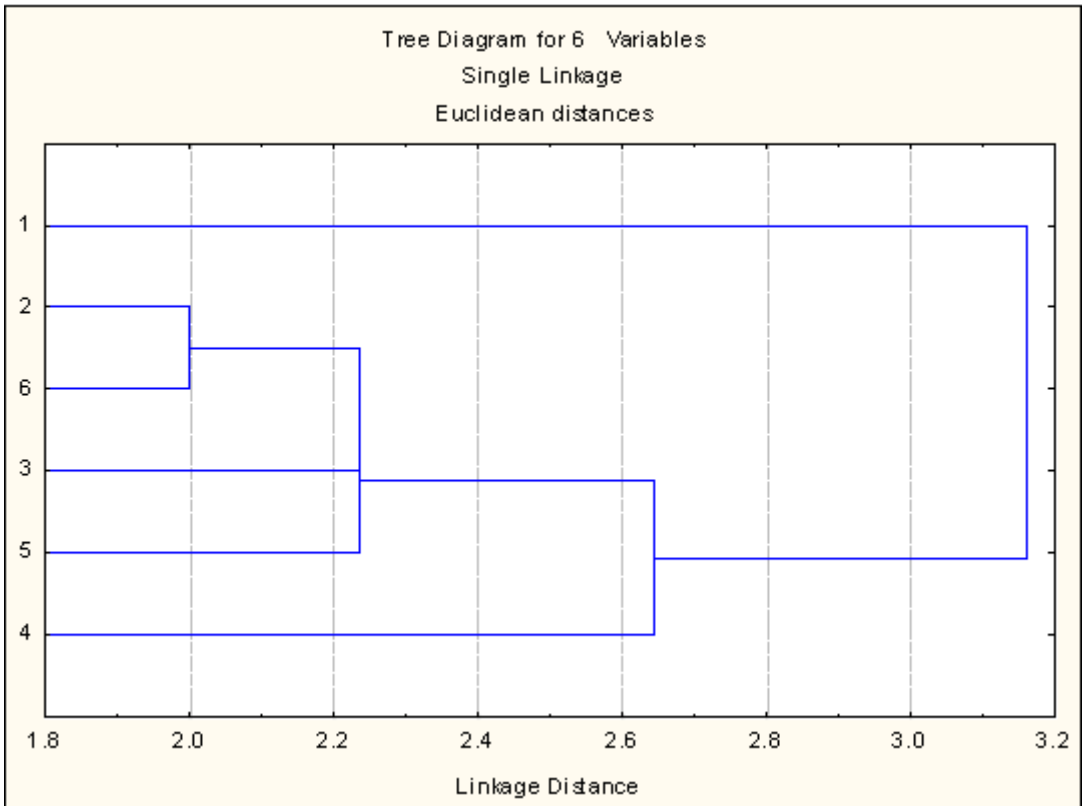

Fig. 5: UPGMA dendrogram showing the relationship for the six lablab cultivars

4. CONCLUSIONS

Cultivar 11640T had the highest plant height (91.87cm), while cultivar 11609T and 147Twere the lowest. Number of leaves at 60 days showed high differences between cultivars at late stage (60 days). Cultivar 11609T had the highest number of leaves (127.41). Cultivar (11609T) gave the highest fresh matter yield, whereas, the local cultivar gave the lowest yield. Cultivar 11640T showed the highest crude fiber and cultivar 11609T had the lowest crude fiber. Cultivar 147I contained the highest crude protein, and cultivar 11640I contained the least crude protein.

Molecular diversity analysis for the six lablab cultivars using inter simple sequence repeats (ISSRs) analysis generated polymorphic alleles from 4 primers. UPGMA method for genetic relationships among the six cultivars using data of four ISSR markers was found very suitable for discrimination among closely related species. Knowledge on genetic divergence among the genotypes and their relatives as revealed by the Inter cluster distances calculated using the dissimilarity values will enable the breeders to choose the parents for hybridization to get high heterotic hybrid combination which would allow wide spectrum of recombination's and also facilitate transfer of wild genes to cultivate varieties through conventional breeding.

REFERENCES

Arif I, Khan HA, Bahkali AH, Al Homaidan AA, Al Farhan AH, Al Sadoon M, Shobrak M (2011). DNA marker technology for wildlife conservation. Saudi Journal of Biological Sciences, 18: 219-225.

Abdel alhafez ME (2002). Effect of partially acidulated phosphate rocks and triple super phosphate and their combinations on growth, minerals composition of wheat. Ph.D. thesis, Sudan University of Science and Technology.

Adam HS (2002). The agricultural climate second edition. Gezria university press. Wad- Madani, Sudan. In Arabic. pp 119

AOA, Assoc. of Official Analytical Chemist (1990). Official methods of analysis,Washington D.C, USA.

Botstein D, White RL, Skolnick M, Davis RW (1980). Construction of a genetic linkage map in man using restriction fragment length polymorphisms. American Journal of Human Genetics, 32: 314–31.

Chen X, Sullivan PF (2003). Single nucleotide polymorphism genotyping: biochemistry, protocol, cost and throughput. Pharmacogenomics J., 3: 77-96.

FAO (2012). Grassland index. *Lablab purpureus*. http://www.fao.org/ag/AGP/AGPC/doc/Gbase/ DATA/Pf000047.HTM (accessed 6 June 2012).

Kilian A, Huttner E, Wenzl P, Jaccoud D (2005). The Fast and the Cheap: SNP and DArT-based Whole Genome Profiling for Crop Improvement. In: Proceedings of the International Congress in the Wake of the Double Helix: From the Green Revolution to the Gene Revolution (Tuberosa R, Phillips RL and Gale M, eds.). May 27-31, Bologna, 443-461.

Kimani F, Wachira M, Kinya L (2012). Molecular Diversity of Kenyan Lablab Bean (*Lablab purpureus* (L.) Sweet) Accessions Using Amplified Fragment Length Polymorphism Markers. American Journal of Plant Sciences, 3: 313-321.

Maass BL, Knox MR, Venkatesha SC, Angessa TT, Ramme S, and Pengelly BC (2010). *Lablab purpureus*-a crop lost for Africa? Trop. Plant Biol., 3(3):123–135.

Valenzuela H, Smith J (2002). Sustainable agriculture green manure crops. SA-GM-7. Cooperative Extension Service, College of Tropical Agric. and Human Resources, Univ. of Hawaii at Manoa.

Rallo P, Dorado G, Martin A (2000). Development of simple sequence repeats (SSRs) in olive tree (*Olea europaea* L.). Theor. Appl.Genet, 101: 984−989.

Saravanon P.; N Senhil, P.veer Badhira . (2013) comparison of genetic relatedness among lablab bean (*Lablab purpurues* (L.)sweet genotypes using DNA markers Schulman, Euphytica. 158: 313-321.

Tautz D (1989). Hypervariability of simple sequences as a general source for polymorphic DNA markers. Nucleic Acids Res. 17: 6463-6471.

Vos P, Hogers R, Bleeker M, Reijans M, Van der Lee T, Hornes M, Frijters A, Pot J, Peleman J, Kuiper M and Zabeau M (1995). AFLP: a new technique for DNA fingerprinting. Nucleic Acids Res. 23: 4407-4414.

Welsh J, McClelland M (1990). Fingerprinting genomes using PCR with arbitrary primers. Nucleic Acids Res. 18: 7213-721883.

Williams JG, Kubelik AR, Livak KJ, Rafalski JA (1990). DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. Nucliec Acid J., 212-220

Zietkiewicz E, Rafalski A, Labuda D (1994). Genome fingerprinting by simple sequence repeat (SSR)-anchored polymerase chain reaction amplification. Genomics, 20: 160-167.

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