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Inter simple sequence repeat (ISSR) analysis of Ethiopian white lupine (*Lupinus albus* L.)

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White lupine (*Lupinus albus* L.) collected from two zones (West Gojjam and Awi) of Amhara region and one zone (Metekel) of Benishangul - Gumuz regional state of Ethiopia were studied using inter simple sequence repeat (ISSR) markers in an attempt to assess the genetic diversity. Four ISSR primers of which three were dinucleotide repeats and one, a penta nucleotide repeat amplified a total of 39 clear and reproducible bands. Both unweighted pair- group method with arithmetic average (UPGMA) phenograms and a neighbor joining (NJ) trees were constructed for the individuals and populations using Jaccard's similarity coefficient. The dendrogram clearly indicated four distinct groups/populations based on the area of origin. The principal coordinates (PCO) analysis also recovered UPGMA and neighbor joining tree groups, although Amhara region white lupine were intermixed with each other. The genetic diversity among white lupine population considered in the present study indicated that Merawi was the highest (0.223) followed by Addis Kidam, Sekela and Wembera with genetic diversity of 0.198, 0.189 and 0.167, respectively. Generally, Amhara region white lupine (0.203) population shows higher genetic diversity than white lupine population of B-Gumuz region (0.167). Analysis of molecular variance (AMOVA) in both grouping and without grouping revealed larger genetic diversity within the populations (74.6%) than among populations (25.4%). Shannon's diversity index also confirmed the existence of higher genetic diversity in Amhara region lupine populations than in Benishangul-Gumuz. Furthermore AMOVA demonstrated highly significant (P = 0.00) genetic differences among populations within groups, among groups and within populations. Of the total variation, 64.64% was attributable to within populations, 27.23% to among groups and the least, 8.13% to among populations within groups. Generally, on the basis of samples of 39 bands in the four populations, ISSR was able to reveal moderate to high levels of genetic diversity within and among Ethiopian white lupine population.

Key words: Amhara, Benishangul - Gumuz, Ethiopia, genetic diversity, ISSR, white lupine.

INTRODUCTION

The genus *Lupinus* belongs to the family Fabaceae (Leguminosae) subfamily Papilionoideae tribe Genisteae (Gladstons, 1998). Originally, the name lupine was derived from the Latin "*Lupus*" (wolf) because it was

thought that they deplete soil nutrients, but the opposite is true as they are legumes and replenish the soil by nitrogen fixation (Christou, 1992). The Ancient Greeks used to call lupine as Thermes, while it is called Turmus in most Arab countries and India (Belteky and Kovacs, 1984). The species of the genus *Lupinus* are distributed in two centers of origin; one in the Mediterranean basin and the other extends through South America (Cowling et al., 2000). There are over 300 species in the genus *Lupinus* but only five are cultivated: white lupine (*Lupinu salbus),* blue lupine (*Lupinus angustifolius*), yellow lupine (*Lupinus luteus*), variable lupine (*Lupinus mutablis*) and garden lupine (*Lupinus polyphillus*). Only four of these have gained agricultural importance. These are *L. albus*, *L. angustifolius* and *L. luteus* of the "Old World" lupine species, and one "New World" species namely *L. mutabilis.* The first three species originated in the Mediterranean area, while *L. mutabilis* belongs to South America. The genus is comprised of geographically separated centers of diversity (Hondelmann, 1984). Borek et al. (2009) reported that the main fatty acid in yellow lupine cotyledons was linoleic acid; in white lupine it was oleic acid, and in Andean lupine, it was both linoleic and oleic acids. The white lupine is an old world species mainly distributed around the Mediterranean and along the Nile valley, where it has been traditionally cultivated for several thousands of years. These cultivated populations constitute the genetic resources of the species. Based on the modification in agricultural practices, genetic erosion in these areas has been extremely rapid (Huyghe, 1997).

Lupines have digitate leaves. The inflorescence is a raceme and the plant height can be up to 1.5 m. The flowers are quite distinctive and mainly self-pollinating but can be occasionally pollinated by bees. The inflorescence bearing the flowers varies 10 to 60 cm long depending on the species. Depending on the species the flowers can be white, pink or blue and are 12 to 16 mm in size. The seed pods are green and silky, up to 13 cm long and often constricted between the seed (Clapham et al., 1987). Lupines are cultivated in the world for three main uses: (1) for human nutrition because of their high protein and oil contents; (2) as green manure contributing to improved soil structure, with an increase of the organic matter content and through nitrogen and phosphorus accumulation in poor sandy soils; and (3) as ruminant feed either as green forage in the areas of traditional cultivation or, more often, as grains introduced as protein supplements in the diets of ruminants (Faluyi et al., 2000).

White lupine is adapted to well drained, light to medium textured, moderately acidic or neutral soils with a pH range of 4.5 to 7.5 (Gladstones, 1998). Currently, it is a minor crop in central Europe, while it is being widely grown in America. Lupine is also a traditional pulse crop, grown around the Mediterranean and in the Nile valley, extending to Sudan and Ethiopia. It is also grown in some parts of South-eastern and Southern Africa (Jansen, 2006). The white lupine in Ethiopia is locally known as “Gibto”. It is produced by small holder subsistent farmers mainly in two regional states of Ethiopia; Amhara and Benishangul-Gumuz, the former being the largest producer. It is grown in elevations ranging between 1500 to 3000 m.a.s.l. In the main production season (June to December) of the year 2008, a total of 17, 241 tons of lupines, with a mean productivity of 0.84 t/ha, were produced in these two major lupine producing regional states (ECSA, 2009). According to Francis (1999), the white lupine variety grown in North-western Ethiopia is bitter due to its high alkaloid content. He also reported that, though the variety is bitter, it is relatively non-shattering, high yielding and most importantly resistant to lupine anthracnose disease which is currently a problem for the cultivation of white lupine in some parts of Western Australia and Europe. Lupine production by small holder farmers in Ethiopia is targeted for its grain and soil fertility maintenance values. Its grain is used as snack and for the preparation of local alcoholic drink, Areki and local sauce called Shiro (made of lupine flour) (Likawent et al., 2010). White lupine (2n = 50) and other *Lupinus* species have been fully domesticated only recently when compared with most other crops. Genetic variability especially for extreme temperatures and drought tolerance, and disease and insect resistance in the cultivated germplasm is very low (Raman et al., 2008).

The genetic diversity of white lupine and other species of *Lupinus* have been characterized using morphological and agronomical attributes (Gonzalez et al., 2007), isozymes/proteins (Vaz et al., 2004) and molecular markers including random amplified polymorphic DNA (RAPD), amplified fragment length polymorphism (AFLP) and inter simple sequence repeats (ISSR). Assessment of genetic diversity on the basis of morphological traits is not very reliable, as it may be influenced by the environment, and the number of traits with known inheritance is small. Molecular markers have the distinct advantages of being independent of climatic variables

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Abbreviations: ISSR, Inter simple sequence repeats; **UPGMA,** unweighted pair- group method with arithmetic average; **NJ,** neighbor joining; **PCO,** principal coordinates; **AMOVA,** analysis of molecular variance; **RAPD,** random amplified polymorphic DNA; **AFLP,** amplified fragment length polymorphism.

Table 1. List of *Lupinus albus* populations with regions and site coordinates.

Populationcode	Region	Zone, District (Wereda)	Sample size	Average Longitude/Latitude	Average altitude (m.a.s.l)
Mrw 1-10	Amh	W.Gojjam, Mecha	10	11° 38' 82" N/37° 14' 85"E.	2059
Skl 11-20	Amh	W.Gojjam, Gish Abay	10	10° 59' 07"N/37° 12'10" E.	2736
Adk 22-30	Amh	Awi, Fagita Lekoma	09	11° 5' 20" N/36° 54' 16" E.	2494
Wbr 31-40	BG	Metekel, Wembera	10	10° 34' 60" N/35° 47' 60" E	2539
Total			39		

Mrw - Merawi, Skl - Sekela, Adk - Addis Kidam, Wbr - Wembera, Amh - Amhara and BG - Benishangul-Gumuz.

Table 2. List of primers used for the analysis.

Primer code	Annealing T° (°C)	Primer sequence	Amplification pattern
UBC - 810	45	$(GA)_8T$	Very Good
UBC - 818	48	$(CA)_8G$	Poor
UBC - 824	48	$(TC)_8G$	Poor
UBC - 834	45	$(AG)_8YT$	Excellent
UBC - 835	48	$(AG)_8YC$	Poor
UBC - 844	48	$(CT)_8RC$	Very Good
UBC - 860	45	$(CT)_8RA$	Poor
UBC - 880	45	$(GGAGA)_3$	Very Good

Y = Pyramidine (C or T) and R = Purine (A or G).

and very numerous (Raman et al., 2014), and one of the most convenient and popular methods to identify and study of intraspecific genetic polymorphism is the ISSR-PCR method (Grishin et al., 2011). Although, lupine has immense potential for feed, food and soil fertility maintenance perspective (Anokhina et al., 2012) the Ethiopian lupine cultivation, genetic improvement and utilization remains far behind the other pulse crops. The unavailability of detailed information about the production system, current uses, genetic status and potentials, etc. are limitations of the Ethiopian white lupine. Therefore, this study is aimed at detailing the genetic diversity of an underutilized indigenous crop plant as revealed by a DNA marker.

MATERIALS AND METHODS

Plant materials and sampling strategy

A total of 39 individual plants of *L. albus* L. representing four populations were collected from four districts or weredas each from Wembera in Benishangul-Gumuz (Metekel zone), and Merawi and Sekela (West Gojjam zone) and Addis Kidam (Awi zone) of Amhara reginal state. Lupine plants growing on an individual farmers plot of land were considered as a single population. Hence, young leaves from individual lupine plants were selected randomely with approximately 10 m distance from each and collected separetely. The four populations are indicated in Table 1 along with altitude and site coordinates of each of the locality they are collected from.

DNA extraction

Genomic DNA extraction was done based on the method described in Borsch et al. (2003) which involves a modified triple CTAB extraction method to yield optimal amounts of DNA. All DNA extractions were carried from silica gel dried leaf sample and ground by liquid nitrogen at Genetics Research Laboratory, Department of Biology Addis Ababa University (AAU).

Genomic test gel and electrophoresis

An agarose gel (100 ml, 1xTBE and 0.98 g agarose) was prepared and 2 µl of each genomic DNA samples mixed with 6 µl loading dye (1X bromophenol blue) was loaded on the gel and electrophoresed at constant voltage of 80 V for 45 min. The gel was stained for 30 min with 50 µl ethidium bromide (10 mg/ml) after well mixed with 450 ml distilled water. Then, it was destained/ washed for 30 min with distilled water. Gel picture was taken under UV transilluminator by BiodocAnalyse 2.0 with digital canon camera. From the two extractions following the protocol given by Borsch et al. (2003), those with high band intensity and less smear were selected for PCR, and this was commonly the case for the second extractions.

Primer selection and optimization

A total of eight ISSR primers used by Talhinhas et al. (2003) and Mustafa et al. (2009) were used for the initial testing of polymorphism and reproducibility of PCR products. DNA from three individual plants was selected from each population to screen the primers. Based on polymorphism and reproducibility, four primers were selected for the study (Table 2).

PCR amplification and electrophoresis

The polymerase chain reaction was done using Biometra 2000 T3 Thermo cycler. PCR amplification was carried out in a 25 µl total reaction mixture containing 1 µl template DNA, 13.2 µl ddH_20, 5.6 µl

Table 3. Selected ISSR primers with their amplification and banding pattern.

ISSR primer	Repeat motif	Amplification pattern	Number of scorable bands
UBC – 810	$(GA)_8T$	Very Good	10
UBC – 834	$(AG)_8YT$	Excellent	12
UBC – 844	$(CT)_8RC$	Very Good	8
UBC – 880	$(GGAGA)_3$	Very Good	9
Total			39

dNTP (1.25 mM), 2.6 µl PCR buffer (10xThermopol reaction buffer), 2.0 µl $MgCl_2$ (2 mM), 0.4 µl primer (20 pmol/µl) and 0.2 µl Taq Polymerase (5 U/µl). The amplification program was 4 min preheating and initial denaturation at 94°C, then 39 x 15 s at 94°C, 1 min primer annealing at (45/48°C) based on primers used, 1.30 min extension at 72°C. The final extension for 7 min at 72°C followed. The PCR products were also stored at 4°C until loaded on gel for electrophoresis. An agarose gel (1.67 gm agarose with 100 ml 1xTBE) was prepared and 8 µl amplification product of each sample with 2 µl loading dye (bromophenole blue 6X) was loaded on gel. DNA seizer of 1 kb DNA ladder was used to estimate molecular weight of ISSR fragments. The electrophoresis was done for 2 to 3 h at constant voltage of 100 V. The gel was stained with ethidium bromide (10 mg/ml) for 30 min and destained for a further 30 min. Gel picture was taken with Biometra Biodoc analyzer.

Data scoring and analysis

Each bands that was amplified using ISSR primers, was treated as a unit character and scored as '0' for absence, '1' for presence and '?' for missing or ambiguous. POPGENE version1.32 software (Yeh et al., 1999) was used to calculate genetic diversity for each population as number of polymorphic loci, percent polymorphism, gene diversity and Shannon diversity index. Shannon–Weaver diversity index (H) was calculated as H= -Σpilog2pi; where, pi is the frequency of a given band for each population (Lewontin, 1972). Shannon's index of diversity was used to measure the total diversity (Hsp) for the species as well as the mean diversity per population (Hpop). The proportion of diversity within and between populations was then calculated as Hpop/Hsp and (1-Hpop/Hsp), respectively. Analysis of molecular variance (AMOVA) (Excoffier et al., 2006) was used to calculate variation among and within population using Areliquin version 3.01 (Excoffier et al., 2006). NTSYS- pc version 2.02 (Rohlf, 2000) and Free Tree 0.9.1.50 (Pavlicek et al., 1999) softwares were used to calculate Jaccard's similarity coefficient which is calculated as:

$$S_{ij} = \frac{a}{a+b+c}$$

Where, "a" is the total number of bands shared between individuals i and j, "b" is the total number of bands present in individual i but not in individual j and "c" is the total numbers of bands present in individual j but not in individual i.

NTSYS- pc version 2.02 Rohlf (2000) was used to generate the unweighted pair group method with arithmetic mean (UPGMA) phenogram to analyze and compare the individual genotypes. The NJ method (Saitou and Nei, 1987; Studier and Keppler, 1988) was used to compare individual genotypes and evaluate patterns of genotype clustering using Free Tree 0.9.1.50 Software (Pavlicek et al., 1999). The major difference between the two algorithms is that UPGMA assumes equal rates of evolution (molecular clock assumption) along all branches, whereas neighbor joining assume variations in the rate of change (Saitou and Nei, 1987; Studier and Keppler, 1988; Nei and Kumar, 2000; Lan and Reeves, 2002). To further examine the patterns of variation among individual samples, a principal coordinate analysis (PCO) was performed based on Jaccard's coefficient (Jaccard, 1908). The calculation of Jaccard's coefficient was made with PAST software version 1.18 (Hammer et al., 2001). The first three axes were later used to plot with STATISTICA version 6.0 software (Hammer et al., 2001; Statistica Soft, Inc. 2001).

RESULTS AND DISCUSSION

Banding patterns and ISSR primers

Out of the eight primers tested initially, four of them gave relatively clear banding pattern and they were selected and used in this study (Table 3). The size of the fragments amplified using the four primers were in the range of 450 bp to 4 kb. A total of 39 fragments were amplified by the four ISSR primers of which 32 (82%) were polymorphic. The highest number of bands (12) was recorded for primer UBC - 834 followed by UBC 810 and UBC 880 which generated 10 and 9 scorable bands respectively. The least number of bands (8) were amplified by primer UBC - 844 (Figures 1 and 2).

Polymorphism

The number of polymorphic loci ranges from 5 for UBC-844 to 10 for UBC-834, where all are SSR with di-nucleotide repeat motif (Table 4). The only penta-nucleotide repeat primer UBC-880 and di-nucleotide UBC-810 showed the polymorphism of 8 and 9. Considering the percent polymorphism, UBC-844 showed the least polymorphism with 62.5%, while UBC-810 showed the highest with 100% polymorphism.

Among all the populations, studied Merawi and Addis Kidam from Amhara were found to show higher percentage polymorphism with 53.85 and 48.72%. This might be due to transfer of genes by effectors such as wind, insect, birds and or human (seedling movement) e.t.c. since these two weredas (Merawi and Addis Kidam) are near each other and in the main road of Bahir - Dar to Addis Ababa. Sekela of Amhara region has a percent polymorphism of 46.15%. The least percent

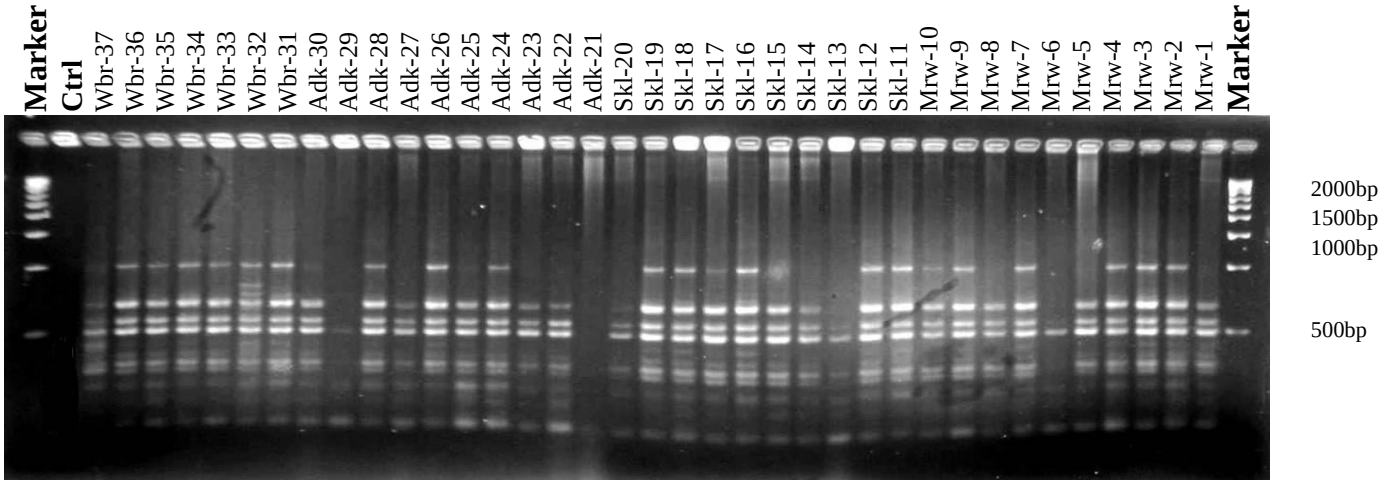

Figure 1. Banding pattern of lupine DNA samples by primer UBC **-** 834.

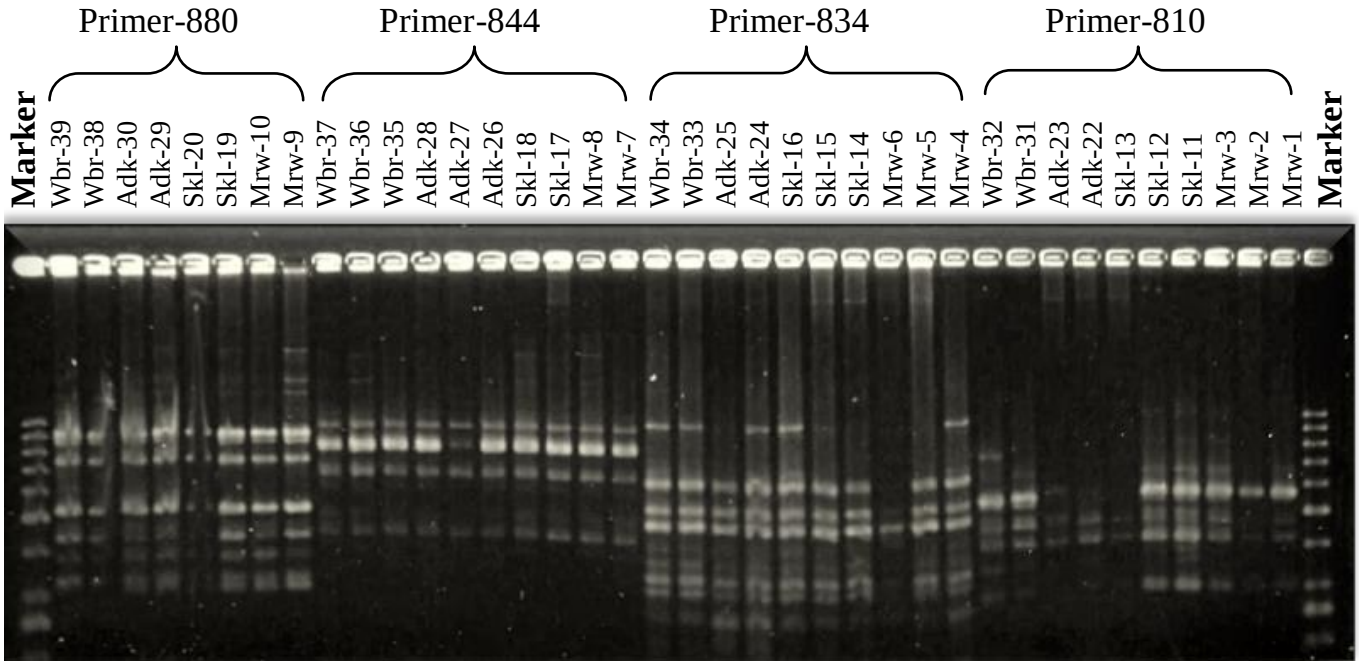

Figure 2. Banding pattern of lupine DNA samples by primers UBC **-** 810, UBC **-** 834, UBC **-** 844 and UBC **-** 880.

polymorphism was observed in Wembera of B-Gumz region population with 43.59%. Generally, Amhara region white lupine population has higher percent polymorphism (49.57%) than that of B-Gumuz region (43.59%) (Table 4).

Genetic diversity

Among white lupine populations considered in the present study, genetic diversity was higher for Merawi population (0.223) followed by those of Addis Kidam, Sekela and Wembera populations with genetic diversity of 0.198, 0.189 and 0.167, respectively. From this work it seems that, Amhara region white lupine (0.203) population shows higher genetic diversity than white lupine population of B-Gumuz region (0.167) (Table 4). This might be also due to transfer of genes by effectors like that of the reason for polymorphism.

Partitions of genetic variation

Levels variations can be attributed to within and between

Table 4. Number of polymorphic loci (NPL), percent polymorphism (PP) and genetic diversity (GD) of white lupine with each population and all primers.

Population	With all primers		
	NPL	PP (%)	GD
Amhara Region			
Merawi	21	53.85	0.223
Sekela	18	46.15	0.189
Addis Kidam	19	48.72	0.198
Sum	58	148.72	0.61
Average	19.3	49.57	0.203
B-Gumuz Region			
Wembera	17	43.59	0.167
Over all	75	192.31	0.777
Average over all	18.75	48.078	0.194
For individual primers			
UBC – 810	9	100	0.301
UBC – 834	10	83.33	0.319
UBC – 844	5	62.5	0.140
UBC – 880	8	80.0	0.233
Sum	32	325.83	0.993
Average	8	81.46	0.248

Table 5. Shannon's diversity index within and among white lupine populations with di and penta-nucleotide primers.

Population	Shannon's diversity index (H)		Over all H
	Di-nucleotide repeat primers	Penta-nucleotide repeat primers	
Merawi	0.291	0.389	0.324
Sekela	0.308	0.131	0.272
Addis Kidam	0.456	0.286	0.289
Wembera	0.309	0.111	0.247
Hpopn	0.341	0.229	0.283
Hsp	0.405	0.405	0.405
Hpopn/Hsp	0.842	0.565	0.698
1- Hpopn/Hsp	0.158	0.435	0.302

Hpopn = mean genetic variation for population, Hsp = mean genetic variation for the entire data, Hpopn/Hsp = proportion of genetic variations within white lupine populations and 1-Hpopn/Hsp = proportion of genetic variations between white lupine populations.

population components. Shannon's diversity index and AMOVA were used to partition the existing genetic variation in to different components.

Shannon Weaver's diversity indices

The overall analysis with both di and penta-nucleotide primers indicated that the white lupines collected from Merawi were found to be more diversified compared to the rest of the populations collected from other weredas of Amhara and B-Gumuz region as shown in Table 5. Generally, Amhara region white lupine populations show higher Shannon`s diversity indices than the population of B-Gumuz region. The partitioning of the mean Shannon weaver diversity index for the species revealed that white

Table 6. AMOVA of white lupine populations, A; without grouping. B; with groups.

Source of variation	d.f	Sum of squares	Variance components	Percentage of variation	Fixation indices	P
A						
Among populations	3	26.630	0.69986	25.38	0.25382	0.00
Within populations	35	72.011	2.05746	74.62		0.00
Total	38	98.641	2.75732			

B

Source of variation	d.f	Sum of squares	Variance components	Percentage of variation	P
Among groups	1	17.517	0.86686	27.23	0.00
Among populations within groups	2	9.113	0.25883	8.13	0.00
Within populations	35	72.011	2.05746	64.64	0.00
Total	38	98.641	3.18315		

lupines are more variable among individuals of a population (0.698) than among the different populations (0.302). This result is similar with the work of Solomon (2007), who works in genetic diversity analysis of the wild *Coffea arabic*a L., populations from Harenna Forest, Bale Mountains of Ethiopia, using inter simple sequence repeats (ISSR) and Tesfaye (2006) Genetic diversity of wild *C. arabica* L., populations in Ethiopia as a contribution to conservation and use planning. The mean genetic diversity for populations was higher with di-nucleotide repeat primers as compared to penta-nucleotide repeat primers but equal value for mean genetic variation for the entire data (Table 5).

Analysis of molecular variance (AMOVA)

Analysis of molecular variance was carried out in two phases; one was done using the populations grouped into Amhara and B-Gumuz and the other was done for the entire populations (that is, using the four populations as it is without grouping) over all loci by considering them as one geographic region. The analysis was carried out by computation of the distance between "haplotypes", each individual's data pattern as one "haplotype" and computing variance components for each level (Excoffier et al., 2006). Partitioning of genetic diversity by analysis of molecular variance using grouped populations (Table 6B) revealed that out of the total genetic diversity, most of the ISSR diversity was distributed between individual plants within the populations (64.6%), with the remaining diversity being distributed among populations within groups (8.13%) and among groups (27.23%). Similarly, partitioning of genetic diversity by analysis of molecular variance without grouping populations revealed that out of the total genetic diversity, most of the ISSR diversity is due to differences between individual plants within the populations (74.6%), while the remaining is due to differences among populations (25.4%) (Table 6A). In both cases, the results of AMOVA revealed the same patterns of genetic diversity and indicated larger genetic diversity within the populations rather than among populations. This result is similar with the work of Solomon (2007) Tesfaye (2006), on wild *C. arabic*a L.

Genetic similarity

In Figure 3, it is indicated that an UPGMA dendrogram of white lupine populations was constructed based on Jaccard's similarity coefficients (Table 7). High similarity was observed between Sekela and Merawi white lupine populations (0.741) followed by the value between Addis Kidam and Merawi (0.734). The least similarity was observed between Wembera and Merawi. Sekela and Merawi which were collected from geographically close weredas of west Gojjam zone of Amhara region form their own group confirming the correlation of genetic distance with geographic distance. The Wembera population shares relatively smaller similarity values with all the populations from the Amhara region. Thus, the Wembera lupine population had similarity values of 0.715, 0.703 and 0.679 with the population of Addis Kidam, Sekela and Merawi, respectively. As it is shown on the UPGMA tree, Wembera is isolated from the other three and this is also correlated with the geographic distance separating these populations.

Cluster analysis

Jaccard's similarity coefficients were also used to construct UPGMA and NJ dendrograms for 39 individuals based on the bands obtained with the four primers (Figure 5).

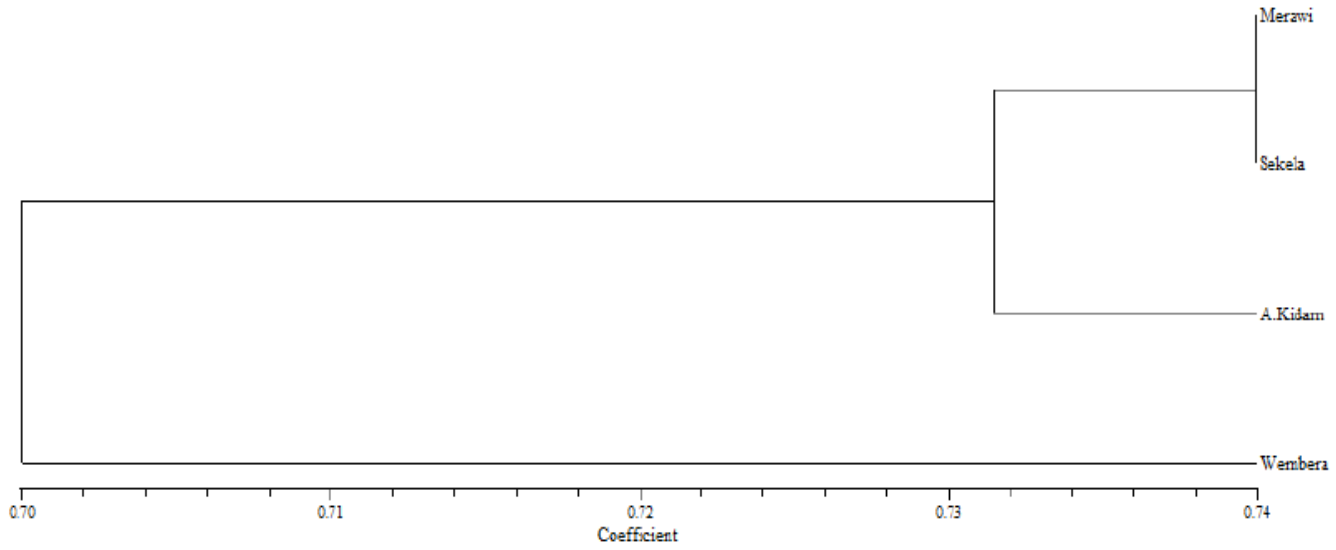

Figure 3. UPGMA based dendrogram for 4 white lupine populations using 4 ISSR primers.

Table 7. Similarity matrix for Jaccard's coefficients for 4 white lupine populations based on the bands obtained with ISSR primers.

Population	Merawi	Sekela	Addis Kidam	Wembera
Merawi	1.000			
Sekela	0.741	1.000		
Addis Kidam	0.734	0.730	1.000	
Wembera	0.679	0.703	0.715	1.000

Accordingly, individuals collected from Wembera wereda of B-Gumuz region located farther west than the other populations tend to form strong separate group in both UPGMA and NJ dendrogram. However, populations collected from Amhara region were observed to form moderate grouping based on their place of origin (Figure 4). Generally, both trees recovered almost the same tree topology with similar groupings, although few individuals appeared to escape from groups in case of NJ analysis.

Conclusion

Currently, a number of molecular markers have been widely used to study diversity in many plants (Karp et al., 1997). Given the proliferation of molecular markers, a comparison between the markers seems highly inevitable on the basis of study objectives and the nature of the markers. Of the many desired qualities of molecular markers, automation (PCR-based), polymorphisms and reproducibility are the highly demanded features of the molecular techniques to be used in the intraregional diversity analysis. ISSR markers are thus one of the molecular markers that have these characteristics to study variability in different crops (Zietkiewicz et al., 1994, Wolf and Liston, 1998). ISSR markers are observed to be highly variable within the species and reveal many more polymorphisms since they use longer primers that allow more stringent annealing temperatures (Hillis et al., 1996). Moreover, this marker observed to be very useful in detecting genetic diversity and population structure of Coffee (Aga, 2005); (Tesfaye, 2006), Tef (Assefa, 2003), and rice (Gezahegn, 2007) collected from all over Ethiopia.

In this study, also the ISSR markers observed to be an appropriate molecular marker for generating the detailed intraspecific genetic diversity data to evaluate extent and distribution of genetic diversity within and among *L. albus L*. Out of the total 39 scorable bands produced with the total of four; 3 di- and 1 penta-nucleotides, 32 bands were polymorphic. In terms of number of polymorphic fragment detected and percentage of polymorphic loci, per class of primer, di-nucleotides were found to be superior. In general, the detection of high levels of polymorphisms makes ISSR analysis with di-nucleotides primers a powerful technique for measuring the genetic diversity in white lupine. Among white lupine populations considered in the present study, Merawi has higher genetic diversity (0.223) than Sekela (0.186), Addis Kidam (0.161) and Wembera (0.167). Generally, Amhara region white lupine (0.203) populations show higher genetic diversity than white lupine population of B-Gumuz region.

Partitioning of genetic diversity by analysis of molecular variance using grouped populations revealed that out of the total genetic diversity, most of the ISSR diversity was distributed between individual plants within the populations (64.6%), with the remaining diversity being distributed among populations within groups (8.13%) and

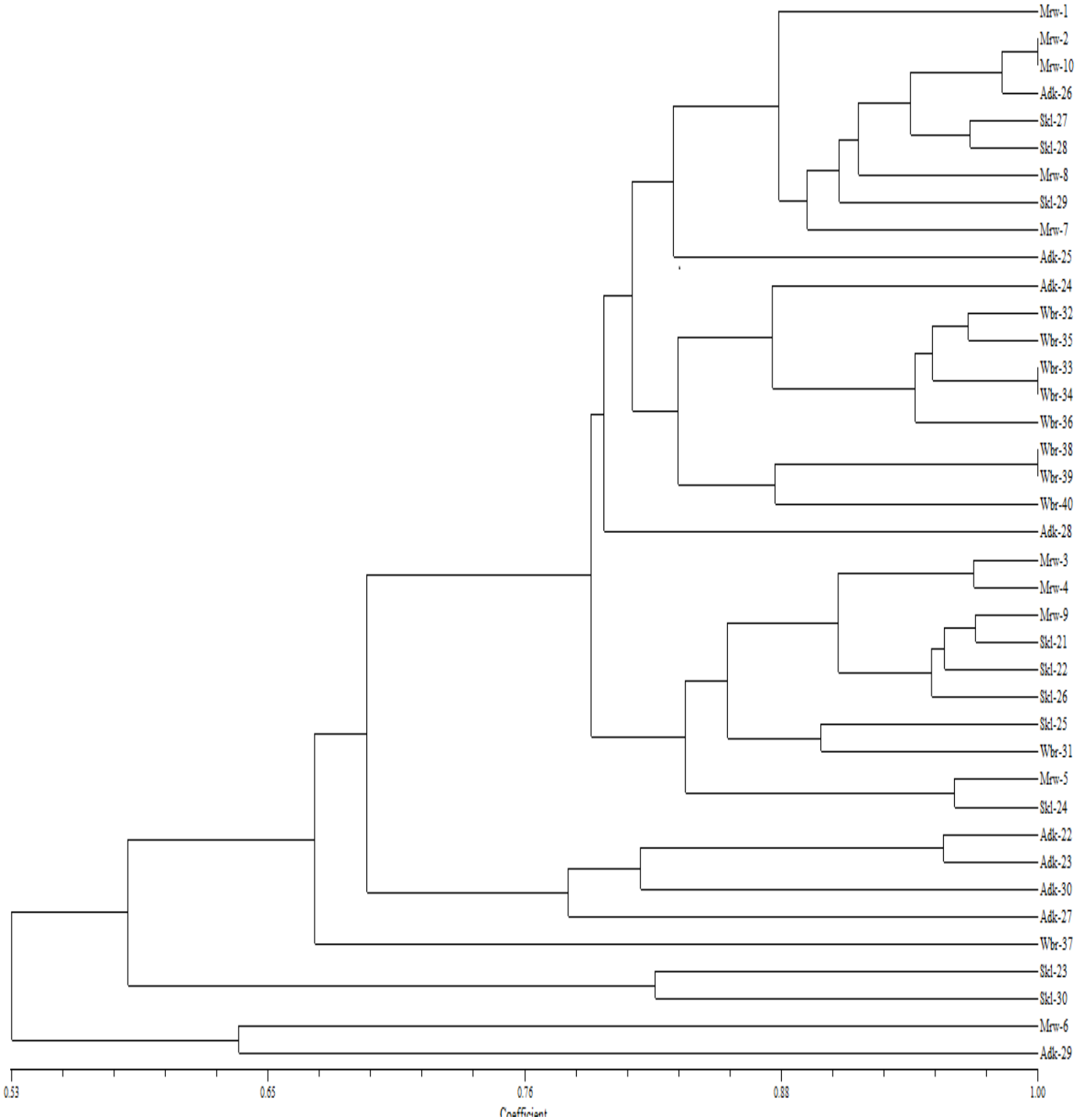

Figure 4. UPGMA dendrogram depicting clustering patterns for 39 individuals of white lupine based on Jaccard's similarity coefficient.

among groups (27.23%). Similarly, partitioning of genetic diversity by analysis of molecular variance without grouping populations revealed that out of the total genetic diversity, most of the ISSR diversity is due to differences between individual plants within the populations (74.6%), while the remaining is due to differences among populations (25.4%). In both cases, the results of analysis of molecular variance revealed the same pattern of genetic diversity and supports the larger genetic diversity found within the populations rather than among populations Based on Jaccard's coefficients of similarity, high genetic similarity was observed between Sekela and

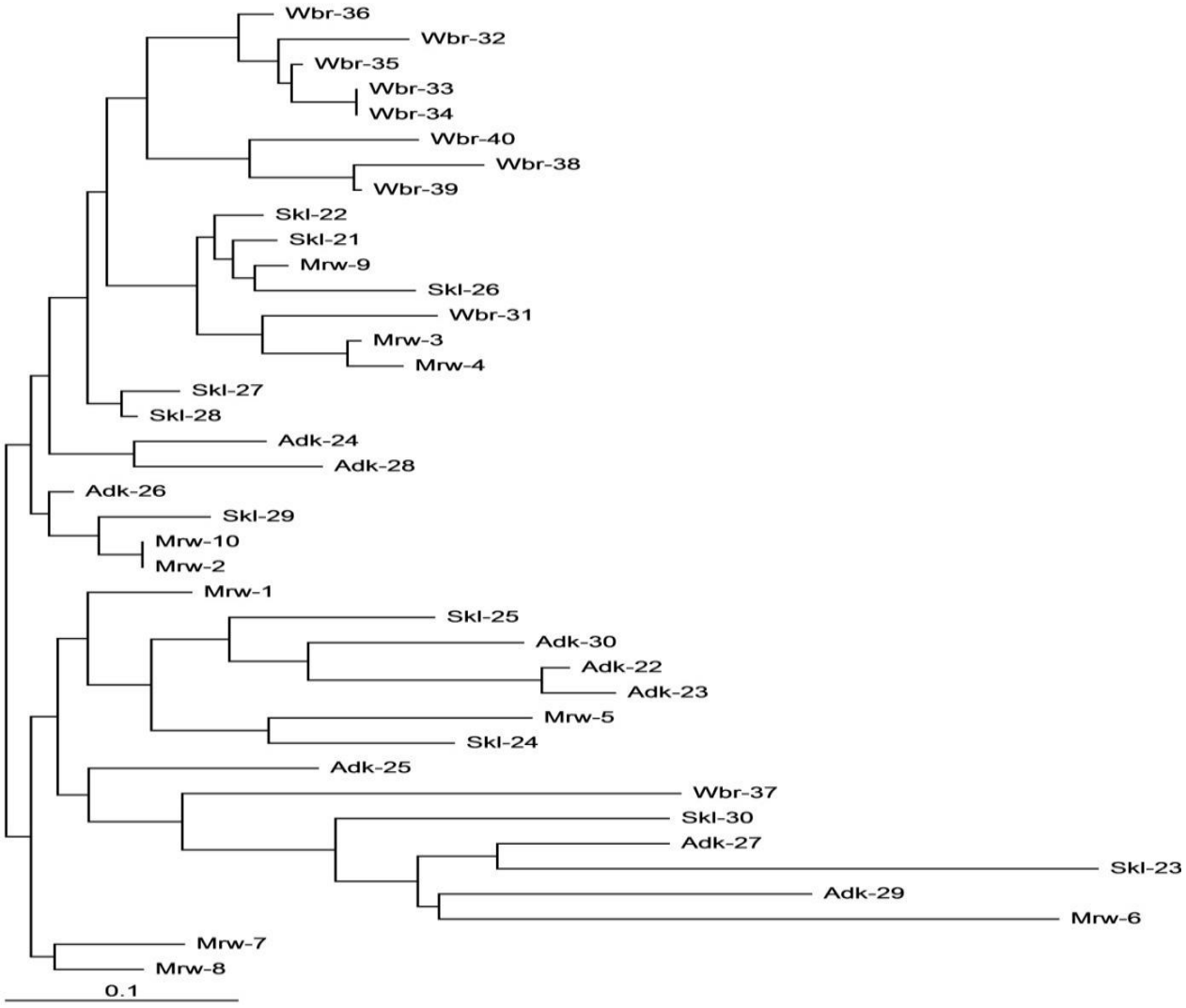

Figure 5. NJ analysis of 39 individuals based on Jaccard's similarity coefficient.

Merawi white lupine populations (0.741) followed by the value between Addis Kidam and Merawi (0.734). The least similarity was observed between Wembera populations with Merawi populations (0.679). The Wembera population shares relatively smaller similarity values with all the populations from the Amhara region. Thus, the Wembera lupine population had similarity values of 0.715, 0.703 and 0.679 with the population of Addis Kidam, Sekela and Merawi, respectively.

In the present study, four ISSR primers; 3 di- and 1 penta- nucleotides were employed. They were able to reveal that genetic diversity ranged from moderate to high levels and identified the highly diverse and least diverse populations in Ethiopian white lupine collected from Amhara and B-Gumuz region. While, high genetic diversity was observed between two regions, moderate levels of variation were shown in Amhara region populations. The Shannon's diversity index also confirmed the existence of higher diversity in two regions population and genetic similarity based on Jaccard's coefficients of similarity was observed high value between Sekela and Merawi white lupine populations (0.741) followed by white lupine populations from Addis Kidam and Merawi (0.734), the least similarity was found between Wembera population with Merawi and Sekela having the value of 0.679 and 0.703. Though a limited number of ISSR markers were used in the study, the results confirm that ISSR markers are efficient in detecting polymorphism within and among populations of white lupine found in close geographic proximity. Until the present day information available on the reproductive biology of *L. albus* L., suggested that it is a predominantly self-pollinating plant. However, the result of this study might be attributed to two reasons: one against and the other in favor of the self-pollinating nature of the *L. albus* plant. In the former case, the result obtained could be

accounted to mixed type of mating, typical of plant species, in which there is a gene flow, and thus there might be moderate gene flow among the local populations by effectors such as wind, insect, human (seedling movement) and birds. The other is that they might have preferential or diverse adaptive genes that are not fixed through self-pollination until the present day.

However, this study used a small sample size, geographic range and limited primers. Therefore, to find clear patterns of diversity for the whole country and reach a sound conclusion, further studies should be conducted with large sample sizes and geographic range using many ISSR primers.

Conflict of interests

The authors did not declare any conflict of interest.

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