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RESEARCH ARTICLE

GENETIC DIVERSITY OF YEHEB (*CORDEAUXIA EDULIS* HEMSL.) FROM SOUTHEASTERN ETHIOPIA USING INTER SIMPLE SEQUENCE REPEAT (ISSR) MARKERS

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ABSTRACT: Yeheb (*Cordeauxia edulis* Hemsl.) is a multipurpose and evergreen shrub plant endemic to southeastern corner of Ethiopia and extends through central Somalia. In spite of its importance as a food security plant, there is no information on the genetic diversity of this plant. Therefore, the objective of this study was to analyze genetic diversity of *C. edulis* using inter simple sequence repeat (ISSR) markers. Genetic diversity of six populations of yeheb, each represented by ten to twelve individuals, was analyzed. Eleven primers were tested and four primers were selected. The four primers amplified 37 loci of which 32 (86.49%) were polymorphic bands with eight polymorphic bands per primer. There were high levels of polymorphism at the population level with polymorphic bands ranging from 32.43% to 59.46%. The highest level of diversity with Jaccard's similarity coefficient of 0.490 was observed between Gambare and Maned populations whereas the lowest level of diversity with similarity coefficient of 0.620 was observed between Godir Woyis and Mirafadle populations. The Nei's gene diversity and Shannon's information index were 0.278 and 0.423, respectively. Analysis of molecular variance (AMOVA) showed that within populations genetic variation (71.03%) was found to be much higher than among populations (28.97%). Gambare population showed the highest genetic diversity indicating that this population should be considered as the primary sites in designing conservation areas for this plant as well as for its future improvement.

Key words/phrases: AMOVA, Cluster analysis, Genetic similarity, Multipurpose tree.

INTRODUCTION

Cordeauxia edulis Hemsley, locally called yeheb, is a small, multi-branched shrub that belongs to the family Leguminoseae. It is the only species within the genus Cordeauxia (Bally, 1966) and a diploid plant having chromosome

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number of 2n=24 (Miège and Miège, 1978). Yeheb is endemic to Somali Regional State of Ethiopia and the semi-arid central Somalia where it is used as edible seed crop (Seegeler, 1983; Zimsky, 1990) although it is reported to be grown as a minor edible seed crop at experimental scale in Israel, Kenya, Sudan, Tanzania and Yemen. Yeheb is drought-tolerant and used as a source of food for both animals and humans. It grows in areas of erratic rainfall as low as 150–200 mm and at altitudes of 300–1000 m above sea level (Drechsel and Zech, 1988). This plant is adapted to frost-free areas and nutrient impoverished soil (Seegeler, 1983; Drechsel and Zech, 1988).

The height of yeheb varies depending on the environmental conditions of the growing area. A highly branched shrub grows to an average height of 1.6 m although sometimes it may reach up to 4 m depending on the growing conditions, when not overbrowsed (FAO, 1988). The bushes blossom during the rainy season once or twice if rainfall is abundant (Gaertner *et al*., 1982).

Yeheb produces nutritious and tasty seeds having a chestnut flavour. They contain 24% low molecular weight sugar, 37% starch, 13% proteins, 11% fats and various mineral salts (Gutale and Ahmed, 1984). The nut is the staple food for the local people and also has medicinal value (Kazmi, 1979; Booth and Wickens, 1988). Yeheb has other multiple uses including as fodder, firewood, house construction, nectar source for bees, mulch, soil conservation, live fence, dyes etc. (Azene Bekele *et al*., 1993).

The population of yeheb in southeastern Ethiopia is very low and declining rapidly, owing to habitat fragmentation, overexploitation and low rate of regeneration. As a result, it is in the list of endangered species by the International Union for Conservation of Nature (Brink, 2006). Analysis of genetic diversity at intra-specific level is important for development of conservation strategies, exploration of plant genetic resources, and future breeding programs (Hamrick and Godt, 1996). The basis of species conservation is the maintenance of genetic diversity in populations (Yeh *et al*., 1996). Analyses of genetic diversity have potential uses in evolution, breeding and conservation of genetic resources (Wu *et al*., 1999). There is no information on the genetic diversity of yeheb using any of the molecular markers. Studying the genetic diversity of yeheb is important to design its conservation strategy and genetic improvement. Therefore, the objective of this research was to analyze genetic diversity of yeheb (*Cordeauxia edulis*) populations in southeastern Ethiopia using ISSR markers.

MATERIALS AND METHODS

Plant material

Young leaves were collected from 64 individual plants of yeheb representing six populations scattered throughout Afardod, Maned, Dabhabalan, Gambare, Mirafadle and Godir Woyis from southeastern Ethiopia, Somali Regional State of Ethiopia (Table 1 and Fig. 1). The young leaf samples were dried over silica gel in zip-lock plastic bags until subsequent genomic DNA extraction.

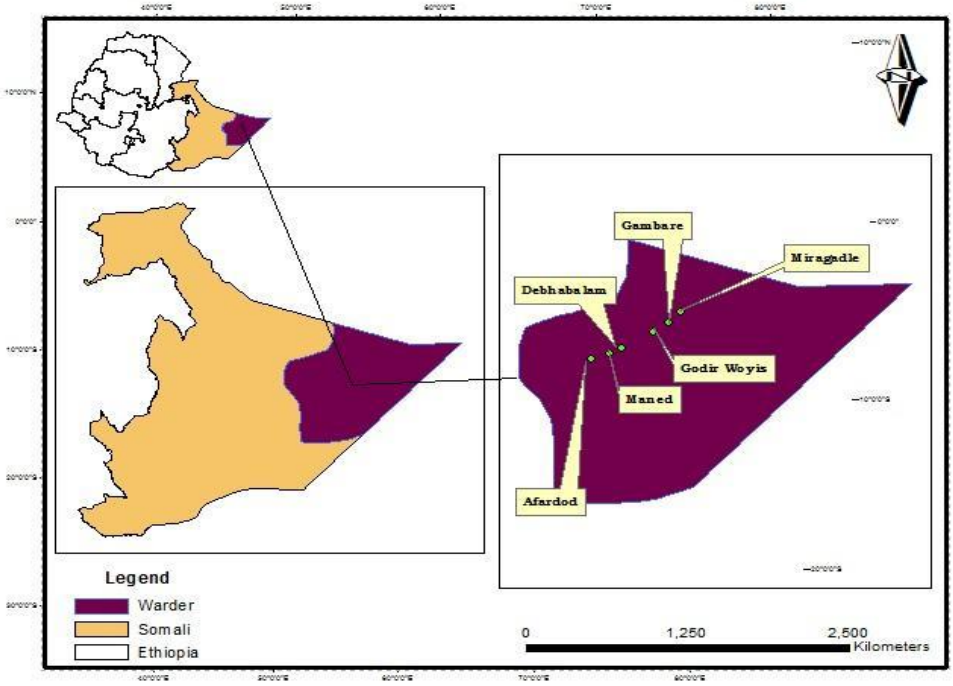

Fig. 1. Map of Ethiopia showing collection sites of yeheb leaf samples.

Table 1. Collection sites of populations of yeheb along with altitudes and site coordinates.

Populations	District (Locality)	Sample size	Geographical locations		
			Latitude (N)	Longitude (E)	Altitude (m)
Afardod	Afardod	12	07°52′79″	046°89′37″	541
Maned	Maned	10	07°22′42″	046°80′22″	472
Dabhabalan	Dabhabalan	12	07°32′25″	046°98′30″	430
Gambare	Gambare	10	07°32′27″	046°98′57″	441
Mirafadle	Mirafadle	10	07°39′81″	046°92′26″	464
Godir woyis	Godir woyis	10	07°28′59″	046°87′43″	486

DNA extraction

Eighty milligram of dried leaves from 64 individuals of yeheb was ground with pestle and mortar using combination of liquid nitrogen and quartz sand. DNA was extracted using CTAB method of Borsch *et al*. (2003) with minor modification, which employs triple extractions. Two microlitre of DNA and 2 µl of 1x loading dye were mixed and loaded on 0.8% agarose gel and electrophoresed in 1x TBE buffer running at 80 V for 45 min. The test gel was stained with 50 µl ethidium bromide (10 mg/ml) in 450 ml distilled water followed by washing in 500 ml distilled water for 30 min. The gels were photographed with digital camera mounted on Gel Doc system.

Polymerase chain reaction

For ISSR primers screening and optimization, DNA of three individuals per population was used. A total of 11 primers were screened for polymorphism and reproducibility of bands. Four primers showed high level of polymorphism and reproducibility and were used for further analyses (Table 2).

DNA amplification was performed in a final volume of 25 µl reaction mixture containing 5.0 µl dNTPs (1.25 mM), 2.5 µl Taq buffer (10x Thermopol reaction buffer), 2.0 µl $MgCl_2$ (2.0 mM), 0.3 µl primer (20 pmol/µl), 0.2 µl Taq Polymerase (5U/µl), 1.0 µl template DNA and the final volume was adjusted with 14 µl H_2O. The amplification program was set at initial denaturation at 94°C for 4 minutes, followed by 40 cycles of 15 seconds at 94°C, 1 minute primer annealing at 45°C/48°C based on the type of primers, 1 minute and 30 seconds extension at 72°C and the final extension at 72°C for 7 minutes. The PCR was conducted using Biometra 2000 T3 Thermocycler. The amplification products were separated by electrophoresis using agarose gel (1.67% agarose with 50 ml 1x TBE) and 8.0 µl amplification product of each sample with 2.0 µl of 6x loading dye. Hundred base pair DNA ladder was used to estimate size of the fragments. Electrophoresis was done for 2 h at constant voltage of 100 V. The gel was stained with 50 µl of 10 mg/ml ethidium bromide mixed with 450 ml distilled water for 30 min followed by washing in 500 ml distilled water for 30 min.

Table 2. List of ISSR primers screened for polymorphism and reproducibility of the amplified bands.

Primers	Annealing temperature (°C)	Sequence[a]	Amplification pattern	Repeat motif
UBC-810	45	(GA)8T	Polymorphic, Reproducible	Di-nucleotide
UBC-812	45	(GA)8A	Polymorphic, Reproducible	Di-nucleotide
UBC-818	48	(CA)8G	Not reproducible	Di-nucleotide
UBC-824	48	(TC)8G	Not amplified	Di-nucleotide
UBC-827	48	(AC)8G	Not amplified	Di-nucleotide
UBC-834	45	(AG)8YT	Polymorphic, Reproducible	Di-nucleotide
UBC-841	48	(GA)8YC	Not amplified	Di-nucleotide
UBC-844	48	(CT)8RC	Polymorphic, Reproducible	Di-nucleotide
UBC-860	45	(TG)8RA	Not amplified	Di-nucleotide
UBC-873	48	(GACA)4	Not amplified	Tetra-nucleotide
UBC-880	45	(GGAGA)3	Not amplified	Penta-nucleotide

[a]Sequences = R = (A, G); Y = (C, T)

Data analyses

ISSR bands were scored as present (1), absent (0) and missing (?). The data were analyzed using POPGENE version1.32 software (Yeh *et al*., 1999) to measure percentage of polymorphic loci (PPL), gene diversity (h), and Shannon's information index (I). The genetic structure was estimated using Analysis of Molecular Variance (AMOVA). The AMOVA analysis was done using the software ARLEQUIN version 3.01 (Excoffier *et al*., 2006) to estimate genetic variability within and among populations without grouping. Dendrograms were constructed on the basis of Jaccard's similarity coefficient using unweighted pair group method with arithmetic average (UPGMA) (Sneath and Sokal, 1973) and neighbour joining (NJ) method (Saitou and Nei, 1987; Studier and Keppler, 1988). NTSYS- pc version 2.02 (Rohlf, 2000) and Free Tree 0.9.1.50 (Pavlicek *et al*., 1999) software were used for these cluster analyses, respectively. Jaccard's similarity coefficient was calculated using PAST software version 1.18 (Hammer *et al*., 2001). Principal coordinate analysis (PCO) was performed based on Jaccard's coefficient (Jaccard, 1908). The first three axes were later used to plot with STATISTICA version 6.0 software (Hammer *et al*., 2001; Statistica Soft, Inc. 2001).

RESULTS

ISSR markers and banding patterns

The four di-nucleotide primers selected for further analyses were UBC-810, UBC-812, UBC-834, and UBC-844. A total of 37 clear bands were generated by these primers with the band size ranging from 100 to 1000 bp. Primer UBC-810 produced the highest (11) number of bands while primer

UBC-834 produced the least (8) with an average of 9.25. The amplification pattern of bands produced by primer UBC-810 is shown in Fig. 2.

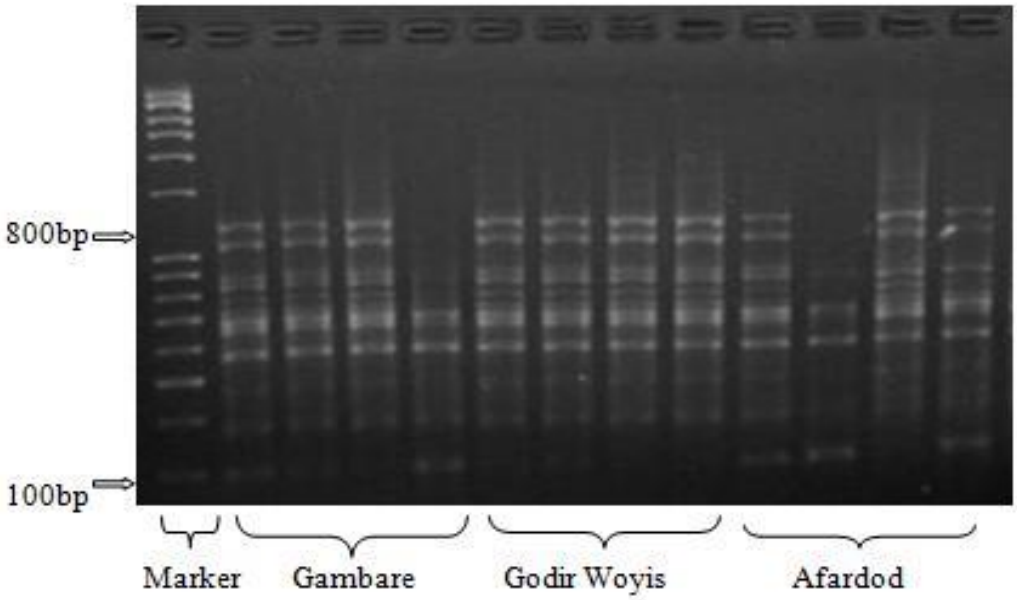

Fig. 2. Banding profiles of 12 yeheb individuals selected from three populations using primer 810.

Genetic diversity of yeheb populations

Thirty seven discernible bands were generated among which 32 (86.49%) were polymorphic in six populations, Afardod, Maned, Gambare, Dabhabalan, Mirafadle, and Godir Woyis. Among these, 100% of polymorphic loci were obtained by primer UBC-834, followed by primers UBC-812 and UBC-844 which showed 88.89% polymorphism. Primer UBC-810 generated the least percentage of polymorphic loci (72.73%) (Table 3). Each primer exhibited Nei's gene diversity (*h*) ranging from 0.21 to 0.37 and Shannon's information index (*I*) ranging from 0.32 to 0.55. The highest Nei's gene diversity (0.37) and Shannon information index (0.55) were exhibited by primer UBC-834. In contrast, primer UBC-810 showed the least Nei's gene diversity (0.21) and Shannon's information index (0.32).

Table 3. Number of scorable bands, number of polymorphic bands, percent of polymorphic loci, Nei's genetic diversity (*h*) and Shannon's information index (*I*) for each primer.

Primers	NSB	NPL	PPL (%)	h ± SD	I ± SD
UBC-810	11	8	72.73	0.21 ± 0.191	0.32 ± 0.267
UBC-812	9	8	88.89	0.30 ± 0.187	0.45 ± 0.250
UBC-834	8	8	100	0.37 ± 0.996	0.55 ± 0.121
UBC-844	9	8	88.89	0.26 ± 0.182	0.40 ± 0.240
Overall	37	32	86.49	0.27 ± 0.176	0.42 ± 0.238

NSB = Number of scorable bands; NPL = Number of polymorphic loci; PPL = Percent of polymorphic loci; *h* = Nei's gene diversity; *I* = Shannon's information index; SD = Standard deviation

Gambare population showed the highest genetic diversity with 59.46% PPL, 0.236 Nei's gene diversity and 0.343 Shannon's information index followed by Godir Woyis population with 43.24% PPL, 0.162 Nei's gene diversity and 0.239 Shannon's information index indicating that probably these places are centres of genetic diversity for yeheb (Table 4). The three populations were collected from close locations relatively with dense natural vegetation cover. On the other hand, Mirafadle population showed the lowest genetic diversity with 32.43% PPL, 0.121 Nei's gene diversity and 0.180 Shannon's information index.

Table 4. Number of polymorphic loci, percent of polymorphic loci, Nei's genetic diversity and Shannon's information indices for the studied populations.

Populations	NPL	PPL (%)	h ± SD	I±SD
Afardod	14	37.84	0.156 ± 0.214	0.225 ± 0.305
Maned	15	40.54	0.156 ± 0.200	0.231 ± 0.290
Dabhabalan	16	43.24	0.142 ± 0.190	0.215 ± 0.275
Gambare	22	59.46	0.236 ± 0.220	0.343 ± 0.310
Mirafadle	12	32.43	0.121 ± 0.188	0.180 ± 0.273
Godir Woyis	16	43.24	0.162 ± 0.208	0.239 ± 0.296
Overall	32	86.49	0.27 ± 0.176	0.42 ± 0.238

NPL = Number of polymorphic loci; PPL = Percent of polymorphic loci; *h* = Nei's gene diversity; *I* = Shannon's information index; SD = Standard deviation

Analysis of molecular variance

Analysis of molecular variance was carried out by using the entire six populations without grouping. The analysis was carried out by computation of the distance between haplotypes, each individual data pattern as haplotype and computing variance components for each level. The total genetic variation among populations (*Gst*) was 0.4147. Partitioning of genetic diversity by AMOVA without grouping populations showed that out of the total genetic diversity, 71.03% were within population while among population genetic diversity was 28.97% (Table 5).

Table 5. Analysis of Molecular Variance (AMOVA) of six yeheb populations from southeastern Ethiopia using four ISSR primers.

Source of variation	Degree of freedom	Sum of squares	Variance components	Percentage of variation	P-value
Among populations	5	47.42	0.72	28.97	P<0.001
Within populations	58	102.97	1.76	71.03	P<0.001
Total	63	150.39	2.45		

Cluster analysis

UPGMA and NJ showed comparable results with an indication of moderate clustering of individuals of the populations (Figs. 4 and 5). The analyses also revealed intermixing of individuals. The UPGMA-based dendrogram (Fig. 3) of the six populations generated four major groups, I, II, III and IV. The first cluster contains Afardod and Dabhabalan while the second cluster divided into two subgroups, Godir Woyis and Mirafadle and the third and fourth groups contain Gambare and Maned, respectively. On the basis of UPGMA most individuals of the respective populations were observed to form moderate grouping with few intermixing with other populations. In the case of NJ, each individual of the respective population was observed to form small clusters by intermixing with individuals of other populations. Generally, on the basis of UPGMA, most individuals of the respective population were observed to form moderately clustered groups with few intermixing from the other populations. Furthermore, Afardod population tends to form separate cluster but with some intermixing from the other populations. Dendrogram of UPGMA showed Mirafadle and Godir Woyis populations are closely related. Individuals of Afardod and Dabhabalan populations were closely clustered. The Gambare and Maned populations had their own lineage far from the other populations.

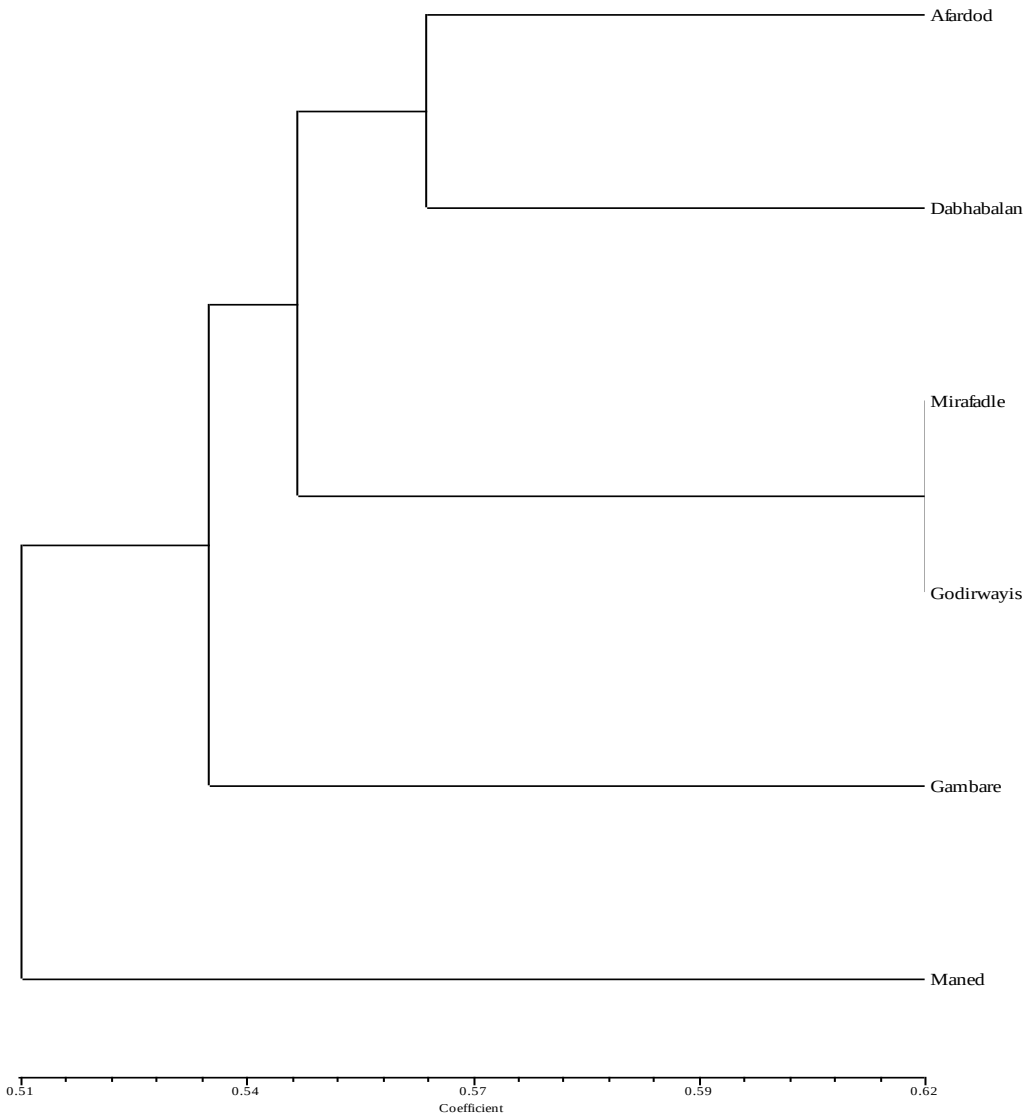

Fig. 3. Dendrogram generated based on UPGMA demonstrating the genetic similarity between six populations of yeheb.

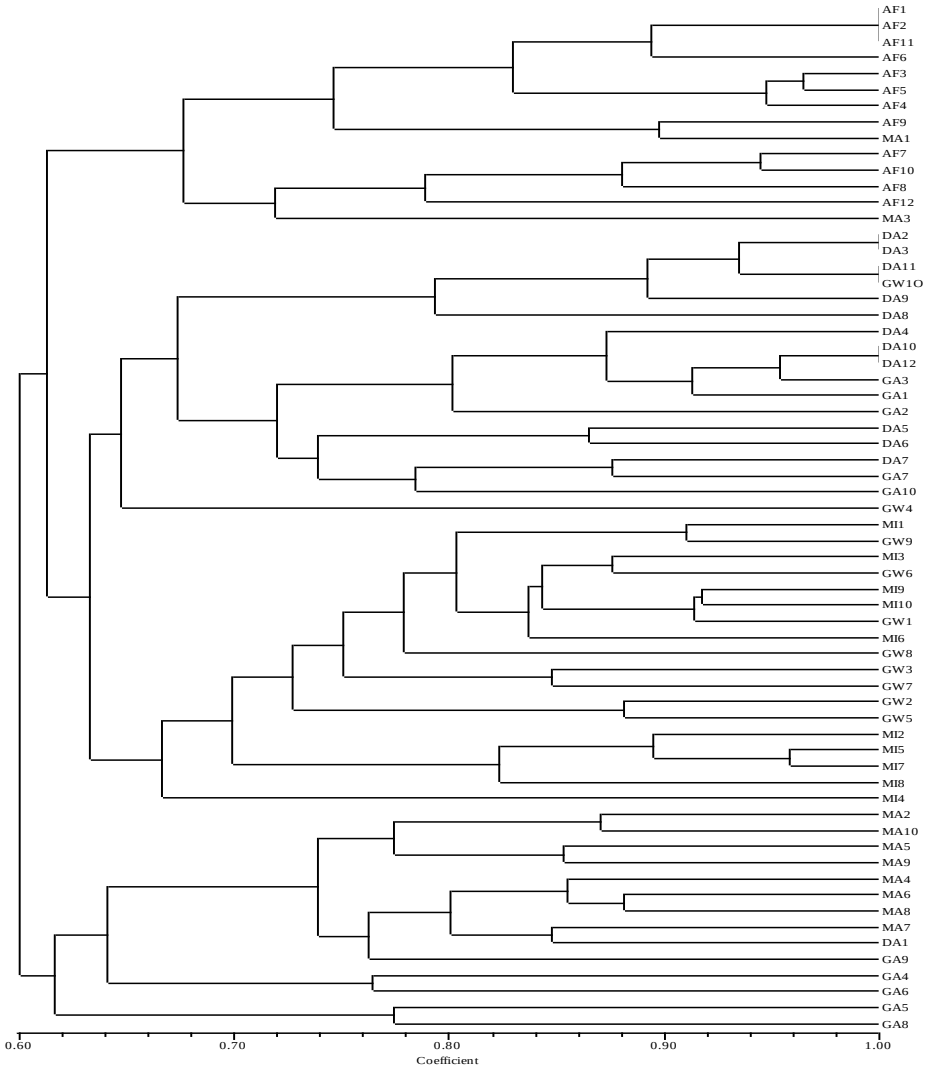

Fig. 4. Dendrogram generated based on UPGMA analysis demonstrating the genetic similarity among 64 individuals of yeheb using the four ISSR primers based on Jaccard's coefficients of similarity.
Key: GA = Gambare, MA = Maned, GW = Godir Woyis, DA = Dabhabalan, MI = Mirafadle, AF = Afardod

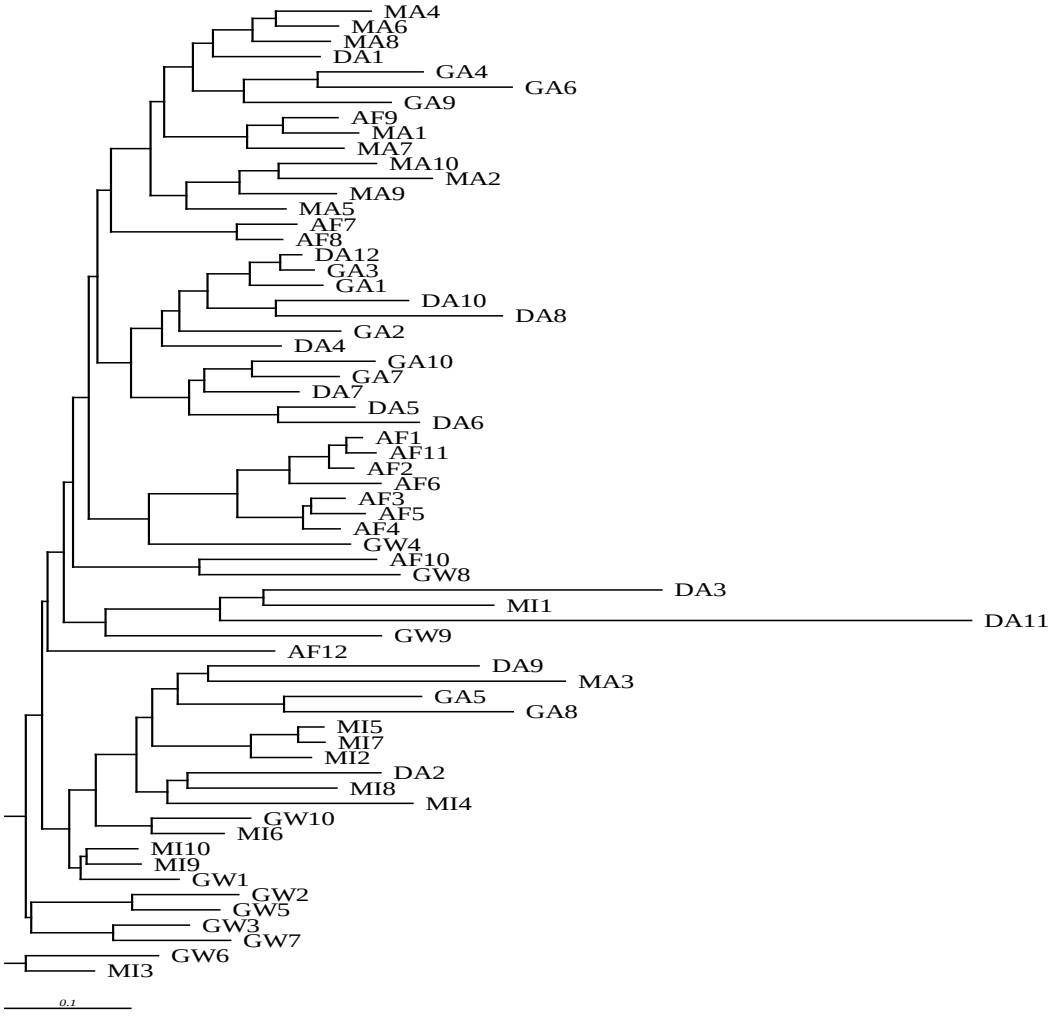

Fig. 5. Neighbor-joining analysis of 64 individuals based on Jaccard's coefficient of similarity.
Key: GA = Gambare, MA = Maned, GW = Godir Woyis, DA = Dabhabalan, MI = Mirafadle, AF = Afardod.

Genetic similarity

Based on Jaccard's coefficient of similarity, the highest genetic similarity (0.620) was observed between Godir Woyis and Mirafadle populations followed by the second highest genetic similarity (0.589) between Godir Woyis and Afardod populations (Table 6). On the other hand, the least genetic similarity was detected between Gambare and Maned with Jaccard's similarity coefficient of 0.490.

Table 6. Similarity matrix of six populations of yeheb based on Jaccard's coefficient of similarity.

Populations	Afardod	Maned	Dabhabalan	Gambare	Mirafadle	Godir Woyis
Afardod	1.000					
Maned	0.558	1.000				
Dabhabalan	0.561	0.515	1.000			
Gambare	0.534	0.490	0.551	1.000		
Mirafadle	0.526	0.492	0.537	0.516	1.000	
Godir Woyis	0.589	0.519	0.531	0.539	0.620	1.000

Principal Coordinate Analysis

The first three eigen-values, 3.88, 3.31 and 2.20 of two dimensional PCO with percentage of 17.00%, 14.52% and 9.69%, respectively were used to show groupings of individuals (Figs. 6 and 7). In two dimensional PCO, the individuals of some populations tended to group together except intermixing of few individuals of Afardod, Maned and Dabhabalan populations while the individuals from Mirafadle, Godir Woyis and Gambare scattered all over the plot. Moreover, individuals from Afardod are relatively separated from other populations. Using three coordinates, almost all individuals of each population were spread all over the plot except Godir Woyis individuals which were intermixed with each other and formed separate group from the rest. Although results of two dimensional coordinates are similar to the three dimensional coordinates, the two dimensional coordinate's analysis showed better resolutions than three dimensional PCO. Overall, no clear grouping was observed among individuals collected from different localities.

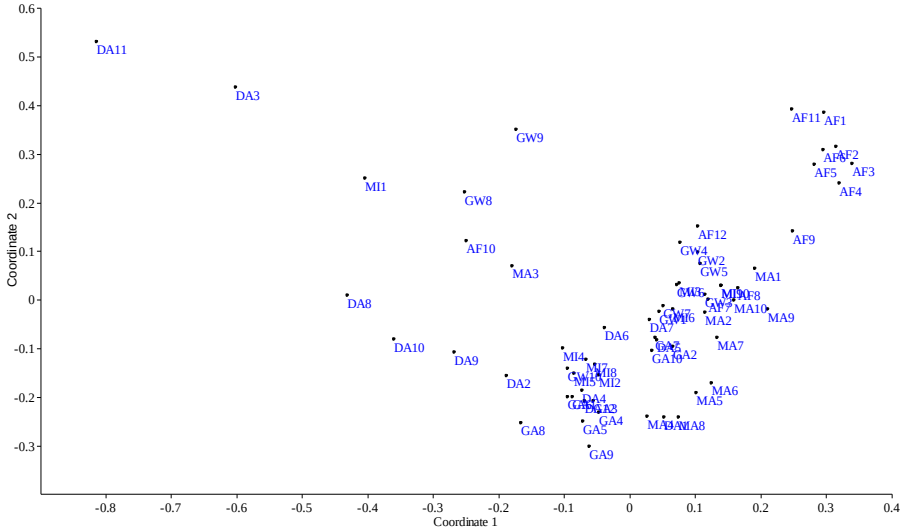

Fig. 6. Two dimensional representations of 64 individual samples of yeheb populations based on Jaccard"s similarity coefficients.

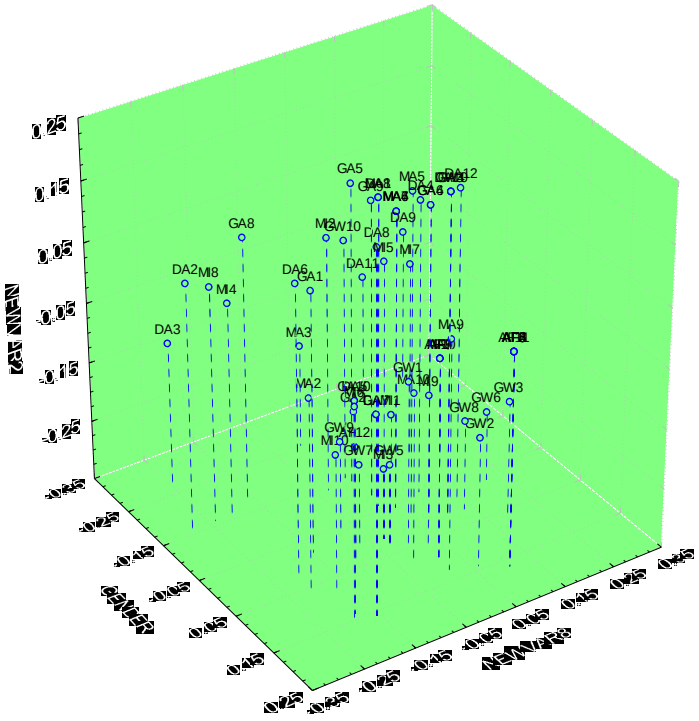

Fig. 7. Three dimensional representations of principal coordinate analysis of genetic relationship among 64 individual samples of yeheb populations based on Jaccard's similarity coefficient.

DISCUSSION

Molecular marker-based analyses of genetic diversity in plant species have become important tools in plant breeding, genecology and conservation (Nybom and Bartish, 2000; Weising *et al*., 2005; Tileye Feyissa *et al*., 2007). Generally, genetic diversity of a species is strongly associated with phyletic group, geographic range, life form, regional distribution, seed dispersal mechanism, breeding system, mode of reproduction and successional status (Hamrick and Godt, 1989; Bhat *et al*., 1999). Among these parameters, breeding system appears to be the most important factor, followed by life form, seed dispersal and successional status (Nybom, 2004).

The present study reports the molecular genetic diversity analyses of yeheb for the first time to the best of our knowledge. Thirty seven discernible bands were generated among which 32 (86.49%) were polymorphic in six populations. In the present study, each of the four ISSR primers showed Nei's gene diversity (*h*) ranging from 0.21 to 0.37 and Shannon's information index (*I*) ranging from 0.32 to 0.55. Gambare population showed the highest genetic diversity with 59.46% PPL, 0.236 Nei's gene diversity and 0.343 Shannon's information index followed by Godir Woyis population with 43.24% PPL, 0.162 Nei's gene diversity and 0.239 Shannon's information index indicating that probably these places are centers of genetic diversity for yeheb. The three populations were collected from close locations relatively with dense natural vegetation cover. On the other hand, Mirafadle population showed the lowest genetic diversity with 32.43% PPL, 0.121 Nei's gene diversity and 0.180 Shannon's information index. This low genetic diversity might be due to anthropogenic destruction of natural habitats, deforestation, overgrazing by livestock, and homogeneity as a result of limited seed sources. Esayas Aga *et al*. (2003) also observed similar pattern of diversity among Ethiopian coffee populations with Shannon-Weaver diversity index, whereby high genetic differentiations (80%) within zones of sample collection sites were observed.

The percentage of total genetic variation among populations (*Gst*) was 0.4147, which is higher than the mean *Gst* value (0.129) reported in the 220 genera, 662 species (Hamrick *et al*., 1992). In addition, the percentage of total genetic variation among yeheb populations (*Gst* = 41.47%) was between the average of out crossing plant species (*Gst* = 10%) and the average of self-pollinating plant species (*Gst* = 51%) (Hamrick *et al*., 1992).

This indicates yeheb is most likely self-pollinating plant. However, information available on the reproductive biology of yeheb suggests it is pollinated by insects. Thus, the obtained result could be accounted to mixed type of mating, in which there is a gene flow, and thus there may be moderate gene flow among the local populations by effectors such as wind, insect, human (seedling movement) and birds. They might also have preferential or diverse adaptive genes that are not fixed through self-pollination until the present day.

The mating system is the most critical factor to affect genetic diversity (He and Liu, 2003). Yeheb is produced with the low seed set and small amounts of viable seeds. At present, there have been no comprehensive studies on yeheb mating system. The higher within population genetic diversity than among population genetic diversity might be accounted for two contrary reasons. Like any other plant species, the population genetic diversity of yeheb is affected by multiple evolutionary forces which operate within historical and biological context of the plant species. This includes the mating types, gene flow, and mode of reproduction, pollinators like winds, birds, insects, water and natural selection (Hamrik and Godt, 1989). For these reasons, it could be speculated from the result that yeheb might have mixed mating system (pollinated by insects and self-pollinating) for which some extent of gene flow is expected that could result in high within genetic diversity.

In contrary to the above, it could also be explained that the high genetic diversity observed within populations in our investigation might be due to preferential adaptive gene complexes adapted to environmental change. It might have evolved during long evolutionary period in the region. This might be due to the location of the sample collection from southeastern Ethiopia where it is believed to be one of the primary diversification centre and origin for yeheb. The fact that yeheb genetic resources have decreased recently due to over-exploitation for different purposes and shrinking of their natural habitat indicates that the threats to the survival of the species mainly come from human activity.

The proportion of genetic variation depends on the type of pollination that the species undergoes. If the species has large proportion of cross pollination, then high genetic variation within population and less divergence among populations is expected. In addition to pollination, behavior of insects and market exchange could facilitate gene flow among regions which could result in higher percent variation within population and

less genetic structure (Quirós and Cárdenas, 1998). Results of UPGMA, NJ and PCO also support this.

Partitioning of genetic diversity by AMOVA without grouping populations showed that out of the total genetic diversity, 71.03% were within population while among population was 28.97%. This could be due to high seed exchange among local community and insect pollination. Jiang *et al*. (2012) who studied the genetic diversity of *Chimonanthus grammatus* populations using ISSR marker showed that there was 73.6% within population variation whereas the rest (26.4%) was due to among population variation. When compared to the present study, there was the same gene flow. Jiang *et al*. (2012) recommended that gene flow, genetic drift and evolutionary history might have important influence on genetic structure and diversity of a given population.

Therefore, the same conclusion was drawn in the present study where high within population variation might be due to preferential adaptive gene complexes adapted to environmental changes being evolved during long evolutionary period in the regions. This implies the need for conservation of individuals in all populations. In addition, it would be wise to conserve populations in different regions in order to limit population decline caused by large scale environmental catastrophes.

On the basis of UPGMA, most individuals of the respective populations were observed to form moderate grouping with few intermixing with other populations. The intermixing occurred among individuals having geographic proximity which could facilitate seed and seedling flow and population migration. For instance, the intermixing of Dabhabalan, Godir Woyis, Mirafadle with Maned and Gambare could be because of their geographical proximity and interaction among people. In the case of NJ, each individual of the respective population was observed to form small clusters by intermixing with individuals of other populations. This lack of clustering may indicate the vulnerability or exposure of the plants to human intensive management, exchange of seeds via marketing and limited gene flow due to long distance.

Generally, UPGMA showed most individuals of the respective population were observed to form moderate clustering with few intermixing from the other populations. Genetic distance is a measure of the allelic substitutions per locus that have occurred during the separate evolution of two populations or species. Smaller genetic distances indicate a close genetic relationship whereas large genetic distances indicate a more distant genetic

relationship. Crosses between distantly related individuals are expected to give better offspring than those between closely related genotypes. Therefore, prior knowledge of the genetic distance between genotypes or population is important in designing breeding program.

The first three eigen-values of PCO, 3.88, 3.31 and 2.20 of two dimensional PCO with percentage of 17.00%, 14.52% and 9.69%, respectively were used to show groupings of individuals. In two dimensional PCO, the individuals of some populations tend to group together except intermixing of few from Afardod, Maned and Dabhabalan populations. This implies the relative degree of similarity in terms of floristic composition or other complex environmental variables; and indicates the history of these plots might be similar while the individuals from Mirafadle, Godir Woyis and Gambare scattered all over the plot, which could show the levels of variation among these individuals. The results suggest that some individuals did not show distinct cluster based on their geographic location. Moreover, relatively individuals from Afardod are separated from other populations. Similarly, in the three dimensional coordinates almost all individuals of each population were spread all over the plot except Godir Woyis individuals which are intermixed with each other and formed separate group from the rest.

CONCLUSION

The genetic diversity analyses showed the existence of high genetic diversity in yeheb. Gambare population showed the highest genetic diversity and Mirafadle population showed the least genetic diversity. Therefore, Gambare population is the primary target site for yeheb conservation and urgent conservation strategy is required for future use and breeding program. Considering very limited distribution of the species, it is worth giving due attention to conservation of all the populations before genetic erosion of this valuable species. AMOVA analysis showed higher genetic variation within populations than among populations and PCO also showed grouping of individuals of some populations based on geographical locations but with some intermixing, indicating the existence of high seed exchange among local community and insect pollination.

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