International Journal of Bio-resource and Stress Management 2016, 7(1):080-087 DOI: HTTPS://DOI.ORG/10.23910/IJBSM/2016.7.1.1528

Full Research Article

Assessment of Genetic Diversity in Two Endemic Aloe Germplasm Populations from Ethiopia Using Morphological Markers

Tewodros Kahsay[1] and Hewan Demissie Degu[2*]

[1]Dept. of Biology, Mizan Tepi University, Bench Maji, SNNPR, Ethiopia
[2]School of Plant Science and Horticulture, College of Agriculture, Hawassa University, Hawassa, SNNPR (1417), Ethiopia

Article History

Manuscript No. AR1528
Received in 19th January, 2016
Received in revised form 28th January, 2016
Accepted in final form 5th February, 2016

Correspondence to

E-mail: hewan.dd@gmail.com

Keywords

Aloe adigratana, *Aaloe percrassa*, cluster analysis, genetic diversity, morphology

Abstract

Thirteen Aloe germplasm population; *A. adigratana* and *A. percrassa* available at the Ganta Afeshum District (GAD) found in Easter Zone of Tigray were subjected analysis in relation to morphological parameters for estimating the extent of diversity within and between species. The naturally occurring populations at Ganta afeshum District used as data source. Thirteen plots, each measuring 5×20 m^2 (100 m^2) $population^{-1}$, were laid down in the study sites. Three individuals per population from each species were randomly sampled to cover variation in growth habit, leaf length, leaf weight, stem length, stem diameter, growth and plant type, leaf color, leaf spot, teeth color, flower color and gel yield. All the genetic individuals and vegetative daughters were sorted, marked and recorded for selected population attributes. Data on the species populations were gathered by using focused group discussions, semi-structured interviews and field observation. The community identified two landraces for *Aloe percrassa* and four landraces for *Aloe adigratana* as indicated by their folk taxonomy. Morphological evaluation (of the 13 accessions for selected characters showed qualitative variation among the studied landraces. Maximum similarity of 100% was observed between Population 2 and Population 11 (*A. adigratana*), Population 12 and Population 5, 7, 8 and 9. While minimum similarity of 11% was observed between Population 3 and Population 6 *A. percrassa* and *A. adigratana*. Thus, Aloe landraces maintained at Ganta afeshum District showed high genetic diversity between and within the species. The present study would be useful in genetic improvement and authentication of species and genotype of this medicinally and economically important genus.

1. Introduction

Aloe vera is one of ancient plant used for many traditional purpose. It is a cactus like plant with fleshy, tapering, spiny dagger- shaped leaves which are filled with a clear viscous gel. It is believed that *Aloe vera* (L.) is the plant of old civilization since it was used as folk medicine which dates back thousands year. It belong to family of Liliaceae mainly adapting the tropical area of South Africa and South Arabia (Baker, 1880).

The origin of aloe species which includes 360 species is still undetermined. The genus has its main distribution in Africa south of the Sahara, including Madagascar, the Mascarene. Few species reach the Arabian Peninsula and Socotra. The plant is highly valued for its gel (O'Donovan et al., 2015; Padmaja et al., 2015; Ramachandra and Rao, 2008; Reynolds and Dweck, 1999; Richardson et al., 2005). Since the key constituent of the gel are Anthraquinones (Wang et al., 2016) (aloin, *Aloe*-emodin), Resins (Sun et al., 2016), Tannins (Taukoorah and Mahomoodally, 2016), Polysaccharides (Taukoorah and Mahomoodally, 2016; Yagi, 2015). The gel is used for many purposes in industrialized countries. Some of the benefits of the gel are drugs (for TB, AIDs (Virani et al., 2016; Yagi, 2015)), Cosmetics (lotion, antiaging, Shampoo (Pilz et al., 2016)), Soft drinks and food preparations (as the gel contains eight of the essential amino acids and Vitamins, for animal health care etc., some species of aloe, especially *Aloe trichosantha* (Oumer et al., 2014), *A. pubscens* (Serrano et al., 2006; Shree et al., 2015), *A. citrine* (Esmaeili and Ebrahimzadeh, 2015), *A. bertemariae, A. eumassawana* and *A. schoelleri* are used in the health care and drug industry.

Sub-Saharan Africa, including the Island of Madagascar accounts for over 90% of the 450 or so taxa (*species,subspecies* or *varieties*) of the genus Aloe known today, with concentration in Southern Africa (over 260 taxa), in Eastern Africa (over 180

taxa) and on the Island of Madagasar (Ca 77 taxa) (Hargreaves et al., 2008). A few species have been recorded in the Arabian Peninsula and on small Islands off the Coast of Africa. Kenya is a home about 60 species (Wabuyele et al., 2006), five of which have been identified as being commercially exploited for aloe bitter gum trade and in South Africa, *Aloe ferox* is highly valued and used in a similar way as *Aloe vera.*

In recent years Aloe has become common in most tropical and subtropical regions (Rowley, 2015). United States of America together with several other countries in North and South America is the main growing and production center for aloe (Starr, 2014). Hundreds of known cultivars has been isolated in the last few hundred years in several aloe growing countries, mainly in USA, and in the Pacific islands. These cultivars are adapted western environment (Menale et al., 2013) by breeding to a thick gel content for the growing cosmetics industry. However, there is still some demand for cultivar improvement, and several breeding programs are active in Australia, South Africa, Brazil and Israel. Germplasm collections are important for genotypic and phenotypic analyses, and as a genetic resource in breeding programs. Knowledge of the diversity and the genetic structure of these collections is fundamental for association studies and controlled breeding.

Despite Ethiopia is endowed for o 80% of the 40 aloe species endemism and long history for the aloe species studies in the country (Demissew, 1996; Demissew et al., 2011; Gilbert and Demissew, 1997), in these past studies have been considered in a broad context. Furthermore, thorough survey on plants with qualitative and quantitative characters has not been done. In the present communication, an attempt has been made to collect information on the geographical distribution and similarity of different accession of the two endemic aloe species available in Ganta-Afeshum District, Tigray Floristic Region of Ethiopia. The objectives of the present study were to: (i) describe the folk taxonomy of the accessions by the local peoples on Aloe; (ii) assess test a significance variation of Aloe accession from the two species based on qualitative and quantitative characters in Ganta-Afeshum Districts of Tigray Regional state.

2. Materials and Methods

Ganta Afeshum District (GAD) is found in Easter Zone and is one of the 36 District in the Tigray National regional state. The GPS record for the District is coordinating between 1558527 m-1588942 mN and 523630 m-559875 m E and the altitudinal gradient of the District ranges from 1853 m- 3314 m.a.s.l. The District is divided into 20 rural Sub-districts. It is bordered on the north by Gulomekada, on the south by Hawzen, on the east by Saesi Tsaedaemba and on the west by the Central Zone. The administrative center of the District is Adigrat, which is situated 900 km far north

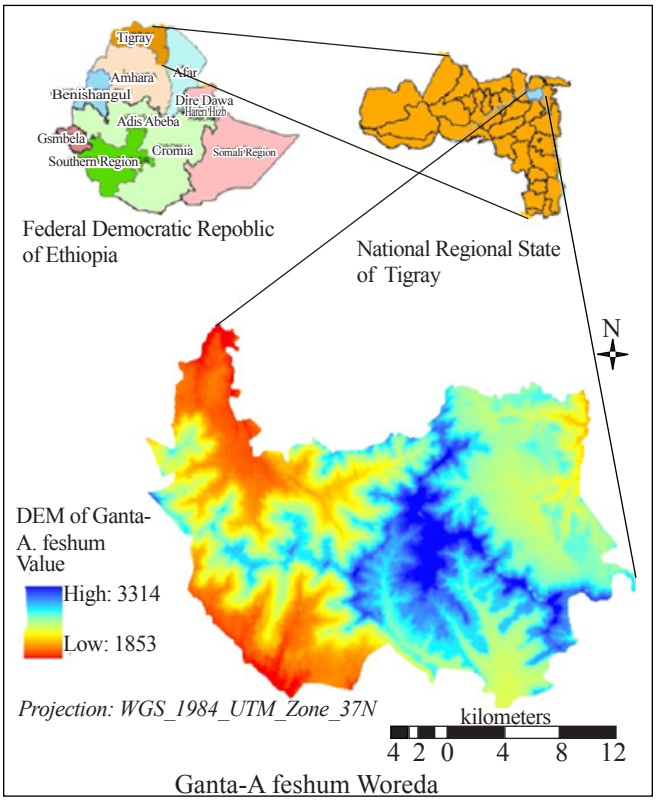

Figure 1: Map showing the study area-Ganta-Afeshum District-(GAD) (Developed using ArcGIS version 9.2)

of Addis Ababa. Based on the 2007 national population and house census conducted by the CSA, the District has a total population of 88,644 of which 85% live in rural areas. GAD has a population density of 54.17.

2.1. Climate and soil type

In accordance with altitudinal gradient, GAD has comprised three agro-climatic zones: '*woinadega*' (56%), '*dega'* (20%) and '*kola*' (24%). The rainfall is uni-modal type and occurs from June to September. It has erratic type of rainfall with high variation between and within the year. The average annual rainfall is approximately 350-700 mm per year and the mean maximum temperature is 20 °C and the minimum is 4 °C .The total land area of the District is 53,035.3 ha, out of which 12,444.3 ha is under crop production. The soil texture of the District is Sandy loam (70%) and Sandy (30%).

2.2. Population distributions

This study was conducted between December 2011 and June 2012 focusing on two endemic species of Aloe GAD, Tigray regional state. The selection of the study area was based on the information obtained by reconnaissance (2° data) about the plant distribution in the District. Of the available *Aloe* germplasm accessions at GAD, 13 population (eight for *A. adigratana* and five for *A. percrassa*) representing different

geographical regions of the study area were used for the study. Data recorded from field observations aided by GPS were consulted to trace the distribution map of the species to construct map for the analyses of population distribution using ArcGIS ver. 9.2.

2.3. Morphological diversities and folk taxonomy.

In each locality a plot of 5×20 m², altogether 13 plots (eight for *A. adigratana* and five for *A. percrassa*) were established in each population sites. The plots were circumscribed using plastic rope and four wooden pegs fixed at each corner of the plots during data recording. Each individual clone consisting of one genet and one to several ramets was sorted and marked. Marking was made on the leaves with double numbers by a water proof marker starting bottom left corner of the plots. In each plot, every genet and number of ramets per genet were counted and recorded. For every ramet in the plots, rosette diameter (RD) was measured and recorded.

Fresh specimens of the two studied *Aloe* species were collected from their natural habitats. Three individuals per population from each species were randomly sampled to cover variation in habit, leaf, stem structure and flower characters.

Morphological traits considered in this study include both qualitative and quantitative characters in vegetative (for both studied species of *A. adigratana* and *A. percrassa*) and reproductive organs (only for *A. adigratana* populations).

In the analyses and presentation of population status data, descriptive and inferential statistics using R statistical package (version 3.2.2) is used.

3. Results and Discussion

3.1. Folk taxonomy of Aloe plants by local people in GAD

Local people in GAD describe and identify different varieties of *Aloe* plants by using ecology, morphology, and some utility; however, some names have no apparent meaning attached to them or have lost meaning over the years (Table 1). Some characters used by the local people to organize the locally available *Aloe* plants include: the presence and absence of stem, leaf size, color and shape, type and color of spines, distribution of the species, flowering period and the sensitivity to leaf necrosis. Local landraces of *A. adigratana* (Reynolds) are identified by the length of stem, leaf color, leaf thickness, the number of buds, the presence and absence of spines etc. The local name for this species is '*Ere habesha'* (Tig.). Based on the characters, there are four local varieties of '*ere habesha'* identified in the study area. These are '*Tselim ere*','*Ere senay*','*Zafizafo ere*' and'*Lemats ere*' (Table 1, Figure 2 C, D, E and F, respectively).

Among the four varieties, '*Tselim ere*'is the most widely distributed type of variety in the study area (Figure 2 F). This variety is believed to grow in high altitudinal range in GAD (Figure 3). While the two varieties named as '*Zafizafo ere*' (Figure 2 E) and'*Lemats ere*' (Figure 2 C) are the least distributed varieties in the study area.

Similarly the different local landraces of *A. percrassa* (Todaro) were identified based on leaf shape, color, size, leaf fragility and thickness. The local name for this species is 'Tsaeda*ere'*. Furthermore there are two accessions identified by local people. These are *'Ere gimel*' and '*Ere ashenda*'. '*Ere gimel*' is endemic to the study area (Figure 2 B). The local people easily recognized those landraces due to the ethno-cosmetic purpose, ethno-ecology use and habit of growing. '*Ere ashenda*' is found only in few sub-districts (Figure 2 A). The name is derived since local people use it for decorating ladies during the local festival called '*Ashenda'*. Compared to 'Ere *ashenda*''*Ere gimel'is widely* distributed.

Local people in GAD also have their own systems of naming and categorizing for their natural surrounding plants. They have recognized the matured local plants by their habit architecture. However, it does not mean that the local people in GAD are familiar with the seedlings of various species since seedlings of many plant species take on a different phenotypes as they pass through various stages of development. Descriptive naming in local culture is common and influences the local knowledge transmission from generation to generation (Bennett, 2007; Van Andel et al., 2014). Furthermore the detail descriptive naming of the two endemic taxa with their

Table 1: Local accession of the two species and their local name and meaning in GAD

Scientific name	Local name	Local Variety	Meaning
A. adigratana	*'Ere habesha'* (Tigrigna.)	*'Tselim ere'*	To mean deep green color of leaf
		'Ere senay'	Very fragile leaves to be cut/ peeled by hand easily
		'Zafizafo ere'	Multi-leaves like dread-locked hair hang down
		'Lemats ere'	The first name refers to the less distribution of marginal teeth of leaf
A. percrassa	*'Tsaeda ere'* (Tigrigna)	*'Ere gimel'*	Eaten by camel, but no camel is available in the study area (GAD)
		'Ere ashenda'	The leaves are used to decorate ladies during the 'Ashenda' Festival (in every August, 26)

Figure 2: Typical morphotypes shown by two species of Aloe in the present study. (A): *A. percrassa*: Tsaeda ere (Tigrigna): Ere ashenda-; (B): *A. percrassa*: Tsaeda ere' (Tigrigna Ere gimel-; (C): *A. adigratana*: Ere habesha: Tselim ere; (D): *A. adigratan*a : Ere habesha : Ere senay; (E): *A. adigratana*: Ere habesha: Zafizafo ere; (F): *A. adigratana* : Ere habesha: Lemats ere

own native language indicated the long awaited wisdom of the people and knowledge of their environment. This indicated the people have subsistence agricultural experience because of their exposure to the surrounding environment. Also the detail description also indicated their economic value of the plants for the people which might have been transferred through oral tradition (Leonti et al., 2003).

3.2. Geographic distribution of different accession of the two species

The distribution of the accessions in GAD corresponds with the altitudinal range of the region. In this study, we found that 61% of the accession were from the altitude of 2079 to 2789 (Figure 3). The accessions in this population are from the areas with the average annual temperature and with small average rainfall distribution (Grace et al., 2015). This population is genetically most diverse of the three inferred populations, and it probably encompasses a broad range of adaptations to past and present climates. The species occurs in two strata: in the first stratum, it grows in large populations intermingled with Acacia woodland, in hedges and field marginal slopes with altitudinal distribution of 2063- 2527 m.a.s.l. and in the second stratum, the species grows on rocky hills elevated at 2580- 2725 m.a.s.l.

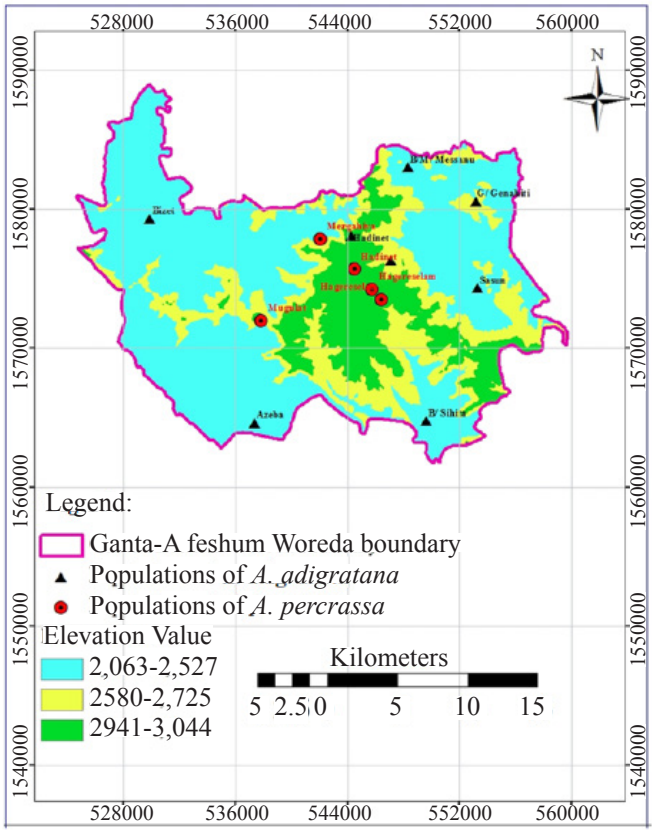

Figure 3: Maps showing the distribution of Aloe Accession of *A. percrassa* and *A. adigratana* in GAD- Tigray regional state

in small populations having scattered individuals associated with open Acaciaspecies, *Shines molasis*, Olea species etc., It was also observed that *A. adigratana* (Reynolds) populations found growing near rivers, plain lands and mountain hills (e.g. Mitsnah Mountains, Azeba Lowlands Dibla-Si'at slops and Ziban Adi uplands Qanda'ero Hills). Furthermore individuals of *A. adigratana* are transplanted by local communities to their farmlands, rangelands, home compound and home garden indicated domestication of the species for different purposes. In general the two endemic taxa fall more or less into two main geographical areas in the District. The first pattern is occupied by *A. adigratana* distributed in Eastern part of GAD indicating more population are distributed in the high altitude areas. Whereas the second pattern was occupied by the populations' of *A. percrassa* which are confined to the Middle-east part of the District. Moreover, distribution of the two endemic *Aloe* species became diminishing to the West indicating the population of the two endemic species are more confined to the high altitude. This can be explained by the interaction of different edaphic and environmental interaction read directed the distribution of the two endemic population in the high and mid altitude (Salman, 2015).

3.3. Morphometric analysis

Significant variations were observed for various characteristics studied among *Aloe* accessions. *A. adigratana* was found to be the tallest (85 cm) as it possesses a distinct stem (caulescent) with long internodes. The leaf color is brown-green with spot. The flower color was red in this species compared to that of deep green colored, without spotted leaf and orange colored flower of *A. percrassa* (Table 2 and 3). Minimum leaf length (42.7 cm) and width (12.0 cm) were recorded in this species among all *Aloe* accessions and therefore, it contained a lesser amount of gel. Among all accessions, *A. percrassa* was considered as a deep green variety because of its deep-green appearance in leaf (Table 3). It is also acaulescent. Leaf width and length were slightly deviated from other *A. adigratana* accessions (Table 2). Among *A. adigratana* accessions, conspicuous variations were observed in leaf length, leaf width, stem length and stem diameter., leaf size, thickness and width while very little variations were observed in leaf color (Table 3). In terms of Leaf length, accession P8 showed the leaf length (656.7 cm) than other accessions. The presence of significant difference among the population of the two species might be attributed to select relevant character based on their adaptation in the District. Nayanakantha et al. (2010) identified significant variation on leaf length and thickness. This confirms that the different ability of utilization of resources from different altitudinal range. It also can be confirmed for the different geographical origin of the aloe species.

Significant inter and intra specific variations could be visualized as evident from the similarity coefficients (Table 4) developed

Table 2: Morphological (quantitative) description of Aloe accessions used in the present study

Accession	Species	SL	SD	LL	LW
P_1	*A. percrassa*	42.3	15.0	46.7	13.3
P_2	*A. percrassa*	45.0	13.5	47.7	13.5
P_3	*A. percrassa*	4.7	9.3	46.2	13.2
P_4	*A. percrassa*	5.0	9.8	42.7	12.0
P_5	*A. percrassa*	10.7	12.3	44.0	12.3
P_6	*A. adigratana*	70.8	5.8	636.7	151.7
P_7	*A. adigratana*	29.8	4.7	506.7	125.0
P_8	*A. adigratana*	85.5	8.6	656.7	171.7
P_9	*A. adigratana*	37.5	5.2	536.7	121.7
P_{10}	*A. adigratana*	44.8	6.0	538.3	131.7
P_{11}	*A. adigratana*	66.8	7.6	611.7	153.3
P_{12}	*A. adigratana*	27.2	4.3	428.3	128.3
P_{13}	*A. adigratana*	49.3	6.6	533.3	136.7

SL: Stem length; SD: Stem diameter; LL: Leaf length; LW: Leaf weight

Table 3: Morphological description (Qualitative) of *Aloe* accessions used in the present study (Number given for leaf color is according to New Ornamentals Society (NOS) color chart)

Accession	Species	Habit	Plant type	Growth type	Leaf color	Leaf spot	Teeth color	Flower color
P_1	*A. percrassa*	Herb	Acaulescent	Clustered	002000	Present	Red	Orange
P_2	*A. percrassa*	Herb	Acaulescent	Clustered	008000	Present	Red	Orange
P_3	*A. percrassa*	Herb	Acaulescent	Clustered	008000	Absent	Red	Orange
P_4	*A. percrassa*	Herb	Acaulescent	Solitary	008000	Absent	Brwon	Orange
P_5	*A. percrassa*	Herb	Acaulescent	Clustered	008000	Present	Brwon	Orange
P_6	*A. adigratana*	Herb	Caulescent	Solitary	004000	Absent	Brwon	Red
P_7	*A. adigratana*	Herb	Caulescent	Clustered	004000	Absent	Brwon	Red
P_8	*A. adigratana*	Herb	Caulescent	Clustered	004020	Present	Brwon	Red
P_9	*A. adigratana*	shrub	Caulescent	Clustered	004000	Present	White	Orange
P_{10}	*A. adigratana*	shrub	Acaulescent	Clustered	004020	Present	White	Orange
P_{11}	*A. adigratana*	Herb	Caulescent	Clustered	004020	Present	Brown	Red
P_{12}	*A. adigratana*	Herb	Acaulescent	Clustered	004020	Present	Brown	Red
P_{13}	*A. adigratana*	shrub	Acaulescent	Solitary	004020	Present	White	Orange

on the basis of quantitative and qualitative data among all possible pairs. Maximum similarity of 100% was observed between Accession11/2 and Accession12/5, 7, 8, 9 while lowest similarity of 11% was observed between accessions 6 and 3 (*A. adigranta*/ and *A. perrassa*). The similarity values ranging from 100 to 11% indicate that there is a remarkable genetic variation among *Aloe* accessions used in the present study. The highest similarity and dissimilarity recorded in the present study indicates that the accession all possess a genetic diversity to a great extent.

Cluster analysis generated from this matrix classified these accessions in to three major clusters. *A. adigratana* branched out from the main cluster containing other species (*A. perrassa*) with distant similarity value of 11% and 33%, respectively. *Aloe vera* accession obtained from *Mt. Aloqa* and *Tsaida Adi* formed a major cluster with other *A. vera* accession form *A. adigranta* obtained from Azeba, B/sihita, Helli Hills and M/Kerseber with intra-specific diversity ranging from 80% to 33%.

Furthermore Cluster analysis clearly branched out two accession of *A.adigranta* from rest of aloe accessions suggesting that these two are more divergent from other accessions. Hence, phenotypic characters analysis provided a better relationship to identify these species. *Aloe percrassa* formed a separate cluster with accession of *A.adigranta* but with a distanced similarity of 11%. This cluster combined with the other major clusters containing *A. adigranta (*accession 13 and 6) with a distanced similarity of 21%. Thus, it is evident that *A. percrassa* suggesting that they all might have originated from common ancestor but grown indifferent geographical localities as evidences in the altitudinal range of the five population.

The analysis of diversity among species is the prerequisite for breeding and conservation purpose. However the tool used for

Table 4: Genetic similarity matrix for Jaccard's Coefficient. Accession numbers 1-5 (*A. percrassa*) and accession numbers 6-13 (*A. adigratana*)

	P_1	P_2	P_3	P_4	P_5	P_6	P_7	P_8	P_9	P_{10}	P_{11}	P_{12}
P_2	0.50											
P_3	0.33	0.67										
P_4	0.75	0.60	0.70									
P_5	0.89	0.83	0.70	0.40								
P_6	0.25	0.63	0.11	0.67	0.80							
P_7	0.86	0.75	0.78	0.80	0.80	0.75						
P_8	0.89	0.83	0.70	0.40	0.40	0.67	0.80					
P_9	0.80	0.71	0.50	0.57	0.33	0.60	0.67	0.33				
P_{10}	0.60	0.78	0.30	0.67	0.50	0.40	0.75	0.50	0.25			
P_{11}	0.75	1.00	0.70	0.67	0.67	0.67	0.80	0.67	0.75	0.50		
P_{12}	0.50	0.80	0.67	0.83	1.00	0.63	1.00	1.00	1.00	0.8	0.6	
P_{13}	0.57	0.60	0.56	0.86	0.86	0.67	0.80	1.00	0.75	0.7	0.9	0.6

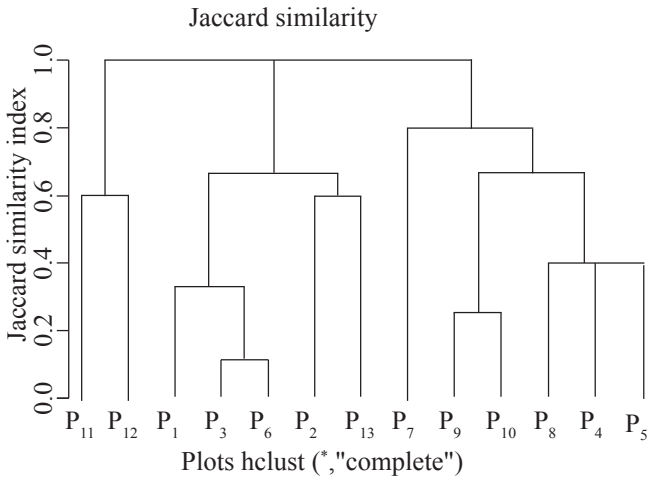

Figure 4: Cluster analysis-based dendrogram depicting genetic relationships among 13 accessions of Aloe. Accession numbers 1-5: *A. percrassa*, 6-13: *A. adigratana*

diversity analysis is depending on the available resource. Thus this research clarified the diversity of the thirteen accession of the two endemic species of important horticultural crops. The research clarified that there is environmental influence which cause the variability of the population. This shows the horticultural traits are easy, fast and important tools for diversity study in the region where little molecular tools are provided.

4. Conclusion

Based on the findings from morphological character and geographic distribution of two aloe species, it is possible to state that the accession of *A. adigranta* and *A. percrassa* population are diverse and grouped into three clusters. The species has current and potential benefits to the locality community and their immediate environment. It is suggested that this useful and endemic species studied using molecular marker for assessing the diversity and future use.

5. Acknowledgement

We are grateful to everyone who kindly shared their knowledge and time. We hope to have contributed to saving and spreading their valuable knowledge. We also thank the Ethiopian Ministry of Education for the grants made to Tewodros Kahsay during the study.

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