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Full Length Research Paper

Evaluation of morphological diversity of tamarind (*Tamarindus indica*) accessions from Eastern parts of Kenya

Mercy Liharaka Kidaha[1*], Wariara Kariuki[3], Fredah K. Rimberia[1] and Remmy Kasili Wekesa[2]

[1]Horticulture Department in Jomo Kenyatta University of Agriculture and Technology, Kenya.
[2]Institute for Biotechnology Research in Jomo Kenyatta University of Science and Technology, Kenya.
[3]School of theology, Scott Christian University, Kenya.

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Tamarind is native to tropical parts of Africa and Asia. It shows considerable phenotypic variation in morphological and horticultural traits that can be utilized in its genetic improvement. In Kenya, there exists a wide range of tamarind germplasm that has not been characterized. Initial characterization is based on morphological descriptors. The objective of this study is to evaluate morphological diversity of tamarind germplasm from Eastern parts of Kenya. Tamarind germplasms were collected from Kitui, Mwingi, Masinga, Embu and Kibwezi and then characterized using morphological descriptors based on seed, fruit and stem. Morphological characters were recorded and data from eighty-nine accessions were submitted to principal component and hierarchic ascendant analysis (HAC) and Euclidian average distance. Accessions from Kibwezi, Embu and Kitui showed the greatest diversity while accessions from Masinga and Mwingi had the least diversity. Trunk diameter at ground, pod weight, number of seeds/pod, height to the first branch and pod width showed greatest variation in principal component analysis. High morphological diversity obtained in these regions can be used to initiate new breeding and conservation programmes in tamarind for improved fruit and tree crop.

Key words: Tamarind, morphology, diversity, accessions, principle component.

INTRODUCTION

Tamarind (*Tamarindus indica* L) belongs to the family *Leguminoseae* (Khanzada et al., 2008). It is an evergreen tree that is native to tropical and subtropical regions of Africa and South Asia (Doughari, 2006). It is primarily used for it fruits that are either processed into juices, eaten directly, used in preparation of recipe and preservatives (Gullipalli and Kasiviswanatham, 2013). The leaves, bark, and pulp have extensively been used in ethnobotany (Gupta et al., 2014). The tree is widely used as an ornamental tree due to its availably and wide use (Doughari, 2006). It has significant importance in the cosmetics, paints and varnishes industries (Santos et al., 2012).

Morphological descriptors have been used as basic character in identification of plants, in breeding, commercialization, conservation of plant resources,

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*Corresponding author. Email: mlihaaraka@gmail.com. Tel: +254731519093.

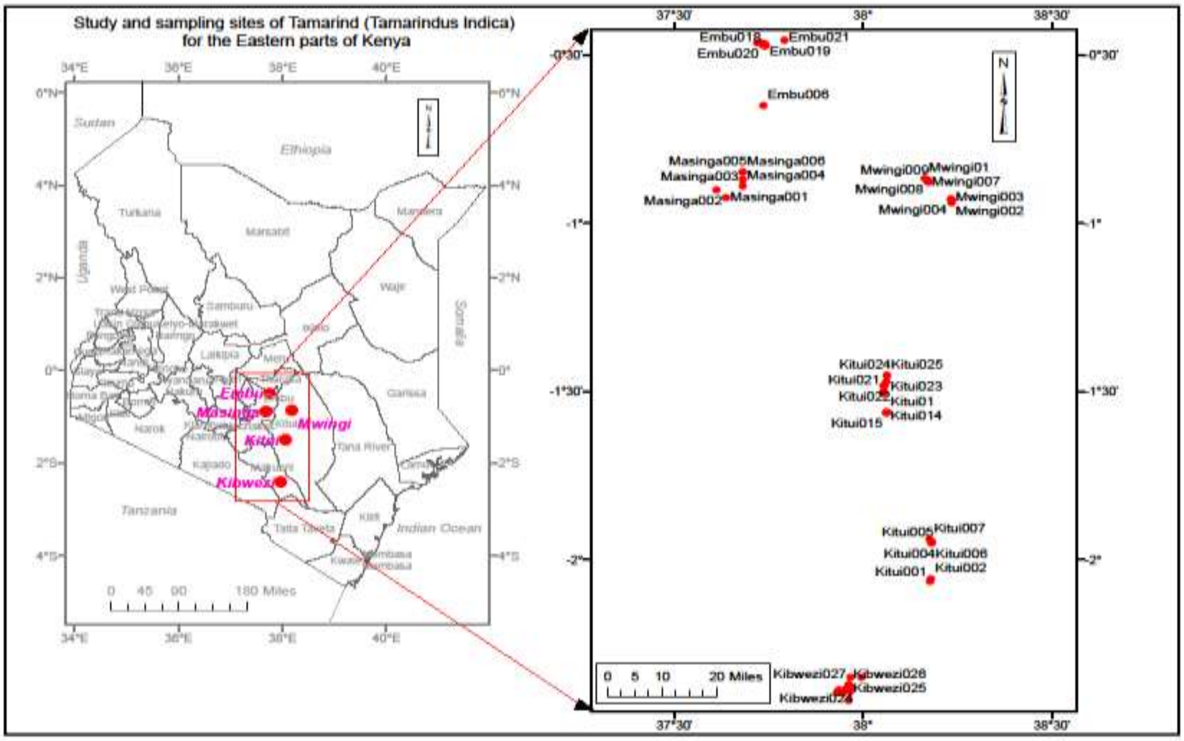

Figure 1. study and sampling sites from Eastern parts of Kenya.

cluster analysis understanding genetic similarities and dissimilarities (Santos et al., 2012; Cervantes and Diego, 2010). Components of fruits such as fruit size, shape, color and general appearance are important in plant description (Nasution and Chinawat, 2017). Morphological descriptors have limitations in distinguishing sub families and tribes as the traits are similar (Swenson and Anderberg, 2005).

T. indica trees are morphologically different in terms of fruit, crown, foliage, trunk, seed and flower characteristics (Nadine et al., 2011). In Thailand Nasution and Chinawat (2017) clustered sweet tamarind based on fruit characters. In a study Algabal et al. (2010) reported differences in pulp color used to distinguish the cultivars. Gunasena et al. (2007) reported that reproductive and fruit traits differ among different population of tamarind and they are influenced either by the environment or genetic make-up.

Morphological traits have been used to study Asian tamarind populations and the results revealed that there existed both morphological and genetic differences (Fandohan et al., 2010). These descriptors enabled them to choose superior cultivars for the market in terms of taste, pulp and thickness (El-Siddig et al., 2006). In West Africa most studies focused on biochemical compounds of tamarind (Adeola and Aworh, 2012). In Eastern parts of Kenya no studies have been carried out to compare morphological differences among tamarind populations in different places. The objective of this study is to evaluate morphological differences among tamarind accessions from the Eastern parts of Kenya. The results from morphological evaluation and clustering of tamarind will be useful in cultivar selection and improvement of breeding programmes for tamarind

MATERIALS AND METHODS

Collection of tamarind germplasm

Sampling was done in December 2015 to August 2016. Study sites included: Kitui, Mwingi, Masinga, Embu and Kibwezi (Figure 1). Tamarind farms were identified using key informants and random sampling was done randomly in the farms.

Morphological characterization

Characterization was done according to International Union of Plant Protection of New Vegetal Variants (UPOV 1987), International Committee of Genetic Resources of Plant for the description of tropical plants (IPGRI, 1991; Fandohan et al., 2010). Twenty descriptors were used for characterization (Table 1).

Data collection

Data collected from tamarind germplasm surveys included trunk diameter at ground, trunk diameter at the neck, height to the first branch. Pod length was determined as an average of five pods from

Table 1. Morphological descriptors used in characterization of Tamarind from Eastern parts of Kenya.

Plant part	Quantitative	Qualitative
Stem	Terminal shoot length (cm)	Growth habit
	Trunk diameter at ground (cm)	
	Trunk diameter at neck(cm)	
	Height to first branch (cm)	
	Number of primary branches	
	Number of secondary branches	
Seed	Number/pod	Shape, color, brightness, roughness
	Weight (g)	
Fruit	Length(cm) width(cm) weight(g)	Shape and color
Pulp	Weight(g)	color

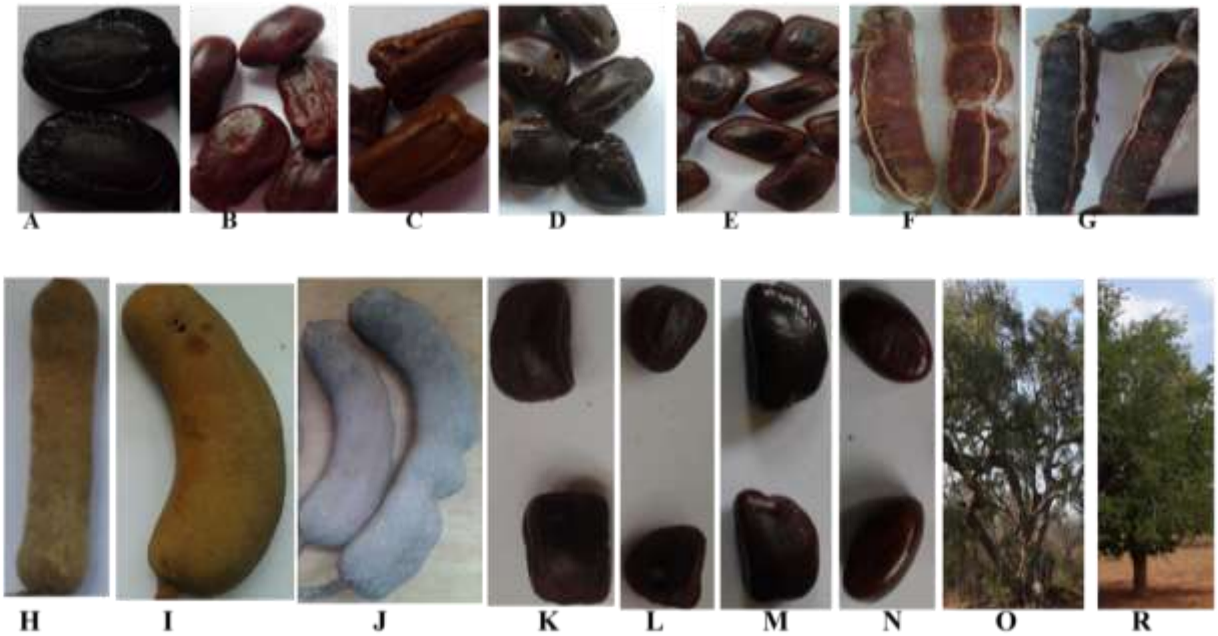

Plate 1. Qualitative morphology observed in in tamarind seed color: Black (A), brown (B), light brown (C), dark brown (D) and dark brown and brown (E); pulp color: brown (F) dark brown (G). Pod color: rusty brown (I), Velvety brown (J). Pod shape: straight (H), semi curved (I)and curved (J). Seed shape: quadrant (K), irregular (L), D shape (M), ovate(N). Growth habit: Plagiotropic (O), orthotropic (R)

pole to pole. Pod width was determined as mean of five pods from the equator of the cross section of the fruit and pod weight was determined as a mean of five pods of the same tree. Seed weight was determined as an average of seeds in an entire pod. Pulp weight was determined as the average of pulp in 5 pods. Seed number was determined as an average of seeds in 5 pods. Growth habit was either orthotropic or plagiotropic

Data analysis

Quantitative data were submitted to principal component analysis (PCA), using the R software statistical package. Cluster analysis was carried out on the principal components with SS loadings 0f 0.98 to 2.93 using the hierarchic ascendant analysis (HAC).

RESULTS

Morphological diversity of tamarind accessions from Eastern parts of Kenya

Tamarind from Eastern parts of Kenya showed a wide variation in tree characteristics including seed color, seed shape, seed number per pod, seed weight, pod shape, pod color, pod length, pod width, pod weight, pulp color, pulp weight, tree habit, terminal shoot length, trunk diameter at ground, trunk diameter at neck, height to the first branch, number of primary branches, number of secondary braches (Plate 1 and Table 2).

Table 2. The standard deviation calculated by measured morphological traits from Eastern parts of Kenya.

S/N	Variable	Maximum	Minimum	Mean	Std deviation
1	Terminal Shoot length (TSL)	2400	340	842.7	339.76
2	Trunk diameter at ground (TDG)	590	54	203.2	114.39
3	Trunk diameter at neck (TDN)	590	43	196.4	119.45
4	Height to first Branch (HB)	420	28	148.7	61.22
5	Number of primary branches	3	1	1.11	0.38
6	Number of secondary branches	12	1	3.39	1.94
7	Number of seeds/pod	12	1	6.87	1.76
8	Seed weight	1.16	0.27	0.65	0.19
9	Pulp weight	2.5	0.28	0.76	0.33
10	Pod length	20.83	3.3	11.49	2.78
11	Pod width	10.7	2.6	5.97	1.98
12	Pod weight	41.59	3	15.3	6.73
13	Seed shape	4	1	1.86	0.89
14	Seed color	5	1	1.87	1.1
15	Seed brightness	1	1	1	0
16	Seed roughness	1	1	1	0
17	Pod color	2	1	1.11	0.3
18	Pod shape	3	1	1.22	0.54
19	Growth habit	2	1	1.1	0.3
20	Pulp color	2	1	1.16	0.37

Principal component analysis

The first five components of principal components in quantitative analysis explained 76% of total variations (Table 3). Eleven traits contributed to PC1 with trunk diameter at the ground contributing more positively. In PC2 eleven traits contributed positively to the component with pod weight having a significant positive contribution to the PC. In PC3 eight traits contributed positively to the component with number of seeds per pod having a significant positive contribution. PC4 had six traits that contributed positively with height to the first branch having the highest positive contribution. In PC5, six traits had positive contribution with pod width having the greatest contribution.

Correlation among characters showed three clusters. In the first cluster, trunk diameter at the neck (TDN), trunk diameter at ground (TDG), number of secondary branches and terminal shoot length are highly correlated. In the second cluster, number of seeds per pod, pod length, pod weight (PPWT), seed weight and pulp weight were highly correlated. In the third cluster height to the first branch (HB) and number of primary branches were highly correlated (Figure 2).

Cluster analysis

HAC distinguished two major clusters when truncated at 1000.Cluster 1 consisted of 50 accessions while cluster 2 had 39 accessions. Each cluster had two sub-clusters. Most diversity was observed from accessions in Kibwezi, Embu and Kitui while least diversity was observed in Mwingi and Masinga. The accessions were distributed across the clusters (Figure 3).

DISCUSSION

Morphological descriptors have been used in initial identification of organism (Piyasundura et al., 2008). In this study high morphological diversity was found among accessions collected from Eastern parts of Kenya. This variation is similar to reports by Nyadoi et al. (2014) who reported there was a great diversity among the tamarind populations collected in Maasai region in Kenya. Fandohan et al. (2011) reported 3-8 number of primary branches and 30-60 number of secondary branches but this study revealed that the number of primary braches ranged from 1-2 and secondary branches from 2-12. In this study trunk diameter varied greatly with the trees from intercrop farm having shorter trees than those that grew widely. This is in agreement with reports by Nyadoi et al. (2014) who reported that the diameter greatly varied with the type of vegetation in the habitat. This is different as the study sites were farm lands, savanna and forests and this was more on forests and farm land. Growth habit of orthotropic and plagiotropic was similar to findings by Ali et al. (2010) He studied tamarind from Southern India indicating that the growth habit is not influenced by

Table 3. PCAs, SS loadings, proportion variation and cumulative variation of 12 quantitative variables performed using R software used to study morphological differences in tamarind accessions from Eastern parts of Kenya.

Variable	PC1	PC2	PC3	PC4	PC5
Terminal shoot length	0.79	0.04	0.23	-0.24	-0.19
Trunk diameter at ground	**0.88**	-0.01	0.08	-0.24	-0.11
Trunk diameter at neck	0.86	0.04	0.15	-0.23	-0.15
Height to first branch	0.31	0.11	0.14	**0.66**	-0.26
No of primary branches	0.53	0.27	-0.02	0.59	0.11
No. of secondary branches	0.31	0.03	0.37	0.39	0.59
No of seeds/pod	0.23	0.35	**0.77**	-0.18	-0.13
Sd. weight	0.02	0.64	-0.55	0.02	0.27
Pulp weight	0.22	0.72	-0.51	0.00	0.06
Pod length	-0.39	0.60	0.47	0.03	-0.18
Pod width	0.21	0.31	-0.10	-0.38	**0.60**
Pod weight	0.16	**0.83**	0.21	-0.09	0.12
SS Loadings	**2.93**	**2.29**	**1.66**	**1.30**	**0.98**
Variability %	**0.24**	**0.19**	**0.14**	**0.11**	**0.08**
Cumulative variability %	**0.24**	**0.44**	**0.57**	**0.68**	**0.76**

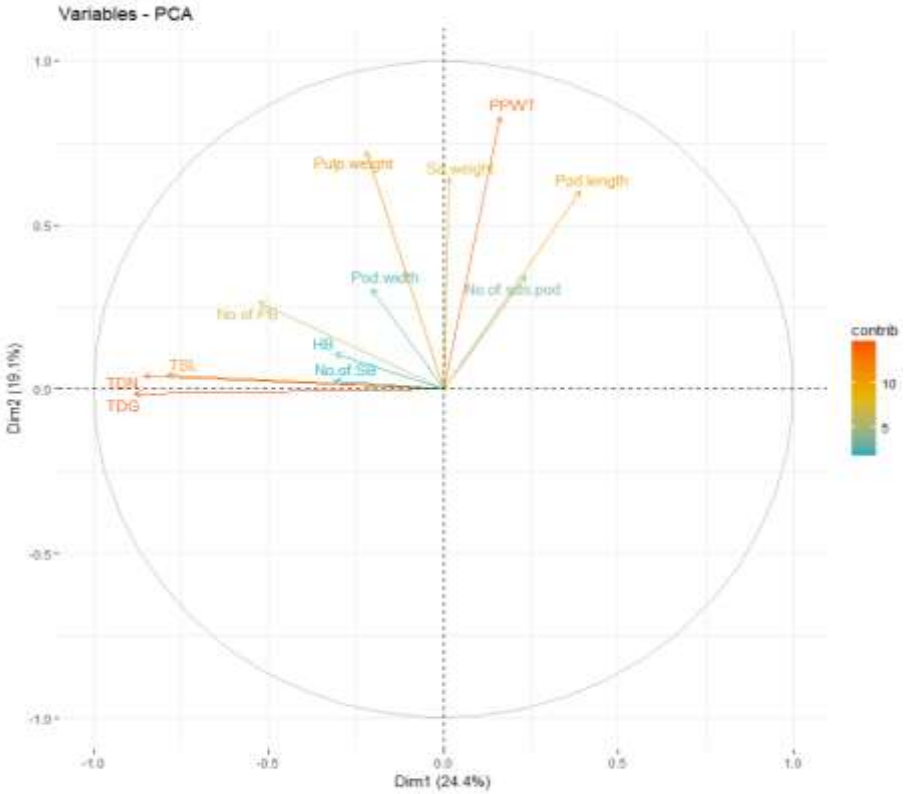

Figure 2. Correlation among characters associated with the first and second principal components. The closer the attributes are to each other in the PCA plot, the higher the correlation.

changes in environment and cultural practices.

Three pod shapes observed (curved, semi curved and straight) were similar to the findings by Algabal et al. (2011) but Fandohan et al. (2010) had only two pod

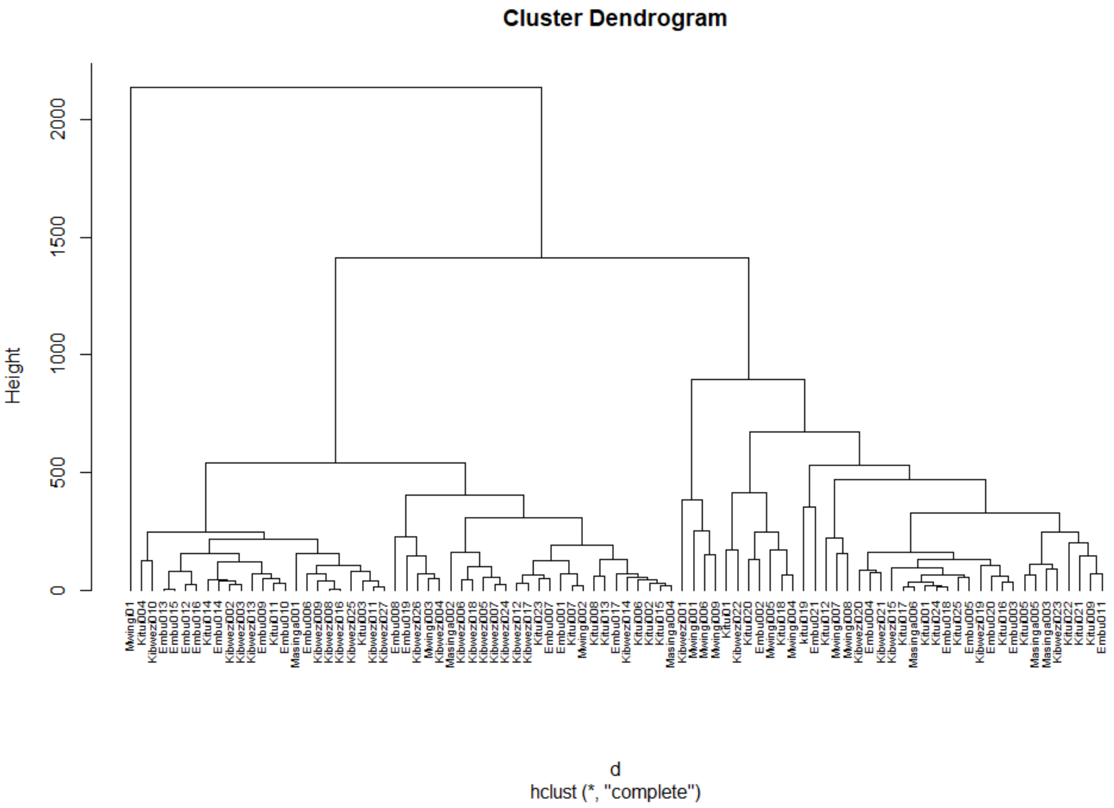

Figure 3. Dendrogram constructed based on morphological characters of 89 tamarind accessions from Eastern parts of Kenya and Euclidian average distance. C1 and C2, are the clusters (1 to 2) generated in the cluster analysis.

shapes (curved and the straight). The shapes are affected by the seed number and seed shapes which are influenced by its genetics. Pod color was either velvety brown or rusty brown that coincides with the findings of Ayala-Silva et al. (2016). Variations in pod color are highly influenced by the age of the pod and environmental changes. Pulp color varied from light brown to dark brown which slightly varies from the findings by Ayala- Silva et al. (2016) who reported colors of reddish brown and brown. The pulp color is highly influenced by genetic make-up of the plant. Highest diversity was observed in seed color; Fandohan et al. (2011) only recorded three seed colors of black, brown and dark brown but this study revealed other colors of dark brown at the center and brown outside, light brown. the colors reported in this study were also evident in the reports by Fawzi (2011.) This trait is inherited and affected by the environment, and in different environment different colors were observed. Fandohan et al. (2011) also recorded seed shape of quadrant, bowl shape and irregular while Fawzi (2011) reported more of oblong, asymmetrical, ovate and rhomboid but from the studies seed shapes of ovate and D shape were observed. The shape is inherited and also affected by the environment

In this study, the pod weight was 3-31.4 g, while Prerak et al. (2013) reported pod weight of 5.49- 24.55 g. This is directly correlated with pulp weight and seed number. Pulp weight ranged from 0.28-1.92 g while Van den et al. (2014) reported pulp weight of 1.96-4.65 g. Pulp weight is a factor of management practices given to the tree. Van den et al. (2014) also reported that the number of seeds per pod ranged from 5-7 while this study depicted seed range of 1-12 per pod. This is highly influenced by nutrition available for the plant and the management practices that also influence directly the length of the pod

Diversity was not observed in fibre color, seed roughness, seed brilliance and pulp taste. Fibre color observed was yellow brown, all seeds were rough, were non brilliant and pulp was sour. These factors were not altered by different environments. Fandohan et al. (2011) reported both brilliant and non-brilliant seed and rough and polished seeds and this could be affected by different environmental factors. HAC clustering grouped the accessions into two major clusters and two sub clusters. The samples were from across the regions indicating that the diversity was not based on the origin. This is also confirmed by reports of Iddi Garba et al. (2015).

According to Chatfield and Collins (1980) components with eigenvalues less than one should be eliminated; so those with eigenvalues of one and above are used for they are considered to be more significant. The eigenvalues decreased in this from PC1 to PC5 showing a decrease in variation. The first five components of principal components in quantitative analysis explained 76% of total variations among the accessions (Table 3). PCA identified eleven traits namely; trunk diameter at the ground, trunk diameter at the neck, height to first branch, number of primary branches, number of secondary branches, pulp weight, pod weight, seed weight and terminal shoot length that contributed positively to PC1. However, trunk diameter at the ground contributed more positively than the rest of the traits.

PC2 identified eleven traits that contributed positively to the component with pod weight having a significant positive contribution to the PC. PC3 identified eight traits that contributed positively to the PC; number of seeds per pod had significant positive contribution to PC3. PC4 had eight traits that contributed positively, with height to the first branch having the highest contribution. In PC5, eight traits had positive contribution with pod width having the greatest contribution. This research reveals morphological diversity and factors of pod weight, pod width and pulp weight that significant and directly correlated to the fruit should be considered for conservation and future improvement

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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Full Length Research Paper

Ecological assessment of plant diversity and associated edaphic and topographic variables in the Gra-Kahsu forests of Ethiopia

Tesfay Atsbha[1]*, Anteneh Belayneh Desta[2]

[1]Tigray Agriculture Research Institute, Alamata Agriculture Research Center, P. O. Box, 56, Alamata, Ethiopia.
[2]School of Biological Sciences and Biotechnology, Collage of Natural and Computational Sciences, Haramaya University, Ethiopia

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The study was conducted to assess the influences of edaphic and topographic variables on plant diversity on the slopes of Gra-kahsu forest area. Collection of vegetation data was made using systematic sampling methods, laying 19 transects and 62 quadrats, each with 20 m × 20 m for trees and 5 m × 5 m for shrubs. In each quadrat, heights (≥1.5 m), diameters (≥2.5cm) and numbers of woody species, level of grazing intensity, level of human disturbance and topographic variables were recorded. Analysis of one way using R-software was used to analyze the mean of plant diversity across the edaphic and topographic variables. The highest species richness and diversity appeared in the upper altitude gradients and the variation was significant ($p<0.05$). The nil and slightly grazed sites had significantly higher woody plant species diversity, density and basal area compared to the heavy grazed sites ($p<0.001$). Grazing pressure and human disturbance had an effect on the density and number of woody species as well as on the vegetation structure. This research concluded that, changes in woody plant vegetation, density and regeneration status are caused by interactions edaphic and topographic variables and these interactions determine the ecological of plant diversity.

Key words: Altitudinal pattern, disturbance, plant species diversity, slope.

INTRODUCTION

Patterns of plant species diversity are influenced by latitudinal, altitudinal and soil gradients (Huston, 1994). Understanding vegetation and species diversity patterns is fundamental for conservation of natural areas; these patterns have frequently been the focus of ecological studies (Loreau et al., 2001; Fetene et al., 2006; Muhumuza and Byarugaba, 2009). Several different patterns of plant species diversity have been noted in response to altitudinal gradients, with plant species diversity being reported to: (i) decline with higher altitude; (ii) increase with higher altitude; (iii) bulge at mid-altitude; (iv) dip at mid-altitude; or (v) have no clear relationship with altitude (Chang et al., 2005). Vegetation patterns are determined by environmental factors that exhibit heterogeneity over space and time, such as climate and topography as well as human disturbances (Alexander

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*Corresponding author. Email: atsbhatesfay@yahoo.com or atsbhatesfay@gmail.com. Tel: +251914505057.

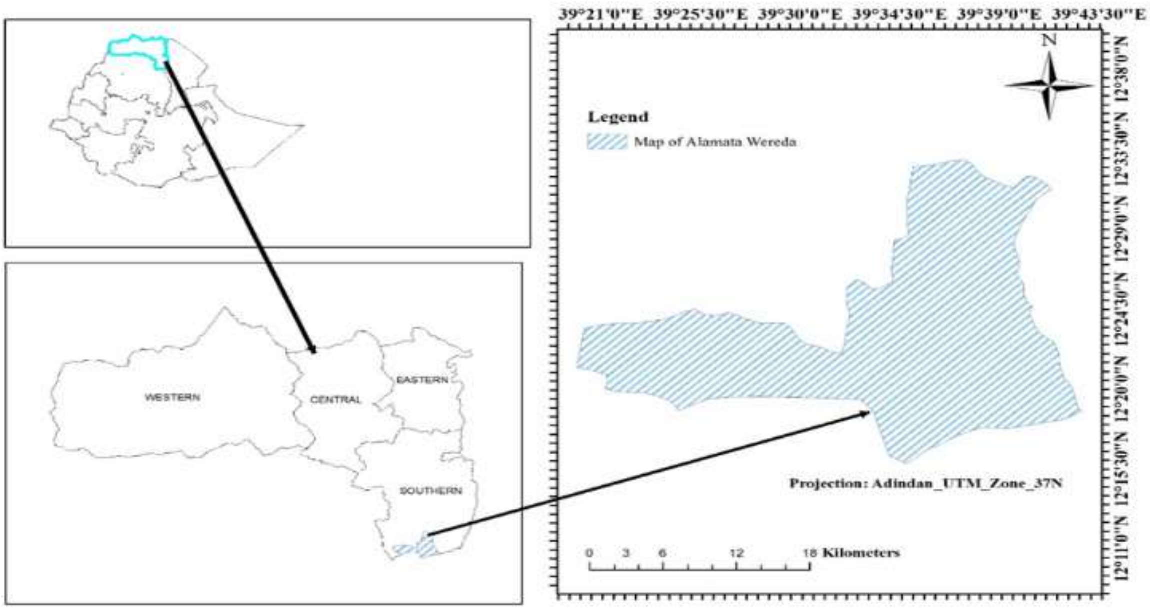

Figure 1. Location map of the study area.

and Millington, 2000). Topography has climatic influence with respect to prevailing sunshine, air currents and water bodies. Altitude has a marked influence on the kind, nature and productivity of rangeland and it creates ecologically diverse vegetation (Herlocker, 1999).

In Southern Tigray, Northern Ethiopia, there are some of the studies conducted earlier (Tefera et al., 2005; Wolde et al., 2011a, b, c; Yayneshet, 2011) were specifically tried to estimate the biodiversity without considering environmental gradients like altitude and slope and edaphic variables. Some of them were only tried in comparing the natural vegetation with that of other land uses like communal grazing. However, environmental factors such as altitude and slope play significant roles in influencing species diversity (Acharya et al., 2011). Therefore, there is high demand on information about biodiversity of natural forests considering edaphic and topographic variables. Such baseline information in return will help for proper land use planning of large watershed area, considering agro ecology, topography and altitude. To minimize loss of forest fragments, understanding the ecological and anthropogenic factors involved in the process are highly important. Studying species richness patterns at different scales is very important both for ecological explanations and for effective conservation design (Feyera et al., 2014). Similarly, the forest of the study area has been exploited by surrounding communities for agricultural land expansion, timber harvesting, firewood collection, woodcutting for construction and other purposes (Leul et al., 2010).

Even though there are many natural vegetation areas in southern Tigray including forests in different agro-ecology and land use, their potential regarding species diversity have not been studied and documented. Especially regarding edaphic and topographic variation of species were not being previously assessed. These data will enable local communities and Forestry Administration to design appropriate intervention strategies for managing the remnant forest of the study area (Leul et al., 2015). Gra-Kahsu forest presents an ideal location to test the hypotheses of altitudinal patterns of plant species diversity because it integrates several distinct vertical vegetation zones and the status of the vegetation and the plant species habitats are natural. There have been a few studies on the altitudinal pattern of plant diversity on Gra-Kahsu forest (Leul et al., 2010), but a more detailed investigation on this subject had not been reported. Therefore, I investigated the effect of edaphic and topographic variables on the plant diversity distribution in Gra-Kahsu forest. The research identifies (1) the significant difference in tree species richness, structure and regeneration status between associated edaphic and topographic variables; (2) the key associated edaphic and topographic variables influencing plant species diversity.

MATERIALS AND METHODS

Description of the study Area

Gra-Khasu forest is located in the southern zone of Tigray at about 600 km north of Addis Ababa or some 160 km south of Mekele, the capital of Tigray Regional State. It is located between 120 22’ and 120 42’N latitude, 390 28’ and 390 40’ E longitude (Figure1) at an altitudinal distribution from 1560 m to 2688 m. The total area of the forest is more than 3500 ha. The study area, Gra-Kahsu forest was designed to conserve long lasted unique natural features, historical interests and other natural values with legal and administrative supports on the upper part of Alamata town and is endowed with different natural resources such as wildlife and other biodiversity, which contribute great potent source as important pillars for future local development. The natural vegetation coverage of the district is very small. It is the Gra-Kahsu forest, which accounts good cover of the district's forest cover. The lowland parts of the district are dominated by *Acacia* species (WAOARD, 2017).

Data collection

Systematic sampling was employed for vegetation. The results were based on a complete inventory of temporary plots, stratified at altitude intervals from 1620 to 2298 m, were set up, and 2–4 quadrats were established around each sampling point in September 2017 (When most plants were in flowering stage) and species data were recorded. The quadrat size was 20 × 20 m for trees, within which 5 × 5 m quadrats were used to record shrubs. The plant height was measured using a height meter, and diameter at breast height was measured using a caliper. In each major plot, subplots (1 m^2) were established at the center and corner for seedlings and saplings data. The number of seedlings (height < 1.3 m) and saplings (height > 1.3 m, diameter at breast height < 5 cm) were counted and trees (height >1.3 m, diameter at breast height ≥ 5 cm) were measured at breast height (1.3 m) by a diameter tape in each quadrat.

Altitude, slope, disturbance and grazing intensity were also recorded for each quadrat. The altitude in each quadrat was measured using a global positioning system, the slope were measured from the center of the plot using a compass meter. The state of human interference at each quadrat was estimated following Hadera (2000). A 1 to 4 subjective scale was taken into consideration to record the presence or absence of stumps, logs and signs of fuel wood collection. Therefore, the magnitude of the impact was quantified as follows:1 = no obvious disturbance, 2 = low disturbance, 3 = medium disturbance, and 4 = heavy disturbance). Grazing intensity was estimated following Kebrom et al*.,* (1997): 0= nil; 1= slight; 2= moderate and 3= heavy. Three altitudinal gradients: lower altitude (1620-1699 masl.); middle altitude (1700-1891 masl) and upper altitude (≥1892– 2298 masl). The ranges given are the minimum and maximum elevation of samples (Bruun et al*.,* 2006). Slope location: 1, 2 and 3 for lower (0-25%), middle (26-45%) and upper (≥46-65%) position at a slope respectively.

Data analysis

The following formula was used to determine density and basal area of wood species in the study sites (Mueller-Dombois and Ellenberg, 1974).

$$\text{Density} = \frac{\text{number of individual}}{\text{area sampled}} \tag{1}$$

$$\text{Basal Area(BA)} = \left(\frac{\pi D^2}{4}\right) \tag{2}$$

Where "D" is diameter at breast-height in meter and π =3.14

Species diversity indices were used to calculate diversity values as follows (Shannon and Wiener, 1949):

$$H' = -\sum_{i=1}^{s} P_i \ln(P_i) \tag{3}$$

Where; H'= Shannon diversity indices, S= the number of species, Pi=proportion of individual species and InPi=log proportion of individual species. The following formula was used to determine species evenness of woody species in the study sites (Magurran, 2004).

$$E = \frac{H'}{H \max} = \frac{\sum_{i=1}^{s} \text{Pi} \ln \text{pi}}{\ln s} \tag{4}$$

Where; H'= Shannon diversity indices, S= the number of species and Hmax = is the maximum level of diversity possible within a given population.

Regression analyses were performed to establish the relationships between species diversity and environmental variables. All statistical analyses were performed in the statistical software package R (R Development Core Team, 2015).

RESULTS AND DISCUSSION

Species diversity and evenness

The three-altitudinal gradients had significantly different value of Shannon and Weaner diversity index and species richness. Upper altitude had significantly higher Shannon and Weaner diversity index value (2.2) than the other two-altitudinal gradients (Table 1). Thus, such result with higher diversity in the upper altitude might be due to relatively favourable climatic, environmental condition and soil characteristics specially soil texture, soil organic matter (Desalegn and Carl, 2010) and slope (De Lafontaine and Houle, 2007). Altitude is a factor that determines the distribution of climatic factors and land suitability; this influences the natural vegetation types and their species diversity (Alemayehu, 2003) and influencing much of other abiotic and biotic component like temperature, soil, topography and vegetation (Otypkova et al., 2011; Tibebu and Teshome, 2015). Studies on the variations in species richness along altitudinal gradients indicate a variety of results under different biotic and abiotic condition. Most of the studies suggests that species richness decline with increasing altitude (Sharma et al., 2009). However, in most cases findings stress that species richness decrease with altitude (Bruun et al., 2006). Nevertheless, several studies have also documented a non-monotonic pattern of species richness (Vetaas, 2002). The most commonly observed pattern of diversity is a mid-altitudinal bulge (Rahbek, 2005; Zhao et al., 2005). This may arise as the upper altitudes have typical and difficult climatic condition than the lower ones, so these conditions may restrict the spread of species in the upper limit of altitude. Therefore, this will create favorable condition to sustain their fitness in the upper limit of altitude (Ole et al., 2002). Some have argued that whether species diversity will increase or decrease with

Table 1. Mean ±SE of Species richness, diversity and evenness of the three altitudinal and slope gradients in Gra-Kahssu forest area.

Altitudinal gradients	Species richness	Shannon and Weaner diversity index (H')	Evenness (J)
Upper altitude	16.28^{a}	$2.2(\pm0.06)^{a}$	0. 79(±0.02)
Middle altitude	12.21^{b}	$1.91\ (\pm0.08)^{b}$	0.79 (±0.01)
Lower altitude	10.04^{b}	$1.87(\pm0.07)^{b}$	0.82 (±0.02)
P value	<0.001	0.009	0.28
Slope gradients			
Lower	11.24(0.84)	$1.90(\pm0.06)^{a}$	0.82(0.01)
Middle	13.64(0.90)	$2.04\ (\pm0.07)^{b}$	0.79(0.01)
Upper	13.62(1.87)	$2.04(\pm0.12)^{b}$	0.81(0.01)
P value	0.13	0.33	0.42

Table 2. Mean ±SE of Species richness, diversity and evenness of the four human disturbance and three grazing intensity in Gra-Kahssu forest area.

Human disturbance level	Species richness	Shannon and Weaner diversity index (H')	Evenness (J)
No obvious disturbance	$17.5\ (\pm0.95)^{a}$	$2.22(\pm0.08)^{a}$	0.79(±0.09)
Lower disturbance	$13.41\ (\pm1.54)^{b}$	$2.05\ (\pm0.08)^{ab}$	0.82(±0.01)
Medium disturbance	$10.56\ (\pm0.59)^{c}$	$1.89\ (+0.07)^{bc}$	0.81(±0.02)
Heavy disturbance	$9.76\ (\pm0.99)^{c}$	$1.78(\pm0.1)^{c}$	0.80(±0.02)
P value	<0.001	0.009	0.76
Grazing intensity			
Nil	$17.84(0.95)^{a}$	$2.27(\pm0.09)^{a}$	0.79(±0.02)
Slight	$13.37(1.15)^{b}$	$2.05\ (\pm0.06)^{ab}$	0.81(±0.01)
Moderate	$9.09(1.04)^{c}$	$1.73(\pm0.1)^{c}$	0.80(±0.02)
Heavy	$10.45(0.60)^{c}$	$1.88(\pm0.07)^{bc}$	0.81(±0.02)
P value	<0.001	<0.001	0.78

increasing altitude or will peak at an intermediate altitude depends largely on specific patterns of interactions among plant communities, species, and environmental factors (Brown, 2001; Körner, 2007).

Altitude gradients are one of the most commonly determinative factors in shaping the spatial patterns of species richness (Acharya et al., 2011; Ru and Zhang, 2012; Zhang et al., 2013). The reason of high species richness, Shannon diversity index in the upper altitude might be its climatic conditions that allow many species to coexist and due to the topographical nature where upper altitude is almost steep slope made itself away from human disturbance. On the other hand, lower altitude is more prone to arable land due to gentle slope nature made to coexist less plant species. Yohannes et al. (2015) conclude that, mountain forest mostly affects by environmental variables due to change in species structure and composition.Species evenness did not show a strong response to slope gradient as there is no significant difference in its value in all the three slope gradients (P>0.05). This indicates that the distribution of species in the natural vegetation was not affected by slope. Even though it had slight variation among the three slope gradients, noticeably the upper and middle had slightly highest species richness value with 13.6 as compared with lower (11.24) (Table 1). However, there is a significant difference (P<0.001) in species richness and Shannon and Weaner diversity index between the four human disturbance and grazing intensity (Table 2). This indicates human disturbance and animal grazing intensity significantly influence species richness as well as Shannon and Weaner diversity index of an area. This idea was also in line with other similar report (Jin et al., 2013). Similarly, species richness, diversity, and evenness decreased with increasing disturbance intensity, reaching a maximum at no obvious disturbance.

The mean of Shannon and Weaner diversity index for no obvious disturbance, low and moderate human disturbance were 2.22±0.08, 2.05±0.08 and 1.78±0.1, respectively. However, areas having heavy and moderate

Table 3. Mean ±SE of BA and number of individuals of the three altitudinal and slope gradients in Gra-Kahssu forest area.

Altitudinal gradients	**BA(m^2/ha)**	**Individuals/ha**
Upper altitude	10.15(±0.60)^a	334.94(±26.87)^a
Middle altitude	6.48(±0.96)^b	186.36(±15.13)^b
Lower altitude	6.40(±1.38)^b	155.28(±12.79)^b
P value	0.001	<0.001
Slope gradients		
Lower slope	5.97(±0.81)	163.37(±13.51)b
Middle slope	7.81(±0.96)	249.80(±20.94)a
Upper slope	7.25(±1.89)	308.62(±55.42)a
P value	0.37	<0.001

grazing intensity had significantly lower species richness coverage than the other two grazing intensity followed by lower grazing intensity. However, nil grazing intensity had significantly higher species richness coverage (Table 2). This result could be related to the decreasing of animal grazing pressures and human activities on plant damaged with increased distance from settlements (Brinkmann, 2009). These differences are primarily a function of differences in site productivity, habitat heterogeneity and/or disturbance factors (Maestre, 2004). For example, the low species richness in the heavy and moderate disturbance are due to anthropogenic disturbances such as burning, grazing, and wood collection, which have significantly reduced species richness (Maestre, 2004; Feyera et al., 2014). The low values of the Shannon diversity index of heavy and moderate grazing intensity also support the hypothesis of dominances of few early successional species and/or few species due to selective cutting of other species (Bone et al., 1997). Generally, the difference in the values of Shannon diversity among the studied human and grazing intensity are an indicator of disturbance level difference. Also, Gunnar and Ove (2001), Oba (2011), Tessema et al. (2011), and Angassa (2014) conclude that grazing is a key process for maintaining biodiversity.

Species richness, Shannon–Wiener index and evenness showed a quadratic decreasing pattern along the disturbance gradient, that is, the highest diversity appearing at the lowest disturbance intensity (Xiang and Zhang, 2009). Some researchers have argued that the highest diversity appeared at medium disturbance (grazing) (Pavlu et al., 2003; Smet and Ward, 2005). The maximum diversity appeared under the weakest disturbance (Jin-Tun et al., 2016). Similarly, Amsalu (2000) suggested that heavy grazing might cause reduction of plant species composition and diversity over time. Grazing is also considered essential to maintain the structure, functionality and diversity of plant communities (Hoshino et al. 2009). Similarly, mean species richness in undisturbed treatments was significantly higher than disturbed treatments (Leul et al., 2015). A parallel result was observed by Aynekulu et al. (2009) in Northern Ethiopia, demonstrating the impact of disturbance on species richness. Main factors responsible for destruction of natural forests are combination of agricultural expansion, commercial harvest, free livestock grazing, unsustainable firewood collection and charcoal production regimes (Alemayehu, 2007). Adane (2011) correspondingly observed that both mean number of species and species richness decrease along disturbance gradient.

There is a significant difference in basal area between the three altitudinal gradients (P=0.001) (Table 3). This indicates altitude significantly influence basal area of an area. This finding was also in line with other similar report (Markos and Simon, 2015). The mean basal area for lower altitude, middle altitude and upper altitude were 6.40 m^2/ha, 6.48 m^2/ha and 10.15 m^2/ha, respectively. Therefore, upper altitude had significantly higher basal area coverage than the other two gradients followed by middle altitude. Based on the report the basal area of the lower altitude and lower slope, indicating the woody species were thin. The greater difference in basal area between the environmental variables could be due to the high number of multi-stemmed trees in the upper altitude and slope, leading to bigger diameters. This directly relational trend indicated that the value of basal area starts to grow higher as altitude increase. This might be due to the presence of relatively higher proportion of larger and aged trees in higher altitudinal classes, owing to little intervention by human activities like tree cutting, farming, grazing, etc. In addition, Markos and Simon (2015) suggest that altitude affects basal area due to its influence on light radiation, temperature, moisture, runoff and infiltration. Similarly, Kumelachew and Tamrat (2002) and Teshome et al., (2004) confirmed the results. The mean basal area increases with altitude but does show a significant trend (Carpenter, 2005).

The mean basal area of trees and shrubs per hectare and number of individuals in the four human disturbance as well as grazing intensity difference was highly significant (P<0.001). Areas with low human disturbance

Table 4. Mean ±SE of BA and number of individuals of the four human and grazing intensity in Gra-Kahssu forest area.

Human disturbance	BA(m^2/ha)	Individuals/ha
No obvious disturbance	11.30(±1.58)^a	371.14(±26.57)^a
Lower disturbance	6.54(±0.62)^b	211.50(±20.40)^b
Medium disturbance	5.48(±1.38)^b	161.17(±12.85)^b
Heavy disturbance	4.56(±0.89)^b	154.69(±15.75)^b
P value	<0.001	<0.001
Grazing intensity		
Nil	11.73(±1.65)^a	382.30(±26.04)^a
Slight	6.67(±0.63)^b	211.68(±15.81)^b
Moderate	5.29(±1.07)^b	160.22(±13.41)^c
Heavy	4.49(±0.97)^b	142.72(±14.97)^c
P value	<0.001	<0.001

and nil grazing intensity had higher number of individual per hectare (371.14+26.57 and 382.30+26.04), respectively (Table 4). The stands sampled in this community are located in an area with low human interference in the form of firewood collection and selective cutting and cattle is encountered. This might be due to being far from the farmer's settlement area. However, areas with heavy human disturbance and grazing intensity had lower mean of basal area, this may be due to highly influenced by people collecting firewood, charcoal making and grazing animals. This is due to its being nearby to community and having species of plants suitable for charcoal making and firewood. This finding is dissimilarity with what was reported by Althof (2005) and Rembold (2011) where basal area is inversely related to disturbance. Higher basal area means there are large number of individuals in bigger diameter classes. However, when a given forest is heavily disturbed most of the trees with bigger diameter are removed for various reasons and result in low basal area (Adane, 2011).

Regeneration status of plants across edaphic and topographic variables

Human disturbance and grazing intensity of Gra-kahsu forest were significantly affected the regeneration status of species. Of all the edaphic factors studied, human and grazing intensity were determined the distribution of the density of mature trees, sapling and seedling identified in the study. For instance, the highest of number of mature trees, seedlings and saplings/ha appeared in no obvious human disturbance; this suggests that human disturbance was important factor for regeneration status of plant species (Table 5). Lower number of seedlings from trees might attributed to poor seeding of matured trees due to age, livestock grazing (can destroy seedlings by trampling and browsing), seed and/or fruit predation, etc. or a combination of two or more of these factors (Adane, 2011).

Disturbances such as intensive removal of trees for timber, construction and forest grazing have placed significant pressure on forest regeneration (Leul et al., 2010). Leul et al. (2015) also conclude that, human intervention is high in the disturbed sites accompanied with uncontrolled grazing that affected regeneration success of the species. High density of seedlings/ saplings in the undisturbed sites is a sign of successful seed germination and establishment compared to disturbed sites (Ganesan and Siddappa, 2004). The influence of slope and altitude affects regeneration status of the vegetation. It is also similar to McDonald et al. (2010) idea, which states that the sensitivity of plants to moisture availability renders the regeneration of the dry forests. The regeneration of the forest is affected not only by environmental factors but also by anthropogenic activities (Markos and Simon, 2015). Adane (2011) conclude that the main causes of regeneration of the forest are mainly due to the geographical location of the forests and/or altitudinal difference type of disturbance (grazing and cuttings) as well as the intensity of disturbance or combination these factors. Several types of disturbances such as logging, landslides, herbivores, etc. can affect the potential regenerative status of species composing the natural vegetation stand spatially and temporally (Guarino and Scariot, 2012). Disturbance intensity directly affects plant growth, development and regeneration by trampling, bending shoots, cutting stems and grazing (Austrheim, 2002; Zhang et al., 2013; Sproull et al., 2015).

In the upper altitude, far distance was significantly higher in number of mature tree, sapling as well as seedling than lower and middle altitude (Table 6). This was happened as relatively favorable biotic and abiotic condition of the upper altitude for natural growth and reproduction of a variety of most plant species including the one found in the other altitudinal gradients (Melese and Wendawek, 2016). The lowest density for sapling

Table 5. Mean ±SE of mature tree, sapling and number seedlings of the four human and grazing intensity in Gra-Kahssu forest area.

Human disturbance	Mature	Sapling	Seedling
No obvious disturbance	1494.64(±93.18)^{a}	3950.00(±393.73)^{a}	3085.71(±289.34)^{a}
Lower disturbance	852.08(±108.29)^{b}	2375.00(±259.11)^{b}	1533.33(±243.50)^{b}
Medium disturbance	655.76(±88.07)^{bc}	1523.07(±268.93)^{c}	1169.23(±128.79)^{b}
Heavy disturbance	502.17(±61.95)^{c}	1117.39(±148.33)^{c}	1308.69(±109.96)^{b}
P value	<0.001	<0.001	<0.001
Grazing intensity			
Nil	1478.84(±99.18)^{a}	4100.00(±393.21)^{a}	3230.76(±270.41)^{a}
Slight	920.31(±107.82)^{b}	2362.50(±205.92)^{b}	1506.25(±182.68)^{b}
Moderate	552.27(±58.52)^{c}	1254.54(±250.22)^{c}	1081.81(±138.05)^{b}
Heavy	509.09(±64.43)^{c}	1109.09(±154.99)^{c}	1313.63(±114.96)^{b}
P value	<0.001	<0.001	<0.001

Table 6. Mean ±SE of mature tree density/ha, sapling and number seedlings of the three altitudinal and slope gradients in Gra-Kahssu forest area.

Altitudinal gradients	Mature/ha	Sapling/ha	Seedling/ha
Upper altitude	1386.11(±98.08)^{a}	3605.55(±346.45)^{a}	2683.33(±290.17)^{a}
Middle altitude	721.05(±75.95)^{b}	1968.42(±233.96)^{b}	1384.21(±169.21)^{b}
Lower altitude	503.00(±57.08)^{c}	1080.00(±142.94)^{c}	1292.00(±108.61)^{b}
P value	<0.001	<0.001	<0.001
Slope gradients			
Lower slope	628.44(±80.99)^{b}	1379.31(±170.86)^{b}	1313.79(±129.20)^{b}
Middle slope	924.00(±92.78)^{a}	2484.00(±310.73)^{a}	2032.00(±216.08)^{a}
Upper slope	1237.50(±194.45)^{a}	3400.00(±611.49)^{a}	2250.00(±519.27)^{a}
P value	0.003	<0.001	0.01

seedling and matured plants were detected in lower gradient might arise due to illegal grazing and tree cutting in lower altitude. The main reason for this difference could be low seed production due to heavy logging for charcoal and timber production and physical damage on seedlings and sapling during cuttings (Adane, 2011). In addition, in the upper and middle slope; the number of mature tree, sapling and seedling were recorded significantly higher than middle lower slope gradients. This is because, the quadrats in this community were might not be suitable for grazing and browsing by animals of the community due its topography. Besides, Soromessa et al. (2004) ensured effect of slope on runoff, drainage, and moisture. The influence of slope and altitude of vegetation on moisture availability affects regeneration status of the vegetation. The anthropogenic disturbances in the study area are higher at the lower altitude (for example, logging and wood collection) and lower at the higher altitude. This is could be the main reason for the increasing trend of regeneration status along altitudinal gradient. The regeneration of the forest is affected not only by environmental factors but also by anthropogenic activities (cutting of trees for charcoal production, constructing wood and fence (Deribe, 2006).

Population structure of plants across edaphic and topographic variables

Population structure is one of the forest characteristics that can be easily influenced by disturbance. Different disturbance factors also affect population structure differently (Adane, 2011). Plant height and diameter at breast height was significant differences (p<0.001) along human disturbance and grazing intensity. It was observed that the higher plant height and diameter at breast height was recorded in no obvious disturbance with mean of 5.74± 0.14 cm and 9.94 ± 0.32 cm, respectively. On the other hand, the lower plant height and diameter at breast height was recorded in the heavy disturbance with the mean of 4.07 ± 0.12 and 6.18±0.21 cm and the higher plant height and diameter at breast height was computed

Table 7. Mean ±SE of plant height and DBH (cm) of the four human and grazing intensity in Gra-Kahssu forest area.

Human disturbance	**Height (cm)**	**DBH(cm)**
No obvious disturbance	5.74(±0.14)^{a}	9.94(±0.32)^{a}
Lower disturbance	5.47(±0.10)^{a}	6.89(±0.16)^{b}
Medium disturbance	4.28(±0.14)^{b}	6.63(±0.31)^{bc}
Heavy disturbance	4.07(±0.12)^{b}	6.18(±0.21)^{c}
P value	<0.001	<0.001
Grazing intensity		
Nil	5.75(±0.14)^{a}	9.94(±0.33)^{a}
Slight	5.62(±0.10)^{a}	7.06(±0.17)^{b}
Moderate	4.40(±0.15)^{b}	7.49(±0.40)^{a}
Heavy	4.06(±0.10)^{b}	5.84(±0.17)^{c}
P value	<0.001	<0.001

Table 8. Mean ±SE of plant height and DBH (cm) of the three altitudinal and slope gradients in Gra-Kahssu forest area.

Altitudinal gradients	**Height (cm)**	**DBH(cm)**
Upper altitude	5.68(±0.13)^{a}	9.80(±0.31)^{a}
Middle altitude	5.16(±0.08)^{b}	6.76(±0.14)^{b}
Lower altitude	4.31(±0.12)^{c}	6.34(±0.22)^{b}
P value	<0.001	<0.001
Slope gradients		
Lower slope	4.49(±0.08)^{c}	6.34(±0.14)^{b}
Middle slope	5.13(±0.12)^{b}	6.75(±0.20)^{b}
Upper slope	5.76(±0.12)^{a}	9.11(±0.26)^{a}
P value	<0.001	<0.001

in nil and slight grazing intensity with significant differences (p<0.001) along grazing intensity (Table 7). The reason greater plant height recorded in the no obvious disturbance and nil grazing could be due to the presence of higher species density, less human and livestock interference than in the others. The possible reason for decreasing the mean value of diameter at breast height in the heavy disturbance and heavy grazing site might be due to illegal cutting system used by the local people for construction materials and fuel wood consumption. This study is in line with the study made by Getaneh (2007). Tefera et al. (2015), who showed that the local people for construction and charcoal preparation harvested woody species with diameter at breast height > 30cm. It is also clear those cuttings (be it for charcoal or timber) affect basal area as it targets bigger diameter trees (Adane, 2011). Disturbance regimes may play a considerable role in structuring the pattern of diversity distribution (Jentsch et al., 2002).

Due to the altitudinal gradient, the values of plant height and diameter at breast height varied (Table 8). The upper altitudinal class had the highest plant height of 5.68m, whereas the lower altitudinal class had the lowest plant height with the recorded value of 4.31m. In addition, the diameter at breast height was significantly lower in the lower altitudinal class compared to the other altitudinal class (p<0.001) (Table 8). The diameter at breast height was significantly larger in the upper slope class compared to the others slope class (p<0.001).The average value of diameter at breast height at the lower slope class was 6.34cm. Similarly, the mean plant height was varied in classes of lower, middle and higher slope with mean value of 4.49±0.08, 5.13±0.12 and 5.76±0.12cm, respectively (Table 8). Generally, the present study shown distinct pattern of variation of plant height and diameter at breast height in altitudinal and slope class although the variation has significant difference.

Relationship between vegetation patterns and environmental factors

Vegetation patterns are determined by environmental

Table 9. Pearson correlations of diversity with environmental gradients, human disturbance and grazing intensity.

Variable	BA		Individual		H		S		E	
	r	p-value	r	p-value	r	p-value	r	p-value	r	p-value
Altitudinal gradients	0.44	<0.001	0.78	<0.001	0.44	<0.001	0.64	<0.001	-0.15	0.25
Slope gradients	-0.05	0.72	0.46	<0.001	0.21	0.09	0.28	0.03	-0.11	0.4
Grazing intensity	-0.3	0.01	-0.69	<0.001	-0.41	<0.001	-0.59	<0.001	0.09	0.48
Human disturbance	-0.3	0.01	-0.67	<0.001	-0.4	<0.001	-0.57	<0.001	0.08	0.5

r=Correlation coefficient value.

factors that exhibit heterogeneity over space and time, such as topography as well as human disturbances (Alexander and Millington, 2000). In the present study, the variation of plant diversity was closely related to environmental factors, including altitude, disturbance intensity and slope among which altitude and disturbance were the most important. Species richness and diversity all showed significant relationships with altitude and disturbance intensity (Jin et al., 2013). Species richness was negatively correlated with grazing intensity (r =−0.59, p < 0.001), and number of individuals/ha was positively correlated with slope (r =0.46, p < 0.001). Disturbance intensity was significantly correlated with number of individuals/ha, species richness and Shannon diversity index (r =−0.67,-0.4 and -0.57) respectively (Table 9). Species richness, Shannon diversity index and evenness were significantly correlated with altitude and disturbance. Altitude is a key variable affecting species diversity in mountains (Zhang et al., 2006; Muhumuza and Byarugaba, 2009; Chawla et al., 2008). The effects of slope on vegetation were also significant, confirming the results of many other studies (Lovett et al. 2006; Zhang and Zhang, 2007 and Jin-Tun et al., 2016). Changes in slope may lead to changes in humidity and temperature, all of which affect species diversity (Virtanen et al., 2010). Basal area was negatively correlated with grazing intensity and human disturbance (r =−0.3, p =0.01), and positively correlated with altitude (r =0.44, p < 0.001) (Table 9). Altitude and slope have an equally strong significant (p< 0.0001) effect on basal area of species (Markos and Simon, 2015). Although the linear trend explains a significant amount of the variability in basal area on the altitude gradient (Carpenter, 2005).

Altitude have strong correlation with species richness and Shannon diversity index of plant and have great impact especially on woody vegetation distribution (Zewde, 2014). Forest diversity was most significantly correlated with altitude and disturbance (Jin-Tun et al., 2016). Species richness, Shannon diversity index and evenness all significantly correlated with elevation and disturbance gradients. The study of Wana and Carl (2010) in the southwest Ethiopian highlands found that the correlation coefficients for woody species richness and altitude were relatively lower (0.41). Forest composition, diversity and distribution pattern generally are significantly correlated with environmental gradients that exhibit heterogeneity over space and time, such as topography and disturbances (Korner, 2007; Brinkmann et al., 2009; Zhang et al., 2015). This was proven true in the Gra-Kahsu forest. The variation of forests was closely correlated with environmental variables such as altitude, grazing, disturbance and slope.

Conclusion

The decline in species richness and Shannon and Weaner diversity index of woody vegetation was an outcome of the edaphic and topographic variables. Therefore, this study indicated that different edaphic and topographic variables has great role for the variation of plant species diversity in the forest. Altitudinal gradients, human disturbance and grazing intensity played key roles in plant species diversity. This is because, with altitudinal gradients, human disturbance and grazing intensity play major roles and composition tends varies; and this affects the diversity. A reduction in woody plant species diversity parameters was observed as a direct effect of human disturbance, grazing pressure and altitudes in the Gra-Kahsu forest and this consequently affected the growth, diversity, density, regeneration and population structure of woody plant species. Measures such as controlling grazing intensity and human disturbance, monitoring forest diversity change, and effective management should be taken serious in this forest.

CONFLICT OF INTERESTS

The author has not declared any conflict of interests.

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