Genetic diversity and population structure of Vernonia [*Vernonia galamensis* (Cass.) Less] populations from Ethiopia revealed by SSR markers

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Abstract

Background

Vernonia (*Vernonia galamensis*) is a potential novel industrial crop due to high demand for its natural epoxidised oil, which can be used for the manufacturing of oleochemicals such as paints, plastic formulations (polyvinyl chloride), and pharmaceutical products. This study is initiated for the systematic and intensive genetic diversity assessment of *V. galamensis* accessions by SSR molecular markers to minimize the existing research gaps, provide a clue for germplasm conservation and further research.

Results

Twenty SSR markers were used for genetic diversity analyses of 150 individual *V. galamensis* accessions representing 10 populations, from which a total of 79 bands were identified across the entire loci. All the loci used showed high polymorphism that ranged from 0.50 to 0.96, while the mean observed heterozygosity (Ho) was 0.15 across all the 20 markers evaluated. The molecular variance analysis (AMOVA) showed significant variations despite low differentiation among populations which accounted for only 11% of the total variations. Populations clustering showed that the dendrogram and principal coordinate's analysis roughly classified the 150 accessions into four groups. However, the Bayesian model-based clustering (STRUCTURE) grouped into 6 (K = 6) major gene pools. These analyses showed accessions collected from the same region of origin did not often grouped entirely together within a given major groups.

Conclusions

The result suggested that the markers applied to ten populations, in which East Showa and East Harerghe revealed higher genetic diversity, signaled that these areas are the hotspots for in-situ conservation of *V. galamensis*. In addition, the values of SSR markers such as heterozygosity, Shannon's index, polymorphic information content, and population clusters are important baseline information for future *V. galamensis* cultivation, breeding and genetic resource conservation endeavors in Ethiopia.

Background

Vernonia (*Vernonia galamensis* (Cass.) Less.; 2n =18) belongs to the family Asteraceae (Compositeae), a potential novel industrial crop due to the high demand for its natural epoxidised oil [1-5]. *Vernonia galamensis* subsp. *galamensis* variety *ethiopica* M. Gilbert was first identified by Perdue in 1964 in Eastern Ethiopia [6, 7]. Seeds of *Vernonia* species are the major sources of naturally occurring epoxidised fatty acids and other essential fatty acids such as linoleic acid, oleic acid, palmitic acid, stearic acid and trace amounts of arachidic acid [9, 10, 11-14].

Studying genetic diversity of crop plants including *V. galamensis* is a valuable tool for effective utilization, conservation, management, and improvement during breeding and selection [15, 16]. Molecular markers are valuable tools for discrimination between accessions, easy and cost-effective to measure and not influenced by environmental factors. Microsatellites (SSR) markers have efficient advantages over other DNA markers that they are highly polymorphic, co-dominant inheritance with high mutation rates, relatively abundant and good genome coverage, which used to investigate taxonomical, genetic diversity and evolutionary relationship [17, 18]. However, SSR markers have limitations such as genomic sequencing is needed to design specific primers; it is also not very cost-effective and requires much discovery and optimization for each species before use [19]. To date, this study was the first report that used SSR markers to study the genetic diversity of *V. galamensis*.

In Ethiopia, geo-ecological conditions are favorable for the cultivation of *V. galamensis* for the use as a source of raw material for agro-processing industries. However, the plant is neglected and considered only as a wild weed colonizing disturbed and bare agricultural lands [3]. As a result, the crop is not cultivated in any of the collection sites and/or elsewhere in the country. Moreover, lack of attention, negligence in research and conservation, priority has been given to other major crop plants while the potential industrial values of *V. galamensis* is underestimated and underexploited. The plant is also under threat of continued genetic erosion. This study is, therefore, initiated for the systematic and intensive genetic diversity evaluation and characterization of *V. galamensis* accessions by molecular analysis using SSR markers to minimize the existing research gaps and provide a clue for germplasm conservation and further research.

Methods

Plant material

A total of 150 *V. galamensis* accessions, representing 10 populations, were randomly collected from their diverse agro-ecologies of the three-potential growing regional states of Ethiopia (Figure 1; Table 1). The samples identified were confirmed with the descriptions available in the Flora of Ethiopia and Eritrea. Most of the study materials were collected from the field and, others were assembled from the Ethiopian Biodiversity Institute and Wondo Genet Agricultural Research Center

At each collection area, seed samples were collected from plants and kept in separate bags to ensure that the distance between any two collection sites was about 5-10 Km. From collection areas observations, *V. galamensis* naturally grows in hilly/depression, along the roadside, in the valley, in farmlands, in the forest, in the compounds of mosques and churches. The collections were done by taking either seed samples of the individual flower heads or seeds from plants with all matured flowers, and then accessions were threshed, cleaned and documented. *V. galamensis* was not cultivated in any of the collection sites.

Plant material collection and DNA extraction

Fresh young leaves of 150 *V. galamensis* accessions were collected from individual plants that were grown at experimental sites, representing 10 populations. Collected leaf samples were put in a sealed bag envelope and dried with silica gel (with 1:10 ratio of leaf samples to silica gel), then kept under room temperature until used for later DNA extraction according to Gilbert et al. [20]. The dried leaf samples were transported to Huazhong Agricultural University, China for genetic analysis. The total genomic DNA extraction was made according to the modified CTAB protocol of Doye and Doye [21]. Extracted DNA was visualized on a 1% (w/v) agarose gel and quantified spectrophotometrically using a Nanodrop® 2000 (Thermo Scientific, USA). Finally, it was stored at -20 °C for further use.

Polymerase Chain Reaction (PCR) Amplification

The twenty SSR markers used for this study were selected based on their high polymorphism from the work of Narina et al. [22] (Table 2). The amplification reaction was performed with a thermal cycler using 96-well plates (T100^{TM} Thermal Cycler) in a total volume of 10 µl reaction mixture, containing 100 ng/ml of template DNA, 5 µl 2 x Taq PCR master mix (Vazyme P213-01, China), 1 µl of forward and reverse primers and 3.0 ml of double-distilled water. The PCR amplification was programmed at an initial denaturation step of 5 minutes at 94 °C followed by 35 cycles of 30 s denaturation at 94 °C, annealing at 56/58 °C (depending on primers) for 30 s, initial extension at 72 ^{o}C for 1 minute and final extension at 72 °C for 5 minutes. The amplified DNA samples were stored at 4 °C until it was loaded on 3% agarose gel electrophoresis for visualization.

Band Scoring and Analysis

The amplified products were visually scored based on their migration in comparison with the standard size DNA (100 bp DNA ladder) photographed under UV gel illumination (Gel Doc^{TM} with Image Lab^{TM} software, BIO-RAD). The genetic diversity for each allele such as the number of different alleles (Na), the effective number of alleles (Ne), Shannon's diversity index (I), observed heterozygosity (Ho), expected heterozygosity (He), F-statistics values (F_{is}, F_{it} and F_{st}), polymorphic information content (PIC), random segregation and distribution (Hardy-Weinberg equilibrium) of each genotype within the populations for each locus, Nei's genetic identities (Ji), genetic distances (Ds) and gene flow (Nm) in *V. galamensis* populations were performed using GeneAlex version 6.503 software [23].

Simple matching dissimilarity coefficient-based Unweighted Pair Group Method with Arithmetic Mean (UPGMA) and Neighbor-Joining (NJ) tree were computed using DARwin version 6.0.19 software [24], and the significant was tested based on 1000 bootstraps. The resulting trees were displayed using Fig Tree version. 1.4.4 [25]. Each of the four clusters comprised collections of geographic regions of origin. A Bayesian model-based cluster analysis was performed using STRUCTURE version 2.3.4 software [26]. Each of the groups of populations composed of individual plants from different collection zones/geographic regions of origin. To determine the most likely number of populations (K), a burn-in period of 50,000 was used in each run, and data were collected over 500,000 Markov Chain Monte Carlo (MCMC) replications for K = 1 to K = 10 using 20 iterations for each K. The optimum K value was determined according to Evanno et al. [27] using the web-based (http://tyloro.biology.ucla.edu\structure Harvester\) STRUCTURE HARVESTER ver. 0.6.92 [28]. The results generated by this software were visualized in a graphical bar plot using Clumpak beta version (http://www.clumpak.tau.ac.il/) [29].

Results

SSR Polymorphism

Twenty SSR markers were used for the characterization and genetic diversity analysis of the 150 *V. galamensis* accessions, all of which were polymorphic (Table 3). The results of diversity parameters showed a high level of polymorphism among the 20 SSR markers. Polymorphic information content (PIC) values ranged between 0.50 and 0.96 with an average of 0.76. Microsatellite markers such as Vg-002 and Vg-011 showed the highest polymorphism with 0.96 and 0.93, respectively (Table 3). A total of 79 alleles were identified, varied from 2 to 6 with an average of 3.9 alleles per locus. The maximum number of effective alleles (Ne) was 4.79 (Vg-003) and the least number of effective alleles was 1.99 (Vg-016). The highest major allele frequency (MAF) (0.85) was recorded by locus Vg-001 and the least MAF was (0.45) recorded by locus Vg-003. The observed heterozygosity (Ho) values were quite low ranging between 0.05 (Vg-021) and 0.36 (Vg-003) with an average of 0.16 across all the 20 markers evaluated. The expected heterozygosity (*He*) mean was 0.50 (Table 3). Fixation index (F) ranged from 0.25 (Vg-001) to 0.89 (Vg-021) with an average of 0.68. Finally, Shannon-Weaver's information indices (I) ranged from 0.86 to 1.67 (Table 3). On the other hand, East Showa and East Harerghe had the highest in effective alleles, heterozygosity, genetic diversity (Shannon diversity index) and fixation index (Table 4).

Analysis of molecular variance (AMOVA)

The analysis of molecular variance (AMOVA) indicated that the genetic variation within populations contributed more to genetic diversity than that among populations (Table 5). The AMOVA showed 67% of the total variation was attributed to genetic variability among individuals from different populations, whereas 22% was due to variation among individuals within the same population. In contrast, a smaller portion (11%) was among the populations variations.

Genetic distance between populations

The Nei's [30] standard genetic distance (GD) between populations ranged from 0.24 to 0.57. The highest pairwise genetic distance was observed between populations of Borena and East Harerghe (0.57), followed by between populations of Sidama and West Harerghe (0.54). The minimum pairwise genetic distance was observed between populations of Borena and Konso (0.24) (Table 6).

Cluster, principal co-ordinate analysis (PCoA) and population structure

Clustering analysis, which was performed based on the allelic frequency, grouped the 150 accessions into four (4) major clusters from the main node using neighbor-joining, with the DARwin 6.0.19 software programs. Each of the four clusters comprises individual plants from different zones (geographic regions). The first and the third cluster further divided into sub-clusters according to their geographic origin (Figure 2).

The first cluster constituted 39 accessions, the second cluster contained 42 accessions, while cluster three was characterized as the smallest group in clustering comprising of 15 accessions and the fourth cluster was the major group of clustering and comprised of 54 accessions (Figure 2). The cluster analysis revealed that accessions from different populations (collection sites) clustered together with no clear pattern of geographic origins. The principal coordinate analysis (PcoA) showed that the majority of samples were placed at the center of a two-dimensional coordinate plane and roughly forms four groups (Figure 3). The first three axes of the PCoA together accounted for 33.02% of the total variation.

Population structure analysis

Analysis of population structure distinguished the 150 *V. galamensis* accessions using a model-based Bayesian approach with the highest ΔK Value that ranged from K = 1 to K = 10 and 20 iterations for each K. According to Evanno et al. [27] and Gilbert et al. [31], STRUCTURE outputs were predicted K = 6, most likely selected to describe the genetic structure of the 150 *V. galamensis* accessions (Figure 4). Based on this value, population structure (Clumpak result) revealed that accessions collected from the same region of origin did not often grouped entirely together within a given major group. There was a wide admixture in structuring of *V. galamensis* populations.

Discussions

Determination of SSR-markers based genetic diversity with genetic parameters

Vernonia galamensis is a potential novel industrial crop containing naturally occurring epoxidized oil. However, its potential values are neglected, underestimated and underexploited. In addition, it is also exposed to genetic erosion. Therefore, assessment of genetic diversity with SSR markers generally in plants and particularly in *V. galamensis* is important for in-situ and ex-situ conservation and for efficient management of genetic diversity, for selection and improvement of the available genetic resource. The SSR marker-based study showed considerable genetic diversity. A total of 79 alleles with an average of 3.9 alleles were detected in this study, which was higher than the one reported by Ramalema et al. [13] in *V. galamensis*. The author also stated that less genetic diversity was observed among different *Vernonia* lines. According to Aikpokpodion et al. [32], a total of 29 bands were detected using five RAPD primers in genus *Vernonia*. Hence, the large number of alleles detected in the present study indicated the suitability of microsatellites for genetic linkage and QTL mapping of desirable traits applied to marker-assisted selection (MAS) in breeding programmes.

Polymorphic information content (PIC) is generally used for the characterization of marker polymorphism. In this study, the PIC values ranged between 0.50 (Vg-005) and 0.96 (Vg-002) with an average of 0.76, which was higher than that reported by Ramalema et al. [13] using RAPD markers in *V.galamensis*. Microsatellite markers such as Vg-002 and Vg-011 showed the highest polymorphism with 0.96 and 0.93, respectively. Aikpokpodion et al. [32] also indicated that PIC was ranged from 0.27 to 0.78 in *V. galamensis* accessions. This indicates that the majority of markers were able to distinguish differences among the studied *V. galamensis* accessions. For most of the loci, expected heterozygosity (H_e) values were higher than that of observed heterozygosity (H_o), revealing a high homozygosity at the given loci among the accessions. The diversity parameters showed high level of polymorphism among the 20 SSR markers, favoring the genetic variation within *V. galamensis* collection. In addition, East Showa and East Harerghe had the highest in effective alleles, heterozygosity, genetic diversity (Shannon diversity index) and fixation index. Hence, the two sites are the hotspot for in-situ and ex-situ conservation of *V.galamensis*.

Genetic Differentiation and Gene Flow

AMOVA demonstrated that *V. galamensis* had low variation among the population (11%). On the other hand, 67% of the total variation was attributed to genetic variability among individuals from different populations and 22% was due to variation among individuals within the same population. The result is similar to the previously reported in *Vernonia* species [33]. According to IPGRI and Cornell University [34], *F*st value ranging from 0 to 0.05 is small in genetic differentiation, from 0.05 to 0.15 is moderate, and from 0.15 to 0.25 is large, and greater than 0.25 is very large genetic differentiation among populations in terms of allele frequencies. In line with this, the extent of genetic differentiation among the ten populations in terms of allele frequencies measured was moderate (F_{st} = 0.101), which implies the presence of moderate gene flow among populations in different regions.

Genetic distance is the measure of the allelic substitutions per locus that have occurred during the separate evolution of two populations. In this study, the largest genetic distance was observed between Borena and East Harerghe (0.57) populations, while the minimum genetic distance was observed between Borena and Konso (0.24). The overall magnitude of the pairwise population matrix of Nei genetic distance was relatively low [30].

Clustering and principal co-ordinates analysis among *Vernonia galamensis* accessions

In the present study, a dendrogram tree was constructed based on the 150 accessions of *V. galamensis* collected from different geographic and agro-ecological regions. *V.galamensis* accessions were clustered into four (4) major clusters based on the allelic frequency. Cluster 1 was comprised of 39 accessions, the second cluster characterized as the second major clustering, contained 42 accessions, the third cluster composed of 15 accessions, and the fourth groups consisted of the major clustering which comprised 54 accessions that were collected from different regions of origin. The cluster analysis revealed a poor clustering pattern was observed. Hence, accessions collected from different geographic regions/zones of origins were clustered together, since clusters did not follow a clear pattern of geographic origins, which may imply the presence of gene flow between and within populations/regions/collection sites. Similarly, Nwakanma et al. [33] reported that 49 *Vernonia* lines fingerprinted using RAPD markers were grouped into four major clusters with no clear-cut separation among accessions related to their origin. Aikpokpodion et al. [32] also reported that genus *Vernonia* grouped into four major clusters and showed the existence pattern of relationships between geographical origins and genetic diversity.

Principal components (PC) analysis explores complex data sets and transforms a number of associated variables into a smaller number of PCs. In the present investigation, the principal component analysis revealed that the majority of samples were placed at the center of a two-dimensional coordinate plane and roughly forms four groups with a total variation of 30.04%. This, in turn, agrees with the results of the NJ dendrogram in which there was no unique clustering among accessions from the same population/collection areas. The presence of gene flow between and within populations/collection areas, accompanied by the prevalence of inter-gene pool introgressions/hybrids between the gene pools of origin may be the most probable explanation behind the mixed clustering of accessions from different populations/collection areas together. Although UPGMA and PCoA analyses also showed a certain level of population clustering according to their geographical regions, the clustering pattern is weak, not clustered by their regions of origin.

Populations genetic structure in *Vernonia galamensis*

The structure analyses of 150 *V. galamensis* accessions using a Bayesian-model based approach with the highest ΔK Value, according to Gilbert et al. [31] and Evanno et al. [27] method. The samples from 10 collection zones were inferred into six (K = 6) structure outputs. The structure analysis revealed a close relationship (weak sub-division) of the population from 10 collection zones, and in general, six inferred groups (K = 6) with potential admixtures have been observed. It is interesting to indicate that all individual plants analyzed have alleles originated from the six clusters, which supports the presence of a strong gene flow that led to poor population differentiation. Hence, accessions collected from the same region of origin did not often group entirely together within a given major groups. There was a wide admixture in the structuring of *V. galamensis* populations, and population genetic structure study using SSR markers, which is the first report in *V.galamensis*. Most of the magnitudes identified for the SSR markers were important information for *V. galamensis* cultivation, breeding and genetic resource conservation.

Conclusions

The present molecular genetic diversity analysis by using SSR markers was the first report in *V. galamensis*. All the twenty markers used were polymorphic in the population studied. The markers detected a large number of alleles and higher expected heterozygosity than observed heterozygosity. Generally, the results of the present study showed that there was ample allelic diversity among the *V. galamensis* accessions studied. The UPGMA and PCoA showed a certain level of populations clustered according to their geographical regions, but the pattern of clustering was weak as it did not show clustering by their regions of origin. Most of the values identified for the SSR markers were important baseline information for future *V. galamensis* cultivation and breeding/genetic resource conservation endeavors in Ethiopia. As the present study used only 20 SSR markers, it was not enough to cover all the actual genetic diversity in *V. galamensis*. Hence, more such markers, as well as other appropriate and up-to-date molecular marker systems are important to generate reliable and exploitable data in this regard for the use in breeding and conservation programs.

Abbreviations

AMOVA: Analysis of molecular variance; CTAB: Cetyltriethyl ammonium bromide; He: Expected heterozygosity; Ho: observed heterozygosity; NJ: Neighbor joining; PCoA: Principal coordinate analysis; PIC: Polymorphic information content; SSR: simple sequence repeat; UPGMA: Unweighted pair group with arithmetic mean

Declarations

Ethics approval and consent to participate

Not applicable

Consent for publication

Not applicable

Availability of data and materials

Pass port data of 150 *Vernonia galamensis* samples representing the 10 populations used in the current study are provided in Additional file 1. Unweighted neighbor joining based clustering of 150 *Vernonia galamensis* accessions for 20 polymorphic SSR

markers are provided in Additional file 2. AMOVA variation pie chart for 150 *Vernonia galamensis* accessions from ten populations in Ethiopia are provided in Additional file 3.

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

AM, KT and KD designed the study. AM and XH coordinate and carried out the laboratory work. AM performed statistical data and wrote the manuscript. All the authors read and approved the final manuscript.

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Tables

Table 1. *Vernonia galamensis* samples used in the current study

Population	Sample size	Altitude ranges (m)	Co-ordinates	
			Latitude ranges	Longitude ranges
Borena	15	1090-1200	4°88’- 4°90’N	39°35’- 39°40’E
East Showa	15	1630-1643	7°56’- 8°90’ N	38°43’- 39°70’E
West Arsi	15	2000-2143	7°15’- 8°90’ N	38°38’- 38°42’E
East Harerghe	16	1574-2750	9°06’- 9°25’ N	41°25’- 41°38’E
West Harerghe	14	1393-1889	8°56’- 9°13’ N	40°52’- 42°27’E
West Gojjam	14	1205-2560	10°27’- 10°30’ N	38°12’- 39°09’E
South Wollo	16	1866-2630	9°51’- 11°08’N	39°10’- 39°38’E
Sidama	15	1708-1780	6°51’- 7°15’ N	37°45’- 38°27’E
Konso	15	1500-1650	5°15’- 5°20’ N	37°27’- 37°40’E
Derashie	15	1395-1450	6°18’- 6°25’ N	36°53’- 37°09’E

Table 2. Primer sequences, annealing temperature and amplicon sizes which were used for genetic diversity studies in *Vernonia galamensis*

SSR primers	SSR motifs (5’-3’)	Forward Sequence (5’-3’)	Reverse Sequence (5’-3’)	Annealing tem	Product Size
Vg-001	(AT)12	CTTGATTTTGTGGGGACCTAAGTG	TAGGAATGGAATAGAATGGATCGG	56	135
Vg-002	(TC)12	GGGTTGTGGGGAGAGATAGAGATA	AGCCAAGTTACGCATAGACATCTG	56	146
Vg-003	(TC)24	GTGAGCGGGGATCTTCACTTC	GAGAAAGCGAGCATCAACAGACTT	56	142
Vg-004	(CCA)12	ACCATACAGTCCCGCATGAATATC	GCTCCTGGAAATGGAGGATAGAAT	58	145
Vg-005	(AAG)12	AGCTTAAACAAGAACAAACCGCTG	TGCGAAGGCTTACCAGTTACAAAC	56	157
Vg-006	(ACA)12	ATCAGCGTTGCTTGAAAAGAGTG	AAACTCATCGCTCAAACTCAAACG	58	101
Vg-007	(TCT)18	ATGACGATGCAACTCACCGTT	CGGAGAGGTTTGGTAGGGTAAGAT	56	136
Vg-008	(GAT)24	ATGTCTTCCAAATCGAGGATGGTA	AATTTTTGCAGCTAAGCCAGTGAG	58	101
Vg-009	(GGA)15	ATTGAAGGATGAACGGACAGAGTC	GTATCACATCACGTCGTCCACATC	58	127
Vg-010	(ATG)15	CAAAGGGAAGATGCACCTAGAGAA	ATCAAACCTGCTGCTTTTCAAGTC	58	130
Vg-011	(TGA)12	GCACAATCAGACTTGAGACCAAGA	GCAGTGATCAGCCATAGTGCATAC	58	130
Vg-012	(CGC)12	GGGCTGAGCAAATACAGCAGAC	AGGATCTTCTTGTTGGTGTGGAAA	58	150
Vg-013	(CAG)15	GGGGCGTTTCCTTGATTTTG	CTCTTCACCTGCCATCTTTTCTGT	56	93
Vg-014	(CAA)12	GTAGCAGCAGCAGTTCACTACCAC	CAAAATCCTCACAAACTTCACACG	56	132
Vg-015	(GGC)18	GTGCTACAACGGTGGTACATCAAG	TCATTGATTCCATGCTGAAATAGC	56	159
Vg-016	(GGT)12	GTTAGAGATGGGTTTGAAGAGCGA	CCTTACCAACTCCAACACCACTTG	58	139
Vg-019	(CTTCAC)24	GGGTCTCCATCTATTCACCTTCAA	AAGGAGCGTGAGCTAGAAGAAGC	56	158
Vg-021	(GTC)15	TGAAGAAGAAGGTTTCCCAAATCA	GATGCATTGACATCACGTAGAAGC	56	155
Vg-024	(ATC)15	TTGGATGTCGAAAAAGATGAGGTT	TTCTCCCTCTGTTTCAACACCTTC	56	144
Vg-030	(CT)12	TCAAACACACTCCCCAATTTCTCT	GCTGCCGATTGATCAAATTACACT	56	100

Table 3: Summary of genetic parameters revealed by using 20 SSR markers for *Vernonia galamensis* populations collected from different regions of Ethiopia

Locus	MAF	Na	Ne	I	Ho	He	uHe	F	PIC
Vg-01	0.85	3	2.80	1.06	0.25	0.48	0.49	0.25	0.80
Vg-02	0.77	5	3.26	1.36	0.13	0.50	0.51	0.73	0.96
Vg-03	0.45	6	4.79	1.67	0.36	0.65	0.64	0.77	0.62
Vg-04	0.52	3	2.28	0.90	0.07	0.59	0.61	0.88	0.64
Vg-05	0.64	3	2.01	0.89	0.13	0.60	0.63	0.78	0.50
Vg-06	0.54	5	3.69	1.44	0.13	0.44	0.45	0.70	0.86
Vg-07	0.61	4	3.46	1.31	0.20	0.58	0.60	0.66	0.57
Vg-08	0.59	5	3.26	1.36	0.20	0.61	0.63	0.67	0.89
Vg-09	0.61	3	2.60	1.01	0.23	0.45	0.47	0.49	0.87
Vg-10	0.60	4	3.81	1.36	0.07	0.46	0.48	0.86	0.86
Vg-11	0.56	4	2.03	0.95	0.13	0.23	0.24	0.42	0.93
Vg-12	0.57	3	2.53	1.01	0.07	0.46	0.48	0.86	0.84
Vg-13	0.49	4	3.57	1.33	0.13	0.48	0.50	0.72	0.73
Vg-14	0.60	3	2.27	0.95	0.27	0.48	0.50	0.44	0.77
Vg-15	0.76	4	2.68	1.16	0.08	0.50	0.52	0.85	0.88
Vg-16	0.64	2	1.99	0.86	0.07	0.36	0.37	0.81	0.78
Vg-19	0.58	4	3.46	1.31	0.33	0.64	0.67	0.48	0.70
Vg-21	0.51	5	4.41	1.55	0.05	0.60	0.62	0.89	0.86
Vg-24	0.67	5	3.81	1.46	0.27	0.62	0.65	0.57	0.53
Vg-30	0.61	3	2.53	1.01	0.07	0.28	0.29	0.76	0.55
Mean	0.61	3.9	3.06	1.20	0.16	0.50	0.52	0.68	0.76

Key: MAF = major allele frequency, Na = number of different alleles, Ne = number of effective alleles, I = Shannon's information index, Ho = observed heterozygosity, He = expected heterozygosity, uHe = unbiased expected heterozygosity, F = fixation index, PIC = polymorphic information content.

Table 4. Important allelic values recorded in the ten populations of *Vernonia galamensis*

Population	N	Na	Ne	I	Ho	He	Uhe	F
Borena	15	3.00	1.98	0.76	0.13	0.48	0.50	0.74
Sidama	15	4.00	2.03	0.74	0.12	0.47	0.51	0.72
East Showa	15	5.00	4.74	0.82	0.15	0.51	0.55	0.68
West Arsi	15	3.00	2.07	0.70	0.13	0.45	0.47	0.70
E. Harerghe	16	4.00	3.68	0.79	0.12	0.49	0.53	0.73
West Harerghe	14	4.00	3.12	0.77	0.16	0.48	0.50	0.73
Gojjam	14	3.00	2.33	0.70	0.13	0.47	0.49	0.64
Wollo	16	4.00	2.74	0.72	0.14	0.48	0.52	0.68
Konso	15	3.00	2.21	0.71	0.13	0.45	0.48	0.72
Derashie	15	2.00	1.74	0.75	0.09	0.43	0.47	0.81
Average across population	15	3.50	2.70	0.75	0.13	0.47	0.50	0.72

Key: Na = number of different alleles, Ne = number of effective alleles, I = Shannon's diversity index, Ho = observed heterozygosity, He = expected heterozygosity, uHe = unbiased expected heterozygosity, F = fixation index, PIC = polymorphic information content

Table 5. Analysis of molecular variance (AMOVA) showing the distribution of genetic diversity within and among populations and among individuals of *Vernonia galamensis* collected from different regions of Ethiopia

Source	Df	SS	MS	Est. Var.	% Variation	F-Statistics	Value	P
Among Populations	9	322.71	35.86	0.76	11%	F_{st}	0.101	0.001
Among Individual	140	1842.01	13.16	6.39	67%	F_{is}	0.946	0.001
Within individual	150	55.21	0.37	0.37	22%	F_{it}	0.951	0.001
Total	299	2219.93		7.52	100%			

Df = Degrees of Freedom; SS = Sum of Squares; MS = Mean Square; Est. Var. = Estimated Variability

Table 6. Pairwise population matrix of Nei standard genetic distance (GD)

Population	BOR	SID	ESH	WAS	EHG	WHG	GOJ	WOL	KON	DER
BOR	**	0.47	0.37	0.43	0.57	0.40	0.46	0.36	0.24	0.41
SID		**	0.59	0.37	0.39	0.54	0.51	0.44	0.50	0.36
ESH			**	0.49	0.44	0.47	0.34	0.29	0.42	0.27
WAS				**	0.44	0.38	0.45	0.36	0.35	0.47
EHG					**	0.47	0.47	0.40	0.39	0.25
WHG						**	0.41	0.35	0.31	0.41
GOJ							**	0.36	0.37	0.38
WOL								**	0.37	0.42
KON									**	0.35
DER										**

Keys: BOR = Borena, SID = Sidama, ESH = East Showa, WAS = West Arsi, EHG = East Harerghe, WHG = West Harerghe, GOJ = Gojjam, WOL = Wollo, KON = Konso, DER = Derashie

Figures

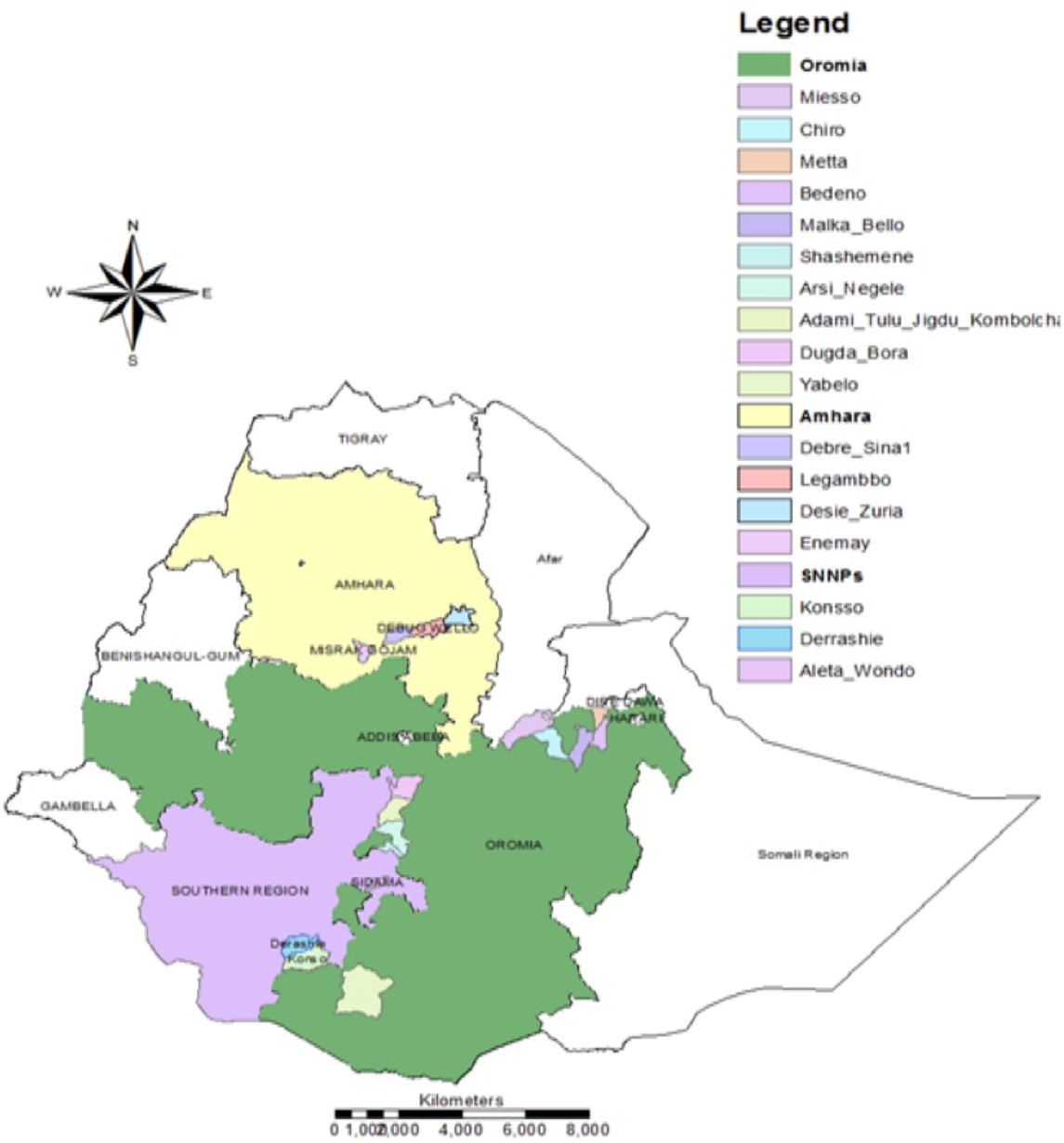

Figure 1

Map of Ethiopia showing Vernonia galamensis collection sites within three of the Federal Regions. The map was original and constructed using geographic coordinates and elevation data gathered from each collection sites using global positioning system (GPS) that represent the 10 populations.

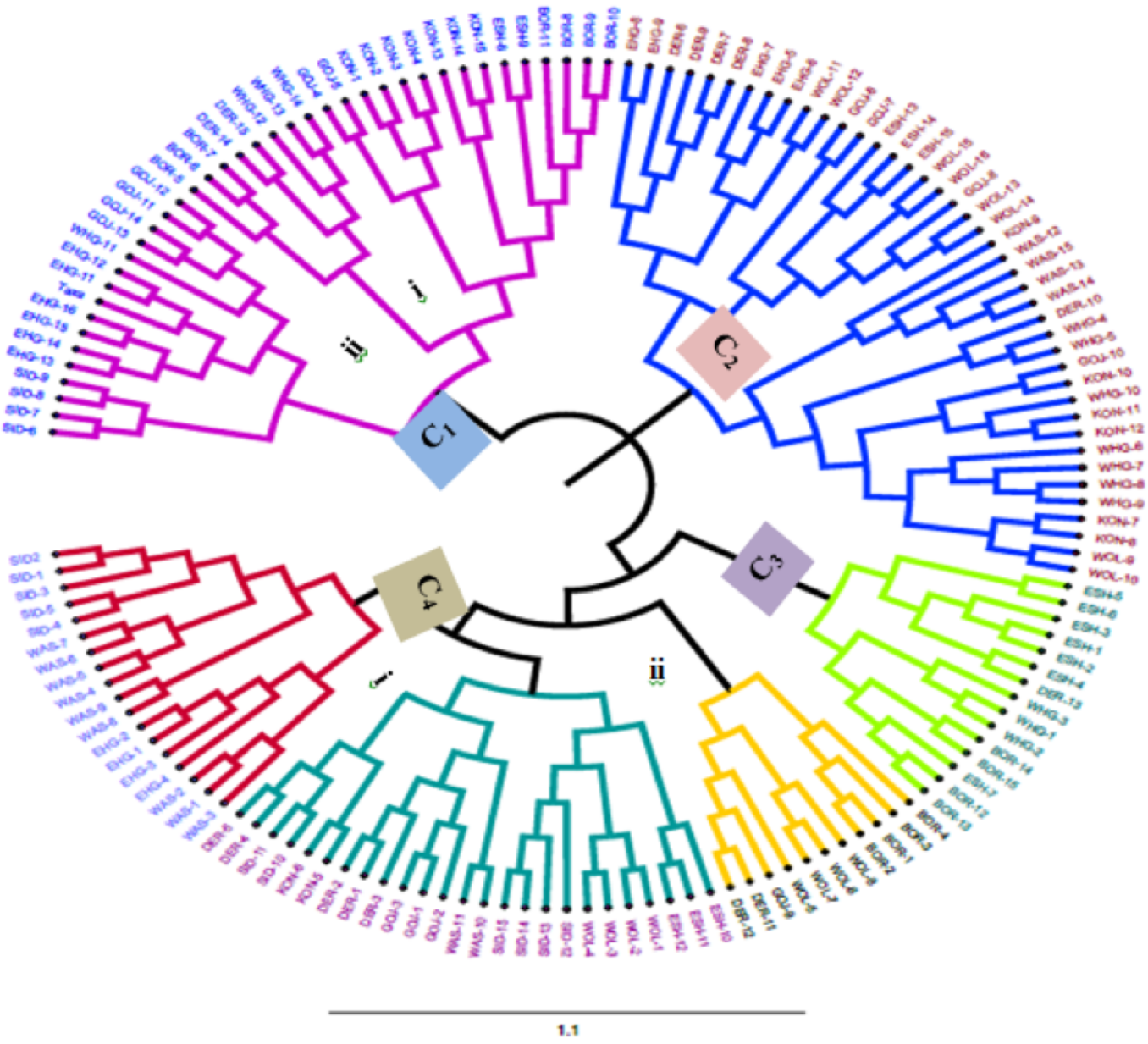

Figure 2

Neighbor-joining tree of the 150 Vernonia galamensis accessions constructed by Darwin software program. Keys: BOR = Borena, SID = Sidama, ESH = East Showa, WAS = West Arsi, EHG = East Harerghe, WHG = West Harerghe, GOJ = Gojjam, WOL = Wollo, KON = Konso, DER = Derashie

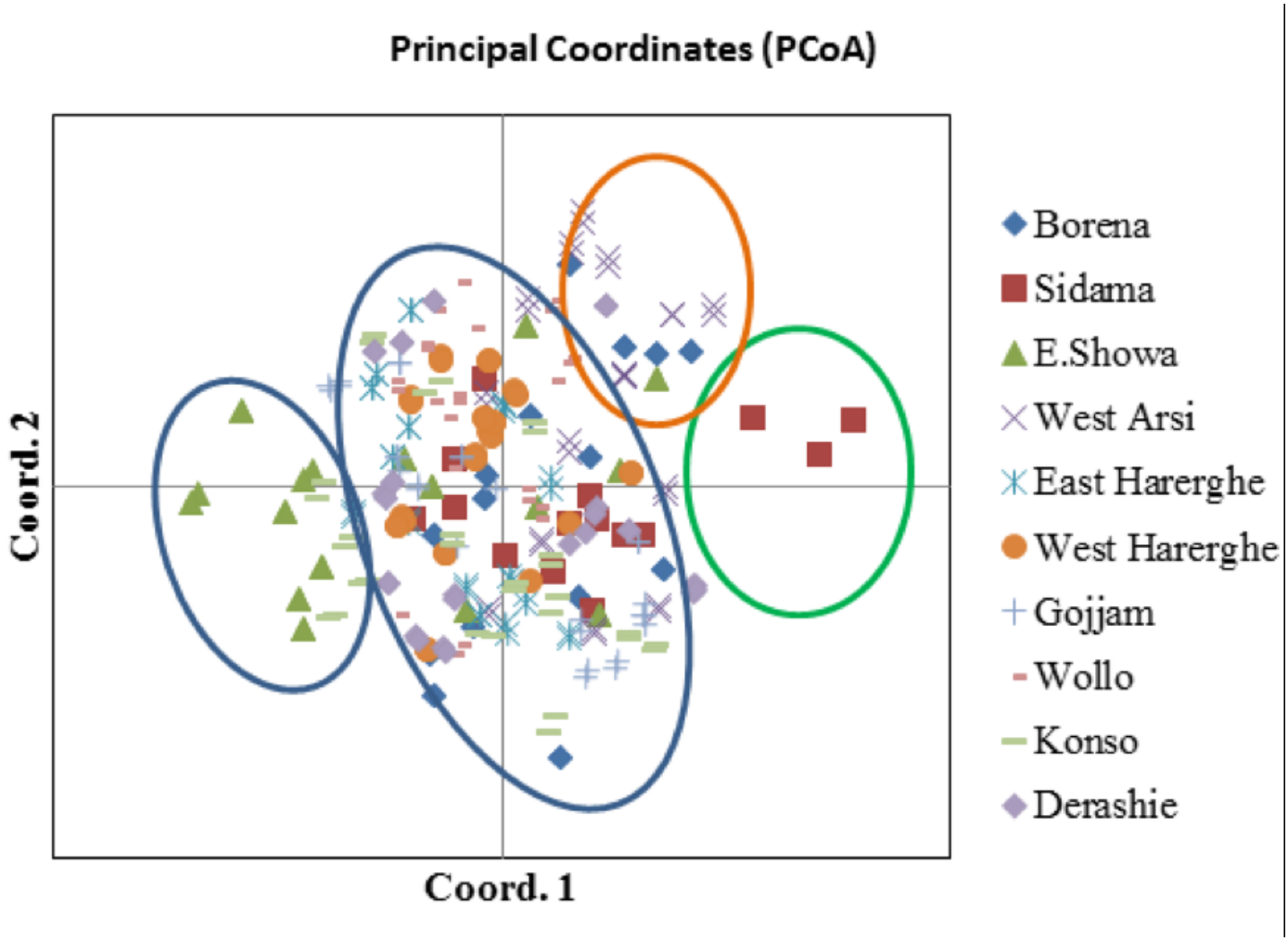

Figure 3

Two dimensional scaling principal coordinate analyses of 150 Vernonia galamensis accessions of ten populations.

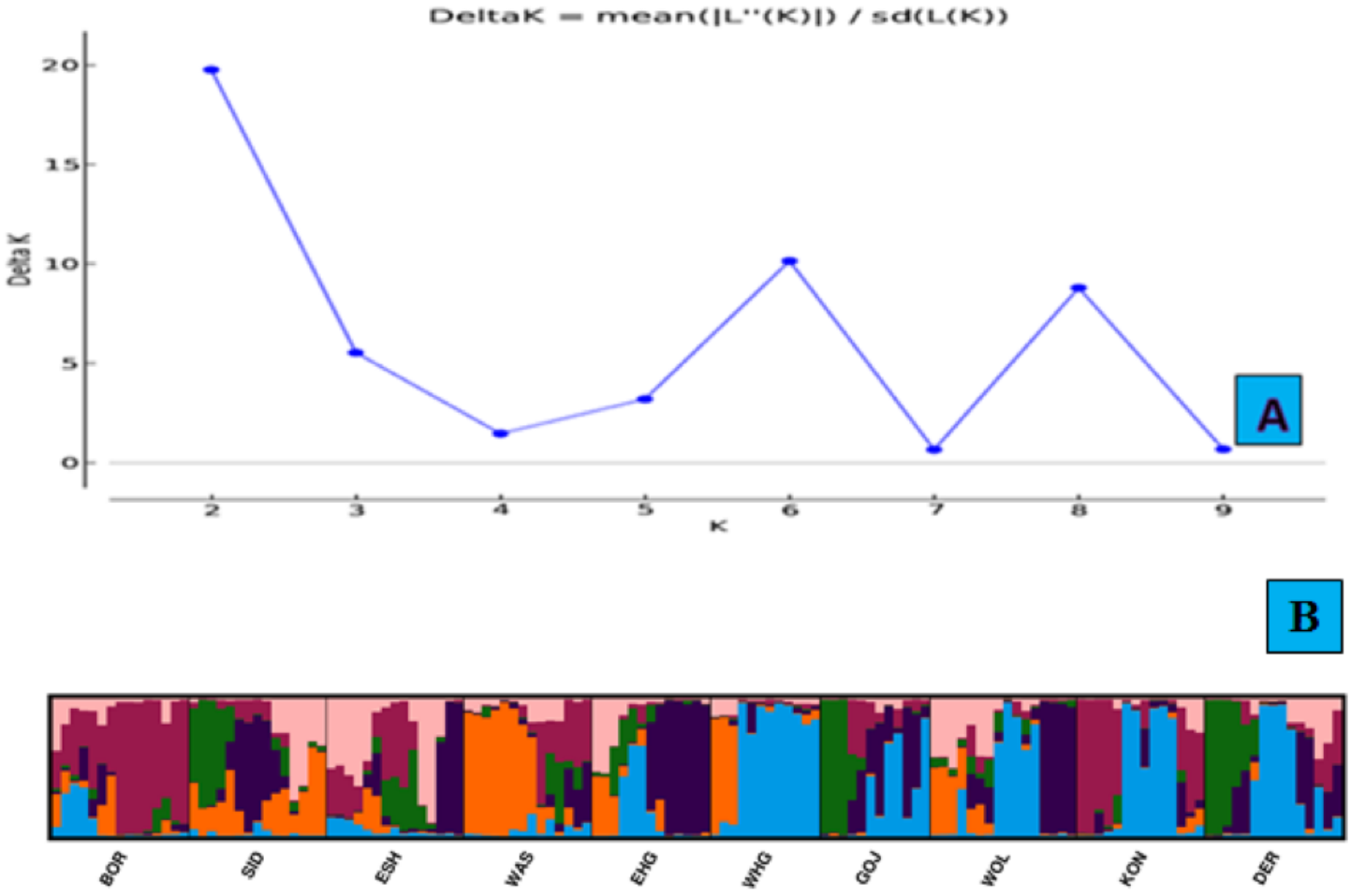

Figure 4

Delta K values estimated according to Evanno et al. 2005 Method (A) and Bayesian model-based estimation of population structure (K = 6) (B) for 150 Vernonia galamensis accessions from different growing regions of Ethiopia. Keys: BOR = Borena, SID = Sidama, ESH = East Showa, WAS = West Arsi, EHG = East Harerghe, WHG = West Harerghe, GOJ = Gojjam, WOL = Wollo, KON = Konso, DER = Derashie

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