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Research Article

Morphological Characterization of Ethiopian Sweet Basil (*Ocimum basilicum* L.) Accessions for Qualitative Traits

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Sweet basil (*Ocimum basilicum*) exhibits wide morphological variability because of centuries of cultivation, long-term traditional uses, selection, and breeding efforts. But the extent of morphological diversity is not well investigated. The objective of this study was to assess the morphological variability of sweet basil accessions using qualitative morphological traits. Forty-nine accessions of basil were used in a 7×7 simple lattice design. Shannon–Wiener diversity indices showed the existence of wider genetic variability among the accessions for qualitative traits. Shannon–Wiener diversity indices showed a maximum value (0.96) for leaf blade glossiness and flower color of the corolla and the lowest value (0.53) for the color of style. The first four principal components were able to explain 75.58% of the total variation. Cluster analyses based on qualitative traits revealed four distinct clusters. The first, second, third, and fourth clusters consisted of 46.94%, 6.12%, 42.86%, and 4.08% of the accessions, respectively. Thus, the current study reveals the existence of substantial levels of phenotypic variability that could be exploited as a source of a valuable gene pool for future breeding programs.

Keywords: diversity; germplasm; medicinal; morphological; qualitative; sweet basil

1. Introduction

Sweet basil (*Ocimum basilicum* L.) is an important group of aromatic and medicinal plants that belongs to the Lamiaceae family [1]. The typical characteristics of this family are a square stem and opposite and decussate leaves with many gland dots. Sweet basil is a nonwoody perennial that is widely distributed almost all over the temperate and tropical regions of the world [2]. The origin of *O. basilicum* is uncertain; some reported that it is originated in Egypt and East Mediterranean [3], but Hossein Gholami basil is supposed to be originally native to India and other tropical regions of Asia, having been cultivated for more than 5000 years [4]. The genus *Ocimum* is characterized by great variability among its constituent species, including morphology, growth habit, the color of flowers, leaves, stems, and chemical composition [1, 5].

Sweet basil's essential oils are used in perfume, pharmacy, cosmetic, food industries, and aroma therapy [4, 6, 7]. Medicinally, it is useful in a variety of human and animal diseases treatment such as malaria, colic, vomiting, common cold, cough, and skin diseases [8–10]. Externally, they have been applied for the treatment of acne, loss of smell, insect stings, snake bites, and skin infections [11, 12].

In Ethiopia, basil has been used for thousands of years as a spice and medicinal herb. The leaves and flowers are dried, ground, and added to different kinds of locally prepared foods (Jansen, 1981). Sweet basil is cultivated and widely used throughout the country; it is cultivated in every provenance of Ethiopia. Farmers use this crop for house consumption as well as to provide it for the local market [13–15]. It has strong domestic market demand for essential oil in Ethiopia with an estimated value of USD 5–7 million per year [16].

Evaluation and documentation of existing diversity are essential to maintain the exploration of the genetic variability in sweet basil breeding programs. According to different scholarly reports on basil accessions, there is wide genetic diversity throughout the world [13, 17–23]. The morphological diversity within basil is a result of centuries of cultivation, long-term traditional uses, selection, and breeding efforts [24]. In Ethiopia, there is a high genetic diversity of sweet basil in qualitative characters [13]. However, the previous study was done on only a few accessions and within a limited geographic region. So, this study gives more information about the genetic diversity of basil in Ethiopia.

Morphological characterization is the first and the cheapest step in the description and classification of the accessions. In Ethiopia, scant trials have been conducted on morphological, chemical, and molecular trait diversity [13, 22, 23, 25]. However, limited effort has been made in the evaluation of qualitative morphological trait variability. However, analyzing qualitative morphological traits and knowing the extent and patterns of morphological variation among accessions and populations is vital for efficient management, enhancement, and utilization of the crop. Given the scanty studies regarding the characterization of the available basil accessions and the significance of the study toward its use in the collection, conservation, and breeding programs; this experiment was designed to determine the morphological variability of Ethiopian basil accessions using standard qualitative morphological descriptors.

2. Materials and Methods

2.1. Description of the Study Area. The study was conducted at the experimental field of Wondo Genet Agricultural Research Center from 2019 to 2020. Wondo Genet is located in Sidama Regional State, which is 264 km south-east of Addis Ababa. The geographical coordinates of the research site are 7°19′N and 38°38′E with an altitude of 1780 m above the sea level. The site has mean maximum and minimum temperatures of 26°C and 12°C, respectively. The mean annual rainfall is 1120 mm. The soil type of the experimental area is Nitosols, and it has a textural class of sandy clay loam with a pH of 6.4 [26].

2.2. Planting Material and Experimental Design. A total of forty-nine (49) basil accessions were used in this particular study (Table 1). These included twenty-two accessions obtained from the Ethiopian Biodiversity Institute (EBI), two released varieties of sweet basil from Wondo Genet Agricultural Research Center, 23 accessions collected from different parts of the country (SNNP, Oromia, and Harari), and two accessions obtained from abroad (one from Norway and the other from Israel) (Figure 1). The experimental fields were arranged in a 7×7 simple lattice design with two replications. Spacing of 1.5 m and 1m was maintained between replications and plots, respectively. The experimental field consisted of 2.4 m × 3.6 m plots each containing six rows with 40 cm intrarow and 60 cm inter-row spacings. Basil seed was seeded in a 10 cm polyethylene bag in 2019 in the Wondo Genet Agricultural Research Center greenhouse. Watering was done twice a week after all of the seedlings had emerged. After five weeks, the seedlings were transplanted in a lath house for hardening off for one week. Before transplanting the seedlings to the main field, the experimental plot was prepared by plowing, hoeing, and leveling. Well-performed seedlings were transplanted to the main experimental field after six weeks. Each experimental plot contained a total of 36 plants. All horticultural management was done as required.

Table 1: Codes and descriptions of the characters used for the study.

Characters	Code	Description
Growth habit	1	Rounded
	2	Intermediate
	3	Erect
Stem: anthocyanin coloration	1	Absent
	9	Present
Stem: intensity of anthocyanin coloration	3	Weak
	5	Medium
	7	Strong
Stem: hairiness	1	Absent
	9	Present
Leaf blade: shape	1	Broad ovate
	2	Ovate
	3	Elliptic
Leaf blade: anthocyanin coloration	1	Absent
Leaf blade: intensity of anthocyanin coloration	3	Weak
	5	Medium
	7	Strong
Leaf blade: distribution of anthocyanin	1	Few mottles
		Many
	2	Mottles
	3	Total surface
Leaf blade: green color	3	Light
	5	Medium
	7	Dark
Leaf blade: glossiness	1	Absent
	3	Weak
	5	Medium
Leaf blade: profile in cross section	1	Convex
	3	Concave
	4	V-shaped
Leaf blade: serration of margin	1	Absent
	9	Present
Leaf: hairiness	1	Absent
	9	Present
Flower: color of corolla	1	White
	2	Pink
	3	Dark violet
Flower: color of style	1	White
	2	Light violet
Flowering stem: total length	3	Short
	5	Medium
	7	Long
Flowering stem: hairiness of bracts	1	Absent
	9	Present

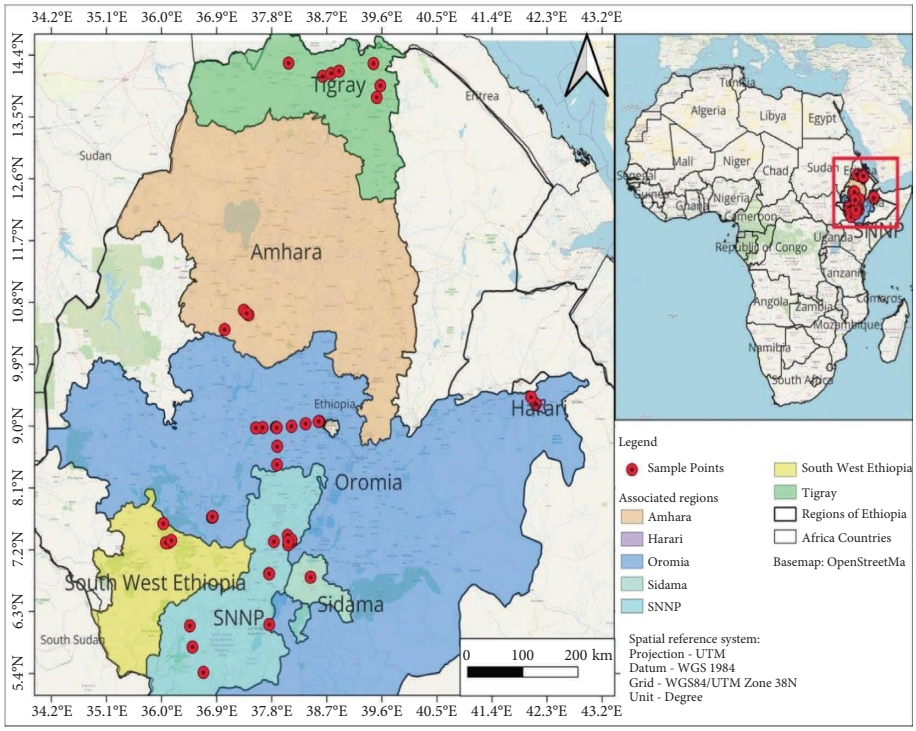


Figure 1: Map of Ethiopia showing the collection region of basil.

2.3. Data Collection. After sixty days, 17 qualitative morphological traits data were recorded using International Union for the Protection of New Varieties of Plants descriptors given for basil [27] (Table 1). Ten representative samples per plot were selected, and data were recorded as described in Table 2.

2.4. Data Analysis. To identify the patterns of morphological variation, principal component analysis (PCA) was conducted. PCA with eigenvalues of more than 1 was considered for interpretation as recommended by [28].

Hierarchical clustering, the ward method of analysis, was performed to group accessions and construct a dendrogram using [29] R software Version 4.1.0., 2021. The diversity index (H') of [30] Shannon and Wiener was used to measure the level of genetic variation for qualitative traits. The index (H') was estimated as follows:

$$H' = \frac{-\sum_{i=1}^{n} P_i * \ln(P_i)}{\ln(n)}, \quad (1)$$

where H' = Shannon diversity index; P_i = the proportion of accessions in the ith class of an n–class character; and n = the number of phenotypic classes of traits. To normalize and keep the value between 0 and 1, each diversity index value was divided by Ln (n).

3. Results and Discussion

3.1. Frequency Distribution of Qualitative Characters. Frequency distribution, percent of proportion, and Shannon–Wiener Diversity Index were estimated for 49 basil accessions from 17 qualitative characters and results are presented in Table 2.

The major (67.34%) growth pattern among the accessions was erect, 20.41% of the accessions showed an intermediate pattern of growth habit, and only 12.24% showed rounded growth habit.

The intensity of stem anthocyanin coloration was also distributed with the percent of weak (69.39%), medium (14.28%), and strong 16.33%) (Figures 2(a), 2(b), and 2(c), respectively).

Stem and leaf hairiness were present in most of the stems (65.31%) and leaves (69.38%) (Figures 3(a) and 3(b)) of the collected accessions. Egata et al. [13] also reported that most Ethiopian basil accessions have dense hair on their leaves. Most of the leaf blade shapes of the collected accessions were ovate (53.06%), a few accessions broad ovate (4.08%), and the rest of the accessions elliptic (42.86%) leaf blade shapes (Figures 4(a), 4(b), and 4(c)).

The intensity of leaf blade anthocyanin coloration is weak in 59.18%, medium in 36.73%, and strong in only 4.08% of the accessions. Most leaf blade colors are medium green color (61.22%), light green (16.32%), and dark green (22.45%). The leaf blade glossiness is absent (14.28%), weak (55.1%), and medium (30.61%) of the collected accessions. Most of the flower color of Ethiopian basil was pink (55.10%). The other accessions had dark violet (30.61%) and white (14.28%) colors (Figures 5(a), 5(b), and 5(c)).

Patel et al. [31] reported that most of the *Ocimum* genotypes showed erect growth patterns and ovate leaf shape. Singh et al. [20] also reported that *Ocimum* species show variability in leaf shape and color of flower. This result is in line with the report of Egata et al. [13] which indicated that the flower color of major Ethiopian basil accessions was pink and also most of the leaves had dense hair. Malav et al. [18]

Table 2: Qualitative traits, descriptor, code, frequency, percent, and Shannon–Wiener diversity index (H') of qualitative traits of 49 Ethiopian basil accessions.

Qualitative traits	Descriptor	Code	Frequency	Percent	H'
Growth habit	Rounded	1	6	12.24	0.85
	Intermediate	2	10	20.41	
	Erect	3	33	67.34	
Stem: anthocyanin coloration	Absent	1	19	38.77	0.67
	Present	9	30	61.22	
Stem: intensity of anthocyanin coloration	Weak	3	8	14.28	0.83
	Medium	5	34	69.39	
	Strong	7	7	16.33	
Stem: hairiness	Absent	1	17	34.69	0.65
	Present	9	32	65.31	
Leaf blade: shape	Broad ovate	1	2	4.08	0.83
	Ovate	2	26	53.06	
	Elliptic	3	21	42.86	
Leaf blade: anthocyanin coloration	Absent	1	27	55.1	0.69
	Present	9	22	44.9	
Leaf blade: intensity anthocyanin	Weak	3	29	59.18	0.81
Coloration	Medium	5	18	36.73	
	Strong	7	2	4.08	
Leaf blade: distribution of anthocyanin	Few mottles	1	31	63.26	0.9
	Many mottles	2	11	22.45	
	Total surface	3	7	14.28	
Leaf blade: green color	Light	3	8	16.32	0.93
	Medium	5	30	61.22	
	Dark	7	11	22.45	
Leaf blade: glossiness	Absent	1	7	14.28	0.96
	Weak	3	27	55.1	
	Medium	5	15	30.61	
Leaf blade: profile in cross section	Convex	1	30	61.22	0.9
	Concave	3	9	18.38	
	V-shaped	4	10	20.4	
Leaf blade: serration of margin	Absent	1	33	67.34	0.63
	Present	9	16	32.65	
Leaf: hairiness	Absent	1	15	30.61	0.62
	Present	9	34	69.38	
Flower: color of corolla	White	1	7	14.28	0.96
	Pink	2	27	55.1	
	Dark violet	3	15	30.61	
Flower: color of style	White	1	11	22.45	0.53
	Light violet	2	38	77.55	
Flowering stem: total length	Short	3	29	59.18	0.81
	Medium	5	18	36.73	
	Long	7	2	4.08	
Flowering stem: hairiness of bracts	Absent	1	15	30.61	0.62
	Present	9	34	69.38	

examined the genetic diversity of 49 holy basil accessions using 18 qualitative morphological traits. Yildiz and Camlica [32] reported three leaf colors (green, purple, and purple-green) of basil. Shannon–Wiener diversity index (H') was used to compare diversity among qualitative traits. The value of the Shannon diversity index ranged from 0.53 to 0.96. The higher value of H' was observed for flower color (0.96), leaf blade glossiness (0.96), and leaf blade color (0.92). The lowest value was recorded for the trait style color (0.53). The higher value indicated more diversity in that qualitative trait and vice versa. Carović-Stanko et al. [24] reported a higher Shannon information index for leaf blade glossiness and the lowest value obtained for stem hairiness and leaf blade serration of margin.

3.2. PCAs for Qualitative Traits. PCA was done on 17 qualitative traits of the 49 basil accessions. Four principal components (PCs) were retained based on eigenvalues and scree plot (Table 3 and Figure 6). The first four PCs captured

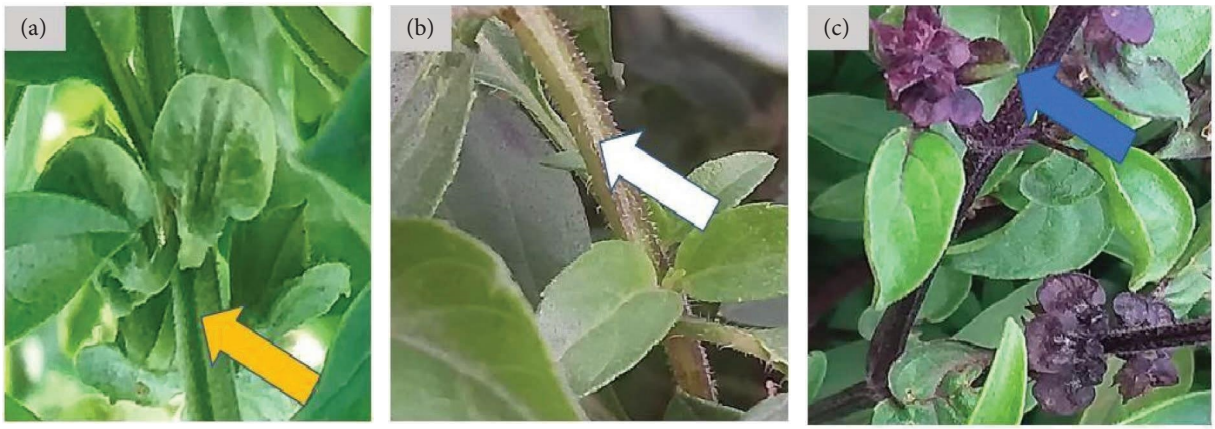


Figure 2: Weak (a), medium (b), and strong (c) intensity of stem anthocyanin coloration of basil accessions.

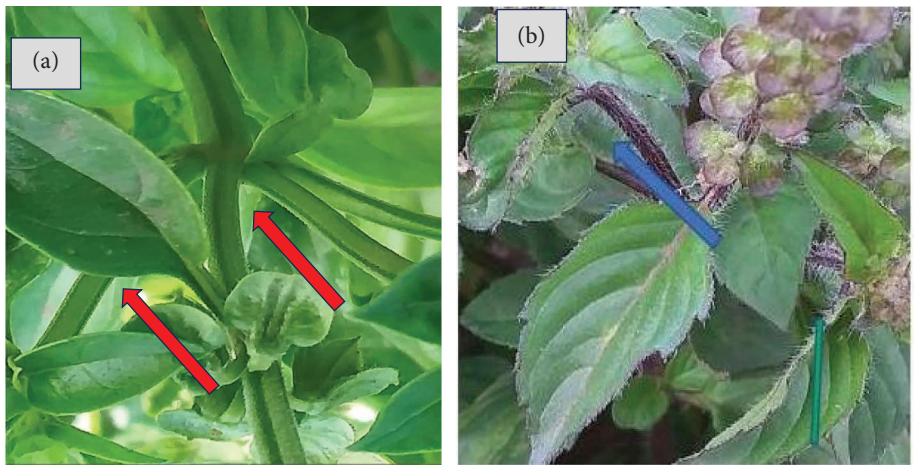


Figure 3: Stem and leaf blade hairiness absent (a) and present (b).

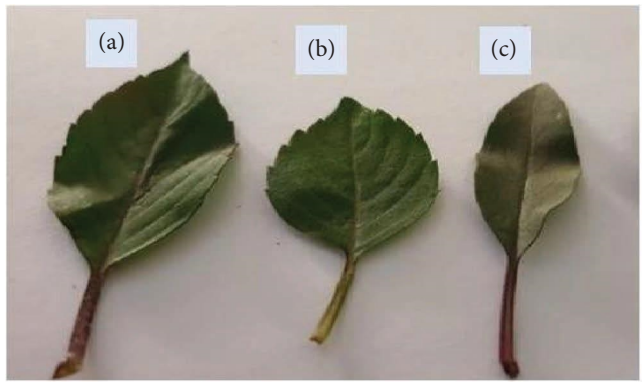


Figure 4: Leaf blade shape ovate (a), broad ovate (b), and elliptic (c).

75.58% of the variability in the collected basil accessions. The qualitative traits showed that the first four PCs explained 34.99%, 18.39%, 15.23%, and 6.97% of the total variance, respectively. Maximum variability was contributed by the first PC (34.99). Similarly, the first four PCs gave about 75.53% of the variability based on the morphological quantitative traits of similar accession to this work in Ethiopia [23]. Likewise, Brisibe et al. [33] reported that the first three PCs contributed 71.05% of the total variability using 28 accessions of *O. gratissimun* using nine quantitative traits in Nigeria. As opposed to this, Malav et al. [18] reported that the first three components explained very high portion (91.55%) of the total variability in *O. tenuiflorum* L. from 49 accessions in India. Chowdhury et al. [19] also

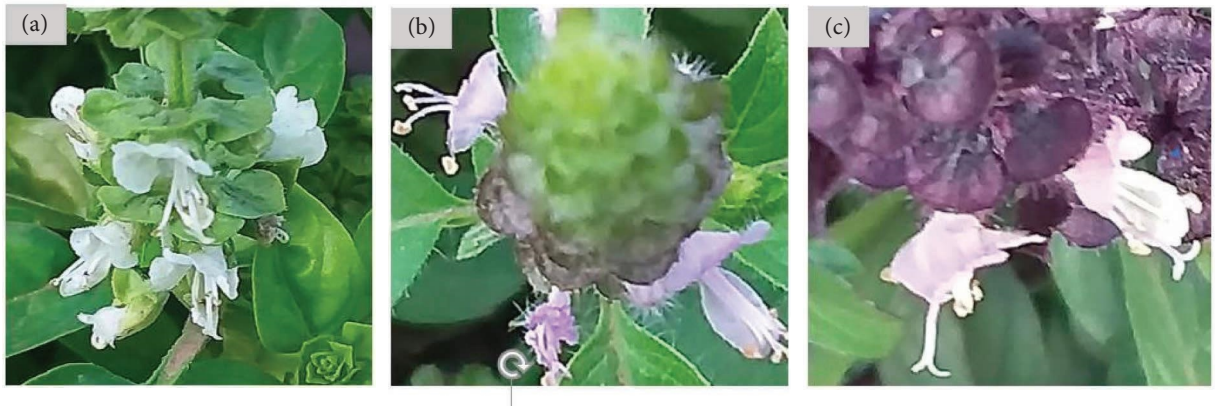


Figure 5: Flower color of basil accessions: white (a), pink (b), and dark violet (c).

Table 3: Eigenvectors and eigenvalues of the first four principal components (PCs) for 17 qualitative traits of basil accessions.

Trait	PC1	PC2	PC3	PC4
Eigenvalue	5.24	2.76	2.28	1.04
Proportion	34.99	18.39	15.23	6.97
Cumulative	34.83	53.38	68.61	75.58
Eigenvectors				
Leaf blade: shape	0.47	0.05	−0.24	0.63
Stem: anthocyanin coloration	0.25	0.57	0.23	−0.03
Stem: intensity anthocyanin coloration	0.77	0.04	0.51	−0.06
Growth habit	−0.97	0.13	0.01	0.04
Flower: color of corolla	0.36	0.02	0.45	0.52
Leaf blade: anthocyanin coloration	0.26	0.92	0.01	−0.08
Leaf blade: intensity anthocyanin coloration	0.22	0.9	0.00	−0.15
Leaf blade: distribution of anthocyanin	0.87	−0.08	−0.48	0.21
Leaf blade: green color	−0.04	0.66	−0.42	0.29
Leaf blade: profile in cross section	−0.67	−0.04	0.00	0.13
Leaf: hairiness	−0.71	0.24	0.56	0.05
Stem: hairiness	−0.97	0.12	0.01	0
Leaf blade: glossiness	0.51	0.15	−0.6	−0.38
Flower: color of style	0.1	0.22	0.85	−0.06

Abbreviation: PC = principal component.

reported that the first three PCs contributed 82.85% of the total variability using nine genera of *Ocimum* using nine quantitative traits in India.

The criterion of Raji [34] was chosen to decide the cutoff limit for the coefficients of the proper vectors; this criterion treated coefficients greater than 0.3 as having a large enough effect to be considered important, while traits having a coefficient less than 0.3 were considered not to have important effects on the overall variation observed in the study. In this study, traits with eigenvectors > 0.3 comprising leaf blade distribution of anthocyanin (0.87), stem: intensity of anthocyanin coloration (0.77), leaf blade glossiness (0.51), leaf blade shape (0.47), and flower color (0.36) contributed maximum toward the first PC (Table 3).

In the second PC, leaf blade anthocyanin coloration (0.92), leaf blade intensity anthocyanin coloration (0.90), leaf blade green color (0.66), and stem anthocyanin coloration (0.57) contributed more while style color (0.85), leaf hairiness (0.56), and stem intensity anthocyanin coloration (0.51) and flower color of corolla contributed more for the third PC. The fourth PC was influenced more only by the leaf blade shape (0.63) and the flower color of the corolla (0.52).

3.3. Cluster Analyses for Qualitative Traits. The distribution pattern of qualitative traits of basil accessions was shown by cluster analysis. From the hierarchical ward method, four distinct groups were obtained based on the qualitative morphological traits using Euclidean distance by Ward's method (Figure 7). Cluster I was the largest cluster containing 23 accessions (46.94%), followed by Clusters III, II, and IV which contain 21, 3 and 2 accessions, respectively. Among the 23 accessions grouped in Cluster I, 39.13%, 21.74%, 8.69%, 8.69%, 8.69%, 8.69%, and 4.35% of the accessions were collected from Oromia, Tigray, SNNP, Amahara, Harari, SWE, and Sidama regional states, respectively. Likewise, in Cluster II, 66.67% and 33.33% of the accessions were collected from SNNP and Amahara regional states, respectively. In Cluster III, 47.62%, 28.57%, 14.29%, 4.76%, and 4.76% of the accessions were collected from

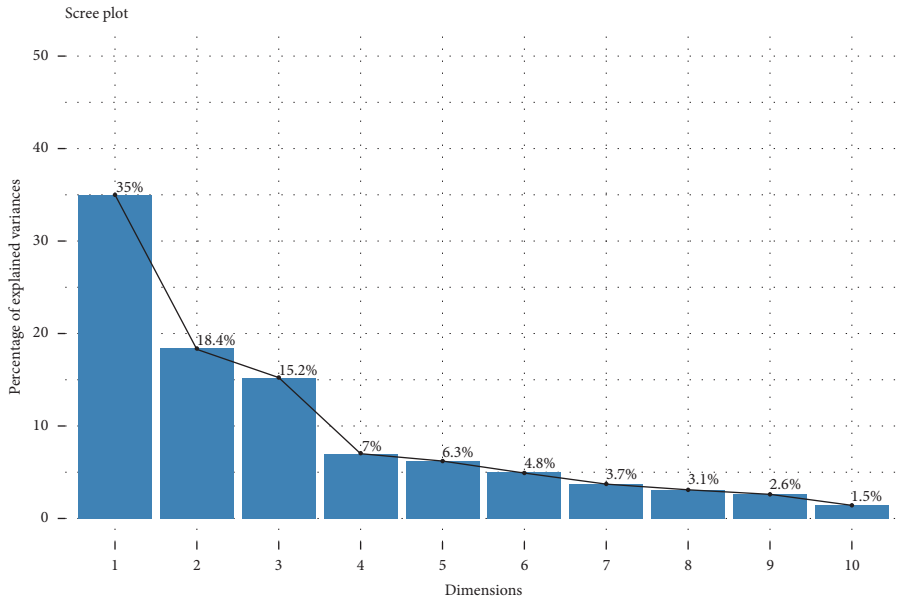


Figure 6: Accessions plot of principal components (PCs) for 17 qualitative traits in 49 basil accessions.

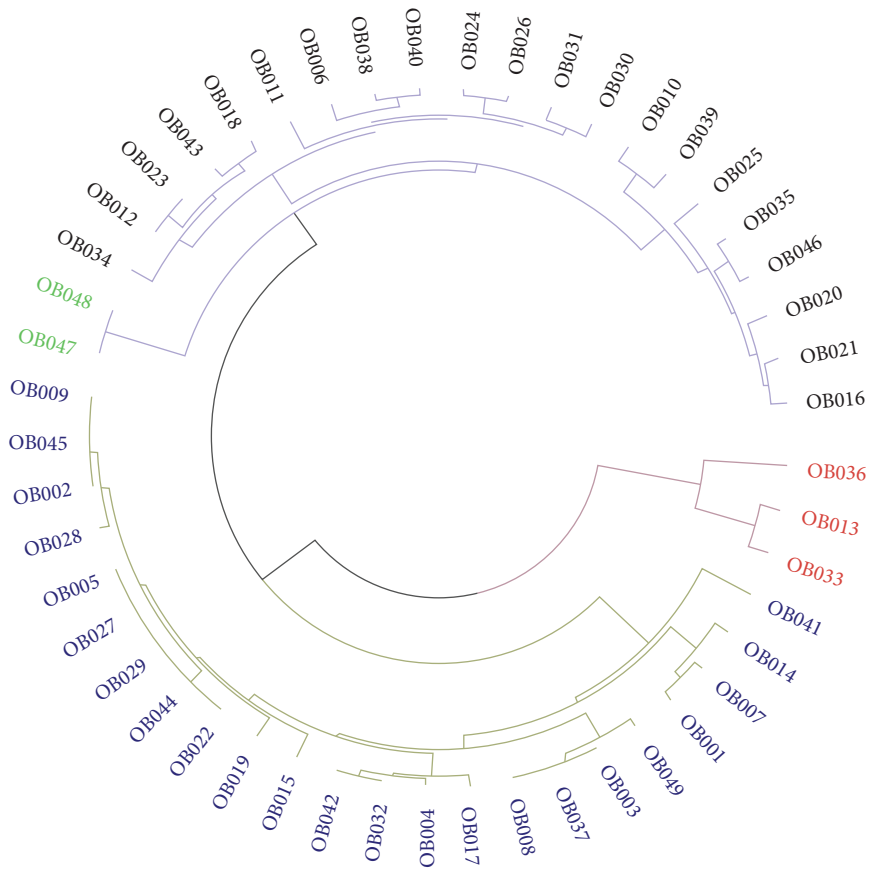


Figure 7: Dendrogram showing clustering patterns for 49 accessions based on different qualitative traits of *O. basilicum* Cluster I (blue), Cluster II (red), Cluster III (green), and Cluster IV (black).

Oromia, SNNP, SWE, Amahara, and Harari regional states, respectively. Cluster IV contains only two accessions, one from Norway (50%) and the other from Israel (50%). From the cluster analysis, accessions collected from different regions fall into a similar cluster and vice versa. In Clusters I and III, the basil accessions that originated from the same regions were clustered into different clusters, indicating the absence of relationships between genetic diversity and

geographic origin. For instance, the accessions from Oromia, Amhara, SNNP, and Hariri regions were grouped into two distinct clusters (Figure 7).

This may be due to the exchanges of accession by farmers among neighboring regions, natural and artificial selection, and environmental variation. Latif et al. [35] reported that geographical and genetic distributions did not follow the same trend. In addition, Lal [36] reported no parallelism between geographic distribution and genetic diversity. Therefore, the difference in geographic origin cannot be used as an indication of genetic diversity. Contrary to these, the accessions from Norway and Israel fall in one cluster indicating that the accessions in Ethiopia have evolved distinctly from those of Europe.

4. Conclusion

Morphological characterization based on qualitative traits exhibited the existence of high genetic variability among basil accessions of Ethiopia. Shannon–Wiener diversity indices showed a maximum value (0.96) for leaf blade glossiness and flower color of the corolla and the lowest value (0.53) for the color of style show the existence of high morphological variation among qualitative traits. The first four PCs were able to explain 75.58% of the total variation observed among basil accessions.

Leaf blade distribution of anthocyanin, the intensity of anthocyanin coloration, glossiness and shape, and flower color of the corolla were the most important traits that contributed to the major PCs accounting for a large portion of the morphological diversity. Cluster analyses based on qualitative traits revealed four distinct clusters. The first, second, third, and fourth clusters consisted of 46.94%, 6.12%, 42.86%, and 4.08% of the accessions, respectively. The clustering of the accessions from different regions of Ethiopia did not follow patterns of geographical distribution due to high martial transfer among regions. In general, the result of this study indicated the existence of high genetic diversity among basil accessions and this brings many opportunities to improve the crop through breeding by crossing and selection of the most diverse accessions from the clusters.

Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Disclosure

This study is the continuation of the previously published paper as a preprint in Flora Journal by Gossa et al., 2023.

Conflicts of Interest

The authors declare no conflicts of interest.

Author Contributions

Aynalem Gebre Gossa: conceived, designed, performed the experiments, analyzed the data, and wrote the original draft, Bizuayehu Tesfaye Asfaw: conceptualization and reviewing and editing the paper.

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