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Agro-Morphological and Chemical Traits-Based Diversity Assessment in Rosemary (*Salvia Rosmarinus* Schleid.) Accessions

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Abstract

The study was conducted to examine the extent of diversity in Ethiopian rosemary accessions for morphological and chemical characters. Forty landraces, three released varieties, and two commercial varieties were tested at Wondo Genet Agricultural Research Center using Randomized Complete Block Design with three replications. Analysis of variance displayed a highly significant variation ($p \leq 0.001$) among accessions for all traits, indicating the presence of diverse genotypes that make selection possible. Mean performance analysis confirmed the presence of elite accessions over the released varieties for leaf yield, essential oil content, and essential oil yield. Principal component analysis showed that 85% of the total variation was captured by the first five components, and the first two components were able to explain 61.3% of the total variation. The accessions were grouped into five distinct clusters with significant inter-cluster distance (6.3-249). Cluster means performance also showed that accessions in the third cluster can be used as a source of desirable genes to improve leaf and essential oil yields of rosemary. The results confirmed the presence of adequate genetic variability in Ethiopian rosemary accessions for morphological and chemical traits that could be utilized for future breeding activities.

Introduction

Rosemary (*Salvia rosmarinus* Schleid.) is an aromatic, evergreen and highly branched perennial shrubby herb belonging to the Lamiaceae family [1,2]. Previously, it was known by the botanical name of *Rosmarinus officinalis* L. But recently, the species was embedded in the genus *Salvia* with the denomination of *Salvia rosmarinus* Schleid. [3]. Rosemary is a significant crop all over the world and originally native to the Mediterranean region [4,5]. It is the most exploited species of the genus due to the medicinal quality of its essential oils [6], and cultivated around the world mainly as medicinal, food, flavor and ornamental [7,8]. Rosemary grows both for its leaves and essential oils extracted from its leaves, flowers, buds, and bark [9-11], and can be used fresh, dried and as extracts or as essential oils [12-14].

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It is one of the prominent crops in Ethiopia and successfully grown in various ecologies of the country [15]. Farmers of Ethiopia cultivate rosemary and are traditionally used as a food flavoring agents and as a spice for flavoring different food preparations. Large scale rosemary essential oil and biomass production has also been conducted in various areas of the country [16,17]. In spite of its wider adaptability and tremendous potential for local and export markets, research intervention is limited to the characterization and improvement of rosemary in Ethiopia. This could be the major constraint for future improvement programs and large-scale production of the crop in the country.

Assessing diversity and knowing detail appraisal of the collected germplasms are an essential step and prerequisite in plant breeding, and could give valuable information for determining distinctness of genotypes for conservation, evaluation and utilization of genetic resources [18-21]. Morphological, chemical, and molecular procedures are being used in the characterization and evaluation of crop variability [22-24]. In these methods, morphological characterization is the first step to assess genetic diversity and is used as a basis for plant classification and collection of plant genetic resources [25-28]. Quantitative morphological characters have been used in the analysis of genetic diversity and identification of suitable genotypes for production purposes [29]. They are an essential tools in identifying desirable types with useful characters either for direct use or for future breeding and improvement programs.

Several studies have been conducted and showed the existence of wide variability in rosemary genotypes for various quantitative, morphological and chemical traits elsewhere [30-35]. In Ethiopia, limited effort was made to characterize and evaluate rosemary germplasm [36]. But the study was conducted on limited samples and dealt only with the description of some agro-morphological traits which cannot give a full picture of the genetic variation in the germplasm collections. Thus, this work was conducted to investigate quantitative morphological and chemical traits based on genetic divergence and pattern of variability in Ethiopian rosemary accessions, which will provide useful information for selecting desirable genotypes for production and improvement programs.

Materials and methods

Description of the experimental site

The experiment was conducted at Wondo Genet Agricultural Research Center from 2018 to 2019 both under rain-fed and irrigation conditions. Wondo Genet Agricultural Research Center is situated at 287 km south of Addis Ababa, the capital city of Ethiopia. Geographically the site is located at $7^0$19’ N latitude and 38^0 38’E longitudes with an altitude of 1780 m.a.s.l. The area is categorized under tropical semi-humid zone with long-term average annual rainfall of 1128 mm. The main rainy season is from July to September, while the minimum rainfall is from November to January. The annual mean, minimum and maximum temperatures vary from 11.8 to 15.1°C and from 25.1 to 29.7°C, respectively. The soil textural class of the experimental area is sandy loam with a pH of 6.4 [37].

Table 1: Codes and area of collection of rosemary accessions evaluated for morphological and chemical traits in Ethiopia.

Accessions No.	Code	Area of collection	Accessions No.	Code	Area of collection
1	Ros02	Wolaita	24	Ros40	Arssi
2	Ros03	Wolaita	25	Ros41	Arssi
3	Ros05	Wolaita	26	Ros26	Arssi
4	Ros14	Wolaita	27	Ros27	Arssi
5	Ros35	Wolaita	28	Ros12	Arssi
6	Ros36	Wolaita	29	Ros20	North Shewa
7	Ros01	Hadiya	30	Ros21	North Shewa
8	Ros04	Hadiya	31	Ros22	North Shewa
9	Ros15	Hadiya	32	Ros23	North Shewa
10	Ros37	Hadiya	33	Ros24	North Shewa
11	Ros16	Hadiya	34	Ros25	North Shewa
12	Ros08	Gurage	35	Ros06	Gonder Zuria
13	Ros30	Gurage	36	Ros07	Gonder Zuria
14	Ros31	Gurage	37	Ros09	Harari
15	Ros33	Gurage	38	Ros10	Harari
16	Ros38	Gurage	39	Ros11	Harari
17	Ros39	Gurage	40	Ros34	Harari
18	Ros32	Gurage	41	Ros17	Harari
19	Ros13	Sidama	42	Ros18	Harari
20	Ros42	Sidama	43	Ros19	Harari
21	Ros43	Sidama	44	Ros28	Commercial farm, Ethiopia
22	Ros44	Sidama	45	Ros29	Commercial farm, Ethiopia
23	Ros45	Sidama			

Ros01, Ros05 and Ros08 are released varieties, while Ros28 and Ros29 are commercial verities obtained from commercial farms.

Composition of plant materials and field experimentation

In this experiment, a total of 45 rosemary accessions composed of local collections (n = 40), commercial varieties (n = 2), and released varieties (n = 3) were used (Table 1). The local accessions were collected from farmers' fields and two commercial verities were obtained from commercial farms in Ethiopia, which were introduced from abroad by private investors. For field experimentation, well-performing seedlings were transplanted to the main experimental plots using Randomized Complete Block Design (RCBD) with three replications. Each plot consisted of 16 plants in four rows of 1.8 m length with inter-and intra-raw spacing of 60 cm. The distances between the plots and blocks were 1 m and 1.5 m, respectively. During the experimentation, all nursery and field management practices were executed properly.

Data collection

Data on morphological traits were recorded following the descriptor lists given for rosemary by the International Union for the Protection of New Varieties [38]. Additionally, data on fresh leaf yield ha^{-1}, dry leaf yield ha^{-1}, essential oil content and essential oil yield ha^{-1} were calculated as described in Table 2.

Data analysis

Quantitative traits were subjected to Analysis Of Variance (ANOVA) using SAS 9.4 version software package [39]. All data were standardized to a mean of zero and a variance of unity to avoid bias due to the difference in measurement scale before multivariate analysis. Principal Component Analysis (PCA) and cluster analysis were performed using MINITAB version17 software. To determine the number of clusters, the procedures given in SAS software were followed [40] and the true number of clusters was determined by the points where small values of t^2 statistic join with local peaks of the pseudo F statistic followed by a larger pseudo t^2 value [41,42]. After the true number of clusters was determined, a dendrogram was generated based on Euclidean distance with the average linkage clustering method. Distances between clusters were calculated using Mahalanobis's D^2 [43], statistics as:

$$D^2ij = (xi - xj)' \text{ cov-1 } (xi - xj)$$

Where, D^2ij = the distance between genotypes i and j; xi and xj = vectors of the values of the variables for cases i and j; and cov-1 = the pooled groups variance covariance matrix. The obtained values of D^2 were considered as calculated values of chi-square and their significances were tested against tabulated chi-square ($\chi2$) values at 'p-1' degree of freedom, where p is the number of characters considered in the analysis [44]. Cluster means, standard error, and coefficient of variation of traits were calculated to estimate variability among the clusters and to understand the characteristics of the accessions in each cluster.

Table 2: Descriptors of quantitative, morphological, and chemical traits used to evaluate rosemary accessions in Ethiopia.

No.	Parameters	Descriptors
1	Plant height (cm)	It is the mean length of plants from soil level to the tip of the longest leaf
2	Branch number per plant	Is the mean number of branches arising from the main stem
3	Stem diameter (mm)	Is the mean thickness of main stem at 20 cm of the height from ground level
4	Internodes number on the main stem	Is the total number of internodes of the main stem from the ground level to the apex
5	Length of internodes on the main stem (mm)	Average length of each inter nodes on 20 cm of main stem
6	Canopy width (cm)	Is the average width of individual plant canopy measured at the widest point
7	Leaf length (mm	Is the mean length of 50 leaves $plant^{-1}$
8	Leaf width (mm	Is the average of 50 leaves $plant^{-1}$
9	Leaf fresh weight per plant (g)	Measured after leaves separated from stems and branches
10	Stem fresh weight per plant (g)	Is the mean fresh stem weight of sampled plants
11	Leaf dry weight per plant (g	It was taken after drying 100 g of leaf sample in hot oven at 100°C for 24 hours until constant weight is reached
12	Stem dry weight per plant (g)	Estimated by the same procedure as of leaf dry weight
13	Leaf to stem ratio	Is the mean of dry leaf weight to dry stem weight
14	Fresh leaf yield (tha^{-1})	Is the yield obtained from harvestable plot and converted in to yield ha^{-1}
15	Dry leaf yield (tha^{-1})	It was estimated by taking composite sample of leaves from harvestable plots and dried in hot oven
16	Essential oil content (%)	Is the oil content determined from central plants by taking 300 g fresh leaf of composite samples and subjected to hydro distillation in a Clevenger apparatus for 4 hrs.
17	Essential oil yield (kg ha^{-1})	Is the oil yield obtained from harvestable rows of plots and converted in to yield ha^{-1} based on essential oil content and leaf biomass

Results

Diversity based on morphological and chemical traits

The statistical analysis showed the presence of a highly significant variation ($p \leq 0.001$) among the accessions for all the studied traits (Table 3). The existence of a wide range of variation between the tested accessions for growth and yield attributes reflected the potential of the germplasm for further selection and improvement activities. Higher variations in terms of fresh leaf yield $plant^{-1}$ (257.1 g - 1291g), dry leaf yield $plant^{-1}$ (74.9 kg - 367.4 kg), fresh leaf yield ha^{-1} (7.1 t - 35.9 t), dry leaf yield ha^{-1} (2.08 t - 10.21 t), essential oil content (0.77% - 2.22%) and essential oil yield ha^{-1} (26.56 kg - 178.08 kg) were recorded among the accessions under study (Table 4). Accession Ros41 was superior to the others in leaf and essential oil yields. On the contrary, accession Ros34 has the lowest values of leaf and essential oil yields. Higher essential oil content was recorded for Ros26, whereas a lower value (0.77 %) was obtained for Ros43 (Table 4). Mean performance analysis also demonstrated the presence of superior accessions over the released and introduced varieties (Table 5).

Table 3: Analysis of Variance for morphological and chemical traits of rosemary accessions evaluated at WGARC in Ethiopia from 2018 to 2019.

Sources of variation	Df	FW	DW	FY	DY	EOC	EOY
Replication	2	58003	4922	45	3.79	2.57	15506
Accessions	44	166385.27***	16111.23***	128.39***	12.43***	0.37***	4676.37***
Error	88	26038	2321	20	1.79	0.01	470.7

Df: Degree Of Freedom; FW: Fresh Leaf Weight Plant-1; DW: Dry Leaf Weight Plant-1; FY: Fresh Leaf Yield Ha-1; DH : Dry Leaf Yield Ha-1; EOC: Essential Oil Content; EOY: Essential Oil Yield Ha-1; WGARC: Wondo Genet Agricultural Research Center.

Table 4: Analysis of Variance for morphological and chemical traits of rosemary accessions evaluated at WGARC in Ethiopia from 2018 to 2019.

Accessions	FW(g)	DW(g)	FY(t)	DY(t)	EOC(%)	EOY(kg)	Accessions	FW(g)	DW(g)	FY(t)	DY(t)	EOC(%)	EOY(kg)
Ros01	677.1	200.1	18.8	5.56	1.62	89.95	Ros25	322.1	106.0	8.9	2.95	1.19	34.96
Ros02	995.4	280.2	27.6	7.78	1.38	108.17	Ros26	478.8	144.4	13.3	4.01	2.22	89.28
Ros03	1005.0	277.9	27.9	7.72	1.63	126.05	Ros27	724.0	195.9	20.1	5.44	1.48	79.62
Ros04	943.8	278.4	26.2	7.73	2.01	156.30	Ros28	493.1	140.8	13.7	3.91	1.69	66.55
Ros05	488.1	148.3	13.6	4.12	1.03	42.31	Ros29	435.6	128.8	12.1	3.58	1.97	70.75
Ros06	732.9	188.8	20.4	5.25	1.32	69.60	Ros30	498.4	140.9	13.8	3.91	2.15	83.78
Ros07	941.7	285.1	26.2	7.92	1.48	120.32	Ros31	630.7	183.5	17.5	5.10	1.68	85.88
Ros08	1089.1	308.5	30.3	8.57	1.91	162.89	Ros32	827.7	220.5	23.0	6.13	1.44	88.20
Ros09	1004.0	278.2	27.9	7.73	1.73	131.36	Ros33	836.1	226.7	23.2	6.30	1.79	113.48
Ros10	1065.6	324.0	29.6	9.00	1.80	160.45	Ros34	257.1	74.9	7.1	2.08	1.26	26.56
Ros11	988.8	320.4	27.5	8.90	1.37	121.10	Ros35	879.4	272.5	24.4	7.57	1.45	113.92
Ros12	884.6	284.1	24.6	7.89	1.38	105.47	Ros36	1047.3	298.9	29.1	8.30	1.54	128.17
Ros13	973.8	319.1	27.0	8.86	1.35	119.14	Ros37	586.3	161.2	16.3	4.48	1.79	80.62
Ros14	1017.9	334.9	28.3	9.30	1.72	165.35	Ros38	713.4	190.5	19.8	5.29	1.41	74.64
Ros15	1014.5	350.7	28.2	9.74	1.63	155.96	Ros39	744.5	209.4	20.7	5.82	1.22	73.58
Ros16	864.5	279.8	24.0	7.77	1.46	112.58	Ros40	723.6	197.7	20.1	5.49	1.52	86.75
Ros17	735.7	257.6	20.4	7.16	1.05	77.75	Ros41	1291.0	367.4	35.9	10.21	1.73	178.08
Ros18	709.6	256.8	19.7	7.13	0.80	57.32	Ros42	743.7	205.4	20.7	5.71	1.02	58.28
Ros19	603.9	167.2	16.8	4.65	1.85	86.24	Ros43	649.3	188.6	18.0	5.24	0.77	43.10
Ros20	860.6	242.4	23.9	6.73	1.56	107.21	Ros44	583.1	162.9	16.2	4.53	1.78	83.02
Ros21	544.3	157.9	15.1	4.39	0.97	42.47	Ros45	619.1	176.3	17.2	4.90	1.36	64.70
Ros22	449.5	140.2	12.5	3.90	0.86	34.66	Mean	745.4	221.0	20.7	6.14	1.48	92.43
Ros23	404.2	126.1	11.2	3.50	1.01	34.96	LSD (5%)	261.8	78.2	7.3	2.17	0.12	35.20
Ros24	462.1	145.2	12.8	4.03	1.17	47.83	CV (%)	21.6	21.8	21.6	21.80	5.04	23.47

WGARC, Wondo genet agricultural research center; FW, Fresh leaf weight plant^{-1}; DW, Dry leaf weight plant^{-1}; FY, Fresh leaf yield ha^{-1}; DY, Dry leaf yield ha^{-1}; EOC, Essential oil content; EOY, Essential oil yield ha^{-1}.

Table 5: Comparison of mean performance of the best performed accessions with released and introduced varieties for leaf and essential oil yields.

	Best performed accessions							Released varieties			Introduced varieties	
Traits	Ros04	Ros10	Ros14	Ros15	Ros26	Ros30	Ros41	Ros01	Ros05	Ros08	Ros28	Ros29
FW (kg)	-	1.07	-	-	-	-	1.29	.68	0.49	1.09	0.49	0.44
DW (kg)	-	-	0.33	0.35	-	-	0.37	0.20	0.15	0.31	0.14	0.13
FY (t)	-	29.56	-	-	-	-	35.86	18.81	13.56	30.25	13.70	12.10
DY (t)	-	-	9.30	9.74	-	-	10.21	5.56	4.12	8.570	3.91	3.58
EOC (%)	2.00	-	-	-	2.22	2.15	-	1.62	1.03	1.90	1.69	1.97
EOY (kg)	-	-	165.35	-	-	-	178.08	89.95	42.31	162.89	66.55	70.75

FW, fresh leaf weight plant^{-1}; DW, dry leaf weight plant^{-1}; FY, fresh leaf yield ha^{-1}; DY, dry leaf yield ha^{-1}; EOC, essential oil content; EOY, essential oil yield ha^{-1}.

Principal component analysis

Principal Component Analysis (PCA) was performed using 15 quantitative morphological and chemical traits. The first five components with Eigenvalues greater than 1 were selected as meaningful components and examined further [45]. These components together explained 85% of the total variation among the accessions (Table 6). The first two components were able to explain about 61.3% of the total variations with the first and the second PC accounting for 40% and 21.3% of the variations, respectively, and hence were the most meaningful components. The other three components (PC3-PC5) explained 23.7% of the total variability. The number of PCS formed and the total variability (85%) captured indicated the presence of adequate variation among rosemary accessions.

A given trait is considered as an important contributor to the variability in a component if its vector loading has an absolute value closer to unity [46,45]. Based on this, the characters with high positive loadings that contributed to variability on the first PC are branch number plant^{-1}, plant canopy width, and fresh and dry leaf weight plant^{-1}, fresh and dry leaf yield ha,$^{-1}$ and essential oil yield ha^{-1} (Table 6). Thus, this component is mainly related to yield traits and those accessions with wider canopy width, higher branch number, and higher leaf and essential oil yields contributed to the variability of this component. High negative loadings of plant height, stem diameter, internode number, and leaf width; and high positive loading of leaf to stem ratio were contributed to the variation in the second PC. Therefore, the variability in this component is related to agronomic characteristics and contributed by those accessions with shorter plant height, narrow stem diameter, narrow leaf width, lower number of internodes, and higher leaf -to-stem ratio. Accessions with lower percentages of essential oil content contributed to the variability in PC3 with higher negative loading of this character. The variability in the fourth and fifth principal components was contributed by accessions with shorter internode length and longer leaf length, respectively.

Cluster and distance analysis among rosemary accessions

Clustering using the average linkage criterion method based on Euclidean distance grouped the 45 rosemary accessions into five major clusters on the basis of quantitative, morphological and chemical traits (Table 7, Figure 1). Among all clusters, cluster II contains the largest number of accessions (n=22) from all collection areas except those from commercial farms. The second largest number of accessions (n=13) were found in cluster III. One released variety (Ros08) and landraces from all collection regions, except North Shewa and commercial farms, were grouped in this cluster. Cluster one (C-I) and cluster five (C-V) contained an equal number of accessions (n=4, each) and the third largest number of accessions. One released variety (Ros01) and three landraces (from Hadiya and Arssi) formed the first cluster, whereas the fifth cluster was made up of one released variety (Ros05) and three landraces (from Harari). Commercial farm varieties (n=2) formed the forth cluster (C-IV) exclusively, showing greater similarity among them for the studied traits but more divergence from the local collections. The commercial varieties were introduced from abroad for large-scale production purposes. The difference in place of origin may favor accumulation of different alleles, and this might increase genetic dissimilarity between these groups and the local collections.

Table 6: Eigen vector and proportion of variance explained by the first five principal components for morphological and chemical characters of rosemary accessions.

	Principal components				
	PC1	**PC2**	**PC3**	**PC4**	**PC5**
Eigen values	6	3.20	1.31	1.15	1.08
Proportion of variance (%)	40	21.3	8.8	7.7	7.2
Cumulative variance (%)	40	61.3	70.1	77.8	85
Traits	**Eigen vectors**				
Plant height	0.021	**-0.847**	0.044	-0.464	0.019
Branch number	**0.833**	0.377	-0.155	0.135	0.024
Stem diameter	-0.130	**-0.476**	0.109	0.106	0.078
Internode number	0.036	**-0.642**	-0.104	0.081	0.156
Internode length	-0.159	-0.255	-0.031	**-0.928**	-0.170
Canopy width	**0.726**	-0.063	-0.174	0.028	0.075
Leaf length	-0.251	-0.107	0.015	0.159	**0.941**
Leaf width	-0.082	**-0.881**	0.258	-0.060	0.253
Fresh leaf weigh plant^{-1}	**0.976**	0.002	-0.055	0.040	-0.121
Dry leaf weigh plant^{-1}	**0.987**	-0.050	0.015	0.023	-0.097
Leaf to stem ratio	-0.015	**0.912**	0.127	0.076	0.104
Fresh leaf yield ha^{-1}	**0.976**	0.002	-0.055	0.040	-0.121
Dry leaf yield ha^{-1}	**0.987**	-0.050	0.015	0.023	-0.097
Essential oil content	0.188	0.057	**-0.972**	-0.027	-0.009
Essential oil yield ha^{-1}	**0.883**	0.013	-0.447	0.038	-0.069

Table 7: Distribution of 45 rosemary accessions from different collection region into each cluster.

	Clusters					
Collection areas	**I**	**II**	**III**	**IV**	**V**	**Total**
Wolaita	0	1	4	0	1	6
Hadiya	3	1	1	0	0	5
Gurage	0	6	1	0	0	7
Sidama	0	4	1	0	0	5
Arssi	1	2	2	0	0	5
North Shewa	0	6	0	0	0	6
Gonder	0	1	1	0	0	2
Harari	0	1	3	0	3	7
Commercial	0	0	0	2	0	2
Total	4	22	13	2	4	45

Pairwise generalized square distance (D^2) based on mahalanobis's statistics displayed significant genetic distance between all pairs of clusters except between clusters II and III (Table 8). The significant distance variation revealed the presence of considerable diversity among accessions and the potential of the groups to serve as a source of unique traits for future crop improvement activities. Thus, crossing the genotypes in different clusters could increase the probability of obtaining unique desirable traits. Lower distance variation between clusters II and III indicated the presence of common genetic material shared between these groups.

Table 8: Pairwise Generalized Squared Distance (D2) between clusters.

Clusters	I	II	III	IV	V
I	0				
II	26.16*	0			
III	32.9**	6.3^{ns}	0		
IV	65.1***	144.1***	139.1***	0	
V	144.1***	186.4***	167.5***	249***	0

$\chi 2$ = 23.69, 29.14 and 36.12 at 5%, 1%, and 0.1%, respectively. * = significant at $p \leq 0.05$, **= highly significant at $p \leq 0.01$, ***= very highly significant at $p \leq 0.001$, ns= non-significant ($p \geq 0.05$).

Clustering criterion of accessions as assessed by cluster means analysis

The mean and coefficient of variation for the traits in each cluster are presented in Table 9. It is observed that the first cluster is composed of accessions with short plant height (84.2 cm), lower internode length (10.5 mm), narrower leaf width (2.5 mm), longer leaf length (31.5 mm) and higher essential oil content (1.9%). While, accessions in cluster II showed average values for most of the characters but demonstrated a higher leaf to stem ratio (1.7) and lower values of internode number (53.4).

Most of the accessions in cluster III were characterized by wider canopy width (85.8 cm), higher branch number plant^{-1} (50.6), higher fresh and dry leaf yield plant^{-1} (1024.5 g and 310 g, respectively), higher fresh and dry leaf yield ha^{-1} (28.5 t and 8.6 t, respectively) and higher essential oil yield ha^{-1} (137.1 kg). These yields and yield-related traits were, therefore, the main factors to group the accessions from different growing areas together in cluster III. The fourth cluster was marked by accessions with lower stem diameter (16.7 mm) and higher internode length (21.3 mm). Accessions with lower essential oil content (1.0% w/w) were grouped in cluster V.

Cluster mean analysis would help to identify groups of accessions with desired traits. In this analysis, cluster III might be useful to select accessions with superior leaf and essential oil yields. Due to the closer genetic distance among accessions in clusters III and II, accessions in cluster II could also serve as a source of important genes for improving the economic traits of rosemary.

Table 9: Mean and Coefficient of Variation (CV) of morphological and chemical traits among five clusters of rosemary accessions.

Clusters	Values	PH	BN	SD	IN	IL	CW	LL	LW	FLW	DLW	LSR	FLYH	DLYH	EOC	EOY
I	Mean	84.2	43.9	26.1	64.3	10.5	72.6	31.5	2.5	671.5	196.0	1.7	18.7	5.4	1.9	104.0
	SE	0.28	2.91	0.23	0.68	0.74	2.90	0.28	0.01	99.40	29.80	0.02	2.70	0.83	0.13	17.50
	CV	0.7	13.3	1.8	2.1	14.2	8.0	1.8	0.9	29.6	30.4	2.7	29.6	30.4	13.7	33.7
II	Mean	109.8	50.6	18.6	563	13.7	85.8	28.1	3.2	1024.5	310.0	1.5	28.5	8.6	1.6	137.1
	SE	3.02	1.49	1.38	1.13	0.41	1.81	0.44	0.11	26.50	8.13	0.06	0.73	0.22	0.05	6.69
	CV	9.9	10.6	26.8	7.2	10.8	7.6	5.6	12.6	9.3	9.5	13.7	9.3	9.5	11.7	17.6
III	Mean	146.5	24.6	36.8	74.9	15.6	60.9	30.3	5.5	547.6	184.4	1.0	15.2	5.1	1.0	51.0
	SE	4.43	2.52	0.68	2.65	0.18	2.85	0.19	0.28	112.00	44.60	0.06	3.10	1.24	0.09	10.90
	CV	6.1	20.5	3.7	7.1	2.4	9.4	1.3	10.1	40.8	48.4	12.6	40.8	48.4	18.2	42.8
IV	Mean	101.1	36.5	20.3	53.4	14.5	70.4	27.7	2.9	655.3	187.5	1.7	18.2	5.2	1.4	73.6
	SE	2.46	1.15	1.01	0.98	0.33	1.62	0.48	0.06	34.00	9.42	0.05	0.94	0.26	0.07	5.54
	CV	11.4	14.8	23.2	8.7	10.8	10.8	8.1	9.0	24.3	23.6	15	24.3	23.6	24.9	35.3
V	Mean	143.5	26.2	16.7	54.8	21.3	73.0	29.8	3.6	464.4	134.8	1.2	12.9	3.7	1.8	68.7
	SE	0.5	0.17	2.03	0.17	0.47	1.5	0.15	0.03	28.8	5.99	0.07	0.8	0.17	0.14	2.1
	CV	0.5	0.9	17.2	0.4	3.1	2.9	0.7	1.1	8.8	6.3	7.8	8.8	6.3	10.8	4.3

PH, plant height(cm); BN, branch number; SD, stem diameter (mm); IN, internode number; IL, internode length(mm); CW, canopy width (cm); LL, leaf length (mm); LW, leaf width (mm); FLW, fresh leaf weight plant^{-1}(g); DLW, dry leaf weight plant^{-1} (g); LSR, leaf to stem ratio; FLYH, fresh leaf yield ha^{-1}(t); DLYH, dry leaf yield ha^{-1}(t), EOC, essential oil content (%), EOY, essential oil yield ha^{-1}(kg).

Discussion

Variability of accessions for morphological and chemical characters

The studied rosemary accessions displayed variation for all morphological and chemical traits evaluated in the present work (Table 3). Wider ranges between the minimum and maximum values were also observed for the majority of the characters (Table 4), reflecting the existence of ample amounts of variation among the tested accessions for growth and yield attributes. Significant variations among rosemary genotypes for various agronomic and yield traits have also been reported by different researchers [47,32,30,48].

Rosemary is cultivated worldwide for its fresh and dry leaves as a culinary herb and for its essential oil for application in cosmetic, pharmaceutical, and food industries [14,35], thus leaf and essential oil yields are important economic traits and are the targets of selection in rosemary improvement activities. Broader variations in terms of fresh leaf yield, dry leaf yield, essential oil content, and essential oil yield were recorded among the accessions (Table 4). Fresh and dry leaf weight plant^{-1} varied from 257 g to 1291 g and from 74.9 g to 367.4 g with mean yields of 745.36 g and 221 g, respectively. The accessions gave 7.1 t-35.9 t and 2.1 t-10.2 t of fresh and dry leaf yield ha^{-1} with the mean value of 20.7 t and 6.1 t, respectively. The leaf yields obtained for the tested accessions in this study were far better than the fresh leaf yield of 191.82 to 293.23 g plant^{-1} and 5.33 to 8.15 t ha^{-1} reported for Ethiopian rosemary accessions in another study [36]. It was also better than the leaf yield of 5.56 t-11.81 t ha^{-1} obtained for rosemary genotypes in India

[49]. The variation in leaf yields might be due to environmental, genetic, or interaction among the two factors. It also indicates the potential of the local collections for use as a source of desirable traits for improvement. By considering at least 5% of the studied accessions, two accessions, namely, Ros41 (from Arssi) and Ros10 (from Harari) and one released variety (Ros08) were the best performed genotypes for fresh leaf weight plant^{-1} and fresh leaf yield ha^{-1}. Three accessions from Arssi (Ros41), Wolaita (Ros14), and Hadiya (Ros15) were the top performed and superior to all released varieties for dry leaf weight plant^{-1} and dry leaf yield ha^{-1} (Table 5).

Wider variation among accessions was also observed for the other economic traits of rosemary, namely, essential oil content and essential oil yield. The essential oil content and yield ranged from 0.77% to 2.22% and from 26.56 kg to 178.08 kg ha^{-1} with mean values of 1.48% and 92.43 kg, respectively (Table 4). The observed variation between the minimum and maximum values and the overall mean values recorded in the present study was generally higher than the values reported in previous studies [50,49,51,36]. The highest essential oil yield was obtained for Ros41 (from Arssi), followed by Ros14 (from Wolaita) and Ros08 (released variety). Three accessions, namely, Ros26 (from Arssi), Ros30 (from Gurage), and Ros04 (from Hadiya), exceeded the released varieties for essential oil content and were among the best performed of the accessions (Table 5). The significant variability observed for the agronomic characters, leaf and essential oil yields among the studied accessions implies the presence of broader genetic diversity that could be utilized for future selection and improvement of desirable characters.

As a result of this study depicted, there are elite accessions over the released varieties for leaf yield, essential oil content, and essential oil yield; indicating the availability of desirable traits in accessions collected from different areas that could be used for future improved variety development through selection and hybridization. The results also revealed the presence of superior local collections than the introduced varieties for the majority of the economic traits, showing the potential of Ethiopian rosemary accessions for large-scale commercialization. Therefore, any conservation and improvement program aimed at the exploitation and commercialization of the crop should give focus and priority to accessions available at different geographic locations of the country.

Pattern of diversity based on multivariate analyses

Analysis of morphological and chemical traits diversity using principal components and cluster analyses showed the presence of high variability among rosemary accessions. The first five principal components were explained 85% of the total variation, and all quantitative morphological and chemical traits were found to measure different constructs and were efficient to capture the variability among the studied accessions.

A dendrogram generated from cluster analysis grouped the 45 rosemary accessions into five distinct groups and provided a more understandable sense of relationship among the accessions. Accessions collected from different areas were clustered based on their similarity in morphological and chemical characteristics irrespective of their collection regions. All accessions and released varieties were dispersed into more than one cluster, except accessions from North Shewa, which fell into one cluster (Table 7). But the commercial varieties showed divergence from the rest and clustered together in one group. Even though there were accessions from similar collection regions grouped together, the overall clustering pattern of the accessions indicated the presence of a weak association between genetic distance and area of growing. Grouping of accessions from different growing regions into a similar clusters designated the existence of genetic closeness among accessions of different regions. Presence of gene flow between geographic regions due to planting material exchange, similar evolutionary forces such as mutation and genetic drift, and/or similar ancestral gene pools might result in genetic resemblance among growing regions. Lack of strict grouping of rosemary accessions in relation to their growing region was also defined in this study at the molecular marker level [52].

On the other hand, dispersed clustering of accessions from similar growing regions into different groups showed that the existence of high variability among accessions within growing regions. Our result was in agreement with that of [53,54], who found higher within-population morphological variability for Sicilian rosemary germplasms. Similar observations were also reported for other medicinal and spice crops [55-58]. In general, the variability observed in ANOVA, which showed a wider variability of accessions for the examined traits, reaffirmed the results demonstrated by principal component and cluster analyses, and revealed the presence of a broader genetic base among Ethiopian rosemary germplasm.

Conclusions

The results of this study revealed the presence of significant variation for all studied morphological and chemical characters among rosemary accessions collected from different parts of Ethiopia. Mean performance analysis confirmed the presence of superior accessions in leaf and essential oil yields than the released and introduced varieties. Most of the studied traits were found important in characterizing and evaluating rosemary accessions since they measured different constructs and contributed at large to the first five principal components that explained 85% of the variation.

Cluster analysis partitioned the accessions into five mean full groups with significant distance variation among them, which signifies the existence of morphological and chemical diversity that could be used for the rosemary breeding program. Besides, cluster analysis helped to identify a group of accessions that can serve as a source of desirable genes for future breeding and improvement programs. From the cluster mean values, it is possible to highlight that cluster III can serve as a source of important genes for improving the most desirable traits of rosemary such as leaf yield and essential oil yields. Due to the high values of leaf to stem ratio of group II accessions and the closer genetic distance between clusters III and II, accessions in cluster II could also serve as a source of important genes and crossing of accessions in these groups with others might be advantageous to improve yield and yield related characters in the rosemary breeding program.

Generally, the result confirms the existence of sufficient diversity in Ethiopian rosemary which could support the breeders for designing conservation strategies and improvement programs. The presence of elite accessions over the released and introduced varieties could suggest the possibility of direct selection for variety release and commercialization of local accessions. To exploit the existing diversity and harmonize the breeding work, it is recommended to conduct further characterization and evaluation studies on different agro-ecologies.

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