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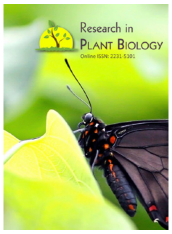

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Morpho-agronomic variability, traits association and path analysis in Rosemary (*Salvia rosmarinus* Schleid.) genotypes from Ethiopia

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ABSTRACT

Forty-five rosemary genotypes collected from different parts of Ethiopia were evaluated for quantitative morphological traits to estimate the genetic variability, heritability and association of characters. The genotypes displayed significant differences for all of the studied traits, showing the presence of wide variability among the tested genotypes. The genotypic and phenotypic coefficients of variation were found to be medium and high for all growth and yield traits except for leaf length. Moderately high to high broad-sense heritability (0.66 -0.97) coupled with moderate to high genetic advance as a percent of the mean (10.37 -79.14) values were found for all traits. Correlation coefficient and path coefficient analysis revealed that characters vis. branch number plant^{-1}, fresh leaf weight plant^{-1}, dry leaf weight plant^{-1}, fresh leaf yield ha^{-1}, and essential oil content had a high positive direct effect and significant positive association with essential oil yield ha^{-1}. Thus direct selection for these traits would be quite effective for essential oil yield enhancement in rosemary. In general, the studied accessions were diverse in nature and could be exploited in the conservation, breeding and commercialization of the crop.

KEYWORDS: Accessions, Ethiopian rosemary, genetic variability, *Salvia rosmarinus*

INTRODUCTION

Rosemary (*Salvia rosmarinus* Schleid.) is an evergreen perennial shrubby herb of the Lamiaceae family and endemic to the Mediterranean region (Ali *et al*., 2000; Li *et al*., 2016). It is a diploid (2n = 24) with hermaphrodite flowers, on which insect mediated pollination is common both resulting in allogamy and self-fertilization (Pottier-Alapetite, 1981; Zaouali *et al*., 2010; Nunziata *et al*., 2019). The previously used botanical name of rosemary, *Rosmarinus officinalis* L. has become a synonym of the actual name *Salvia rosmarinus* since evidence from molecular investigation nested *Rosmarinus* into the genus *Salvia* (Drew *et al*., 2017).

Rosemary leaves are widely used for cooking as a flavoring agent and as tea infusion (Sasikumar, 2012; Ribeiro-Santos *et al*., 2015). The essential oils and extracts of rosemary are a source of antioxidants, which are used in food packaging, aromatherapy, preservatives in the cosmetic industry, and in medicinal treatment against many illnesses (Navarrete *et al*., 2011; Amaral *et al*., 2013; Barreto *et al*., 2014; Borras-Linares *et al*., 2014). Furthermore, studies on the biological activities of its essential oil showed significant antimicrobial, anti-cancerous, pain relief, blood circulation and anti-lipid peroxidation activities (Ngo *et al*., 2011; Jordan *et al*., 2011).

In the recent period, food and cosmetic destinations are becoming economically predominant for rosemary products and the optimization of rosemary production is an arising issue in the market (Nunziata *et al*., 2019). In Ethiopia, it is abundantly growing in the wild, around homesteads and on farmer's fields as well as on commercial farms (Engels *et al*., 1991; Banjaw *et al*., 2016). Despite its wider application and high market potential, technological intervention is scanty on the production and improvement of rosemary in Ethiopia.

For efficient and effective crop improvement work, investigation and a better understanding of the variability existing in a crop population are mandatory. The degree of crop improvement depends on the magnitude of the available beneficial genetic variability. The information as well as the assessment of genotypic and phenotypic coefficient of variation is useful in detecting the amount of variability present in the available genotypes (Deepthi *et al.*, 2016). Furthermore, the success of any crop improvement work depends not only on the amount of variation that exists in a crop population but also on the extent to which the desirable characters are heritable from the parent to the progeny (Bello *et al.*, 2012). Therefore, assessment of heritability is necessary to predict the expected gain that could be achieved through the selection process. High heritability alone does not give full information to make efficient selection unless accompanied with a considerable amount of genetic advance (Johnson *et al.*, 1955). Genetic advance shows the degree of the gain obtained in a character from one cycle of selection, and high genetic advance coupled with high heritability estimates offers the most suitable condition for the selection of a particular character (Larik & Rajput, 2000; Syukur *et al.*, 2012).

The ultimate goal of any crop breeding work is increasing economic yield. But yield itself is the product of many traits and is influenced by the interactions of these traits directly or indirectly. Therefore, it is important to understand the relationship between yield and its component traits for selecting desirable genotypes for yield improvement programs (Malek *et al.*, 2014). Determination of correlation coefficients is an important procedure for making the best use of these relationships in selection. To split the correlation coefficient into direct and indirect effects, path coefficient analysis is a useful technique which permits a critical examination of components that influence a given association (Sabaghnia *et al.*, 2010). Hence, estimates of correlation coefficients in combination with path coefficient analysis help to explain the direct and indirect contribution of one character to another.

Although rosemary has got diverse use and is cultivated in different parts of Ethiopia, knowledge on genetic variability of the local genotypes and the extent of heritability and association of characters are scanty. In this context, the present investigation was conducted to estimate the magnitude of genetic variability, heritability, genetic advance, character association and the direct and indirect effect of different characters on the essential oil yield of rosemary accessions collected from different parts of Ethiopia.

MATERIALS AND METHODS

Experimental Site

The experiment was carried out at Wondo Genet Agricultural Research Center experimental field from 2018 to 2019. 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 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 (Abayneh *et al.*, 2006).

Plant Material and Experimental Design

A total of 45 rosemary accessions comprising forty local collections, two commercial varieties, and three released varieties were used for this experiment (Table 1). The experiment was laid out in a randomized complete block design with three replicates. Plot-to-plot and block-to-block distances were maintained at 1 m and 1.5 m, respectively, with a plot size of 1.8 m x 1.8 m. Seedlings were planted with inter and intra row spacing of 60 cm. During experimentation, all necessary intercultural operations were done for proper growth and development of plants in each plot.

Table 1: List of rosemary accessions used for the study and their places of collection

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

WGARC, Wondo Genet Agricultural Research Center; Ros01, Ros05 and Ros08 are released varieties, while Ros28 and Ros29 are commercial verities obtained from commercial farms

Data Collection

Data were recorded from all central plants as described in Table 2.

Statistical Analyses

The data collected for each character was subjected to analysis of variance (ANOVA) using Statistical Analysis System (SAS) version 9.4 software (SAS Institute Inc, 2013).

Phenotypic and genotypic variances and genotypic and phenotypic coefficients of variations were calculated as described by Kwon and Torrie (1964), Falconer (1981), Singh and Chaudhary (1985) and Syukur *et al.* (2012) as follows:

$$\sigma^2_G = (MSG - MSE)/r$$

$$\sigma^2_P = \sigma^2_G + \sigma^2_E$$

$$\sigma^2_E = MSE/r$$

Genotypic coefficient of variation (GCV) = $[\sqrt{\sigma^2_G}/X] \times 100$

Phenotypic coefficient of variation (PCV) = $[\sqrt{\sigma^2_P}/X] \times 100$

Where σ^2_G, Genotypic variance; σ^2_P, Phenotypic variance; σ^2_E, environmental variance, MSG is an estimate of the mean square of tested accession, MSE is an estimate of the mean square of error, r refers to the number of replications and *X* is grand mean of a character.

Broad sense heritability (h_b^2) of all traits was calculated based on the formula described by Allard (1960) as follows:

$h_b^2 = (\sigma^2_G/\sigma^2_P) \times 100$, where: h_b^2 = heritability in broad sense; σ^2_G = Genotypic variance; σ^2_P, Phenotypic variance.

Genetic advance (GA) and genetic advance as percentage of the mean (GAM) were calculated according to the formula given by Johnson *et al.* (1955) as follows:

$$GA = K(\sigma_P)\, h_b^2$$

$$GAM = \frac{GA}{X} \times 100$$

Where: GA, genetic advance; GAM, genetic advance as percentage of the mean K, the selection differential (K = 2.06 at 5% selection intensity); σ_P, the phenotypic standard deviation of the character; h_b^2, broad sense heritability and x, grand mean of a character

Genotypic and phenotypic correlation coefficients for all possible comparisons were calculated using META-R software (Alvarado *et al.*, 2016). For path coefficient analysis, essential oil yield ha^{-1} was taken as a dependent variable while the rest variables were considered as contributory factors. The direct and indirect effect of the independent characters on essential oil yield was done using the formula outlined by Dewey and Lu (1959).

Table 2: Parameters taken and method of measurement for 45 rosemary accessions evaluated at WGARC from 2018 to 2019

No.	Traits	Method of measurement
1	Plant height (cm)	Is the mean vertical length of plants from the soil level to the tip of the longest leaf measure using measuring tape
2	Branch number plant^{-1}	Is the mean number of branches arising from the stem of sampled plants
3	Stem diameter (mm)	Is the mean thickness of main stem at 20cm of the height from ground level measured using caliper at harvest form all central raw plants
4	Internodes number on the main stem	The total number of internodes of the main stem from the ground level to the apex counted at harvest from all central raw plants per plot
5	Length of internodes on the main stem (mm)	Average length of each inter nodes on 20cm of main stem
6	Canopy width (cm)	Is the average width of individual plant canopy measured twice, North-South and East-West sides at harvest from all central raw plants per plot
7	Leaf length (mm	Is the mean length of 50 leaves per plant taken from all central raw plants
8	Leaf width (mm)	Is the mean width of 50 leaves per plant taken from all central raw plants
9	Leaf fresh weight plant^{-1} (g)	Is the mean fresh leaf weight of sampled plants measured after separated from stems and branches
10	Stem fresh weight plant^{-1} (g)	Is the mean fresh stem weight of sampled plants
11	Leaf dry weight plant^{-1} (g)	Is the mean leaf dry weight of sampled plants estimated by taking 100g leaf sample from each sampled plant and dried in hot oven at 100oC for 24 hours until constant weight is reached
12	Stem dry weight plant^{-1} (g)	Is the mean stem dry weight of sampled plants estimated by taking 100g stem sample from each sampled plant and dried in hot oven at 100oC for 24 hours until constant weight is reached
13	Leaf to stem ratio	Is the mean of dry leaf weight to dry stem weight of sampled plants
14	Fresh leaf yield (tha^{-1})	Is the yield obtained from harvestable plot and converted in to yield per hectare and expressed in ton per hectare
15	Dry leaf yield (tha^{-1})	Is the yield obtained from harvestable plot and converted in to yield per hectare. It was estimated by taking composite sample of leaves and dried in hot oven
16	Essential oil content (%)	Is the oil content determined from central plants by taking 300g 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 per hectare based on essential oil content and leaf biomass

WGARC, Wondo Genet Agricultural Research Center

RESULT AND DISCUSSION

Analysis of Variance

Highly significant variation among the genotypes was observed for all the characters under study (Table 3), which revealed the existence of an enormous amount of variability among the tested genotypes. A wider range of variation was observed for fresh leaf weight plant^{-1} (257 – 1291 g), dry leaf weight plant^{-1} (74.85 – 367.4 g), essential oil yield ha^{-1} (26.58 –178.1 kg), fresh leaf yield ha^{-1} (7.1 –35.9 t), dry leaf yield ha^{-1} (2.1 – 10.2 t), and essential oil content (0.77 – 2.22%) (Table 4). The significant range of variation observed for the majority of the economic traits of rosemary reflecting the presence of ample scope for identifying superior and desired genotypes by plant breeders for future rosemary improvement programs. The wide range of variations in quantitative traits of rosemary has also been reported (Flamini *et al*., 2002; De-Mastro *et al*., 2004; Kassahun *et al*., 2013; Cervelli & Masselli, 2013).

Variance Components

The extent of variability present in the accessions was measured in terms of genotypic variance (σ^2_G), phenotypic variance (σ^2_P), genotypic coefficient of variation (GCV) and phenotypic coefficient of variation (PCV) (Table 5). The values of genetic variance ranged from 0.08 for leaf to stem ratio to 46782.42 for fresh leaf weight plant^{-1}. Similarly, phenotypic variance ranged from 0.09 to 55461.76 for leaf to stem ratio and fresh leaf weight plant^{-1}, respectively. The magnitudes of genetic variances were higher for fresh leaf weight plant^{-1}, dry leaf weight plant^{-1}, essential oil yield ha^{-1}, and plant height. Thus, the higher proportion of phenotypic variation observed in these traits is due to the larger contribution of genotypic variation.

The genotypic coefficients of variation ranged from 6.19 to 40.51 for leaf length and essential oil yield ha^{-1}, respectively. The values of the phenotypic coefficient of variation also varied from 7.6 for leaf length to 42.72 for essential oil yield ha^{-1} (Table 5). According to Deshmukh *et al*. (1986), GCV and PCV values less than 10% are considered to be low, values between 10% and 20% to be medium and values greater than 20% are regarded as high. Accordingly, medium GCV and PCV values were noted for plant height, internode numbers, internode length, canopy width and leaf to stem ratio, while low GCV and PCV were recorded for leaf length only. The highest values of GCV and PCV were obtained for the majority of the characters vis. branch number plant^{-1}, stem diameter, leaf width, fresh and dry leaf weight plant^{-1}, fresh and dry leaf yields ha^{-1}, essential oil content and essential oil yield ha^{-1}. The highest value of the genotypic coefficient of variation for most of the characters signified the existence of a broad genetic base in the Ethiopian germplasm for these traits. The highest magnitudes of GCV coupled with the highest value of PCV indicated the presence of a wide range of genotypic and phenotypic variability, and hence insured ample scope of improvement of these traits through selection. The result is in agreement with previous findings for other medicinal and oil bearing crops; in osmium for plant height, number of branches plant^{-1}, fresh herb yield and oil content (Smita & Kishori, 2018), in brassica for number of branches plant^{-1} (Naznin *et al*., 2015) and in sesame for number of branches per plant (Parameshwarappa *et al*., 2009).

In the present study, the values of PCV are relatively higher than GCV for all traits; however, there was a close correspondence between the phenotypic and genotypic coefficients of variation for traits like plant height, internode number, canopy width, leaf length, leaf width, leaf to stem ratio and essential oil content. It indicated that these characters were influenced by the environment to a lesser extent and there was a high contribution of genotypic effect for the phenotypic expression of these characters. Therefore, selection based on the phenotypic performance of these characters would be reliable.

Table 3: Analysis of variance for different quantitative traits of rosemary accessions evaluated at WGARC from 2018 to 2019

Traits	Replication mean square (df=2)	Genotypes mean square (df=44)	Error mean square (df=88)
Plant height (cm)	88.11	1109.46***	75.6
Branch number	23.79	290.56***	61.09
Stem diameter (mm)	41.26	135.71***	27.7
Internode number	8.21	173.1***	17.28
Internode length (mm)	17.05	17.28***	4.3
Canopy width (cm)	216.24	322.08***	49.72
Leaf length (mm)	0.96	14.05***	4.75
Leaf width (mm)	0.18	1.99***	0.14
Leaf to stem ratio	0.00	0.26***	0.03
Fresh leaf weight plant^{-1} (g)	58003.45	166385.27***	26038
Dry leaf weight plant^{-1} (g)	4921.69	16111.23***	2321
Fresh leaf yield ha^{-1} (t)	44.756	128.39***	20
Dry leaf yield ha^{-1} (t)	3.79	12.43***	1.79
Essential oil content (%)	2.57	0.37***	0.01
Essential oil yield ha^{-1}(kg)	15505.66	4676.37***	470.7

WGARC=Wondo Genet Agricultural Research Center, df=degree of freedom

Table 4: Estimates of range, mean and coefficient of variation for 15 quantitative traits among rosemary accessions

Traits	Range			
	Minimum	Maximum	Mean±SE	CV (%)
Plant height (cm)	83.30	159.3	108±2.87	8.05
Branch number	18.30	61.00	39.7±1.47	19.68
Stem diameter (mm)	11.60	38.64	21.63±1.00	24.35
Internode number	43.70	82.30	57.2±1.13	7.25
Internode length (mm)	8.68	21.79	14.32±0.36	14.43
Canopy width (cm)	52.33	97.83	74.3±1.54	9.49
Leaf length (mm)	23.90	32.24	28.46±0.32	7.66
Leaf width (mm)	2.37	6.28	3.21±0.12	11.73
Leaf to stem ratio	0.84	2.30	1.5±0.04	11.75
Fresh leaf weight plant^{-1} (g)	257	1291	745.36±35.10	21.65
Dry leaf weight plant^{-1} (g)	74.85	367.4	221±10.90	21.80
Fresh leaf yield ha^{-1} (t)	7.10	35.9	20.7±0.97	21.65
Dry leaf yield ha^{-1} (t)	2.10	10.20	6.1±0.30	21.80
Essential oil content (%)	0.77	2.22	1.48±0.05	5.04
Essential oil yield ha^{-1} (kg)	26.58	178.1	92.43±5.89	23.47

CV, Coefficient of variation

Table 5: Estimates of variance components, heritability and genetic advance for 15 agro-morphological traits of rosemary accessions

Characters	GV	PV	GCV (%)	PCV (%)	Hb^2 (%)	GA	GAM
Plant height (cm)	344.62	369.82	17.19	17.81	0.93	36.92	34.18
Branch number	76.49	96.85	22.03	24.79	0.79	16.01	40.33
Stem diameter (mm)	36.00	45.24	27.74	31.09	0.80	11.03	50.98
Internode number	51.94	57.70	12.60	13.28	0.90	14.09	24.63
Internode length (mm)	4.33	5.76	14.53	16.76	0.75	3.71	25.93
Canopy width (cm)	90.79	107.36	12.82	13.95	0.85	18.05	24.29
Leaf length (mm)	3.10	4.68	6.19	7.60	0.66	2.95	10.37
Leaf width (mm)	0.62	0.66	24.46	25.37	0.93	1.56	48.59
Leaf to stem ratio	0.08	0.09	18.46	19.63	0.88	0.54	35.76
Fresh leaf weight plant^{-1} (g)	46782.42	55461.76	29.02	31.60	0.84	409.22	54.90
Dry leaf weight plant^{-1} (g)	4596.74	5370.41	30.68	33.16	0.86	129.22	58.47
Fresh leaf yield ha^{-1} (t)	36.13	42.80	29.04	31.60	0.84	11.38	54.96
Dry leaf yield ha^{-1} (t)	3.55	4.14	30.68	33.16	0.86	3.59	58.47
Essential oil content (%)	0.12	0.12	23.42	23.75	0.97	0.70	47.60
Essential oil yield ha^{-1}(kg)	1401.89	1558.79	40.51	42.72	0.90	73.15	79.14

GV, genotypic variance; PV, phenotypic variance; GCV, genotypic coefficient of variation; PCV, phenotypic coefficient of variation; Hb^2, broad-sense heritability; GA, genetic advance; GAM, genetic advance as % of the mean

Heritability and Genetic Advance

The genotypic coefficient of variation indicates the contribution of the genetic factors for the observed phenotypic variability; though, it does not give the full scope of the variation that is heritable or not. Thus, an estimate of heritability is necessary to predict the expected gain through selection process. In this study, heritability estimates in a broad-sense ranged from 66% for leaf length to 97% for essential oil content (Table 5). According to Singh *et al.* (2001), heritability values greater than 80% were considered as high, values from 60 to 79% were moderately high, values from 40 to 59% were medium and values less than 40% were low. Hence, the estimates of broad-sense heritability obtained in the present study were high for all traits except branch number, internode length and leaf length which had moderately high broad-sense heritability. Higher values of heritability of these characters reflected a relatively small contribution of the environmental factor to the phenotype and selection based on phenotypic performance for such characters would be easy due to the high additive gene effect. The high estimates of broad-sense heritability have been also reported in other medicinal plants, for branch number plant^{-1} in bottle gourd (Deepthi *et al.*, 2016) and for plant height in *Gossypium hirsutum* (Ahsan *et al.*, 2015). Moderately higher broad–sense heritability for leaf length and essential oil content was also reported by Mengesha and Alemaw (2010) in coriander.

An estimate of heritability alone does not indicate the expected gain in the next generation. Therefore, knowledge of heritability coupled with genetic advances will provide a clear base on the reliability of the particular traits for efficient selection. Estimates of genetic advances for all the studied traits are displayed in Table 5. The magnitude of genetic advance for fresh leaf weight plant^{-1}, dry leaf weight plant^{-1}, fresh leaf yield ha^{-1}, dry leaf yield ha^{-1}, essential oil content and essential oil yield ha^{-1} were 409.22, 129.22, 11.38, 3.59, 0.7 and 73.15, respectively. This indicates that whenever we select the best genotypes, 5% high yielding as a parent, mean values of the offspring could be improved as large as 409.22 g, 129.22 g, 11.38 t, 3.59 t, 0.7% and 73.15 kg for these respective characters.

The utility of information on heritability estimate would be increased when used in combination with genetic advance expressed on a percentage of mean (Allard, 1960; Deepthi *et al.*, 2016). According to Jonhson *et al.* (1955), the value of genetic advance as a percent of the mean is categorized as high (> 20%), moderate (10–20%) and low (< 10%). Based on this category, all characters assessed in the present study possess high genetic advance as a percent of the mean except leaf length, which had a moderate value (Table 5). Characters that exhibit high heritability estimates along with high genetic advance as a percentage of mean are controlled by additive gene action and could be used as a powerful tool in the selection process (Panes & Sukhatme, 1995). In the present study, the majority of evaluated traits exhibited high heritability estimates along with high genetic advance as a percent of the mean, reflecting the presence of additive gene action for the expression of these traits which is fixable for the next generations. Therefore, selection in the next generation based on these characters would be effective. Similar findings have been reported in different medicinal and oil crops (Pandey & Dobhal, 1993; Parameshwarappa *et al.*, 2009; Adityaa *et al.*, 2011; Sangwan *et al.*, 2013; Kumar *et al.*, 2014).

Association Among Characters

Yield is a complex character resulting from the interaction of various characters, which contribute through positive or negative association. Correlation studies provide reliable information about the nature and direction of selection to combine high yield potential with desirable traits (Srivastava *et al.*, 2018). To assess the magnitude of association of various traits with the most important economic yields of rosemary, genotypic and phenotypic correlation coefficients were computed (Table 6). Significant correlation between leaf and essential oil yield with various agro-morphological traits was found. The genotypic correlation coefficients were higher in magnitude than the corresponding phenotypic correlation coefficients for most of the characters, signifying a strong inherent association between the characters studied. Lower phenotypic correlation coefficients than the genotypic correlation coefficient for the

Table 6: Genotypic (below diagonal) and phenotypic (above diagonal) correlation coefficients of 15 quantitative traits of 45 rosemary accessions tested at WGARC during 2018-2019

Traits	PH	BN	SD	IN	IL	CW	LL	LW	FLW	DLW	LSR	FLYH	DLYH	EOC	EOY
PH		-0.38**	0.45**	0.67***	0.59***	0.07	0.10	0.75***	-0.01	0.05	-0.82***	-0.01	0.05	-0.06	-0.02
BN	-0.45**		-0.37*	-0.22	-0.34*	0.66***	-0.24	-0.45**	0.83***	0.78***	0.27	0.83***	0.78***	0.31*	0.80***
SD	0.46**	-0.46**		0.66***	-0.02	-0.28	0.21	0.58***	-0.18	-0.10	-0.58***	-0.18	-0.10	-0.21	-0.19
IN	0.63***	-0.26	0.78***		0.07	-0.03	0.24	0.65***	0.02	0.07	-0.69***	0.02	0.07	0.06	0.06
IL	0.69***	-0.45**	-0.01	0.02		-0.15	-0.17	0.21	-0.19	-0.18	-0.29	-0.19	-0.18	0.04	-0.17
CW	0.05	0.71***	-0.36*	-0.06	-0.18		-0.11	-0.17	0.78***	0.70***	-0.10	0.78***	0.70***	0.36*	0.72***
LL	0.06	-0.35*	0.31*	0.40**	-0.44**	-0.17		0.39**	-0.35*	-0.33*	-0.04	-0.35*	-0.33*	-0.11	-0.29*
LW	0.81***	-0.53***	0.65***	0.67***	0.27	-0.19	0.36*		-0.16	-0.07	-0.70***	-0.16	-0.07	-0.32*	-0.23
FLW	-0.01	1.00***	-0.25	0.01	-0.21	0.93***	-0.5**	-0.2		0.97***	-0.03	1.00***	0.97***	0.25	0.90***
DLW	0.06	1.00***	-0.15	0.07	-0.19	0.83***	-0.5**	-0.1	0.96***		-0.05	0.97***	1.00***	0.17	0.88***
LSR	-0.90***	0.30*	-0.69***	-0.75***	-0.37*	-0.12	0.0	-0.8***	-0.04	-0.06		-0.03	-0.05	-0.06	-0.05
FLYH	-0.01	1.00***	-0.25	0.01	-0.21	0.93***	-0.5***	-0.2	1.00***	0.96***	-0.04		0.97***	0.25	0.90***
DLYH	0.06	1.00***	-0.15	0.07	-0.19	0.83***	-0.5**	-0.1	0.96***	1.00***	-0.06	0.96***		0.17	0.88***
EOC	-0.07	0.36*	-0.23	0.08	0.02	0.39**	-0.1	-0.3*	0.27	0.18	-0.06	0.27	0.18		0.60***
EOY	-0.03	0.99***	-0.25	0.06	-0.18	0.84***	-0.39**	-0.3	0.89***	0.87***	-0.05	0.89***	0.87***	0.63***	

PH, plant height; BN, branch number; SD, stem diameter; IN, internode length; CW, canopy width; LL, leaf length; LW, leaf width; FLW, fresh leaf weight plant^{-1}; DLW, dry leaf weight plant^{-1}; LSR, leaf to stem ratio; FLYH, fresh leaf yield ha^{-1}; DLYH, dry leaf yield ha^{-1}; EOC, essential oil content; EOY, essential oil yield ha^{-1} WGARC, Wondo Genet Agricultural research Center

majority of the traits indicated reduced expression of phenotypic association due to environmental influence. Similar trends were reported for related species by different researchers (Kassahun *et al.*, 2013; Sangwan *et al.*, 2013; Kumar *et al.*, 2014; Naznin *et al.*, 2015). In few cases (internode numbers, canopy width and leaf length), phenotypic correlation coefficients were higher than their respective genotypic correlation coefficients showing that both environmental and genotypic correlations acted in the same direction, and maximized phenotypic expression.

Fresh leaf weight plant^{-1} was significantly and positively correlated with branch number plant^{-1} (r_g =1, r_p = 0.83) and canopy width (r_g = 0.93, r_p = 0.78) but negatively correlated with leaf length (r_g = -0.5, r_p =-0.35) both at the genotypic and phenotypic level. Dry leaf weight plant^{-1} correlated positively with branch number plant^{-1} (r_g = 1.00, r_p = 0.78), canopy width (rg = 0.83, rp = 0.7) and fresh leaf weight plant^{-1} (r_g = 0.96, r_p = 0.97), while leaf length was negatively correlated with dry leaf weight plant^{-1} (r_g = -0.5, r_p = -0.33) both at the genotypic and phenotypic level. Therefore, any change in these traits will have a considerable effect on dry and fresh leaf weight plant^{-1}.

Branch number plant^{-1} (r_g =1, r_p = 0.83), canopy width (r_g = 0.93, r_p = 0.78), fresh leaf weight plant^{-1} (r_g =1, r_p =1.00) and dry leaf weight plant^{-1} (r_g = 0.96, r_p = 0.97) were found to be significantly and positively correlated with fresh leaf yield ha^{-1} both at genotypic and phenotypic correlation level. However, leaf length was negatively correlated with fresh leaf yield ha^{-1} (r_g =-0.5, r_p =-0.35). Similarly, dry leaf yield ha^{-1} showed positive and significant association both at genotypic and phenotypic levels with branch number plant^{-1} (r_g 1 =, r_p = 0.78), canopy width (r_g = 0.83, r_p = 0.70), fresh leaf weight plant^{-1} (r_g = 0.96, r_p = 0.97) dry leaf weight plant^{-1} (r_g =1, r_p =1) and fresh leaf yield ha^{-1} (r_g = 0.96, r_p = 0.97). But, its association with leaf length was negative (r_g = -0.5, r_p =-0.33). The negative association of leaf length with fresh and dry leaf yields implies that selection for plants with longer leaf would result in decreased leaf yields.

Essential oil content showed positive interaction with branch number plant^{-1} (r_g = 0.36, r_p = 0.31) and canopy width (r_g = 0.39, r_p = 0.36) both at the genotypic and phenotypic level, suggesting that essential oil content can be improved through selection for these yield components. But the association of essential oil content with leaf width was negative (r_g =-0.3, r_p = -0.32) indicating that plants with narrow leaves should be exploited to enhance essential oil content in rosemary. Essential oil yield ha^{-1} was significantly and positively correlated with branch number plant^{-1} (r_g = 0.99, r_p = 0.80), canopy width (r_g = 0.84, r_p = 0.72), fresh leaf weight plant^{-1} (r_g = 0.89, r_p = 0.90), dry leaf weight plant^{-1} (r_g = 0.87, r_p = 0.88), fresh leaf yield ha^{-1} (r_g = 0.89, r_p = 0.90), dry leaf yield ha^{-1} (r_g = 0.87, r_p = 0.88), and essential oil content (r_g = 0.63, r_p = 0.60). Therefore, selection for these attributes would lead to higher essential oil yield ha^{-1}. However, leaf length was negatively correlated with essential oil yield ha^{-1} (r_g =-0.4, r_p =-0.29). The significant and positive association of branch number plant^{-1} and canopy width with leaf, essential oil content and essential oil yield indicates the practicality of considering these traits in the improvement program. The positive and significant associations among fresh leaf yield, dry leaf yield, essential oil content, and essential oil yield reflect the possibility of simultaneous improvement of these traits.

Path Coefficients Analysis

The estimates of correlation coefficients indicate the interrelationship of different characters, but it does not measure the relative importance of the direct and indirect influence of each component on yield (Nazin *et al.*, 2015). Path analyses partitioned the correlation coefficients into direct and indirect effects and help to find out a clear picture of the inter-relationship between yield and other yield attributes (Rukhsar *et al.*, 2018). To assess the magnitude of the contributions of various characters to essential oil yield ha^{-1} in the form of cause and effects, path coefficient analysis was performed (Table 7). Out of the 14 characters which were considered as causal

Table 7: Path coefficient analysis showing direct and indirect effect of yield components on essential oil yield ha^{-1} in 45 rosemary accessions

	PH	BN	SD	IN	IL	CW	LL	LW	FLW	DLW	LSR	FLYH	DLYH	EOC	EOY
PH	**0.0043**	-0.8017	0.1682	-0.4736	0.6035	-0.0600	0.0643	-0.0871	-0.0217	0.0127	0.6913	-0.0076	-0.1069	-0.0164	-0.03
BN	-0.0019	**1.7841**	-0.1673	0.1969	-0.3887	-0.8320	-0.3972	0.0565	1.8312	0.2161	-0.2269	0.6414	-1.8105	0.8166	0.99
SD	0.0020	-0.8142	**0.3666**	-0.5890	-0.0122	0.4223	0.3477	-0.0696	-0.4537	-0.0322	0.5247	-0.1589	0.2695	-0.5258	-0.25
IN	0.0027	-0.4643	0.2855	**-0.7564**	0.0192	0.0742	0.4576	-0.0718	0.0233	0.0157	0.5759	0.0082	-0.1318	0.1770	0.06
IL	0.0030	-0.7974	-0.0052	-0.0167	**0.8697**	0.2131	-0.5002	-0.0294	-0.3803	-0.0421	0.2806	-0.1332	0.3524	0.0044	-0.18
CW	0.0002	1.2624	-0.1317	0.0478	-0.1577	**-1.1757**	-0.1906	0.0203	1.7092	0.1800	0.0897	0.5987	-1.5077	0.0924	0.84
LL	0.0002	-0.6219	0.1118	-0.3038	-0.3817	0.1966	**1.1396**	-0.0385	-0.9091	-0.1008	0.0179	-0.3184	0.8448	-0.2426	-0.39
LW	0.0035	-0.9379	0.2374	-0.5050	0.2374	0.2224	0.4077	**-0.1075**	-0.3303	-0.0166	0.5911	-0.1157	0.1390	-0.7692	-0.25
FLW	-0.0001	1.7839	-0.0908	-0.0096	-0.1806	-1.0973	-0.5657	0.0194	**1.8313**	0.2075	0.0270	0.6414	-1.7383	0.6166	0.89
DLW	0.0003	1.7839	-0.0546	-0.0550	-0.1693	-0.9789	-0.5318	0.0083	1.7581	**0.2161**	0.0443	0.6158	-1.8105	0.0436	0.87
LSR	-0.0039	0.5296	-0.2517	0.5699	-0.3192	0.1379	-0.0267	0.0832	-0.0646	-0.0125	**-0.7643**	-0.0226	0.1051	-0.1379	-0.05
FLYH	-0.0001	1.7839	-0.0908	-0.0096	-0.1805	-1.0973	-0.5657	0.0194	1.8312	0.2075	0.0270	**0.6415**	-1.7382	0.6167	0.89
DLYH	0.0003	1.7839	-0.0546	-0.0551	-0.1693	-0.9790	-0.5317	0.0083	1.7581	0.2161	0.0444	0.6158	**-1.8107**	0.0436	0.87
EOC	-0.0003	0.6382	-0.0844	-0.0587	0.0160	-0.4579	-0.1211	0.0362	0.4947	0.0397	0.0462	0.1733	-0.3326	**0.2374**	0.63

PH, plant height; BN, branch number; SD, stem diameter; IN, Internode length; CW, canopy width; LL, Leaf length; LW, Leaf width; FLW, Fresh leaf weight plant^{-1}; DLW, Dry leaf weight plant^{-1}; LSR, Leaf to stem ratio; FLYH, Fresh leaf yield ha^{-1}; DLYH, Dry leaf yield ha^{-1}; EOC, Essential oil content; EOY, Essential oil yield ha^{-1}

factors, nine characters had a positive direct influence, whereas the remaining five characters had a negative direct effect on essential oil yield ha^{-1} (Table 7).

Fresh leaf weight plant^{-1} had the highest positive direct effect (1.8313) on essential oil yield ha^{-1}. It also had a positive indirect effect on essential oil yield ha^{-1} through branch number plant^{-1}, leaf width, dry leaf weight plant^{-1}, leaf to stem ratio, fresh leaf yield ha^{-1}and essential oil content. Due to its high positive direct and indirect effects, the negative indirect effect through various traits was counterbalanced and resulted in a highly significant positive correlation with essential oil yield ha^{-1} (r_g = 0.89***). Therefore, fresh leaf weight plant^{-1} is found to be an important component and direct selection for this trait may be rewarding for the improvement of rosemary essential oil yield. Similarly, branch number plant^{-1} scored a high positive direct effect (1.7841) on essential oil yield ha$^{-1.}$ It also exerted a positive indirect effect via various traits (internode number, leaf width, fresh leaf weight plant^{-1}, dry leaf weight plant^{-1}, fresh leaf yield ha,$^{-1}$ and essential oil content) on essential oil yield ha^{-1} and resulted in a highly significant positive genotypic correlation with essential oil yield ha^{-1} (r_g = 0.99***). Thus, considering genotypes with high branching ability would be effective in essential oil yield improvement program.

The direct effect of leaf length on essential oil yield ha^{-1} was high and positive (1.1396). It also demonstrated positive indirect influence through plant height, stem diameter, canopy width, leaf to stem ratio and dry leaf yield ha^{-1}. But, its negative indirect effect via branch number plant^{-1}, internode number, internode length, leaf width, fresh leaf weight plant^{-1}, dry leaf weight plant^{-1}, fresh leaf yield ha,$^{-1}$ and essential oil content caused a negative association of leaf length with essential oil yield ha^{-1} (r_g =-0.39**). Even though the correlation of leaf length with essential oil yield ha^{-1} was negative, it might be important to consider this trait in the selection of genotypes for essential oil yield improvement due to its high positive direct effect on essential oil yield ha^{-1}.

Path coefficient analysis also showed that fresh leaf yield ha^{-1} had a high positive direct effect on essential oil yield ha^{-1} (0.6415) and a positive indirect effect through branch number plant^{-1}, leaf width, fresh leaf weight plant^{-1}, dry leaf weight plant^{-1}, leaf to stem ratio, and essential oil content. Even though its effect on essential oil yield ha^{-1} via the remaining characters was negative, the high and positive direct and indirect influences made the overall genotypic correlation of fresh leaf yield ha^{-1} with essential oil yield ha^{-1} positive and highly significant (r_g = 0.89***). Hence, this trait is important and directly attributed to the improvement of essential oil yield in rosemary.

The direct effect of internode length, stem diameter and plant height on essential oil yield ha^{-1} were positive (0.8697, 0.3666 and 0.0043, respectively). But the negative indirect influence of these characters through the various traits made the net overall correlation of these traits with essential oil yield ha^{-1} negative and non-significant (Table 7). Even though the correlation of internode length, stem diameter and plant height with essential oil yield was non-significant, path analysis revealed a positive direct contribution of these traits for essential oil yield ha^{-1}. Therefore, giving allowance for these traits might be useful in the selection of desirable genotypes for essential oil yield improvement.

Essential oil content had a positive direct contribution to essential oil yield ha^{-1} (0.2374). It also had a positive indirect contribution on essential oil yield ha^{-1} through branch number plant^{-1}, internode length, leaf width, fresh leaf weight plant^{-1}, dry leaf weight plant^{-1}, leaf to stem ratio, and fresh leaf yield ha^{-1}. On the other hand, the indirect effect of essential oil content on essential oil yield ha^{-1} through the rest traits was negative. However, due to high indirect and direct positive effects, the genotypic correlation of essential oil content with essential oil yield ha^{-1} was positive and highly significant (r_g = 0.63***). Therefore, selection would be effective for this trait for essential oil yield improvement.

Dry leaf weight plant^{-1} had a positive direct effect on essential oil yield ha^{-1} (0.2161) and a positive indirect effect through plant height, branch number plant^{-1}, leaf width, fresh leaf weight plant^{-1}, leaf to stem ratio, fresh leaf yield ha,$^{-1}$ and essential oil content. Due to these positive effects, the genotypic correlation of dry leaf weight plant^{-1} with essential oil yield ha^{-1} was positive and highly significant (r_g = 0.87***). Thus, dry leaf weight plant^{-1} was found an important character to be considered in essential oil yield enhancement mainly due to its positive direct and indirect contribution on essential oil yield ha^{-1}.

Dry leaf yield ha^{-1} had a direct negative effect (-1.8107) on essential oil yield ha^{-1}. It also demonstrated an indirect negative effect on essential oil yield through stem diameter, internode number, internode length, canopy width, and leaf length; and an indirect positive effect through the rest characters. Even though the genotypic correlation of dry leaf yield ha^{-1} with essential oil yield ha^{-1} was positive and highly significant (r_g = 0.87***), it had a direct negative influence on essential oil yield ha^{-1}. Hence, selection for dry leaf yield ha^{-1} depending only on its association would not be effective for the improvement of essential oil yield. Canopy width also had a high negative direct effect (-1.1757) and a negative indirect effect on essential oil yield ha^{-1} through stem diameter, internode length, leaf length and dry leaf yield ha^{-1}. However, due to its positive indirect influence via the majority of the remaining traits, the genotypic correlation with essential oil yield ha^{-1} was positive and highly significant (r_g = 0.84***). As this significant and positive association was not directly contributed by canopy width, considering only this trait in the selection of better genotypes for essential oil yield enhancement in rosemary may not be practical.

The direct effect of leaf to stem ratio (-0.7643), internode number (-0.7564) and leaf width (-0.1075) on essential oil yield ha^{-1} was negative. These traits also presented negative and positive indirect effects through various characters which nullified each other and made the final association non-significant (Table 7). Therefore, considering these traits would not be important for essential oil yield improvement due to their negative direct contribution and non-significant correlation.

Overall, traits such as branch number plant^{-1}, fresh leaf weight plant^{-1}, dry leaf weight plant^{-1}, fresh leaf yield ha,$^{-1}$ and essential oil content were found the most important contributors for essential oil yield ha^{-1} due to their high positive direct effect and strong positive association. Hence, these characters should be considered as important selection criteria in the improvement program and direct selection for these traits would be suggested for essential oil yield enhancement in rosemary.

CONCLUSION

Analysis of variance displayed a highly significant difference among the accessions for all the studied characters. Medium to high genotypic and phenotypic coefficients of variation were observed for all growth and yield traits except for leaf length, reflecting the existence of wide range of variability among the studied accessions. The estimates of heritability were observed to be moderately high and high for all characters studied, showing that higher contribution of genetic factors than environmental factors for the observed variability. It was also noticed that all the evaluated characters exhibited high to moderately high heritability coupled with high to moderate genetic advance as a percentage of the mean, which indicated the presence of ample scope of improvement through selection. The presence of high genotypic coefficient of variation, high heritability and high heritability coupled with high genetic advance as a percentage of mean in this study insures that the possibility of simple selection for these traits to enhance genetic improvement in the desired direction.

The magnitudes of genotypic correlation coefficients were higher than the corresponding phenotypic correlation coefficients for the majority of the studied traits, suggesting the existence of a strong inherent association among the characters. A strong and positive association of branch number plant^{-1}, canopy width and leaf weight plant^{-1} with leaf and essential oil yields ha^{-1} was observed. This indicated that selection for these traits would result in leaf and essential oil yield improvement. In path coefficient analysis, positive direct effects on essential oil yield ha^{-1} were exerted by branch number plant^{-1}, fresh and dry leaf weight plant^{-1}, fresh leaf yield ha^{-1} and essential oil content. Therefore, these characters could be used as good criteria and simple selection could be possible in rosemary essential oil yield improvement program. The study demonstrated the presence of heritable broad genetic diversity among the tested accessions that could be exploited for the future improvement program.

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