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MORPHOLOGICAL AND ESSENTIAL OIL CONTENT DIVERSITY OF IRANIAN MINTS (*MENTHA SPP*)*

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Abstract – There is little information available on morphological and agronomic traits, as well as chemical compositions of Iranian mint landraces. This study was conducted to investigate the morphological, agronomic and oil content characteristics of Iranian mint landraces using twelve landraces, three of which belong to *Mentha longifolia,* and the remaining, *Mentha spicata* L, originated from central regions of Iran. Days to 50% and 100% flowering, plant height, number of lateral branches, leaf length, leaf width, herbage yield per plant, number of nodes per main stem, number of nodes per lateral branch, spike length of main stem, number of spikes per plant, height of lateral branches and spike length of lateral branches were recorded and used in this study. The results showed the significant differences among landraces for all tested traits with the exception of the number of nodes per lateral branch. Two accessions, including Mzin3 with 1.9 ml/100g essential oil content, and Mzin6 with 2.1 ml/100g essential oil content, produced the highest essential oil content. Mzin3, with15.9 g/plant herbage dry yield and Mzin6 8.1 g/plant, produced the highest and lowest herbage yield. Genotypic and phenotypic coefficients of variation were high for the spike length of the main stem (32.3%, 29.7 %), the number of spikes per plant (41.7%, 28.5%), and the essential oil content (38.5%, 34.1%), and was low for days to 50% flowering (11.9%, 10.7 %), days to 100% flowering (9.2%, 6.5%) and number of nodes per main stem (9.7%, 6.9%). Stepwise regression analysis indicated that leaf length justified 53 percent of the total variation and was accounted as the most important component of essential oil content. Cluster analysis divided the landraces into 3 groups, each of which having 5, 3 and 4 clones. The number of nodes per main stem, number of lateral branches, spike width of main stem, leaf width, days to 50% flowering, plant height and nodes per lateral branches were the major sources of diversity among the mint clones. Strong association was observed between leaf length and essential oil content (r = 0.73). Therefore, under tested environmental conditions, leaf length may be used as a morphological criterion for selecting clones with high essential oil content in mint.

Keywords – Mint, *mentha spicata*, *mentha longifolia*, essential oil content, diversity

1. INTRODUCTION

Mentha species is commercially grown for its essential oil content and herbage yields [1]. It has a variety of applications in the pharmaceutical, perfumery, food, confectionery and cosmetics industries [1-3]. Genetic improvement in *Mentha species* leading to a wider adaptation, higher herbage yield, higher essential oil content and better quality of oil will allow economical production of mint related commodities [4].

Currently, much emphasis is being laid on conserving plant germplasm as valuable bio-resources because obscure genes from these plants may provide solutions to new diseases, insects,

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environmental or crop production problems [4, 5]. Selection between and within accessions for a high level of essential oil content, herbage yield and other characters require an effective tool to be employed by mint breeders [6].

Cluster analysis has been used to assess similarities among landraces in plant breeding programs where genotypic and phenotypic repetitions of several characters were found among a set of populations, lines, or accessions from which parents were selected for hybridization [7]. Ward's cluster and principal component analyses were used to investigate the nature and degree of divergence using peal millet populations [8]. Four principal components were found to explain 92% of the total variation. Days to 50% flowering, plant height, stem diameter and grain and spike yield per plot were the major sources of diversity among the landraces [8]. Multivariate statistical methods such as principal component and cluster analysis are very useful for summarizing and describing the variation found either in natural or breeding populations, especially if based on morphological data showing continuous distributions [9]. Multivariate analysis has been used to evaluate and classify chemical composition genotypes of *Mentha species* [10].

Correlation analysis of several oil components of mints was conducted and a significant correlation was found between the components [6]. Mirzaie-Nodoushan et al. [6] applied path analysis and reported the association between essential oil content and stem diameter, stem length, leaf length and leaf width. Essential oil content can be increased in *Mentha species* by indirect selection using the correlated characters [6].

There is little information available on morphological and agronomic traits, as well as essential oil content of Iranian mint landraces. The objective of this study was to evaluate morphological, agronomic, and essential oil content variability among 12 Iranian mint landraces.

2. MATERIALS AND METHODS

The aerial parts of twelve fully flowered accessions of Iranian mint (Mzin1-12) collected from central regions of Iran were identified as *Mentha longifolia* (L.), Hudson (Mzin5, Mzin6 and Mzin11) and *Mentha spicata* L. (Mzin1, Mzin2, Mzin3, Mzin4, Mzin7, Mzin8, Mzin9, Mzin10 and Mzin 12) by R. Olson at the W.P. Fraser Herbarium. Voucher specimens were deposited at the W.P. Fraser Herbarium, University of Saskatchewan, Saskatoon, Canada.

Clones of twelve Iranian mint landraces were grown at the Agriculture Research Farm of Isfahan University of Technology located in Shervedan, Isfahan, in April, 2001 (Table 1). The mint clones were provided by Isfahan Agricultural Research Center. A randomized complete block design with three replications was used, where the clones were planted at a density of ten plants per m^{-2}. Before planting, plots were fertilized with 50 kg P/ha and 50kg N/ha. After planting, 100 kg N/ha was top dressed in two equal splits (30 and 50 days after planting).The plots were irrigated every ten days. Days to 50% flowering, days to 100% flowering, plant height, number of lateral branches, leaf length, leaf width, herbage yield per plant, number of nodes on main stem, number of nodes on lateral branches, spike length of main stem, spike width of main stem, number of spike per plant, length of lateral branches and spike length of lateral branches were scored using ten randomly selected plants. Plots were harvested at full flowering stage, where the net area of 1.5 m^2 of each plot was harvested after discarding marginal borders. The aerial parts of the plants at flowering stage were dried at room temperature. The samples were then grossly pulverized and submitted to hydrodistillation for 2 hrs using a Clevenger-type apparatus and then essential oil content was extracted in ml/100g herbage dry matter (DM) (11).

Data were subjected to analysis of variance, and mean comparison was conducted using an LSD test. Genotypic and phenotypic coefficients of variation were calculated according to the method of Burton (12). Heritability estimates were made by the formula suggested by Robinson et al (13). The Pearson correlation coefficients were calculated using main economic traits of essential oil content and herbage yield per plant with other studied traits. Stepwise regression analysis was inducted on essential oil content and herbage yield per plant as dependent variables. Variance between accessions was further studied by using the principal component analysis concerned with explaining the variance-covariance structure through a few linear combinations of the original variables. The variables were standardized and its principal component was given as described by Quendeba (14). Cluster analysis was conducted with 12 clone means of the variables according to Ward's minimum variance method using the cluster procedure of SAS computer software (SAS Institute, 1996) [15]. The clones' means were standardized with a mean of zero and a standard deviation of one.

Table 1. The accession number, name, species, origin and source of 12 Iranian mint landraces used in this study

Accession	Landraces name	Species	Origin	Source
Mzin1	American mint	*Mentha spicata*	Kashan	IARC
Mzin2	M.pipertia 1	*Mentha spicata*	Kashan	IARC
Mzin3	Isfahan poneh mint	*Mentha spicata*	Isfahan	IARC
Mzin4	Isfahan mint	*Mentha spicata*	Isfahan	IARC
Mzin5	M.piperita 2	*Mentha longifolia*	Kashan	IARC
Mzin6	Poneh	*Mentha longifolia*	Isfahan	IARC
Mzin7	Sosanber	*Mentha spicata*	Kashan	IARC
Mzin8	Mahalaty mint	*Mentha spicata*	Mahalat	IARC
Mzin9	Gozestany poneh mint	*Mentha spicata*	Isfahan	IARC
Mzin10	Ghazwen mint	*Mentha spicata*	Kashan	IARC
Mzin11	Spearmint	*Mentha longifolia*	Kashan	IARC
Mzin12	Badrody	*Mentha spicata*	Isfahan	IARC

IARC: Isfahan Agriculture Research Center

3. RESULTS AND DISCUSSION

The Iranian mint landraces differed significantly for all of the traits with the exception of number of nodes per lateral branch (Table 2). Accession means for days to 50 percent flowering varied from 66 to 89.6. The differences of days to 50 percent flowering among Mzin1, Mzin2, Mzin3, Mzin5, Mzin6, Mzin8, Mzin9 and Mzin11 were small and not significant. Plant height varied from 49.2 cm for Mzin1 to 86.1 cm for Mzin3. The leaf width varied from 1.3 cm for Mzin6 to 2.33 cm for Mzin3. Leaf length varied from 3.3 cm for Mzin2 to 5.1 cm for Mzin8. Mzin3 produced the highest herbage yield of 15.9 g/plant and Mzin6 had the lowest (8.1 g/plant). Mzin3 with 1.9 ml/100g DM and Mzin6 with 2.1 ml/100g/DM produced higher essential oil content than other accessions. Considering both herbage yield per plant and essential oil content (Table 3), Mzin3 could be suggested for growing in the region.

Table 2. Mean and Mean squares of 15 tested characteristics of Iranian mint landraces

Characters	Means ±SE	Mean of squares
Days to 50% flowering	72.91 ±8.11	197.04^{**}
Days to 100% flowering	86.86 ±6.49	126.63^{**}
Plant height	67.84 ±9.88	293.37^{**}
Number of nodes on main stem	14.59 ±1.16	4.06^{**}
Number of nodes on lateral branches	127.15 ±18.86	1067.26
Number of lateral branches	24.61 ±3.17	30.22^{**}
Spike length of main stem	8.99 ±2.75	22.75^{**}
Number of spikes per plant	28.83 ±9.65	279.58^{**}
Spike width of main stem	0.85 ±.17	0.08^{**}
Height of lateral branches	27.97±5.28	83.89^{**}
Spike length of lateral branches	5.05±1.33	5.36^{**}
Leaf width	1.87±.33	0.33^{**}
Leaf length	3.97±.49	0.74^{**}
Essential oil content	1.26± .45	0.61^{**}
Yield per plant	10.73±2.23	15.01^{**}

*, ** Significant at the 5% and 1% levels of probability, respectively

Table 3. Mean comparison of 12 Iranian mint landraces for 15 tested traits using LSD test

Accession	1	2	3	4	5	6	7	8
Mzin1	66.3^{c}	85^{c}	49.2^{g}	13.2^{dc}	96.5^{b}	20.4^{c}	7.1^{ed}	10.7^{ab}
Mzin2	66.0^{c}	83.6^{c}	61.9^{ef}	14.4^{a-d}	136.8^{ab}	24.2^{b}	10.1^{bc}	9.3^{b}
Mzin3	66.3^{c}	83.3^{c}	86.1^{a}	13.9^{b-d}	133.8^{ab}	24.5^{b}	11.6^{b}	15.9^{a}
Mzin4	89.6^{a}	100^{a}	66.1^{dec}	16.2^{a}	149.5^{ab}	26.8^{ab}	9.9^{bc}	8.7^{b}
Mzin5	69.3^{bc}	77.6^{c}	65.2^{d-f}	14.5^{a-d}	159.1^{a}	25.4^{ab}	7.5^{c-e}	12.8^{ab}
Mzin6	73.3^{bc}	83^{c}	72.4^{b-d}	15.3^{a-c}	137.2^{ab}	28.4^{a}	6^{e}	8.1^{b}
Mzin7	77^{b}	86.6^{bc}	74.4^{bc}	14.8^{a-d}	123.2^{ab}	25.5^{ab}	7.2^{ed}	11.0^{ab}
Mzin8	72.3^{bc}	86.6^{bc}	65.3^{d-f}	15.7^{ab}	137.2^{ab}	25.9^{ab}	5.6^{e}	11.9^{ab}
Mzin9	69.3^{bc}	84.3^{c}	76.2^{b}	15.2^{a-c}	124.5^{ab}	25.9^{ab}	8.9^{b-d}	12.3^{ab}
Mzin10	68^{c}	85^{c}	57.3^{gf}	12.5^{d}	114.1^{ab}	19.7^{c}	10.7^{b}	8.9^{ab}
Mzin11	69.3^{bc}	87.6^{a-c}	63.3^{ef}	13.4^{dc}	99.8^{b}	19.5^{c}	15.4^{a}	9.1^{b}
Mzin12	88^{a}	99.3^{ab}	76.6^{b}	16.1^{ab}	114.1^{ab}	29^{a}	7.9^{c-e}	9.8^{b}

Table 3. Continued

Accession	9	10	11	12	13	14	15
Mzin1	46.5^{a}	.8^{b-d}	22.3^{bc}	4.2^{c-e}	2.1^{ab}	3.7^{cd}	1.2^{e-d}
Mzin2	23.6^{b}	.8^{b-d}	20.2^{c}	6.2^{a-c}	1.7^{a-d}	3.3^{d}	.9^{e}
Mzin3	44.3^{a}	1.0^{a-c}	31.3^{a-c}	7.1^{ab}	2.3^{a}	4.3^{bc}	1.9^{ab}
Mzin4	17.8^{b}	.6^{ed}	37.3^{a}	5.2^{a-d}	1.4^{cd}	3.9^{cd}	.8^{e}
Mzin5	26.4^{ab}	.9^{a-c}	24.4^{bc}	4.9^{a-e}	2.2^{ab}	3.8^{cd}	.8 ^{e}
Mzin6	17.8^{b}	.8^{e-c}	32.6^{ab}	3.7^{de}	1.3^{d}	4.8^{ab}	2.1^{a}
Mzin7	35.8$^{a\ b}$	.8^{dc}	31.4^{a-c}	4.5^{c-e}	2.1^{ab}	3.9^{cd}	1.4^{e-d}
Mzin8	32.1^{ab}	.5^{e}	27.5^{a-c}	2.7^{e}	1.7^{b-d}	5.1^{a}	1.5^{b-d}
Mzin9	22.7^{b}	.9^{a-c}	32.4^{ab}	4.8^{b-e}	2.0^{a-c}	3.9^{cd}	1.6^{cb}
Mzin10	31.8^{ab}	1.0^{ab}	21.9^{bc}	5.7^{a-d}	2.2^{ab}	3.6^{cd}	.9^{ed}
Mzin11	27.6^{ab}	1.1^{a}	24.5^{bc}	7.3^{a}	1.9^{a-d}	3.5^{cd}	.9^{e}
Mzin12	19.5^{b}	.7^{ed}	29.9^{a-c}	4.4^{c-e}	1.6^{b-d}	3.8^{cd}	1.0^{e-d}

1=day to 50% flowering; 2=day to 100% flowering; 3=plant height; 4=number of nodes on main stem; 5=number of nodes on lateral branches; 6=number of lateral branches; 7=spike length of main stem; 8=yield per plant; 9=number of spikes per plant; 10=spike width of main stem; 11=height of lateral branches; 12=height of spikes of lateral branches; 13=leaf width; 14=leaf length; 15=essential oil content. Means with the same letter are not significantly different

The genotypic and phenotypic coefficient of variations is given in Table 4. Phenotypic coefficient of variation for spike length of the main stem (32.3%), number of spikes per plant (41.7%), spike height of lateral branches (31.3%) and essential oil content (38.5%) were high. Phenotypic coefficient of variation for days to 50 percent flowering (11.9%), days to 100 percent flowering (9.1%) and number of nodes on main stems (9.7%) were low. In general, phenotypic coefficients of variation were higher than the corresponding genotypic coefficients of variation (Table 4).

Table 4. Coefficient of variability and broad sense heritability of studied traits using 12 Iranian mint landraces

Characters	Coefficient of variability (%)		Heritability broad sense (%)
	Phenotypic	Genotypic	
Days to 50% flowering	11. 9	10. 7	80.0
Days to 100%flowering	9. 1	6. 5	50.1
Plant height	15. 3	14. 2	86.5
Number of nodes on main stem	9. 7	6. 9	51.7
Number of nodes on lateral branches	21. 1	10. 3	23.9
Number of lateral branches	14. 0	12. 3	77.2
Spike length of main stem	32. 3	29. 7	84.6
Number of spikes per plant	41. 7	28. 5	37.9
Spike width of main stem	22. 0	18. 6	46.5
Height of lateral branches	23. 8	15. 8	44.5
Spike length of lateral branches	31. 3	23. 6	56.8
Leaf width	21. 4	15. 5	52.8
Leaf length	14. 2	11. 5	66.5
Essential oil content	38. 5	34. 1	78.3
Yield per plant	27. 2	16.7	71.4

Broad sense heritability estimated for days to 50% flowering (80.0%), plant height (86.5%), number of lateral branches (77.2%), spike length of main stem (84.6%), herbage yield per plant (71.4%) and essential oil content (78.3%) were relatively high (Table 4). Table 5 shows the correlation coefficients for all traits with herbage yield and essential oil content. Essential oil content had only positive and significant correlation with plant height (0.56) and leaf length (0.73) (Table 5).

Table 5. Phenotypic correlation coefficient between essential oil content and herbage yield plant with other traits

Characters	Essential oil content	Herbage yield plant
Days to 50% flowering	-0.21	-0.35
Days to 100% flowering	-0.30	-0.39
Plant height	0.56*	0.49
Number of nodes on main stem	0.14	-0.08
Number of nodes on lateral branches	0.07	0.22
Number of lateral branches	0.36	0.05
Spike length of main stem	-0.35	-0.01
Number of spikes per plant	0.21	0.57*
Spike width of main stem	-0.09	0.19
Height of lateral branches	0.44	0.09
Spike length of lateral branches	-0.26	0.13
Leaf width	-0.05	0.65*
Leaf length	0.73**	0.21
Essential oil content	1	0.36

**, *Significant at the 1% and 5% levels of probability, respectively

Results of stepwise regression analysis for essential oil content as dependent variable, and the rest of the traits as independent variables, showed that leaf length accounted for 53 percent of the total variation in essential oil content (Table 6). This suggested that leaf length is the most important component of essential oil content. Therefore, selection for a higher leaf length would result in the improvement of essential oil content. Stepwise regression analysis, using herbage yield per plant as dependent variable, showed that leaf width is the most important component accounting for 42 percent of the total variation of herbage yield per plant. Afterward, the number of nodes per main stem and essential oil content were applied into the model and totally justified 84 percent variation of herbage yield per plant (Table 6).

Table 6. Stepwise regression analysis for 15 studied traits of Iranian mint landraces using essential oil as a dependent variable

Interred variable to model	intercept	regression coefficient	R^2
Leaf length	-1.37	0.66	0.53**

Using herbage yield per plant as a dependent variable

Interred variables to model	intercept	Regression coefficients b1	b2	b3	R^2
Leaf width	2.45	4.40			0 .42*
Number of nodes of main stem	-27.23	8.25	1.53		0.31**
Essential oil of yield	-27.02	8.05	1.41	1.63	0 .11*

**, * Significant at the 1% and 5% levels of probability, respectively

Principal component analysis revealed four components, which justified 86 percent of the total variation among traits (Table 7). Principal component 1 (PC1) justified 42 percent of the total variation and was equally associated with days to 50 percent flowering, number of nodes on the main stem and number of nodes on lateral branches. PC2 mainly consisted of essential oil content and herbage yield per plant, and accounted for 21 percent of total variation. PC3 explained 14 percent of the total variation and was mainly associated with plant height, spike length of main stem and lateral branches. PC4 accounted for 8 percent of the total variation and consisted of days to 100 percent flowering and number of lateral branches.

Table 7. Principal component coefficients of 15 tested traits in 12 Iranian mint landraces

Characters	PC1	PC2	PC3	PC4
Days to 50% flowering	0.30	-0.26	0.14	0.23
Days to 100% flowering	0.21	-0.34	0.17	0.44
Plant height	0.16	0.27	0.49	0.05
Number of nodes on main stem	0.38	-0.02	0.08	-0.06
Number of nodes on lateral branches	0.19	0.14	0.09	-0.63
Number of lateral branches	0.36	0.11	0.12	-0.16
Spike length of main stem	-0.23	-0.21	0.44	0.04
Number of spikes per plant	-0.24	0.28	-0.13	0.43
Spike width of main stem	-0.32	0.03	0.28	-0.13
Height of lateral branches	0.29	0.09	0.32	0.21
Spike length of lateral branches	-0.25	-0.11	0.47	-0.09
Leaf width	-0.31	0.23	0.08	0.09
Leaf length	0.22	0.34	-0.14	0.13
Essential oil content	0.11	0.45	0.03	0.19
Yield per plant	-0.07	0.44	0.21	0.04
Percent variation	0.42	0.21	0.14	0.08
Cumulative percent of total variance	0.42	0.63	0.77	0.85

Figure 1 shows the dendrogram of similarities among 12 mint landraces using Ward's minimum variance method of cluster analysis of the tested quantitative traits. The results revealed three groups, (clusters) Mzin7 and Mzin9, having the smallest variance, and the Mzin1 and Mzin4 having the highest variance. Cluster I contained mainly 3 clones from Kashan and 2 clones from Isfahan. Cluster II included 3 clones from Kashan and cluster III had 3 clones from Isfahan and 1 clone from Mahalat. A question arises why clone number 9 from Isfahan clustered with clones 7, 2 and 5 from Kashan or clone number 8 from Mahalat clustered with clones 4, 12 and 6 from Isfahan. This can be due to the exchange of germplasm within these regions located in central Iran. Analysis of variance among clusters revealed the significant differences for the number of nodes on the main stem, number of lateral branches, spike width of main stem and leaf width ($P< 0.01$). Days to 50 percent flowering, plant height and number of nodes on the main stem also differed significantly ($P<0.05$). These traits were major sources of diversity among mint clones (Table 8). According to the dendrogram, the clones can also be divided into two major groups, (clusters) which join together at 165 Euclidean distance. The first group comprised 8 clones (7, 9, 2, 5, 3, 10 11 and 1) and the second group consisted of 4 clones (4, 12, 6 and 8). These results could be useful in choosing clones for intercrossing to develop improved cultivars, synthetics and hybrids for use in the tested region.

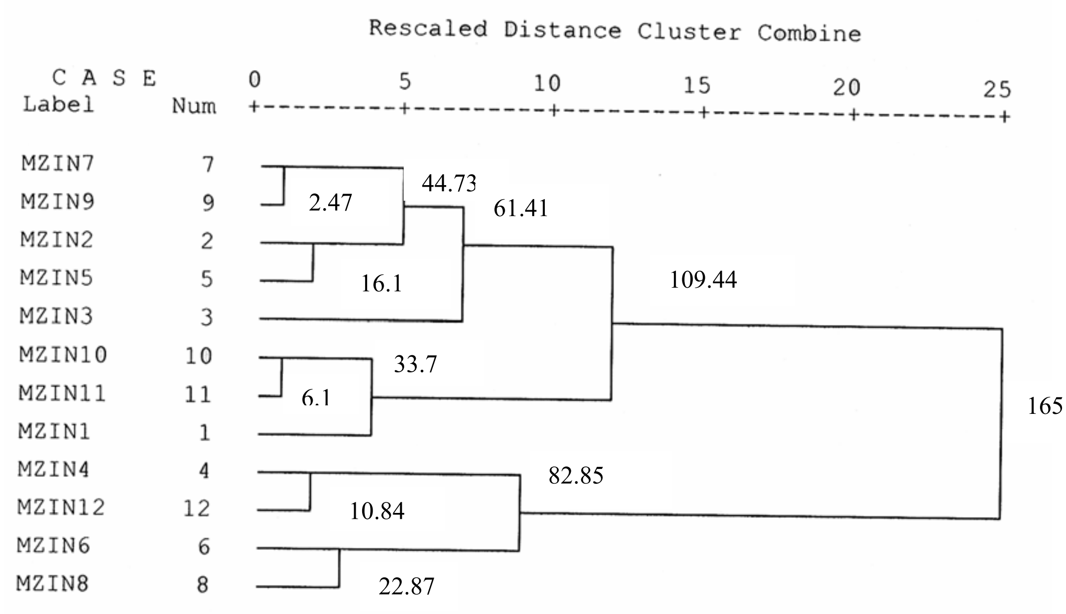

Fig.1. Dendrogram of the similarities among 12 Iranian mint landraces using Ward's minimum variance method of cluster analysis

Table 8. Analysis of variance and mean comparison of clusters for 15 tested traits of Iranian mint landraces

Characters	Mean squares	Mean of cluster I	II	III
Days to 50% flowering	190.76*	69.60[b]	67.88[b]	80.83 [a]
Days to100% flowering	94.25	83.13 [a]	85.88 [a]	92.25 [a]
Plant height	260.59*	72.76[a]	56.59 [b]	70.14 [a]
Number of nodes of main stem	6.45**	14.55[b]	13.06[c]	15.81 [a]
Number of nodes of lateral branches	1123. 97*	135.49[a]	103.46 [b]	134.51 [a]
Number of lateral branches	51.13**	25.11[b]	19.88[c]	27.52 [a]
Spike length of main stem	11.75	9.06 [a]	11.06 [a]	7.37 [a]
Number of spikes per plant	170.34	30.58 [a]	35.32 [a]	21.77 [a]
Spike width of main stem	0.10**	0.91 [a]	0 .99[a]	0.67[b]
Height of lateral branches	68.09*	27.93[ab]	22.91[b]	31.81[a]
Spike length of lateral branches	3.46	5.51[a]	5.72 [a]	3.98 [a]
Leaf width	0.44**	2.06 [a]	2.07 [a]	1.49 [b]
Leaf length	0.54*	3.84[ab]	3.65 [b]	4.38 [a]
Essential oil of content	0.13	1.33 [a]	1.01 [a]	1.37 [a]
Yield per plant	10.36	12.28 [a]	9.58 [a]	9.64 [a]

*, ** Significant at 5% and 1% levels of probability, respectively

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