Mentha viridis lavanduliodora Sacco Essential Oils: State of the Art

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Mint essential oils are commonly used in the food-flavoring and fragrance industries. The essential oils exploited globally in commercial ventures include peppermint *(Mentha x piperita* L.), spearmint *(Mentha spicata* L.), cornmint *(Mentha arvensis* L. var. piperascens), *Mentha x gracilis* Sole and *Mentha pulegium* L.[1]

Minty fragrances differ greatly in their organoleptic properties, going from fresh and cool long-lasting tastes (as those of spearmint) to sweeter flavors and fragrances (such as peppermint and cornmint). As a result of these differences, mint essential oils are responsible for a wide variety of applications in food, cosmetics and pharmaceutical preparations. The organoleptic characters of these oils rely mainly on the presence of *p*-menthan C-3 oxygenated monoterpenes, like pulegone, menthone and menthol in peppermint and cormint, or C-6 oxygenated derivatives present in carvone-rich essential oils such as spearmint and its relatives.[2]

With regards to mint oil chemical composition, discovery and selection of new land races or chemotypes of *Mentha* species bring forth novel and unique flavor profiles. These are unique model systems for the study of essential oil biosynthetic pathway regulation and can be applied commercially, fulfilling some special market requirements.

In 1956 Sacco[3] selected and described a new form of mint of the Spicatae section, bearing morphological characters very closed to those of *Mentha viridis* L., but with a pleasant lavender aroma owing to its high linalool content, an uncommon characteristic for the genus.[4]

Such a finding was worth noting since until that time, few mint species were known to produce essential oils so rich in alcoholic and esterified functions with the exception of lemon mint (*Mentha citrata* Ehrh.). Ketones were also present in very low levels.

Mentha viridis (L.) cultivar *lavanduliodora* Sacco n. cult. has been extensively propagated at the Department of Plant Biology, University of Torino and subjected to morphological and cytotaxonomical analysis. Such analysis has definitively stated its taxonomical identity and cariologycal status (2n = 96) (Figure 1).[3,5]

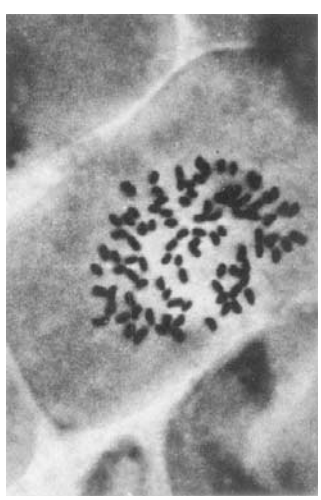

Figure 1. Metaphasic plate in root tip of *Mentha viridis lavanduliodora* Sacco, 2n = 96 (x1500).

This new clone mint is a vigorously growing plant that has proved to be resistant to the most common mint pests such as *Verticillium* wilt disease and rusts. Its cycle is of 110-120 days with possible early regrowth, yielding more than 50 kilos of oil per hectare.[6] Owing to its day-neutral behavior, flowering and oil production is not limited by geographical photoperiodic conditions, thus allowing intensive cultivation in Moldavia, Japan, Brazil and Argentina (Figure 2).[6,7]

Mentha viridis lavanduliodora leaf secretory structures are similar to those described for *Mentha x piperita*, belonging to both the peltate and capitate-type (Figure 3).

A precise analysis of the relationships existing between trichome number, essential oil composition and yield has shown how these parameters can be profoundly influenced by the status of leaf development and seasonal period of

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harvest. Trichome density decreases along with leaf elongation, specifically the total number of both trichome types drop during bloom (Figure 4).[8]

Mentha viridis lavanduliodora essential oil is characterized by a high percentage of both linalool and linalyl acetate. GC-MS analysis has confirmed previous observations about this species. A great variation in linalool and linalyl acetate percentage composition resulted while distilling leaves at different times before, during and after bloom. Linalool is present in considerable quantity, 46.5%, before bloom, then drops to 39.3% during and after bloom. Linalyl acetate, on the other hand, shows a steady increase from a minimum of 20.21% before bloom to a maximum of 33.23% after bloom (Table 1). *Mentha viridis lavanduliodora* essential oil showed significant percentages of aliphatic hydrocarbons along with menthol and pulegone only before bloom, while in flowering plants aliphatic hydrocarbons dropped and at the same time increasing oxygen-containing compounds (Table 2).[8]

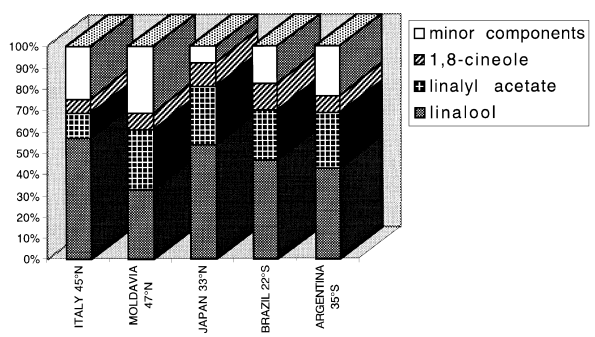

Figure 2. Plot of linalool, linalyl acetate and 1,8-cineole responses in different parts of the world

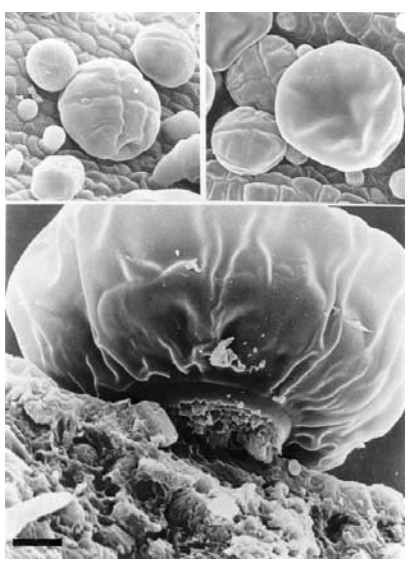

Figure 3. Scanning electron micrographs of *Mentha viridis lavanduliodora* trichomes (metric bar = 14m)

This study suggests the existence of a mechanism that diverts metabolic energy from glandular structures to florets formation, thus drastically changing the profile of terpene derivatives.

Changes in terpene composition due to environmental and seasonal factors have been thoroughly documented in the genus *Mentha*.[9,10] For example, changes in light intensity, temperature and day length may affect mint essential oils' composition through variations in plant photosynthetic activity.[11,12]

The developmental stage of plants, with special regard to the differentiation level of leaf secretory structures, is another key factor in mint essential oil biogenesis. Leaf expansion during plant growth and relative trichome number and density have been reported to directly influence menthone, menthol and menthyl acetate percentages in *Mentha x piperita*.[13]

All these factors are clearly plastic phenomena in which environmental and/or developmental changes may influence the expression of plant characteristics as well as terpene composition, both at phenotypic and genotypic levels. Plasticity and genotypic variation in *Mentha* spp. has been demonstrated and efficaciously employed as a means of selecting plants producing important secondary metabolites.[14]

Flowering F_1 hybrids originating from a seed population of *Mentha viridis lavanduliodora* were studied by the authors in regards to their essential oil composition during two growing seasons, 1989 and 1990 (Table 3).[15]

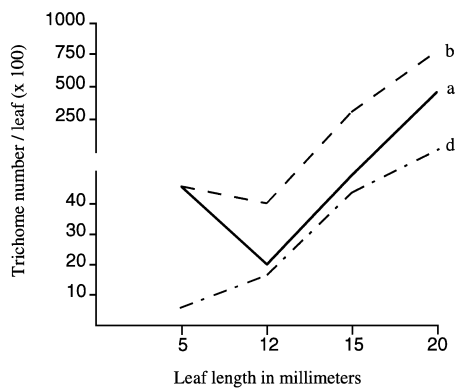

Figure 4. Total capitate and peltate trichome number per leaf at the three collecting periods (b = before bloom; d = during bloom; a = after bloom)

Table 1. Main components (%) of the essential oil of *Mentha viridis lavanduliodora* distilled from leaves before (B), during (D) and after (A) bloom; flowers (FL); whole plants (WP)

Components	B	D	A	FL	WP
linalool	46.54	39.28	41.67	46.3	49.72
linalyl acetate	20.21	31.99	33.23	33.40	23.91

Table 2. Minor components of *Mentha viridis lavanduliodora* essential oils distilled from leaves before (B), during (D) and after (A) bloom. Relative percent (R%) and percent with respect to the essential oil content (T%)

Compounds	T%(B)	R%(B)	T%(D)	R%(D)	T%(A)	R%(A)
α-pinene	0.01	0.06	0.03	0.14	0.01	0.03
β-pinene	0.01	0.06	0.05	0.25	0.58	3.17
sabinene	0.10	0.46	0.04	0.17	0.03	0.15
myrcene	0.36	1.63	0.17	0.83	0.14	0.78
limonene	1.20	5.38	0.54	2.58	0.67	3.64
1,8-cineole	5.15	23.19	3.50	16.62	2.93	16.00
β-fellandrene	0.65	2.95	0.40	1.88	0.40	2.20
ocimene	0.50	2.24	0.26	1.23	0.36	1.99
terpinolene	0.14	0.64	0.11	0.52	0.13	0.69
3-heptyl acetate	0.18	0.83	0.21	1.00	0.22	1.18
1-octyl-3-yl acetate	0.47	2.11	0.62	2.96	0.56	3.04
menthone	0.07	0.32	0.46	2.18	0.04	0.20
1-octen-3-ol	0.12	0.56	0.17	0.78	0.04	0.24
dihydroedulane	0.30	1.35	0.23	1.11	0.12	0.66
β-caryophyllene	2.23	10.04	3.04	14.44	1.75	9.56
menthol	1.08	4.85	0.88	4.18	0.21	1.16
pulegone	0.63	2.83	0.40	1.92	0.22	1.20
2,6-dimethyl-5,8-octadiene	0.64	2.89	0.25	1.21	0.44	2.42
aromadendrene	0.69	3.13	0.28	1.33	0.21	1.17
α-terpineol	2.85	12.83	3.33	15.81	3.05	16.65
neryl acetate	1.10	4.96	0.96	4.57	1.50	8.18
α-terpinene	1.28	5.77	1.10	5.22	1.51	8.24
geranyl acetate	1.26	5.67	1.18	5.59	1.48	8.10
nerol	0.20	0.92	0.29	1.39	0.18	0.98
geraniol	0.67	3.03	1.36	6.47	1.11	6.16
elemol	0.28	1.29	1.19	5.64	0.40	2.21
Total	22.20	100.00	21.08	100.00	18.32	100.00

Limonene, 1,8-cineole, linalool, linalyl acetate, β-caryophyllene, α-terpineol and geraniol, all major constituents of essential oils, were statistically processed to evaluate genotypic variability and phenotypic plasticity in the F_1 population, taking in account terpenes' statistical linkages and their partition among plants. When variations of each terpene in each plant were evaluated between both growing seasons, high degrees of phenotypic plasticity were shown by linalyl acetate and geraniol. Additionally, high levels of variation among individuals (genotypic variation), regardless of the year, were found for linalool (Figure 5). These results were also confirmed by cluster (CA) and principal component analysis (PCA). The latter was calculated by pooling the data from both growing seasons.[15] A good discrimination amongst plants was possible with the characterization of F_1 hybrids regarding their linalool, geraniol and linalyl acetate content.

By means of statistical techniques it was possible to state which terpenes are more stable (linalool and, to a lesser degree, limonene and the other minor constituents) and strictly fixed in the genotype of the individual. This enabled selection of essential oils for these useful traits. Those more plastic oils (linalyl acetate and geraniol) are greatly influenced by environmental changes and growing conditions.

Studies conducted while intercrossing different species of the genus *Mentha* have

Table 3. Oil chemical composition of *M. viridis lavanduliodora* mother plant (M.P.) and F_1 hybrids distilled during two growing seasons (values are expressed as area percent; C.V. = coefficient of variation)

Compounds	M.P.	ML-103	ML-104	ML-109	ML-110	ML-113	ML-115	ML-124	ML-127	ML-131	ML-132	ML-133	Means	C.V.
1989														
limonene	0.70	0.26	0.38	0.18	0.26	0.10	0.10	0.10	0.10	0.54	0.35	0.10	0.26	76.92
1,8-cineole	5.32	0.46	0.40	1.94	2.64	0.20	0.21	0.90	0.22	6.01	2.18	0.13	1.72	118.60
linalool	40.53	30.06	36.31	53.79	54.55	9.27	20.42	29.98	12.52	57.61	52.25	21.65	34.91	48.73
linalyl acetate	34.20	48.07	27.61	27.86	26.24	15.57	26.63	37.63	28.68	19.50	22.93	29.65	28.71	29.50
β-caryophyllene	2.57	1.23	1.99	2.06	1.22	4.17	2.11	0.10	6.61	0.37	1.33	1.70	2.12	83.02
α-terpineol	1.27	1.96	3.25	1.39	2.16	2.79	8.81	2.10	3.14	0.88	1.49	1.77	2.58	81.01
geraniol	2.88	3.40	5.76	0.10	0.11	29.80	1.79	10.82	15.09	0.13	3.77	21.15	7.90	120.76
1990														
limonene	0.89	0.30	0.10	0.71	0.10	0.10	0.10	0.10	0.30	0.59	0.34	0.10	0.31	90.32
1,8-cineole	4.42	2.98	3.21	6.19	4.46	0.50	0.59	0.30	2.35	5.68	2.26	1.48	2.87	69.69
linalool	49.72	40.38	47.60	47.89	43.09	31.92	19.15	26.55	38.42	47.49	48.50	31.24	39.31	25.56
linalyl acetate	23.91	42.11	37.07	29.34	41.79	35.59	55.96	43.86	30.99	31.33	29.02	35.24	36.35	23.71
β-caryophyllene	1.44	2.34	2.53	2.19	2.19	2.96	4.14	2.96	2.91	1.75	2.48	2.69	2.55	27.06
α-terpineol	4.26	1.18	1.63	1.11	1.58	2.81	4.79	2.31	2.23	0.97	1.57	2.29	2.23	54.26
geraniol	1.92	1.19	1.54	0.10	0.10	6.38	1.05	1.94	1.32	0.20	0.43	0.10	1.36	127.21

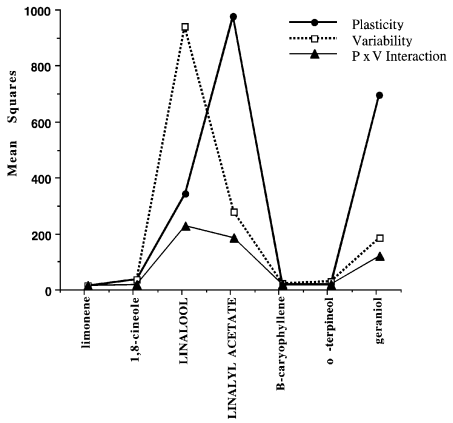

Figure 5. Plot of the mean squares (MS) values vs. oil componenet of the *Mentha viridis lavanduliodora* F_1 hybrids

highlighted the existence of specific gene coding for most of the monoterpene biosynthetic enzymes. Gene expression, alleles distribution and segregation patterns are thought to be heavily involved in the modulation of the qualitative profile of mint essential oils.[2]

In several *Mentha* species the action of the dominant "E" gene causes esterification of alcohols to yield acetates. Changes in temperature and day length cause activation of this gene with consequent overproduction of menthyl acetate starting from menthol.[11,16] The latter monoterpene expresses high levels of activation of a *p*-menthanone-3-cheto-reductase, controlled by an "R" gene. This expression is high only in full expanded mature leaves.[2]

The biochemical pathway that leads to linalool is known to go through a transformation of geranyl-pyrophosphate (GPP) to yield linalyl-pyrophosphate (LPP) and then successive dephosphorylation and C-3 hydroxylation.[15] Early works of Murray and Lincoln[17] in *Mentha citrata* suggested the action of a dominant "I" gene for linalool production. Thus an accumulation of geraniol would potentially divert GPP for further transformation to LPP and then to linalool.

In *Mentha viridis lavanduliodora*, geraniol content was found to be strictly dependent on environmental factors, creating suspicion of the presence of a gene controlling GPP reduction to geraniol under strict climatic control similar to the "E" gene of peppermint.[15]

The essential oil of *Mentha viridis lavanduliodora* has a pleasant fresh and fragrant aroma because of its high percentage of linalool and linalyl acetate and low levels of ketones. The high percentage of both linalool and linanyl acetate make this plant an ideal tool for studying their biosynthetic pathways. Work is in progress on the cloning of the genes involved. Its pest resistance and day-neutral characteristics make this plant an interesting chemical resource for linalool. Its potential application could include pest-risk areas and in parts of the world where photoperiodic and climatic conditions are primary limiting factors for other essential oil crops.

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