Microchemical Journal 133 (2017) 13–21

Contents lists available at ScienceDirect

Microchemical Journal

journal homepage: www.elsevier.com/locate/microc

Geographical patterns of *in vivo* spontaneously emitted volatile organic compounds in *Salvia* species

Roberta Ascrizzi [a,*], Pier Luigi Cioni [a], Lucia Amadei [b], Simonetta Maccioni [b], Guido Flamini [a]

[a] Dipartimento di Farmacia, University of Pisa, Via Bonanno 6, 56126 Pisa, Italy
[b] Orto e Museo Botanico dell'Università di Pisa, Via Luca Ghini 13, 56126 Pisa, Italy

ARTICLE INFO

Article history:
Received 8 November 2016
Received in revised form 1 March 2017
Accepted 1 March 2017
Available online 07 March 2017

Keywords:
Salvia
HS-SPME
Geographical patterns
Spontaneous volatile emission
Volatile organic compounds

ABSTRACT

Salvia is the largest genus in the Lamiaceae family: such a wide number of species is due to the almost ubiquitous distribution of this genus, but also to the cultivars selection carried out by botanists and private collectors during centuries. It shows a wide variety of characteristics in the specimens, both in the external appearance and the volatile organic compounds (VOCs) profiles. We analysed the spontaneous volatile emission profiles of living samples of leaves taken from 30 different species of Salvia using the Head Space – Solid Phase Micro Extraction technique coupled with Gas Chromatography – Mass Spectrometry analysis. The aim was to evaluate the existence of possible geographical patterns in the volatile composition of the spontaneous emission profiles. The selected samples belong to three geographical areas: Central and South America, Mediterranean Europe and Middle East, and South Africa. The major identified compounds (in over 70% of the samples) are α-pinene, limonene, α-β- and copaene, β-caryophyllene, α-humulene, germacrene D and *n*-heptadecane. The Multivariate Statistical Analyses (HCA and PCA) show a sharp tendency of the samples to gather in groups with a behavior that significantly matches the geographical origin of the analysed specimens.

1. Introduction

The genus *Salvia*, with its nearly 1000 different species, both from the Old and the New World, is the largest in the Lamiaceae family. Among the Mentheae tribe, *Salvia* genus is characterized by the number of the stamens and their peculiar structure. Whereas most Mentheae have four stamens, *Salvia* genus only expresses two: in both the stamens, the two thecae are separated by an elongate connective, which enables the formation of the peculiar lever mechanism. This is associated with *Salvia*'s unusual nototribic (dorsal) pollination syndrome, in which the pollinator, looking for nectar in a male stage flower, pushes the posterior anther theca, causing the lever mechanism to move the stamens, depositing the pollen on the back of the pollinator. When the pollinator leaves the flower, the lever lets the stamens return to their original position. This peculiar stamens structure, and the pollination method that comes with it, have long been considered as evolved only once within the Mentheae tribe, thus making *Salvia* genus monophyletic [1].

Walker et al. [1] investigated the monophyly of the genus using the chloroplast DNA regions *rbcL* and *trnL-F*: the study concluded that *Salvia* genus is not monophyletic, but comprises at least two, possibly three, distinct clades that evolved independently. Among these clades, much of the diversification of *Salvia* fits along biogeographical lines. *Salvia* clade I is largely distributed in the Old World but with one New World lineage; *Salvia* clade II is a lineage of New World only species; *Salvia* clade III is an independent Asian lineage.

In this study, we investigated the Volatile Organic Compounds (VOCs) patterns in *Salvia* species from different geographical areas, all comprised in the first two Salvia clades' areas of origin: the aim was to determine whether the profile of the Volatile Organic Compounds, spontaneously emitted *in vivo* by leaves taken from living specimens, reflects the geographical distribution of the two clades.

Spontaneously emitted VOCs are secondary plants metabolites which comprise both terpene and non-terpene derivatives (apocarotenoids, phenylpropanoids, alkanes, *etc.*). As products of the secondary metabolism, they are not of vital importance to plants survival, but they confer peculiar abilities, like predator defense and pollinator attraction, and environmental-induces changes. Other than seasonal variations and injuries response, variations in the volatile emission profile of plants are a direct result of the environment the specimens are subjected to: different geographical areas are characterized by different pollinators, predators, humidity, temperature and climatic conditions, which ultimately lead to secondary metabolism changes [2].

The Head Space Solid Phase Micro-Extraction sampling followed by Gas-Chromatography coupled with Mass Spectrometry analysis (HS-SPME-GC/MS), coupled with chemometrical analyses, can be used as

* Corresponding author at: Dipartimento di Farmacia, Università di Pisa, Via Bonanno 6, 56126 Pisa, Italy.
E-mail address: roberta.ascrizzi@for.unipi.it (R. Ascrizzi).

http://dx.doi.org/10.1016/j.microc.2017.03.002
0026-265X/

an investigation method to assess the changes in the emission profiles induced by the geographical origin of specimens. Mesquita et al. [3] investigated three biotypes of *Eugenia uniflora* L. from different regions of Brazil, each with a peculiar fruit color: HS-SPME-GC/MS results analysed by means of Multivariate Statistical Analyses showed the ability of this method to discern between the three biotypes. Farag et al. [4] reported the identification of target compounds in *Glycyrrhiza* species to assess the geographical impact on their volatile emission. The reliability of this method to identify different plant origins is also reported by Mustafa et al. [5]: they discriminated wild-grown from cultivated and commercially bought *Gentiana lutea* species. In Ouni et al. [6], the analysis of the Head Space of olive oils from seven different Tunisian geographical areas allowed the detection of significant differences in the proportion of volatile constituents from oils of different geographical origins.

The analysed specimens come from three different geographical areas: Central and South America, Mediterranean Europe and Middle East, and South Africa (Table 1).

The Central and South America area is a wide geographical region, in which climate conditions vary according to latitude and altitude. The mountain climate itself varies according to the region: *Salvia discolor* area of origin is represented by the dry slopes of the Peruvian Andes, while humid mountain forests of southern Mexico are home to *S. elegans*, *S. cinnabarina* and *S. karwinskii*. Other species from this area have their native environment in tropical climate conditions: this is the case for *S. leucantha*, *S. leucantha* 'Midnight', *S.* 'Waverly', *S. dorisiana*, *S. squalens*, *S. confertiflora*, and *S. splendens*. *S. urica, S. tubiflora*, and *S. guaranitica* are more widespread throughout the entire Central and South America region.

Mediterranean Europe and Middle East regions share the Mediterranean climate conditions, with rainy winter seasons and dry summer months. *S. officinalis* and *S. officinalis* 'Purpurascens' have their native environment in Western Mediterranean Areas, where they are mainly distributed in Italy and Spain. *S. desoleana* is an endemic species of Sardinia, in Italy. *S. aethiopis* is a species whose home environment is the southern region of Europe. Some species included in this geographical area come from Canary Islands, which show climate conditions very similar to those of the Mediterranean area, with warmer winters and hotter summers, but with a lower amount of humidity as they are situated in the Atlantic Ocean: this is the case of *S. canariensis*, *S. fruticosa*, and *S. libanotica*. *S. amplexicaulis*, *S. austriaca*, and *S. transsylvanica* come from the Balkans Peninsula, which has Mediterranean climate conditions on its coastal regions. Turkey is the home region of both *S. chionantha* and *S. heldreichiana*.

S. aurita has its home environment in the coastal regions of South Africa, whose subtropical position makes the climate conditions very similar to those of the Mediterranean region, with temperate climate and abundant rainfalls. The other specie coming from South Africa is *S. africana-lutea*, which has its home environment in continental areas, where the climate is more similar to the tropical regions of South America.

Table 1
Legend of the samples for the studied *Salvia* species.

Species[a]
A1 – *S. cinnabarina* M. Martens & Galeotti
A2 – *S. confertiflora* Pohl
A3 – *S.* 'Waverly'
A4 – *S. discolor* Kunth
A5 – *S. dorisiana* Standl.
A6 – *S. elegans* Vahl
A7 – *S. guaranitica* St.-Hil. Ex Benth.
A8 – *S. karwinskii* Benth.
A9 – *S. leucantha* Cav.
A10 – *S. leucantha* Cav. 'Midnight'
A11 – *S. splendens* Sellow ex Schult.
A12 – *S. squalens* Kunth
A13 – *S. tubiflora* Sm.
A14 – *S. urica* Epling
M1 – *S. aethiopis* L.
M2 – *S. amplexicaulis* Lam.
M3 – *S. austriaca* Jacq.
M4 – *S. canariensis* L.
M5 – *S. candelabrum* Boiss.
M6 – *S. chionantha* Boiss.
M7 – *S. desoleana* Atzei & V. Picci
M8 – *S. fruticosa* Mill.
M9 – *S. heldreichiana* Boiss.
M10 – *S. lavandulifolia* Vahl
M11 – *S. libanotica* Boiss. & Gaill.
M12 – *S. officinalis* L.
M13 – *S. officinalis* L. 'Purpurascens'
M14 – *S. transsylvanica* Schur
S1 – *S. africana-lutea* L.
S2 – *S. aurita* L. f.

[a] Voucher number for all (except *S. officinalis* L.) the species: Herbarium Horti Botanici Pisani (PI) Nuove Acquisizioni, N. 7290/1; for *Salvia officinalis* L.: Herbarium Horti Botanici Pisani (PI) Nuove Acquisizioni, N. 7290/4.

2. Materials and methods

2.1. Plant material

The fresh leaves were collected from species that are part of a wide collection located at the Botanic Garden of the University of Pisa, Italy. This collection has been certified by the Italian Botany Society, that declared it a "Collection of National Relevance" in December 2012, for both the number and the variety of the represented specimens. The samples were collected at the Botanic Garden of Pisa from January to March 2014. Once cut, the leaves were put in glass vials and immediately covered with aluminum foil and left to equilibrate for 60 min at room temperature before the insertion of the fibre. The equilibration time was chosen as the ideal one after several trials at different intervals.

2.2. Sample analysis

Supelco SPME (Solid Phase Micro-extraction) devices coated with polydimethylsiloxane (PDMS, 100 μm) were used to sampling the headspace. SPME sampling was performed using the same new fibre, preconditioned according to the manufacturer instructions, for all the analyses. Sampling was accomplished in an air-conditioned room (22 ± 1 °C) to guarantee a stable temperature. The 22 °C sampling temperature was chosen to avoid the thermal damage of the superficial glandular hairs of the leaves and, thus, any artificial-induced volatiles release. After the equilibration time, the fibre was exposed to the headspace for 30 min. The sampling time was experimentally determined to obtain an optimal adsorption of the volatiles, to avoid both under- and over-saturation of the fibre and of the mass spectrometer ion trap. Once sampling was finished, the fibre was withdrawn into the needle and transferred to the injection port of the GC-MS system. All the SPME sampling and desorption conditions were identical for all the samples. Furthermore, blanks were performed before each first SPME extraction and randomly repeated during each series. Quantitative comparisons of relative peaks areas were performed between the same chemicals in the different samples.

2.3. GC/MS analysis

The GC/EI-MS analyses were performed with a Varian CP-3800 apparatus equipped with a DB-5 capillary column (30 m × 0.25 mm i.d., film thickness 0.25 μm) and a Varian Saturn 2000 ion-trap mass detector. The oven temperature was programmed rising from 60 °C to 240 °C at 3 °C/min; injector temperature, 220 °C; transfer-line temperature, 240 °C; carrier gas, He (1 ml/min).