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***AROMATIC & MEDICINAL PLANTS***
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***Varazdin, Croatia, 11-12/12/2-19***

# *“Research activities on Medicinal and Aromatic Plants*

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# The campus

# Institute of Plant Breeding and Genetic Resources
# Research activities

- ✓ **Maintenance, evaluation of plant genetic resources, breeding for new varieties with improved performance, quality and adaptability.**
- ✓ **Plant protection: Weed management, insect-plant-microbe interactions, chemical ecology, integrated pest management, insecticide resistance, invasive species**
- ✓ **Research on individual sectors focuses on field crops (cotton, tobacco, wheat, rice, industrial plants etc), vegetables, aromatic and medicinal plants along with wild and native plants of the Greek flora, fruits and nut trees.**
- ✓ **Research also focuses on sustainable and precision agriculture, integrated management, modern crop protection systems, optimal use of inputs aiming to lower production costs and smaller environmental footprints.**
- ✓ **Collection, conservation, propagation (basic or advanced *in vitro*, sexual and asexual methods), valorization, cultivation and utilization of Greek flora species (endangered, endemic etc).**

## Medicinal Aromatic plants / cosmetic or perfume plants

**Medicinal plants have been essential resources for human health from ancient times to the present day.**

**Nowadays they play a key role due to their numerous uses in the pharmaceutical, cosmetic, flavor and food industry.**

***Industrial crops***

Essential Oils

Pharmaceuticals (phytotherapy, food supplements)

Cosmetics

Plant protection

Animal feeding

Chemical industry

## PLANT SOURCES

**WILD GROWN**

**/ OR CULTIVATED PLANTS**

# Conservation and Sustainable Use of Medicinal and Aromatic Plants

The market for herb products is growing rapidly, while Europe holds a dominant role in the world trade in MAPs.

Harvesting from the wild is one of the sources of raw material.

From the 2000 species in European medicinal plant trade, at least 90% are still wild-collected, resulting in some cases, in loss of genetic diversity, local extinctions and habitat degradation.

About 150 species were reported to be threatened in at least one European country

# Some overharvested plants in Greece

***Sideritis* sp. –Mountain Tea**
**Wild grown at altitudes higher than 1000 m**

***Primula veris***

*Main conservation strategies for MAP species*

- In situ (protection of their habitats)
- Ex situ (conservation at species and germplasm level through field collections and gene banks)
- Introduction into cultivation

Domestication of wild medicinal plants is necessary to allow intensive production to meet high demand- especially in the period of global trade, massive and expanding markets for medicinal and herbal products

Companies involved in trade and production of herbal remedies and other botanical products report that 60-90% of the volume of medicinal plants are from cultivated production

## Research activities
## 1. Conservation and Sustainable exploitation

### *SEEERAPLUS-135 MOUNTEA-CONSE Project*

### *"Conservation and sustainable exploitation of indigenous Medicinal and Aromatic plants traditionally used in the SEE, WB countries.* A model approach for *Sideritis* spp. (Mountain tea)*"*

***In situ* conservation**

•Data on the threatening status of *Sideritis* populations in the studied areas were recorded and evaluated.

•A protocol illustrating the basic rules for the sustainable collection (defining the time and frequency of harvesting, the specific parts of the plant to be collected, means used etc.) of *Sideritis* plants and germplasm from the wild has been elaborated.

***Ex situ* conservation**

Seeds were deposited to the Greek Gene Bank, and two accessions have been planted in ex situ field collection

In the frame of the **"*on farm conservation***" small scale plantations were established

**Protocol for the Sustainable exploitation of MAPs through domestication –** emphasizing on Sideritis case".

Assessment of conditions for introduction into cultivation *Sideritis* and other MAPs

Main breeding aims and objectives

The principles for the Organic cultivation of Sideritis and other MAPs.




## *MOUNTEA-CONSE Project*

Phytochemical analysis (essential oils, phenolics, flavonoids, antioxidant potential of the collected populations)

Nutritional value and mineral content

Assessment of genetic variability

**The exchange of knowledge and know how on *Sideritis* conservation, can also promote similar research conservation actions and ethnopharmacobotanical surveys on other traditionally used MAPs in the SEE countries.**

***Domestication***

*Introduction of plants into the agriculture is a time consuming process and presumes the knowledge on the basic production-biological information of the species. Introduction strategies include research on ecophysiology, genetics, chemical features as well as production potential of the chosen population.*

## ***Main principles for successful introduction of MAPs***


- ***Specific ecological requirements***
- ***Agrotechnological techniques***
  - **Propagation**
  - **Cultivation practices**
- ***Selection and Breeding***


# Factors affecting the Quality of cultivated MAPs

Propagation material
(Germplasm, variety)

Harvesting season

Pedoclimatic conditions

Agrotechnological practices

Harvesting

Post harvest treatments

**2. Research on effective MAPs' propagation methods**

Sexual (seeds)

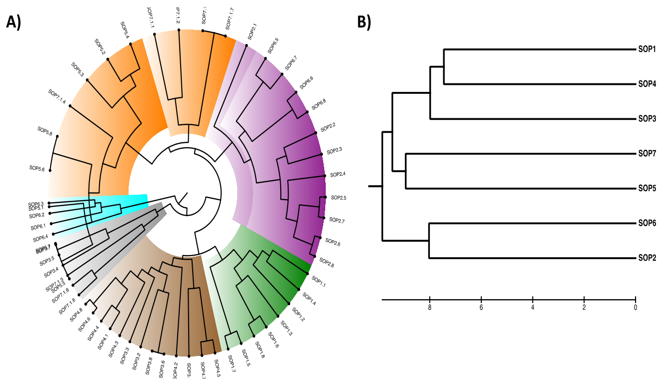
Asexual (cuttings, roots, division), in vitro


**3. Sustainable production of Medicinal and Aromatic Plants (MAPs)**
Agrotechnology: Cultivation procedures and protocols- Organic Agricultural practices

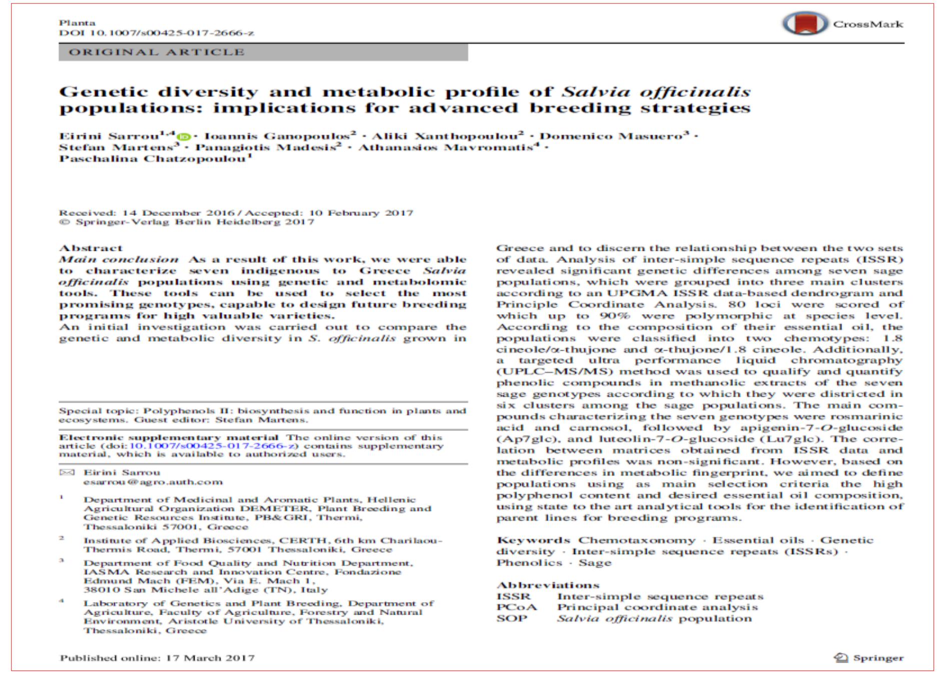
*Evaluation of mulching systems (organic and inorganic) in Tanacetum parthenium and Basil*

**4. Screening of genetic- and chemo-variability, by using modern tools of genomics and metabolomics, for breeding purposes**

**Genetic diversity and metabolomic profile of cultivated *S. officinalis* populations**




**Genetic relationships of seven populations of *Salvia officinalis* based on ISSR markers**


Planta
DOI 10.1007/s00425-017-2666-z

ORIGINAL ARTICLE

# Genetic diversity and metabolic profile of *Salvia officinalis* populations: implications for advanced breeding strategies

Eirini Sarrou[1,4] · Ioannis Ganopoulos[2] · Aliki Xanthopoulou[2] · Domenico Masuero[3] · Stefan Martens[3] · Panagiotis Madesis[2] · Athanasios Mavromatis[4] · Paschalina Chatzopoulou[1]

Received: 14 December 2016 / Accepted: 10 February 2017


**Abstract**
***Main conclusion*** **As a result of this work, we were able to characterize seven indigenous to Greece *Salvia officinalis* populations using genetic and metabolomic tools. These tools can be used to select the most promising genotypes, capable to design future breeding programs for high valuable varieties.**
An initial investigation was carried out to compare the genetic and metabolic diversity in *S. officinalis* grown in Greece and to discern the relationship between the two sets of data. Analysis of inter-simple sequence repeats (ISSR) revealed significant genetic differences among seven sage populations, which were grouped into three main clusters according to an UPGMA ISSR data-based dendrogram and Principle Coordinate Analysis. 80 loci were scored of which up to 90% were polymorphic at species level. According to the composition of their essential oil, the populations were classified into two chemotypes: 1.8 cineole/α-thujone and α-thujone/1.8 cineole. Additionally, a targeted ultra performance liquid chromatography (UPLC–MS/MS) method was used to qualify and quantify phenolic compounds in methanolic extracts of the seven sage genotypes according to which they were districted in six clusters among the sage populations. The main compounds characterizing the seven genotypes were rosmarinic acid and carnosol, followed by apigenin-7-*O*-glucoside (Ap7glc), and luteolin-7-*O*-glucoside (Lu7glc). The correlation between matrices obtained from ISSR data and metabolic profiles was non-significant. However, based on the differences in metabolic fingerprint, we aimed to define populations using as main selection criteria the high polyphenol content and desired essential oil composition, using state to the art analytical tools for the identification of parent lines for breeding programs.

**Keywords** Chemotaxonomy · Essential oils · Genetic diversity · Inter-simple sequence repeats (ISSRs) · Phenolics · Sage

**Abbreviations**
ISSR Inter-simple sequence repeats
PCoA Principal coordinate analysis
SOP *Salvia officinalis* population

Special topic: Polyphenols II: biosynthesis and function in plants and ecosystems. Guest editor: Stefan Martens.

**Electronic supplementary material** The online version of this article (doi:10.1007/s00425-017-2666-z) contains supplementary material, which is available to authorized users.

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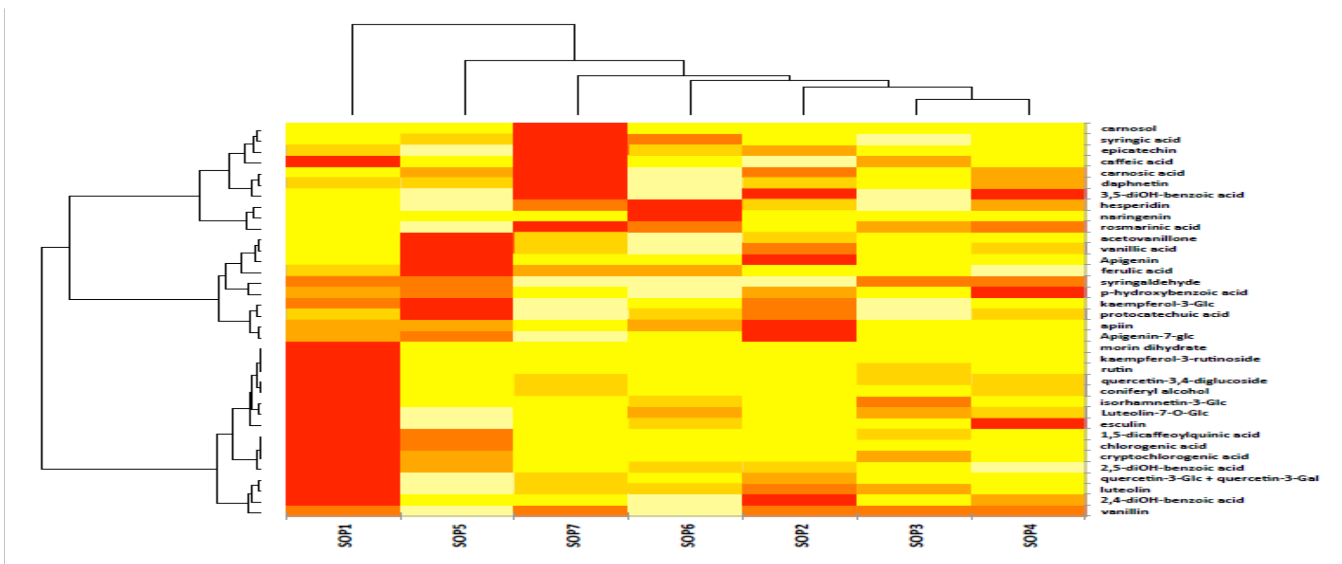
Published online: 17 March 2017

Springer



# Variation of bioactive compounds in wild grown and cultivated populations of *Hypericum perforatum*

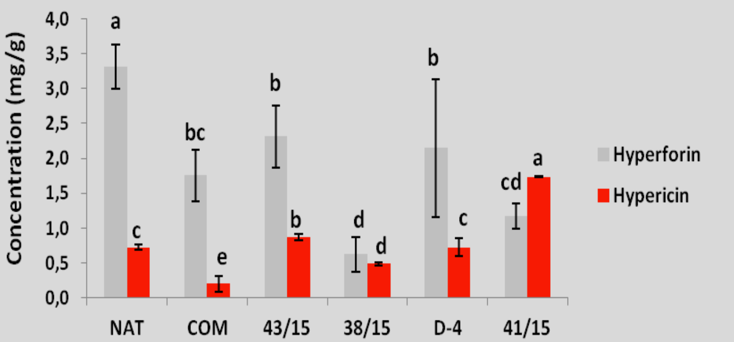



Fig. 3. Variability of hyperforin and hypericin content (mg/g dry extract) among the methanolic crude extracts from wild (38/15, 41/17, 43/17), cultivated (NAT, D-4) and commercial (COM) *Hypericum* herba.


Food and Chemical Toxicology 114 (2018) 325–333

Contents lists available at ScienceDirect

Food and Chemical Toxicology

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

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Metabolomics assisted fingerprint of *Hypericum perforatum* chemotypes and assessment of their cytotoxic activity


Eirini Sarrou[a,*], Lefki-Pavlina Giassafaki[b], Domenico Masuero[c], Daniele Perenzoni[c], Ioannis S. Vizirianakis[b], Maria Irakli[a], Paschalina Chatzopoulou[a], Stefan Martens[c]

# Variation of phenolic compounds in cultivated *Oregano*, *Thyme*, *Satureja*, *Rosemary* and *Lemon balm*

## High rosmarinic content in Greek Oregano (*Origanum vulgare* ssp. *hirtum*)

Journal of Applied Research on Medicinal and Aromatic Plants 6 (2017) 62–69

Contents lists available at ScienceDirect

Journal of Applied Research on Medicinal and Aromatic Plants

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

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JARMAP

### Analysis of phenolic compounds in Greek plants of Lamiaceae family by HPLC

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ARTICLE INFO

*Article history:*
Received 18 September 2016
Received in revised form 2 January 2017
Accepted 5 February 2017
Available online 21 February 2017

*Keywords:*
Antioxidant activity
Aromatic medicinal plants
HPLC
Diode array detection
Lamiaceae family
Phenolics

ABSTRACT

The objective of the current study is the simultaneous determination of 24 phenolics including carvacrol and thymol, in plants of Lamiaceae family by developing a simple and reliable reversed-phase high-performance liquid chromatographic method involving diode array detection. The separation of 24 compounds was achieved on a reversed phase $C_{18}$ column kept at 30 °C by gradient elution with a flow rate of 1.3 mL/min. The method was validated for specificity, linearity and intra- and inter-day precision. The limit of detection and quantification for all analytes were small, ranging from 0.002 to 0.16 and 0.01 to 0.48 μg/mL, respectively. This method was successfully applied to detect and quantify phenolics present in the plants. It was found that the amount of phenolics in both extracts (water, and methanolic) decreased in the following order: *Origanum vulgare* ssp. *hirtum* > *Thymus capitatus* > *Satureja thymbra* > *Melissa officinalis* > *Rosmarinus officinalis*. The limit of quantification values are low enough to detect and quantify successfully small amounts of 24 phenolics in the plants of Lamiaceae family. Generally, *Melissa officinalis* exhibited the highest antioxidant activity among the samples followed by *Origanum vulgare* ssp. *hirtum*. The developed method, is simple and reliable with satisfactory sensitivity and precision and is ideally suited for routine analysis.



**Table 3**
Content (μg/g) of phenolic compounds on dry-weight basis in the Lamiaceae family species based on the proposed method [a].

| Analyte | 70% aqueous methanol | | | | | water | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Oregano | Thyme | Satureja | Lemon balm | Rosemary | Oregano | Thyme | Satureja | Lemon balm | Rosemary |
| phenolic acids and their derivatives | | | | | | | | | | |
| 4-HBA | 9.9 | 14.0 | 42.8 | 28.8 | 40.8 | 12.1 | 42.1 | 75.1 | 4.9 | 33.0 |
| GA | 1.9 | ND | 0.3 | 3.4 | 2.1 | 7.2 | 1.3 | 0.9 | 161.5 | 1.2 |
| PRCA | 36.3 | 19.4 | 27.6 | 105.3 | 71.5 | 41.6 | 15.3 | 17.4 | 35.9 | 38.4 |
| SRA | 1.3 | 8.1 | 0.9 | 6.6 | 5.2 | 2.2 | 30.8 | 6.8 | 108.9 | 4.8 |
| VA | 41.9 | 56.1 | 68.3 | 33.4 | 69.4 | 31.9 | 71.4 | 41.8 | 45.3 | 48.1 |
| CIN | 2.0 | 2.8 | 6.2 | ND | 5.0 | 9.3 | 2.0 | 2.9 | ND | ND |
| CA | 98.8 | 50.7 | 131.0 | 150.0 | 131.3 | 214.3 | 119.5 | 116.2 | ND | 5.7 |
| FA | 27.6 | 257.4 | 66.9 | 19.5 | 14.0 | ND | 25.3 | 7.0 | ND | ND |
| SA | 8.9 | ND | ND | ND | ND | 14.8 | ND | ND | ND | ND |
| PCA | 7.9 | 5.9 | 7.2 | ND | 20.3 | 9.9 | 95.9 | 14.5 | 20.9 | 51.5 |
| CLA | 4.0 | 21.7 | 5.5 | 37.6 | 19.4 | 27.6 | 28.6 | 1.9 | 97.2 | 56.3 |
| RMA | 12036.2 | 3262.0 | 8181.9 | 8335.1 | 4941.5 | 983.0 | 734.8 | 116.2 | 458.9 | 518.7 |
| Total | 12276.7 | 3697.9 | 8538.6 | 8719.6 | 5320.4 | 1354.0 | 1167.0 | 400.7 | 933.6 | 737.7 |
| Flavonoids | | | | | | | | | | |
| LUT | 91.5 | 503.9 | 364.9 | 37.3 | 72.3 | 19.7 | ND | 31.0 | LLQ | ND |
| API | 18.0 | 36.2 | 111.6 | 48.8 | ND | ND | ND | ND | ND | ND |
| QUE | ND | 12.2 | ND | 2850.7 | 1811.9 | ND | 22.6 | ND | 35.8 | ND |
| KAM | 92.5 | 48.5 | 49.1 | 77.9 | ND | 56.9 | 21.8 | 23.6 | ND | ND |
| MYR | ND | ND | 27.4 | 100.3 | 244.5 | ND | ND | 30.0 | 52.1 | ND |
| RUT | 503.4 | 8.0 | 33.1 | ND | 201.8 | 44.8 | ND | ND | ND | ND |
| NAR | 8171.4 | 8291.7 | 7096.9 | ND | 23.1 | ND | 49.3 | 37.1 | ND | ND |
| CAT | 6.2 | 36.5 | 93.5 | 92.6 | 31.1 | 49.6 | 51.5 | 273.4 | ND | ND |
| EPI | ND | 121.9 | 25.2 | 29.5 | ND | 183.6 | 233.4 | 331.4 | ND | 9.8 |
| EPIG | 19.1 | 26.3 | ND | 59.6 | ND | 137.9 | 281.2 | ND | 901.5 | ND |
| Total | 8902.0 | 9085.1 | 7801.7 | 3296.8 | 2384.6 | 492.4 | 659.7 | 726.4 | 989.4 | 9.8 |
| Phenolic monoterpenes | | | | | | | | | | |
| CAR | 28804.3 | 28269.3 | 23590.8 | ND | ND | 1855.6 | 1466.5 | 1406.0 | ND | ND |
| THY | 10.6 | 13.8 | ND | ND | ND | ND | ND | ND | ND | ND |
| Total | 28814.9 | 28283.2 | 23590.8 | 0.0 | 0.0 | 1855.6 | 1466.5 | 1406.0 | 0.0 | 0.0 |
| Total phenolics | 49993.6 | 41066.2 | 39931.1 | 12016.4 | 7705.0 | 3702.0 | 3293.1 | 2533.1 | 1923.0 | 767.4 |

# Selection and Breeding:
# Objectives and goals in MAPs

| | |
|---|---|
| growth form | better homogeneity |
| proportion of organs | biomass yield |
| tolerance against abiotic and biotic factors | increased level of desired compounds decreased in toxic |



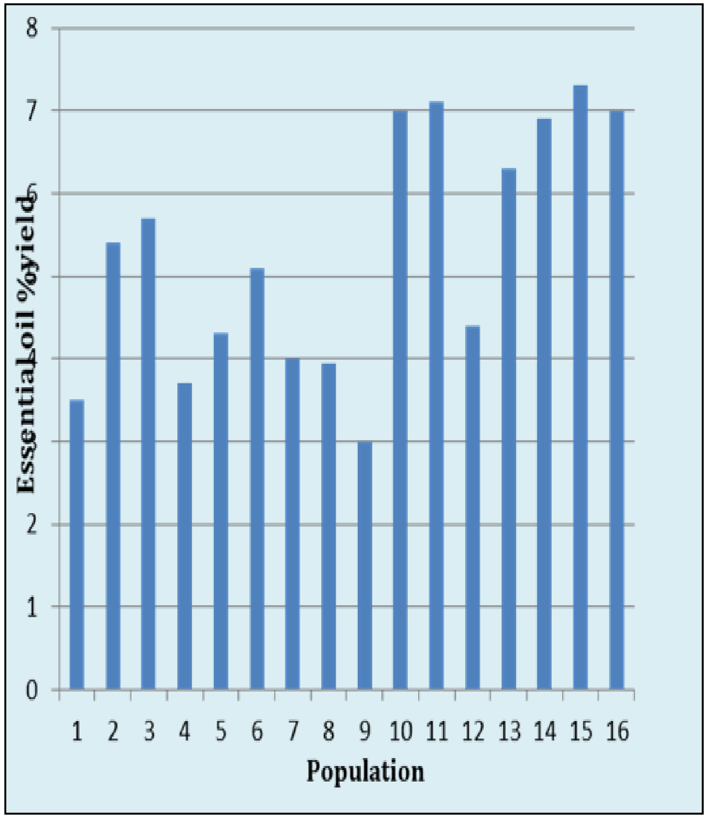
## *5. Selection and breeding*
## Improvement of Greek Oregano *(Origanum vulgare ssp. hirtum)*

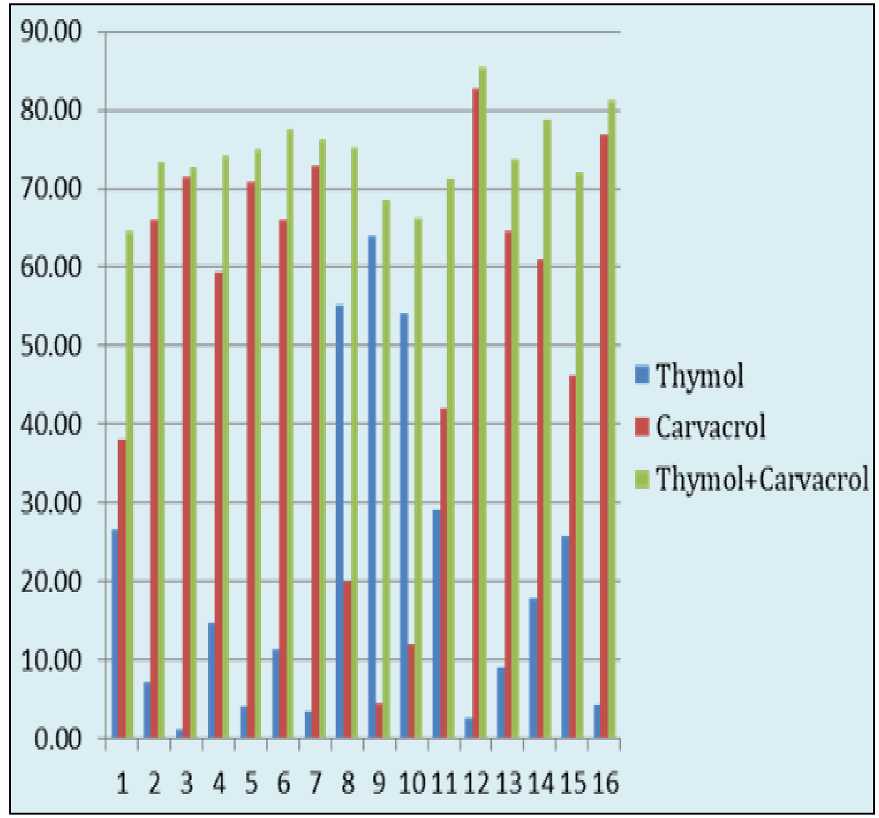
- **Greek oregano grows wild throughout nearly all Greece, particularly in mountain and semi-mountain districts.**
- **The plant is widely used as spice, containing an essential oil, rich in the phenols carvacrol and thymol, which are responsible for the antimicrobial and antifungal properties of the oil *in vitro* and in animals.**
- **The yield and the quality of the essential oil is dependent on the plant origin and the environmental conditions**
- **The species presents a great variability both in phenotypical and qualitative features (biomass yield, essential oil content and composition).**

- **Because of the great commercial importance of Greek oregano (ssp. *hirtum*), we aim at the selection of "elite" genotypes , based on the following criteria:**

***Selection criteria***

- **High essential oil yield and desirable compounds content (high carvacrol )**
- **Biomass yield**
- **Agronomical traits; Plant habit (erect), plant height, uniform blooming**



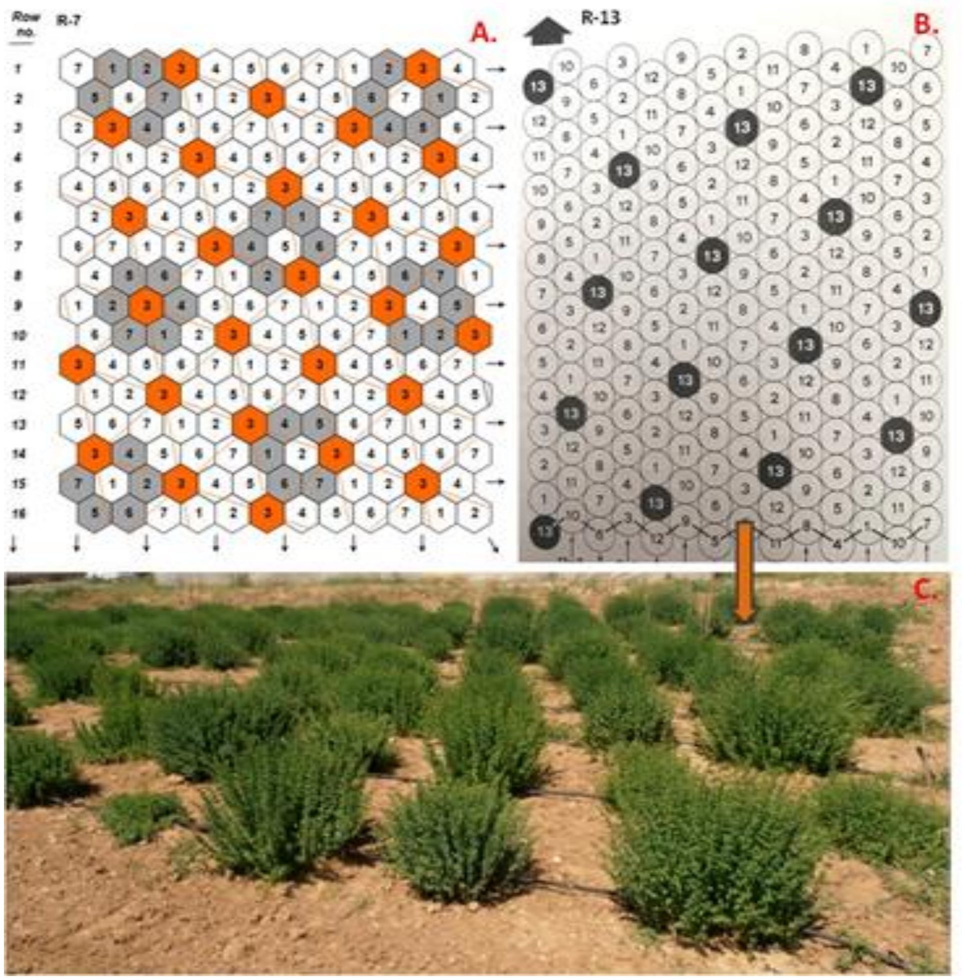




**Variation of Oregano essential oil content and its main costituents in wild grown oregano populations**


**Selected Oregano clones**

**Experimental plot =**

**Honeycomb design**




**Selection in Greek oregano: high biomass yield, essential oil and carvacrol**

min max

| Mode of Polination | Family | Selected plants | essential oil content (%) | cavacrol (%) | leaves+flowers (g) | Ratio leaves+flowers/stems |
|---|---|---|---|---|---|---|
| SP | 1 | 1 | 8,2 | 73,8 | 345,3 | 1,0 |
| | | 2 | 9,8 | 81,9 | 169,1 | 1,0 |
| | | 3 | 7,9 | 85,4 | 291,8 | 0,8 |
| | | 4 | 8,3 | 84,8 | 241,8 | 0,8 |
| | | 5 | 7,8 | 82,9 | 134,4 | 0,7 |
| OP | 1 | 6 | 10,2 | 85,1 | 218,7 | 0,8 |
| | | 7 | 8,8 | 84,1 | 259,6 | 0,7 |
| | | 8 | 8,9 | 81,0 | 374,0 | 1,3 |
| | | 9 | 7,8 | 85,8 | 147,1 | 1,0 |
| | | 10 | 8,9 | 85,5 | 234,6 | 0,8 |
| SP | 2 | 11 | 6,9 | 79,5 | 165,1 | 0,8 |
| | | 12 | 8,1 | 80,7 | 146,0 | 0,8 |
| | | 13 | 8,3 | 82,4 | 167,2 | 0,7 |
| | | 14 | 7,4 | 77,6 | 228,7 | 0,8 |
| OP | 2 | 15 | 10,7 | 84,5 | 347,2 | 0,8 |
| | | 16 | 6,0 | 76,2 | 290,0 | 0,6 |
| | | 17 | 8,1 | 83,2 | 285,7 | -1,2 |
| | | 18 | 9,5 | 82,4 | 461,8 | 0,9 |
| | | 19 | 7,8 | 52,0 | 168,3 | 0,8 |
| OP | 3 | 20 | 9,2 | 82,1 | 346,1 | 0,8 |
| OP | 4 | 21 | 8,3 | 84,8 | 269,6 | 0,8 |
| | | 22 | 9,2 | 81,1 | 425,4 | 0,8 |
| | | 23 | 8,7 | 84,2 | 368,0 | 0,9 |
| OP | 5 | 24 | 6,6 | 81,8 | 327,9 | 0,8 |
| | | 25 | 9,3 | 81,4 | 296,4 | 0,7 |
| | | 26 | 7,8 | 84,6 | 389,5 | 0,7 |
| OP | 6 | 27 | 8,7 | 80,9 | 390,3 | 0,7 |
| | | 28 | 11,1 | 82,5 | 380,6 | 0,8 |
| OP | 7 | 29 | 8,8 | 75,8 | 262,0 | 0,8 |
| | | 30 | 8,0 | 81,3 | 337,2 | 1,0 |
| | | 31 | 8,6 | 78,5 | 186,5 | 1,0 |
| OP | 8 | 32 | 9,8 | 83,5 | 257,3 | 0,6 |
| | | 33 | 8,1 | 87,3 | 195,5 | 0,6 |
| | | 34 | 8,3 | 79,6 | 305,8 | 1,0 |
| OP | 9 | 35 | 8,9 | 81,8 | 265,8 | 0,9 |
| | | 36 | 8,4 | 62,9 | 171,4 | 0,5 |
| OP | 11 | 37 | 7,4 | 80,9 | 217,9 | 0,5 |
| | | 38 | 8,2 | 80,0 | 247,6 | 0,8 |
| | | 39 | 6,8 | 78,2 | 204,2 | 0,8 |
| | | 40 | 8,0 | 80,8 | 326,3 | 0,9 |
| | | CV% | 12,62 | 7,71 | 31,3 | 46,4 |

**After 4 consecutive years of selection**

**1. Higher essential oil content (9-10%)**
**2. Carvacrol remains high (> 80%)**
**3. Better homogeneity**
**4. High yield potential**

Euphytica (2017)213:104
DOI 10.1007/s10681-017-1889-1

**Conventional breeding of Greek oregano (*Origanum vulgare* ssp. *hirtum*) and development of improved cultivars for yield potential and essential oil quality**

Eirini Sarrou · Nektaria Tsivelika · Paschalina Chatzopoulou · George Tsakalidis · Georgios Menexes · Athanasios Mavromatis

Received: 15 January 2017 / Accepted: 27 March 2017


**Abstract** This study is an attempt to describe a conventional breeding program on Greek oregano (*Origanum vulgare* ssp. *hirtum*). First of all, a descriptor list including the most significant morphological traits was developed for the starting genetic material. The program aimed to select the elite self-plants obtained from the initial population originated from Samothraki Island, in purpose to generate the ideotype of a new cultivar, characterized by desirable

inflorescences and their components of yield
In addition, the effect of the mode of pollin
plants' performance from two types of
obtained from self (SP) and open pollinati
was assessed. The evaluation for the compo
yield potential, the composition of essential
carvacrol content, was detected in selected se
revealing genotypes with high yield poten
target of this breeding program was the sele

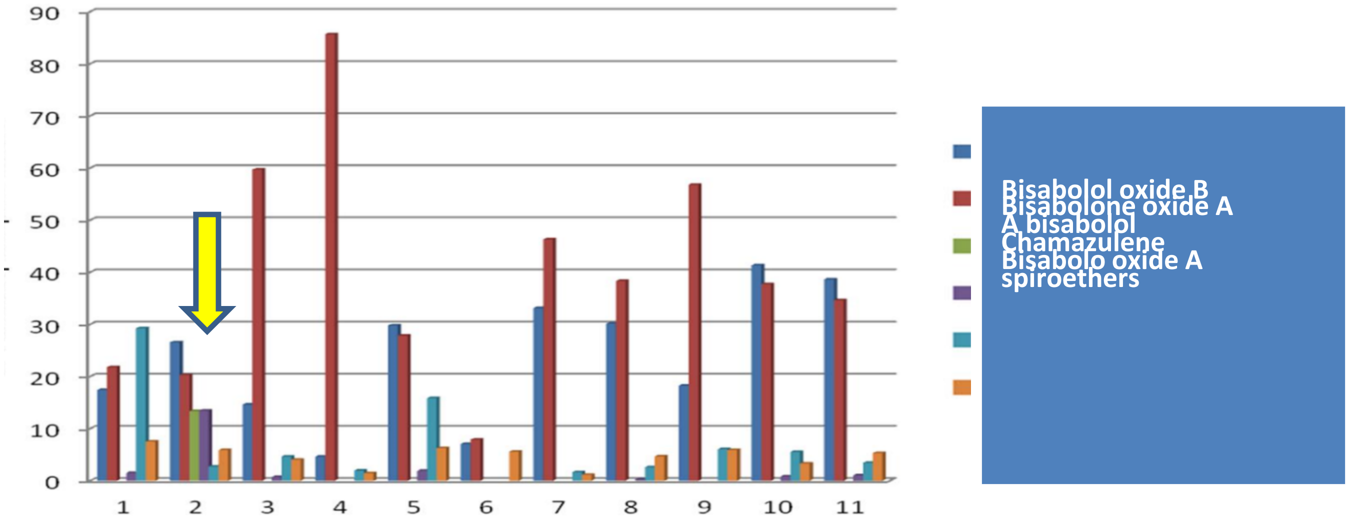
# Improvement of *Matricaria chamomilla*

•Chamomile is used worldwide as a medicinal plant and is one of the most important crops for pharmaceutical and cosmetic properties.

•The pharmaceutical properties are mainly due to chamazulene, a -bisabolol and different flavonoids.

•Chamomile raw material traded are <u>extremely variable in quality; essential oil content and the main components, and is depended on the source of the plant (wild  diploid types, cultivated diploid or tetraploid varietes etc)</u>

# Chemodiversity in wild Chamomile (*Matricaria chamomilla*) populations




**Variation of the main essential oils' constituents in wild Chamomile populations**

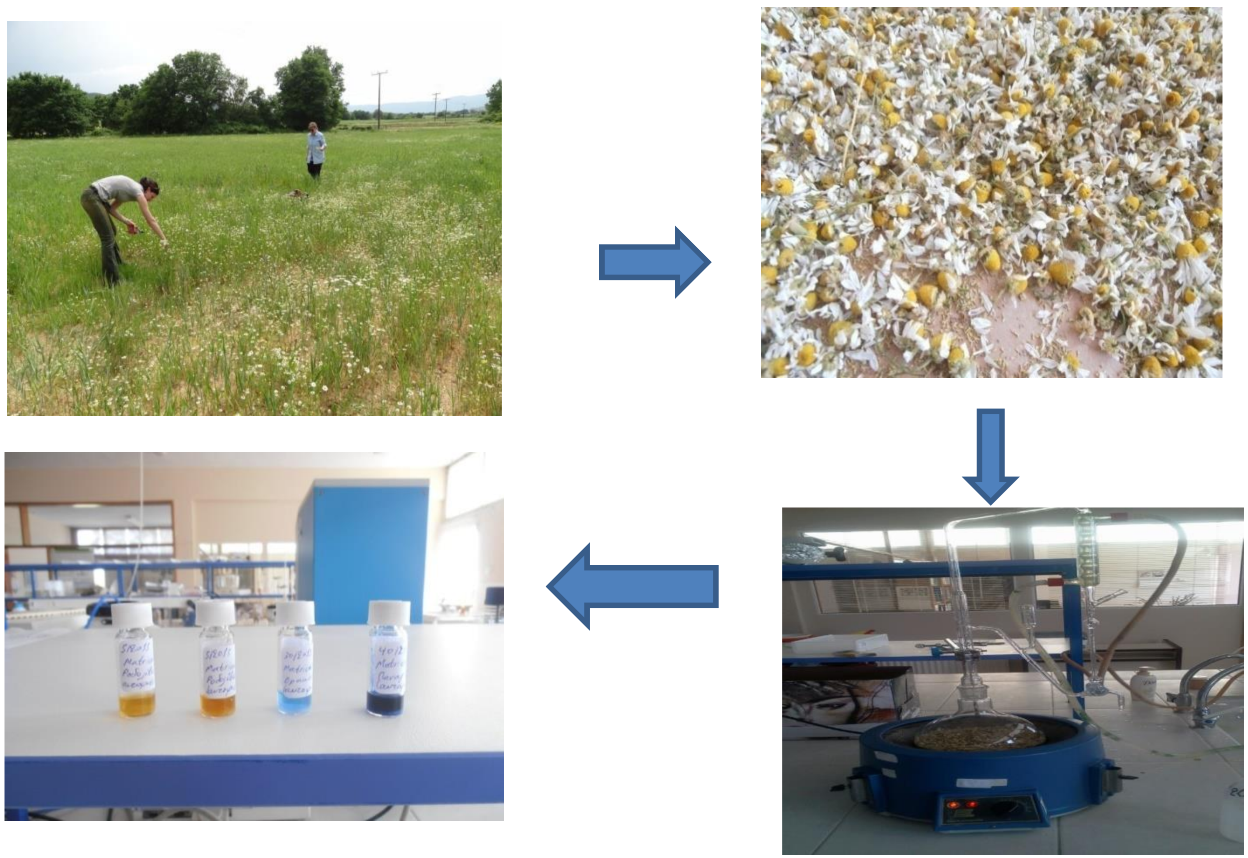
**Chamomile essential oil specifications (E.U PHARMACOPOEIA)**

- **Bisabolol oxides (sum) > 29-81%**
- **Chamazulene >1%**
- **a bisabolol >**10%

## AIMS OF BREEDING WORK

- **Wild populations as starting material in breeding programs**

- **Greek Chamomile varieties (adjusted to official Pharmacopoeias specifications)**



## Chamomile experimental fields
## 3 consecutive years


**Selection plants with desired agronomic traits, high yield, and high content in  chamazoulene and a-bisabolol**

# 7. Ex situ field collection

Preservation in *ex situ* field collection and experimental fields more than 200 accessions of different taxa of Medicinal and Aromatic Plants, native to Greece






## 1. National List of Varietes of registered Aromatic and Medicinal Plants (Ministerial decision 2266/2014)

Oregano, Thyme, Chamomile, Basil, Anethum

Examinations in the laboratory and in the field comprise tests for Distinctness, Uniformity and Stability (DUSH), according to UPOV Guidelines

VARIETES

CERTIFIED GERMPLASM

## 2. Technical Regulation for MAPs propagation material trading

***(Government Gazette 2663/2014***

It regulates the terms and requirements for trade

Phytosanitary

## Good Agricultural Practices

**Within the overall context of quality assurance, the guidelines on good agricultural and collection practices (GACP) for medicinal plants are primarily intended to provide general technical guidance on obtaining medicinal plant materials of good quality for the sustainable production of herbal products classified as medicines. (WHO)**
**They apply to the cultivation and collection of medicinal plants, including certain post-harvest operations. Quality control measures are also described (from the primary producers to traders).**

**ORGANIC CERTIFICATION**

**CERTIFICATION OF MAPs**
***complicated***

**Different end users (Food, medicines, cosmetics, flavours etc) –**
**specific quality specifications**

**The EU does not have a centralised authorisation procedure for the use of botanicals and derived preparations in food. Nonetheless, the use of botanicals and derived preparations in food has to comply with the general requirements set out in Regulation (EC) No 178/2002, which lays down the general principles and requirements of food law in the EU.**

**The EFSA documents for safety assessment of botanicals and botanical preparations intended for use as ingredients in food supplements (EFSA, 2009)**

***The Scientific Committee of EFSA published the Compendium of Botanicals reported to contain naturally occurring substances of possible concern for human health when used in food and food supplements.(EFSA 2012)***

Some botanicals are considered as traditional herbal medicinal plants and are used both in medicinal products and in food supplements. The **European Medicines Agency (EMA) is responsible for assessing both the safety and efficacy of herbal preparations <u>when used as medicines</u>.**
**Guideline on specifications: test procedures and acceptance criteria for herbal substances, herbal preparations and herbal medicinal products /traditional herbal medicinal products 31 March 2011 EMA/CPMP/QWP/2820/00 Rev. 2 (***Committee on Herbal Medicinal Products (CHMP)*

**Quality control:**

**Identity of plant material /part of plant used /origin / wild grown or cultivated**

**Processing /Extraction/ conditions/ solvents etc**

**Standardization (EU Pharmacopoeia criteria)**

**Specifications**

**Nutritional ingredients**

**Health ingredients**

**Groups of ingredients**

**Limits or absence of undesirable or toxic substances (residues, pesticides, heavy metals, mycotoxins etc)**

**Thank you for your attention!**