P 16: Evaluation of agronomical and qualitative characteristics of Greek Oregano (*Origanum vulgare ssp. hirtum*) germplasm for breeding purposes.

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Abstract
Oregano (*Origanum vulgare*) is one of the most commercially valued species with remarkable biological properties, while its world trade and consumption is steadily increased. In order to identify a standardized plant material of Greek Oregano (*Origanum vulgare* ssp. *hirtum*), sixteen native populations collected from different regions of Greece, were evaluated for their essential oil yield and composition. A breeding program was initiated for the most effective populations, concerning the most desirable biochemical, agronomic and morphological characteristics, using pedigree method and honeycomb design for plant selection.

Keywords: *Origanum vulgare*, breeding, honeycomb, carvacrol, thymol

Introduction

Greek Oregano (*Origanum vulgare* ssp. *hirtum*) is widely grown and collected from native wild populations throughout Greece. In addition, essential oil from Greek Oregano has been suggested as one of the best quality worldwide, with high concentration of the major compounds: carvacrol and thymol, accompanied by *p*-cymene and *γ*-terpinene (KINTZIOS, 2002).

Despite the highly increasing consumption and the great commercial value of Oregano, the excessive and uncontrolled collection from wild has significantly reduced the native populations, which indeed are a natural source of biodiversity. CANTER et al. (2005) reported the growing concern about diminishing populations, loss of genetic diversity, local extinctions and habitat degradation. Therefore, bred populations and controlled cultivation instead of wild collection, has been proposed as a safe way to balance human demands and production, as well as to conserve biodiversity of Greek Oregano.

Last decades, efforts have been started in the area of domestication and systematic cultivation of Oregano (BERNÁTH, 1997, GOLIARIS et al. 2002). Taking into consideration the demands of growers, producers and consumers, it is necessary to develop oregano breeding programs which should be directed to the improvement of yield components (e.g. growth habit, leaf/stem ratio) and quality related parameters (e.g. essential oil content and composition) (FRANZ and NOVAK, 1996).

Therefore the aim of this study was the selection of efficient oregano genotypes, from native Greek populations, to use them further as starting material for breeding purposes, focused on the development of commercial oregano varieties.

Materials and Methods

Plant material and experimental design

Sixteen native populations of oregano (*Origanum vulgare* ssp. *hirtum*), collected from different geographical areas of Greece, were evaluated for oil content and the most characteristic chemical compounds (carvacrol and thymol). The populations indicating higher concentration of essential oil and carvacrol (No. 2, 3, 6, 7, 11, 12, 14, 15, 16) were propagated by seeds and cultivated in the experimental field of IPB&GR (40°34'35'' N 22°57'19'' E). These selected populations were evaluated under the same ecological conditions for two consequent growing seasons, for certain agronomi-

cal and biochemical characteristics. Among them, population No. 16, with desirable characteristics, was propagated with stem cuttings and seeds and was experimentally cultivated. Plants from the specific population, derived both from open (OP) and self pollination (SP), were used for the establishment of an R-13 honeycomb experimental design (Figure 1).

Figure 1. The R-13 honeycomb design used for the breeding program of *Origanum vulgare* ssp. *hirtum* (population No 16). Each entry is allocated in such a way that is always surrounded by plants of all the other twelve entries.

The principal breeding objectives were: a) qualitative characteristics; high essential oil and carvacrol, thymol yield, b) agronomical characteristics; early and uniform blooming and high biomass production and c) morphological characteristics; plants with straight and high stems and broad inflorescences.

Essential oil isolation

The Essential oil content was determined using the European Pharmacopoeia apparatus (Clevenger-type). The dried aerial parts (leaves and flowers) of oregano were subjected to hydrodistillation for 1.30 hours with a distillation rate of 3 to 3.5 mL min^{-1}. The oil content was estimated on the basis of dry weight plant material (mL 100 g^{-1} of dried leaves).

Analysis of essential oil

The essential oils were analyzed by GC-MS on a fused silica DB-5 column, using a Gas Chromatograph 17A Ver. 3 interfaced with a mass spectrometer Shimadzu QP-5050A supported by the GC/MS Solution Ver1.21 software, using the method described previously (Sarrou et al., 2013). The identification of the compounds was based on comparison of their retention indices (RI) relative to n-alkanes (C_7-C_{22}), with corresponding literature data and by matching their spectra with those of MS libraries (NIST 98, Willey) (Adams, 1995).

Results

Selection of genetic material

The essential oil content and composition varied among the 16 different native populations of Oregano (Figure 2). More specifically, four of them (No. 10, 11, 15, 16) presented high essential oil yield (> 7 %), while populations 12 and 16 exhibited the higher concentration in carvacrol (82.7 and 76.86 %) and thymol (2.7-4.3 %) (Figure 3).

Further evaluation of their morphological characteristics indicated that population 16 is the most desirable for starting breeding material, since it is characterized from high and straight stems, high leaf/stem ratio, broad inflorescences, early and uniform blooming (Figure 4).

Breeding methodology & Honeycomb design

Pedigree method was applied using individual plants from population No 16 in R13 honeycomb design (KOUTSOS, T. V. and M. KOUTSIKA-SOTIRIOU, 2001).The first year of plant's development, agronomical and morphological characteristics were determined (% area/plant, fresh and dry biomass production/plant, existence of blooming plants and seed production). The evaluation of these data in combination with the observations from the second year of experimentation, will determine the most preferable plant row material from this population.

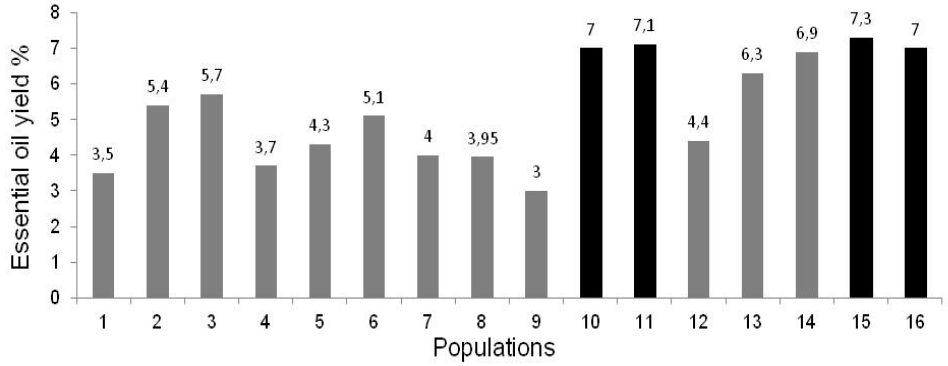

Fig. 2 Essential oil yield (%) of native populations of *Origanum vulgare* ssp. *hirtum,* collected from different geographical areas of Greece.

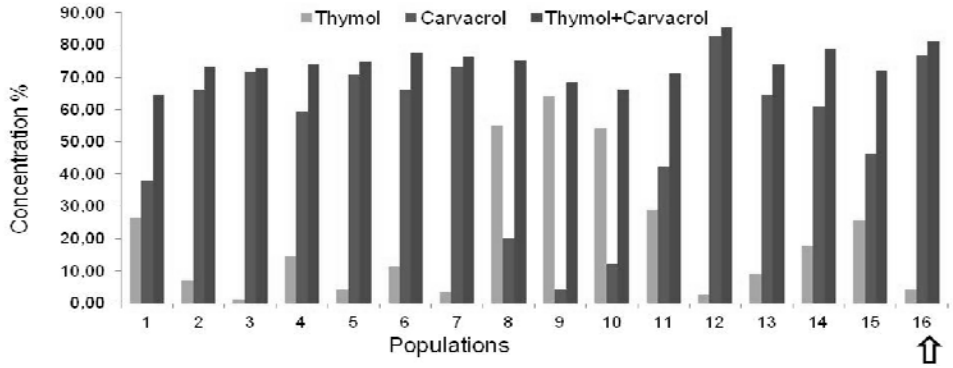

Fig. 3 (%) Composition of thymol and carvacrol of the essential oil from native populations of *Origanum vulgare* ssp. *hirtum* collected from different geographical areas of Greece.

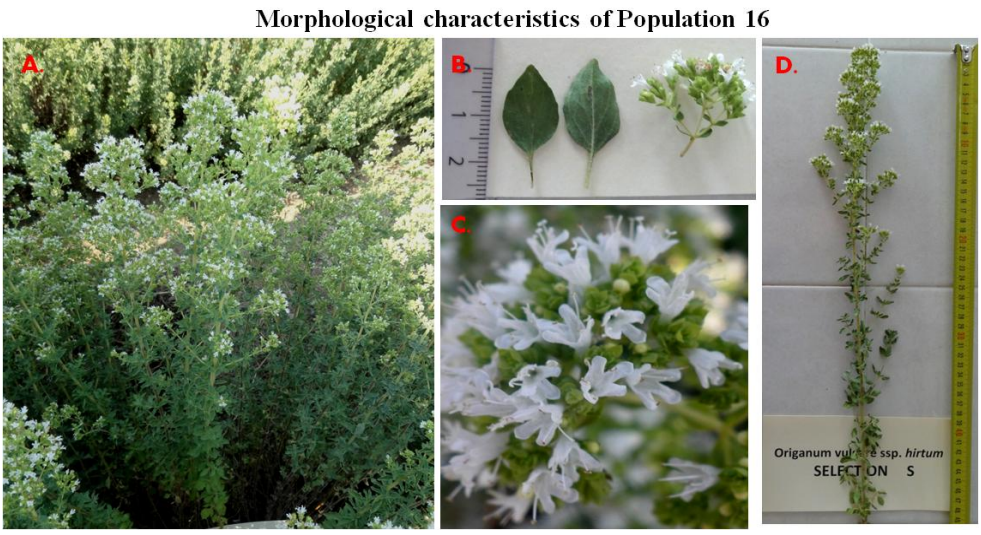

Fig. 3 Morphological characteristics of selected plants from population (No 16) of *Origanum vulgare* ssp. *hirtum*.

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