

P 24: Clone Selection for High Quality Types of Oregano (*Origanum dubium* Boiss.)



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Abstract

Origanum dubium Boiss. is one of the economically important wild oregano species and it is intensely collected from the natural flora of Antalya in Turkey. Carvacrol chemotype of *Origanum dubium* is used mainly for essential oil production due to its high essential oil content. In this study, chemical diversity of *Origanum dubium* was investigated in order to develop new cultivars with improved essential oil yield and carvacrol content using clone selection method under cultivated condition. Essential oils obtained by hydrodistillation of the aerial parts of *Origanum dubium* were analysed by GC-MS and 24 different components were identified. In these genotypes, carvacrol was the major component and followed by p-cymene, γ -terpinene, myrcene and α -thujene. Among the selected genotypes, essential oil yields were varied between 5.0% to 14.0%; carvacrol rates were varied between 73.76% to 88.21%; and p-cymene rates were varied between 3.16% to 9.10%.

Key words: *Origanum dubium*, essential oil, carvacrol, diversity

Introduction

Origanum dubium Boiss. is one of the economically important wild oregano species in Turkey. The most important characteristic of this oregano is high essential oil content (6-8%). It is intensely wild crafted from the upland of Alanya and Gazipasa towns of Antalya province and used for essential oil production. However, there are various genotypes and chemotypes within the wild populations such as high carvacrol and high linalool types. Also, natural populations have been decreased year after year (TURGUT et al., 2013). Therefore, cultivation of *Origanum dubium* seems to be the most convenient way for conservation of wild populations and production of stable drugs.

Origanum dubium Boiss. is grown in the wild flora of Turkey, Greece and Cyprus. Recently, Lukas et al. ² reported a new basis about the taxonomic uncertainties concerning section *Majorana* (LUKAS et al., 2013). They assessed the taxonomic status of *O. onites*, *O. syriacum*, *O. dubium* and *O. majorana* and discuss evolutionary relationships in section *Majorana* by considering molecular, morphological and phytochemical evidence. According to their results, "cymyl" chemotype of *Origanum majorana* L. is classified as *Origanum dubium* Boiss. The biological activities of essential oil of *Origanum dubium* Boiss. such as fungicidal (AHMAD et al., 2011), insecticidal (TANG et al., 2011) and antimicrobial (NOSTRO and PAPALIA 2012) properties associated with carvacrol content.

Recently, utilization of oregano oils has been increased significantly in various sectors (food, health, agriculture, cosmetics etc.). Thus, the yield of essential oil and carvacrol in oregano is very important due to its biological activity. *Origanum dubium* is used mainly for essential oil production not for culinary herb or herbal tea production because of high essential oil rate. Therefore, development of new genotypes with much higher essential oil yield and carvacrol content will be very valuable for the industry. The aim of the study was to improve chemical content of *Origanum dubium* Boiss. in order to develop new cultivars.

Materials and Methods

Plant material

In the preliminary studies, high essential oil and carvacrol type *Origanum dubium* Boiss. populations were identified and their seeds were collected from the natural flora of Gazipasa towns of Antalya province in Turkey (1372 m above sea level and N36 26.749 E32 28.266). Seeds were germinated in the greenhouse and then 2200 healthy seedlings were transferred to the experimental plot for individual plant selection. One hundred plants (genotypes) were selected clonally according to their agronomic features and essential oil yields. The study was conducted in Antalya located in Mediterranean Region of Turkey (33 m above sea level and 36 ° 53' N; 30° 38' E). This location was characterized by a Mediterranean climate with 1068 mm total rain fall, 19.7 °C mean air temperature, 13.6 °C minimum air temperature and 24.2 maximum air temperature. Terra-rossa type soil characteristics of the experimental field were clay loam, very high in lime, low in salt, and light alkaline (pH 7.7). The layer of 0-30 cm soil had low concentrations of organic material and sufficient amount of nitrogen. Available phosphorus content of the soil was low and useful potassium content was high.

Essential oil isolation

Plant rows which consist of 10 plants (clones) were harvested separately and then aerial parts were dried at room temperature. For the study, 100 g of samples from each plant row were subjected to water distillation for 3 h using a Clevenger type apparatus. As a result, percentage of essential was measured by the volumetric method (v/w) for each sample.

Analysis of the essential oils

Samples were diluted 1:50 with hexane for analyses. GC-MS analyses were performed on a gas chromatography (Agilent 7890A)-mass detector (Agilent 5975C) GC-MS system operating in the EI mode at 70 eV, equipped with a split/splitless injector (250 °C). The identification of the components of *Origanum dubium* Boiss essential oil was confirmed by comparison of their relative retention times and mass spectra with OIL ADAMS, NIST and Wiley libraries. Retention indices of all the components were determined by the Kovats method.

Results

In this study, yield and composition of the essential oil from aerial parts of 100 selected *Origanum dubium* Boiss. genotypes were determined. In total, 24 different components were identified representing 99.68% of the essential oil by GC-MS analysis. Quantitatively, carvacrol was the major component and followed by p-cymene, γ -terpinene, myrcene and α -thujene. All compounds except carvacrol were found in much lower amounts. They were rich in the active monoterpene phenols such as carvacrol and monoterpene hydrocarbon precursors such as p-cymene and γ -terpinene. According to the results, essential oil yields were varied between 5% to 14%. Actually, 24 genotypes out of one hundred were produced more than 10.0% essential oil yield; 62 genotypes produced at least 8.0% essential oil yield. These essential oil yields were found to be much higher than earlier studies which were reported as 7.6% (SARER et al., 1982) and 6.5 - 7.7% (BASER et al., 1993). Among the selected genotypes only one genotype produced extremely high (14%) essential oil yield with high carvacrol (84.65%) content.

The main constituent was carvacrol in all of the genotypes and carvacrol rates were varied between 73.76% to 88.21% with average rate of 83.29%. These results showed presence of significant variations in carvacrol content of *O. dubium*. Carvacrol contents of the genotypes were found to be higher than previous studies on *O. majorana* from Turkey 78.27 - 79.46% (BASER et al., 1993) and *O. dubium* from Cyprus 69.5 - 71.3% (KARIOTI et al., 2006). Carvacrol, linalool, linalool-carvacrol chemotypes as well as thymol chemotypes were found in *O. dubium*. Thymol, linalool and carvacrol

chemotypes were reported in Turkish *O. dubium* populations, while Cyprus ones revealed only carvacrol chemotype (FIGUÉRÉDO et al., 2006).

Second major constituent was appeared to be p-cymene in all samples and it varied between 3.16% to 9.10%. According to the results, the highest p-cymene (9.10%) rate was obtained from genotype G which had also the lowest carvacrol rate (73.76%). These results showed that carvacrol and p-cymene ratios were inversely correlated. In all samples, γ -terpinene was found to be third abundant component which varied from 1.14% to 5.17%.

In conclusion, considerable variations in essential oil yield and constituents were found within the population of *O. dubium* which were collected from the wild flora and then grown in the field. These variations proved that wild *O. dubium* populations are genetically and chemically heterogeneous. Selection and cultivation of oregano genotypes with high essential oil yield and carvacrol content are believed to be very important for essential oil producers and other related sectors.

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