Assessment of genetic variation and chemical composition of Origanumspp

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Abstract: The study was carried out on 23 entries of Origanumcollected from different areas of south Italy. The 23 entries were characterizedvia determining the chemical composition of their essential oils and genetic variability. The gas-chromatography of the essential oils of oregano accessions allowed the detection of 44 components with the predominance of carvacrol, thymol, linalyl acetate, γ-terpinene, o-cimene, s-caryophylleneand cis-ocimene. A high variability in the main components concentration was revealed except in the case of the accessions 13, 14 and 15 where the linalyl acetate ranging between 51.27 and 60.93%, outlining a new oregano chemotype.Using hierarchical cluster analysis, four main groups of samples were observed. Genetic variability using the RAPD analysis was not able to reveal clear polymorphism PCR patterns useful to distinguish the entries.So that, we decided to conduct further molecular analyses to determine the genetic variation among the entries under investigation, using AFLPs a powerful tool to perform phylogenetic analysis. This technique shows a high capability in detecting genetic variation. Combination between fluorescent system and polyacrylamide gels allows obtaining large number of bands (225 to 557). Finally,

thecurrent study shows thatthe Dendrogram of genetic similarity of Origanum is widely variable amongst genotypes of this plant.

Keywords: Origanumspp, Essential oil, Gas chromatography–mass spectrometry,Random Amplification of Polymorphic DNA,Amplified fragment length polymorphism.

Introduction

The genus of Origanumcomprises a large number of species (1). Most of the species grow in the mountainous areas inthe Mediterranean region (2,3). The property of secreting an essential oil gives this genus of plants thecharacteristic ofbeing widely used to make traditional food and medications. The chemical analysis of these plants shows that theycontain more than 60 chemical compounds (4-6). Recent studies revealed that plants belong to the genus Origanum have antimicrobial, antifungal, insecticidal and antioxidant properties (7,8). Despite the medical importance of theseplants, their genetic diversity has not been deeply investigated. In the present work molecular characterization of 23entries was performed using two classes of molecular marker: random amplified polymorphic DNA (RAPD) andamplified fragment length polymorphism (AFLP).

Materials and methods

Plant material: The study was carried out on 23 entries of Origanumcollected from different areas of south Italyand previously characterized in relation to their main morphological and agronomical features (9). The 23 entries werecharacterized both determining the chemical composition of the essential oil and investigating their genetic variability.

Gas chromatography–mass spectrometry:the essential oil was extracted from 40-gram samples of driedleaves collected for each genotype. Gas chromatography analysis (GC) was then performed using gas chromatograph Agilent coupled with mass spectrometer and equipped with stationary phase of HP-5MS with Helium gas a carrier. The data were acquired and instrument was controlled using Chem-Station software. Determining of constituents was obtained via comparing their determined relative retention index with published data and computer library (9).

DNA extraction and Random Amplified Polymorphic DNA (RAPD) analysis:DNA was extracted from youngleaves using the Sigma's GenElute^{TM} plant Genomic DNA

kit. The amount of obtained DNA was then quantified andqualified using Nano drop spectrophotometer and confirmed with 1% agarose gel electrophoresis run. Finally, RAPDanalyses were conducted to all genotypes following a modified protocols published by Williams et al. (10).

Amplified Fragment Length Polymorphic analysis (AFLP) and phylogenetic analysis:AFLP analysiswhich was carried out following a modified reported by Voset al. (11). DNA was digested with two restriction enzymes, EcoRI and MseI, ligated with site-specific adapters and pre-amplified with no selective primers. Finally, 10 selective primer combinations with fluorescent dyes were used to carry out PCR analyses. PCR products were analyzed using the ABI PRISM 3100 sequencer and the geno-typer software (Applied Biosystems Crop, Norwalk, Connecticut, U.S).

Statistics analysis: The percent concentrations of the components in the different oils were used as matrix elements to perform the hierarchical cluster analysis. All PC analyses were carried out using SAS software (SAS Institute Inc., Cary,NC) procedures. Polymorphic data were analyzed by means of NTSYS 2.0 software.

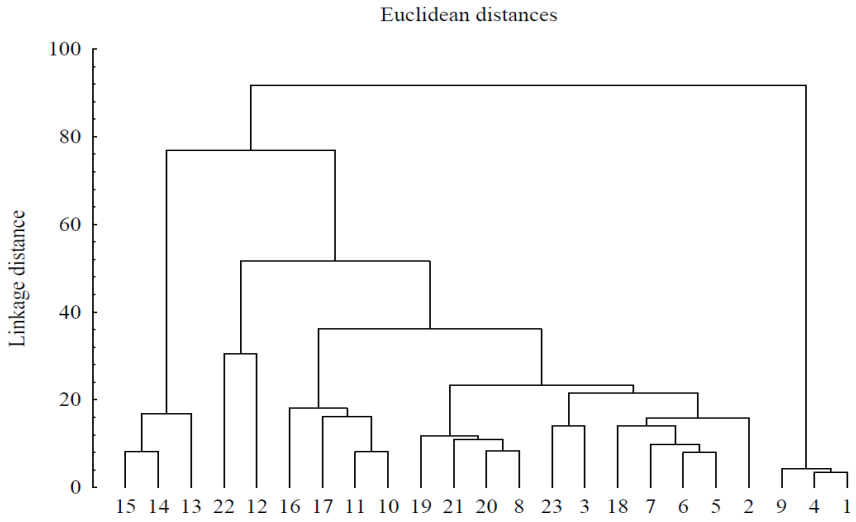
Results

The gas-chromatography of the essential oils of oregano accessions allowed the detection of 44 components with thepredominance of carvacrol, thymol, linalyl acetate, γ-terpinene, o-cimene, s-caryophylleneand cis-ocimene (Table 1).A high variability in themain components concentration wasrevealed except in thecase of the accessions 13,14 and 15 where thelinalyl acetate rangingbetween 51.27 and60.93%, outlining a new oreganochemo-type.

Tab. 1 - Main essential oil components (% v/v) in 23 oregano biotypes.

Major volatile oil constituents	Biotipes																						
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23
carvacrol	66,15	-	2,69	66,74	0,20	1,38	0,39	0,18	66,68	4,01	1,10	-	-	-	-	1,28	2,22	1,18	1,62	-	-	-	1,65
thymol	0,27	11,03	13,19	0,20	18,36	18,63	15,89	22,47	0,20	38,16	43,68	3,88	-	-	-	28,56	33,64	15,67	30,24	29,15	24,17	-	15,67
linalyl acetate	-	6,97	-	-	-	-	-	-	0,06	-	-	-	60,93	51,27	53,00	-	-	-	-	-	-	-	-
γ - terpinene	8,93	24,69	11,61	11,31	21,69	21,57	18,53	25,69	9,78	11,90	13,48	24,25	-	-	1,60	6,96	16,12	25,70	19,71	25,92	22,49	-	12,99
o - cimene	3,20	6,17	2,15	4,68	5,57	3,58	3,01	3,31	2,30	6,79	4,94	2,37	-	-	-	3,10	3,52	2,83	6,44	3,92	2,43	-	13,41
β - caryophyllene	2,81	2,80	1,34	3,08	3,41	7,27	6,37	2,52	2,78	2,90	0,77	9,77	3,73	3,16	4,65	4,65	1,57	2,41	4,97	3,59	3,02	21,59	3,54
cis - ocimene	1,48	3,48	1,56	-	3,19	4,78	3,87	2,10	0,15	5,58	3,91	13,04	1,98	13,68	7,70	5,88	3,83	5,96	2,33	5,07	4,51	6,30	2,29

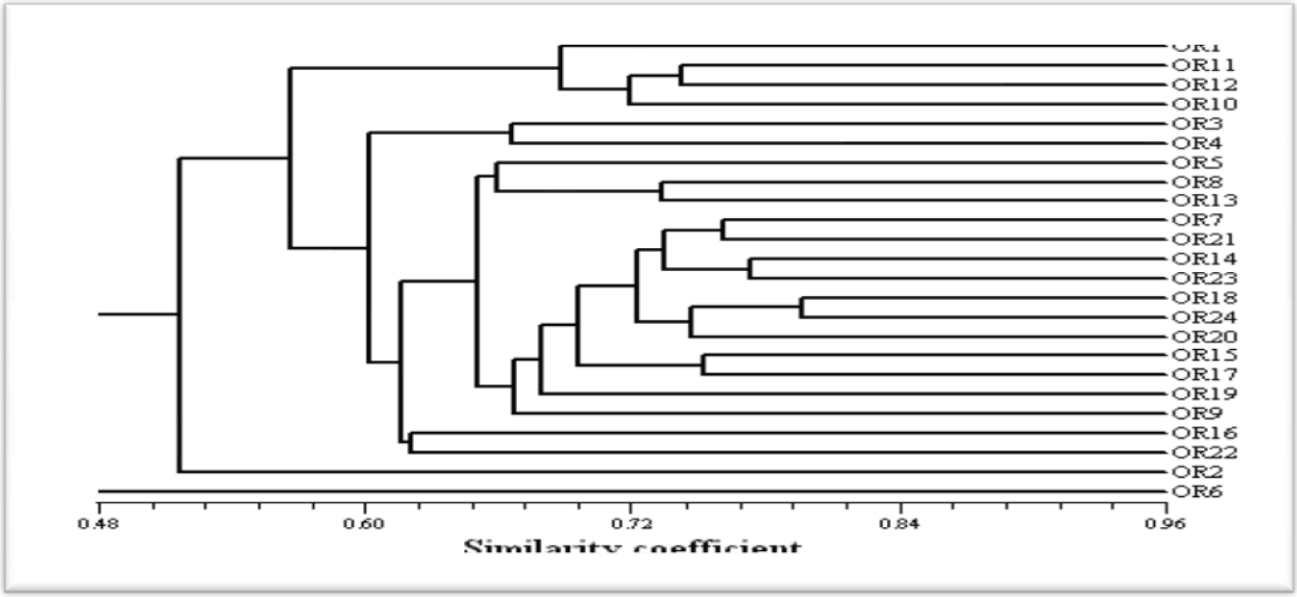
Figure 1: Cluster analysis of essential oils composition determined on 23 entries of Origanum.



Using hierarchical cluster analysis, four main groups of samples were observed (Figure 1). It is, thus, possible to distinguish clearly the linalyl acetate chemo-type, in the group including biotypes 13-14-15; the subsequent group, including accessions12 and 22, is less well-defined since there is a predominance of the precursors (γ-terpinene and of the two main mono-terpenic phenols (thymol/carvacrol). The third most numerous group represents the thymol chemo-type with so high variations (11.03-43.68%) such to allow a distinction in high and low thymol concentration types. The last group represented by the accessions 1, 4 and 9 defines distinctly the carvacrolchemo-type. The quality and quantity of the DNA obtained using the commercial kit was satisfactory and can be used to carry out molecular analyses. However, RAPD analysis was not able to reveal clear polymorphism PCR patterns useful to distinguishthe entries.This result is consisted with other bibliographic data, reporting that the use of RAPD analysis to identify the genetic variation among entries results in low reproducibility.So that, we decided to conduct further molecular analyses to determine the genetic variation among the entries under investigation, using AFLPs a powerful tool to perform Furthermore, the use of the fluorescent system to detect the polymorphic bands associated with applying polyacrylamide gels allowed to obtain large number of bands ranging from 225 to 557. A total of 3315 bands were scored after the amplification and 1179 showed polymorphism (35.8%). The high capability of AFLP in detecting genetic variation is similar to that reported in the

literature (8, 12). Polymorphic bands were analyzed to obtain a dendrogram of genetic similarity (Figure 2), showing a wide variability among genotypes. As shown in Figure 2, the 23 entries were grouped into two clusters at a similarity level of 0.45: the first one includes entries from OR1 to OR10 while the second one included only the OR4 and OR6 entry.

Figure 2: Dendrogram of genetic similarity obtained using Jaccard coefficient determined on 23 entries of Origanum.



Discussion

In the current study, four main chemo-type groups were found amongst 23 entries of Origanumand distinguished clearly.A study performed to determine the morphological and chemical variability amongst oregano spp.showed that there is a relationship between the morphological traits and chemical contents amongst the species populations. In addition the study reported that the total content of essential oil ranged from 0.35 to 0.87%; Sabinene, 1,8-cineole, linalool, p-cymene, β-caryophyllene and caryophyllene oxide were the dominant compounds in essential oil (14).

RAPD technique is a suitable technique to be used to differentiate between various

genotypes but it is proven to be difficult to clearly differentiate amongst Origanumgenotypes. The reasons for this result are the low annealing temperature, the small size of primers and essential oil residues phylogenetic analysis (12).So that, AFLP technique was chosen to perform gento-typing analysis because it has been found that AFLP technique is informative and efficient technique to discover Origanum and thymus genomes. Additionally, this technique is found to be helpful to determine the genetic variations and phylogenetic relationships within the studied species (13).Thus, we conclude that AFLP and phylogenetic are practical, simple, time-effective and cost-effective techniques and can be efficiently used to detect genetic variations amongst medicinal plants.

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