SHORT COMMUNICATION

Agronomic, metabolomic and lipidomic characterisation of Sicilian *Origanum vulgare* (L.) ecotypes

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**ABSTRACT**

Although *Origanum vulgare* (L.) has been deeply analysed at phytochemical level, poor knowledge is available regarding non-volatile compounds such as lipids. The aim of this work was to characterise five wild Sicilian *Origanum* ecotypes from an agronomic, metabolomic and lipidomic perspective. Serradifalco presented higher dry weight and inflorescences/plant than the others while Favara had a significantly higher number of branches per plant and more extensive flowered stratum. Metabolomic analysis, performed with LC-MS-TOF, allowed a preliminary characterisation of the non-volatile metabolome of the five oregano ecotypes *Origanum vulgare* ssp. *hirtum*. Twenty-five metabolites were identified belonging to organic acids, amino acids, lysophosphatidylcholines, carnithines, nucleic bases and lysophosphatidylethanolamines. Lipidomic analysis identified 115 polar plant membrane glycerolipid species. Thirteen of them were differentially present in the two chosen ecotypes. The role of these metabolites in plant physiology from a qualitative and pharmacological point of view was discussed.

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1. Introduction

Crop species from the Lamiaceae family have a peculiar importance due to their wide use as culinary herbs and flavourings for food and beverages (Tuttolomondo et al. 2009). *Origanum vulgare* (L.) belongs to the Lamiaceae family; well known for the aromatic and medicinal properties of the essential oils found in large quantities in the leaves and inflorescences. This species is widespread in North Africa, Western Asia and Europe particularly in Sicily (Tuttolomondo et al. 2013). Oregano subspecies have been extensively characterised from a phytochemical perspective (Leto et al. 1996; Tuttolomondo et al. 2012; Tair et al. 2014; Baranauskaitė et al. 2015). In contrast to volatile components, the non-volatile compounds have been studied much less.

Cellular lipids play a fundamental role in cell structure, metabolism and regulation. Lipid profiling using UPLC-ESI QTOF MS/MS involves direct infusion of lipid extracts into a mass spectrometer and thus requires an easy extraction protocol. Lipidomics is a branch of metabolomics focusing on the large-scale analysis of pathways and networks of cellular lipids in cells (Wenk 2005). Untargeted ‘omic’ approaches have been extensively used to characterise the metabolome of different plant tissues such as fruits and flowers (Martinelli et al. 2013; Noutsos et al. 2015).

The aim of this study was to characterise five *O. vulgare* (L.) ecotypes from Sicily at an agronomic, metabolomic and lipidomic level. These data will help valorize germplasm of this medicinal and aromatic species, identifying key features which could be used in the food and pharmaceutical sectors.

2. Results and discussion

2.1. Agronomic characterisation

S (Serradifalco) showed a higher dry weight and inflorescences/plant value than V (Villalba), N (Naro) and LZ (Linguaglossa). F was characterised by a significantly higher level of branches per plant (Table S1). Relating to stem weight, significant differences were observed only between S and LZ. S and LZ had similar total leaf weight, while V, N and F (Favara) were characterised by a lower weight. N showed a lower percentage of plants in flower than S and B3. Regarding percentages of stems and of leaves per plant, F was found to have the highest and lowest values, respectively. Conversely, N had the lowest percentage of stems and the highest of leaves. S, V and LZ presented a significantly higher stems/clump than N and F. This latter ecotype F has the most extensive flowered layer. S, V and LZ had a high level of stem/clump, while N, LZ and F were distinguished by a high length of stem per plant. Taken together, it seems that F and S may be more suitable for local development of this crop in Sicily.

2.2. PCA analysis of agronomic data

Principal component analysis (PCA) of the 12 agronomic parameters was performed to determine differences and similarities between the five ecotypes (Figure S1). The two PC components accounted for 89% of the total variation. Agronomic parameters were slightly correlated with both PCs. The PC1 clearly separated S from F. PC 2 was effective to separate F from V, N and LZ. Flower stratum and plant ramifications mostly contribute to the distinctive profile of F.
2.3. Metabolomic analysis

The 25 identified components of the extracted essential oils of the inflorescences belonged to organic acids, amino acids, lysophosphatidylcholines, carnitines, nucleic bases and lysophosphatidylethanolamines (Table S2). The identified compounds are interesting from a food and/or health perspective.

PCA analysis of the metabolomic data showed a quite clear separation between the five *Origanum* ecotypes (Figure 1). The five ecotypes showed distinctive patterns although S and N metabolomic compositions were found to be more similar to each other than the others. As far as it concerns, the amount of acetylcholine was higher in Serradifalco and Naro than Villalba. This metabolite is an organic metabolite with the function of a neurotransmitter in different organisms, including humans. The presence of this compound in oregano may be related to the well-known ACh properties of increasing sensory perceptions and maintaining attention in humans (Jacob et al. 2013). Serradifalco showed high amounts of three carnitine-derived metabolites (carnitine C0:0, carnitine C2:0 and carnitine C3:0). L-carnitine has been characterised by antioxidant and antiradical properties (Gülçin 2006). Favara exhibited higher levels of glycerophosphocholine than the other four ecotypes. Interestingly, L-alpha glycerylphosphorylcholine (alpha-GPC) is a natural choline that seems to have positive effects on the treatment of Alzheimer’s disease (Pernetti et al. 2007) and dementia (Doggrell & Evans 2003). Lysophosphatidylcholines (LPC), also called lysolecithins, are a class of chemical compounds which are made by hydrolysis of phosphatidylcholines catalysed by phospholipase A2. Some differences were observed among ecotypes regarding the specific amounts of each of the analysed lysolecithins: Serradifalco showed higher LPC 18:2 than Naro, Linguaglossa and Favara. LPC 20:0 was higher in Naro than in Serradifalco. In medicine, LPCs can be employed to simulate the effects of demyelinating diseases such as multiple sclerosis. Their anticancer properties seemed to be related to their insertion in plasma membrane inducing apoptosis, affecting signal pathways and not linked with the proliferation of cancer cells.
state of the tumor cell (van Blitterswijk & Verheij 2008). Some significant differences were observed for lysophosphatidylethanolamine (LPE) among the ecotypes. LPE is interesting since it can be used in agriculture to regulate plant growth, such as colour increase, sugar content increase, plant health increase and storability increase without any known disadvantages. As far as it concerns Serradifalco presented significantly higher levels of phenylalanine than the other four ecotypes. An extremely high variability was observed among the five ecotypes for proline content. Linguaglossa had the highest levels, while Favara showed the lowest. It is well known that proline is abundant in the reproductive organs of different plant species, such as florets, pollen, siliques and seeds (Chiang & Dandekar 1995). The levels of proline may be related to flowering time due to the stress-induced factor. This metabolite in apical meristems is suggested to be essential for the timing of flowering in response to varying environmental conditions (Mattioli et al. 2008). We observed a significant variability in tryptophan content among the five ecotypes. S and LZ had the highest content while V showed the lowest level. Tryptophan is also involved in the biosynthesis of secondary metabolites such as terpenoid indole alkaloids, indole glucosinolates and indolic phytoalexins (Tohge et al. 2013).

2.4. Lipidomics

The present lipidomic analysis identified 114 polar plant membrane glycerolipid species of the major phospholipid classes in the two analysed ecotypes (S and V): phosphatidylcholine (PC), phosphatidylethanolamine (PE), phosphatidylglycerol (PG), phosphatidylinositol (PI), galactolipid classes digalactosyldiacylglycerol (dGdG), lysophosphatidylcholine (lyso PC), lysophosphatidylethanolamine (lyso PE) and triacylglycerols (TG), as well as minor acyl species within each head group class (Table S3). Of these glycerolipid species, only 13 were significantly varied (Figure S2). We observed that LPC 20:4 and PC 38:2 and PE 36:1 varied significantly in abundance between the two chosen Origanum species. LPC analogues are metabolically stable, and a number of them, such as edelfosine, miltefosine and perifosine, are under research and development as drugs against cancer and other diseases (Houlihan et al. 1995). Lyso PC and lyso PE have been found in higher levels in flowers than in other organs. The specific activity of acyltransferases and desaturases has led to specific desaturation of acyl chains depending on the different plant organs (Wang et al. 2006). Six of the fifty-four detected TGs significantly varied in abundance between the two ecotypes. TG 52:0, TG 52:2 and TG 56:6 were higher in V while TG 51:4, TG 53:3 and TG 54:6 were more abundant in S.

Triglycerides (TG), usually more unsaturated, are the main components of vegetable oil. As in animals, plants use TG as energy reserves. They allow plants to maintain great levels of energy in the smallest spaces.

In conclusion, this study increased the primary knowledge of oregano wild populations’ agronomic and qualitative perspectives. Particularly, the lipidomic approach was capable of generating quantitative data for 114 polar glycerolipid species. This provides the most updated complete analysis of molecular species of polar lipids in O. vulgare. Indeed, this study showed that wild oregano present important compounds that render this medicinal crop very interesting not only for culinary but also for healthy perspectives. Data obtained from metabolomic and lipidomic perspectives may be integrated into transcriptome analysis using approaches such as RNA-seq. This will allow deciphering which genes play an important role in the different accumulation of these metabolites among ecotypes.
Disclosure statement

No potential conflict of interest was reported by the authors.

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