EDIBLE, AROMATIC AND MEDICINAL PLANTS OF THE ALPS: A RESOURCE TO VALUE


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The increased interest of the consumers towards natural products, has involved a growing request of aromatic and medicinal plants by processing industries. Such demand became so high to raise the preoccupation of the WHO concerning the conservation of natural resources of these plants, from which, even nowadays, the health of 80% of the human population (mainly that of developing countries) is depending.

In Trentino, numerous medicinal and aromatic species are naturally growing. The collecting of these species was widespread up to about 50 years ago, but since 30 years ago the Provincial Law for the protection of the wild flora (n. 17/73) allows their collection only to authorized professionals. According to recent studies, however, several wild plants species, some of whose medicinal and aromatic plants, are endangered in Trentino as well, mainly due to the disappearance of traditional farming.

The aim of this project is to exploit some species of the alpine environment which are especially promising because of their taste (*Cicerbita alpina* (L.) Wallr), and/or medicinal properties *Euphrasia rostkoviana* Hayne, *Hieracium pilosella* L., *Lytrum salicaria* L. e *Rhodiola rosea* L. (*Sedum roseum* Scop.). The above species, after they have been characterised both from the chemical and the molecular sides and having been domesticated, could be cultivated locally with sustainable methods and they could form the row material which some local herbal/cosmetic workshops could utilise and transform into products of added value.
CHARACTERIZATION OF HELICHRYSUM STOECHAS HAIRY ROOT-REGENERATED PLANTS

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Agrobacterium rhizogenes, everlasting flowers, plant architecture, secondary metabolites

Regenerated plants from hairy roots, induced by wild Agrobacterium rhizogenes strains, exhibited the typical alterations due to T-DNA gene expression: dwarfing, increased rooting, advanced flowering, increased branching, reduced apical dominance and small leaves (Christey M.C., 2001). These altered phenotypic features show potential applications for plant propagation and for improving secondary metabolites production. Helichrysum stoechas (fam. Asteraceae) is an aromatic wild species of the Mediterranean region. The plant is an evergreen shrub, growing in arid soil and flowering from May to August. The bright yellow flower heads contain principally essential oils, flavonoids (helichrysin A and B) and tannins. H. stoechas has been used in folk medicine because of its antibacterial, antitoxic, diuretic and antiallergic properties. The flowering stems are also used dried as "everlasting flowers".

Tissue cultures from in vivo germinated seedlings of H. stoechas (L.) Moench, provided by the Siena Botanical Garden, were established. Shoot induction was obtained from leaf tissue of micropropagated plant clones, on a medium supplemented with thidiazuron (Giovannini et al., 2003). A. rhizogenes 15834 wild type strain was effective to induce hairy roots in one H. stoechas plant clone (CL 7). Shoots developed spontaneously from hairy roots on hormone-free medium, in light conditions. T-DNA rolC gene was detected by PCR analysis in four hairy root-regenerated plant lines (B, E, M, N), originated from independent transformation events (Amoretti et al., 2003). About twenty rooted plants deriving from each of the hairy root lines E, M and N and from the control were acclimatized in greenhouse. Untransformed plants of clone 7 were used as the source of control. After one year of in vivo culture, several cuttings were obtained (October 2004) and cultivated into a cold greenhouse (November 2004). The experiment was arranged in 4 blocks with 15 pots (14 cm diameter) per block and one plant per pot.

From the end of April 2005, the following data were collected for each sample: plant height, number and length of primary branches, leaf length and width, onset of flowering and number of flower heads per inflorescence. Data were subjected to analysis of variance (ANOVA). Means were compared by Student-Newman-Keuls multiple range test (P ≤ 0.05). H. stoechas hairy root-regenerated plants showed a more compact plant habit (plant height significantly lower by 32% as compared to the control) and early flowering in two lines. Moreover, primary branch length was significantly reduced (42%), whereas the number of primary branches resulted not affected in two lines and significantly higher in one line.

VIVIPAROUS PLANTLETS FORMATION IN KALANCHOE: TOOLS FOR MOLECULAR ANALYSIS OF RESPONSIBLE GENES

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asexual reproduction, epiphyllous bud, vivipary

The formation of viviparous plantlets is a trait common to several families (e.g. Graminaceae, Crassulaceae, Liliaceae). The plantlets are produced over organs such as leaves, roots and flower stalks.

The Kalanchoe plantlets develop on leaf margin and are organised with an inverse polarity to that of the mother plant. Inverse polarity was already reported for the extra flower developing on the distal part of the lemma in Hooded mutant barley (Müller et al., 1995). The Hooded mutation is caused by a duplication involving intron 4 of the gene Bkn-1 (Müller et al., 1995). Furthermore epiphyllous bud has been observed over Nicotiana leaves over-expressing Bkn-1 e Bkn-3 genes (Lin & Müller, 2002). These observations show that genes of the Knotted family, which control the fate of meristematic cells (Kobayashi et al., 2000), could play a role on the viviparous plantlet formation.

Two strategies have been addressed in order to identify genes involved in bud formation in Kalanchoe:

1) Identification of heterologous Knotted genes.
2) Construction of a subtracted cDNA library.

Knotted class I genes were identified applying in Kalanchoe the methodology used by Kobayashi et al., (2000) in Pharbitis. The first step was carried to amplify the 3’ region of Knotted class I candidate genes by 3’ Rapid Amplification of cDNA Ends (RACE) PCR. 3’ RACE was carried using as template cDNA obtained through RT-PCR of mRNA, two nested degenerated forward primers, which sequence was based on a conserved domain (homeobox), and a reverse poly-A primer. 4 different genes have been identified. Bioinformatics analysis confirmed their homology to Knotted like genes. 5’ region is currently under analysis.

The subtracted cDNA library was prepared utilising poly(A)+ RNA extracted from the internal lamina (reference) and from the leaf margin, which contains putative transcripts involved in viviparous plantlets formation. The subtracted and enriched DNA fragments were directly cloned into T/A cloning vector. Screening of the colonies is in progress.
BIODIVERSITY OF SARDINIA AND CALABRIA MYRTLE (*MYRTUS COMMUNIS*) ASSESSED THROUGH MORPHOLOGICAL CHARACTERS AND AFLP MARKERS

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*Myrtus communis, AFLP, morphological characters, biodiversity, genetic variation*

Myrtle (*Myrtus communis*), a shrub widespread in the Mediterranean area, is the only species of Myrtaceae family growing in Europe. In the last years it is appreciated for its pharmacological and aromatic properties. The use of myrtle as aromatic plant is traditionally rooted in coastal areas of Mediterranean Sea, especially in the Italian regions of Sardinia and Calabria, where it represents a potential in food and medicinal herbs industry.

In this work an evaluation of biodiversity in 14 populations of Sardinia and Calabria myrtle was carried out by means of morphological characters and AFLP markers.

Principal Component Analysis (PCA) done on morphological characters showed that phenotypic variability was primarily related to branch diameter, seed characteristics and leaf dimensions.

Genetic diversity, assessed with AFLP markers revealed a clear separation between populations of Sardinia and Calabria. AMOVA analysis indicated that genetic variation was greater within populations (51.86%) than among populations (16.99%), as reported for outcrossing species. A significant amount of variation (31.15%) was attributable to variation between the two groups including populations of Sardinia and Calabria, suggesting a genotypic differentiation between myrtle collected in these two regions.

Genetic diversity within populations was assessed estimating expected unbiased heterozigosity (Hₑ) that ranged from 0.0595 to 0.2595. These values resulted correlated with population extension (r= 0.918, p<0.01) and with two reproductive parameters: seed germinability (r=0.793; p<0.01) and number of seeds per fruit (r=0.631; p< 0.05). A moderate gene flow within Sardinia and Calabria myrtle (1.2719 and 1.0478 respectively) counteracts the loss of genetic variation observed in some populations and avoids their differentiation and isolation.
ELICITATION OF PHENOLIC AND TERPENOID COMPOUNDS IN CELLS AND HAIRY ROOTS OF SALVIA SCLAREA

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One interesting strategy to boost biosynthesis of plant natural bioactive molecules is the use of external compounds or compound mixtures, able to mimic in vitro the effect of a pathogen attack or other physical stresses and force plant cells to synthesize secondary metabolites.

Different elicitors were used to enhance the biosynthesis of bioactive secondary metabolites in Salvia sclarea cells and hairy roots:

a) salicylic acid and methyl-jasmonate (MeJ), known to activate in plant cells the signal transduction pathway leading to switch on transcription of genes involved in plant defense, including genes belonging to biosynthetic pathway of secondary metabolites;

b) yeast extract, a mixture of elicitors of different chemical nature, extracted from yeast cells, known to be very effective in increasing the synthesis of tanshinone, an anti-tumoral plant diterpene.

Liquid chromatography-mass spectrometry (LC-MS) was used for simultaneous detection and identification of phenolic and terpenoid compounds from elicited S. sclarea cells and roots.

The most effective eliciting compounds in S. sclarea cell suspension culture were methyl-jasmonate (MeJ) and yeast extract, that enhanced significantly the content of rosmarinic acid, but a negligible effect on terpenoid compounds.

In S. sclarea hairy roots, major metabolic changes were induced by MeJ treatment, that enhanced significantly the content of several diterpenoid compounds.