



Genetic background of Dalmatian pyrethrum (*Tanacetum cinerariifolium* /Trevir./Sch. Bip.) insecticidal potential (PyrDiv) (IP-06-2016-9034)

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Dalmatian pyrethrum

(*Tanacetum cinerariifolium* /Trevir./Sch. Bip.)

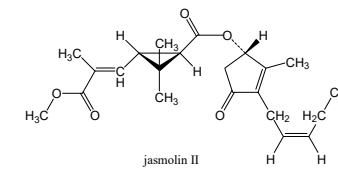
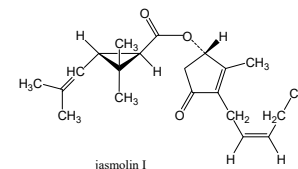
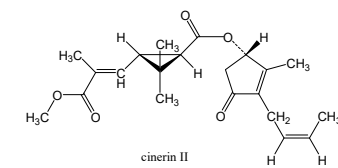
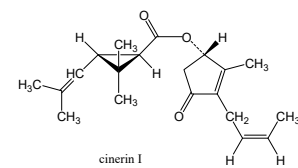
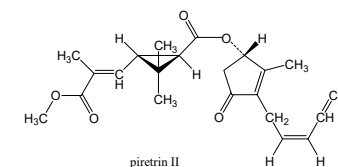
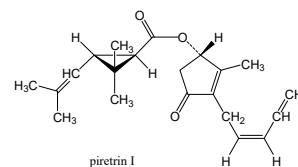


- perennial, outcrossing plant species
- indigenous to the Eastern coast of the Adriatic Sea
- natural habitat: extremely degraded habitats with shallow rocky soils
- strictly protected plant species
- permission of Ministry of Nature protection and Energy

Dalmatian pyrethrum (*Tanacetum cinerariifolium* /Trevir./Sch. Bip.)

PYRETHRIN - natural insecticide

- pyrethrin I i II, cinerin I i II, jasmolin I i II
- pyrethrin I i II - the most active
- contact insecticide
- acting on the nervous system of insects
- causing *knock-down* effect and death



Dalmatian pyrethrum (*Tanacetum cinerariifolium* /Trevir./Sch. Bip.)

- documented history in Croatia
- cultivation and use of Dalmatian pyrethrum and its products in households and agricultural systems
- 1850. beginning of cultivation in Dubrovnik
- '*Flores Crisanthemi*' - dried flowers powder
- from 1930. rapid decrease in production
- 1945. Kenya leading producer of Dalmatian pyrethrum
- in the 60's attempts have been made to revitalize pyrethrum production in Dalmatia - not success



Objectives

- (1) Evaluation of genetic diversity and structure of natural Dalmatian pyrethrum populations
- (2) Evaluation of biochemical diversity of natural Dalmatian pyrethrum populations
- (3) Determination of genetic background of pyrethrin synthesis - Association mapping

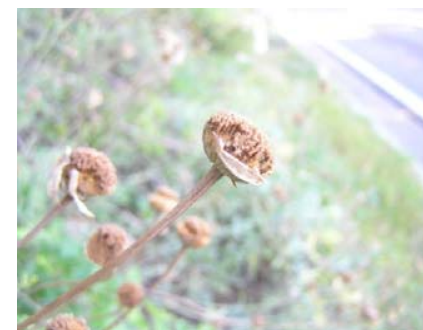
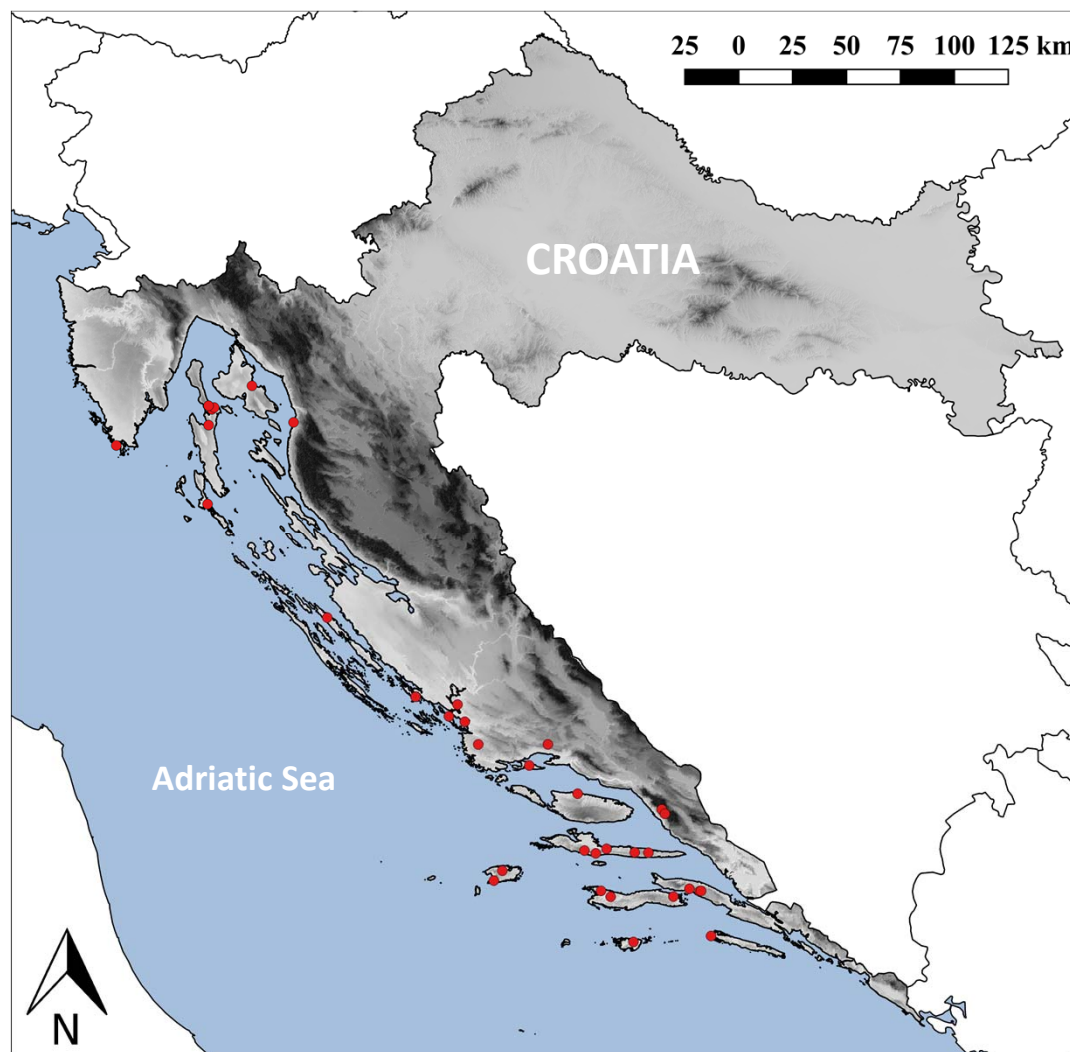


Methods

- (1) Seed sampling**
- (2) Field trial**
- (3) Genetic analysis (SSR, AFLP, cpDNA)**
- (4) Biochemical analysis (MSPD, HPLC-DAD)**
- (5) Association mapping**



(1) Seed sampling



(2) Field trial

- 10 population, 3 repetitions; total of 300 samples
- plant material for further analysis
- Collection of Medicinal and Aromatic Plants, University of Zagreb, Faculty of Agriculture
- voucher specimens in ZAGR, FCD



(2) Genetic analysis

(A) Development of SSR primers (*SSR; Single Sequence Repeats*)

- multiallelic, inherited co-dominantly, usually abundant
- valuable tools in many research areas
- species-specific, the major drawback of microsatellite markers is that for most species, microsatellites must be developed *de novo*
- **Next Generation Sequencing**
 - enables the efficient generation of a large amount of genome sequence data from which microsatellite motifs can be identified and suitable primers developed

(A1) Utilization in assessing genetic diversity



(2) Genetic analysis

(B) AFLP markers (*Amplified Fragment Length Polymorphism*)

- universal and no prior sequence information is needed for their amplification
- simultaneous detection of various polymorphisms in different genomic regions
- require small amounts of DNA for the analysis, and have high reproducibility, resolution, and sensitivity at the whole genome level

(C) Chloroplast DNA diversity



(3) Biochemical analysis

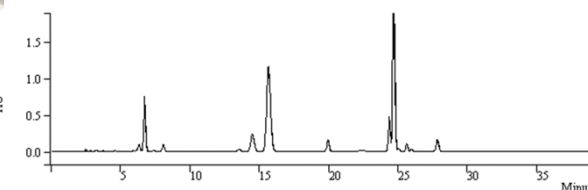
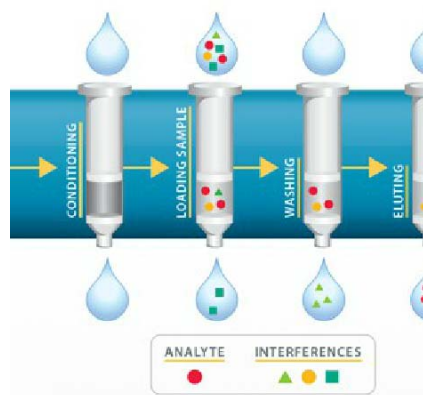
- insecticidal potential of Dalmatian pyrethrum

(1) Optimization of Matrix Solid Phase Dispersion (MSPD): sorbent type, elution solvent and volume of elution solvent, sorbent/plant ratio

- MSPD has never been optimized and applied to obtain pyrethrum extract

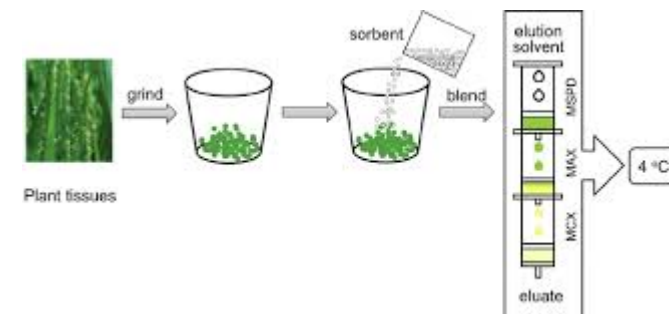
(1) Extraction of pyrethrins from flower samples

(2) Determination and quantification of 6 extracted pyrethrin components (HPLC)



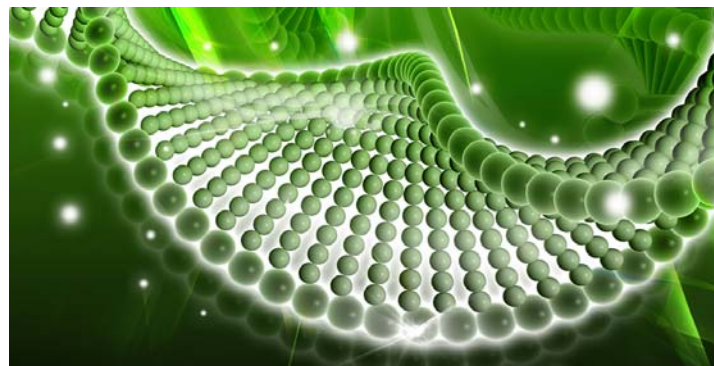
(3) Biochemical analysis

- MSPD procedure includes mechanical blending of the plant material with a solid sorbent to achieve sample disruption and its homogenous distribution around the sorbent particles
- homogenous material is then transferred to a column or a cartridge and analytes are eluted with an appropriate solvent
- **Advantages:** significant reduction in solvent consumption, rapid, less manual-intensive and eco-compatible



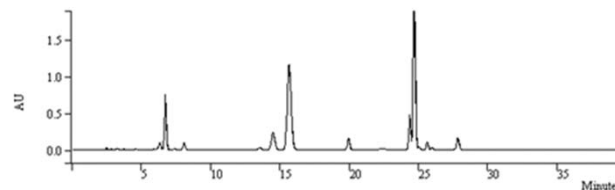
(4) Association mapping

- new insights into the genetic control of pyrethrin synthesis in Dalmatian pyrethrum flowers
- an integrated approach will be undertaken that will allow the characterization of pyrethrin content variability and the inherent genetic diversity as assessed by AFLP markers, while the effect of population structure will be controlled by SSR markers
- crucial information for identification of genes involved in biochemical pathways of pyrethrin synthesis and the selection of genotypes with higher pyrethrin content



Expected results i contribution

- (1) Developed SSR primers for Dalmatian pyrethrum
- (2) Developed MSPD extraction technique for the routine analysis of pyrethrum flowers
- (3) Valuable knowledge about genetic and biochemical diversity of natural Dalmatian pyrethrum populations needed for planning and development of appropriate conservation strategies



Expected results i contribution

- (4) Background for future breeding and agricultural exploitation of Dalmatian pyrethrum
- identification of genes involved in biochemical pathways of pyrethrins and their corresponding molecular markers will accelerate efficient breeding of Dalmatian pyrethrum for higher pyrethrin content by marker assisted selection (MAS)



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