

Utilization of Developed Single Sequence Repeats Markers for Dalmatian Pyrethrum (*Tanacetum cinerariifolium*) in Preliminary Genetic Diversity Study on Natural Populations

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Abstract : Dalmatian pyrethrum (*Tanacetum cinerariifolium* (Trevir.) Sch. Bip.; Asteraceae), a source of the commercially dominant plant insecticide pyrethrin, is a species endemic to the eastern Adriatic. Genetic diversity of *T. cinerariifolium* was previously studied using amplified fragment length polymorphism (AFLP) markers. However, microsatellite markers (single sequence repeats - SSRs) are more informative because they are codominant, highly polymorphic, locus-specific, and more reproducible, and thus are most often used to assess the genetic diversity of plant species. Dalmatian pyrethrum is an outcrossing diploid ($2n = 18$) whose large genome size and high repeatability have prevented the success of the traditional approach to SSR markers development. The advent of next-generation sequencing combined with the specifically developed method recently enabled the development of, to the author's best knowledge, the first set of SSRs for genomic characterization of Dalmatian pyrethrum, which is essential from the perspective of plant genetic resources conservation. To evaluate the effectiveness of the developed SSR markers in genetic differentiation of Dalmatian pyrethrum populations, a preliminary genetic diversity study was conducted on 30 individuals from three geographically distinct natural populations in Croatia (northern Adriatic island of Mali Lošinj, southern Adriatic island of Čiovo, and Mount Biokovo) based on 12 SSR loci. Analysis of molecular variance (AMOVA) by randomization test with 10,000 permutations was performed in Arlequin 3.5. The average number of alleles per locus, observed and expected heterozygosity, probability of deviations from Hardy-Weinberg equilibrium, and inbreeding coefficient was calculated using GENEPOP 4.4. Genetic distance based on the proportion of common alleles (DPSA) was calculated using MICROSAT. Cluster analysis using the neighbor-joining method with 1,000 bootstraps was performed with PHYLIP to generate a dendrogram. The results of the AMOVA analysis showed that the total SSR diversity was 23% within and 77% between the three populations. A slight deviation from Hardy-Weinberg equilibrium was observed in the Mali Lošinj population. Allele richness ranged from 2.92 to 3.92, with the highest number of private alleles observed in the Mali Lošinj population (17). The average observed DPSA between 30 individuals was 0.557. The highest DPSA (0.875) was observed between several pairs of Dalmatian pyrethrum individuals from the Mali Lošinj and Mt. Biokovo populations, and the lowest between two individuals from the Čiovo population. Neighbor-joining trees, based on DPSA, grouped individuals into clusters according to their population affiliation. The separation of Mt. Biokovo clade was supported (bootstrap value 58%), which is consistent with the previous study on AFLP markers, where isolated populations from Mt. Biokovo differed from the rest of the populations. The developed SSR markers are an effective tool for assessing the genetic diversity and structure of natural Dalmatian pyrethrum populations. These preliminary results are encouraging for a future comprehensive study with a larger sample size across the species' range. Combined with the biochemical data, these highly informative markers could help identify potential genotypes of interest for future development of breeding lines and cultivars that are both resistant to environmental stress and high in pyrethrins. Acknowledgment: This work has been supported by the Croatian Science Foundation under the project 'Genetic background of Dalmatian pyrethrum (*Tanacetum cinerariifolium* /Trevir./ Sch. Bip.) insecticidal potential' - (PyrDiv) (IP-06-2016-9034) and by project KK.01.1.1.01.0005, Biodiversity and Molecular Plant Breeding, at the Centre of Excellence for Biodiversity and Molecular Plant Breeding (CoE CroP-BioDiv), Zagreb, Croatia.

Keywords : Asteraceae, genetic diversity, genomic SSRs, NGS, pyrethrum, *Tanacetum cinerariifolium*

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