

Development of Microsatellite Markers for Dalmatian Pyrethrum Using Next-Generation Sequencing

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Abstract : Microsatellites (SSRs) are highly informative repetitive sequences of 2-6 base pairs, which are the most used molecular markers in assessing the genetic diversity of plant species. Dalmatian pyrethrum (*Tanacetum cinerariifolium* /Trevir./ Sch. Bip) is an outcrossing diploid ($2n = 18$) endemic to the eastern Adriatic coast and source of the natural insecticide pyrethrin. Due to the high repetitiveness and large size of the genome (haploid genome size of 9,58 pg), previous attempts to develop microsatellite markers using the standard methods were unsuccessful. A next-generation sequencing (NGS) approach was applied on genomic DNA extracted from fresh leaves of Dalmatian pyrethrum. The sequencing was conducted using NovaSeq6000 Illumina sequencer, after which almost 400 million high-quality paired-end reads were obtained, with a read length of 150 base pairs. Short reads were assembled by combining two approaches; (1) de-novo assembly and (2) joining of overlapped pair-end reads. In total, 6.909.675 contigs were obtained, with the contig average length of 249 base pairs. Of the resulting contigs, 31.380 contained one or multiple microsatellite sequences, in total 35.556 microsatellite loci were identified. Out of detected microsatellites, dinucleotide repeats were the most frequent, accounting for more than half of all microsatellites identifies (21,212; 59.7%), followed by trinucleotide repeats (9,204; 25.9%). Tetra-, penta- and hexanucleotides had similar frequency of 1,822 (5.1%), 1,472 (4.1%), and 1,846 (5.2%), respectively. Contigs containing microsatellites were further filtered by SSR pattern type, transposon occurrences, assembly characteristics, GC content, and the number of occurrences against the draft genome of *T. cinerariifolium* published previously. After the selection process, 50 microsatellite loci were used for primer design. Designed primers were tested on samples from five distinct populations, and 25 of them showed a high degree of polymorphism. The selected loci were then genotyped on 20 samples belonging to one population resulting in 17 microsatellite markers. Availability of codominant SSR markers will significantly improve the knowledge on population genetic diversity and structure as well as complex genetics and biochemistry of this species. Acknowledgment: This work has been fully 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).

Keywords : genome assembly, NGS, SSR, *Tanacetum cinerariifolium*

Conference Title : ICEBPGA 2021 : International Conference on Evolutionary Biology and Population Genetic Analysis

Conference Location : Istanbul, Türkiye

Conference Dates : March 22-23, 2021