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DNA GENOTYPING OF *CHRYSANTHEMUM* SPP. FORENSIC SAMPLES TO ASCERTAIN THEIR VARIETAL IDENTITY

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The genus *Chrysanthemum* of the family of Asteraceae includes several economically important species. Indeed, a very large number of chrysanthemum cultivars have now ornamental and medicinal uses all over the world.

As for other vegetatively propagated ornamental plants, new varieties can be obtained from conventional breeding through hybridization and selection or from variants of existing varieties through spontaneous or induced mutation events. In particular, variant types enlarge the choice of commercial genotypes without the need to alter production and multiplication systems. However, they inherit the expensive and long-time breeding effort needed to create a completely new variety. Under the rules defined by the UPOV convention of 1991 any new variant type should be considered as an "Essentially Derived Variety" (EDV). In this case the breeders' rights may be eventually licensed to growers who wish to use protected plants upon the payment of royalties.

We report the implementation and application of a multi-locus molecular assay based on SSR markers for the genotyping of *Chrysanthemum* forensic samples, including interspecific hybrids and polyploids, in order to ascertain their varietal identity. After their discovery, microsatellites soon became DNA markers of choice for forensic analysis of human DNA, whereas they have been much less applied in the DUS testing for plant breeders' rights (PBR). DNA markers can help granting these rights to the breeder of a new plant variety, giving also the breeder exclusive control over the propagating and harvested materials of that variety for a number of years.

A total of 20 genomic DNA microsatellites, selected on the basis of the PIC index and organized into five multiplex PCR assays, were used to genotype a total of 38 forensic samples collected from two different propagation/cultivation sites. The resulting electropherograms were compared to those of 29 reference varieties of *Chrysanthemum* spp. chosen on the basis of their morphology. Genetic identity or diversity estimates were computed in all possible pairwise combinations of SSR loci for all DNA sample comparisons, using 1 for identity and 0 for diversity. Moreover, genetic similarity estimates were also computed between DNA samples across all SSR loci. As many as 19 forensic samples scored genetic similarity higher than 95% when compared with the references and 14 of them completely matched, with 100% genetic identity, registered varieties.

This case study provides an original insight into the use of SSR markers to protect the PBR. To detect either essentially derived varieties or commercial frauds, conventional criteria based on morphological descriptors are known to be insufficient. Our findings enable to shed light on the possibilities and the limitations of the use of molecular markers in disputes on frauds and essential derivations of ornamental plants as *Chrysanthemum* spp. From this framework, we intend to discuss

what benefits molecular markers could provide to plant variety (*i.e.* clones) registration and protection of Plant Breeders' Rights.