

PERSPECTIVES OF AMPLIFIED FRAGMENT LENGTH POLYMORPHISM IN RHODODENDRON FINGERPRINTING

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Plant identification and plant breeding are central and basic processes of plant improvement. Typically, plant breeders perform crossing between the best specimens available and screen the progeny to recover individuals who combine as much positive traits as possible from their parents. Once a good genotype has been identified, it is multiplied on a large scale (conventionally or using in vitro techniques) and commercialisation can start. At this point, it is crucial to authenticate the plants and to guarantee their stability. For woody species whose principal interest is the flower, it can take years to confirm a misidentification or to detect a genetic instability.

Today, extremely powerful molecular markers substitute classical morphological markers. The recent molecular methods are based on the study of DNA and allow probing DNA variability (polymorphism) between individuals.

AFLP (Amplified Fragment Length Polymorphism) is a novel DNA fingerprinting technique. It needs minute amounts of DNA, is easily automatable, gives robust, reliable and reproducible markers that do not require prior sequence knowledge. (Lin *et al.*, 1995; Breyne *et al.*, 1997). It is based on the selective PCR amplification of restriction fragments from a total digest of genomic DNA (Vos *et al.*, 1995). The technique involve three steps : restriction of DNA and ligation of oligonucleotide adapters, selective amplification of a subset of restriction fragments and finally gel analysis of the amplified fragments. PCR amplification of restriction fragments is achieved by using the adapters and restriction site sequences as target sites for primer annealing. The selective amplification is achieved by the use of primers that extend into the restriction fragments, amplifying only those fragments in which the primer extensions match the nucleotides flanking the restriction sites.

We used the EcoRI and MseI restriction enzymes and the CTA-MseI and AAG-EcoRI selective primers on genomic DNA from a large collection of Rhododendron cultivars. This allows authentication of cultivars and the establishment of a phylogenic tree.

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References

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