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GENETIC DIVERSITY OF A COLLECTION OF ROSE SPECIES AND CULTIVARS EVALUATED BY FLUORESCENT AFLP

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Genetic diversity of a collection of rose species and cultivars evaluated by fluorescent AFLP

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Summary

Fluorescent AFLP and automated data analysis of rose species, varieties and cultivars was conducted on plants of the rose collection of the Department of Plant Genetics and Breeding (DvP-CLO). A very clear distinction between species and cultivars could be observed related to their molecular marker profiles. Relationships known among the chosen plants could be observed.

Introduction

Depending on the taxonomist, between 240 and 300 rose species are being distinguished, it has been estimated that only 10 to 20 of these species contributed to about 20000 modern rose cultivars (De Vries & Dubois, 1996). AFLP has been used on roses for variety identification (De Riek *et al.*, 1997; Zhang *et al.*, 2000). Molecular markers have also become a tool in taxonomic studies in the genus *Rosa* (Debener *et al.*, 1996; Millan *et al.*, 1996; Moreno *et al.*, 1996; Reynders-Aloisi & Bollero, 1996; Debener *et al.*, 1997) and in genetic linkage maps (Debener & Mattiesch, 1999).

An AFLP study on the genetic relationship of roses was conducted on 88 plants. The plants were chosen between species, varieties and cultivars of different breeders. Within the species the plants belonged to the sections: Caninae (Can.), Cinnamomeae (Cin.), Gallicanae (Gal.), Pimpenellifoliae (Pim.) and Synstylae (Syn.). Of some of the plants the history is clear and relationships towards other roses are known. The plants used differed in ploidy range from 2n=2x to 2n=6x. Most of the cultivars were 2n=4x.

Material and methods

Plant material:

Plant material was collected from the gene pool of the Department of Plant Genetics and Breeding (DvP-CLO), Melle, Belgium. Collection material was maintained in open air and in glasshouses.

DNA extraction:

At harvest of the youngest leaves, the material was immediately immersed in liquid nitrogen and lyophilised for 48h. Dry material was ground using a Culatti mechanical mill.

A CTAB protocol, based on Weising *et al.* (1995) was used for most of the plants. As some plants showed some difficulties in obtaining DNA of good quality, another method according to De Riek *et al.* (1999) was used.

AFLP analysis:

AFLP was performed using the commercially available kit from Perkin-Elmer Biosystems for fluorescent fragment detection (Perkin Elmer, 1995). *Eco*RI and *Mse*I were used for DNA digestion. Selective amplification was done with 6 fluorescent labelled *Eco*RI - *Mse*I primer combinations with 6 selective bases: E-AAC/M-CAT (1), E-ACT/M-CAT (1), E-AAG/M-CTA (2), E-AGC/M-CTA (2), E-ACA/M-CAG (3) and E-AGC/M-CAG (3). These primercombinations were used multiplexed in the couples (1), (2) and (3).

Statistical analysis:

After export of the Genescan data to Microsoft Access a scoring table (1/0) was generated. Calculation of similarity coefficients, construction of dendrograms (UPGMA) and principle coordinates analysis were performed by the module SIMIL, CLUSTER and PCOORD of the 'R package' (Legendre & Vaudor, 1991).

Results and discussion

A considerable degree of genetic variation was observed in the gene pool. According to the AFLP data, a clear distinction could be made between the species group with closely related varieties and the group with only cultivars (Fig.1 and Fig. 2). The only species occuring in the cultivars group is *R. chinensis minima*. *R. chinensis* and *R. multiflora* are those species with the largest influence during more recent breeding of modern rose cultivars (19th century) (De Vries & Dubois, 1996). Next to *R. chinensis minima*, *R. multiflora* can be seen in Fig. 1 to be more related to the cultivars than other species. Relationships between subspecies are revealed in the dendrogram. With exception of the *Caninae* section also a clustering of the sections can be seen in the dendrogram of the species group. Relationships between plants are proven, e.g. *R. hugonis* which is one of the parents of *R. x pteragonis*.

In the group of the cultivars some known relationships (not all ancestors are known) can be mentioned. The clustered plants: 'Ravel', 'Rossini', 'Pavarotti', 'Vivaldi' and 'Timeless' are all cut roses of the same breeder. Other plants of this breeder in the analysis are 'Hollywood', 'Peach Unique', 'Orange Unique' and 'Yellow Unique'. A very close relationship was found between 'Païline' and its sport. 'Melflor' x 'Melglory' resulted in the cultivars 'Professor Boesman' and 'Melrose', these show a close relationship in the analysis.

References

- Debener T, Bartels C, Mattiesch L (1996) RAPD analysis of genetic variation between a group of rose cultivars and selected wild rose species. Mol. Breeding 2: 321-327
- Debener T, Bartels C, Spethmann W (1997) Parentage analysis in interspecific crosses between rose species with RAPD markers. Gartenbauwissenschaft 64:180-184
- Debener T, Mattiesch L (1999) Construction of a genetic linkage map for roses using RAPD and AFLP markers. Theor. Appl. Genet. 99: 891-899
- De Riek J, Dendauw J, Mertens M, Van Bockstaele E, De Loose M (1997) Use of AFLP for variety protection: some case studies on ornamentals. Med. Fac. Landbouww. Univ. Gent 62: 1459-1466
- De Riek J, Dendauw J, Mertens M, De Loose M, Heursel J, Van Bockstaele E (1999) Validation of criteria for the selection of AFLP markers to assess the genetic variation of a breeders' collection of evergreen azaleas. Theor. Appl. Genet. 99:1155-1165
- De Vries DP, Dubois, LAM (1996) Rose breeding: past, present, prosects. Acta Horticulturae 424: 241-248
- Legendre P, Vaudor A (1991) The R package: multidimensional analysis, spatial analysis. Département de sciences biologiques, Université de Montréal, 142p.
- Millan T, Osuna F, Cobos S, Torres AM, Cubero JI (1996) Using RAPDs to study phylogenetic relationships in *Rosa*. Theor. Appl. Genet. 92: 273-277
- Moreno MT, Torres AM, Millan T, Armada J, Cubero, JI (1996) Use of molecular markers in taxonomic studies of *Rosa sp.* Acta Horticulturae 242: 293-295

- Perkin-Elmer (1995) AFLPTM Plant Mapping Kit: protocol.
- Reyenders-Aloisi S, Bollereau, P (1996) Characterisation of genetic diversity in genus *Rosa* by randomly amplified polymorphic DNA. Acta Horticulturae 424: 253-259
- Weising K, Nybom H, Wolff K, Meyer W (1995) DNA-fingerprinting in plants and fungi. Boca Raton, CRC press inc, 332p.
- Zhang D, Germain E, Reynders-Aloisi S, Gandelin MH (2000) Development of amplified fragment length polymorphism markers for variety identification in rose. Acta Horticulturae 508: 113-120

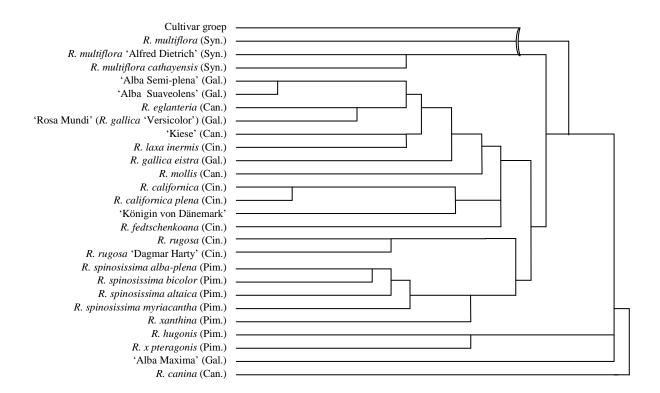


Figure 1 : Ordination of the species group gene pool based on AFLP data (Jaccard similarity coefficient, UPGMA clustering)

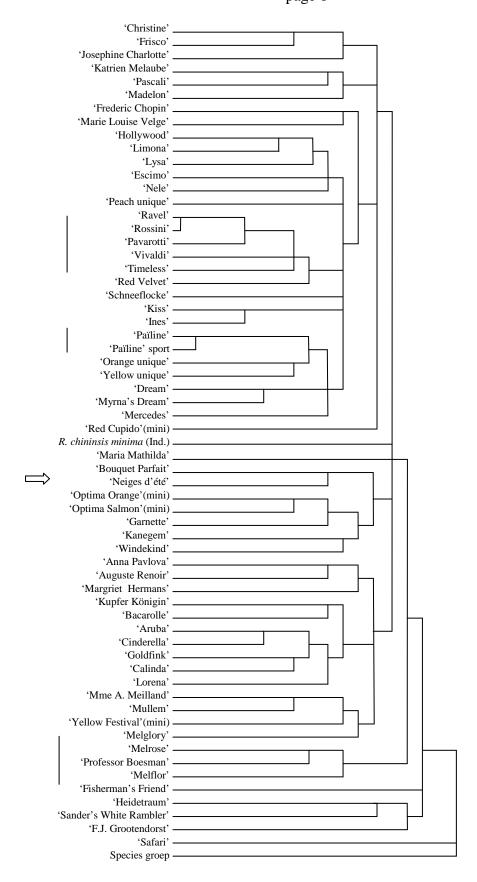


Figure 2 : Ordination of the cultivar group gene pool based on AFLP data (Jaccard similarity coefficient, UPGMA clustering)

PCO-plot showing an overview of the complete dataset

