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EVALUATION OF AFLPS FOR VARIETY IDENTIFICATION IN MODERN ROSE

Document prepared by experts from France

Evaluation of AFLPs for variety identification in modern Rose

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Overview of previous work on molecular techniques in rose

RAPD

RAPD markers have been developed for genetic diversity studies or variety identification by Ballard *et al*. (1995), Cubero *et al*. (1995), Debener T. and Mattiesch L.(1995), Debener T. *et al*. (2000), Gallego F.J. and Martinez I. (1996), Matsumoto S.and Fukui H. (1996), Reynders-Aloisi S. and Bollereau P. (1995), and Torres *et al*. (1993). The results obtained by these researchers can be summarized as following:

- high degree of RAPD polymorphism between cultivars,
- no polymorphism between sports and their original variety, reported by Debener T *et al*. (2000),
- sometimes there was polymorphism between sports and their original variety (Ballard *et al*.,1995, Cubero *et al*., 1995)
- no intra-variety variability **(**Gallego F. J. and Martinez I. (1996).

RFLP

Ballard *et al*. (1995) and Rajaapakse *et al*. (1992) reported the easy identification of rose cultivars by RFLP markers. The same type of results were published by Vainstein A. and Ben-Meir H. (1994), using oligonucleotides as probes.

AFLP

Debener *et al*. (2000) reported the following results by analyzing sports and seedlings of several rose varieties, with AFLP markers:

- no polymorphism between sports and their original variety for a cut-rose,
- 5 differences out of 629 AFLPs between the sports and their original variety for a garden rose,
- easy differentiation between variety and its seedlings.

Zhang *et al.* (1999) also developed AFLP markers for variety identification in rose.

SSR

There are no published results of SSRs in rose until now. However, some research programs are in progress, especially by the team of Dr Rajaapakse S. (personal communication, Clemson University, USA). There are also private SSRs in rose developed (Dr B. Vosman, personal communication).

Results obtained by GEVES (France) with AFLP markers

Identification

106 French rose varieties were analyzed by 11 primer sets which generated a total of 394 AFLPs. These varieties have 4 types of relationships:

- mutants and their original varieties
- genotypes sharing one or two parents
- parents and offspring
- genotypes not related.

Each primer set can generate from 25 to 45 AFLPs, with an average of 36. The Polymorphism Information Content (PIC) varied from 0.29 to 0.39 per primer combination, with a mean of 0.34.

Genetic distance estimates (Nei & Li, 1979) by type of relationships between genotypes are showed in the following table.

Relations between genotypes	Number of pairs	Distance Range	Mean
Genotypes non related	5493	0.16 –0.64	0.37
Genotypes related (parents and descendants)	40	0.14 – 0.42	0.26
Genotypes of half-sib	3	0.23 – 0.31	0.26
Genotypes of full-sib	12	0.07 – 0.21	0.14
Sports and their original varieties	14	0.0 – 0.05	0.02

Genetic relationships between rose genotypes:

* easy identification and big distance values between varieties from selection involving a hybridisation program,

* between varieties from mutation, no systematic differentiation of sports from their original varieties; even if there is differentiation sometimes, the distance values are very small in general.

Uniformity & Stability

Three studies have been carried out to evaluate the uniformity and stability of AFLPs in roses.

Trial 1 (1 variety) aimed to analyze 6 plants and 3 shoots / plant. No AFLP variability was observed between the different plants and shoots, with 194 band positions generated by 4 primer sets.

Trial 2 (1 variety) aimed to study 5 plants having different combinations between:

- plant origin (NL, Fr and SP)
- root-stock (R. canina inermis, R. indica major and R. manetti)
- root-stock origin (NL, D, SP and Marocco)

No AFLP variability was detected between the 5 samples, with 227 band positions generated by 4 primer sets

Trial 3 (5 varieties) aimed to test 3 types of organs (petals, sepals and leaves) with 2 repetitions each. No AFLP variability was observed between the different organs for all the 5 varieties and repetitions, analyzed by 4 primer sets.

Repeatability and reproducibility of AFLPs in rose

Three trials have been performed to test the repeatability and reproducibility of AFLPs in rose.

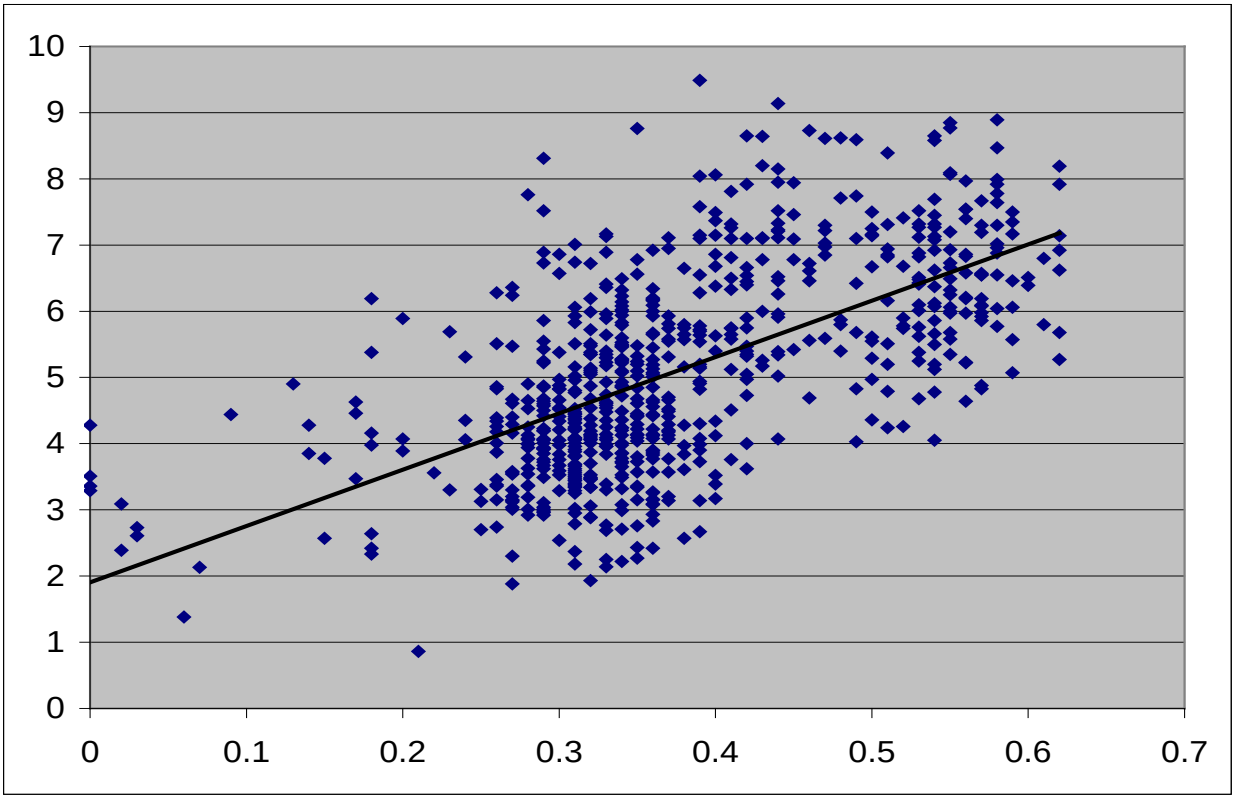
Trail 1 aimed to test the repeatability by using the same DNA extract: 3 repeats from the DNA digestion step on 7 varieties X 4 primer pairs. We observed 8 variable band positions among 1664 (0.5%).

Trial 2 aimed to test 2 DNA isolation techniques (CTAB and Qiagen Dneasy Plant Mini Kit) on 6 varieties X 4 primer pairs. We noted 58 variable band positions out of 1239 (4.7%). The percentage of variable band positions varied according to varieties (from 1.7% to 8.1%) as well as primer pairs (from 1.6 % to 11%)

Trial 3 aimed to control the reproducibility of the DNA extract technique (pre-treatment by Triton + Qiagen DNeasy Plant Mini Kit) that we used finally for assessing the 106 genotypes. The experiment was realized on 18 genotypes, 2 DNA extractions per genotype, with the 11 primer pairs. The variable band positions recorded were 11 out of 14148 data points.

Relationships to phenotype

Morphological data are available in GEVES, on 75 of the 106 varieties analyzed. The data correspond to the observations and notations of 16 principal traits used for DUS testing in France. A standardized *euclidian* distance was computed on a subset of 38 varieties. The coefficient of correlation between AFLP and morphological distances on this subset of varieties was 0.62 (see the figure below).



Relations between AFLP(abscissa) / Morphology (ordinate), r = 0,62

Conclusion

- Good AFLP polymorphism among modern rose varieties,
- No AFLP variability within variety,
- Easy identification of rose varieties obtained from hybridization using AFLP markers,
- Not easy to differentiate mutants from their original varieties,
- Genetic relationships among rose genotypes revealed by AFLP showed a good concordance with the genealogical information,
- Good reproducibility of AFLPs can be achieved by controlling the DNA extract procedure.

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References

Ballard, R.E., J., Rajapakse, S., Abbott, A.G., and Byrne, D. (1995) DNA markers in rose and their use for cultivar identification and genome mapping. Acta Hort. 424 ISHS : 265-268.

Cubero, J.I., Millan, T., Osuna, F., Torres, A.M., Cobos, S.(1995) Varietal identification in *Rosa* by using isozyme and RAPD markers. Acta Hort. 424 ISHS : 261-264.

Debener, T., and Mattiesch, L.,(1995) Genetic analysis of molecular markers in crosses between diploid roses. Acta Hort. 424 ISHS : 249-252.

Debener T., Mattiesch L. (1998) Effective pairwise combination of long primers for RAPD analysis in roses. Plant Breeding 117: 1-5

Debener, T., and Mattiesch, L., (1999) Construction of a genetic linkage map for roses using RAPD and AFLP markers. Theor Appl Genet 99: 891-899.

Debener T., Janakiram T., Mattiesch L. (2000) Sports and seedlings of rose varieties analysed with molecular markers. Plant Breeding 119: 71-74

Gallego F. J., Martinez I. (1996) Molecular typing of rose cultivars using RAPDs. Jounrnal of Horicultural Science 71: 901-908

Hubbard, M., Kelly, J., Rajapakse, S., Abbott, A.G., and Ballard, R.E., (1992) Restriction fragment length polymorphisms in rose and their use for cultivar identification. HortScience. 27 : 172-173.

Millan T., Osuna F., Cobos S., Torres A. M. (1996) Using RADPs to study phylogenetic relationship in Rosa. Theor Appl Genet 92: 273-277

Matsumoto S., Fukui H. (1996) Identification of rose cultivars and clonal plants by random amplified polymorphic DNA. Scientia Horticulturae 67: 49-54

Matsumoto S., Wakita H., Fukui H. (1997) Molecular classification of wild roses using organelle DNA probes. Scientia Horticulturae 68: 191-196

Matsumoto S., Kouchi M., Yabuki J., Kusunoki M., Ueda Y., Fukui H. (1998) Phylogenetic analyses of the genes Rosa using matK sequence: molecular evidence for the narrow genetic background of modern roses. Scientia Horticulturae 77: 73-82

Nei M., and Li W.H., 1979. Mathematical model for studying genetic variation in terms of restriction endonuclease. Proc. Natl. Acad . Sci. U.S.A. 76 : 5269-5273.

Rajaapakse S, Hubbard, M., Kelly J.W., Abbott A. G., and Ballard R.E., 1992. Identification of rose cultivars by restriction fragment length polymorphism. Scientia Horticulturae, 52: 237-245.

Rajaapakse S and Ballard R.E., 1998. Genetic mapping of blackspot tesistance in roses. American Rose, July 1998: 20-21.

Reynders-Aloisi, S., and Bollereau, P., (1995) Characterization of genetic diversity in genus *Rosa* by random amplified polymorphic DNA. Acta Hort. 424 ISHS : 253-260.

Torres, A., Millan, T., Cubero, J.I., (1993) Identifying rose cultivars using random amplified polymorphic DNA. HortSience 28 : 333-334.

Vainstein, A., and Ben-Meir, H., (1994) DNA fingerprint analysis of roses. J. Amer. Soc. Hort. Sci. 119 (5) : 1099-1103.

Zhang, D., Germain, E., Aloisi, S., and Gandelin M.H., (1999) Development of Amplified Fragment Length Polymorphism markers for variety identification in rose. Acta Hort., N°508: 113-120.

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