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USE OF RAPD MARKERS TO DETERMINE THE GENETIC DIVERSITY OF PEACH VARIETIES

Document prepared by experts from France

Use of RAPD markers to determine the genetic diversity of peach varieties.

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Introduction

French peach orchard is characterised by a great diversity of varieties with a fast evolution. The Fruit Tree and Grape Research Station of Bordeaux in relation with the Mediterranean Fruit Research Station of Avignon, is in charge of fruit tree breeding programmes that need introduction of new peach varieties from the whole world. Each year, about 130 new hybrids are introduced in the national network of experimentation (stade A orchards) in order to test the DHS (Distinction-Homogeneity-Stability) necessary for the inscription to the French official catalogue and application for protection. It is necessary to develop molecular markers in order to be able to use then in addition in pomological studies for the varieties identification. A previous study analysed the genetic distances between 10 peach varieties using isoenzyme (Reynders and Monet, 1987). As most of these varieties have lot of parents in common, the polymorphism of the isoenzyme was to low to be able to distinguish them. In the present study, we have used RAPD markers to test if they can be useful to detect intraspecific variations in peach varieties.

Materials and methods

Forty five peach varieties were analysed. They were chosen in the peach conservatory of Bordeaux. They are reference varieties, some of them are still registered in the catalogue.

DNA was extracted from young expanding leaves using a CTAB procedure (Saghai-Maroof et al. 1984. RAPD reactions were performed as described by Williams et al. (1990) using ten-base primers from Operon Technologies Kits A-B-I-O-P (Operon Technologies, Alameda, Calif.). Reactions were performed in a final volume of 12 µl per sample in a 96-well Perkin Elmer GeneAmp PCR System 9600. RAPD fragments were separated on 1.5 % agarose gels, stained with ethidium bromide.

For each individual amplification, data were scored on the presence or absence of amplification products. If a product was present in a genotype it was designed as 1, if no share product was present in other genotypes they were design as 0. This type of scoring was done for each amplification product. The genetic distance was calculated using D:

$$D = 1 - \frac{N(00) + N(11)}{N(T) - N(MD)}$$

N(00) is the number of double absence of the band between two individuals
N(11) is the number of double presence of the band between two individuals
N(T) is the total number of band analysed for all the individuals
N(MD) is the number of missing data occurring in at least one individual
D was used to build a dendrogram by hierarchic classification using Splus software (Version 3.1).

Results and discussion

Among the 100 primers tested, only 47 revealed polymorphism for the 45 peach varieties. This level of polymorphism is very low and is in agreement with the results obtained with isoenzyme (Mowrey et al. 1990, Monet and Gibault, 1991) and with RAPD (Chaparro et al. 1994, Dirlewanger and Bodo, 1994). 130 RAPD polymorphic markers were identified with the 47 primers used.

The genetic distance are range from about 15 to 50 and all the varieties can be distinguish to each other (Fig1). Six groups can be identified at a distance of about 35. The dendrogram confirm the relation between related varieties: for example 10 is an offspring of 28, as well as 9 and 12, 2 and 20, 4 and 14; and 5 and 11 are full sib.

This result shows that RAPD markers can be used for identification and distinction of peach varieties. This method is quite fast when the technical process is well defined and when the polymporphic primers have been detected.

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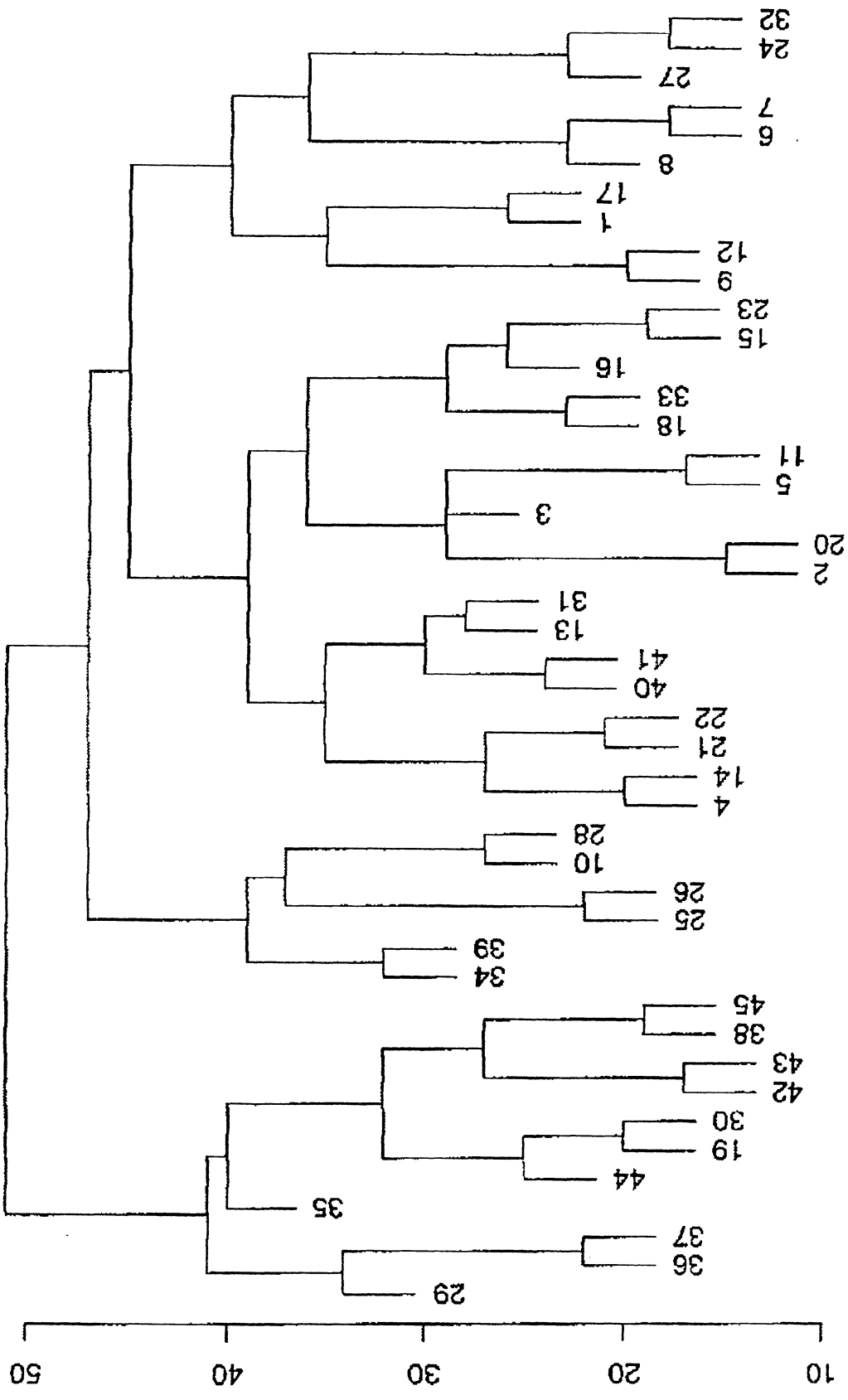

FIGURE 1 : DENDOGRAM CONSTRUCTED WITH GENETIC DISTANCES MATRIX

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