THE USE OF CLONED DNA MARKERS TO ASSESS THE GENETIC VARIABILITY OF RAPESEED AND RELATED CRUCIFEREAE

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INTRODUCTION.

Molecular cloning has made available to the plant geneticist a large number of cloned sequences which can be used as genetic markers. During the recent years, we have isolated several recombinant clones corresponding to radish DNA sequences. Most of them cross-hybridize with *B. napus* and other related species. In this paper we present results illustrating the use of such markers to assess for rapeseed nuclear genome variability and to search for foreign sequences in a species.

MATERIALS AND METHODS.

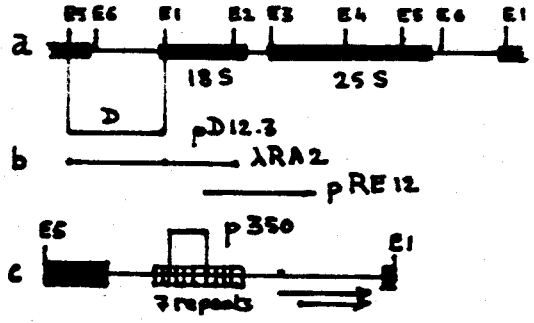
Seeds were obtained from Dr Michel RENARD (INRA, RENNES) and from Mr Fernand ARNAUD (CETIOM, PARIS). They were germinated in a green house and DNA was purified from leaves either according to a minipreparation protocole (DELLAPORTA et al., 1983) or by an already described method (DELSENY et al., 1984). Isolation and characterization of the recombinant clones have been reported previously (GOLDSBROUGH and CULLIS, 1981; CROUCH et al., 1983; DELSENY et al., 1984; GRELLET et al., 1986; LAROCHE-RAYNAL and DELSENY, 1987; DELCASSO-TREMOUSAYGUE et al., 1987). DNA was digested with various restriction enzymes, separated by agarose gel electrophoresis, blot-transferred to nitrocellulose sheets and hybridized with radioactively labelled probes.

RESULTS.

General organization of cruciferae ribosomal RNA genes.

We have analysed in detail the structure and organization of radish ribosomal genes. This species contains 6500 gene copies/diploïd genome. These copies are not all

identical on the basis of their restriction pattern, some sites being absent in some units. The general organisation of a tandem repeat is shown in figure 1a. Some of the Eco RI sites are not present in all the units so that when genomic DNA is digested with Eco RI and hybridized with a probe such as fragment D multiple fragments are detected. Also indicated in figure 1b are indicated several recombinant clones which have been used. The Eco RI fragment D has been subcloned and completely sequenced (DELCASSO-TREMOUSAYGUE et al., 1987), its organization is show in figure 1c. Seven short repeats $\simeq$ 100 bp long occur within this intergenic region. Hybridization between genomic DNA and a probe specific for the repeat revealed that it is species specific.



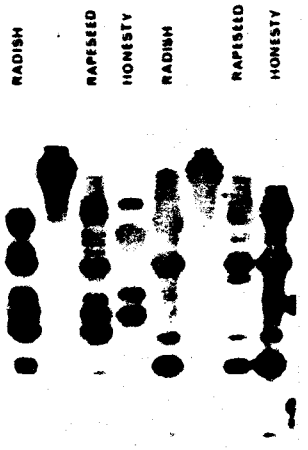


Fig. 1. a) - Eco RI restriction map of r DNA tandem repeat. b) Recombinant radish rDNA clones, c) Organization of the intergenic region contained in ECO RI fragment D; arrow indicate the 5' end of the most abundant pre-rRNA.

Fig. 2. Southern blot of Eco RI (1, 3,4) and Bam HI (5,7,9) digests of radish rapeseed and honesty DNA hybridized with a rDNA probe.

rDNA probes discriminate between crucifer species.

Figure 2 shows hybridization of Eco RI and Bam HI digest from radish, rapeseed

and honesty DNA with a full length flax rDNA probe (GOLDSBROUGH and CULLIS, 1981). Some fragments are obviously common, others are not and discriminate between various species. The discriminating fragments correspond to the intergenic region. Other similar experiments with fifteen cruciferae, belonging to various tribes, demonstrated that each species can be distinguished from one another and that it was even possible to distinguish between populations.

The next step was to use more restricted probes to assay for variability within rapeseed cultivars. The result of such an experiment is shown in Figure 3. Using the radish Eco RI D (pD 12.3) fragment as a probe reveals that most rapeseed cultivars have very similar ribosomal genes. Only cultivar, Lembkes, shows an additional fragment. This result was confirmed with other restriction enzymes.

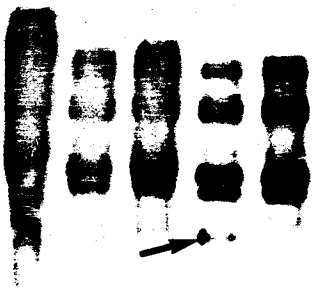


Fig. 3.- Southern blot of Bgl II digests of DNA from various rapeseed cultivars hybridized with plasmid pD12-3. From left to right:Lithuanie, Pradel, Nemert-chanski, Lembkes and Lingot.

We also analysed a large number of individual plants from cultivar Orleans and found them to be identical. But on the other hand, we observed variation in a CMS line derived from this cultivar. Whether the changes are induced by CMS or result from the regeneration step from protoplast fusions is not clear.

Satellite DNA sequences are species specific.

We isolated another type of tandemly repeated sequence 177 bp long (GRELLET et al., 1986). In hybridization experiments with various cruciferae (Fig. 4) this sequence turns out to be species specific when stringent condition are used.

When less stringent conditions are used then this probe detects related sequences in most *Brassica* species with an hybridization intensity related to their phylogenetic distance. Similar probes are presently available in other laboratories for *Sinapis alba* and *B. oleracea* (CAPESIUS, 1983; BENSLIMANE et al., 1986).

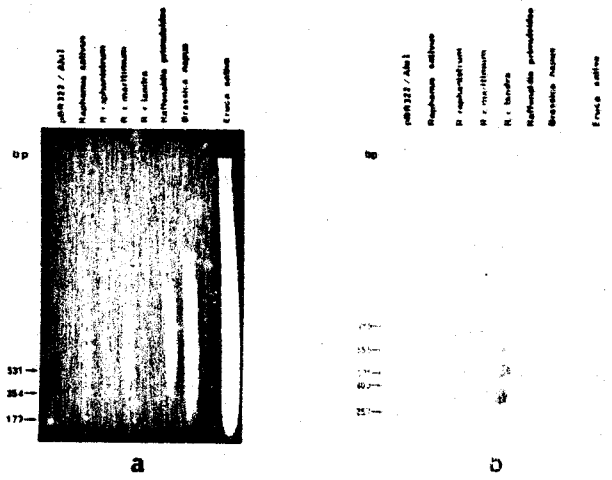


Fig. 4.- Southern blot of Alu I digests of DNA from various crucifers hybridized with a satellite DNA probe.

Napin probes discriminate between rapeseed cultivars.

Recombinant cDNA clones, specific for the napin storage proteins have been isolated from rapeseed (CROUCH et al., 1983) and radish (LAROCHE-RAYNAL and

DELSENY, 1986). Using the cDNA probe from rapeseed we have compared the restriction pattern of 8 cultivars and have been able to distinguish them as illustrated in figure 5.

Fig. 5.- Southern blot of Bam HI digests of DNA from various rapeseed cultivars hybridized with a rapeseed napin cDNA probe. Arrows denote the variable fragments.

DISCUSSION.

We have illustrated how molecular probes can be used to assay for some plant breeding problems. These probes fall into two classes (YEDEL and DELSENY, 1987): a) those which are species specific and give a positive signal with the homologous DNA in stringent conditions; b) those which are not species specific but which detect some polymorphism. The first group of probes can be used for phylogenetic studies and to help in analysing addition lines or interspecific crosses for the presence of an alien chromosome. The second group can be used for linkage study or for cultivar and lines identification. The main results concern the remarkable homogeneity of the ribosomal genes in several rapeseed cultivars and from one plant to another. However we also observed that these patterns are susceptible to rearrangements during the course of a

plant breeding experiment. On the other hand RFLP can be detected in genes for which there is less selective pressure such as napin.

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