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BMT/7/8
ORIGINAL: English
DATE: November 21, 2001

UPOV

INTERNATIONAL UNION FOR THE PROTECTION OF NEW VARIETIES OF PLANTS

GENEVA

WORKING GROUP ON BIOCHEMICAL AND MOLECULAR TECHNIQUES AND DNA-PROFILING IN PARTICULAR

Seventh Session
Hanover, Germany, November 21 to 23, 2001

USE OF ISSR TO STUDY THE GENETIC VARIABILITY OF POPLARS, HYDRANGEA AND PEAS VARIETIES

prepared by experts from France

SLIDE 1

The use of ISSR to study the genetic variability of poplar and hortensien cultivars

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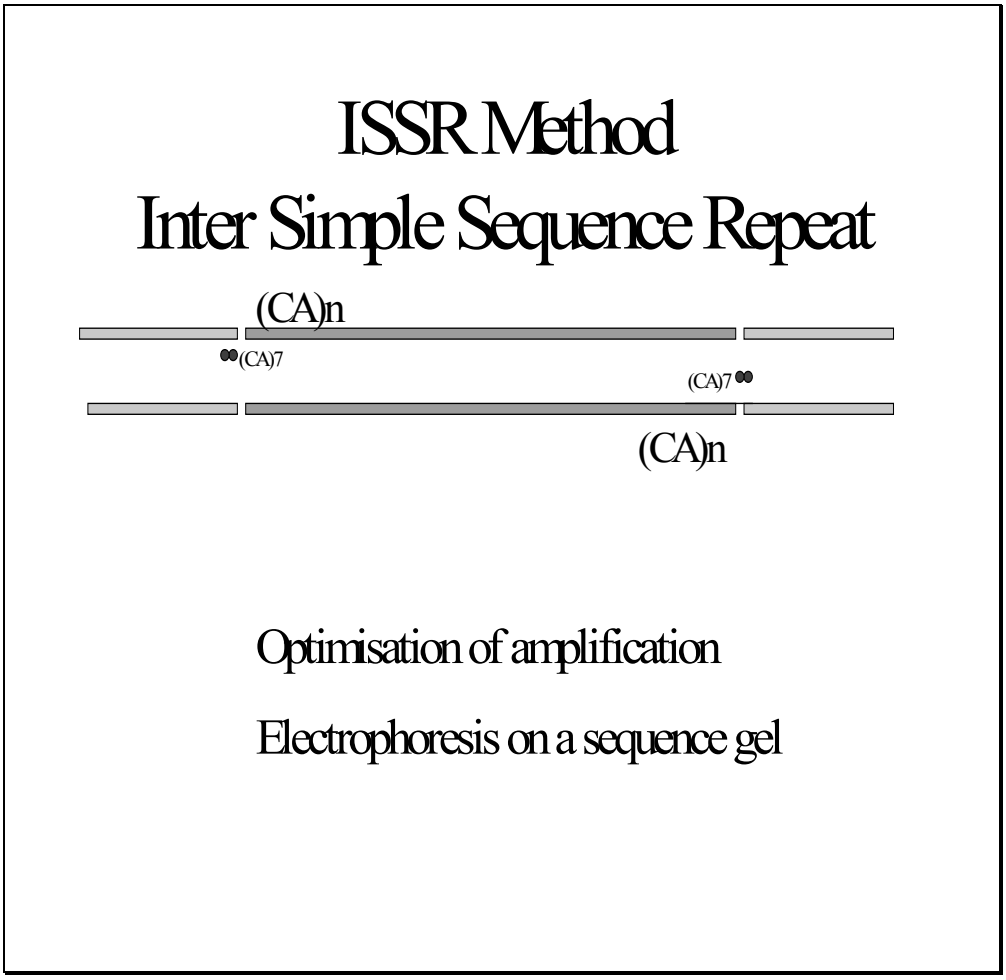
SLIDE 2

AFLP and ISSR

- AFLP
  - digestion
  - ligation
  - préamplification
  - amplification
- ISSR
  - amplification

We were looking for an alternative to AFLP which have the following drawbacks :
They are patented
They can generate artefacts when the quality of DNA is not good enough (it's often the case for ligneous species).
They are time consuming

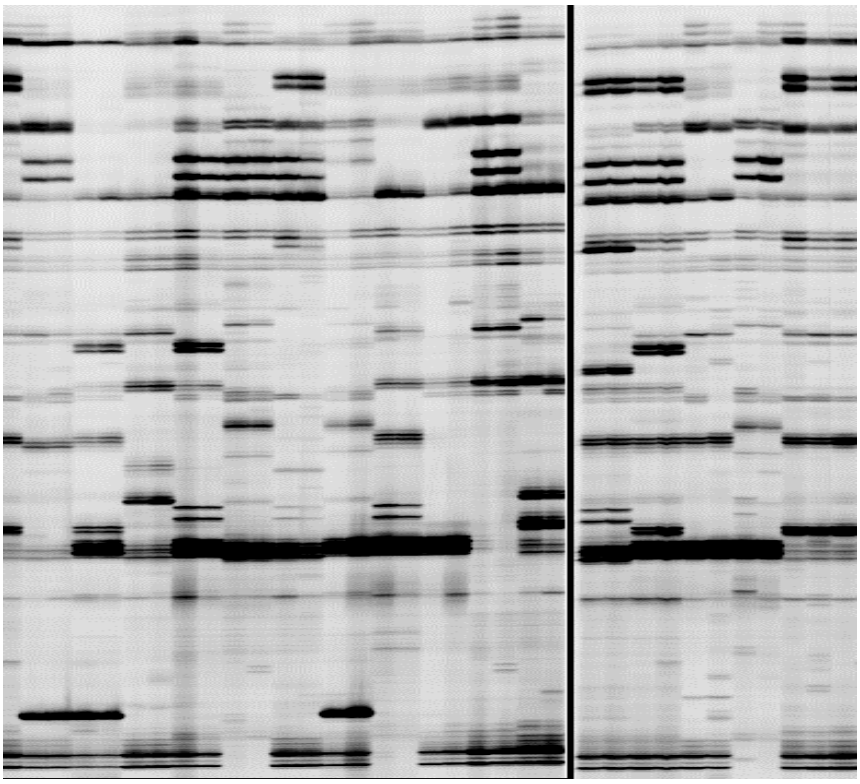
SLIDE 3



The method uses SSRs as primers, anchored by 1 to 3 selective bases at either end. (We generally choose the 5' end since the 3' end is patented). It means that the amplified region of the DNA is comprised between 2 SSRs.
Optimisation of the amplification and separation of the amplification products on a sequence gel enable good quality patterns to be obtained.

SLIDE 4

pea

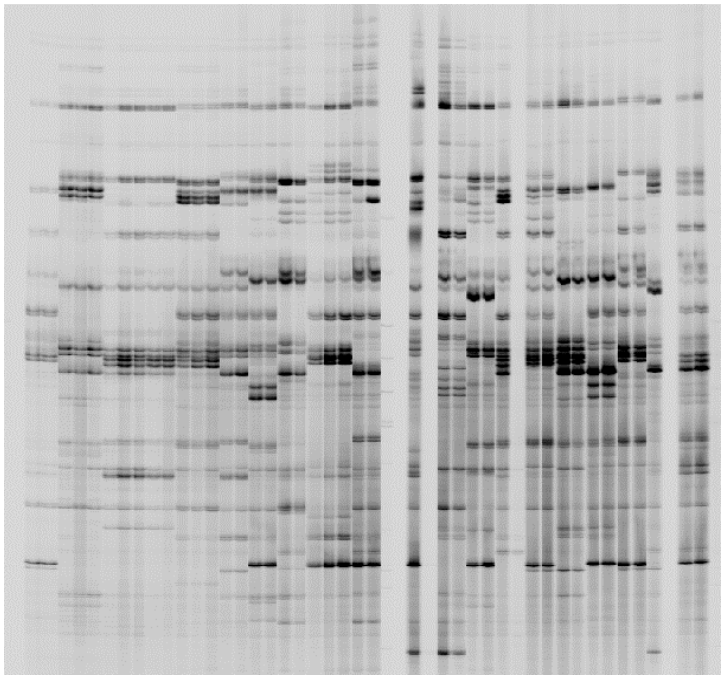


This is an example showing 17 pea cultivars (2 replicates per cultivar)
The polymorphism is high. All cultivars are distinguishable except 2 mutants.

SLIDE 5

Reproducibility

- Extractions
- Organs
  - leaves
  - cambium
  - poplar

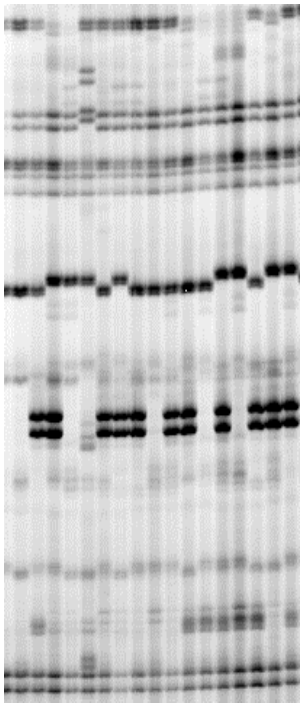


The reproducibility has been checked on different DNA extractions and different organs. The results were reproducible even when the same DNA samples yielded inconsistent results using AFLP.

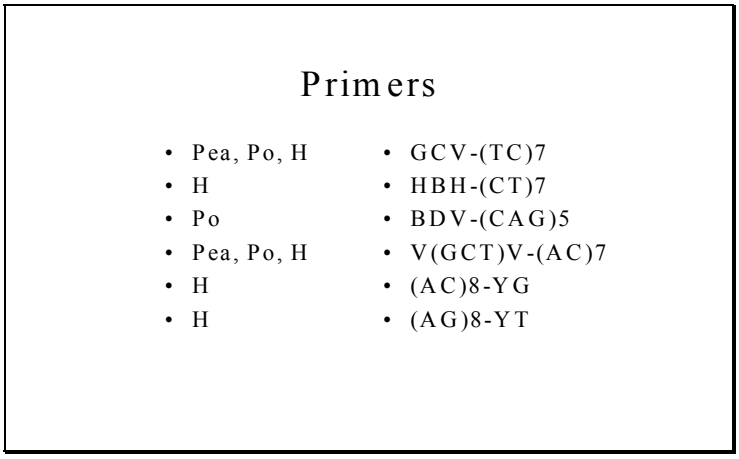
SLIDE 6

Mapping

- Poplar
  - 41 markers
  - 35 linkage groups
  - 23% codominant
  - no distorsion in the ségrégation

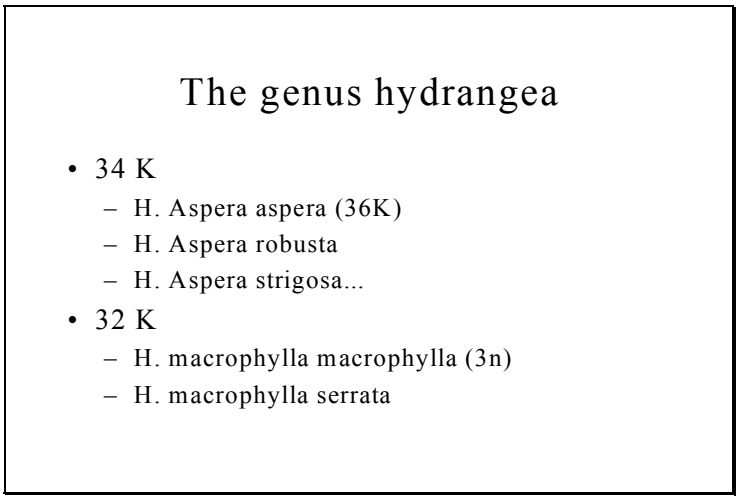


SLIDE 7



These primers have been used on different species (Po = poplar, H=hortensia) and give good information (20 to 25 markers per primer)

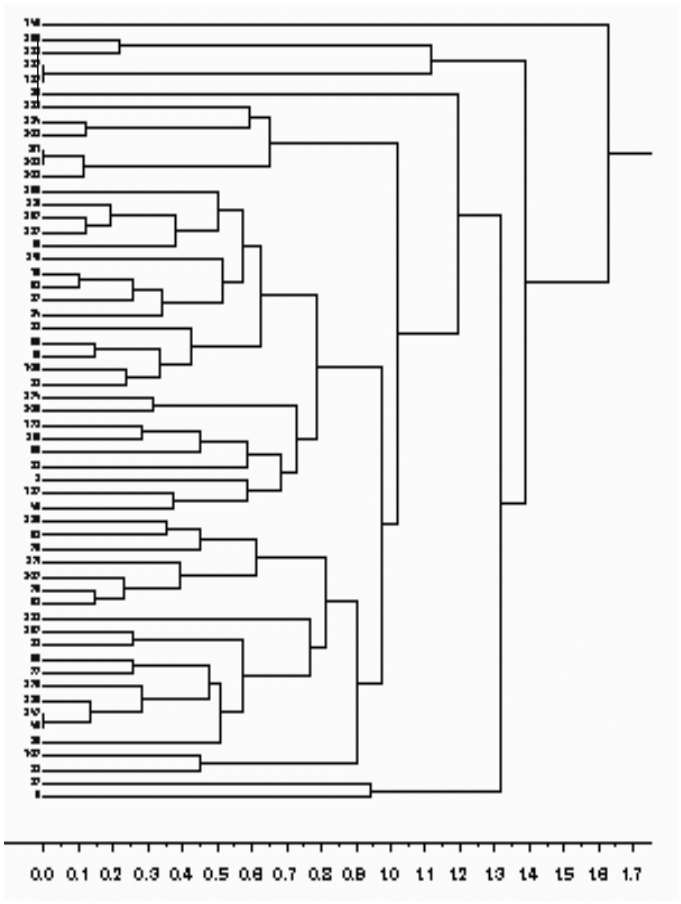
SLIDE 8



The reference collection for Hydrangea is located in Angers and contains over 500 accessions belonging to an uncertain number of species. The taxonomy is still controversial in particular concerning the status of the taxonomic levels : are the subspecies really subspecies or species? We will speak only about asperae and macrophyllae. The primers retained for one group were also suitable for the other. On the other hand, some markers were specific to only one subpecies.
For Hydrangea, no molecular marker is yet available.

SLIDE 9

Pink and white

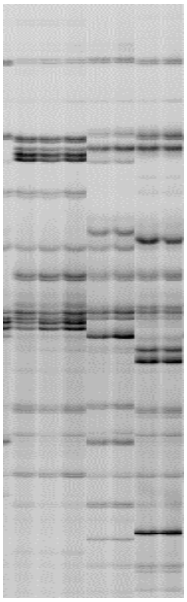


When looking at the dendrogram (here we see macrophylla cultivars), we notice some varieties which are not distinguished (these were suspected duplications of accessions), we also notice that the cultivars are grouped by colours, and all the groups showing short distances are related or share a common ancestor.

SLIDE 10

Mis-labelling

- macrophylla
  - 14 pairs
  - 4 triplets
  - 1 quadruplet
- Serrata
  - 12 pairs



A number of duplications or identification problems were identified. For example, Sea foam and Azisai are synonymous, as well as Iowa and Rotkehlchen. As expected, no difference was found between Merveille and its mutant form Merveille sanguinea.

SLIDE 11

Variability

- High Polymorphism in macrophylla (over 100 markers for 4 primers)
- Polymorphism even greater for serrata
- No or few common bands for the 2 « subspecies »

SLIDE 12

Poplar

- P. x euramericanus = P. deltoides x P. nigra
- P. x interamericanus = P. deltoides x P. trichocarpa
- P. tremula
- P. alba...

Like *Hydrangea*, numerous species or interspecific hybrids are commercialized in the *Populus* genus.
Molecular markers are available for poplar:
RAPD,
AFLP : we first used this method with success, but we the discarded it because of the patent and also because ISSR were easier to perform
SSR : These markers are very interesting, we did not use them because they were specific of one or a few *populus* species, rarely to all.

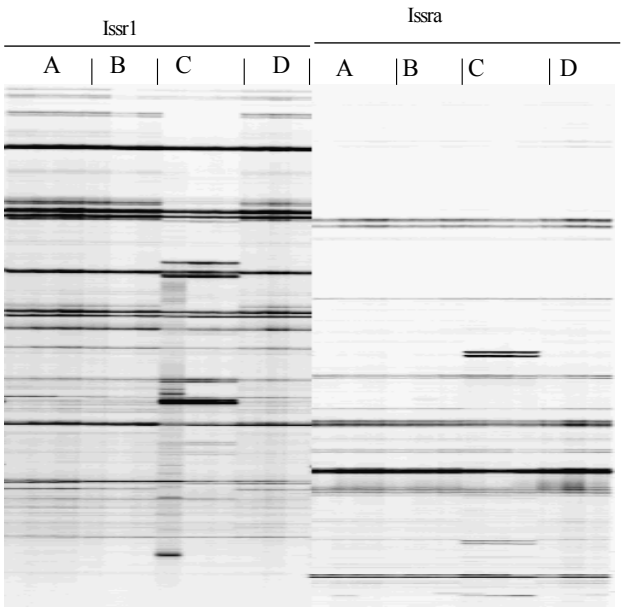
SLIDE 13

Poplar
Identification problems

- diseases
- morphological characters not reliable
- topophysis

Identification is crucial to the growers since the cultivars have different susceptibilities to diseases. On the other hand, diseases can impair identification.
Morphological characters are not a great help and no expert is able to recognize a cultivar on the basis of its description.
Topophysis is the influence of the origin of the cutting on morphological characters. Differences can be observed according to which part of the tree has been used as cutting.

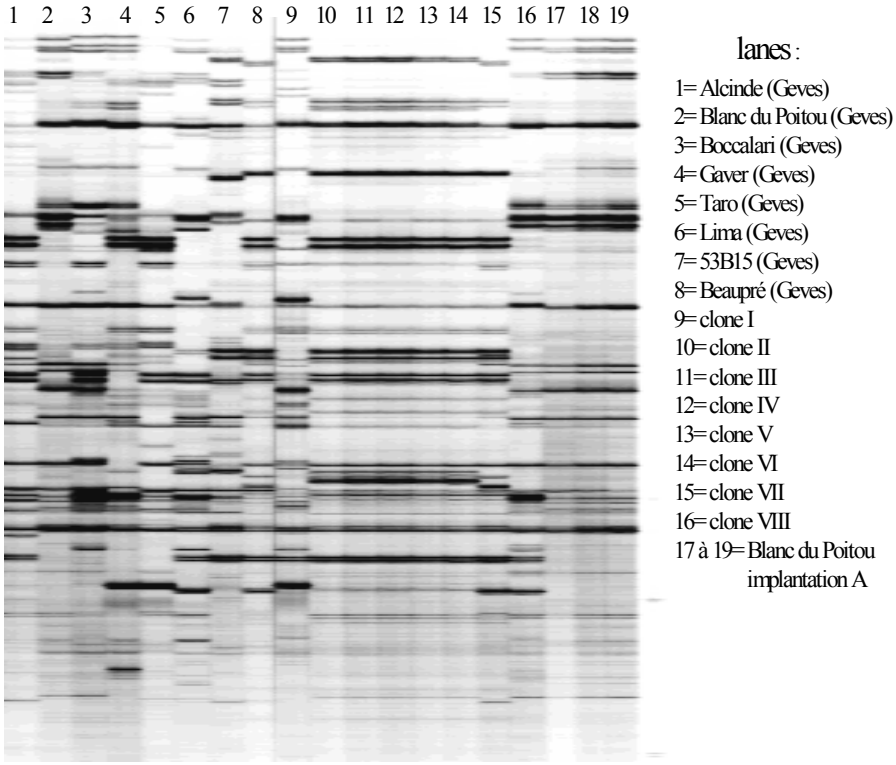
SLIDE 14



Blanc du Poitou

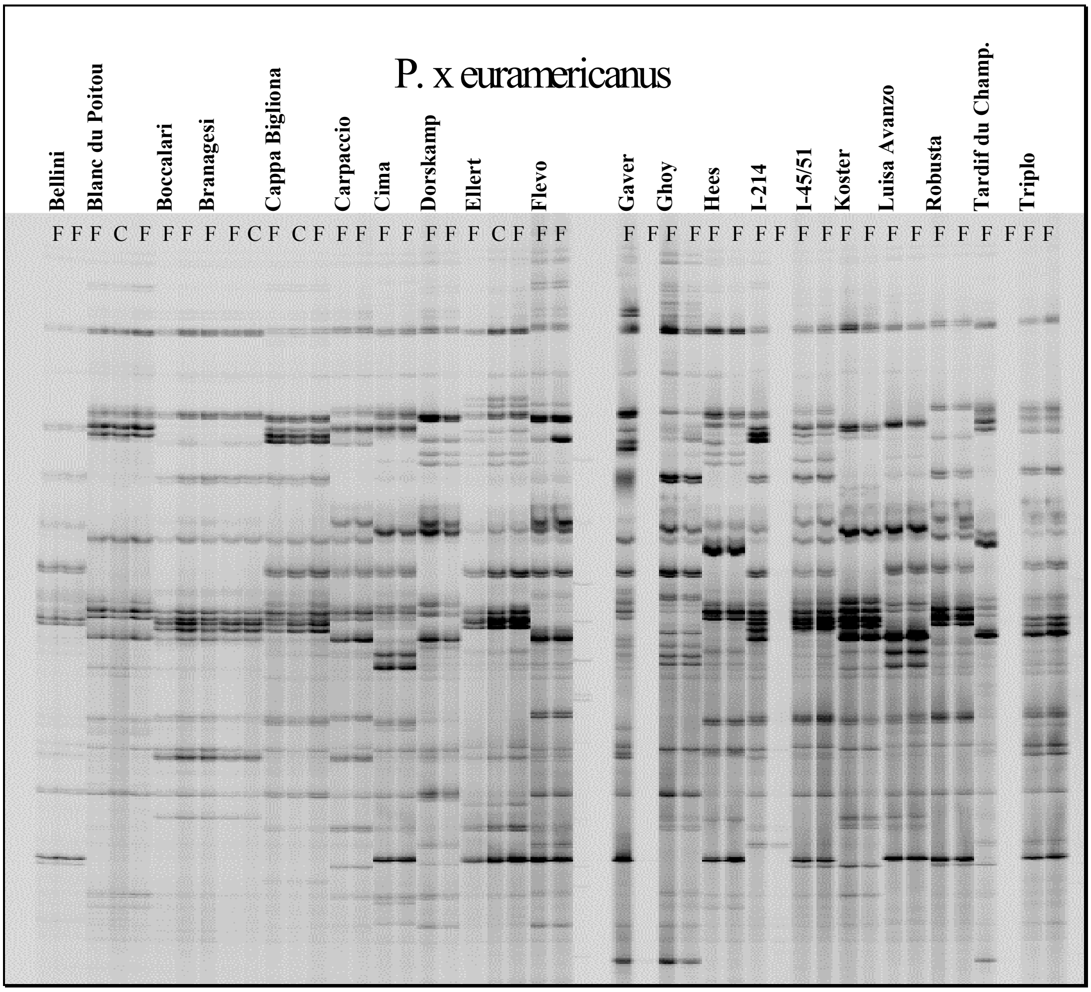
An example of a survey of 25 orchards with different disease occurrences. In only one case was the cultivar different from expected

SLIDE 15



A survey of different species and sections. Some markers are specific to one species.
A blind test was conducted on 15 clones. 2 clones did not belong to our collection. The other 13 were all correctly identified.

SLIDE 16



Within our collection of P. euramericanus, only 2 clones were identical. When the problem was reported to the examining office, it confirmed a suspicion on these clones. A dozen other clones were subsequently analysed and were found to belong to only 2 groups. These clones will be declared synonymous.

SLIDE 17

Conclusion

- Good behaviour of the markers for
  - Genetic studies
  - Identification
  - DUS purposes ?

It is not a surprise that the markers are a help for genetic studies and identification problems.
For DUS purposes, vegetatively propagated species should be easier to deal with, since we do not expect uniformity or stability problems.
Distinctness :
according to our experience, when the varieties were not distinguishable using the ISSR markers, they were found upon enquiries to be identical.
Non identical varieties were never very close to each other, even sibs or one clone to its progeny.

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