shrub becoming 2.5 to 4.0 m tall and about 2 to 3 m broad with few main branches from the bottom, side branches horizontal to upright, with a rather open branching system. Leaves are elliptic, 7 to 20 cm long, 2 to 5 cm broad, upper side dark green, shiny, a little shrinky, the lower side is densely yellow-grey with woolly hair. Flowers are small, white, in large corymbs. It flowers in June, and the fruits are first red and later black, but they are not common, probably due to self-sterility. The species has much in common with *V. rhytidophyllum*.

Genetic Fingerprinting. What Is It? – And What Is it Used For?©

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INTRODUCTION

Methods for DNA fingerprinting were originally developed for use in forensic medicine but are now widely used for many purposes concerning analyses of practically any kind of biological system. This mini-review is intended to introduce the concept of DNA fingerprinting; it is in no way an exhaustive and detailed description of techniques and their applications. Rather, the purpose is to present a few examples of the use of fingerprinting, and thereby hopefully enable the plant breeder or propagator to consider whether a problem may be solved easier by using some kind of DNA fingerprinting.

WHAT IS A GENETIC FINGERPRINT?

In essence, a genetic fingerprint is simply a "bar code" that can be used for identification of a preparation of DNA. It will be necessary briefly to discuss various methods and their strengths and weaknesses, but hopefully this will not blur the main purpose of this paper.

The production of a genetic fingerprint can be divided into three steps:

1) Isolation of DNA from the material under investigation.
2) Performance of some kind of enzymatic reaction using the isolated DNA.
3) Analysis of the outcome of step 2 (usually by gel-electrophoresis).

Isolation of DNA. DNA can be isolated from practically any tissue but generally some kind of soft tissue (leaf, shoot) is preferred. Less than 0.1 µg of DNA is needed for making several fingerprints. As a very broad rule of thumb it can be said that a satisfactory yield of DNA can be obtained from 1 cm^2 of leaf material. One person can handle up to 50 DNA isolations per day.

Enzymatic Reactions. A number of different procedures have been developed for producing genetic fingerprints. Most of the currently used methods are based on the "Polymerase Chain Reaction" (PCR), but the principles of the procedures will not be described here. There is an ever increasing confusion in the names of the various procedures. I will try to compare three principally different methods: RAPD

(Random Amplification of Polymorphic DNA), AFLP (Amplified Fragment Length Polymorphism), and analysis of SSR (Simple Sequence Repeats, also known as microsatellites) (Table 1). Whatever procedure is used, the outcome is a collection of DNA fragments of various lengths.

Analysis. The last step is to separate the DNA fragments according to their size, which — depending on the choice of procedure — may vary from about 50 base pairs to 5000 base pairs. This separation is performed by a procedure known as gel electrophoresis. Finally, the DNA fragments in the gels are made visible, e.g., by staining, and some kind of banding pattern (the "bar code") is observed.

Table 1 summarises the principal advantages and disadvantages of three different fingerprinting methods. The table is purposely held in vague terms, and expensive, specialised equipment may reduce both time and price of the assays. Once the suitable procedures have been established, a standard laboratory without specialised equipment can perform the whole procedure from DNA isolation to inspection of the fingerprint within 2 days (fast) to about a week (slow), and the material cost per fingerprint will vary from about $2 (cheap) to about $20 (expensive).

The SSR analysis will not be discussed further here because it is only available for species that are well characterised at the level of molecular genetics. The main problem with the "poor" RAPD method is that it may be difficult to obtain reproducible results. The method is rather sensitive to impurities in DNA preparations and differences in the concentration of the input DNA. Furthermore, results obtained in one laboratory can be difficult to reproduce in other laboratories due to minor differences in working procedures or equipment. The actual appearance of the fingerprints obtained by a well functioning RAPD procedure and an AFLP procedure is illustrated in Fig. 1. The AFLP procedure generally produces more bands than the RAPD procedure, and bands observed in the AFLP fingerprint are sharp and have almost equal intensities, whereas bands observed in the RAPD fingerprint are more fuzzy and sometimes so weak that they are hard to discern from the background.

WHAT ARE GENETIC FINGERPRINTS USED FOR?

The list below should be considered as a collection of examples of situations where professional plant growers might want to use genetic fingerprinting. It is naturally impossible to make a complete list, but hopefully it helps to give an idea of the wide range of uses for genetic fingerprinting.

Table 1. Comparison of advantages and disadvantages of three different fingerprinting methods.

	RAPD	AFLP	SSR analysis
Time considerations	Fast	Slow	Slow
Price	Cheap	Expensive	Expensive
Quality	Poor	Good	Very good
DNA knowledge necessary	No	No	Yes

Relatedness. Closely related organisms have similar but not identical fingerprints whereas remotely related organisms have different fingerprints. This is illustrated in Fig. 1A, where three different species of *Helianthus* are compared, and Fig. 1B, where two different ecotypes of *Arabidopsis thaliana* are compared. This quality of a genetic fingerprint can be used to optimise the selection of a few plants from a big collection.

Consider a plant breeder who wants to introduce new germplasm into his breeding programme. Naturally, the breeder will still look at the general appearance of the new plants, but might also want to get an idea about how related the new plants are to each other and to the breeder's previously used material. The genetic fingerprint may help him to make a wise selection of new plants.

Similarly, consider a plant propagator who has collected a large number of new cultivars of a species (in nature or from botanical selections) because he/she wants to market a new selection with a quality that is difficult or expensive to analyse. The

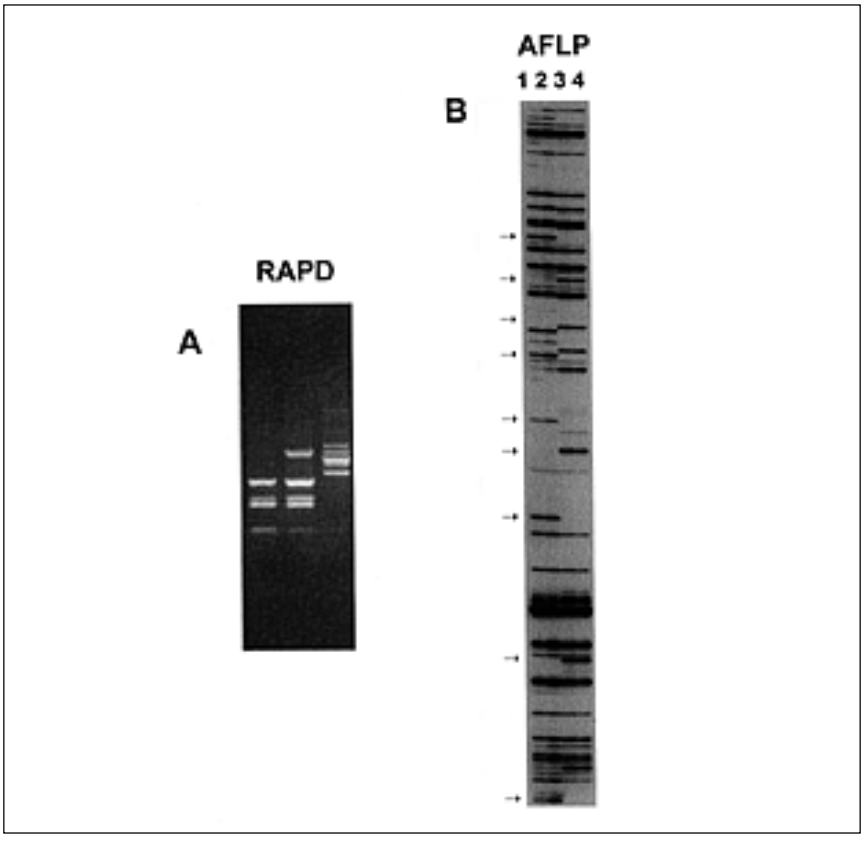

Figure 1. Examples of RAPD and AFLP fingerprints. (A). RAPD fingerprints of three sunflower species (*Helianthus maximiliani, H. giganteus, H. annuus* from left to right). (B). AFLP fingerprints of two ecotypes of *Arabidopsis thaliana* (lanes 1 and 2: Columbia; lanes 3 and 4: Landsberg). [A was taken from the web site of "Qiagen", http:// www.qiagen.com; B is from second reference]

genetic fingerprint may help ensure that the limited number of plants he/she chooses for analysis are indeed different.

Paternity Assays. The performance of paternity assays is naturally a "classic discipline" within forensic medicine, but it may also be very useful for plant breeders. Plant breeders often want to perform crosses between different species. This may be possible if the two species are closely related, but only possible with a very low efficiency if the species are more distantly related. In the latter case, whenever a germinating seed is found, the question arises: "Is this the hybrid I want ? — or an unwanted self-fertilisation I was not able to avoid?" As in forensic medicine: A genetic fingerprint of the two parents and the child will give you the answer.

Paternity assays may also be of great value for other reasons. All that is needed for a fingerprint is about 1 cm^2 of leaf. Thus, it is possible to grow a substantial number of plantlets at limited space until fingerprint testing has been done, and only keep the few wanted cross-fertilised plants for further growth.

Identification. Fingerprinting can be used to identify plant material and thereby constitute important evidence in judicial disputes. However, instead of focusing on the possibility of winning a lawsuit, fingerprinting can be implemented as part of a standard quality control system. In our modern, specialised society it is not uncommon to have a seed company, that sells its seeds to a plantlet producer, who sells the plantlets to a grower. Each link in this chain can test and document the genetic quality of its product by a genetic fingerprint.

There are other cases where even a less strict identification may be important. In some cases, for instance with tree seeds, the geographical origin of the seed may be of great interest. The underlying reason for this interest is, naturally, that the trees in this specific geographic area constitute an isolated subpopulation characterised by specific genetic traits. It may be impossible to identify the underlying genes, but fingerprints from individuals belonging to the same subpopulation must show common features different from those of fingerprints from other populations. The AFLP fingerprints in Fig. 1B can be considered as an example of this kind of testing.

Genetic Maps. The presence or absence of a band in a genetic fingerprint can be treated like any other genetic character. It is therefore possible — and has indeed been done for many plant species — to create genetic maps based on fingerprinting. Various phenotypic characters can be placed on such genetic maps, i.e., genes for specific characters can be linked to specific bands in the fingerprint. This kind of knowledge can be extremely valuable for breeders. In many cases cumbersome and expensive analyses for traits such as keeping quality, temperature tolerance, or disease resistance may be substituted by analyses of genetic fingerprints. A detailed description of this technique, known as "marker-assisted breeding", falls beyond the scope of this presentation, but it has tremendous importance for focused breeding programmes.

The use of genetic fingerprinting has also reduced the time and effort needed to create a genetic map. Genetic fingerprinting enables the investigator to create a usable genetic map with hundreds of markers, without the prior knowledge of the inheritance of a single phenotypic trait. As a consequence, genetic maps are now appearing even for genetically poorly described minor cultures.

CONCLUSION

The methods for genetic fingerprinting are still improving and we must expect that fingerprinting in the near future will be an integral part of many kinds of plant testing and documentation. Genetic fingerprinting will be a natural tool in any kind of selection of new plant material. Fingerprinting is simply no longer a technology restricted to research laboratories working with major cultures, but a logical choice for solving everybody's problems today.

REFERENCES

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