USE OF MOLECULAR AND BIOCHEMICAL METHODS FOR IDENTIFICATION OF PLANT VARIETIES THROUGHOUT THE AGRI-CHAIN

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Introduction

The quality of plant material, and the products which are to be derived from it, has a direct bearing on the choice of the plant variety grown. Many important characteristics, such as morphology, disease resistances, storage behavior (keeping quality), taste of food materials and industrial characteristics such as baking quality, are strongly variety dependent. This relation might be epitomized as "IDENTITY=QUALITY": ensuring the identity of the material grown is essential and, in most cases, sufficient for the assurance of a particular quality expected by the users, whether they be propagator, farmer, trader, processor or consumer. As the power of the consumer increases, food labeling can be expected to increase in prominence, particularly with regard to the marketing of disease-resistant (and thus more environment-friendly) or genetically modified varieties.

Conventionally, varieties are identified using morphological characteristics such as leaf shape, plant height and flower color. Plants have to be grown to full maturity before proper observation can take place, making the process of identification time-consuming and expensive. Furthermore, the expression of these characteristics is often influenced by the environment, necessitating the use of controlled growth cabinets or greenhouses, or extensive field trials. The result of all this is that identification by means of these characteristics proves to be too slow and too expensive to be used as an effective way of checking the identity of plant material throughout the agri-chain.

Biochemical and molecular marker systems, which are based on variety-specific protein and DNA profiles (fingerprints), respectively, have shown their potential for rapid varietal identification in several crops (Cooke[7,8]), including wheat (Jones et al.[10]; Houwing and Van Dreven[11]), barley (Lallemand and Briand[15]; Becker and Heun[3]), rice (Wu and Tanksley[25]), grasses (Gardiner and Forde[9]; Van Dreven et al.[22]; Booy et al.[6]), Gerbera (Booy[4]), tulips (Booy et al.[5]), potatoes (Stegemann and Loeschke[19]), tomatoes (Vosman et al.[24]; Arens et al.[1,2]), cabbages (Kresovich et al.[14]), soybeans (Rongwen et al.[16]), citrus (Kijas et al.[12]) and grapes (Thomas and Scott[20,21]). Unlike morphological characteristics, these markers do not require fully-grown plants but can be established by using

seeds, tubers, bulbs, fruits, or small leaves, thus making the growth of plants redundant. Furthermore, the markers can be analyzed cost-effectively and rapidly, are reproducible and generally unaffected by environmental interactions. In general, biochemical markers are relatively inexpensive to develop and relatively easy to use. Molecular markers have the advantage of an almost unlimited availability. Also, some molecular techniques can provide extremely polymorphic markers capable of high levels of discrimination between varieties.

Depending on the marker system chosen and on the amount of variation present among varieties of a given species, it may be possible to identify either all varieties, or a major part of the assortment of varieties, by a unique fingerprint. Although this implies that it is not always possible to identify a completely unknown sample unequivocally, this is not a problem because, in practice, all questions are of the nature: is this a plant of variety A or of variety B? This question can be answered in almost all cases. For instance, if 90% of the varieties can be identified by a unique pattern, then it will be possible to discriminate between two varieties in more than 99% of the cases; even if only 80% of the varieties obtains a unique pattern, it will still be possible to discriminate in 96% of all pairs of varieties.

Therefore, biochemical and molecular methods can serve as valuable tools to control the identity of the plants or their products in chain quality management systems, from propagative plant material to the end product, both within and between companies. At the very beginning of the chain, the markers can be used during plant breeding and for varietal evaluation used for granting plant breeders' rights. Over the last decade, biochemical and molecular methods for these purposes have been developed world-wide, and such methods are being used in our institute for routine identifications in ornamental, vegetable and arable plant species (e.g., Booy et al.[5]). Applications for four species are discussed here.

Isozyme and protein profiles as a means of quality assurance in the flower production and trade

The Tulip crop

The tulip crop is the most important flower bulb crop grown in the Netherlands. To guarantee varietal identity during dry sale, it is important that cultivars can be identified at the time they are sold, which is in the summer and autumn. The conventional method for identification is based on the morphological characteristics of the flowering plant. These characteristics are not suitable for use in an efficient quality control system, because by the time the variety can be identified, the plants are flowering in the gardens of the consumers.

We have developed an identification system based on polymorphisms in the different forms of the enzyme esterase from tulip bulb scales (Booy et al.[5]). Out of the more than 300 varieties analyzed (excluding mutants), 90% showed a unique fingerprint, which makes it possible to discriminate between

two varieties in 99% of the cases. The method is now applied routinely by the Flower Bulb Inspection Service. Inspectors who run this service take random samples, which are tested for their identity. The method appears also to be very useful for proving the hybrid nature of small bulbs resulting from interspecific crosses, which is of great importance to tulip breeders.

For flower traders, this identification system makes it possible, for the first time, to guarantee that bulbs sold are of the variety that was asked for. The aim for the near future is to arrive at a hallmark, issued by an independent organization, which will provide a guarantee for the retailer and the consumer that the tulip bulb will flower in the expected color.

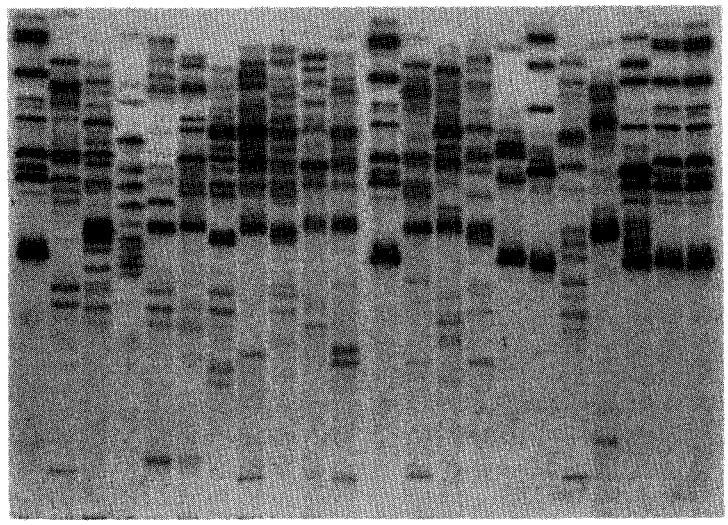

Figure 1. Esterase patterns of 20 tulip varieties. Lanes 1, 11 and 22 (numbered from the lefthand side) contain the reference variety Couleur Cardinal. Lane 2 contains Rococo, a mutant of Couleur Cardinal. All other varieties can be distinguished. This test takes approx. four hrs.

The Lily crop

The lily is propagated vegetatively. Early propagation is done by specialized companies using tissue culture. They then deliver their material to other companies that grow the bulbs to maturity. During this propagation process, the chance mixing of different varieties should be kept to a minimum. To assure, amongst other things, that the right variety is produced, the 'Registration Bureau for Lily Tissue Culture' was recently established. Samples are taken from different lots of plants during tissue culture propagation and tested for their identity.

This test is conducted in our laboratory. Since most lily varieties show hardly any morphological differences in tissue culture, we have developed a method based on differences in protein profiles, to check the identity and homogeneity of tissue culture lots. By regular random tests during propagation of the protein profiles of plantlets taken from one lot, accidental mixing and interchanging of lots can be prevented.

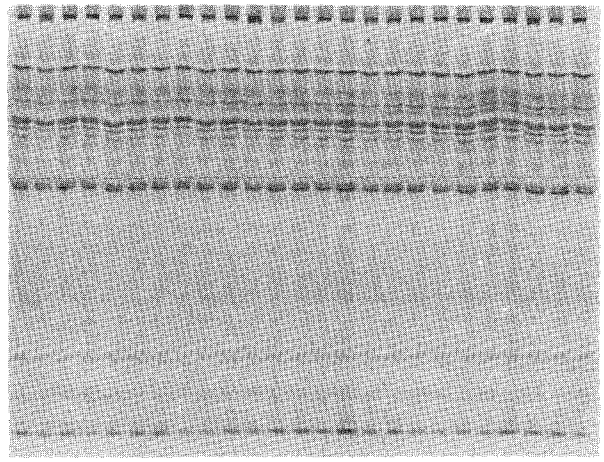

Figure 2. Protein patterns of 25 lily bulbs sampled from a batch of lily bulbs propagated in vitro. This test for homogeneity takes approximately six hours.

Varietal identification in the potato chain

Potato varieties differ in a large number of important characteristics, and therefore knowing the identity of the potato variety being grown or traded is essential. For farmers, the difference in disease resistance levels between varieties is important information determining the use (or non-use) of chemical protection agents. For the processing industry, the difference in baking quality between varieties is very important. The baking protocol to be used depends on the characteristics of the variety that is being processed. Moreover, not all varieties are equally well suited to all uses. For the consumer, the differences in taste and constitution are important.

For varietal identification of the potato the analysis of tuber proteins (Stegemann and Loeschke[19]) is being successfully used. The majority of the about 600 varieties analyzed so far has a unique protein pattern. This method is applied by our institute on a routine basis to check the identity or the homogeneity of potato lots. These tests are performed in all stages of the Agri-

chain: for breeders, traders, the potato industry and for the General Inspection Service.

In the near future, we intend to use potato protein patterns to speed up registration research for the purpose of establishing Plant Breeders' Rights. By analyzing the protein patterns of the varieties submitted by the breeder for testing for Plant Breeders' Rights, reference varieties may be selected on the basis of their homology with the applicant, thus saving space in field trials.

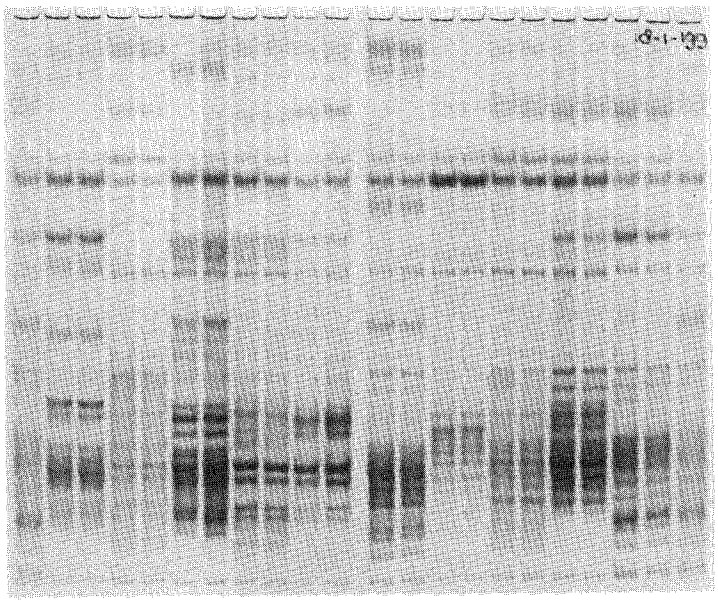

Figure 3. Protein patterns of 11 potato varieties. Lanes 1 and 22 contain the reference variety Bintje. For each of the other 10 varieties, two independent samples are loaded next to each other. This test takes approximately eight hours.

DNA-based identification methods distinguish between closely related varieties

For plant breeding companies it is of vital importance that they are able to protect the high quality germplasm that they have developed and the varieties that they have produced. This is made possible by the granting of Plant Breeders' Rights. Breeders receive royalties from the sale of plant material of protected varieties. If they are to efficiently detect encroachments on their rights, the breeders have to be able to identify their varieties and the potential infringing varieties efficiently.

In the case of the tomato, protein patterns are not sufficient to identify the variety, since the genetic basis of the cultivated tomato is very small, and therefore variation in proteins among varieties is rare (Vosman et al.[24]; Rus-Kortekaas et al.[17]; Smulders et al.[18]). In such cases, DNA fingerprinting using special types of repetitive DNA can be helpful, since these types of DNA are more polymorphic. In humans and animals, this technique is already being applied routinely. In humans, for instance, DNA fingerprinting is used in forensic cases, and in cases of disputes regarding parentage (Kirby[13]).

In the case of the tomato, we used an analysis of microsatellite DNA to identify varieties (Vosman et al.[24]; Rus-Kortekaas et al.[17], Arens et al.[2]). A multilocus fingerprint, i.e. a fingerprint combining a large number of bands, yielded 91% unique fingerprint patterns among almost 50 different varieties of tomato (Arens et al., in preparation). The disadvantage of this method is that it requires skilled technicians, a well-equipped laboratory, and still takes approximately 3-5 days. Even so, the method is now being applied in legal cases regarding infringements of Plant Breeders' Rights.

Currently, we are converting this method into a PCR-based method, i.e. one based on a reaction that multiplies the desired bands on the basis of minute quantities of sample DNA. In this way, tiny samples can be used with less handling, and this reduces the requirements for equipment and personnel. Several of these STMS (Sequence-Tagged Microsatellite Site) reactions can be combined to obtain the same level of discrimination (Arens et al.[2]; Bredemeijer et al., in preparation). Since the detection of bands and the analysis of results can be improved further, the method will not only be fast but also become relatively inexpensive. This method is therefore well suited for identity tests throughout the chain.

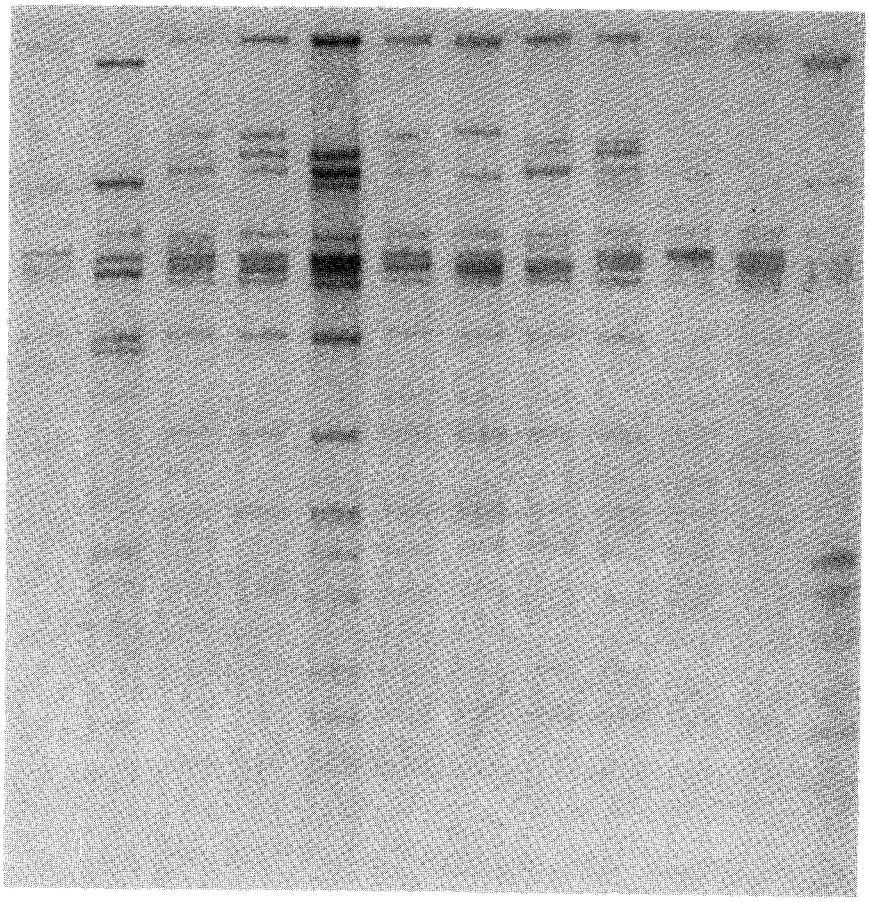

Figure 4. DNA fingerprint of 12 tomato varieties. Although some closely related varieties resemble each other, all can be distinguished by a unique fingerprint.

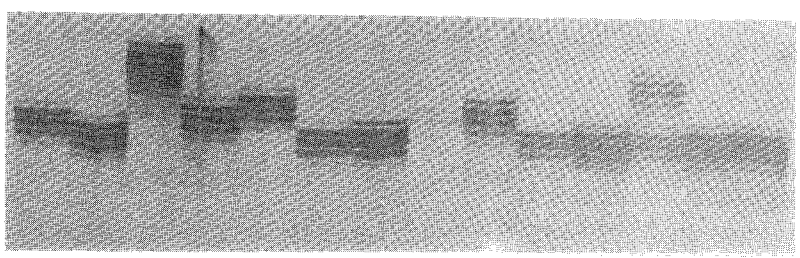

Figure 5. Microsatellite (STMS) analysis of 14 tomato varieties.

Conclusion

Ensuring the identity of the material grown is essential to guarantee specific quality levels expected by the end-user, who may be a farmer, processor or consumer. For this, it is essential that varietal identity be established rapidly and accurately. The laboratory-based identification techniques and computerized databases of varietal profiles being developed now, will be of direct benefit to the process of quality assurance. Such marker systems can be used readily to trace varieties (and the products derived from them) throughout 'total quality management' systems, from propagative material to the end product, both within and between companies.

It is foreseen that technological improvements will make DNA-based fingerprint techniques cheaper and easier to use in the near future (Vosman [23]). Therefore, faster, and cheaper methods for the determination of the identity of plant material will become available, and should be integrated into quality assurance systems.

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