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THE USE OF MOLECULAR TECHNIQUES FOR THE IDENTIFICATION OF TOMATO CULTIVARS

Document prepared by experts from the Netherlands

The use of molecular techniques for the identification of tomato cultivars.

In the past decade several molecular techniques have been developed that can be used for cultivar identification. For tomato only RFLP, RAPD and DNA fingerprinting with oligonucleotide probes have been applied and the results published. In these studies the number of different cultivars used has been limited (less than 20) although the cultivars were often chosen to represent the whole spectrum from cherry to beef tomato and from old to relatively modern.

RFLP probes

Two reports have been published in which exclusively RFLP probes were used for cultivar identification (1, 2). The probes used were selected on their ability to show polymorphisms between L. esculentum and L. pennellii. Miller and Tanksley (1) tested 9 tomato cultivars with 40 RFLP clones and used 5 different restriction enzymes. Up to 20% of the probes detected polymorphisms among the cultivars used, which included fresh market, processing, and cherry tomatoes as well as some older, obsolete, varieties. Within these subtypes, the amount of polymorphism is likely to be lower.

Van der Beek et al (2) compared 3 different cultivars using 195 RFLP probes and six different restriction enzymes. The cultivars used were two introgression free obsolete cultivars (Moneymaker and Premier) and a modern cultivar (Sonatine) which carries at least five introgressed resistance genes. Only 3 probes detected polymorphisms between Moneymaker and Premier, whereas 11 probes detected polymorphisms between Moneymaker and Sonatine. Analysis of the results showed that part of the polymorphisms found between Moneymaker and Sonatine were linked to the introgressed resistance genes.

RAPDs

Two papers have been published that describe the use of RAPDs for identification of tomato cultivars (3, 4). In the study of Williams and st. Clair (3) eight vintage and eleven modern cultivars were used. Primers were selected on several criteria that maximized band robustness of the amplification. The criteria were met by 24 of the 100 primers tested. Utilizing these primers they could distinguish nine out of the 19 cultivars used. The study also compared the results obtained with the RAPD primers to RFLP probes. The level of polymorphism detected was considered identical for RAPD primers and RFLP probes.

Foolad et al (4) used one breeding line and two primitive, introgression free cultivars from arid regions. In this study RAPDs were compared to Isozymes and RFLPs. All isozymes, RFLP probes and RAPD primers used were capable of detecting polymorphisms between the breeding line and L. pennellii. None of the 16 isozymes was able to detect a polymorphism in pair wise comparisons of the breeding line and the two cultivars. Sixteen percent of the 25 RFLP probes could detect polymorphisms between the breeding line and either of the cultivars, but none could detect a polymorphism between the cultivars. In contrast, approx 60% of the 313 RAPD primers detected polymorphisms between the breeding line and either of the cultivars, 16% of primers could detect a polymorphism between the two cultivars. Rus-Kortekaas et al. (5) also analyzed tomato cultivars with RAPD primers. From the 89 primers initially tested, 85 showed polymorphisms between L. esculentum and L. pennellii, but only four distinguished among three L. esculentum cultivars. These four were

subsequently tested on 15 cultivars from which 11 could be identified by a unique combination of RAPD patterns.

Microsatellites

Oligonucleotide probes complementary to microsatellite sequences have also been used for fingerprinting of tomato cultivars (6). All 15 cultivars tested could be discriminated with either (GATA)4 or (GACA)4 as probe. To test the potentials of the (GATA)4 probe on modern cultivars, representative plants of all applications for plant breeders rights that were being studied in the Netherlands in 1992, together with a number of cultivars used as comparison, were fingerprinted (7). Using TaqI-digested DNA, 29 of the 36 plants could be distinguished by a unique fingerprint. Among the remaining plants were one representative of a cultivar, and two plants propagated from this cultivar by tissue culture procedures. It was not surprising, therefore, that these plants had an identical fingerprint. Two other plants with an identical fingerprint were from one breeding company, and had one parent in common. We do not know if there is any relationship between the second parent of the two plants. Finally, nothing is known about a possible relationship between the two other plants that shared one fingerprint. These results show that fingerprinting with (GATA)4 can distinguish well between modern tomato cultivars, but that it is not possible to distinguish all of them. However, relationships between cultivars can be identified.

Conclusions

For efficient DNA profiling it is essential that the probes used detect reasonable levels of polymorphism. This is especially important in tomato, where the amount of variation present between modern cultivars is low. The level of polymorphism detected by RFLP probes is probably too low to allow an efficient cultivar identification system (to many probes would be needed). On the other hand, RFLP are codominant markers that can be scored reliably.

RAPD primers detect probably more, but at least equal amounts of polymorphisms. Since RAPDs are PCR based markers the technique is easy to handle. However, RAPDs markers are dominant and therefore less informative than RFLPs. Questions have been raised with respect to the reproducibility of RAPDs (8, 9, 10 ,11). Different RAPD profiles may result from the use of different PCR machines or different batches of polymerase and/or primers. It is also not clear whether profiles produced on a particular PCR machine, can be reproduced on the same machine some time later.

Microsatellites are highly polymorphic DNA sequences that can be detected, by Southern hybridization, with complementary oligonucleotide probes. Rus-Kortekaas et al (5) showed that the average band sharing percentage in a pair-wise comparison of 15 cultivars with four selected RAPD primers was 82.7%, while it was 50.8% with a probe that detected GACA containing microsatellites, indicating that the latter probe detects more polymorphic DNA. For closely related cultivars the detection of microsatellite sequences appears to be the most promising method, eventhough the bands detected are mostly dominant. A most promising alternative is the detection of microsatellite polymorphisms by PCR with primers based on flanking sequences (the sequence tagged microsatellite technique (12)).

Unfortunately, this technique has been developed very recently and experience with it is lacking.

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