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A MODEL STUDY IN TOMATO TO ASSESS THE USABILITY OF MOLECULAR MARKER IN DETERMINING ESSENTIAL DERIVATION

Document prepared by the Tomato-EDV Study Group of ASSINSEL

A model study in tomato to assess the usability of molecular marker in determining essential derivation

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Background

Tomato was chosen for a model study of essentially derived, organized and funded by ASSINSEL with support from ASTA, NVZP, FNPSP, Japan Seed Trade and Israel Seed Trade. Tomato was selected because it represents a difficult challenge which severely tests the development of the practical implication of EDV in a well-developed commercial crop. Also, this crop is a good model because it is extraordinarily well characterized with very complete genic and molecular maps. It has been a model crop for molecular research for many years at numerous laboratories.

Genotypes and Analysis

For this study, clusters of about 15 varieties were developed for Moneymaker, Montfavet and California Processing ideotypes. The varieties in these clusters trace the development of the ideotype to modern varieties. Phenotypic data were collected from registration information or by breeders. Molecular analyses were conducted by Keygene (Marc Zabeau) and the Department of Plant Breeding, Cornell University (Steve Tanksley), using AFLP, RAPD and microsatellite technologies. Molecular and phenotypic data were analyzed at the University of Wisconsin (James Nienhuis) and the Centre of Plant Breeding and Reproductive Research, Wageningen (F.A. van Eeuwijk).
In order to get a better overview of potential bottle necks of the statistical analyses of the data, two independent statisticians were selected. The two selected ones have a different background. Nienhuis has a breeding background and has specialised since in using statistical approaches to organise germplasms. Van Eeuwijk is a theoretical statistician who is applying and developing statistical approaches to identify and tackle problems in measuring genetic distances.

Results

Independent polymorphic bands detected by AFLPs, RAPDs and microsatellites have equal information contents per locus, useful to discriminate tomato genotypes. All of these genetic marker methods generated similar ordination patterns, indicative for the relative organisation of the germplasm. Reliability of these estimations appears to be independent of the molecular marker method used and to be dependent on the number of bands only. Thus, the precision of the ability to discriminate between genotypes is limited by the number of polymorphic bands. There are substantial differences in the number of polymorphic bands defined with the three molecular methods. The greatest number of polymorphic bands resulted from AFLPs, intermediate levels with RAPDs and low levels with microsatellites where unfortunately only one probe was used. If an appropriate number of polymorphic bands can be discovered with each of the three methods studies, the value for EDV will be equal.

Both statisticians stressed the importance of redundancy of data. Intuitively this seems like an important issue. Nienhuis has deleted in his analyses all complete redundant information. Van Eeuwijk stresses the fact that one may have to consider to correct also for nearly redundant bands. However, there are as yet no statistical methods to decide how to approach the latter. The percentage of completely redundant bands differed among the marker methods. For the AFLP, the percentage of redundant bands was about 60%, for RAPDs about 50%, and for microsatellites about 2%. The redundant bands are probably largely linked with introgressed disease resistance segments. Nienhuis eliminated redundancy and found for the processing genotypes 77 independent AFLP bands, 80 independent RAPD bands (he included RAPD data for the processing cluster from another study on the same genotypes), and 13 independent microsatellite bands. A re-analysis of the resulting combined 170 bands gave 139 independent bands. For the Moneymaker and Montfavet clusters which he combined, he found 127 independent AFLP bands, 35 independent RAPD bands (Tanksley analysis only) and 23 microsatellite bands. Re-analysis of these 185 bands resulted in 170 independent bands. He did subsequent analysis using the combined independent bands. He concluded that at least 200 independent molecular marker bands will be necessary to achieve a co-efficient of variation (an estimate of reliability of the calculated genetic distance with a given number of data points)(variance $^{1/2}$/mean) of 10%. This result indicates that a limiting factor will be finding an adequate number of independent bands. Since the technologies have similar genotypes discriminatory capabilities, it is possible to combine technologies to get the needed number of independent bands.

The precise defining of a threshold is largely limited by the need for a significant increase in reliability which is largely a function of the number of independent marker bands. A potential problem identified by both statisticians is the fact that duplicate samples from the same variety, not always lead to an identical data set. Whether this is due to experimental error or genetic variation within the variety is not known yet. Implication for the EDV concept if the latter is the case needs to be evaluated.
If a sufficient number of independent bands can be obtained with a single technology that will suffice, or technologies can be combined to achieve the required band number. Obviously the distribution of markers throughout the genome will increase as independent band number increases.

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