

Summary Progress Report, 2001:

Development and characterization of simple sequence repeat (SSR) markers and their use in detecting cultivar relationships and in identifying markers linked to resistance to bacterial canker in tomato

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Plant breeding is more and more using molecular techniques to overcome recalcitrant breeding problems. Selection based on phenotype may be ineffective or inefficient due to environmental influences, or because of the complex inheritance of polygenic characters. However, selection can be done for desired genotypes using molecular markers. The advantage of using molecular markers over traditional selection is their ability to predict the presence or absence of the resistance genes in question based on the linkage between the resistance genes and the markers. Therefore, the identification of molecular markers linked to tomato disease resistance could facilitate the screening of breeding lines carrying the resistance gene.

Microsatellites are highly repeated sequence consisting of short nucleotide repeats motifs such as AG, GT, ATT and TTC etc. As genetic markers, they are used in many crop plants since they are codominant, highly polymorphic because of the variation in the number of repeat units and evenly spread throughout a genome. Identification of microsatellite markers can effectively assist selection for disease resistance, other agronomic traits, and assist in cultivar or hybrid identification, thus accelerating the process of tomato breeding.

A list of about 1000 solanaceae microsatellites (the majority were tomato) were searched from the GenBank database for the relevant DNA sequences. The entire DNA sequence for each accession was searched, retrieved from the GenBank database and verified for simple sequence repeat (SSR). In total, over 500 *Lycopersicon esculentum* DNA sequences were searched and checked for the presence of SSRs and analyzed for the design of PCR primers; 158 SSR primers were designed, purchased and used to screen a set of diverse genotypes in tomato; the primers were also used to screen the parental materials of the populations used for developing the genetic markers linked to disease resistance; 129 primers were found producing the expected DNA fragments in their PCR products and 65 of them were polymorphic among 19 tomato cultivars; all the SSR markers were characterized; part of these SSR markers were used to screen the parental materials of the population being used to develop SSR markers linked to the resistance to bacterial canker in tomato.

Nineteen geographically different *Lycopersicon esculentum* varieties from the tomato germplasm at Agriculture and Agri-Food Canada, Harrow, Ontario were used to detect polymorphism of simple sequence repeats. Genomic DNA was isolated from young leaves.

All nineteen genotypes from different geographic origins were used to screen the SSR primers to examine their PCR amplification and to detect possible polymorphism. For primers that produced the expected fragments after PCR reactions, the number of

alleles was recorded and the polymorphism information content (PIC) of the SSRs loci was calculated as described by Saal and Wricke (1999):

$$PIC = 1 - \sum_{i=1}^k p_i^2$$

where p_i is the frequency of the i^{th} allele out of the total number of alleles at a SSR locus, and k is the total number of different alleles for that locus.

For phylogenetic analysis, only the data for the polymorphic SSR loci were entered for all DNA samples and only “1” or “0” was used if an allele was present or absent for a genotype, respectively. The data were analyzed using the computer program TREECON (Van de Peer and De Wachter 1994). The genetic distance estimation was based on the method described by Nei and Li (1979). All nineteen different tomato genotypes were clustered based on the estimated genetic distance and the phylogenetic tree topology was inferred with the clustering method of Unweighted Pair Group Method Using Arithmetic Average (UPGMA).

The 65 polymorphic microsatellite loci, together with the primer sequences flanking the SSR loci, were listed in Table 2. The allele number ranged from 2 to 6 and the expected fragment sizes varied from 100 to 385 bp. The polymorphism information content ranged from 0.09 for the primer AW035051 to 0.67 for the primer X90770. For the polymorphic SSR loci, 32 (24.8%) of them showed 2 alleles and 22 (17.1%) had 3 alleles (Table 3) with an average of 2.7 alleles per locus for the polymorphic primers. Most of the SSR loci had 2 (55.0%) and 3 (38.8%) nucleotides per repeat.

For the study on the resistance to bacterial canker and anthracnose, the populations have been raised to F5 generation. For the bacterial canker population, the two parents, LE982831 and TD900170, are being used to screen SSR primers. So far, 6 SSR primers, i.e. AW034362, AW037257, AW039042, TMS33, TMS42, TMS43, were able to generate polymorphism between the parents and more screening is in progress.