E

BMT-TWV/Tomato/1/2
ORIGINAL: English
DATE: March 16, 2001

INTERNATIONAL UNION FOR THE PROTECTION OF NEW VARIETIES OF PLANTS
GENEVA

AD HOC CROP SUBGROUP ON MOLECULAR TECHNIQUES FOR TOMATO

First Session
Le Magneraud, France, March 19 to 21, 2001

LITERATURE REVIEW

Document prepared by the experts from France

A.1/ Consistency and reproducibility

Isozymes

21 (Miller *et al.,* 1990)

- Fingerprint of tomato cultivar on the basis of protein profiles or isozymes were not entirely successful due to the lack of sufficient variation.

17 (Henn *et al.,* 1992)

- Genetic polymorphism within 2 of the 6 enzymes were sufficient to identify the majority of the studied cultivars. (12/17 varieties)

20 (Mather *et al.,* 1993)

- The advantages of allozymes analyses over RFLP's are the lower relative cost and the shorter time needed for visualising gene markers.

Restriction Fragment Length Polymorphism RFLP

22 (Miller *et al.*, 1990)

Effect of different restriction enzymes, probe source, and probe length on detecting restriction length polymorphism in the genus *Lycopersicon.*

- 40 clones: nuclear sequences from 3 different libraries (cDNA, EcoRI genomic, PstI genomic) were hybridised to total DNA.
- Positive relationship between the average genomic fragment size produced with different enzymes and the level of the polymorphism detected. (Enzymes that cut less frequently tend to detect more polymorphism in the tomato genome)
- cDNA clones detect significantly more variation than random genomic clones (e.g. EcoRI clones) or genomic clones selected on the basis of hypomethylation (e.g. PstI clones)
- No correlation between the size of the probe (ranging from 400 to 5.300 bp) and the amount of polymorphism detected by each clone.

Random Amplified Polymorphism DNA RAPD

13 (Foolad *et al.,* 1993)

- No correlation between the primers size and the number of amplified fragments.

16 (Hashizume *et al.,* 1993)

- Effect of the amount and the quality DNA, and reaction volume
  - Amount of the template DNA/ reaction mixture<0.1 ng : banding pattern non reproducible
  - Band intensity: function of template amount, cannot be easily used for discrimination
  - Results essentially the same between DNA extracted by a standard method (Edward *et al*, 1991) and purified DNA
  - Essentially same banding pattern in a range from 1 to 100ng/reaction volume

28 (Rus-Kortekaas *et al.,* 1994)

RAPD are fast, easily generated by PCR reaction and require small amounts of DNA. However, several primers have o ften to be tested to distinguish among cultivars, both for the level of polymorphism detected and for the level of reproducibility.

11 (Dax *et al.,* 1994)

- Non-specific random sets of primers
- None requirement prior sequence information, blotting or hybridisation

14 (Grandillo *et al,* 1996)

75% of the RAPD loci associated with centromeric region.

30, **29** (Saliba-Colombani *et al.,* 1999, 2000)

- RAPD markers are not randomly distributed, 60 % are clustered around putative centromeric regions.
- Deviation from expected ratio: 8%

Amplified Fragment Length Polymorphism _________________________AFLP

15 (Haanstra *et al.,* 1999)

- The EcoRI + MseI AFLP markers were not evenly distributed over chromosomes (centromeric region), whereas the PstI + MseI AFLP markers showed a more even distribution.
- The restriction enzyme PstI is methylation sensitive and would recognise restriction sites in non methylated euchromatin but not in the methylated heterochromatin. Fewer PstI + MseI markers should be obtained but they should be located in more distal part of the chromosomes.
- The choice of restriction enzymes has implications on the number and the location of the AFLP markers.
- Nevertheless, it has to be stated that methylation varies between genotypes and during plant development. It can influence marker analysis and the predictive value of such markers (PstI + MseI markers) within germplasm.
- For map-based cloning, the choice of AFLP markers is dependent upon the position of the gene of interest.
    - The tomato gene Cf-9 conferring resistance to *Cladosporium fulvum* is located in euchromatin (chromosome 6) and consequently PstI + MseI markers were preferred to define the precise position of this gene.
    - The tomato gene Mi, conferring resistance to *Meloidogyne incognita* is located in heterochromatin and EcoRI + MseI markers were preferred to clone this gene.

30 (Saliba-Colombani *et al.,* 1999)

AFLP markers are clustered around putative centromeres. (Deviation observed from expected ratios)

29 (Saliba-Colombani *et al.,* 2000)

- In the case of AFLP loci, the clustering is in part due to the choice of enzymes or to the choice of the primer combinations.
- Despite the different levels of polymorphism between the 3 classes (RFLP, RAPD, AFLP) of markers when compared between different species, AFLP is still the most advantageous since it allows the simultaneous analysis of a large number of DNA bands per gel.
- Even if AFLP markers appear more interesting because of the large number of bands detected on the same gel, they showed a high level of co-segregation and clustering. The efficiency of AFLP markers is thus reduced, as finally, only 61 out of 231 AFLP loci were retained on the framework map.
- AFLP markers are not randomly distributed, 80 % are clustered around putative centromeric regions.
- Deviation from expected ratio: 18%

Single Sequence Repeat = Microsatellite ______________________________SSR

28 (Rus-Kortekaas *et al.,* 1994)

They are hypervariable DNA sequences, which can be probes with labelled oligonucleotides. The amount of work involved makes the detection of microsatellite containing DNA more time consuming than the amplification of RAPDs.

1 (Arens *et al.*, 1995)

Microsatellites GATA and GACA are not evenly distributed (centromeric region)

14 (Grandillo *et al.,* 1996)

100% of the SSR markers associated with centromeric regions

5 (Broun *et al.,* 1994)

- $(GA)_n$ and $(GT)_n$ sequences were found to occur most frequently in tomato genome, followed by $(ATT)_n$ and $(GCC)_n$.
- Only (AT) and (GA) microsatellites (n>7) were found to be frequent in the GenBank database, suggesting that other motifs may be preferentially located away from genes.
- GATA microsatellites are highly clustered in the region of tomato centromeres. Long dinucleotide (>20 repeats : GA, GT, AT) microsatellites markers shown the same location.

31 (Smulders *et al.,* 1997)

- Sometimes several primers pairs do not generate amplification product in some tomato cultivar or species.
- The lack of amplification of an allele in certain cultivars or accessions can be the result of divergence in the sequences flanking the microsatellite, creating null allele.
- However, it can also result from the production of an undetectable amount of PCR product. In this latter case, the optimum PCR conditions for the amplification of a fragment will differ between genotypes. Optimisation of PCR conditions would be necessary for individual cultivars or species from which no fragments have been yet amplified.

2 (Areshchenkova *et al.,* 1999)

- Because of this uneven distribution, genetic mapping of the entire tomato genome using long dinucleotide microsatellites will be very difficult to achieve and markers will shorter arrays of microsatellites could be more suitable although their lower level of polymorphism.
- Long microsatellite arrays (>20 repeats) detect a higher level of polymorphism in tomato.
- The drawback of short microsatellites is that they are not very polymorphic in *L. esculentum* since only microsatellites with a repeat length of more 12 display a reasonable level of polymorphism.
- The generation of microsatellites should always go in parallel with the genetic mapping so that a possible clustering of markers can be noticed at a point when isolation procedures still can be modified.

Whether this centromere-linkage is a general feature of all tomato microsatellites or whether this is a specific feature of the relatively long array (>30 repeats on average) of simple sequence repeats can not be decided at the moment because no significant mapping data are available for shorter tomato microsatellites.

A.2/ Cost and accessibility

cf. documents extracted from D.Zhang, (BMT Subgroup Maïs)

B.1 Use of gene specific markers linked to traditional characteristics

Availability and usefulness of gene specific markers

35 (Tanksley *et al.,* 1988)
Double tagging of the gene **ms-10** conferring a **genic male sterility**, using a morphological and enzymatic marker gene. This gene is placed in *cis* with 2 flanking selectable markers:
Prx-2 (Peroxydase-2)….0.5cM….**ms-10**….5.0cM….aa (anthocyanin absent)
- These linkages should facilitate the use of genic male sterility in production of hybrid tomato seeds.

39 (Vos *et al.,* 1998)
The tomato **Mi-1** gene confers resistance to both **root knot nematodes** (*Meloidogyne incognita*) and **potato aphids** (*Macrosiphum euphorbiae*, gene Meu-1). It exists selective AFLP markers, which allowed cloning this gene.

41 (Williams *et al.,* 1993)
Gene **Tm-2** conferring the resistance to **Tobacco Mosaic Virus**: RFLP markers highly linked to this gene are detected.

11 (Dax *et al.,* 1994)
A RAPD marker for the **Tm-2** gene in tomato
- The validity of the RAPD markers was corroborated by screening several tomato varieties, and correctly identifying those, which carried Tm-2a, as well as by F2 segregation analysis.
- This RAPD assay was developed to identifying TMV resistants, carrying this gene, in inbred lines and heterozygous F1 hybrids.
- The currently used, month long, inoculation and screening procedure can therefore be replaced by a four-day molecular assay.

32 (Sobir *et al.,* 2000)
Molecular characterisation of the SCAR markers tightly linked to the **Tm-2** locus in the genus *Lycopersicon.*
- Comparison of the polymorphism detected between a susceptible and a resistant line
- Determination of closer markers

10 (Chunwongse *et al.,* 1997)
The **Lv** locus confers the resistance to *Leveillula taurica* (Powdery Mildew)
- It is a single dominant gene, located near the centromere of the chromosome 12, surrounded by two RFLP markers:
  - CT121….0.16cM.…**Lv**….0.68cM.…CT129
- In tomato, on average: 1cM= 900kb
- CT121 is estimated to be 0.13cM from Lv, which corresponds to an expected physical distance of approximately 100kb. This distance is small enough that one might be able to use CT121 in a chromosome-landing approach to isolate a single BAC or YAC containing the Lv resistance gene.

18 (Huang *et al.,* 2000)
Development of PCR markers closely linked to the **powdery mildew** resistance gene **Ol-1** on chromosome 6.
- This resistance is controlled by an incompletely dominant gene, **Ol-1,** in the vicinity of Mi, Cf-2, Cf-5 genes. The RFLP markers TG153 and TG164 surround it.
- 5 RAPD markers are sequenced, mapped and converted into SCAR markers. Some of them are specific of one parent (*L. esculentum*) and the others specific of the other parent (*L. hirsutum*).

12 (Doganlar *et al.,* 1998)

The **py-1** gene confers the resistance to the corky root rot (***Pyrenochaeta lycopersici***)

- A single recessive gene
- Field tests are required because greenhouse inoculation is unreliable (lack of reliable greenhouse screening procedure).
- Molecular markers: an alternative mean of diagnosing and screening large populations for disease resistance.
- Development of CAP markers (Cleaved Amplified Polymorphism), conversion of RFLP or RAPD makers .
- Because these markers are codominant, homozygous and heterozygous individuals can be easily distinguished. The CAPs assay, based on the primer set TG 324R/F, is currently being used for screening of disease resistance to aid the introgression of the **py-1** gene into tomato cultivars.

19 (Kawchuk *et al.,* 1998)

Development of sequence characterised DNA markers linked to a dominant ***Verticilium* wilt** resistance gene: gene **Ve** on chromosome 9.

- Objectives: to avoid unreliable phenotypic test and accelerate accurate identification of genotypes.
- Codominant and allele specific (Ve and ve) SCARs are developed.
- The linkage between the genetic marker and the **Ve** locus is less than 0.67 ± 0.49cM.

6 (Causse *et al.,* 2000)

Breeding to disease and insect resistance achieves to F1 hybrids carrying dominant genes, controlling 7 to 9 resistances:

- **TMV**, **TSWV**, ***Cladosporium fulvum*** , ***Verticilium dahliae, Stemphilium*** spp., ***Fusarium oxysporum*** f. sp. ***lycopersici, Fusarium oxysporum*** f. sp. ***radicis lycopersici, Pseudomonas tomato*** and ***Meloidogyne incognita.***
- Others major genes are used in breeding to enhance **fruit quality** and **plant architecture** in tomato. Some of them are cloned and positioned on the tomato genetic map, such as the gene j (**jointless**), the gene nor (**non ripening**), the gene rin (**ripening inhibitor**), the gene sp (**self pruning**) …

Association with traditional quantitative characteristics

26 (Nienhuis *et al.,* 1987)

Restriction Length Polymorphism Analysis of loci associated with **insect resistance**

- Principal toxic factor: 2-tridecanone (2-TD) and other related methyl ketones
- Identification of RFLP loci associated with QTLs affecting expression of insect resistance
- Additional objectives were to determine the gene effects of the RFLP loci and to develop a predictive model for 2-TD mediated insect resistance using the 3 genotyping classes at each RFLP locus as predictor variables.

34 (Tanksley *et al.,* 1988)

Use of molecular markers (RFLP) in breeding for **soluble solids content**

4, 23, 24, and 25 (Breto *et al.,* 1994); (Monforte *et al.,* 1996a, 1996b)

Salt tolerance in *Lycopersicon* species.

- Detection of quantitative trait loci by means of molecular markers.
- Efficiency of markers-assisted selection
- Effect of the genetic variability of QTL on their analysis
- Pleoitropic action of genes: salt tolerance / earliness on fruit yield

30 (Saliba-Colombani *et al.,* 1999)
Detection of QTLs for **organoleptic quality** in fresh market tomato.

- A program of QTL detection for physical, chemical and sensorial traits has been achieved in order to understand the genetic determinism of tomato organoleptic quality
- Construction of a saturated genetic map with RFLP, AFLP, and RAPD markers
- Significant QTL associations with marker loci were identified for each trait. Some of these QTLs, with large identified effects, will be useful for markers aided selection.

7 (Chague *et al.,* 1996)
RAPD markers are linked to the **Sw-5** gene conferring resistance to **TSWV**.

- It is an incompletely dominant gene, heterozygous plants are less resistant than homozygous plants.
- Phenotyping screening of resistance requires time, plants at the right stage, inoculum and restricted area in which to control virus spread.
- Development of molecular markers:
    - To facilitate its introgression
    - To accelerate breeding process
    - To detect more easily without disease testing
- Definition of RFLP and RAPD markers, on chromosome 9, developed into SCARS and Pseudo SCARs.
- Analysis of 13 F3 and 8 BC2 give confirmation of the linkage of the RAPD markers found (they are used in MAS).

43 (Zamir *et al.,* 1994)
The **TY-1 gene** confers tolerance to **Tomato Yellow Leaf Curl Virus**

- RFLP markers are significantly associated with the level of tolerance on chromosome 6 and 3.
- TY-1: gene with partial dominance, but it is the major TYLCV- tolerance locus

8 (Chague *et al.,* 1997)
Identification of RAPD markers linked to a locus involved in quantitative resistance to **TYLCV**.

- 4 RAPD markers were found to be linked to a QTL resistance responsible for up to 27.7% of the resistance.
- These markers are localised in the same linkage group within a distance of 17.3cM (mapped on chromosome6).
- Two resistance tests:
    - Pathogenicity phenotype scoring
    - Agroincubation of young plants on *Agrobacterium* stain bearing the Jordanian TYLCV isolate
    - Scoring with a symptom severity scale
    - Molecular detection of TYLCV
    - Probe: the whole TYLCV genome
    - Quantification of Dot signals using an image analyser
    - Quantification of TYLCV/18S signal ration
- Positive correlation between the 2 tests: r=0.59 and @ <0.001
- Several environmental factors may influence the pathological test, such as room temperature, water uptake and attacks by other pathogens, the result in TYLCV-like symptoms. This may account the low heritability of this test, rendering the squash test more reliable. In this latter test, a direct estimation of virus multiplication in plant is allowed, and did not appear to depend on morphological variation due to external factor, thus resulting in a better heritability than the pathogenicity test.

B.2 Use of DNA profiles regardless of their linkage with traditional characteristics

Assessment of polymorphism revealed in *Lycopersicon*

21 (Miller *et al.,* 1990)
RFLP analysis of phylogenetic relationships and genetic variation in the genus *Lycopersicon.*

- 40 RFLP probes from 3 libraries (DNA library, 2 genomic library: EcoRI et PstI)
- 8 species
- Confirmation of the current classification
- Genetic distances between species have been calculated from the RFLP results.
- cDNA dendrogramme / genomic dendrogramme: nearly identical in their general grouping of species.
- Classification:

Classification:	1- mating behaviour	a- Self Compatible (SC species)
		b- Self Incompatible (SI species)
	2- Fruit colour	a- red fruit
		b- green fruit

- Most of the diversity within these species exists between populations, rather than within populations.
- Amount of genetic variation of the SI species >> Amount of genetic variation of the SC species.
- More genetic variation could be found within a single accession of one SI species than among all accessions tested of any one of the SC species.
- Modern *L. esculentum* cultivars: less than 10% of variation could be accounted for the within-accession variation. Most of the genetic variation was found between accessions.
- *L. esculentum* var *cerasiforme* and landraces: 2-3 times as much within accession variation.
- Lower diversity in the modern cultivars may reflect popular breeding methods (SSD or pedigree selection). (promotion of the genetic uniformity)
- Even though only a limited amount of variation was found between modern cultivars, it was still possible to distinguish cultivars on the basis of one or more unique RFLP.
- RFLP: method of identifying tomato cultivars for legal and practical purposes. (e.g.: testing seed mixture, establishing the identity of questionable seed material)
- Wild species: source of important genes (disease, insect resistance…)
- While other segments of the genome may have only low levels of polymorphism among modern cultivars, the regions around introgressed genes are highly polymorphic facilitating identification of diagnostic RFLP markers of these genes.
- Modern cultivars: narrow genetic base. So, it is not possible to find RFLP markers segregating overall chromosome region for any given cross between cultivars. In fact, it is likely that large tracts of genomes between cultivars may be common by recent descent. Chromosomal region that does show polymorphism may be the ones that contribute most substantially to the characters differentiating varieties.

28 (Rus-Kortekaas *et al.,* 1994)
Direct comparison of the ability of 4 RAPD primers and GACA containing microsatellite probes to detect genetic variation in *Lycopersicon.*

- Goal of the study:
  - to maximise the performance of individuals primers
  - to select for the fewest possible primers that would distinguish as many cultivars as possible.
- Of the 89 RAPD primers initially tested, 85 showed differences between a representative of *L. pennellii* and *L. esculentum*, but only 4 distinguish among 3 *L. esculentum* cultivars. These 4 primers were subsequently tested on representative of six *Lycopersicon* species. In pairwise comparisons of species, single primers could distinguish all or 14 of the 15 combinations.
- The primers were tested on 15 *L. esculentum* cultivars, the four primers could distinguish 90 of the 105 combinations together.
- Tomato cultivars are practically homozygous and hardly any differences between individual plants can be found using RFLPs. None difference at all in RAPD banding pattern could be found among

24 calli and regenerated derived from separate tomato plants of the cultivar Moneymaker , indicating that no polymorphism exited among these plants.

- None of the 118 tested primers (RAPD and SSR) showed reproducible differences among calli or progeny of regenerants from tissue culture, although some of the plants had inherited morphological mutations.
- Modern and vintage cultivars: 44 % of polymorphism (William and St Clair, (1994): Vintage cultivars : 3%; modern cultivars: 12%) The way RAPD primers are selected may be an important factor in determining the overall performance of the method in finding polymorphism. Values for RAPD were maximised by preselection for primers that could distinguish among species as well as cultivars.
- The four selected RAPD primers were able to detect polymorphic bands among species at a frequency of 80%, and among cultivars at a frequency of 44%.
- The microsatellite probe detected polymorphic bands at a frequency of 100 and 95%, respectively. The GACA-containing probe did not detect any common bands among the representatives of the 6 species; while band sharing with RAPDs was 48%. The performance of the microsatellite DNA probes was not optimised, since e.g. a GATA detecting probe appeared to generate even more polymorphism between cultivars.
- To explain the mutation rate of microsatellite DNA, slipping of the DNA polymerase has been suggested. This may be an exclusive phenomenon for short tandemly repeated sequences.
- RAPD DNA thus appears to be more conserved than DNA detected with the microsatellite probe. Therefore, the choice of whether to use RAPDs or microsatellites to distinguish among cultivars or species should depend on the amount of genetic variation expected and the question to be answered. The higher percentage of band sharing in RAPDs makes them more suitable for pedigree relationship studies of tomato species and accessions.
- Microsatellite DNA is more appropriate for the identification of tomato cultivars because in this way more variation can be detected.

Both methods have their drawbacks:

 - necessity for the RAPDs to test the reproducibility rigorously
 - time and amount of work involved in microsatellite fingerprinting

- The best technical solution might very well be the tagging of microsate llites bands with PCR primers (STS, STMS). RAPD bands may also be sequenced and tagged (SCARs). This would lead to a combination of speed and high levels of reproducible polymorphism.
- The two methods detect two types of DNA that differ in their degree of variability.
- The technique that produces the patterns does not change the nature of the types of DNA detected. It can therefore be expected, that primers derived from RAPD bands will mostly amplified bands that are shared by several *Lycopersicon* species, while primers derived from microsatellite bands will mostly amplify bands that are unique for given species.

40 (Vosman *et al.,* 1992)

Phenetic relationships and level of variability detected by RFLP and RAPD analysis of cultivated and wild accessions of *Lycopersicon esculentum*.

- 46 accessions (vintage cultivars, modern cultivars, South American regional cultivars, wild *L. esculentum* var. *cerasiforme*) + 2 *L. cheesmanii*
- 63% RAPD markers: monomorphic for the 48 accessions
- 65% RFLP markers: monomorphic for the 48 accessions
- The identified alleles distinguished the 5 groups and many of the cultivars.
- RAPDs appears promising for both germplasm fingerprinting and as a predictor of genetic diversity for plant breeding applications.
- The within-group variability in vintage cultivars is less than 2.8%. In contrast, introgression of wild germplasm into modern cultivars has increased the polymorphic loci to 11.6%. Within the group of regional cultivars linkage drag and outcrossing may be responsible for the further increase to 20.3%. In *L. esculentum* var. *cerasiforme*, 24.5% of the loci are polymorphic.

31 (Smulders *et al.,* 1997)

PCR analysis of 44 of the microsatellite-containing *Lycopersicon* loci identified 36 primer pairs that yielded well-scorable fragments, or group of fragments, in *L. esculentum* cultivars and accessions of *Lycopersicon* species.

- 29 of these amplified bands that were polymorphic among the four *Lycopersicon* species. 10 primer pairs generated polymorphic bands among 7 tomato cultivars.
- Upon examining the number of microsatellites and the degree of polymorphism in relation to the repeat type and motif, the type of DNA the microsatellite resided in, the length of the microsatellite, and the presence of imperfections in the microsatellite. Only 2 significant correlations were found.
- Imperfect repeats were less polymorphic among species than perfect repeats
- The percentage of loci polymorphic among cultivars increased from 6% for the shortest loci (with 8 or less repeat units) to 60% for the group with the longest repeats (12 repeat units or longer). The degree of polymorphism increased with the total length of the repeat.
- Between the *Lycopersicon* species there was no clear effect of the length of the microsatellite: most loci generated polymorphic fragments. The fact that longer microsatellites generated polymorphism both among cultivars and among species, while short microsatellites did so only among species, but indicate that longer microsatellites are by nature also able to produce polymorphism at a higher frequency among a genetically very homogenous group.
- At the same time, the lower mutation frequency of short microsatellites may render them useful for phylogenetic studies, for which the average mutation frequency of microsatellites is considered to high.
- Among the species, all length classes contained about 83% polymorphic loci.
- 2-4 alleles were found for each locus among the samples of the test set. In a few cases, up to eight alleles were found.
- A combination of these microsatellite loci can therefore be useful in distinguishing cultivars of tomato, which are genetically very closely related to each other.

2 (Areshchenkova *et al.,* 1999)

Long arrays of tetranucleotide microsatellites containing the motif GATA are highly clustered around the centromeres of the chromosomes.

- Isolation of tomato microsatellites containing long arrays (>20 repeats) of the dinucleotide motifs GA, GT, AT, as well as GATA. Their variability in *Lycopersicon esculentum* varieties has been assessed and they are localised onto a genetic map of tomato.
- The investigated microsatellite markers exhibited between 1 and 5 alleles in a divers set of *L. esculentum* lines.
- GATA microsatellites are highly clustered in the region of tomato centromeres. The same location was found for long dinucleotide microsatellite markers.
- Because of the uneven distribution, genetic mapping of the entire tomato genome using long dinucleotide microsatellites will be very difficult to achieve and microsatellite markers with shorter arrays of microsatellites could be more suitable for mapping experimentation in spite of their lower level of polymorphism.
- The variability of the investigated microsatellite markers is higher than those previously studied in tomato. 80% of the microsatellite markers are polymorphic between *L. esculentum* and *L. pennellii*. This is nearly twice the percentage described by Smulders *et al.* (1997) for tomato microsatellites extracted from databases and four times the rate described by Broun and Tanksley (1996). Within the *L. esculentum* gene pool, the number of alleles is also higher than the rate described in previously for tomato. Not taking presence- absence of amplification into account, most of the microsatellites display more than one amplified allele in the investigated tomato varieties. For repeat numbers >20, longer microsatellites arrays detect a higher level of polymorphism in tomato.
- The large number of alleles in *L. esculentum* is often associated with null alleles in *L. pennellii* and that microsatellites with low levels of variability do more frequently amplify fragments from *L. pennellii*. This is confirmed by the isolation of two microsatellite markers, which amplified only fragments from DNA derived from *L. peruvianum* linked to the TMV resistance gene Tm-2. Because of the relatively high number of alleles in *L. esculentum*, some microsatellites described in this study are highly suitable for the analysis of genetic relationships in the cultivated tomato and variety identification.

29 (Saliba-Colombani *et al.,* 2000)

A tomato genetic linkage map based on intraspecific cross between two inbred lines of *Lycopersicon esculentum* and *Lycopersicon esculentum* var. *cerasiforme*. The map is comprised of one morphological, 132 RFLP, 33 RAPD, and 211 AFLP loci.

- The three types of markers have been compared for their polymorphism, segregation, and distribution over the genome. RFLP, RAPD, and AFLP methods revealed 8.7%, 15.8%, and 14.5% informative bands, respectively. This corresponded to polymorphism in 30% of RFLP probes, 32% of RAPD primers, and 100% of AFLP primer combinations.
- In tomato, they revealed approximately twofold more polymorphism with RAPD and AFLP than with RFLP. Previous studies revealed that only a minority of RFLP probes but more than 60% of the RAPD primers discriminate between old and modern cultivars of tomato.

Distinction of cultivated varieties

17 (Henn *et al.,* 1992)

Isozyme analyses to identify cultivated tomatoes

- 6 isozyme systems of tomato seed extracts were resolved by isoelectric focusing on polyacrylamide gel with narrow pH gradient
- 9 ADH phenotypes   identification of 12 of the 17 cultivars
- + 3 APS phenotypes
- Fewer differences were found for the other isozymes.
- This method could differentiate between breeding parents and their progeny.

20 (Mather *et al.,* 1993)

Cellulose-acetate electrophoresis of individual tomato seeds was used to examine the potential of allozymes as gene markers to differentiate between a random selection of tomato cultivars.

- A sufficient allozyme diversity is present amongst randomly-selected tomato cultivar lines for enzyme markers to be useful for application in breeding programmes.
- 13 enzyme loci of the 29 routinely examined in all cultivars were polymorphic and could be used to distinguish between lines. 4 polymorphic loci (Adh-3, Mdh-3, -4, Pgm-1) provided the best discrimination between individual lines.
- 92% of the pair-wise comparisons between cultivars could be distinguished using the 13 polymorphic loci identified. The 4 loci indicated above on their own could distinguish 77 % of the pair-wise comparisons. Therefore, use of a smaller number of gene markers still provides significant discriminatory power, at least amongst the lines examined here.
- Apart from the randomly-selected cultivars and lines examined in this study, all of which were developed from *L. esculentum* genetic material, a number of related species and interspecies hybrids were deliberately included to provide reference points for any allelic variation recognised amongst the *L. esculentum* lines.

13 (Foolad *et al.,* 1993)

Intraspecific analysis. Comparison of the generated polymorphism by RAPD, isozymes and RFLP techniques.

Investigation of the relative potential to generate polymorphic DNA markers within cultivated varieties:

  - 0% isozymes
  - 16% RFLP markers   revealed polymorphism.
  - 63% RAPD markers

- Isozymes and RFLP markers have potential as genetic markers only in interspecific tomato crosses and do not detect sufficient heterozygosity to genetically distinguish cultivated varieties.

16 (Hashizume *et al.,* 1993)

Determination of genetic purity of hybrid seed in tomato using RAPD.

- Objectives: to discriminate parents and their hybrids using a simple primer for each reaction
- 12 RAPD primers were screened to discriminate 2 inbred lines (Morioka and Ottawa). 4 primers showed polymorphism. ¼ was enabled to distinguish the female line Morioka from the F1 hybrid.
- 4-7% of the total PCR products showed polymorphism, suggesting that there close relationships among parental lines.
- Study of the conditions required for PCR and the method for DNA preparation
- Use of the presence / absence of one or several bands.
- Morphological determination of hybrid purity of seeds is rather difficult. RAPD could be effectively used for detection of genetic differences in the parental lines even if they are closely related .

27 (Philips *et al.,* 1994)
Simple sequence repeat oligonucleotides were used to probe the tomato genome for elements displaying variability amongst commercial cultivars.

- Sequence analysis of cloned element reveals that it is almost entirely composed of GACA or GATA repeats.
- The oligonucleotide $(GACA)_4$ was found to be particularly informative on genotype screening blot, hybridising to a highly polymorphic family of elements.
- The combination of multiple bands, most of which are independently-segregating, together with high levels of variability, yields a probe with considerable discriminatory power. Even a highly restricted set of genotypes, such as 27 Californian processing cultivars display substantial banding differences and, with careful analysis, all can be distinguished from each other, thus demonstrating practical utility as a genetic fingerprinting probe.
- Such a predisposition of GACA/GATA repeats to amplification may explain the high level of polymorphism described here in tomato. Further studies are now in progress to establish the stability of loci within defined tomato cultivar lineages.

5 (Broun *et al.,* 1996)
Tomato genomic libraries were screened for the presence of SSR with 17 synthetic oligonucleotide probes, consisting of 2 to 5 basepair motifs repeated in tandem.

- Set of 10 tomato cultivars
- Surprisingly , only 2 of the 9 microsatellite clones surveyed (5 GT_n, 3 GA_n, and 1 ATT_n) showed length variation polymorphism among these accessions. Polymorphism was also very limited between *L. esculentum* and *L. pennellii*, 2 distant species.
- Most of the 17 oligonucleotide probes, including $GGAT_n$, could not detect clear DNA fingerprints under the described hybridisation conditions. In some instances polymorphic fragments could be seen, but high level of background hybridisation, as well as faint signals, prevented reliable genotypic scoring.
- $GATA_n$ and $GAAA_n$ are identified as useful motifs for the detection of multiple polymorphic fragments among tomato cultivars. These oligonucleotide probes allowed the detection of multiple and discrete bands. $GATA_8$ detected the highest levels of polymorphism with the best reproducibility.
- One striking result from this study is the general lack of polymorphism of both GA_n and GT_n tomato microsatellites. Based on numerous reports, hypervariability of simple sequence repeats is assumed to be a universal feature of eukaryotic genomes.

3 (Bredemeijer *et al.,* 1998)
The use of semi-automated fluorescent microsatellite analysis for tomato cultivar identification.

- Objectives:
  - to evaluate the usefulness of a fluorescent-analysis method for genotyping PCR-based tomato microsatellite markers (or STMSs)
  - to establish the value of these markers to generate unique DNA profiles of tomato cultivars.
- 16 tomato cultivars (representing a large spectrum of tomato differing in the type of fruits and in their resistances) were DNA-types for 20 selected STMS markers using fluorescent approach. (In general, analysis of the tomato STMSs revealed distinct allelic peaks.)
- Length polymorphism among the PCR products was detected with 18 of these markers.
- In total, 58 alleles were found, 15 of which were unique. The number of unique alleles per cultivar varied from 0 to 3 for 15 of 16 cultivars, cultivar Mirabell however, had 5 unique alleles. The number of alleles per microsatellite locus ranged from 2 to 8.
- It appeared that closely related cultivars showed more similarity in STMS fingerprint patterns than cultivars of more remote types. Thus the two closely related cultivarss San Marzano Lampadone and San Marzano differed by only 2 loci, while the more remote cultivarss Moneymaker and Mirabell differed by seven loci. All the F1 cultivars were heterozygous for three or more loci.
- As few as 4 microsatellites were sufficient to differentiate between all 16 cultivars, indicating that these markers are especially suitable for a species like tomato which has low levels of variation as detected by other types of markers.

- An aid for selecting additional loci is the information content of a locus, which can be determined by taking into account its ability to differentiate between cultivars. As a measure of information content they used the gene diversity (D). The values of gene diversity associated with each of the tomato STMS markers were rather low, ranging from 0.06 to 0.74 when calculated based upon the set of 16 cultivars.
- In a first place, some markers may not very appropriate for large-scale application because these loci produce difficult-to-score products. Secondly, linkage of a number of the STMS markers used in this study cannot be excluded. GATA- and GACA-containing microsatellites in tomato seem to cluster in the same chromosomal regions. In order to obtain a better choice of microsatellite loci for tomato identification purposes a selection of additional STMSs will be carried out.

Literature

1 **ARENS P., ODINOT P., van HEUSDEN A.W., LINDHOUT P., VOSMAN B.** (1995). GATA- and GACA- repeats are not evenly distributed throughout the tomato genome. Genome. 38 : 84-90.

2 **ARESHCHENKOVA T., and GANAL M.** (1999). Long tomato microsatellites are predominantly associated with centromeric regions. Genome. 42 : 536-544.

3 **BREDEMEIJER G.M.M., ARENS P., WOUTERS D., VISSER D., VOSMAN B.** (1998). The use of semi-automated fluorescent microsatellite analysis for tomato cultivar identification. Theor Appl Genet. 97 : 584-590.

4 **BRETO M.P., ASINS M.J., CARBONELL E.A.** (1994). Salt tolerance in *Lycopersicon* species. III. Detection of quantitative trait loci by means of molecular markers. Theor Appl Genet. 88 : 395-401.

5 **BROUN P., TANKSLEY S.D.** (1996). Characterization and Genetic mapping of simple repeat sequences in the tomato genome. Mol Gen Genet. 250 : 39-49.

6 **CAUSSE M., CARANTA C., SALIBA-COLOMBANI V., MORETTI A., DAMIDAUX R., ROUSELL P**. (2000). Valorisation des ressources génétiques disponibles pour l'amélioration de la tomate. Cahiers Agricultures. 9 :197-210.

7 **CHAGUE V., MERCIER J.C., GUENARD M., de COURCEL A., VEDEL F.** (1996). Identification and mapping on chromosome 9 of RAPD markers linked to *Sw-5* in tomato by bulked segregant analysis. Theor Appl Genet. 92 :1045-1051

8 **CHAGUE V., MERCIER J.C., GUENARD M., de COURCEL A., VEDEL F.** (1997). Identification of RAPD markers linked to a locus involved in quantitative resistance to TYLCV in tomato by bulked segregant analysis. Theor Appl Genet. 95 : 671-677.

9 **CHETELAT R.T., RICK C.M., de VERNA J.W.** (1989). Isozyme analysi s, chromosome pairing, and fertility of *Lycopersicon esculentum* x *Solanum lycopersicoides* diploid backcross hybrids. Genome. 32 : 783-790.

10 **CHUNWONGSE J., DOGANLAR S., CROSSMAN C., JIANG J., TANKSLEY S.D.** (1997). High-resolution Genetic map of the *Lv* resistance locus in tomato. Theor Appl Genet. 95 : 220-223.

11 **DAX E., LIVNEH O., EDELBAUM O., KEDAR N., GAVISH N., KARCHI H., MILO J., SELA I., RABINOVITCH H.D.** (1994). A random amplified polymorphic DNA (RAPD) molecular marker for the $Tm\text{-}2^{a}$ gene in tomato. Euphytica. 74 : 159-163.

12 **DOGANLAR S., DODSON J., GABOR B., BECK-BUNN T., CROSSMAN C., TANKSLEY S.D.** (1998). Molecular mapping of the py-1 gene for resistance to corky root rot (*Pyrenochaeta lycopersici*) in tomato. Theor Appl Genet. 97 :784-788.

13 **FOOLAD M.R., JONES R., and RODRIGUEZ R.** (1993). RAPD markers for constructing intraspecific tomato Genetic maps. Plant cell Report, 12 : 293-297.

14 **GRANDILLO S., and TANKSLEY S.D.** (1996). Genetic analysis of RFLPs, GATA microsatellites and RAPDs in a cross between *L. esculentum* and *L. pimpinellifolium..* Theor Appl Genet. 92 : 957-965.

15 **HAANSTRA J.P., WYE C., VERBAKEL H., MEIJER-DEKENS F., van den BERG P., ODINOT P., van HEUSDEN A.W., TANKSLEY S.D., LINDHOUT P., PELEMAN J.** (1999). An integrated high-density RFLP-AFLP map of tomato based on two *Lycopersicon esculentum* x *L. pennelii* F2 populations. Theor Appl Genet. 99 : 254-271.

16 **HASHIZUME T., SATO T., HIRAI M.** (1993). Determination of Genetic purity of hybrid seed in watermelon (*Citrullus lanatus*) and tomato (*Lycopersicon esculentum*) using random amplified polymorphic DNA (RAPD). Japan. J. Breed.. 43 : 367-375.

17 **HENN G., NEITZ A.W.H. and LOUW A.I.** (1992). Identification of tomato cultivars (*Lycopersicon esculentum*) by polyacrilamide isoelectric focusing. Euphytica. 62 : 77-82.

18 **HUANG C.C., CUI Y.Y., WENG C.R., ZABEL P., LINDHOUT P.** (2000). Development of diagnostic PCR markers closely linked to the tomato powdery mildew resistance gene Ol-1 on chromosome 6 of tomato.). Theor Appl Genet. 101 :918-924.

19 **KAWCHUK L.M., HACHEY J., and LYNCH D.R.** (1998). Development of sequence characterized DNA markers linked to a dominant verticillium wilt resistance gene in tomato. Genome. 41 : 91-95.

20 **MATHER P.B., HUGHES J.M., McGRATH D.** (1993). Identification of random cultivar lines of tomato (*L. esculentum)* from gene markers developed using cellulose-acetate electrophoresis. Seed Sci. & Technol.. 21 : 643-651.

21 **MILLER J.C. and TANKSLEY S.D.** (1990). RFLP analysis of phylogenetic relationships and Genetic variation in the genus *Lycopersicon.* Theor Appl Genet. 80 :437-448.

22 **MILLER J.C., TANKSLEY S.D.** (1990). Effect of different restriction enzymes, probe source, and probe length on detecting restriction fragment length polymorphism in tomato. Theor Appl Genet. 80 : 385-389.

23 **MONFORTE A.J., ASINS M.J., CARBONELL E.A.** (1996). Salt tolerance in *Lycopersicon* species. IV. Efficiency of markers-assisted selection for salt tolerance improvement.. Theor Appl Genet. 93 : 765-772.

24 **MONFORTE A.J., ASINS M.J., CARBONELL E.A.** (1996). Salt tolerance in *Lycopersicon* species. V. Does Genetic variability at quantitative trait loci affect their analysis ? Theor Appl Genet. 95 : 284-293.

25 **MONFORTE A.J., ASINS M.J., CARBONELL E.A.** (1996). Salt tolerance in *Lycopersicon* species. VII. Pleiotropic action of genes controlling earliness on fruit yield. Theor Appl Genet. 98 : 593-601.

26 **NIENHUIS J., HELENTJARIS, SLOCUM M., RUGGERO M., SCHAEFER A.** (1987). Restriction fragment length polymorphism analysis of loci associated with insect resistance in tomato. Crop Sci.. 27 :797-803.

27 **PHILIPS W.J., CHAPMAN C.G.D., JACK P.L.** (1994). Molecular cloning and analysis of one member of a polymorphic family of GACA- hybridising DNA repeats in tomato. Theor Appl Genet. 88 : 845-851.

28 **RUS-KORTEKAAS W., SMULDERS M.J.M., ARENS P., VOSMAN B.** (1994). Direct comparison of levels of Genetic variation in tomato detected by a GACA- containing microsatellite probe and by random amplified polymorphic DNA. Genome. 37 : 375-381.

29 SALIBA-COLOMBANI V., CAUSSE M., GERVAIS L., PHILOUZE J. (2000). Efficiency of RFLP, RAPD, and AFLP markers for the construction of an intraspecific map of the tomato genome. Genome. 43 : 29-40.

30 SALIBA-COLOMBANI V., CAUSSE M., PHILOUZE J., BURET M., ISSANCHOU S., LEESCHAEVE I. (1999). QTLs for organoleptic quality in fresh market tomato. G.T. Scarascia Mugnozza, E.Poceddu & M.A.Pagnotta (Eds.) Genetics and breeding for Crop Quality and Resistance, 291-299.

31 SMULDERS M.J.M., BREDEMEIJER G., RUS-KORTEKAAS W., ARENS P., VOSMAN B. (1997). Use of short microsatellites from database sequences to generate polymorphisms among *Lycopersicon esculentum* cultivars and accessions of other *Lycopersicon* species. Theor Appl Genet. 97 : 264-272.

32 SOBIR, OHMORI T., MURATA M., MOTOYOSHI F. (2000). Molecular characterization of the SCAR Markers tightly linked to the *Tm-2* locus of genus *Lycopersicon.* Theor Appl Genet. 101 : 64-69.

33 SUURS L.C.J.M., JONGEDIJK E., TAN M.M.C. (1989). Polyacrylamide gradient gel electrophoresis : a routine method for high resolution isozyme electrophoresis of *Solanum* and *Lycopersicon* species. Euphytica. 40 : 181-186.

34 TANKSLEY S.D. and HEWITT J. (1988). Use of molecular markers in breeding for soluble solids content in tomato – a re-examination.. Theor Appl Genet. 75 : 811-823.

35 TANKSLEY S.D., and ZAMIR D. (1988). Double tagging of male-sterile gene in tomato using morphological and enzymatic marker gene. HortScience. 23(2) : 387-388.

36 TANKSLEY S.D.,RICK C.M. (1980). Isozymic gene linkage map of the tomato : Apllications in Genetics and breeding. Theor Appl Genet. 57 : 161-170.

37 TANKSLEY S.D., RICK C.M., VALLEJOS C.E. (1984). Tight linkage between a nuclear male-sterile locus and an enzyme marker in tomato. Theor Appl Genet. 68 : 109-113.

38 van den BERG B.M. (1991). A rapid and economical method for hybrid purity testing of tomato (*Lycopersicon esculentum* L.) F1 hybrids using ultrathin- layer isoelectric focusing of alcohol deshydrogenase variants from seeds. Electrophoresis. 12 : 64-69.

VOS P., SIMONS G., JESSE T., WIJBRANDI J., HEINEN L., HOGERS R., FRIJTERS A., GROENENDIJK J., DIERGAARDE P., REIJANS M., FIERENS-ONSTENK J., de BOTH M., PELEMAN J., LIHARSKA T., HONTELEZ J., ZABEAU M. (1998). The tomato *Mi-1* gene confers resistance to both root-knot nematodes and potato aphids. Nature Biotechnology. 16 : 1365-1369

40 VOSMAN B., ARENS P., RUS-KORTEKAAS W., SMULDERS M.J.M., (1992). Identification of highly polymorphic DNA region in tomato. Theor Appl Genet. 85 : 239-244.

41 WILLIAMS C.E. and ST CLAIR D.A. (1993). Phenetic relationships and levels of variability detected by restriction fragment length polymorphism and random amplified polymorphic DNA analysis of cultivated and wild accessions of *Lycopersicon esculentum*. Genome. 36 : 619-630.

42 YOUNG N.D., and TANKSLEY S.D. (1989). RFLP analysis of the size of chromosomal segments retained around the *Tm-2* locus of tomato during backcross breeding. Theor Appl Genet. 77 : 353-359.

43 ZAMIR D., EKSTEIN-MICHELSON I., ZAKAY Y., NAVOT N., ZEIDAN M., SARFATTI M., ESHED Y., HAREL E., PLEBAN T., van OSS H., KEDAR N., RABINOWITCH H.D., CZOSNEK H. (1994). Mapping and introgression of tomato yellow leaf curl virus tolerance gene, Ty-1. Theor Appl Genet. 88 : 141-146.

[End of document]