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Mapping strategy for resistance genes in tomato based on RFLPs between cultivars: *Cf9* (resistance to *Cladosporium fulvum*) on chromosome 1

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Summary. The contribution of introgressed regions derived from wild species to the genetic variation within the species of *Lycopersicon esculentum* was investigated by comparing the RFLP patterns of 2 introgression-free, obsolete cultivars ('Moneymaker' and 'Premier') and a modern cultivar ('Sonatine') that carries at least 5 introgressed resistance genes. In this analysis 195 mapped nuclear markers were used in combination with 6 restriction enzymes. Among the 1170 probe-enzyme combinations tested, only 3 showed a polymorphism between the 2 introgression-free cultivars. On the other hand 24 probe-enzyme combinations were found to exhibit polymorphisms between 'Moneymaker' and 'Sonatine'. These represented ten polymorphic loci distributed among 5 linkage groups on chromosomes 1, 3, 4, 6, and 9.

On the assumption that most of the polymorphic loci corresponded to introgressed chromosome segments of wild species carrying resistance genes, linkages between these loci and the component resistance genes were examined by RFLP analysis of pairs of near-isogenic lines differing only for one particular resistance gene, and a variety of commercial cultivars having different resistance gene compositions. Two of the polymorphic linkage groups could thus be ascribed to resistance genes whose map positions were already known: *Cf2* on chromosome 6 and *Tm2a* on chromosome 9, whereas another marker, TG301 on chromosome 1, could be assigned to the *Cladosporium fulvum* resistance gene *Cf9* with a hitherto disputable map position. By linkage analysis of a segregating F_2 population the genetic distance between the *Cf9* gene and the marker TG301 was estimated at 5.5 ± 2.3 cM.

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Key words: *Lycopersicon esculentum* – RFLP – Resistance gene mapping – *Cladosporium fulvum* – Genetic variation

Introduction

Until the middle of this century the germ plasm of the domesticated descendants of *Lycopersicon esculentum* var '*cerasiforme*' served as the sole source of variability in the horticultural breeding of tomato. In combination with the self-compatible nature of *L. esculentum* and the extensive inbreeding of the material derived from the small seed samples originally introduced into Europe from Mexico in the 16th century, this set a serious restriction to the genetic repertoire of the cultivated tomato (Rick 1991). With the recognition of wild tomato species as an enormous source of potentially valuable genes for plant breeding, the genetic diversity of tomato has been broadened over the past four to five decades by interspecific crosses, resulting in the introduction of chromosomal segments encoding resistances to pests, diseases and environmental stress conditions (Rick 1982; Boukema and den Nijs 1984; Patterson 1988). Nevertheless, the overall genetic variation of modern tomato cultivars has remained limited.

With the advent of restriction fragment length polymorphism (RFLP) markers, tools have become available that allow genetic variation in tomato to be studied at the molecular level. Initial studies by Helentjaris et al. (1985) showed that in tomato, but not in maize, the level of variation between domestic lines was low. More recently, genetic diversity and phylogenetic relationships in the genus *Lycopersicon* were studied in more detail by Miller and Tanksley (1990) using 40 single-copy nuclear probes

of a known map position. Of the 8 *Lycopersicon* species tested, *L. esculentum* showed the lowest level of variation. Within this species, the highest level of genetic variation was found in var '*cerasiforme*' and other land races, while the modern tomato cultivars ranked the lowest.

Most of the polymorphisms detected among the cultivars are likely to be associated with the introgressed regions from the wild relatives, as has been shown for the regions retained around the genes that confer resistance to tomato mosaic virus (Young and Tanksley 1989; Young et al. 1988), *Fusarium oxysporum* f. sp. *lycopersici* (Sarfatti et al. 1989, 1991), *Meloidogyne incognita* (Klein-Lankhorst et al. 1991a, b), *Pseudomonas syringae* pv. tomato (Martin et al. 1991), *Stemphyllium* (Behare et al. 1991), insects (Nienhuis et al. 1987) and for regions found to increase soluble solids content (Osborn et al. 1987; Paterson et al. 1991).

To gain more insight into the contribution of the introgressed regions to the genetic variation of tomato cultivars, we have made a comparison of the RFLP patterns of 2 introgression-free, obsolete cultivars ('Moneymaker' and 'Premier') and a 'modern' cultivar carrying various disease resistance genes. It is shown here that the introgression-free cultivars are virtually free of polymorphisms. Furthermore, in assuming that the polymorphic loci detected in the modern cultivars are present on introgressed regions around resistance genes and thus could provide a clue as to the position of hitherto unmapped resistance genes, we have been able to map the *Cladosporium fulvum* resistance gene *Cf9* on chromosome 1.

Material and methods

Plant material

All true breeding lines were from the collection of the Centre of Genetic Resources (CPRO, The Netherlands). 'Vetomold' (PI 270250), 'Purdue 135', 'Ontario 7719' (PI 126933) and *Lycopersicon peruvianum* PI 128650 were used as original donor lines for *Cf2*, *Cf4*, *Cf9* and *Tm2a*, respectively. Pairs of near-isogenic lines (NILs) for *Cf2*, *Cf4* and *Cf9* were developed at CPRO by backcrossing (5×) the donor parents carrying the respective resistance genes to 'Moneymaker' as the recurrent parent, and subsequently selfing for five generations. The NIL for *Tm2a* had undergone approximately 15 backcrosses to 'Moneymaker'-types. 'Sonatine', its parental lines, and the pair of NILs for *Tm2a* were obtained from *'De Ruiter Seeds'* (Bleiswijk, The Netherlands). Other commerical cultivars were provided by the seed companies as indicated in Table 3.

DNA extraction

Total DNA was extracted from frozen leaves of tomato plants using a method developed by the group of S.D. Tanksley (Cornell University, Ithaca, N.Y.) with some minor modifications. Frozen leaf tissue (1–4 g) was homogenized for 30 s in the presence of STE-buffer (0.35 *M* Sorbitol, 0.1 *M* TRIS-HCl, 5 m*M* EDTA, 20 m*M* NaBisulfite, pH 7.5) using a polytron. After centrifugation (2000 *g* for 20 min) of the homogenate, the green pellet was resuspended in 1.25 ml STE-buffer, mixed with 1.75 ml of nuclear lysis buffer (0.2 *M* TRIS-HCl, 0.05 *M* EDTA, 2 *M* NaCl, 2% CTAB w/v, pH 7.5) and 0.6 ml of 5% Sarkosyl and incubated at 65 °C for 20 min while inverting gently at 5-min intervals. The mixture was then extracted with chloroform:isoamylalcohol (24:1), and the DNA was precipitated with an equal volume of cold isopropanol. DNA was hooked out of the solution, dried, dissolved in TE buffer (10 m*M* TRIS-HCl, 1 m*M* EDTA, pH 7.5) and centrifuged to spin down starch and residual plant material (5 s at maximum speed). The DNA concentration was measured using a fluorometer (TKO 100; Hoefer Instruments).

RFLP analysis

Plant DNA (5 μg) was digested with the restriction enzymes *Hin*dIII, *Eco*RI, *Eco*RV, *Dra*I, *Xba*I and *Taq*I (Bethesda Research Laboratories). Restriction fragments were separated by electrophoresis on 1.0% agarose gels, denatured and blotted onto Genescreen Plus hybridization membranes. The 195 RFLP markers used in this study have been developed and mapped by the group of Tanksley (Tanksley and Muthschler 1990). The positions of the markers are indicated in Fig. 1. Inserts of RFLP markers were amplified by the polymerase chain reaction (PCR), radiolabeled and hybridized in pools of two to four clones. Hybridizations were carried out at 65 °C as described by Klein-Lankhorst et al. (1991a). When a pool of inserts showed a polymorphism, the inserts were tested individually.

A9 elicitor bioassay for the Cf9 gene

Tomato plants were grown in the greenhouse under a 20°/16 °C day/night regime. When the plants were 3 months old, the leaves were injected with 100 μl apoplastic fluid containing the elicitor from avirulence gene *A9* (provided by Pierre de Wit, Agricultural University, Wageningen, the Netherlands) and prepared from a compatible race-cultivar interaction as described by de Wit and Spikman (1982) and de Wit et al. (1988). Control leaves were injected with water. Three days after inoculation, the leaves were evaluated for chlorosis and necrosis, which demonstrate the presence of the *Cf9* gene.

Results

Polymorphisms between obsolete and modern tomato cultivars

As part of a research project that is exploring the potential of DNA markers in breeding programs of the cultivated tomato, we have conducted an extensive survey of the restriction fragment length polymorphisms (RFLPs) in 2 obsolete ('Moneymaker' and 'Premier') and 1 "modern" ('Sonatine') cultivar. To reveal the RFLPs, DNA of these cultivars was digested with 6 different restriction enzymes and subjected to Southern blot analysis using 195 genomic DNA probes with known map positions (Fig. 1). Of all the 1170 probe-enzyme combinations tested, only 3 exhibited a polymorphism between the 2 introgression-free cvs 'Moneymaker' and 'Premier'. This result corroborates other studies (Helentjaris et al. 1985; Miller and Tanksley 1990) in showing a very low level of genetic variation within the species *L. esculentum*. In contrast, 24 polymorphic probe-enzyme combinations repre-

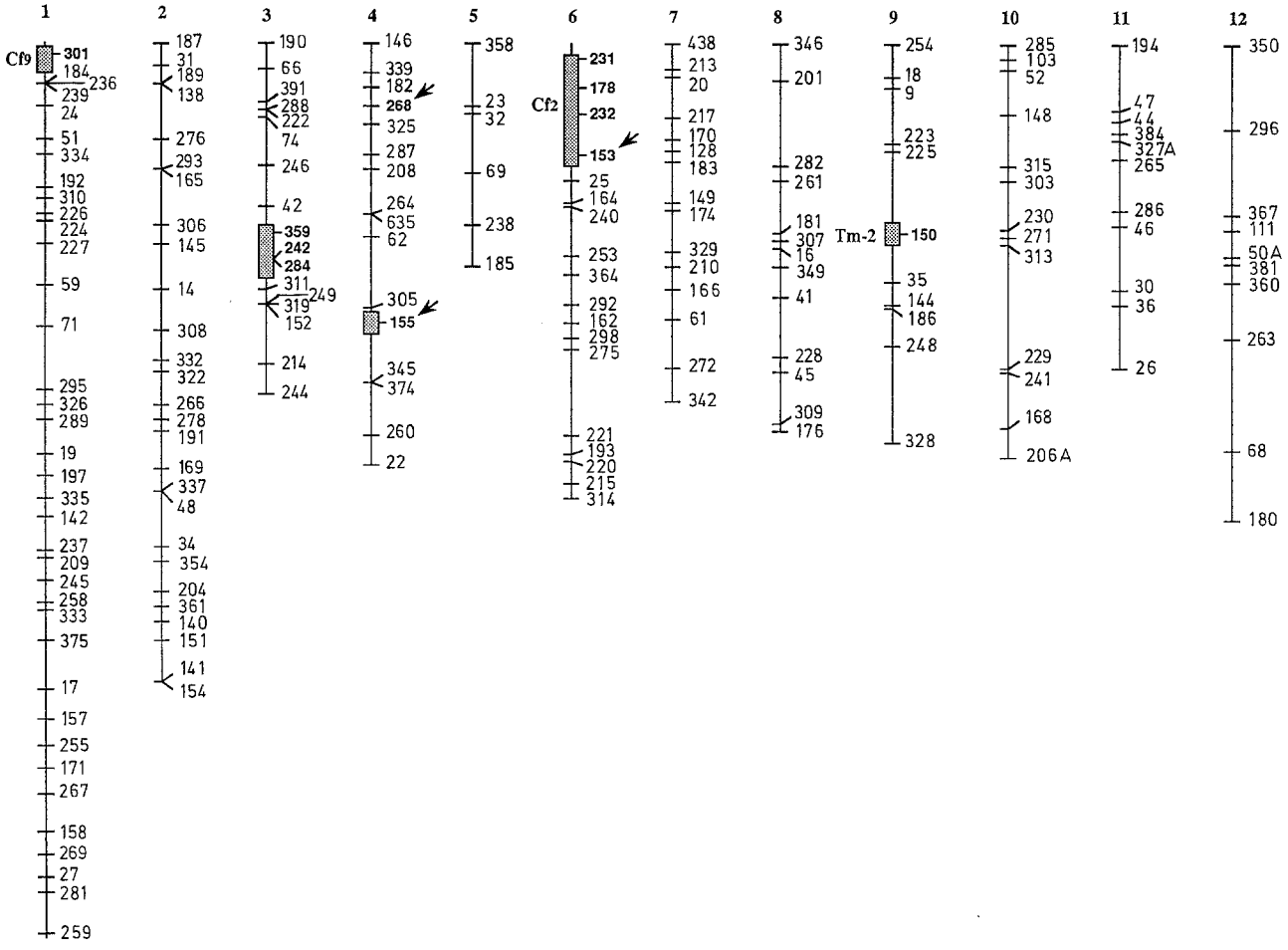

Fig. 1. Genetic map of tomato indicating polymorphic loci among 3 cultivars. The *vertical lines* represent the 12 chromosomes of tomato. The loci examined are indicated with the corresponding *numbers* of the genomic markers (the prefix TG is omitted). The map positions are based on the data of Tanksley with some minor modifications resulting from a recalculation of the dataset from Tanksley by Johan van Ooijen (unpublished). The *arrows* point to polymorphic markers between 'Moneymaker' and 'Premier'. The *boxes* represent chromosomal segments that are polymorphic between 'Sonatine' and 'Moneymaker'. Only resistance genes that have been mapped in the present study are shown

senting ten loci were found between 'Sonatine' and 'Moneymaker' (Table 1). These loci were distributed among five linkage groups on chromosome 1, 3, 4, 6 and 9.

As 'Sonatine' mainly differs from 'Moneymaker' in the presence of 5 or 6 resistance genes (Table 2) that have been introgressed from wild tomato species, the RFLPs detected between the 2 cultivars were likely to be associated with at least some of the resistance genes. To test this hypothesis, pairs of NILs for resistance genes that have already been mapped (*Tm2a* and *Cf2*) were subjected to Southern blot analysis using as probes the RFLP markers exhibiting a polymorphism between 'Sonatine' and 'Moneymaker'. When a comparison was made between 'Moneymaker' and 'Moneymaker-*Tm2a*' which differ for the *Tm2a* gene that confers resistance to tomato mosaic virus (ToMV), TG150 on chromosome 9 uncovered an RFLP between the NILs. Similarly, *TG178* on chromosome 6 revealed a polymorphism between 'Moneymaker' and 'Moneymaker-*Cf2*'.

Further evidence for the linkage of *TG150* to the *Tm2a* resistance gene was obtained by screening a collection of commercial cultivars, which have been independently developed by different breeding companies and which represent various tomato types (Table 3). Among the 28 cultivars tested, all 23 cultivars resistant to ToMV showed the *L. peruvianum*-specific TG150 allele, which was also detected in 'Moneymaker-*Tm2a*' and its donor parent. On the other hand, all 5 susceptible cultivars were found to be homozygous for the *L. esculentum* allele of *TG150*. From these results it is concluded that *TG150* is tightly linked to the *Tm2a* resistance gene on chromosome 9 and thus provides a useful diagnostic marker for this resistance gene. This result is in accordance with that

Table 1. Polymorphic probe-enzyme combinations for tomato cultivars

Marker		Money-maker/ Premier	Moneymaker/Sonatine
Code	Chromo-some		
TG 301	1	–	*Eco*RI
TG 359	3	–	*Hin*dIII
TG 242	3	–	*Hin*dIII, *Eco*RI, *Dra*I, *Xba*I, *Taq*I
TG 284	3	–	*Hin*dIII
TG 268	4	*Eco*RI	–
TG 155	4	*Hin*dIII	*Hin*dIII, *Taq*I
TG 231	6	–	*Eco*RI
TG 178	6	–	*Hin*dIII, *Eco*RI, *Eco*RV, *Dra*I, *Xba*I
TG 232	6	–	*Eco*RI
TG 153	6	*Xba*I	*Xba*I
TG 150	9	–	*Hin*dIII, *Eco*RI, *Eco*RV, *Dra*I, *Xba*I, *Taq*I

Table 2. Some characters of resistance genes in cv 'Sonatine'

Genotype	Resistance gene		Pathogen
	Origin	Chromo-some location	
Cf2cf2	*L. pimpinellifolium*	6[b]	*C. fulvum*
Cf4cf4	*L. hirsutum*	1[c]	*C. fulvum*
Cf9cf9	*L. pimpinellifolium*	10[d]	*C. fulvum*
Ii, I2i2[a]	*L. pimpinellifolium*	11[e]	*F. oxysporum* f. sp. *lycopersici*
Tm2aTm2a	*L. peruvianum*	9[f]	Tomato mosaic virus

[a] It is unknown whether both or one of both genes is present
[b] Langford (1937)
[c] Kerr and Bailey (1964)
[d] Kanwar et al. (1980)
[e] Laterrot (1976)
[f] Hall (1980)

of Young et al. (1988), who showed that *TG101*, which is 3.6 cM from *TG150* (Tanksley and Mutschler 1990), is tightly linked to the *Tm2a* gene.

Similarly, using *TG178* as a diagnostic probe for the *Cf2* gene, we could easily demonstrate the presence of *Cf2* in these cultivars. As indicated in Table 3, the *L. pimpinellifolium* allele of *TG178* was present in 15 of the 17 *C. fulvum* resistant cultivars tested.

Mapping of Cf9

The results described so far indicate that 2 of the 5 polymorphic linkage groups detected between 'Sonatine' and 'Moneymaker' were attributable to the presence of an introgressed region carrying a known resistance gene. By the same token, the other polymorphic loci present on chromosomes 1, 3 and 4 could correspond to other introgressed regions and thus provide a clue as to the location of resistance genes that have not yet been mapped – like *Cf4* and *Cf9*. To test this hypothesis, a pair of NILs for *Cf9* were screened for RFLPs using as probes the genomic clones that uncovered polymorphisms between 'Sonatine' and 'Moneymaker'. The chromosome 1 marker *TG301* detected a distinct RFLP, showing 'Moneymaker-*Cf9*' to contain the *L. pimpinellifolium* allele that was also present in the donor parent for *C. fulvum* resistance, 'Ontario 7719' (Fig. 2). A similar RFLP analysis was performed with the set of NILs for *Cf4*, but in this case none of the RFLP markers tested was found to be discriminatory.

Supportive evidence of linkage between *TG301* and *Cf9* was obtained by analyzing 17 tomato cultivars for the presence of both *Cf9* and the *L. pimpinellifolium*-specific *TG301* allele. The presence of the *Cf9* gene was determined by means of a bioassay that involves the injection of purified elicitor encoded by a virulence gene *A9* and the subsequent scoring for a hypersensitive reaction (de Wit et al. 1988). As shown in Table 2, 6 of the 7

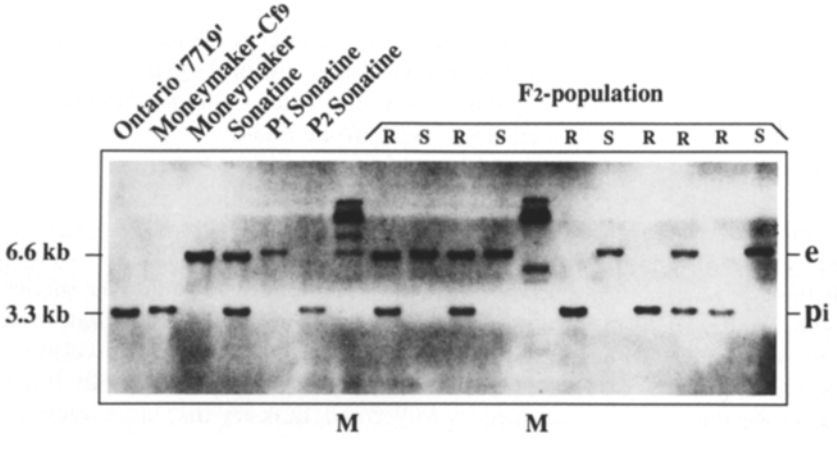

Fig. 2. Linkage analysis between TG301 and *Cf9*. Probe TG301 was hybridized onto blots of *Eco*RI-digested DNA from the genotypes indicated *above* the lanes. The *upper band* of 6.6 kb (*e*) is the *L. esculentum* allele; the *lower band* of 3.3 kb (*pi*) is the *L. pimpinellifolium* allele. Only 10 of the 101 F_2 plants are shown. *R* and *S* refer to the presence and absence of the *Cf9* gene, respectively. *M*, marker

Table 3. Resistance genes and RFLP alleles in tomato cultivars

Cultivar	Origin[a]	Type[b]	Resistance			RFLP-alleles		
			C. fulvum[c,d]		ToMV[e]	TG178[f]	TG150[g]	TG301[h]
Aratino	R	r/o	C5	nd	TmTm	Pi/E	Pe	Pi/E
Belcanto	R	r/o	C5	*Cf9*	TmTm	Pi/E	Pe	E
Bolero	R	r/o	C5	nd	TmTm	Pi/E	Pe	Pi/E
Bornia	RS	r/o	C3	*cf9*	TmTm	E	Pe	E
Caruso	R	o	C5	*cf9*	TmTm	Pi/E	Pe	Pi/E
Cherita	R	c	–	nd	tmtm	E	E	Pi/E
Concreto	R	o	C5	*Cf9*	TmTm	Pi/E	Pe	Pi
Estavette	R	r	C5	nd	TmTm	Pi/E	Pe	Pi
Everset	RS	o	–	*cf9*	TmTm	Pi/E	Pe	E
Floriset	RS	r	C5	*Cf9*	TmTm	Pi/E	Pe	Pi/E
Forlano	R	o	C5	nd	TmTm	Pi/E	Pe	Pi/E
Liberto	R	r/o	C5	*Cf9*	TmTm	Pi/E	Pe	Pi
Mercator	R	r	C5	nd	TmTm	Pi/E	Pe	Pi/E
Moneymaker	RS	r	–	*cf9*	tmtm	E	E	E
Monprecos	RS	r	–	*cf9*	tmtm	E	E	E
Multiset	RS	–	C5	*Cf9*	TmTm	Pi/E	Pe	Pi/E
Narita	RS	r/o	–	*cf9*	Tmtm	E	Pe/E	E
Premier	vN	r	–	nd	tmtm	E	E	E
Ramada	R	r/o	–	nd	TmTm	Pi/E	Pe	E
Restino	R	r/o	C5	nd	TmTm	Pi/E	Pe	E
Robin	RS	o	C	*cf9*	tmtm	E	E	E
Romatos	R	r/o	C5	nd	TmTm	nd	Pe	Pi/E
Royesta	RS	o	–	*cf9*	TmTm	E	Pe	E
Sidonia	RS	r/o	–	*cf9*	TmTm	E	Pe/E	E
Solentos	R	r/o	C5	*cf9*	TmTm	Pi/E	Pe	E
Solido	N	r/o	–	nd	Tmtm	nd	Pe/E	E
Sonatine	R	r	C5	*Cf9*	TmTm	Pi/E	Pe	Pi/E
Wilset	RS	r	C5	*Cf9*	TmTm	Pi	Pe	Pi

[a] Dutch breeding companies: R, de Ruiter, Bleiswijk; RS, Royal Sluis, Enkhuizen; N, Nunhem's Zaden, Haelen; vN, van Noort, Zwijndrecht

[b] Fruit type: c, cherry, 10–15 g; r, round, 70–80 g; r/o, round-slightly oblate, 85–150 g; o, oblate (beefsteak), 160–200 g

[c] –, susceptible to any race of *C. fulvum*; C, C3 and C5 indicate resistance to *C. fulvum* race A, ABC and ABCDE, respectively

[d] *Cf9* and *cf9* indicate the presence and absence, respectively, of *C. fulvum* resistance gene *Cf9* as determined with the elicitor assay; nd, not determined

[e] TmTm, Tmtm and tmtm represent the genotypes *Tm2aTm2a*, *Tm2atm2a* and *tm2atm2a*, respectively. Genotypes with *Tm2a* are resistant to tomato mosaic virus

[f] Pi, *L. pimpinellifolium* allele, 3.0-kb *Eco*RI fragment; E, *L. esculentum* allele, 5.0-kb *Eco*RI fragment; nd, not determined

[g] Pe, *L. peruvianum* allele, 2.5-kb and 1.3-kb *Dra*I fragments; E, *L. esculentum* allele, 3.2-kb *Dra*I fragment

[h] Pi, *L. pimpinellifolium* allele, 3.0-kb *Eco*RI fragment; E, *L. esculentum* allele, 7.0-kb *Eco*RI fragment

The fruit type, *C. fulvum* resistance and tomato mosaic virus resistance data were obtained from the catalogues of the breeding companies. The presence of the *Cf9* gene and the RFLP patterns were determined in this study

cultivars containing the *C. fulvum* resistance gene *Cf9* did indeed reveal the *L. pimpinellifolium TG301* allele. Among the 10 cultivars without the *Cf9* gene, 9 were homozygous for the *L. esculentum TG301* allele. These results provided further evidence that *TG301* is linked to the *Cf9* gene on chromosome 1.

Finally, to determine the genetic distance between *TG301* and *Cf9* an F_2 population obtained by selfing the F_1 hybrid cv 'Sonatine' (heterozygous for the *Cf9* gene) was analyzed for the segregation of *Cf9* and *TG301*. Of the 101 F_2 plants analyzed, 69 were resistant, 65 of which showed the *L. pimpinellifolium TG301* allele. Thirty-one of the 32 susceptible plants were homozygous for the *L. esculentum* allele; in only 1 susceptible plant was the *L. pimpinellifolium* allele found. From these recombination frequency data, it was calculated (Allard 1956) that *Cf9* is located $5.5 \pm 2{,}3$ c*M* from *TG301*.

Discussion

Among the major crop plants studied so far, the species *L. esculentum* is notorious for its limited genetic variation and the paucity of RFLPs detectable between cultivars (Helentjaris et al. 1985; Miller and Tanksley 1990). It has been argued by Miller and Tanksley that large tracks of

the genomes between cultivars may even be common and that the (relatively few) polymorphisms found between obsolete and modern varieties are largely due to introgression from wild relatives. A dramatic example of the virtual absence of polymorphisms between 2 types of obsolete, introgression-free, fresh-market tomatoes is shown in the present paper. Of the 195 RFLP markers tested using 6 different restriction enzymes, only 3 markers were capable of distinguishing the 2 cultivars. Although these 3 markers may turn out to be useful in being associated with the characters differentiating the 2 varieties, it is apparent that in tomato RFLP markers are not the universal, informative molecular markers as originally anticipated. In this respect, it would be worth investigating whether other types of molecular markers (Welsh and McClelland 1990; Williams et al. 1990; Klein-Lankhorst et al. 1991 b; Lindhout et al. 1991) are more appropriate to reveal minor changes in the genomes of related genotypes.

As discussed by Tanksley et al. (1989), the merit of RFLP markers in tomato breeding mainly lies in their ability to diagnose the presence and facilitate the transfer of alien highly polymorphic chromosomal regions containing valuable traits. Indeed, when the comparison was made between 'Moneymaker' and 'Sonatine', the latter cultivar containing a number of introgressed resistance genes originating from various wild species, more polymorphisms were detected. For some of those linkage to a resistance gene could be established: *TG178* to *Cf2* on chromosome 6, *TG150* to *Tm2a* on chromosome 9 and *TG301* to *Cf9* on chromosome 1. However, unlike the relatively high degree of variation encountered among the American cultivars studied by Miller and Tanksley (1990) – up to 20% of the probes were polymorphic using five restriction enzymes – the genetic variation between 'Moneymaker' and 'Sonatine' was nevertheless remarkably low. Only 24 out of 1170 probe-enzyme combinations tested were polymorphic. Apparently the introgressed regions carrying the resistance genes were substantially reduced in size during the breeding of this particular cultivar. These results are in line with those of Young and Tanksley (1989) in showing that the size of the introgressed region retained around the *Tm2* locus is not related to the number of backcross generations. Some breeding programs achieved significant reduction in linkage drag within only a few generations, while others involving large numbers of generations were ineffective in that respect.

In the 1960s gene *Cf2*, which originates from *L. pimpinellifolium* and confers resistance to *C. fulvum*, was introduced into various Dutch tomato cultivars. After breakdown of the *Cf2* resistance by the *C. fulvum* race carrying virulence gene 2, breeding programs were designed to introduce other *Cf* resistance genes, like *Cf4*, *Cf5* and *Cf9*. Nowadays cultivars resistant to *C. fulvum* are either of the so-called C3 or C5 type (Table 2), indicating that they are resistant to races ABC and ABCDE, respectively. Cultivars with C3 resistance carry any *Cf* gene different from *Cf2* and *Cf4*, whereas C5 cultivars carry any *Cf* gene different from *Cf2*, *Cf4* and *Cf5* (Hubbeling 1978; Laterrot and Clergeau 1979). Remarkably, 15 out of the 17 *C. fulvum*-resistant tomato cultivars tested showed the *TG178* allele of *L. pimpinellifolium* that is linked to *Cf2*. Apparently *Cf2* cultivars have frequently been used as recurrent parents in breeding programs aimed at the introduction of other *Cf* genes.

To date, the fate of the *Cf2* gene can not be followed by disease tests in cultivars that also carry the *Cf9* gene since no *C. fulvum* races have been identified that carry virulence gene 9 but not virulence gene 2. In this respect, the marker *TG178*, which allows the *Cf2* gene to be detected irrespective of other *Cf* genes, may serve as a useful diagnostic probe, particularly in breeding for durable resistance. By using diagnostic probes for each of the genes contributing to the resistance to a particular pathogen, the individual resistance genes can be combined into a single cultivar. This should pose a serious delay to the appearance of races carrying new virulence genes.

The mapping of gene *Cf9* on the short arm of chromosome 1 contradicts Kanwar et al. (1980), who reported *Cf9* to be located on chromosome 10 at position 35, but is completely in line with recent mapping data from Jones et al. (1991). In view of the very small F_2 population (36 individuals) used in determining linkage to two morphological markers, the statistical significance of the mapping data of Kanwar et al. is questionable (see also Gerlagh et al. 1989), particularly with reference to our present data that are based on a F_2 population of 101 individuals. As the mapping data obtained with both the NILs for *Cf9* and the set of commerical varieties are, furthermore, in accordance with the linkage analysis, we conclude that *Cf9* is located on chromosome 1, 5.5 ± 2.3 cM from *TG301*.

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