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The assessment of TGGE for the detection of interspecific and intergeneric DNA-marker polymorphism within Solanaceae

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Abstract. RFLP markers are currently the most appropriate marker system for the identification of uncharacterised polymorphism at the interspecific and intergeneric level. Given the benefits of a PCR-based marker system and the availability of sequence information for many Solanaceous cDNA clones, it is now possible to target conserved fragments, for primer development, that flank sequences possessing interspecific polymorphism. The potential outcome is the development of a suite of markers that amplify widely in Solanaceae. Temperature gradient gel electrophoresis (TGGE) is a relatively inexpensive gel-based system that is suitable for the detection of most single-base changes. TGGE can be used to screen for both known and unknown polymorphisms, and has been assessed here, for the development of PCR-based markers that are useful for the detection of interspecific variation within Solanaceae. Fifteen markers are presented where differences between *Lycopersicon esculentum* and *L. pennellii* have been detected by TGGE. The markers were assessed on a wider selection of plant species and found to be potentially useful for the identification of interspecific and intergeneric polymorphism in Solanaceous plants.

Additional keywords: tomato, potato, *Solanum*, capsicum, pepper.

Introduction

RFLP markers are currently the most appropriate marker system for identifying polymorphism at the interspecific and intergeneric level. Given the availability of DNA sequence information and the benefits of PCR, it is now appropriate to develop PCR-based marker systems to replace RFLP procedures. At the start of this investigation, the Solanaceae Genomics Network (SGN) (http://www.sgn.cornell.edu/markers/cos_markers.html) contained sequences for approximately 1000 conserved-ortholog-set markers (COS). COS markers are single-copy tomato EST clones that are highly conserved between tomato (*Lycopersicon esculentum*) and *Arabidopsis*. Increasing amounts of EST sequences for a range of *Lycopersicon* and *Solanum* species are also available at SGN. It is now possible to target fragments conserved within Solanaceae that flank interspecific polymorphisms, for the development of PCR-based markers for use in genetic mapping, comparative genetic mapping, or the identification of introgressed fragments (e.g. via cell fusion).

The power of temperature gradient gel electrophoresis (TGGE) to detect mutations, known and unknown, makes it a useful tool for the identification of single nucleotide polymorphism (SNP), other DNA polymorphisms, and the development of new PCR-based marker systems. TGGE is a relatively inexpensive gel-based system and is suitable for the detection of most single-base changes (Sheffield *et al*. 1989).

The analysis of double-stranded DNA by TGGE (Rosenbaum and Riesner 1987) requires fragments of 100–500 bp. The fragments must possess a high-melt domain (naturally occurring GC-rich region, GC clamp, or chemical clamp) and a low-melt domain. Polyacrylamide gel electrophoresis is used to run fragments through a gradient of increasing temperature. Fragments that have a different sequence in the low-melt domain will reversibly denature into Y-shaped (non-rigid) molecules at different temperatures and therefore at different positions in the gel. Further migration is greatly reduced once the low-melt domain has denatured. Alleles with a different sequence within the low-melt domain may then be identified by their relative positions in the gel.

Further resolution in the separation of allelles can be achieved by heteroduplex analysis (Lessa and Applebaum 1993; Qiagen 1993; Elphinstone and Baverstock 1997). When a mixture of 2 alleles is denatured and annealed, 2 types of hybrid molecules are formed (heteroduplices) along with the reformation of the original 2 molecules (homoduplices). The heteroduplexed products will have mismatched base pairing and will usually denature at lower temperatures

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than the homoduplices. Optimally, up to 4 bands will be seen when 2 alleles are heteroduplexed and analysed by TGGE. Heteroduplex/TGGE analysis could be used to screen for different alleles in populations or DNA samples, and can naturally occur in the analysis of PCR products from a heterozygous individual (Lessa and Applebaum 1993; Elphinstone and Baverstock 1997).

Currently, TGGE and related systems such as denaturing gradient gel electrophoresis (DGGE) and temporal temperature gel electrophoresis (TTGE) are frequently used to assess diversity in complex mixtures of organisms. For example, these systems have been used to study bacterial communities (Gomes *et al*. 2001; Brummer *et al*. 2003; Fouratt *et al*. 2003; Nishida *et al*. 2003; Ramirez-Saad *et al*. 2003; Chen and Banks 2004; Haruta *et al*. 2004; Henri-Dubernet *et al*. 2004; Luo *et al*. 2004; Ogier *et al*. 2004; Xu and Leff 2004), and eukaryotic communities (Carlos *et al*. 2000; Hernan-Gomez *et al*. 2000; Anderson and Cairney 2004; Ikenaga *et al*. 2004; Prakitchaiwattana *et al*. 2004; Savin *et al*. 2004). These systems are also commonly used to screen for polymorphisms in human DNA (Toliat *et al*. 2000; Muller *et al*. 2001; Katzke *et al*. 2002; Lage *et al*. 2004; Mihic-Probst *et al*. 2004; Saarikoski *et al*. 2004; Sekiguchi *et al*. 2004; Soufi *et al*. 2004) and less commonly used in wildlife and animal population genetics (Heslewood *et al*. 1998; Jerry and Baverstock 1998; Houlden *et al*. 1999; Leo and Barker 2002; da Mota *et al*. 2004; Westerdahl *et al*. 2004). The author is not aware of any publications that involve the use of TGGE, or other related systems, for the investigation of vascular plants.

The author has assessed TGGE for the development of PCR-based markers that are widely useful for the detection of interspecific variation within Solanaceae.

Materials and methods

Conserved ortholog set (COS) markers from the Solanaceae Genomics Network (SGN) (http://www.sgn.cornell.edu/markers/cos_markers.html) were identified that possessed orthologous EST sequences for *Lycopersicon esculentum* (tomato), *L. pennellii* (wild tomato relative), *Solanum tuberosum* (potato), and *Arabidopsis thaliana.* GenBank blast searches were performed in order to identify sequences for other species, and other sequences available from SGN were included if available. Sequences were aligned using ClustalW (WWW Service at the European Bioinformatics Institute http://www.ebi.ac.uk/clustalw; Higgins *et al*. 1994). The comparison between *L. esculentum* and *L. pennellii* was used to identify fragments demonstrating interspecific polymorphism. Primers were designed for these fragments if the following criteria were met:

(*a*) the presence of suitable primer sites flanking the polymorphic regions with close to 100% conservation between the tomato species and potato;
(*b*) the primer sites also demonstrated moderate to good conservation with *Arabidopsis*;
(*c*) the primer sites produced a product of 80–500 bp, with preference for product sizes of 150–400 bp;
(*d*) fragments possessed a distinct, single, melting domain, when analysed by the computer program Poland (Steger 1994), at Heinrich-Heine-Universität Düsseldorf, Institut für Physikalische Biologie, http://www.biophys.uni-duesseldorf.de/local/POLAND/poland.html;
(*e*) fragments, with GC clamp, possessed distinct high- and low-melting domains; and
(*f*) the fragment to be amplified did not contain an intron, as identified in the gDNA sequence from *Arabidopsis*.

It was hoped that the resulting fragments would amplify widely within Solanaceae and possibly amplify for some non-Solanaceous plants, possess interspecific variation, and with the addition of a GC clamp be suitable for analysis by TGGE.

PCR primers were designed for 38 ESTs, using the computer program Primer3 (Rozen and Skaletsky 2000). Either the forward or reverse primer was chosen for the addition of the GC clamp so as to reduce steep melting profiles at the end of the fragment, thus producing a relatively flat profile for the low melt domain (the fragment incorporating the GC clamp would possess a high- and low-melting domain). The GC clamp 5′-CGCCCGCCGC GCCCCGCGCCCGGCCCGCCGCCCCCGCCCC-3′ (Muyzer *et al*. 1993) was added to the chosen primer, of a primer pair that possessed higher C content than G (especially for the 3′ end); or the reversed, reverse-compliment sequence 5′-GCGGGCGGCGC GGGGCGCGGGCCGGGCGGCGGGGGCGGGG-3′ if the primer pair had higher G content than C content (especially at the 3′ end). On a few occasions the GC clamp was constructed in reverse order or with slight variations in order to reduce the chance of self-binding or primer pair interaction. Primers were synthesised by Proligo Pty Ltd, SCU, Lismore, NSW, Australia.

Primer pairs were tested for amplification using the following conditions. PCR reactions were carried out on a Perkin Elmer, Gene Amp PCR System 9700. The reaction volume was 20 μL containing 1 × Roche PCR Buffer, approximately 50 ng of genomic DNA, and 188 μM dNTPs. The PCR reaction mixture also included 100 nM of each primer, 5.5 mM total $MgCl_2$, and 0.1 units of Taq DNA Polymerase (Roche). The temperature cycling conditions were 3 min at 94°C; followed by 35 cycles of 94°C for 30 s, 56°C for 30 s, and 72°C for 1 min; with a final hold at 72°C for 1 min. An annealing temperature of 50°C was used when the primers were tested on non-tomato species.

DNA was extracted from approximately 0.03 g of fresh leaf tissue and ground with 1 mL of extraction buffer at 60°C (Edwards *et al*. 1991). The extract was added to 700 μL of chloroform/isoamyl alcohol (24 : 1), mixed, left on ice for at least half an hour, and centrifuged at 11200 rcf for 3 min. DNA was precipitated from the supernatant after adding 1 volume of isopropanol followed by 5 min centrifugation at full speed. The pellet was washed twice with 70% ethanol and resuspended in 100 μL of Tris-EDTA (TE) buffer at pH 8.

The orthologous fragments for 33 primer pairs, from *L. esculentum* (Rouge de Marmande, D.T. Brown & Co. Ltd), *L. pennellii* (LA716), and an F_1 individual resulting from a cross between the 2 species, were analysed by TGGE. Five primer pairs did not amplify DNA.

PCR products were heteroduplexed on a Perkin Elmer, Gene Amp PCR System, 9700 by 2 cycles of 95°C for 5 min, cooling to 50°C at a 5% ramp rate, and 50°C for 30 s. TGGE was performed on a TGGE maxi system, Whatman Biometra. Running conditions included: 8% polyacrylamide gel (37.5 : 1) with 2% glycerol and 8 M urea, 1 × MOPS buffer, 200 V for 6.5 h, and a parallel temperature gradient of 40–60°C. Fragments were detected by silver staining following the procedure recommended by Whatman Biometra.

Three primer pairs, representing COS markers T1359, T1277, and T1212, were tested on a range of plant species, and

analysed for the determination of segregation-ratio in a F_2 mapping population (*L. esculentum*/*L. pennellii*, $n = 60$). The range of plant species included *L. pimpinellifolium*, *L. hirsutum*, *Solanum tuberosum* (potato), *Solanum melongena* (egg plant), *Capsicum annuum*, *Capsicum chinense*, *Nicotiana tabacum* (tobacco), *Petunia* sp. (Order Solanales, Family Solanaceae); *Ipomoea batatus* (sweet potato) (Order Solanales, Family Convolvulaceae); *Impatiens balsamina* (Family Balsaminaceae); *Raphanus sativus* (radish), *Brassica campestris* (turnip), *Brassica oleracea* (cabbage) (Family Brassicaceae); *Pisum sativa* (pea), *Phaseolus vulgarus* (bean) (Family Fabaceae Papilionoideae); *Cucurbita pepo* (pumpkin), *Cucumis sativus* (cucumber) (Family Cucurbitaceae); *Daucus carota* (carrot) (Family Apiaceae); *Beta vulgarus* (beet) (Family Chenopodiaceae); *Abelmoschus esculentus* (okra) (Family Malvaceae); and *Latuca sativa* (lettuce) (Family Asteraceae Lactuceae).

DNA was extracted as previously described; however, carrot and lettuce required a further ethanol precipitation and re-suspension, impatiens and okra were extracted using a Nucleospin column (Macherey-Nagel), and 4% PVP was added to the extraction buffer for sweet potato. All gDNA preparations were tested for suitability as PCR templates through positive amplification using internal transcribed spacer (ITS) primers 1 and 4 (White *et al*. 1990).

The remaining primer pairs were tested on the Solanaceous species and sweet potato. Tomato introgression lines (Eshed and Zamir 1994), possessing single chromosomal fragments from *L. pennellii* (LA716), of known genome location, were used to confirm the expected genetic-map position of the markers. Expected map positions were based on the Tomato_Arabidopsis synteny map (SGN: http://www.sgn.cornell.edu/maps/tomato_arabidopsis/synteny_map.html).

Those markers that amplified for *Capsicum* were analysed by TGGE to identify polymorphisms between *C. annuum* and *C. chinense*.

Results

Fifteen primer pairs (Table 1) produced TGGE results where alleleic differences between *L. esculentum* and *L. pennellii* could be detected. General characteristics of the fragments are provided in Table 2 and the relative positions of DNA bands produced by TGGE analysis are provided in Table 3.

Table 1. Primers specific to conserved ortholog set (COS) markers, Solanaceae Genomics Network (SGN)

Amplified fragments are suitable for TGGE analysis
GC Clamp 1: CGCCCGCCGCGCCCCGCGCCCGGCCCGCCGCCCCCGCCCC
GC Clamp 2: GCGGGCGGCGCGGGGCGCGGGCCGGGCGGCGGGGGCGGGG
GC Clamp 3: CGCCCGCCGCGCCCCGCGCCCGCCCCGCCGCCCCCGCCCG
GC Clamp 4: CCCCGCCCCCGCCGCCCGGCCCGCGCCCCGCGCCGCCCGC

COS marker	Primer name	Oligonucleotide sequence (5′–3′)
T1208	GC-T1208F	(GC Clamp 1) AAAGCCATTATTCTCCCTGTAA
	T1208R	GCACCTCTAGCAAGTCCTT
T1409	GC-T1409F	(GC Clamp 1) CATCTTTCCCTTACTTCATGCT
	T1409R	ATCTGAATAGCTAATGCTTCGGA
T1485	T1485F	TGTCCAGCAAGGATTAGCTGCT
	GC-T1485R	(GC Clamp 2) AGCTTGCGGACAGTGATGAAATT
T1361	GC-T1361F	(GC Clamp 2) CAGAGGATGGAAATATGGATC
	T1361R	TGAACTGCTACATTGATTAGATC
T1308	GC-T1308F	(GC Clamp 2) CTGTAGACATTTCAAGAGGATACTT
	T1308R	TGTCCTGGGTCATATAAAAGATG
T1181	T1181F	GGGAAACTTCCTAAGCATAATA
	GC-T1181R	(GC Clamp 1) CAGGATACTCAATCACACTCCA
T1510	GC-T1510F	(GC Clamp 2) ATCGAATCCATTGCTGTTGG
	T1510R	CCAGCTGAAGAAGGGTAAGAGTT
T1277	GC-T1277F	(GC Clamp 1) GGTAATGTAAATGCACCTTATGAA
	T1277R	TCAACGACAGATGACTGTGC
T1581	T1581F	TGAAGAAAGCCGTGATGCT
	GC-T1581R	(GC Clamp 3) CTCTAGTAGCATACATAATTGC
T1359	T1359F	AAGGAGGAGGAATTTGTGTT
	GC-T1359R	(GC Clamp 4) CGGTTAAACTCATCAGAATCAT
T1212	T1212F	AGAAAATCACAATGGCAGCA
	GC-T1212R	(GC Clamp 1) GAGACAACATCAAGTTTCTTCTGAT
T1482	GC-T1482F	(GC Clamp 1) CACAACTCCTGGCTTGCAAAT
	T1482R	CTGAAACTGGCACTTGTTCACT
T0736	GC-T0736F	(GC Clamp 1) TCTATATCAACCATGTACGGATTC
	T0736R	ATTTTGGAGATAAGGACCGTATT
T1125	GC-T1125F	(GC Clamp 2) ATGATTTTTTGGCTGGGAGACT
	T1125R	CCTGGAGAATGCAAAGTGGTTT
T1460	GC-T1460F	(GC Clamp 1) TTGCAGTGGAGATGAAACAGC
	T1460R	GCTACGTTCCACCAGCTGAA

Table 2. General characteristics of PCR products of conserved ortholog set (COS) based markers, Solanaceae Genomics Network (SGN)

COS marker	Exp. size (bp)[A]	Obs. size as expected[B]	No. of SNP[C]	SNP positions relative to GC clamp (GC)	Tomato chromosome no.	%GC
T1208	410	Yes	5	(GC)-71-80-153-333-357-	1	38
T1409	130	Yes	3	(GC)-32-101-107-	1	46
T1485	243	Yes	4	-221-196-174- 27-(GC)	1	43
T1361	180	Yes	2	(GC)-146-151-	2	41
T1308	135	No[D]	2	Not applicable	3	–
T1181	190	Yes	2	-164-76-(GC)	5	42
T1510	182	Yes	2	(GC)-51-54-	7	39
T1277	124	Yes	2	(GC)-57-104	8	41
T1581	143	No[E]	3	Not applicable	8	–
T1359	149	Yes	3	-99-87-39-(GC)	8	44
T1212	427[F]	Yes	3[G]	-indel(407-393)-333-308-120-(GC)	9	38
T1482	242	Yes	2	(GC)-64-146-	10	38
T0736	192	Yes	3	(GC)-25-45-159-	10	45
T1125	222	Yes	4	(GC)-38-113-165-166-	11	32
T1460	88	Yes	2	(GC)-34-48-	11	45

[A]Expected size does not include GC clamp.
[B]Observed size is based on assessment by agarose gel electrophoresis.
[C]Number of single nucleotide polymorphisms between orthologueous sequence for *L. esculentum* and *L. pennellii* as supplied by the SGN.
[D]>200 bp.
[E]About 250 bp.
[F]427 bp in *L. pennellii*, 412 bp in *L. esculentum*.
[G]Plus 1 indel (insertion/deletion) of 15 bp.

Table 3. TGGE Gel relative positions for PCR-amplified DNA bands

Bands represent alleles and heteroduplices for *L. esculentum* and *L. pennellii*, for various conserved ortholog set (COS)-based markers. The gel position is presented as the temperature for a given position within the experimental gradient of 40−60°C as determined on a single gel. The band positions and relative positions between bands will vary slightly between runs

COS marker	Rel. pos. of *L. esculentum* allele (°C)	Rel. pos. of *L. pennellii* allele (°C)	Rel. pos. of heteroduplex bands (°C)
T1208	47.8	47.1	45.1 and 43.6
T1409	55.9	53.6	44.0 and 42.8
T1485[A]	48.3	49.4	45.0 and 44.4
T1361	47.3	48.8	45.5
T1308	45.4	45.1	42.9 and 42.0
T1181	49.4	50.0	49.1
T1510	48.1	47.8	46.3 and 46.0
T1277[B]	51.6	50.5	49.0 and 47.5
T1581	51.3	50.8	None
T1359	52.9	52.0	48.0 and 47.3
T1212	46.1	46.6	41.6
T1482	47.3	46.6	45.5 and 44.4
T0736	52.9	51.3	49.1 and 47.6
T1125	43.8	43.1	42.3 and 41.9 (double bands)
T1460	54.5	55.3	50.0 and 48.9

[A]A double-banding pattern was occasionally produced for putative alleles of this marker.
[B]A double-banding pattern was produced for putative alleles of this marker.

The results represent a success rate of 39.5% for the conversion of COS DNA sequences into useful TGGE-based markers where differences between orthologous fragments of *L. esculentum* and *L. pennellii* could be detected. The success rate improves to 45.5% if consideration is only given to primer pairs that successfully amplified PCR product.

The results concerning the mapping of markers using introgression lines are presented in Table 4. All analysed markers (no results for T1361) were located at or near the expected genome location, except for the markers representing T1212 and T1277. An important introgression line needed for T1212 was not available, making a positive result impossible. A *L. pennellii* allele was not detected in the introgression line expected to contain the T1277 marker. In fact, all 3 introgression lines analysed, which in total cover all of chromosome 8, delivered negative results. The results suggest that the marker representing T1277 is not on chromosome 8.

The assessment of the markers for PCR amplification in a range of Solanaceous species and sweet potato (order Solanales) is presented in Table 5. The results indicate that the 7 primer pairs representing COS markers T1208, T1485, T1277, T1581, T1359, T1212, and T1482 amplified for all of the Solanaceous species analysed. Another 4 primer pairs (T1409, T1181, T0736, T1125) either did not amplify, or poorly amplified, for *Petunia* sp. but amplified for all other Solanaceous species. Petunias are more distantly related to tomato than the other Solanaceous species analysed.

Table 4. Mapping of markers using introgression lines (IL)

COS marker	Exp. genome location (IL[A])	IL analysed	Obs. genome location (IL with *L. pennellii* alleles)
T1208	1-1 & 1-2	1-1, 1-2, 1-3	1-2[B]
T1409	1-2	1-1, 1-2, 1-3	1-2
T1485	1-3	1-1, 1-2, 1-3	1-3
T1361	2-3	Nil	Nil
T1308	3-4 & 3-5	3-2, 3-4, 3-5	3-4 & 3-5
T1181	5-1	5-1, 5-2, 5-3	5-1
T1510	7-2, 7-3, 7-4	7-1 to 7-4	7-2 & 7-3[B]
T1277	8-1	8-1, 8-2, 8-3	Nil[C]
T1581	8-2 & 8-3	8-1, 8-2, 8-3	8-2 & 8-3
T1359	8-3	8-1, 8-2, 8-3	8-3
T1212	9-3 and/or 9-2	9-1 & 9-3	Nil[D]
T1482	10-1 and/or 10-2	10-1, 10-2, 10-3	10-1 & 10-2
T0736	10-2	10-1, 10-2, 10-3	10-2
T1125	11-1 & 11-2	11-1, 11-2, 11-3	11-1 & 11-2
T1460	11-2 & 11-3	11-1, 11-2, 11-3	11-2[B]

[A]Introgression lines (*L. esculentum*) contain single fragments introgressed from *L. pennellii.* (Eshed and Zamir 1994). The first number represents the chromosome, and the number after the dash represents the introgressed fragment. Lines from individual chromosomes are sequentially numbered so that fragments are adjoining. Adjoining fragments overlap. If 2 or more lines are indicated, the genome location is at the overlapping region.

[B]The marker is close to the expected genome location but the difference between the expected and observed results may be due to imprecision in the genetic map used to position the marker or a problem with the introgression line that unexpectedly returned a negative result.

[C]This result is not conclusive as there may be a problem with introgression line 8-1, or the marker sequence may not correspond to that of the RFLP probe used for the genetic mapping.

[D]Introgression line 9-2 was not available for testing and the marker may be contained within the associated fragment.

Primer pairs representing T1361 and T1510 did not amplify for *Petunia* and the *Capsicum* species. The primer pair of T1460 did not amplify for eggplant and tobacco. Only the primer pair representing T1359 amplified in sweet potato.

The primer pair representing T1359 successfully amplified in all solanaceous samples and for sweet potato, and the resulting PCR products were successfully analysed by TGGE (*Petunia* sp. not included in TGGE analysis) (Fig. 1*a*). The results suggest that more than 2 loci, or 2 loci with more than 2 alleles, amplified in potato and sweet potato, which is consistent with the polyploid nature of these species; and 2 loci or alleles amplified for tobacco and possibly *L. hirsutum*. Only 1 locus was apparent for the other species. No differences in melting behaviour were detected between *L. esculentum* and *L. pimpinellifolium*, and between *C. annuum* and *C. chinense*. Differences were observed for all other comparisons.

The *L. pennellii* and *L. esculentum* alleles (Fig. 1*b*) were found to segregate in a F_2 mapping population (Fig. 1*c*). Segregation ratio was consistent with that produced by a single locus, as tested by χ^2 (0.05, d.f. = 2) analysis ($P > 0.1$). The observed segregation ratio, homozygous for the *L. pennellii* allele : heterozygous : homozygous for the *L. esculentum* allele, was 10 : 33 : 17 ($n = 60$).

The primer pair representing T1277 successfully amplified in all solanaceous samples and for bean, and the resulting PCR products were successfully analysed by TGGE (*Petunia* sp. not included in TGGE analysis) (Fig. 2*a*). A double-banding pattern was observed for all alleles including heteroduplexed products of *L. esculenum* and *L. pennellii*. As no heteroduplices were observed for

Table 5. Test of markers for PCR amplification in various plant species from the order Solanales
All markers amplified for *Lycopersicon esculentum*, *L. pennellii*, *L. pimpinellifolium*, *L. hirsutum*, and *Solanum tuberosum*

COS marker	*Solanum melongena*	*Capsicum annuum*	*Capsicum chinense*	*Nicotiana tabacum*	*Petunia* sp.	*Ipomoea batatus*
T1208	Yes	Yes	Yes	Yes	Yes	No
T1409	Yes	Yes	Yes	Yes	No	No
T1485	Yes	Yes	Yes	Yes	Yes	No
T1361	Yes	Poor	Poor	Yes	Poor	No
T1308	Yes	Yes	Yes	Poor	Yes	No
T1181	Yes	Yes	Yes	Yes	Poor	No
T1510	Yes	Poor	Poor	Yes	Poor	No
T1277[A]	Yes	Yes	Yes	Yes	Yes	No
T1581	Yes	Yes	Yes	Yes	Yes	No
T1359	Yes	Yes	Yes	Yes	Yes	Yes
T1212	Yes	Yes	Yes	Yes	Yes	No
T1482	Yes	Yes	Yes	Yes	Yes	No
T0736	Yes	Yes	Yes	Yes	No	No
T1125	Yes	Yes	Yes	Yes	No	No
T1460	No	Yes	Yes	No	Yes	No

[A]Also amplified in *Phaseolus vulgarus.*

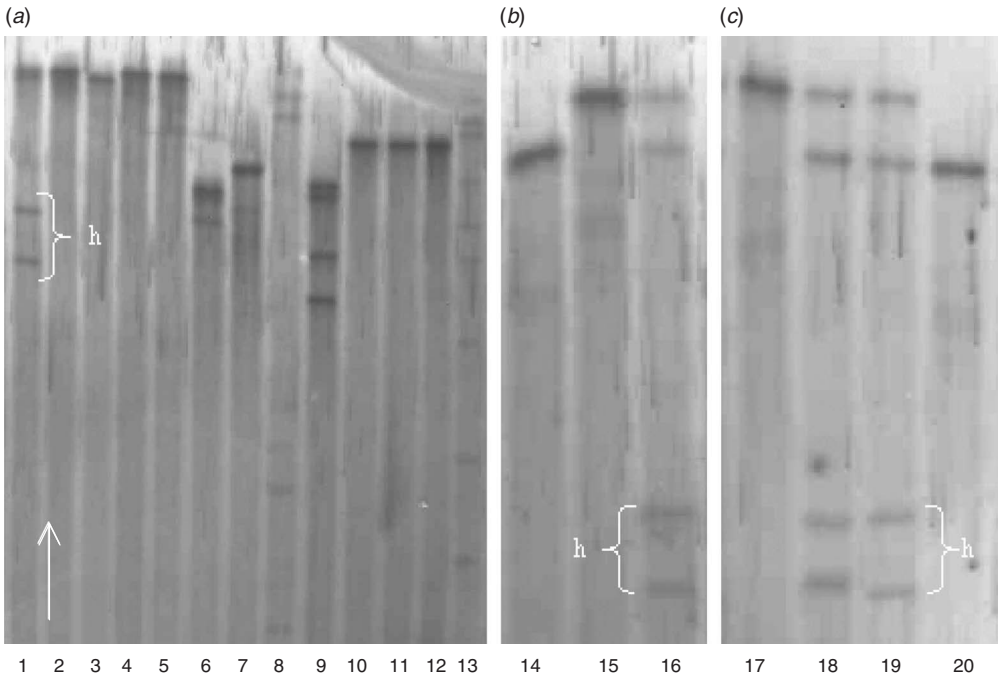

Fig. 1. TGGE analysis of PCR products representing COS-marker T1359. Fragment size excluding GC clamp is 149 bp for *L. esculentum* and *L. pennellii*. There was no apparent difference in fragment size for all species, as observed by agarose gel electrophoresis. The arrow indicates the direction of band migration and of increasing temperature. (h) Heteroduplex bands. (*a*, *b*) (1) F_1 *L. esculentum*/*L. pennellii*, (2) *L. esculentum*, (3) *L. pennellii*, (4) *L. esculentum* heteroduplexed with *L. pimpinellifolium*, (5) *L. pimpinellifolium*, (6) *L. hirsutum*, (7) egg plant, (8) potato, (9) tobacco, (10) *C. annuum* heteroduplexed with *C. chinense*, (11) *C. annuum*, (12) *C. chinense*, (13) sweet potato, (14) *L. pennellii*, (15) *L. esculentum*, (16) F_1 *L. esculentum*/*L. pennellii*. (*c*) (17–20) Alleles segregating in an F_2 mapping population, *L. esculentum*/*L. pennellii.*

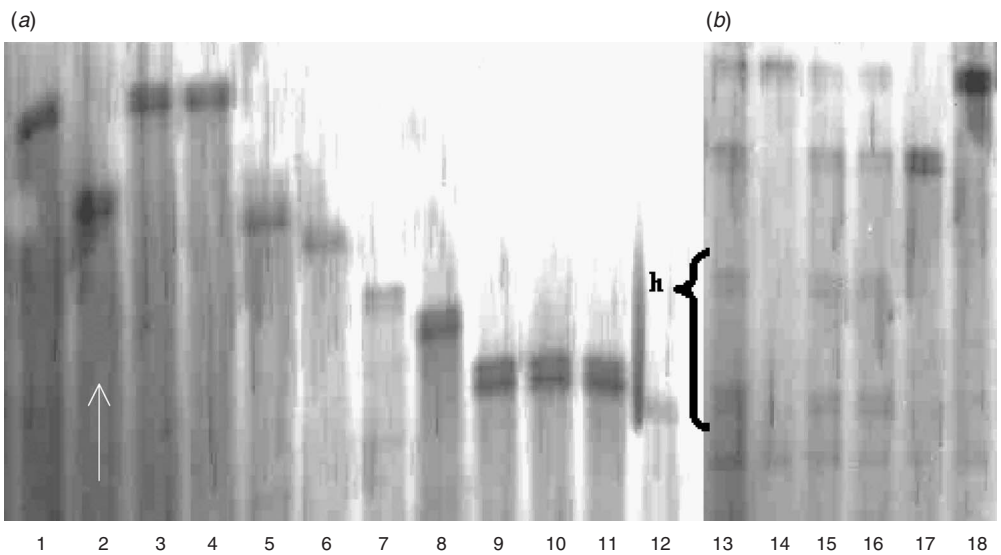

Fig. 2. TGGE analysis of PCR products representing COS-marker T1277. Fragment size excluding GC clamp is 124 bp for *L. esculentum* and *L. pennellii*. There was no apparent difference in fragment size for all species, as observed by agarose gel electrophoresis. The arrow indicates the direction of band migration and of increasing temperature. (h) Heteroduplex bands. (*a*) (1) *L. esculentum*, (2) *L. pennellii*, (3) *L. esculentum* heteroduplexed with *L. pimpinellifolium*, (4) *L. pimpinellifolium*, (5) *L. hirsutum*, (6) egg plant, (7) potato, (8) tobacco, (9) *C. annuum* heteroduplexed with *C. chinense*, (10) *C. annuum*, (11) *C. chinense*, (12) bean. (*b*) (13–18) Alleles segregating in an F_2 mapping population, *L. esculentum*/*L. pennellii.*

individual species, the double-banding pattern is unlikely to be due to the amplification of multiple loci. No differences in melting behaviour were detected between *L. esculentum* and *L. pimpinellifolium*, and between *C. annuum* and *C. chinense.* Differences were observed for all other comparisons (Fig. 2*a*).

The *L. pennellii* and *L. esculentum* alleles were found to segregate in a F_2 mapping population (Fig. 2*b*). Segregation ratio was consistent with that produced by a single locus, although segregation was skewed in favour of the *L. pennellii* allele, as tested by χ^2 (0.05, d.f. = 2) analysis ($P = 0.1$). The observed segregation ratio, homozygous for the *L. pennellii* allele : heterozygous : homozygous for the *L. esculentum* allele, was 22 : 24 : 14 ($n = 60$).

The primer pair representing T1212 successfully amplified product in all solanaceous samples, and the resulting PCR products were successfully analysed by TGGE (*Petunia* sp. not included in TGGE analysis) (Fig. 3*a*). Two distinct bands were observed for eggplant and the 2 *Capsicum* species. No heteroduplex bands were obvious, and therefore it is not apparent if these bands represented 2 separate loci or alleles, or were the consequence of the techniques used and characteristics unique to those DNA samples. Four bands were observed for tobacco, consistent with the amplification of 2 loci or alleles. No differences in melting behaviour were detected between *L. esculentum* and *L. pimpinellifolium.* Differences were observed for all other comparisons including the production of heteroduplicies when PCR products of *C. annuum* and *C. chinense* were mixed.

The *L. pennellii* and *L. esculentum* alleles were found to segregate in a F_2 mapping population (Fig. 3*b*). Segregation ratio was consistent with that produced by a single locus, as tested by χ^2 (0.05, d.f. = 2) analysis ($P > 0.7$). The observed segregation ratio, homozygous for the *L. pennellii* allele : heterozygous : homozygous for the *L. esculentum* allele, was 17 : 30 : 13 ($n = 60$).

Five of the 13 markers that amplified for *Capsicum* were found to be polymorphic between *C. annuum* and *C. chinense* by TGGE analysis (data not shown). These markers were T1212, T1482, T0736, T1125, and T1460.

Discussion

Temperature gradient gel electrophoresis (TGGE) proved to be a successful tool for the identification of expected polymorphism between orthologous fragments representing putative COS-sequences for *L. esculentum* and *L. pennellii*. Of the markers possessing expected polymorphism that amplified by PCR, 45.5% were successfully analysed by TGGE. TGGE therefore provides a PCR-based alternative to RFLP or CAPS-based mapping of COS markers for crosses involving *L. esculentum* and *L. pennellii.*

TGGE also proved its utility for the identification of unknown interspecific-polymorphism for *Lycopersicon* and *Capsicum*, for the markers developed from COS sequences. Differences could be detected between alleles of *L. esculentum* and *L. hirsutum*, and *L. pennellii* and *L. hirsutum*, for the markers T1212, T1277, and T1359. For the 13 markers that amplified in *Capsicum*, 5 recorded a

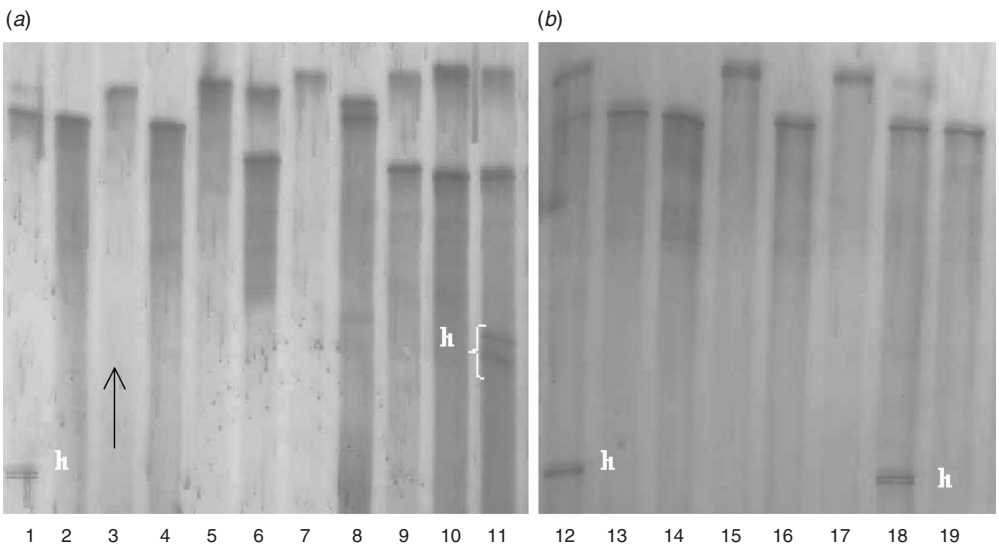

Fig. 3. TGGE analysis of PCR products representing COS-marker T1212. Fragment size excluding GC clamp is 415 bp in *L. esculentum* and 427 bp in *L. pennellii*. Fragment size for the other species is similar, as observed by agarose gel electrophoresis. The arrow indicates the direction of band migration and of increasing temperature. (h) Heteroduplex bands. (*a*) (1) F_1 *L. esculentum*/*L. pennellii*, (2) *L. esculentum*, (3) *L. pennellii*, (4) *L. pimpinellifolium*, (5) *L. hirsutum*, (6) egg plant, (7) potato, (8) tobacco, (9) *C. annuum*, (10) *C. chinense*, (11) *C. annuum* heteroduplexed with *C. chinense*. (*b*) (12–19) Alleles segregating in an F_2 mapping population, *L. esculentum*/*L. pennellii*.

difference between the *Capsicum* species. This study has demonstrated that TGGE is an efficient method for the development of PCR-based markers when DNA sequences are only available for related species or genera, and not available for the species of interest.

With respect to the 3 markers representing T1212, T1277, and T1359, no differences were detected between the closely related species of *L. esculentum* and *L. pimpinellifolium.* Similarly, not all markers tested identified differences between the *Capsicum* species. The results indicate that the marker system will not be useful for all interspecific comparisons.

The potential usefulness of the developed markers for amplification within Solanaceae has also been demonstrated. Seven markers amplified for the 5 Solanaceous genera tested (*Lycopersicon*, *Solanum*, *Nicotiana*, *Capsicum*, *Petunia*), and the 2 markers developed from T1359 and T1277 amplified outside of the family. The marker corresponding to COS marker T1359, amplified in *Ipomoea batatus* (sweet potato), a plant that belongs to Family Convolvulaceae. Solanaceae and Convolvulaceae are contained within the same Order, Solanales. Amplification across these 2 families adds support to the utility of this marker within Solanaceae. In addition, the markers may amplify in a wider range of species with further optimisation.

The marker representing T1277 was the only marker identified as not being located at, or near, the expected genome location. This result should not be considered conclusive, as the supplied introgression line (8-1) may not have contained the appropriate *L. pennellii* fragment. Alternatively, the results may indicate that the sequence used by this author, to design the primers, did not represent the RFLP probe used to genetically map COS marker T1277 (SGN: Tomato_Arabidopsis synteny map).

The general success of this investigation indicates that the 15 markers will provide a useful resource for the identification of interspecific polymorphism in Solanaceae. This is particularly the case for those species for which large amounts of EST data are not available. For example, the markers, and new markers yet to be developed, will provide useful PCR-based markers for tomato mapping and breeding populations involving *L. hirsutum* and probably other wild relatives. In the case of cell fusion lines, the marker system will be very useful for the identification of interspecific or intergeneric fragments.

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