Peiró Barber, RM.; Díez Niclós, MJTDJ.; Pérez De Castro, AM. (2015). A set of PCR-based markers for management of a library of Solanum lycopersicoides introgression lines. The Journal of Horticultural Science and Biotechnology. 90(3):279-284. doi:https://dx.doi.org/10.1080/14620316.2015.11513183.

Journal of Horticultural Science & Biotechnology (2015) **90** (x) xxx-xxx

A set of PCR-based markers for management of a *Solanum lycopersicoides* introgression lines library

By ROSA PEIRÓ, MARÍA JOSÉ DÍEZ and ANA PÉREZ-DE-CASTRO*

Instituto de Conservación y Mejora de la Agrodiversidad Valenciana, Universitat Politècnica de València, Camino de Vera s/n, 46022 Valencia, Spain

(e-mail: anpede1@btc.upv.es)……(*Accepted xxxx 2015*)

Running head: Markers to manage introgression lines of S. lycopersicoides

*Author for correspondence.

SUMMARY

The collection of introgression lines (ILs) of *Solanum lycopersicoides* Dunal in the genetic background of cultivated tomato (*Solanum lycopersicum* L.) is a valuable tool in tomato breeding. Efficient management of the collection requires the use of molecular markers. The objective of this work was to identify polymerase chain reaction (PCR)-based markers that were polymorphic between the parents of the ILs; namely ‘VF36’ and ‘LA2951’. In total, 81 primer pairs were tested on genomic DNA from both parents. Genomic DNA of the inter-specific hybrid between the two parents, from which the IL collection derives, was also tested. Markers used were either cleaved amplified polymorphic sequence (CAPS) markers or were derived from a set of conserved orthologous genes. In both cases, markers were mapped in tomato and described in the SOL Genomics Network. Forty-seven of the markers tested markers provided a single PCR product in ‘VF36’ and ‘LA2951’. Eleven markers revealed polymorphisms as differences in band-sizes between the two parents. At least one restriction enzyme generating polymorphism was identified in 29 of the remaining 36 markers. Among other applications, some of these markers have been used to identify plants carrying the target DNA fragment among segregating generations or to delimit the length of an introgression sequence.

Tomato (*Solanum lycopersicum* L.) breeding relies mainly on exploiting the variability found in wild relatives. These include a group of species native to South America, including 12 species belonging to the 'tomato clade' (i.e., *Solanum* section *Lycopersicon*) and two species each in sections *Juglandifolia* and *Lycopersicoides* (Peralta *et al*., 2008). Although differences in crossability exist, crosses between cultivated tomato and species in the section *Lycopersicon* are possible, facilitating their use in breeding (Díez and Nuez, 2008). Most of the latter species have proved to be important sources of the beneficial traits introgressed into cultivated tomato (Bai and Lindhout, 2007). However, there are strong crossability barriers with species in the sections *Juglandifolia* and *Lycopersicoides*. *S. ochranthum* Dunal and S. *junglandifolium* Dunal (section *Juglandifolia*) appear to be sexually isolated from tomato and the rest of the species of section *Lycopersicon* (Rick, 1988, cited by Albrecht and Chetelat, 2009). Moreover, backcrossing of somatic hybrids between tomato and *S. ochranthum* to tomato have proved unsuccessful (Stommel, 2001). In the case of *S. sitiens* I. M. Johnst, the transfer of chromosomes to tomato was only possible using derivatives of *S. lycopersicoides* as a bridge (Pertuzé *et al*., 2003). *Solanum lycopersicoides* Dunal is the most distant relative of tomato that has been directly crossed with tomato, with the aid of embryo rescue, to obtain fertile progeny (Chetelat *et al*., 1997). A library of introgression lines (ILs) of *S. lycopersicoides* 'LA2951' in the genetic background of cultivated tomato has been developed based on this plant material (Canady *et al*., 2005). This set of ILs represents a useful tool to map genes or quantitative trait loci (QTL).

Solanum lycopersicoides has been shown to harbour resistance or tolerance genes against several diseases that affect tomato (Chetelat *et al*., 1997), as well as extreme tolerance to abiotic stress (Albrecht *et al*., 2010). Specifically, accession

‘LA2951’ has been described as being resistant to *Botrytis cinerea* (Davis *et al*., 2009), to Tomato yellow leaf curl disease, TYLCD (Pérez de Castro *et al*., 2011; Zong *et al*., 2012) and to *Tomato mosaic virus*, ToMV (Soler *et al*., 2012), among other biotic stresses, and tolerant to salinity to the seedling stage (Li *et al*., 2011), among other abiotic stresses.

The set of ILs of *S. lycopersicoides* in the genetic background of cultivated tomato was developed from an initial cross of *S. lycopersicum* ‘VF36’ × *S. lycopersicoides* ‘LA2951’, which resulted in a partially male-fertile F_1 hybrid that was directly back-crossed to tomato to obtain several BC_1 plants (Chetelat *et al*., 1997). After one or two generations of back-crossing, and several generations of selfing, a total of 272 inbred backcross lines were selected to develop the set of ILs (Canady *et al*., 2005).

The IL library is estimated to cover 96% of the *S. lycopersicoides* genome. Two groups of lines were established: a primary subset (56 lines) providing maximum genome coverage and homozygosity, and isogenicity when possible; and a secondary subset (34 lines) chosen as additional recombinants or to represent unique genotypes (Canady *et al*., 2005). Approx. 34% of the lines were sterile in the homozygous condition and had to be maintained as heterozygotes. Reproduction and use of these lines require confirmation of the presence of the *S. lycopersicoides* fragment in each progeny. The DNA markers used during development of the IL library were restriction fragment length polymorphisms (RFLPs) from the tomato RFLP map (Tanksley *et al*., 1992), as well as morphological and allozyme markers (Canady *et al*., 2005). To facilitate genotyping of the ILs, Canady *et al*. (2005) converted 16 of the RFLPs into cleaved amplified polymorphic sequence (CAPS) markers. However, for some regions

of the genome there were no polymerase chain reaction (PCR)-based markers available that were polymorphic between the parents ‘VF36’ and ‘LA2951’.

From the first 1000 RFLP markers mapped on the tomato genome (Tanksley *et al*., 1992), many new markers have now been made available. For example, a collection of PCR-based markers has been developed from a set of conserved orthologous genes (conserved orthologous set II, COSII) in Asterid species, cultivated tomato among them (Wu *et al*., 2006). Moreover, release of the tomato genome sequence provided a valuable tool to identify polymorphisms (The Tomato Genome Consortium, 2012). At present, rapid advances in sequencing technologies have facilitated the discovery of genetic variation in crops by re-sequencing; single nucleotide polymorphisms (SNPs) are the predominant markers developed with this strategy (Deschamps and Campbell, 2010). SNPs can be detected using low-cost markers such as CAPS markers if the polymorphism altered a restriction site. As a result, much information exists concerning easy and cheap PCR-based markers that are polymorphic between tomato and several wild relatives, most of them held by the SOL Genomics Network (http://solgenomics.net/). The transferability of PCR markers among species in the section *Lycopersicon* has been reported because of the high level of sequence conservation among these species (Bai *et al*., 2004). *S. lycopersicoides* is more distantly related to cultivated tomato than species in the section *Lycopersicon*, but a high degree of transferability is still expected, which may be an advantage in detecting higher levels of polymorphism.

The objective of this study was to make use of the available information to identify PCR-based markers that were polymorphic between the parents of the ILs (*S. lycopersicum* ‘VF36’ and *S. lycopersicoides* ‘LA2951’). These markers would then

facilitate more efficient management of the set of ILs. Examples are provided of the use of these markers for different purposes.

MATERIALS AND METHODS

Plant material

The plant material used in this study included both parental genotypes of the IL library, *S. lycopersicum* 'VF36' and *S. lycopersicoides* 'LA2951', as well as their inter-specific hybrid, 90L4178-1. All plant material was provided by the Tomato Genetic Resources Center (TGRC); seeds in the case of 'VF36' and 'LA2951', and clonal replicates maintained *in vitro* in the case of the hybrid 90L4178-1. 'VF36' is a processing cultivar, with determinate growth, which carries the *I* and *Ve* genes that confer resistance to *Fusarium oxysporum* f. sp. *lycopersici* and *Verticillium dahliae*, respectively. 'LA2951' was collected in the Province of Tarapacá (Chile). This accession is maintained combined with LA2954 to get enough seed by the TGRC. The inter-specific hybrid was obtained by Chetelat *et al*. (1997) and was the original plant from which the IL library derives.

Marker analysis

Total genomic DNA was extracted from 75 mg of fresh leaf tissue from each plant following the procedure of Doyle and Doyle (1990), with some modifications. All the PCR markers used (Table I; Supplementary Table SI available on-line at www.jhortscib.com) were CAPS or COSII markers that had been mapped in tomato and described on the SOL Genomics Network (http://solgenomics.net/). TG markers were derived from tomato genomic DNA clones, while CT or CD markers were derived from tomato cDNA clones. Primer sequences were available for all the markers used.

Moreover, for some information existed on experimental data such as PCR product sizes or restriction enzymes that revealed polymorphism between tomato and some wild relatives.

Each 25 μL PCR reaction contained: 1X PCR buffer recommended by the supplier (Roche Diagnostics, Madrid, Spain), 2.5 mM $MgCl_2$, 0.4 mM of each primer, 0.4 mM dNTPs, 1 Unit of *Taq* DNA polymerase (Roche Diagnostics, Madrid, Spain) and 40 ng of template DNA. PCR amplification was carried out in an Eppendorf Martercycler Thermal Cycler (Eppendorf Iberica, Madrid, Spain) under the following conditions: an initial denaturing for 10 min at 94ºC, followed by 35 cycles of 94ºC for 30 s, annealing for 30 s at a defined temperature for each marker (see Table II) and 72ºCfor a time that also depended on the marker (information provided for markers set in Table II), with a final extension step for 10 min at 72ºC. The annealing temperature used was based on information provided on the SOL Genomics Network (SGN; http://solgenomics.net/) for each primer pair. When no PCR amplification product was obtained, gradient PCR was run for the corresponding primer pair on all three samples (both parents and the inter-specific hybrid).

All amplification products were analysed by agarose gel electrophoresis in 1% (w/v) agarose in 1X TBE buffer (Tris-Borate-EDTA) and visualised by staining with 0.5μg/ml ethidium bromide. When no polymorphism between the parental genotypes was observed, the PCR products were digested with different restriction endonucleases (Table II; Supplementary Table II available on-line at www.jhortscib.com). Restriction of 10 μl of the PCR products was performed overnight, in a total volume of 20 μl with 2 Units of the corresponding restriction enzyme, using buffers recommended by the suppliers at the recommended temperature. The digestion products were analysed by agarose gel electrophoresis in 2% (w/v) agarose with 1X TBE buffer and visualised by

staining with 0.5µg/ml ethidium bromide. The restriction enzymes used were mostly those used by Canady *et al*. (2005): *Hae*III, *Dde*I, *Hinf*I, *Rsa*I, *Mse*I, *Hha*I, *Alu*I, *Dpn*I, *Mbo*I, and *Hap*II. In some cases, an enzyme described as revealing polymorphism between tomato and a wild relative was also tested. These were: *Mbo*II, *Eco*RV, *Hind*III, *Acc*I and *Taq*I. Finally, the PCR products of two of the DNA markers for 'VF36' and 'LA2951' were sequenced (by the DNA Sequencing Core Service in the Institute for Plant Molecular and Cell Biology, IBMCP, UPV-CSIC, Valencia, Spain). The results were analysed using the 'Chromas' software (http://www.technelysium.com.au/chromas.html). In these cases, differential restriction sites between both parents were tested to detect polymorphism. *Hind*III and *Nla*III were the enzymes used for detecting the polymorphism between 'VF36' and 'LA2951'for C2-13 and C2-24 markers, respectively.

RESULTS AND DISCUSSION

In total, 81 primer pairs were tested on the genomic DNAs from both parents of the IL collection, 'VF36' and 'LA2951', and their inter-specific hybrid (Table I).

Although the annealing temperature was optimised by running gradient PCR, nine primer pairs failed to amplify in both species. The failure percentage was higher for CT+CD markers, 19.0% (four out of a total of 18 CT and 3 CD markers), if compared with the percentage for TG and COSII markers, 8.0 and 8.6%, respectively. No previous experimental data on PCR product sizes were available for the four CT markers. In this case, the presence of large introns in the genomic DNA may be a cause of such failure. This was not the case for the three COSII and two TG markers, for which a protocol was previously set for tomato in SGN database (http://solgenomics.net/) and for which experimental data existed. Amplification was

thus expected, at least in tomato; differences at the sequence level between ‘VF36’ and the accessions used in the available experiments could explain our results.

Nine primer pairs (1 CT, 6 TG and 2 COSII markers) produced a PCR product for ‘VF36’ but failed to amplify ‘LA2951’ at different temperatures. There was a protocol described for all these markers, with experimental data on tomato and *S. pennellii* for six of them (4 TG and 2 COSII markers). In any case, *S. pennellii* is more closely related to tomato than *S. lycopersicoides*, so the genetic distance between them may be a cause of the lack of amplification in ‘LA2951’. This is especially true for markers corresponding to non-expressed regions of the genome, which are less conserved. Thus, as expected, the failure percentage in ‘LA2951’ was higher for TG markers. Konieczny and Ausubel (1993) found similar results for one of the markers when developing CAPS markers from mapped genes in *Arabidopsis*. The presence of a large introgression was the cause of the amplification failure in one of the ecotypes. These markers could be used as dominant markers, but because of their limitations, were discarded in their study and our study.

Nine primer pairs (1 CT, 4 TG and 4 COSII markers) generated unspecific products in both species when low hybridization temperatures were used. For all of them, higher temperatures were assayed first, with two possible results: amplification only in tomato or no amplification in either species. Amplification was produced only in tomato for two TG markers and one COSII marker, when annealing temperature was the established in the SGN protocol. For these three primer pairs, experimental data were available in the SGN. For the rest of the markers, no amplification was obtained at the annealing temperatures set in the SGN. In these cases, experimental data existed just on TG markers. Lowering annealing temperatures resulted in obtaining several amplification products in both species for all these markers. Probably, amplification in

these conditions was produced for different *loci* in 'VF36' and 'LA2951' genomes, so these markers were rejected.

PCR products were obtained in both species but with different annealing temperatures for markers CD37 and TG217. These conditions are not useful, so these markers were also excluded.

Considering all possible failure causes, 29 primer pairs failed to provide correct amplification in both species (Table I). The failure percentage, 35.8%, was thus lower than that obtained by Bai *et al*. (2004) when converting sequence specific RFLP to PCR markers in tomato and some wild relatives. However, their study included the design of the primers from the information of the probes. A higher success percentage could be expected in our case, given that for some of the markers there were previous PCR experimental results on tomato and some wild relatives. On the contrary, all the wild species used in their study belonged to section *Lycopersicon*, so they were more genetically close to tomato than *S. lycopersicoides*. The higher genetic distance could explain the lack of amplification in 'LA2951'. In this sense, in our study amplification in tomato was obtained for 80.2% of the markers, resulting in a success percentage approaching the percentage obtained in the development of COSII markers from database sequences (Wu *et al*., 2006). The success percentage was lower for TG markers, probably because they can correspond to non-expressed, so less conserved, region, whereas this percentage was higher for COSII markers, most likely because they have been developed from conserved orthologous genes among several Asterid species.

Correct amplification in 'VF36' and 'LA2951' was obtained for 52 primer pairs. Information on annealing temperature was previously available for 45 of them. The temperature finally selected for the protocol was lower than previously established by SGN for 11 of the primer pairs, in order to obtain clear amplification in both genotypes.

The highest success percentage was obtained for COSII markers (65.7%). With only one exception, all COSII markers with previous experimental information for several Asterid species provided correct amplification for both parents, 'VF36' and 'LA2951'.

More than one fragment was amplified in at least one genotype for five of these 52 markers (Table I). In the case of TG254, clear bands were obtained: 2000 bp for 'VF 36' and 1300 and 450 bp for 'LA2951'. Other less intense PCR products appeared in both genotypes. Similar results were found for TG63 and C2-27. In the case of CT75 and C2-21, two bands of the same size were amplified in both genotypes. Probably, more than one *locus* was amplified with these primer pairs. For the first three markers, TG254, TG63 and C2-27, polymorphism was revealed as a difference in PCR product sizes. Even different patterns were detected for CT75 after restriction with some of the enzymes tested. In any case, all these markers were excluded because of the impossibility to distinguish the *loci* responsible for the polymorphism.

Among markers that revealed a single PCR product (47 markers) polymorphism between both parents was obtained as a difference in length for 11 of them (Table II). These markers could then be used as SCARs (Sequence Characterized Amplified Region). In the study of Konieczny and Ausubel (1993), aimed at converting into PCR a set of 18 RFLP markers, one was polymorphic after amplification between two ecotypes of *Arabidospsis thaliana*. The low polymorphism percentage could be explained by the fact that ecotypes of the same species were the genotypes compared. Working in tomato and wild species, Bai *et al*. (2004) obtained polymorphism after amplification in one of the 29 markers successfully designed. As previously stated, the wild species used in their study belonged to section *Lycopersicon*. The higher genetic distance between tomato and *S. lycopersicoides* could be the reason of the higher rate of polymorphism revealed by PCR in our work.

Thirty-six primer pairs amplified a single clear band of the same size in both, 'VF36' and 'LA2951'. These PCR products were digested with different restriction enzymes. At least one enzyme that generated polymorphism between 'VF36' and 'LA2951' was identified for 29 of the markers. For markers C2-13 and C2-24, the enzyme was selected after sequencing PCR product for 'VF36' and 'LA2951'. In all cases, the restriction enzyme revealing a clearer pattern with respect to difference between both alleles was selected for the protocol.

All markers were tested in both parents and their inter-specific hybrid, from which the IL collection derives. This ensured that alleles from both parents were equally amplified. In this sense, for all markers, amplification of both alleles in the hybrid was achieved. However, PCR conditions are critical. In this regard, for some markers (i.e. TG224) an increase of 2ºC in annealing temperature led to the preferential amplification of the tomato allele. For some (i.e., C2-20), unspecific bands were obtained as a result of an increase in template DNA concentration.

The set of 40 markers will be very useful in the management of the IL collection of *S. lycopersicoides* into the genetic background of cultivated tomato constructed by Canady *et al*. (2005). As previously stated, approx.. 34% of the lines must be maintained as heterozygotes to be fertile. Consequently, plants that carry the target fragment must be identified among segregating descendants in each generation. Canady *et al*. (2005) used RFLP, allozyme and morphological markers for this purpose during the development of the IL library. Some of the RFLPs were converted into CAPS markers to facilitate the management of the collection. As stated by the authors of the collection, recombination within the ILs is strongly suppressed; thus they propose the use of one marker per fragment in the genotyping, at least for short introgressions. In any case, there are ILs that carry large introgressions. This is the case of LA4270, which

holds an introgression corresponding to almost all of chromosome 9. This line is homozygous for the final part of the introgression, but maintained as heterozygous for the rest. Canady *et al*. (2005) transformed into a CAPS marker one of the RFLPs in the latter part of chromosome 9. Two of the markers developed in this work, TG18 and C2-23, also correspond to the segregating fragment of the introgression in LA4270. These markers were used to genotype some LA4270 plants; in one of them, recombination had occurred, since it was homozygous for *S. lycopersicoides* allele for TG18 and heterozygous for C2-23. This result confirms the need for a higher density of polymorphic markers when analyzing large introgressions.

Furthermore, markers described in this work could be used with the purpose to more precisely delimit the length of some of the fragments, not only in segregating lines, but also in those provided as homozygotes. As an example, introgression in line LA4233, in chromosome 1, spans between markers TG83 and TG17. The distal end of the introgression is located at some position between markers TG17 and TG267A (Canady *et al*., 2005). CT137, found between both markers, was used to genotype plants of this line and all of them were homozygous for the tomato allele; this means that the introgression ends at some point distal to this marker.

The IL collection is a useful tool to map QTLs responsible for interesting traits. However, the level of resolution provided is relatively low. As a consequence, development of sub-ILs will be necessary to fine map the putative genes/QTLs identified. With this purpose, a higher density of polymorphic markers between 'VF36' and 'LA2951' will be required. Based on the work presented here the use of COSII markers previously tested in several Asterid species would be a strategy to achieve a high success rate in PCR amplification in both species, tomato and *S. lycopersicoides*.

This research was financed by the Ministerio de Educación y Ciencia and Ministerio de Ciencia e Innovación (Madrid, Spain) (Projects AGL2008-05114 and AGL2011-30083).

REFERENCES

ALBRECHT, E. and CHETELAT, R.T. (2009). Comparative genetic linkage map of *Solanum* sect. *Juglandifolia*: evidence of chromosomal rearrangements and overall synteny with the tomatoes and related nightshades. *Theoretical and Applied Genetics*, **118**, 831–847.

ALBRECHT, E., ESCOBAR, M. and CHETELAT, R.T. (2010). Genetic diversity and population structure in the tomato-like nightshades *Solanum lycopersicoides* and *S. sitiens*. *Annals of Botany*, **105**, 535–554.

BAI, Y. and LINDHOUT, P. (2007). Domestication and breeding of tomatoes: what have we gained and what can we gain in the future. *Annals of Botany*, **100**, 1085–1094.

BAI, Y., FENG, X., VAN DER HULST, R. and LINDHOUT, P. (2004). A set of simple PCR markers converted from sequence specific RFLP markers on tomato chromosomes 9 to 12. *Molecular Breeding*, **13**, 281–287.

CANADY, M.A., MEGLIC, V. and CHETELAT, R.T. (2005). A library of *Solanum lycopersicoides* introgression lines in cultivated tomato. *Genome*, **48**, 685–697.

CHETELAT, R.T., CISNEROS, P., STAMOVA, L. and RICK, C.M. (1997). A male-fertile *Lycopersicon esculentum* × *Solanum lycopersicoides* hybrid enables direct backcrossing to tomato at the diploid level. *Euphytica*, **95**, 99–108.

DAVIS, J., YU, D., EVANS, W., GOKIRMAK, T., CHETELAT, R.T. and STOTZ, H. (2009). Mapping of loci from *Solanum lycopersicoides* conferring resistance or

susceptibility to *Botrytis cinerea* in tomato. *Theoretical and Applied Genetics*, **119**, 305–314.

DESCHAMPS, S. and CAMPBELL, M.A. (2010). Utilization of next-generation sequencing platforms in plant genomics and genetic variant discovery. *Molecular Breeding*, **25**, 553–570.

DÍEZ, M.J. and NUEZ, F. (2008). Tomato. In: *Handbook of Plant Breeding*. (Prohens, J. and Nuez F., Eds.). Springer, New York, NY, USA, 249–323.

DOYLE, J.J. and DOYLE, J.L. (1990). Isolation of plant DNA from fresh tissue. *Focus*, **12**, 13–15.

KONIECZNY, A. and ASUBEL, F.M. (1993). A procedure for mapping *Arabidopsis* mutations using co-dominant ecotype-specific PCR-based markers. *Plant Journal*, **4**, 403–410.

LI, J., LIU, L., BAI, Y., ZHANG, P., FINKERS, R., DU, Y., VISSER, R.G.F. and VAN HEUSDEN, A.W. (2011). Seedling salt tolerance in tomato. *Euphytica*, **178**, 403–414.

PERALTA, I.E., SPOONER, D.M. and KNAPP, S. (2008). The taxonomy of tomatoes: a revision of wild tomatoes (*Solanum* section *Lycopersicon*) and their outgroup relatives in sections *Juglandifolium* and *Lycopersicoides*. *Systematic Botany Monographs*, **84**, 1–186.

PÉREZ DE CASTRO, A., DÍEZ, M.J. and NUEZ, F. (2011). Evaluation of a subset of *Solanum lycopersicoides* introgression lines for resistance to Tomato yellow leaf curl disease. *In: Proceedings of the XVII Eucarpia Meeting Group Tomato*, Málaga, Spain. 17.

PERTUZÉ, R.A., JI, Y. and CHETELAT, R.T. (2003). Transmission and recombination of homeologous *Solanum sitiens* chromosomes in tomato. *Theoretical and Applied Genetics*, **107**, 1391–1401.

RICK, C.M. (1988). Tomato-like nightshades: affinities, autecology, and breeders opportunities. *Economic Botany*, **42**, 145–154.

SOLER, S., BELMONTE, I., ARAMBURU, J., GALIPIENSO, L., LÓPEZ, C., SIFRES, A., PÉREZ-DE-CASTRO, A. and DÍEZ, M.J. (2012). Identificación de una fuente de tolerancia al ToMV en una colección de líneas de introgresión derivada de *Solanum lycopersicoides* LA2951. *In: Proceedings of the XVI Congreso de la Sociedad Española de Fitopatología*, Málaga, Spain. 221.

STOMMEL, J.R. (2001). Barriers for introgression of *Solanum ochranthum* into tomato via somatic hybrids. *Journal of the American Society for Horticultural Science*, **125**, 587–592.

TANKSLEY, S.D., GANAL, M.W., PRINCE, J.P., DE VICENTE, M.C., BONIERBALE, M.W., BROUN, P., FULTON, T.M., GIOVANNONI, J.J., GRANDILLO, S. and MARTIN, G.B. (1992). High density molecular linkage maps of the tomato and potato genomes. *Genetics*, **132**, 1141–1160.

THE TOMATO GENOME CONSORTIUM. (2012). The tomato genome sequence provides insights into fleshy fruit evolution. *Nature*, **485**, 635–641.

WU, F., MUELLER, L.A., CROUZILLAT, D., PETIARD, V. and TANKSLEY, S.D. (2006). Combining bioinformatics and phylogenetics to identify large sets of Single Copy Orthologous Genes (COSII) for comparative, evolutinonary and systematics studies: A test case in the Euasterid plant clade. *Genetics*, **174**, 1407–1420.

ZONG, Y.Y., LIU, L., LI, T., SAYED-RASHID, A.S., ZHOU, L.X., SUN, Y.Y., ZHENNG, Z., ZHENG, Q.G. and LI, J.M. (2012). Mapping of QTLs conferring the resistance to *Tomato Yellow Leaf Curl Virus* (TYLCV) in *Solanum lycopersicoides.* *Acta Horticulturae Sinica*, **39**, 915–922.

TABLE I

Markers tested, location in chromosome and position, and result obtained (see footnote for description of the results)

Marker name[#]	Ch.[*]	cM [§]	Result[±]
CT233	1	0.0	CAPS
TG184	1	20.0	T
TG51	1	22.3*	T
TG224	1	53.0	SCAR
TG59	1	62.0	T
CT67	1	120.0	SP
CT137	1	149.0	CAPS
CT190	1	163.5	SP
CD37	2	0.0*	DAT
C2_At1g60640 (C2-19)	2	12.5	CAPS
C2_At2g35130 (C2-20)	2	34.5	CAPS
CT103	2	63.7	T
CT75	2	71.4*	SB
TG337	2	95.5	CAPS
TG151	2	126.0	CAPS
CT24	2	139.5	SP
TG114	3	14.0*	CAPS
TG74	3	74.2	T
C2_At1g80170 (C2-1)	3	122.5	CAPS
CT115	3	118.9*	CAPS
CT229	4	12.0	SP
C2_At3g17040 (C2-2)	4	22.0	SCAR
C2_At3g62940 (C2-3)	4	56.0	SP
C2_At1g78230 (C2-4)	4	82.1	CAPS
CT188	4	88.4	SCAR
CT206	6	92.0	NA
C2_At3g51630 (C2-14)	6	92.5	ULT
C2_At4g29490 (C2-15)	7	22.3	CAPS
TG217	7	43.0	DAT
C2_At1g02180 (C2-16)	7	73.0	CAPS
CT114	7	96.0	SP
TG499	7	97.0	ULT
TG176	8	2.0	SCAR
CD40	8	9.0	SCAR
C2_At4g16440 (C2-35)	8	24.5	NA
TG302	8	37.0	SCAR
CT148	8	67.0	NA
C2_At1g64550 (C2-18)	8	87.0	CAPS
TG294	8	87.0	ULT
TG254	9	4.0	SB
TG18	9	14.0	CAPS
C2_At2g36720 (C2-21)	9	24.0	SB
C2_At3g10070 (C2-22)	9	34.0	CAPS
C2_At2g38025 (C2-23)	9	45.0	CAPS
TG348	9	62.0	ULT
TG424	9	86.0	NA
C2_At1g16590 (C2-24)	9	112.0	CAPS
TG230	10	0.0	CAPS
TG63	10	80.0	SB
CT238	10	87.0*	SP

C2_At1g46480 (C2-5)	4	101.5	CAPS	C2_At5g64730 (C2-25)	11	0.5	ULT
TG498	4	135.7	SCAR	C2_At3g54840 (C2-26)	11	20.5	NA
CT253	4	135.8	NA	TG523	11	29.0	ULT
CT72	5	0.4*	ULT	C2_At5g37290 (C2-27)	11	49.5	SB
C2_At1g14300 (C2-6)	5	20.0	CAPS	C2_At4g33945 (C2-28)	11	49.5	ULT
C2_At1g13380 (C2-7)	5	38.7	CAPS	C2_At5g04590 (C2-34)	11	56.0	ULT
TG504	5	69.0	T	TG400	11	57.0	T
CT172	5	95.0	NA	C2_At5g25760 (C2-29)	11	89.5	CAPS
TG23	5	99.0	NA	C2_At5g58490 (C2-30)	11	97.0	SCAR
C2_At3g54860 (C2-8)	5	112.5	CAPS	TG393	11	103.0	CAPS
C2_At3g46780 (C2-9)	6	4.0	SCAR	C2_At4g26180 (C2-31)	12	32.6	CAPS
C2_At4g32560 (C2-36)	6	8.5	T	CT99	12	53.5	SCAR
C2_At5g05690 (C2-10)	6	24.5	CAPS	C2_At1g17410 (C2-32)	12	64.5	NA
C2_At1g21640 (C2-11)	6	37.0	CAPS	C2_At4g31150 (C2-33)	12	96.5	CAPS
C2_At1g73885 (C2-12)	6	48.0	T	CD2	12	120.0	SCAR
C2_At1g12060 (C2-13)	6	64.5	CAPS				

[#] COSII (conserved orthologous set II) markers (coded in SOL Genomics Network as C2_At…) were recoded as C2-1 to C2-36.

[*] Ch.: Chromosome.

[§] Position expressed as cM (centimorgans). Positions were mainly based in Tomato-EXPEN 2000 map. The exceptions are those with an asterisk, which belong to the Tomato-EXPEN 1992 map. Their relative position with adjacent markers has been respected.

[±] CAPS: cleaved amplified polymorphic sequence marker; SCAR: sequence characterized amplified region marker; T: amplification obtained only in tomato 'VF36'; SP: Polymorphism not found with the restriction enzymes tested; DAT: Different annealing temperature for amplification

in 'VF36' and 'LA2951'; SB: several bands in at least one genotype; NA: no amplification obtained in 'VF36' and 'LA2951'; ULT: unspecific at low annealing temperature.

TABLE II

PCR conditions, restriction enzyme used, if necessary, and fragment sizes in 'VF36' and 'LA2951' DNA

Marker name[#]	Annealing temperature (ºC)	Duration of extensión (s)	PCR product size (bp)[*]	Restriction enzyme	Fragment size (bp)[§]	
					S. lycopersicum 'VF36'	*S. lycopersicoides* 'LA2951'
CT233	58	90	1,500	*Rsa*I	1,100	700+400
TG224	55	90	1,700/1,200	-	1,700	1,200
CT137	53	90	1,500	*Hae*III	850	1,000
C2_At1g60640 (C2-19)	50	60	525	*Alu*I	375	400
C2_At2g35130 (C2-20)	55	60	550	*Alu*I	400	350
TG337	58	90	1,300	*Dde*I	700+550	1,300+550
TG151	55	90	2,000	*Rsa*I	1,400	1,100
TG114	55	90	2,000	*Mbo*II	600	675
C2_At1g80170 (C2-1)	55	90	1,000	*Hap*II	1,000	400+600
CT115	58	60	300	*Mse*I	100	200
C2_At3g17040 (C2-2)	55	60	500/325	-	500	325
C2_At1g78230 (C2-4)	53	30	525	*Alu*I	350+175	300
CT188	55	60	900/350	-	900	350
C2_At1g46480 (C2-5)	55	60	600	*Alu*I	300	400+175
TG498	50	60	375/400	-	375	400
C2_At1g14300 (C2-6)	55	60	650	*Hinf*I	150+350	150+500
C2_At1g13380 (C2-7)	55	60	800	*Mbo*I	200	400
C2_At3g54860 (C2-8)	55	60	300	*Alu*I	200	150
C2_At3g46780 (C2-9)	55	90	1,600/1,400	-	1,600	1,400
C2_At5g05690 (C2-10)	55	60	625	*Hha*I	600	325+275
C2_At1g21640 (C2-11)	53	30	450	*Rsa*I	300	400
C2_At1g12060 (C2-13)	55	30	400	*Hind*III	250	400

C2_At4g29490 (C2-15)	55	60	500	*Dde*I	200+150	300
C2_At1g02180 (C2-16)	55	90	1,700	*Rsa*I	900	700+450
TG176	58	60	350/450	-	350	450
CD40	55	60	800/775	-	800	775
TG302	55	60	750/850	-	750	850
C2_At1g64550 (C2-18)	55	60	450	*Alu*I	450	350
TG18	55	30	500	*Mbo*I	200	500
C2_At3g10070 (C2-22)	50	90	1,600	*Hap*II	600	1,600
C2_At2g38025 (C2-23)	53	90	1,200	*Mbo*I	1,200	1,000
C2_At1g16590 (C2-24)	55	30	450	*Nla*III	200+100	350
TG230	50	90	1,200	*Hha*I	1,100	1,200
C2_At5g25760 (C2-29)	53	90	1,200	*Alu*I	500+450	1,000
C2_At5g58490 (C2-30)	53	60	1,000/500	-	1,000	500
TG393	55	60	700	*Rsa*I	300	600
C2_At4g26180 (C2-31)	55	30	350	*Rsa*I	300	250
CT99	50	60	900/1,000	-	900	1,000
C2_At4g31150 (C2-33)	55	60	500	*Dde*I	300+200	500
CD2	55	60	1,000/900	-	1,000	900

[#] COSII (conserved orthologous set II) markers (coded in SOL Genomics Network as C2_At…) were recoded as C2-1 to C2-33.

[*] Different sizes separated by bars indicated the fragment length for 'VF36' and'LA2951' respectively.

[§] Fragment sizes either at the PCR or after digesting the PCR when necessary.