Acta biol. Colomb., Vol. 16 N.º 2, 2011 47 - 62

LINKAGE MAPPING OF CANDIDATE GENES FOR INDUCED RESISTANCE AND GROWTH PROMOTION BY *Trichoderma koningiopsis* (Th003) IN TOMATO *Solanum lycopersicum*

Mapeo de genes candidatos relacionados con inducción de resistencia sistemica y promoción de crecimiento por *Trichoderma koningiopsis* (Th003) en tomate *Solanum lycopersicum*

JAIME SIMBAQUEBA[1], M.Sc., ALBA MARINA COTES[1], Ph. D.; LUZ STELLA BARRERO[1], Ph. D.
[1] Plant Molecular Genetics Lab, Center for Biotechnology and Bioindustry (CBB), Corporación Colombiana de Investigación Agropecuaria (CORPOICA). Km 14, vía Mosquera, Cundinamarca, Colombia.
Corresponding author: Km 14, vía Mosquera, Cundinamarca, Colombia. Phone: 57 1 422 73 00, ext. 1428 lbarrero@corpoica.org.co

Presentado 8 de julio de 2010, aceptado 9 de marzo de 2011, correcciones 23 de marzo de 2011.

ABSTRACT

Induced systemic resistance (ISR) is a mechanism by which plants enhance defenses against any stress condition. ISR and growth promotion are enhanced when tomato (*Solanum lycopersicum*) is inoculated with several strains of *Trichoderma* ssp. This study aims to genetically map tomato candidate genes involved in ISR and growth promotion induced by the Colombian native isolate *Trichoderma koningiopsis* Th003. Forty-nine candidate genes previously identified on tomato plants treated with Th003 and *T. hamatum* T382 strains were evaluated for polymorphisms and 16 of them were integrated on the highly saturated genetic linkage map named "TOMATO EXPEN 2000". The location of six unigenes was similar to the location of resistance gene analogs (RGAs), defense related ESTs and resistance QTLs previously reported, suggesting new possible candidates for these quantitative trait loci (QTL) regions. The candidate gene-markers may be used for future ISR or growth promotion assisted selection in tomato.

Key words: *Trichoderma koningiopsis*- *Solanum lycopersicum*- induced systemic resistance- growth promotion- candidate genes -genetic linkage map.

RESUMEN

La resistencia sistémica inducida (ISR) es un mecanismo mediante el cual las plantas aumentan sus defensas frente a cualquier condición de estrés. El objetivo de este trabajo fue localizar en el mapa genético de tomate, genes candidatos involucrados en ISR y promoción de crecimiento inducidos por la cepa colombiana nativa Th003 de

Trichoderma koningiopsis. Se realizó una búsqueda de polimorfismos en cuarenta y nueve genes candidatos previamente identificados en plantas de tomate inoculadas con Th003 y la cepa T382 de *T. hamatum*. Diez y seis de estos genes candidatos fueron integrados en el mapa genético de tomate altamente saturado, llamado "TOMATO EXPEN 2000". La ubicación de seis unigenes fue similar a la localización de genes análogos de resistencia (RGAs), ESTs relacionados con defensa y QTLs de resistencia previamente identificados, sugiriendo posibles nuevos candidatos para estas regiones de QTLs. Los genes candidatos o marcadores pueden ser usados en futuros programas de selección asistida relacionados con ISR o promoción de crecimiento en tomate.

Palabras clave: *Trichoderma koningiopsis*, *Solanum lycopersicum*, Inducción de resistencia sistémica, promoción de crecimiento, genes candidatos, mapeo genético

INTRODUCTION

Tomato, *Solanum lycopersicum*, is the most important vegetable crop in the world and constitutes approximately 30% of vegetable consumption with developing countries contributing about 65% of world production; however, phytosanitary constraints are major limiting factors for this production (Peralta and Spooner, 2007). Biological control is an alternative for sustainable disease control and crop production in agriculture. In this context, plant induced systemic resistance (ISR) has aroused great interest. ISR is a mechanism where the defensive capability is enhanced by a specific elicitor by which the innate plant defenses are activated against subsequent biotic targets. It is effective against a broad range of pathogens and is mostly caused by necrotrophic pathogens, herbivorous insects, beneficial microorganisms as growth promoter rhizobacteria (PGPRs) and some strains of *Trichoderma* spp. This mechanism involves production of the plant jasmonic acid (JA) and ethylene (ET) (Van Loon *et al.*, 1998; Hammerschmidt 1999; Harman 2004; Segarra *et al.*, 2009).

Trichoderma ssp. are cosmopolitan soil fungi, widely used to control plant pathogens and pests and promote plant growth (Yedidia *et al.*, 2000; Howell 2003; Harman *et al.*, 2004). For example, in maize, differentially expressed proteins induced by T. harzianum T22 suggested that T22 stimulates both increased growth, which is mediated by an increase in photosynthetic and respiratory rates, and systemic induced resistance (Shoresh and Harman, 2008). Other examples involving pathosystems demonstrate the biocontrol activity of *Thrichoderma*. In cucumber, *T. asperellum* T203 strain induced resistance against *Pseudomonas syringae* pv. *lachrymans* (Shoresh *et al.*, 2005). In *Arabidopsis*, *T. asperellum* T34 has a great inducer effect against *P. syringae*, *Hyaloperonospora parasítica* (biotrophic) and *Plectosphaerella cucumerina* (necrotrophic); (Segarra *et al.*, 2009).

In tomato, studies concerned with the control activity of a native *T. koningiopsis* isolate namely Th003 from Colombia have shown that in addition to its antagonistic activity, this strain induced resistance against *Fusarium oxysporum*, promoted growth and enhanced seed germination rate (Moreno *et al.*, 2009). Using the TOM1-ESTs microarray available at the Solanaceae Genomics Network (SGN); (Mueller *et al.*, 2005) coupled with real-time PCR, (Moreno *et al.*, 2009) also showed differential expression of 45 genes (41 in roots and 4 in leaves) in Th003 treated *vs.* non treated tomato plants. The genes belonged

to the functional categories of transport, signaling, cell wall degradation and hormone responses (auxin and ethylene). Some genes were also differentially expressed by *T. hamatum* T382, which induced resistance against *Xanthomonas euvesicatoria* in tomato (Alfano *et al.*, 2007).

The present study aims to contribute the understanding of ISR and growth promotion triggered by Th003 and T382, through the integration of differentially expressed genes from (Moreno *et al.*, 2009), in the "TOMATO EXPEN 2000" linkage map. This map is based on 80 F2 individuals from the cross *S. lycopersicum* LA925 and *S. pennellii* LA716 and contains about 2506 RFLPs, COS, SSRs, CAPS and other markers; furthermore, this map is anchored to the BAC physical map, used for the tomato genome sequence (Mueller *et al.*, 2005). The location of these candidate genes on the tomato map provides a key starting point for validation through subsequent QTL analyses for ISR or growth promotion, the discovery of novel genes affecting the phenotype not identified by microarray analysis, and their subsequent use in breeding programs related to biological control.

MATERIALS AND METHODS

Plant material and DNA extraction

The 80 F2 plant population along with the parents *S. lycopersicum* and *S. pennellii* (referred as to *Sl* and *Sp*, respectively) of the TOMATO EXPEN 2000 map (Mueller *et al.*, 2005), were kindly donated by professor Steven D. Tanksley, Cornell University, USA, and were propagated *in vitro*. DNA extractions from *in vitro* foliar tissues were carried out according to Fulton *et al.*,2002.

Gene primer design

Forty-five gene sequences were obtained from Moreno *et al.*, 2009 and four from Alfano *et al.*, 2007 (Table 1). Primers derived from candidate genes were designed based on the SGN unigene sequences (EST assembles), and each sequence was compared using *BLASTn* against the SGN unigene and the SGN BAC sequence database (Mueller *et al.*, 2005). Unigenes not found in tomato BACs were compared against *Arabidopsis thaliana* mRNA and genome databases (NCBI, http://www.ncbi.nlm.nih.gov) using *tBLASTx*. Each unigene *vs.* its corresponding tomato BAC or *Arabidopsis* homolog were aligned using Dialign 2.2.1 (Lassmann and Sonnhammer 2002). Primers were designed from a region spanning coding positions and a complete intron using Primer3 v 0.2 (Rozen and Skaletsky 2000).

PCR amplification

Candidate genes were PCR amplified using the designed primers (Table 1) initially in parents, and those amplifying, were screened in the F2 population. Briefly, each amplification was carried out in a 10 µl final reaction containing 1X PCR buffer, 2.5 mM $MgCl_2$, 0.2 µM dNTPs, 0.2 µM each primer, 0.05 U/µl Taq polymerase and 5 ng/µl DNA. All PCR reactions were carried out on an I-cycler (BIORAD) thermocycler programmed for 4 minutes at 94°C (initial denaturation), 30 seconds at 94°C (denaturation), 40 seconds at 56°C (annealing), 2 minutes at 72°C (extension) for 34 cycles, and then, 10 minutes at 72°C (final extension).

Polymorphisms in PCR products

PCR products were run on 2% agarose gel electrophoresis, and visualized with the GeneSnap Windows XP software (SYNGENE). Products showing different sizes in bp (i.e. polymorphic by insertions/deletions, InDels>20pb) in parents, were amplified directly in the F2 population for mapping analysis. PCR products not showing gel size differences in both parents were converted to CAPS markers by using two approaches. The first one was based on in silico analyses of parental sequences, which were cleaned, edited, and aligned to search for polymorphisms (SNPs or small InDels) using PHRED/PHRAP/CONSED (Gordon *et al.*, 2001) and to predict restriction enzymes recognizing the polymorphic region using CAPS designer (Mueller *et al.*, 2005). The second one was performed by using nine randomly chosen restriction enzymes (*Eco*RI, *Eco*RV, *Hind*III, *Hae*III, *Rsa*I, *Msp*I, *Bgl*II, *Dra*I y *Pst*I) (Table 1). PCR digested products were visualized on 2% agarose gel.

Location of candidate genes on the TOMATO EXPEN 2000 map

Linkage analysis was carried out using the MapDisto software for windows (Lorieux, 2007), which uses similar algorithms implemented in MapMaker (Lander *et al.*, 1987). Two-hundred four framework markers were selected from SGN (Mueller *et al.*, 2005), 200 based on location at LOD ≥3 and average distance of 5 cM between markers along the 12 chromosomes of the tomato map and four markers selected from chromosome 11 with LOD≤2 since they served for comparisons with the tomato map developed by (Ashrafi *et al.*, 2009). Candidate genes were located with threshold parameters of LOD≥4, a maximum distance of 30cM, and the Kosambi mapping function (Kosambi, 1944).

RESULTS

Marker development of candidate genes

From 49 primers designed from candidate genes, 33 produced successful PCR amplification products in both parents (66%); of these, two were derived from Alfano *et al.*, 2007, six from Moreno *et al.*, 2009, and 25 from this study. Seven of the 33 (21%) were polymorphic by size and 26 (79%) were monomorphic (Table 1). The CAPS approach based on in silico prediction was useful for three genes (Fig. 1A) in which alignment of good quality sequence (PHRED score >15) had a minimum of 100 base pairs overlap in both parents. The Random CAPS approach was done with nine restriction enzymes on 23 candidate genes of low quality sequence (PHRED score <15), obtaining polymorphisms on eight genes in both parents (Fig. 1B; Table 1). Therefore, marker conversion was performed for 18 of the 49 candidate genes analyzed in this study.

Integration of marker genes on the TOMATO EXPEN 2000 map

To determine the location of the 18 marker genes on the tomato map, each one was PCR amplified in the F2 population and either visualized directly on a 2% agarose gel or digested with the corresponding CAPS enzyme (Table 1). The map was constructed with 204 framework markers, 16 candidate gene markers and either an allele or a duplication of the gene locus *Ara* (Fig. 2; Table 1). One unigene (U586438) out of the 18 was unlinked. The candidate genes integrated on the map were located on

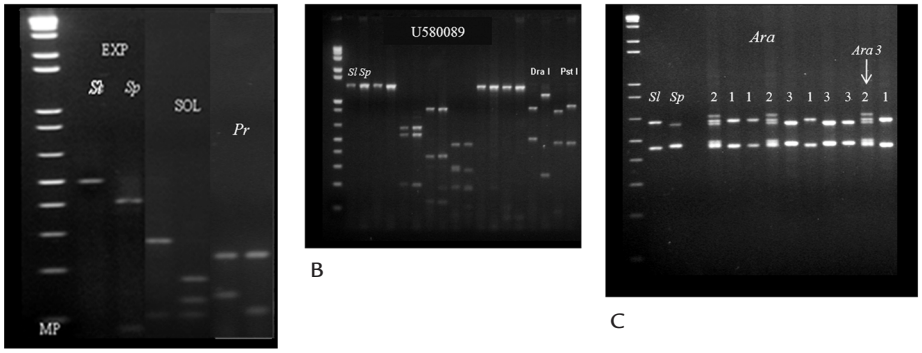
Tissue	SGN Unigen ID	Protein name	Putative Function	Primer – F Primer - R	Size (pb) *Sl*	 *Sp*	CAPS mapping enzyme
	SGN-U585601[6]	*Tr8*	Proteinase inhibitor type [a]	GAATGGGTAAGTGAGGGAGAAA 2 TTCTTAGAACAAACTAGTGTTCCATTT	200	230 [5]	
LEAVES	SGN-U575798	RPS3	Biosynthesis, ribosomal protein [a]	GCGTATCGACGGAAAAGAAA [1] TAATGCGAAGTGATGGGTTG	400	400 [4]	
	SGN-U577360[6]	*PR*	Ethylene induced protein [b]	GAGGCGTTATCATGGACGTT [2] TGGTTCTCATCGTCAGCAAG	350	350 [4]	HhaI
	SGN-U585904		Catalytic activity [a]	GATATCGACCTGGAGGAGCA [1] TGACTGCAGAGCATTTCCAC	300	300 [4]	
	SGN-U580314		Histidin Descarboxilase [a]	GGCACAAATTCTTGGGATGT [1] TTCCATCCTGAAACCACCTC	400	400 [4]	
	SGN-U580745[6]	PL2	Ribosomal complex [a]	TGACCGTGGTGTTTTTGCTA [1] ATGGCTCGACATCCACTAGG	690	690 [4]	*Rsa*I
	SGN-U582344	C-UL	Catalytic activity [a]	TGTTCGAGGTATCCACACCA [1] TGCTACCACACCCAAACTCA	380	380 [4]	
	SGN-U582239	AO1	Catalytic activity, oxide-reduction [a]	CTTTGGAGAGTCCGAGCAAC [1] CCATAAGAGCAGCACGTGAA	200	200 [4]	
ROOTS	SGN-U578305[6]	AB	Sucrose catalysis [a]	GGCCAGTGGAAGAAATTGAA [2] GAGTCTCTGCACGACCATCA	600	620 [5]	
	SGN-U573196 [6]		Lipase [a]	ATAACGGGGCGTTGTTCTAA [1] CCCCTGCTACTGCAAAATTC	230	230 [4]	*Rsa*I
	SGN-U582390 [6]		Chaperon [a]	AAACTTCCAGCTGATCTTCATGT [1] CGACAAAGCCAACAAGGATA	820	800 [5]	
	SGN-U581820	TIR1	Proteolysis [b]	TCGGTGTAATGGTGGTCTGA [1] ATCGTGCCAGAAGGAATTTG			
	SGN-U581328	PTOM13	Ethylene syntesis, defense [b]	TGAGTTGGTGAACCATGGAA [1] TTGAGGAGTTGAAGGCCACT	450	450 [4]	
	SGN-U580403	PE8	Ethylene response, ripening [b]	AAGGCCGGTGTTAAAGGACT [2] TGCAACGTTCTGTCCAAGAC	260	260 [4]	

Tissue	SGN Unigen ID	Protein name	Putative Function	Primer – F Primer - R	Size (pb) *Sl*	 *Sp*	CAPS mapping enzyme
	SGN-U590337	ARG10	Auxin regulation [b]	GCATTTCTCATCTGCCCATT [1] CATTCTAGATGGGGCCTTGA			
	SGN-U578859		Rich prolin protein [c]	TTCAATGTTCAAGCTGCTACAAA [1] TTTTTCAGGATTGCAACACAA			
	SGN-U577960		Membrane binding and transport [c]	TGTATCAAAGACCGCTGCTG [1] TATGCACCACCAGATCCGTA			
	SGN-U579836		Membrane binding and transport [c]	AGTGCCAAACATCCAACACA [1] GCGGAGATCATTGGAACATT	630	630 [4]	
	SGN-U579050	P-ABC	Membrane binding and transport [c]	CCTAGAGGCCTGTGTGTGGT [1] TCATTGCAGAAATCTGCTCCT	250	250 [4]	
	SGN-U569177 [6]		Permease [c]	GAAGGAAGCGTGCAATCAAG [1] CAACGCCTTCCGATATCATT	460	460 [4]	EcoRV
	SGN-U573760		ATPase [c]	GGATGGCGATGGCTGTATTA [1] ATCTTCCGTGCCATCAGATT	920	920 [4]	
	SGN-U584666 [6]	VPS13	Intracellular protein transport [c]	GTTGGGAAGAAGCAATTGGA [1] GCTCCGACCTTGTTCTTTTG	500	500 [4]	PstI
	SGN-U578955		MADS, DNA binding [d]	CGATGCTTCGACGATATGAA [1] CAGAAGCAAGGCTTTTCGAC			
	SGN-U574293		Nucleic acid binding [d]	AAACCTAGGCCAGAGGAACC [1] CTCGACCACGGACATCATAA			
	SGN-U565731		Kinase [d]	TGAAGGATCCCAGCGTTATC [1] CAAAAGCCGCAAAAATCTTC			
	SGN-U580728		Calmodulin [d]	GGATGGCGATGGCTGTATTA [1] ATCTTCCGTGCCATCAGATT	180	180 [4]	
	SGN-U5762506	*ARA* -MYB	Transcriptional regulator [d]	TCCATGGACTGTTGAAGAAGA [2] CAGTTCTCCCTGGCAAATGT	460-580	460-560 [5]	
	SGN-U586438		Transcription factor related to pathogenesis [d]	AGCAACCAAGGCTTACTGGA [1] AGTGAAAGTTCCCAGCCAAA	690	690 [4]	PstI

Tissue	SGN Unigen ID	Protein name	Putative Function	Primer – F Primer - R	Size (pb) *Sl*	 *Sp*	CAPS mapping enzyme
	SGN-U579779[6]		Ca^{++} binding [d]	AGTCAGGTGATGGACGGTTC [1] GGCCCCATACCAGTACCATT	500	480 [5]	
	SGN-U577352[6]	*Asr3*	Transcription factor, hydric stress [d]	AGCACCATAGCCATCTCCAG [1] TCCTTTCTCAGCTGCCTTTT	630	660 [5]	
	SGN-U567693		Kinase [d]	ACTCCTGCCATGAAAACCAC [1] TCACTCCAACGAGAAGCAGA	400	400 [4]	
	SGN-U569983[6]	SRE1B	*S. tuberosum* systemic acquired resistance d	TGACTTGGAAGTTGCTGTGC [1] CACATTCCTTGCACAAATGG	480	480 [4]	PstI
	SGN-U5686166	EXT	Cell wall organization, defense [d]	CACTATGTTTACTCCTCTCCC [3] CATATGGGAGTAGTAATAAC	520	550 [5]	
	SGN-U577557	OSM	Pathogenesis related protein [d]	GACTTACACTTATGGTTCCG [3] CACCGTTTATATTGGCTGTGC			
	SGN-U574735	MYB	Transcription factor, DNA binding [d]	CCTACCAATGATAGAA [3] ATGGTACACACACCTACACG			
	SGN-U565412	CRY1B	Photomorphogenesis [e]	GGATAATTGCGCAAAGGAAA [1] CTCGAGCAAGAAGTGCATTG	420	420 [4]	
	SGN-U580089 [6]		Fructose 1,6 Bisphosphatase [e]	AAAAATCGAAAGCACGATGG [1] ATCCCAGGTTGCAAGACATC	960	960 [4]	DraI
	SGN-U5738966	*SOL*	Photoperiod response	CTCTAATCGCGGTCTCAACC [2] TGTTAGCCCATTTGCCTTTC	400	400 [4]	RsaI
	SGN-U583901		Membrane component [f]	CATCATCTTCGTCTGCGAAA [1] ATCACACAACGGCCAGTACA			
	SGN-U579218		Elastin, Polyprotein [f]	CATATTGCAGTGCGCCTCTA [1] GAAGCCCTTCCACACCTGTA			
	SGN-U5812966	*EXP*	Cell wall structural organization [f]	GTATCGTCCCTGTATCTTTTCG [3] CCTACTCACCCCTTTTATGCC	420	420 [4]	Hsp92II
	SGN-U576195		Hydrolase [g]	AATTGGGGGAGCTAAGGAGA [1] GCAGAGCATTGTATCCACCA	550	550 [4]	

Tissue	SGN Unigen ID	Protein name	Putative Function	Primer – F Primer - R	Size (pb) *Sl*	*Sp*	CAPS mapping enzyme
	SGN-U583548		Uncharacterized [h]	AAGGGAAAAATGGCATCTCA [1] TGCGTATGCTGTCTCTAGCTG			
	SGN-U566136		Uncharacterized [h]	GCCAATGTCCTCCACATTCT [1] AGGACGCAATTTTTGTCACC	400	400 [4]	
	SGN-U568028		Uncharacterized [h]	TTAAGAGGATGGGGGAGCTT [1] AAACGGCTAAGGAAAGCACA			
	SGN-U566629		Uncharacterized [h]	ATGAGAGCAAGTGCCACTGA [1] CAACAGCAGCAGCATTCACT			
	SGN-U577507		Uncharacterized [h]	CCGCCTCACTGACTTGAAAC [1] CCCCTCTATTTTTGGCATCA			
	SGN-U566395		Uncharacterized [h]	AGCTGCAACTGCTCCTAAGC [1] TCTGAACCATCCTCCTCACC			
	SGN-U570158		Uncharacterized [h]	AAAGGAAGGCAAAGATGAGAAC TCGCATTGCCTTAAACATCA	360	360 [4]	

Table 1. Candidate genes analyzed in this study.
Functional categories: a Metabolism, b Hormonal response, c Transport, d Signaling, e light response, f Structural, g enzymatic degradation, h Uncharacterized. Primer sequences: 1Primer design, 2 From Moreno *et al.*, 2008, 3 From Alfano *et al.*, 2007. PCR products without CAPS: 4 Monomorphic, 5 Polymorphic. 6 Genes analyzed for genetic mapping. *Sl*: *Solanum lycopersicum*, *Sp*: *S. pennellii*.



A B C

Figure 1. A. Enzyme digestions for *Exp*, *Sol* and *Pr* with Hsp92II, RsaI and HhaI, respectively based on in silico predictions. B. An example of random digestions with nine restriction enzymes on one (U580089) of the 23 monomorphic PCR products. Polymorphic bands were observed using DraI and PstI. DraI was selected for linkage analysis (Table 1). C. Polymorphic candidate gene (*Ara*) amplified in parents and F2 population. Arrows indicate possible gene duplication events (*Ara1* and *Ara2*); *Ara3* is present only in F2 heterozygotes. *Sl*= *S. lycopersicum*, *Sp*= *S. pennellii*. Homozygote *Sl*= 1, Homozygote *Sp*= 3, Heterozygote= 2.

chromosomes 1 (U573196, U579779), 2 (U569177), 3 (*Ab*), 4 (U580089, *Sol, Ext* and *Asr3*), 6 (*Exp* and *Pr*), 7 (U586360), 11 (U584666 and *Tr8*) and 12 (*Ara1*, *Ara2*, *Sre1b* and *Pl2*). The generated map covered a length of 1083 cM, which is similar to previous tomato maps made from the same species (Grandillo and Tanksley, 1996; Frary *et al.*, 2004; Sharma *et al.*, 2008; Ashrafi *et al.*, 2009).

DISCUSSION

Comparisons with similar marker data obtained by Zhang *et al.*, 2002, Sharma *et al.*, 2008 and Ashrafi *et al.*, 2009, were performed since the authors report a thorough localization of candidate resistance/defense genes and QTLs in tomato. Zhang *et al.*, 2002, constructed a map based on Restriction Fragment Length Polymosphisms (RFLPs) and resistance gene analog (RGAs) markers, Sharma *et al.*, 2008, constructed a map based on RFLPs, ESTs similar to known resistance, defense, signaling, transcription factors and RGAs mainly obtained from Zhang *et al.*, 2002. Whereas, Ashrafi *et al.*, 2009, using an advanced F7 population derived from the same population used by Sharma *et al.*, 2008, constructed a map based on RFLPs, SSRs, CAPS markers and ESTs chosen based on their putative unigene roles in disease resistance and defense-related response.

Furthermore, data obtained in the present map were also compared with the location of other disease and pest resistance QTLs in tomato (Kaloshian *et al.*, 1998; Ammiraju *et al.*, 2003; Brower and St.Clair 2004; Scott *et al.*, 2004 and Davis *et al.*, 2009).

Eleven unigenes mapped to chromosomes 1 (U573196, U579779), 4 (*Sol, Ext* and *Asr3*), 6 (*Pr*), 7 (U586360) and 12 (*Pl2, Sre1b, Ara1* and *Ara2*) in regions were no previous resistance markers or QTLs were reported. However, six unigenes were positioned to chromosomal regions where other resistance QTLs, ESTs or RGAs have been placed in tomato. The chromosomal position of these six unigenes as well as other six not necessarily

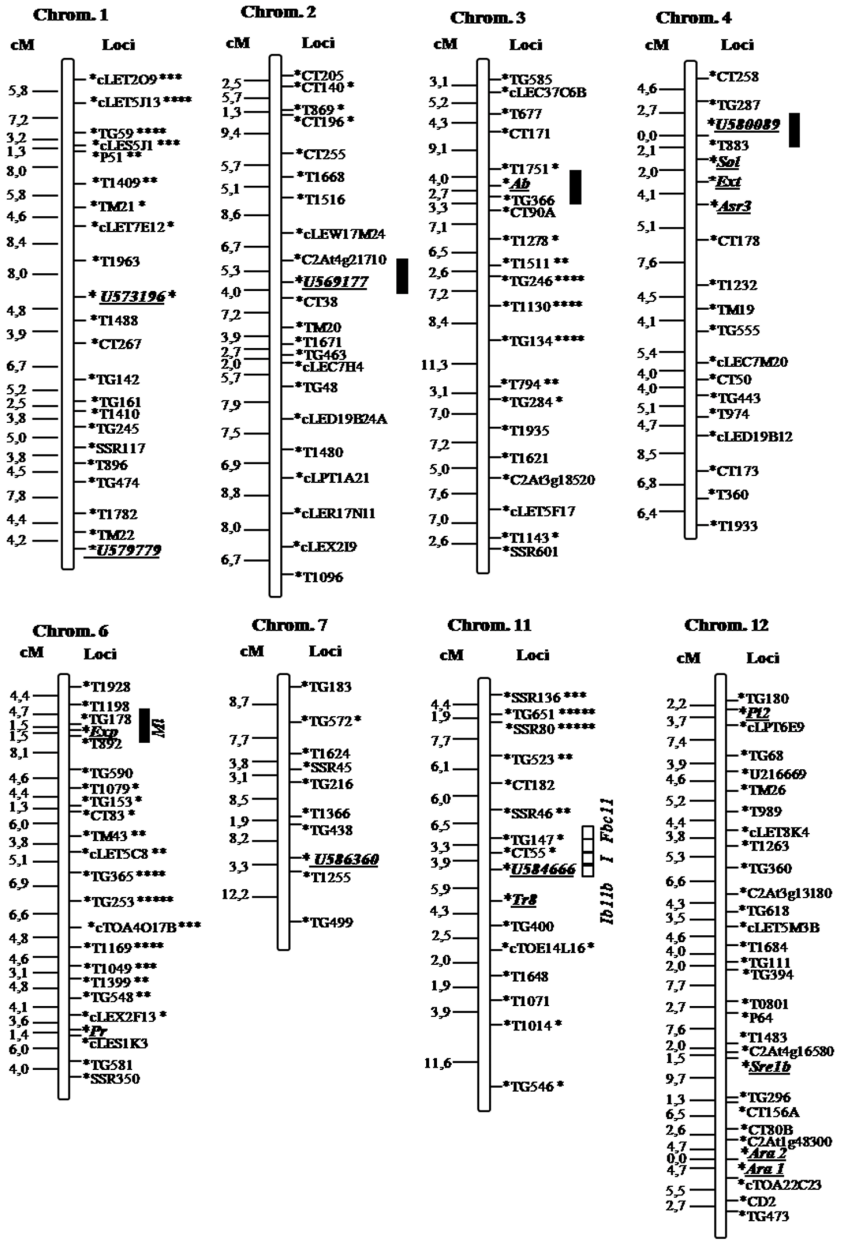


Figure 2. Linkage map generated by MapDisto (Lorieux *et al.*, 2007). Marker names are found at the right of each chromosome and CentiMorgan distances (cM) are shown at the left. Candidate genes are shown in ithalics bold and underlined. The bars at the right of chromosomes indicate approximate positions of similar defense related ESTs (solid bars) or resistance gene analogs RGAs (dashed bars) located on the linkage maps developed by Sharma *et al.*, 2008 and Ashrafi *et al.*, 2009. QTLs are: *Mi= Melodoyine* ssp. resistance (Kaloshian *et al.*, 1998; Ammiraju *et al.*, 2003); I= *Fusarium oxysporum* f.*sp* lycopersici resistance (Scott *et al.*, 2004); Ib11b= *P. infestans* resistance (Brower and St.Clair, 2004); Fbc11= *B. cinerea* succeptibility (Davis *et al.*, 2009).

co-localizing with previous QTLs, RGAs or ESTs related to defense but otherwise interesting because their putative role in resistance or growth is described as follows.

Unigene U569177 was located on chromosome 2, whereas, *Ab* (U578305), was placed on chromosome 3. The former putatively functions in intracellular transport and the latter is related to metabolic function (Table 1). Both co-localized within a 1 to 2 cM EST region reported by Sharma *et al.*, 2008 and Ashrafi *et al.*, 2009, with putative metabolic functions related to ripening.

Unigenes U580089, *Sol* (U573896), *Ext* (U568616) and *Asr3* (U577352) were located on chromosome 4, these unigenes were separated from each other by 2 to 4.5 cM (Fig. 2). U580089 and *Sol* are predicted to be involved in metabolic processes (Mueller *et al.*, 2005; Table 1). Ext belongs to the extensin gene family, whose members are related to structural cell wall function and play important roles in plant defense (Baumberger *et al.*, 2003; Merkouropoulos and Shirsat, 2003; Wei and Shirast, 2006). *Asr3* belongs to the *Asr* gene family of transcription factors expressed in response to abcisic acid (ABA) under drought stress conditions (Rossi *et al.*, 1996; Frankel *et al.*, 2003). The position of *Asr3* agrees with previous studies where three *Asr* genes were placed at about a 19 cM of CD55, which is located at about 1 cM of the T1050 marker close to the *Asr3* mapped in this study (Rossi *et al.*, 1996; Tanskley *et al.*, 1992). Furthermore, the region where U580089 was placed expands an EST related with water/salt stress response described by Ashrafi *et al.*, 2009 (Fig. 2).

Exp (U581296) and *Pr* (U577360) candidates were located on chromosome 6 (Fig. 2). *Exp* belongs to the expansin gene super family involved in growth, expansion of cell wall and is downregulated by growth hormones (auxins, gibberelic acid and ethylene; Catalá.*et al.*, 2000; Cosgrove 2000). *Pr* is related to ISR and functions in response to ethylene. An EST framework marker (cLES1K3) which maps at 1,4 cM of *Pr* mapped in this study is also a member of the unigene construct (SGN-U577360) of *Pr* (Mueller *et al.*, 2005), which could be the result of a *Pr* loci duplication.

Kaloshian *et al.*, 1998; Ammiraju *et al.*, 2003; Sharma *et al.*, 2008 and Ashrafi *et al.*, 2009, located several *Meloidoyine* spp. (*Mi*) resistance QTLs (*Mi-1, Mi-3* and *Mi-9*) close to *Exp* (Figure 2). Plant originated *Exp* expression have been associated with a successful parasitic nematode-plant interaction in tomato (Gal *et al.*, 2006; Fudali *et al.*, 2008); therefore, this co-localization suggest *Exp* as a candidate for *Mi*-tomato interaction.

U584666 and *Tr8* (U585601) were located on chromosome 11. U584666 is related to intracellular transport through vacuoles and *Tr8* functions in cellular metabolism as proteinase inhibitor, related to insects attack (Graham *et al.*, 1985). The position of *Tr8* agrees with previous reports where it was located near TG400, also used in the present study as a frame marker (Taylor *et al.*, 1993; Tanskley *et al.*, 1992; Fig. 2). In addition, a *Fusarium* spp. (*I*) and a *Phytophtora infestans* (*Ib11b*) resistance QTL, as well as a *Botrytis cinerea* susceptible QTL (*Fbc11*) were located between CT182 and TG400 markers, which span the U584666 and *Tr8* region (Scott. *et al.*, 2004; Brower and St.Clair, 2004; Davis *et al.*, 2009; Fig. 2). Moreover, Sharma *et al.*, 2008, located four RGAs and a resistance QTL (*I*) to *Fusarium* spp. in the same region where U584666 was placed.

Ara (U576250) was located on chromosome 12. Two PCR amplified fragments named *Ara1* and *Ara2*, were located at 0 cM from each other (Fig. 2). In addition, a third PCR amplification product (*Ara3*) was observed only in *Ara1* and *Ara2* F2 heterozygote

individuals (Fig. 1C); this observation might suggest either gene duplication events or different alleles of the same locus. A sequence alignment would be important to clarify this. These observations might agree with the fact that *Ara* belongs to one of the largest gene family of plants MYB transcription factors (Yanhui *et al.*, 2006).

Based on this positional information and the results obtained by Moreno *et al.*, 2009, we suggest that some genes analyzed in this study may not only be involved in *T. koningiopsis* Th003 and *T. hamatum* T382 response, but also, may constitute candidates for pathogen resistance QTLs (for example, *Exp* for *Meloidoyine* spp. resistance; U584666 and *Tr8* for *Fusarium* spp., *Phytophtora infestans* and *Botrytis cinerea* resistance or susceptibility). However, these hypotheses must be verified by further studies (e.g. QTL mapping, association to resistance, mutant complementation, gene silencing, among others). Further research on this topic should also consider information derived from the recently released tomato genome sequence draft (Mueller *et al.*, 2005). This sequence is derived from BAC clones anchored to the genetic map of tomato used in the present study. This is a powerful tool to identify several candidates through the whole tomato genome.

Genetic linkage mapping analysis of the differentially expressed candidate genes for Th003 and T382 response in tomato coupled with QTL analyses for specific growth and resistance phenotypes should be assessed. Preliminary measurements of phenotypic differences in response to Th003 in parents *Sl* and *Sp* of the TOMATO EXPEN 2000 population show differences in root volume of Th003 treated *vs.* non-treated plants, suggesting that is possible to evaluate growth promotion or ISR in response to Th003 to further QTL analysis in this population. This will foster future assisted selection for induced resistance or growth promotion triggered by native biocontrol agents in tomato breeding programs.

ACKNOWLEDGMENTS

The authors want to acknowledge Dr. Steven D. Tanksley for providing a copy of the TOMATO EXPEN 2000 population. To Ligia Suescún for assistance in the *in vitro* establishment of this population. To Oscar Bedoya for assistance in primer design. To Carlos Moreno for advice related to Th003. To Drs. Carolina González, Camilo López and Leonardo Mariño for critical reading of the manuscript. Funding was provided by CORPOICA and COLCIENCIAS.

REFERENCES

ALFANO G, IVEY ML, CAKIR C, BOS JI, MILLER SA, MADDEN LV, *et al.* Systemic Modulation of Gene Expression in Tomato by *Trichoderma* hamatum 382. Phytopathology. 2007;97(4):429-437.

AMMIRAJU JS, VEREMIS JC, HUANG X, ROBERTS PA, KALOSHIAN I. The heat-stable root-nematode resistance gene *Mi*-9 from Lycopersicon peruvianum is localized on the short arm of chromosome 6. Theor Appl Genet. 2003;106(3):478-484.

ASHRAFI H, KINKADE M, FOOLAD MR. A new genetic linkage map of tomato based on a *Solanum lycopersicum* x *S. pimpinellifolium* RIL population displaying locations of candidate pathogen response genes. Genome. 2009;52(11):935-956.

BAUMBERGER N, DOESSEGER B, GUYOT R, DIET A, PARSONS RL, CLARK MA, *et al*. Whole genome comparison of leucine-rich repeat extensins in Arabidopsis and rice. A conserved family of cell wall proteins form avegetative and a reproductive clade. Plant Physiol. 2003;131(3):1313-1326.

BROUWER DJ, ST CLAIR DA. Fine mapping of three quantitative trait loci for late blight resistance in tomato using near isogenic lines (NILs) and sub-NILs. Theor Appl Genet. 2004;108(4):628-638.

CATALA C, ROSE JK, BENNETT AB. Auxin-regulated genes encoding cell wall-modifying proteins are expressed during early tomato fruit growth. Plant Physiol. 2000;122(2):527-534.

COSGROVE DJ. Loosening of plant cell walls by expansins. Nature. 2000;407(6802):321-326.

DAVIS J, YU D, EVANS W, GOKIRMAK T, CHETELAT RT, STOTZ HU. Mapping of loci from Solanum lycopersicoides conferring resistance or susceptibility to *Botrytis cinerea* in tomato. Theor Appl Genet. 2009;119(2):305-314.

FRANKEL N, HASSON E, IUSEM ND, ROSSI MS. Adaptive evolution of the water stress induced gene *Asr2* in Lycopersicon species dwelling in arid habitats. Mol Biol Evol. 2003;20(12):1955-1962.

FRARY A, FRITZ LA, TANKSLEY SD. A comparative study of the genetic bases of natural variation in tomato leaf, sepal, and petal morphology. Theor Appl Genet. 2004;109(3):523-533.

FUDALI S, SOBCZAK M, JANAKOWSKI S, GRIESSER M, GRUNDLER FM, GOLINOWSKI W. Expansins are among plant cell wall modifying agents specifically expressed during development of nematode-induced syncytia. Plant Signal Behav. 2008;3(11):969-971.

FULTON TM, VAN DER HOEVEN R, EANNETTA NT, TANKSLEY SD. Identification, analysis, and utilization of conserved ortholog set markers for comparative genomics in higher plants. Plant Cell. 2002;14(7):1457-1467.

GAL TZ, AUSSENBERG ER, BURDMAN S, KAPULNIK Y, KOLTAI H. Expression of a plant expansin is involved in the establishment of root knot nematode parasitism in tomato. Planta. 2006;224(1):155-162.

GORDON D, DESMARAIS C, GREEN P. Automated finishing with autofinish. Genome Res. 2001;11(4):614-625.

GRANDILLO S, TANKSLEY SD. Genetic analysis of RFLPs, GATA microsatellites and RAPDs in a cross between *L. esculentum* and *L. pimpinellifolium*. Theor Appl Genet. 1996;92(8):957-965.

GRAHAM JS, PEARCE G, MERRYWEATHER J, TITANI K, ERICSSON LH, RYAN CA. Wound-induced proteinase inhibitors from tomato leaves. II. The cDNA-deduced primary structure of pre-inhibitor II. J Biol Chem. 1985;260(11):6561-6564.

HAMMERSCHMIDT R. Induced disease resistance: how do induced plants stop pathogens? Physiol. Mol Plant Pathol. 1999;55(2):77-84.

HARMAN GE, HOWELL CR, VITERBO A, CHET I, LORITO M. *Trichoderma* species-opportunistic, avirulent plant symbionts. Nat Rev Microbiol. 2004;2(1):43-56.

HOWELL CR. Mechanisms employed by *Trichoderma* species in the biological control of plant diseases: The history and evolution of current concepts. Plant Dis. 2003;87(1):4-10.

KALOSHIAN I, YAGHOOBI J, LIHARSKA T, HONTELEZ J, HANSON D, HOGAN P, *et al*. Genetic and physical localization of the root-knot nematode resistance locus mi in tomato. Mol Gen Genet. 1998;257(3):376-385.

KOSAMBI DD. The estimation of map distance from recombination values. Ann Eugenic. 1944;12:172-175.

LANDER ES, GREEN P, ABRAHAMSON J, BARLOW A, DALY MJ, LINCOLN SE, *et al*. MAPMAKER: an interactive computer package for constructing primary genetic linkage maps of experimental and natural populations. Genomics. 1987;1(2):174-181.

LASSMANN T, SONNHAMMER EL. Quality assessment of multiple alignment programs. FEBS Lett. 2002;529(1):126-130.

LORIEUX M. MapDisto, A Free User-Friendly Program For Computing Genetic Maps. Computer demonstration (P958) given at the Plant and Animal Genome XV conference, Jan 13-17 2007, San Diego,CA. Available from http://mapdisto.free.fr/.

MERKOUROPOULOS G, SHIRSAT AH. The unusual *Arabidopsis* extensin gene atExt1 is expressed throughout plant development and is induced by a variety of biotic and abiotic stresses. Planta. 2003;217(3):356-366.

MORENO C, CASTILLO F, GONZÁLEZ D, BERNAL A, JAIMES Y, CHAPARRO M, *et al*. Biological and molecular characterization of the response of tomato plants treated with *Trichoderma koningiopsis*. Physiol Mol Plant P. 2009;74(2):111-120

MUELLER LA, SOLOW TH, TAYLOR N, SKWARECKI B, BUELS R, BINNS J, *et al*. The *SOL* Genomics Network: a comparative resource for Solanaceae biology and beyond. Plant Physiol. 2005;138(3):1310-1317.

PERALTA IE, SPOONER DM. History, origin and early cultivation of tomato (Solanaceae). In: Genetic Improvement of Solanaceous Crops. Tomato. M.K. Razdan and A.K. Mattoo (eds), Science Publishers, Enfield, USA; 2007;2:1-27.

ROSSI M, LIJAVETZKY D, BERNACCHI D, HOPP HE, IUSEM N. *Asr* genes belong to a gene family comprising at least three closely linked loci on chromosome 4 in tomato. Mol Gen Genet. 1996;252(4):489-492.

ROZEN S, SKALETSKY H. Primer3 on the WWW for general users and for biologist programmers. Methods Mol Biol. 2000;132:365-386.

SCOTT J, AGRAMA H, JONES J. RFLP-based analysis of recombination among resistance genes to *Fusarium* wilt races 1, 2, and 3 in tomato. J Am Soc Hortic Sci. 2004;129:394-400.

SEGARRA G, VAN DER ENT S, TRILLAS I, PIETERSE CM. MYB72, a node of convergence in induced systemic resistance triggered by a fungal and a bacterial beneficial microbe. Plant Biol. 2009;11(1):90-96.

SHORESH M, HARMAN GE. The relationship between increased growth and resistance induced in plants by root colonizing microbes. Plant Signal Behav. 2008;3(9):737-739.

SHORESH M, YEDIDIA I, CHET I. Involvement of Jasmonic Acid/Ethylene Signaling Pathway in the Systemic Resistance Induced in Cucumber by *Trichoderma asperellum* T203. Phytopathology. 2005;95(1):76-84.

TANKSLEY SD, GANAL MW, PRINCE JP, DE VICENTE MC, BONIERBALE MW, BROUN P. *et al*. High density molecular linkage maps of the tomato and potato genomes. Genetics. 1992;132(4):1141-1160.

TAYLOR BH, YOUNG RJ, SCHEURING CF. Induction of a proteinase inhibitor II-class gene by auxin in tomato roots. Plant Mol Biol. 1993;23(5):1005-1014.

VAN LOON LC, BAKKER PA, PIETERSE CM. Systemic resistance induced by rhizosphere bacteria. Annu Rev Phytopathol. 1998;36:453-483.

WEI G AND SHIRSAT A. Extensin over-expression in *Arabidopsis* limits pathogen Invasiveness. Mol Plant Pathol. 2006;7(6):579-592.

YEDIDIA I, BENHAMOU N, KAPULNIK Y, CHET I. Induction and accumulation of *PR* proteins activity during early stages of root colonization by the mycoparasite *Trichoderma harzianum* strain T-203. Plant Physiol Bioch. 2000;38(11):863-873.

YANHUI C, XIAOYUAN Y, KUN H, MEIHUA L, JIGANG L, ZHAOFENG G, *et al.* The MYB transcription factor superfamily of Arabidopsis: expression analysis and phylogenetic comparison with the rice MYB family. Plant Mol Biol. 2006;60(1):107-124.

ZHANG LP, KHAN A, NINO-LIU D, FOOLAD MR. A molecular linkage map of tomato displaying chromosomal locations of resistance gene analogs based on a *Lycopersicon esculentum* x *Lycopersicon hirsutum* cross. Genome. 2002;45(1):133-146.