Call identifier: H2020 – SFS – 7a-2014
TRADITOM
Grant agreement no: 634561

Traditional tomato varieties and cultural practices: a case for agricultural diversification with impact on food security and health of European population

Deliverable No.D7.2

Linkage drag elimination

Contractual delivery date:
M36

Actual delivery date:
M36 (23/02/2018)

Responsible partner:
P1 CSIC

This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 634561.

Grant agreement no.	H2020 - 634561
Project full title	TRADITOM – Traditional tomato varieties and cultural practices: a case for agricultural diversification with impact on food security and health of European population

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Nature	Report
Dissemination level	Public
Work package number	WP7
Work package leader	P9-HUJI
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Keywords	Recombinant, productive traits, quality traits, sensory analysis

The research leading to these results has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 634561.
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1. Introduction

The objective of WP7, where this deliverable D7.2 fits, is to use marker assisted breeding approaches (non-GM) to improve the resilience of traditional tomato varieties to biotic and abiotic stresses, without affecting any of the unique characteristics and attributes that make these varieties a traditional success. D7.2 'Linkage drag elimination' is the result of work done in Task 7.2 that was directed to analyse the effect of wild species introgressions around resistance genes and to select for genotypes that carry minimal wild species introgressions around resistance genes.

In modern tomato breeding, the most common source of genetic resistance to pests and diseases (DRG) are related wild species. However, introgression of DRG from wild relative often induces negative effects either due to the introgressed genes themselves and/ or to linkage drag (Tanksley et al., 1998; Brouwer and St. Clair, 2004). Recently, Rubio et al. (2016) demonstrated that the introgression of TYLCV resistance (determined by *Ty-1* genes) from a modern hybrid into the traditional tomato cultivars 'De la pera' and 'Muchamiel' affected the agronomic performance of the cultivars, reducing up to 50% the tomato yield. According to these results, the cultivation of these traditional cultivars with *Ty-1* would be only recommended under high levels of virus infection. On the other hand, the effects of other resistance genes (*Tm-2a* to ToMV and *Sw-5* to TWSV) were minor and more variable, increasing or decreasing depending on the trial and the studied parameter. The effects of the introgressed genes are likely due to other wild genes that were dragged during the breeding process. Furthermore, Verlaan et al. (2011) reported the suppression of recombination in the *S. chilense* region containing the *Ty-1* gene due to the occurrence of two chromosomal inversions between the *S. chilense* LA1969 accession (the donor specie of the *Ty-1* gene) and *S. lycopersicum*. Fulton et al. (1997) reported an intrachromosomal translocation of TG9 marker, in chromosome 9 of *S. peruvianum*, where *Tm-2a* gene is located. The consequence of this recombination suppression is that usually the introgression would be large, containing a relatively large number of genes, and the linkage between the R genes and other genes with undesirable effects are very difficult or impossible with classical breeding approaches.

Earlier work using the 8K SolCap Illumina Infinium SNP tomato array (Sim *et al.*, 2012) defined the size of the introgressed chromosome from the wild species, spanning over more than half chromosome: 51 Mb for *Tm-2a* (76 % of chromosome 9) and 45 Mb for*Ty-1* (69% of chromosome 6), whereas for *Sw-5* was only 200 kb. These large introgressed segments harbor thousands of genes which are known to affect important traits, such as fruit size and quality. This situation is found in many other varieties carrying DR, including modern hybrids as the one used as source of DRG in our breeding program. Similar situations can be found in any other program aimed to introduce DRG in traditional tomato varieties.

The objective of the current deliverable is to minimize the linkage drag effects on yield and quality traits on breeding lines derived from traditional varieties lines carrying virus genetic resistance. Due to recombination suppression, we needed to screen a large number of individuals in the recombinant populations using molecular markers flanking the introgressed fragments in order to identify those rare recombination events in the *Ty-1* and *Tm-2a* introgressions. The agronomic performance of the recombinants was assessed to confirm whether the undesirable linkage drag effects were removed in the recombinant genotypes.

2. Description of Activities

The tasks performed during years 2016 and 2017 are shown schematically in Figure 1 and described in more detail below.

Starting plant material

'Muchamiel 'and 'De la pera' breeding lines that were previously developed by P1-CSIC-UMH carrying *Sw-5*, *Ty-1* and *Tm-2a* introgressions were backcrossed to their respective traditional variety to obtain F1 plants. The size of the introgressed chromosome regions containing the resistance genes were 33 Mb for *Ty*-1 gene, 51 Mb for Tm-2a gene and 200 kb for Sw-5. F1 plants were selfed to obtain the number of F2 seedlings that were required for recombinant screening (*Figure 1*).

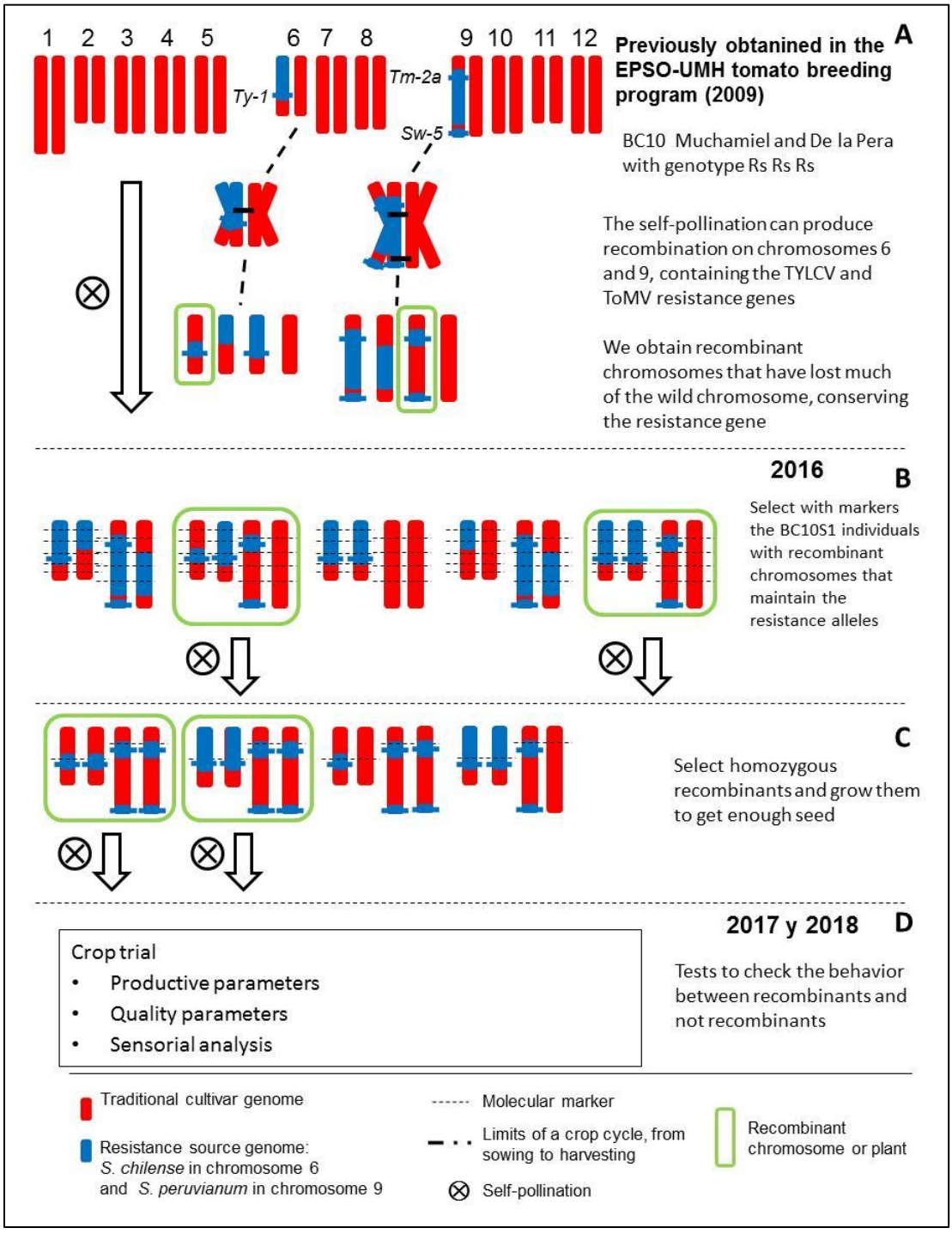


Figure 1: *Recombination and breeding scheme. A) the original genotype of a plant carrying the 3 R genes and that was crossed with the recurernt cultivar and the hybrid selfed. B) F2 seedling were genotyped with an array of markers for each segment and recombinant individuals transferred to greenhouse and selfed. C) Recombination events were fixed and seed multiplied. D) Recombinant genotypes were compared with original varieties in agronomic trials.*

Search for recombinants in the regions of the TYLCV and ToMV resistance genes

Two-hundred 'De la pera' and 800 'Muchamiel' F2 seedlings were screened with SNPs (*Table 1*) distributed around the *Ty-1* and *Tm-2a* genes. Recombinant plants in *Ty-1* and *Tm-2a* regions were transferred to greenhouse for selfing.

Table 1: *Markers used in the selection of recombinant plants and their position in each chromosome. The position is based in the 8K SolCap Illumina Infinium SNP tomato array information.*

Chromosome 6 (*Ty-1*)	
Marker	Position (MB)
Solcap_snp_sl_28362	29.25
Solcap_snp_sl_100298	30.62
Ty-1	
Solcap_snp_sl_44625	30.92
Solcap_snp_sl_44650	31.22
W	
Solcap_snp_sl_44670	31.31

Chromosome 9 (*Ty-1*)	
Marker	Position (MB)
Solcap_snp_sl_45141	7.16
Solcap_snp_sl_41493	13.39
Tm-2a	
Solcap_snp_sl_55219	18.30
Solcap_snp_sl_51514	35.67
Solcap_snp_sl_43269	57.19

Fixation of recombinants

Seeds from the selfing of recombinant plants were obtained, 480 F3 seedlings were screened with SNPs Solcap_snp_sl_28362 and Solcap_snp_sl_45141 (*Table 1*) to identify the homozygous recombinants in *Ty-1* and *Tm-2a* genes regions, respectively. Selected plants were transferred to greenhouse to obtain the amount of seeds sufficient to carry out the proposed trials (at least 200 seeds from each plant).

Figure 2: *Recombinant plants in TYLCV resistance gene region.*

Trials

Recombinant and non-recombinant progenies were grown in the 2017 spring-summer crop cycle in a mesh-covered greenhouse at the School of Engineering of Orihuela, Alicante (Miguel Hernández University, Spain), following the standard growing procedures in the region: plants were allowed to grow vertically with a single stem and were irrigated in accordance with their water demand during

the growing period. Plant density was 2.5 plants/m^2, and black plastic mulch was used to reduce the incidence of weeds. The plants were distributed in four replicates of 5-7 plants each.

Evaluated traits

Recombinant and non-recombinant progenies were evaluated for inflorescence typology, productive and quality traits, in addition to be subjected to sensory evaluation.

Inflorescence typology. The presence of shoot and/ or leaves (0, 1 or 2 points) on the three first inflorescences of each plant were rated.

Productive traits. The number of fruits per plant was determined as the total fruits harvested per plant. Fruit weight, calculated as the average of all the harvested fruits, was expressed in grams. Total yield, considered as the weight of all the fruits (harvested at the commercial ripening state, weekly) per plant, was expressed in g/plant.

Quality traits. Soluble solids content (SSC) was measured in four to six fruits per plant and replicate, in the same ripening state (at least half the fruit surface was red) (*Figure 3*). After the fruits were juiced, the SSC was estimated with an Atago PR-100 digital refractometer per duplicate, and the results were expressed as °Brix. Titratable acidity (TA) was measured in the same samples used for SSC measurements with a CRISON pHmatic 23 with 0.1 mol L^{-1}NaOH to pH 8.1. Data were expressed as the percentage of citric acid.

Figure 3: *Fruits from line 94 selected to measure the acidity and the content of soluble solids, inside the black boxes. The discarded fruits (too ripe or too green) are on the plastic bags.*

Sensory evaluation. The panel was comprised by 9 panelists with previous experience in sensory analysis. Tasting sessions were carried out in a room designed for sensory analyses (ISO, 1988) that was illuminated with green light to mask the color of the samples. After a training session, lines were evaluated in triplicate over 8 sessions with 4 accessions per session. All scoring took place on a semi-structured scale ranging from 0 to 10 with the extremes anchored and labeled with the corresponding descriptors. The evaluated traits were global aroma intensity, green-vine aroma intensity, fruit aroma intensity, earthy aroma intensity, olive oil aroma intensity, sweetness, acidity, tomato taste intensity, skin perception and mealiness.

Due to the difficulty of evaluating a large number of samples using a sensory panel, we selected the six 'Muchamiel' lines where more differences were expected.

To avoid bias in the samples presented to each panelist, samples were presented as a purée of 6 out of 10 tomatoes proceeding from the second, third or fourth truss of different plants, for each accession and session. For each session, including the training session, the fruits were collected in the same ripening state (at least half of the fruit surface was red) in Orihuela at 8:00 AM. They were coded, packaged and sent by express transport to Barcelona, where they arrived before 10:00 AM the next day, with the session being held the same day.

Statistical analysis

For the inflorescence typology, a Kruskal-Wallis probe was used (a non-parametric alternative to ANOVA-one-way). All the other evaluated traits were examined by analysis of variance (ANOVA). Wherever *F*-values were significant, Duncan's multiple range test was used to separate the mean effects. Significance was defined at $P \leq 0.05$. Correlation analysis was performed between the percentage of Anastasia F1 genomes and the evaluated traits. In all cases, the STATGRAPHICS Plus 3.0 software was used (Manugistics, Inc., Rockville, MD, USA).

For the sensory analysis results, a model with two fixed factors (panelist and accession) and one random factor (session) was used. The linear model $x_{ijk}=\mu+\alpha_i+\beta_j+s_k+\alpha\beta_{ij}+\varepsilon_{ijk}$ enabled us to calculate the effects of the accession (α_i), the panelist (β_j), the session (s_k), and the interaction genotype×panelist ($\alpha\beta_{ij}$). Factors with$p \leq 0.05$ were considered significant. Separation of means was done using the Tuckey test.

3. Results

Recombinants in the regions of the TYLCV and ToMV resistance genes

Out of the 1000 BC10S1 plants studied, we obtained only eight recombinants in the *Ty-1* gene region and four recombinants in the *Tm-2a* gene. No simultaneously recombination in the 2 regions was found. To obtain plants with recombination in the 2 regions, crosses have been made between the simple recombinants. Recombinant plants in chromosome 6 (P100, M677 and M902) have 15 Mb of the S. chilense chromosome, so they have been eliminated 30 Mb of the 33 Mb in the starting plant material (*Figure 4*). Recombinant plant in chromosome 9 (M422) have 41 Mb of the *S. peruvianum* chromosome, so it has been eliminated 10 Mb of the 51 Mb in the starting plant material (*Figure 4*).

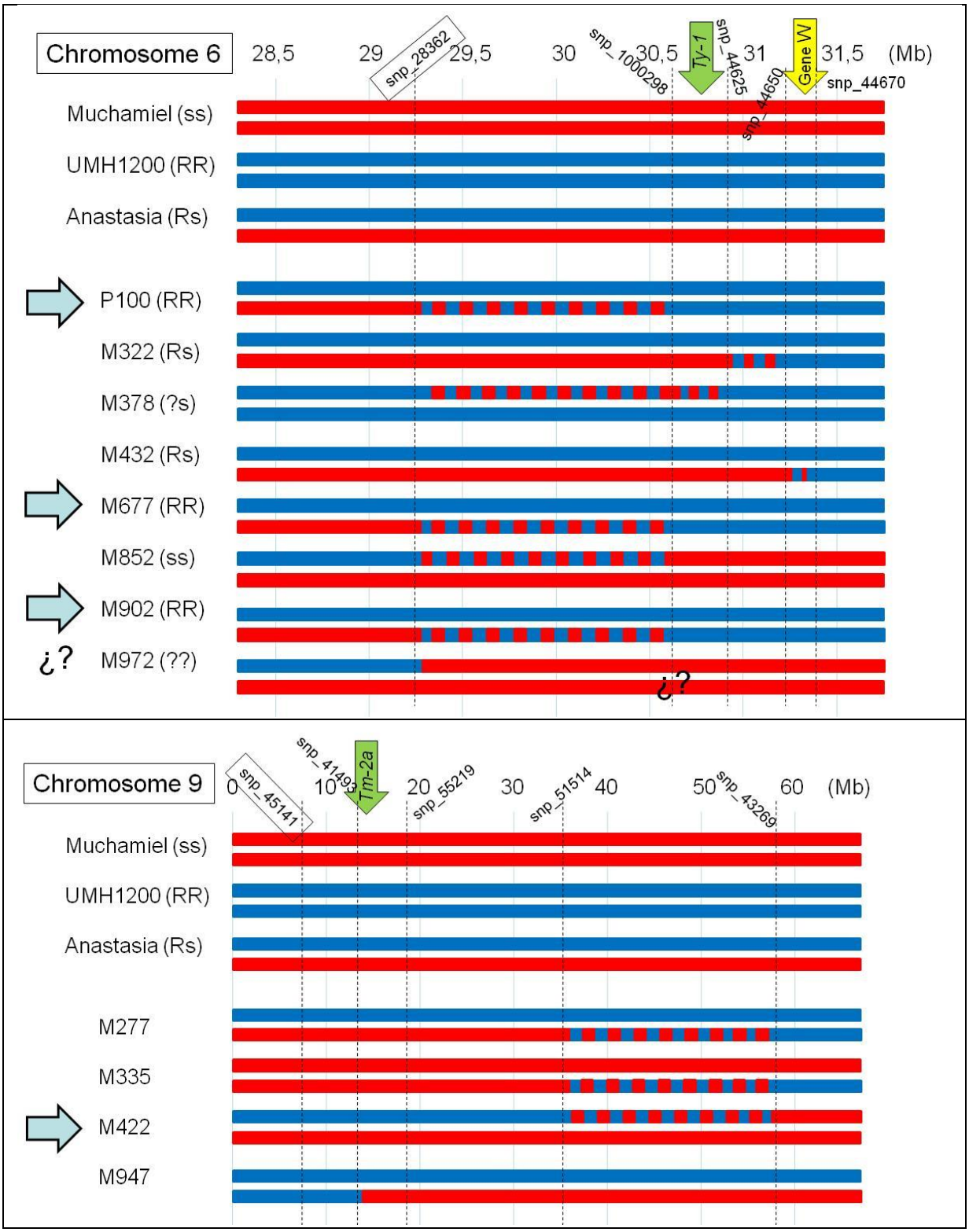
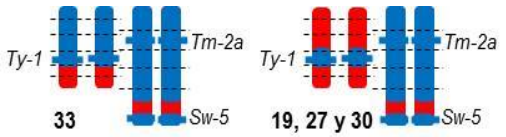
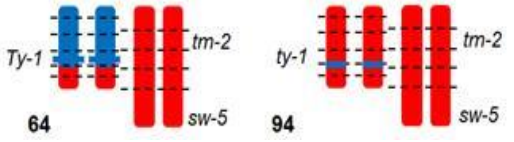
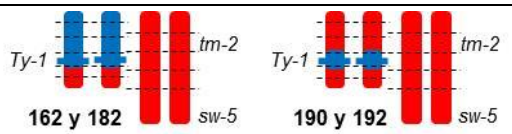
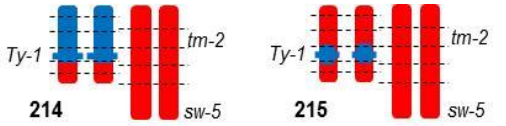
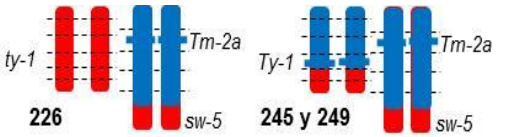
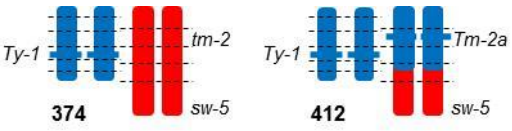
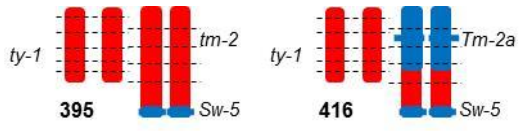
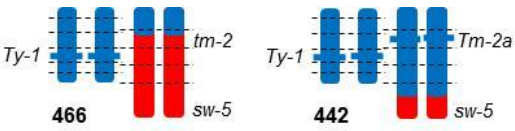


Figure 4: *Recombinant plants in Ty-1 and Tm-2a chromosome regions. Tomato chromosome is represented in red and wild species chromosome is represented in blue. The colored arrows indicate the position of the genes.*

Table 2: Lines obtained including recombinant (underlined) and not recombinant. Tomato chromosome is represented in red and wild species chromosome in blue (*S. chilense* for 6 and *S. peruvianum* for 9). Discontinuous horizontal lines represent the used SNPs.

Family	Line	Virus resistance		Chromosomes 6 and 9 graphic representation
		TYLCV and region size (MB)	ToMV-TSWV	
P100	33	R (35)	R-R	
	19, 27, 33	R (3)	R-R	
M322	64	R (35)	S-S	
	94	S (2)	S-S	
M677	162, 182	R (35)	S-S	
	190, 192	R (3)	S-S	
M902	214	R (35)	S-S	
	215	R(2)	S-S	
226	226	S (0)	R-S	
	245, 249	R (35)	R-S	

Family	Line	TYLCV and TSWV	ToMV and region size (MB)	
M422	374	R (35)-S	S (0)	
	412	R (35)-S	R (50)	
	395	S (0)-R	S (0)	
	416	S (0)-R	R (50)	
M947	466	R (35)-S	R (50)	
	442	R (35)-S	S (0)	

Inflorescence typology

In order to compare recombinant and non-recombinant lines, results were organized in families. After the Kruskal-Wallis probe, notched box-and-whisker plots were used. This plot shows the interquart range of a sample, which notches spanning the 95% confidence limits about the median, allowing pairwise comparation: if notches of any pair of medians overlap, there will be not significant differences between them.

For Ty-1 region (chromosome 6), in 'De la pera' family (P100) differences between the lines were not associated with the size of the region (or the recombination). However, there was a clear association in two 'Muchamiel' families (M322 and M677) with the recombinant lines having the lowest number of shoots and leaves in the three first inflorescences (*Figure 5*).

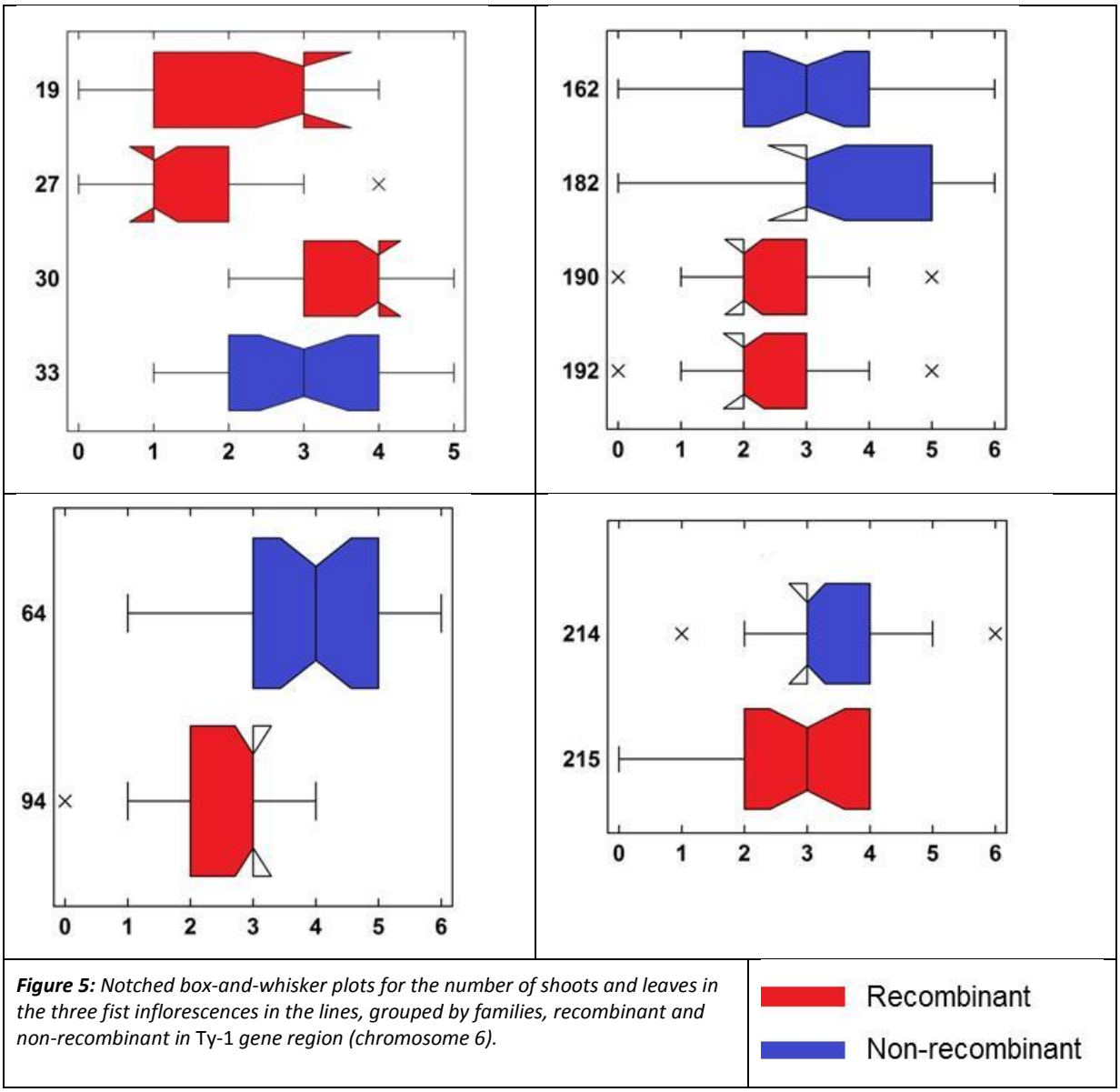


Figure 5: *Notched box-and-whisker plots for the number of shoots and leaves in the three fist inflorescences in the lines, grouped by families, recombinant and non-recombinant in* Ty-1 *gene region (chromosome 6).*

For *Tm-2a* region (chromosome 9), no differences between families (all 'Muchamiel') were found (*Figure 6*). This result suggests that this introgression does not affect the inflorescence typology in 'Muchamiel' tomato plants.

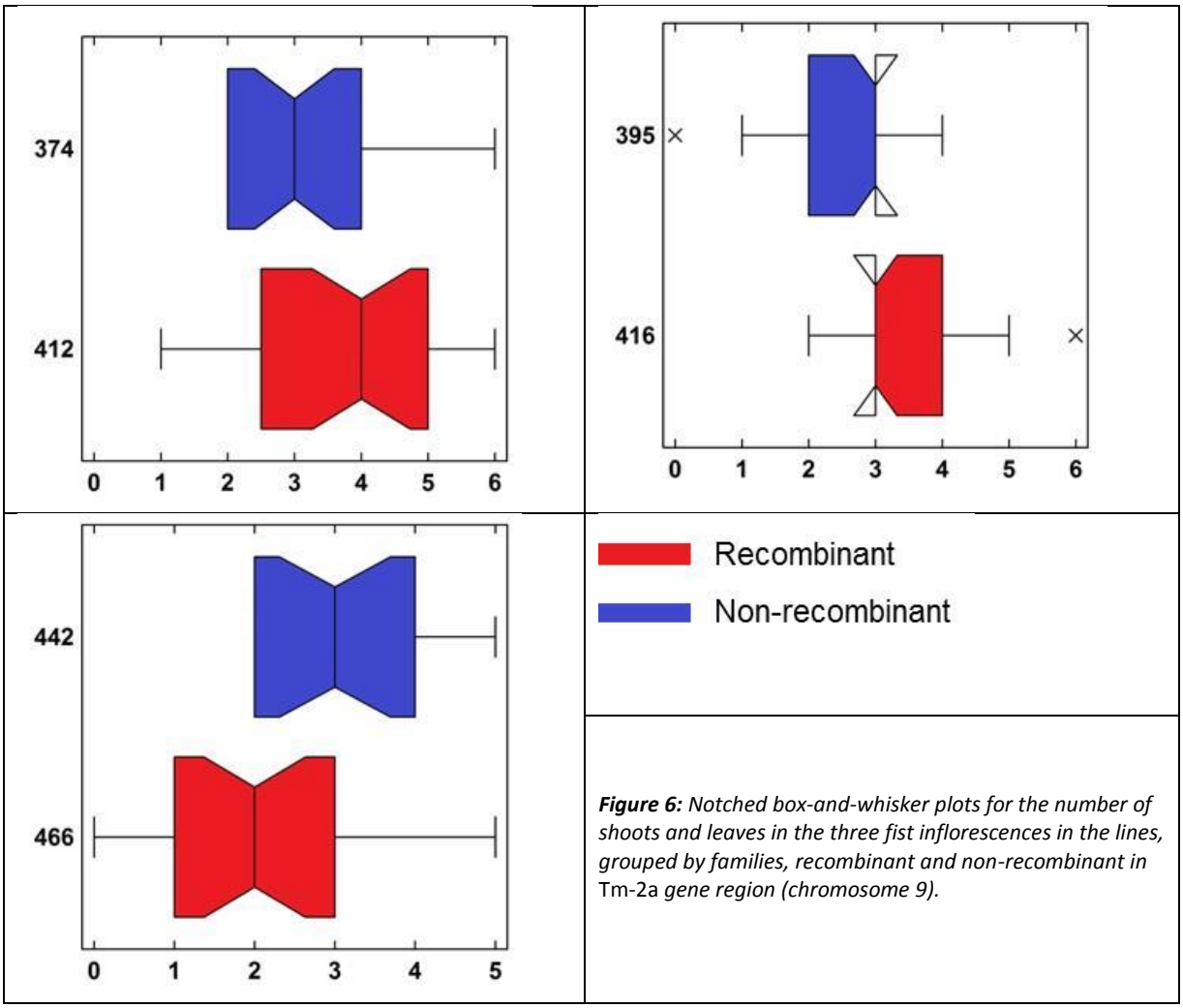


Figure 6: *Notched box-and-whisker plots for the number of shoots and leaves in the three fist inflorescences in the lines, grouped by families, recombinant and non-recombinant in* Tm-2a *gene region (chromosome 9).*

Productive traits

Table 3 contains the productive traits results obtained for the different recombinant and non-recombinant lines in the region of chromosome 6 that contains the *Ty-1* gene. The lines corresponding to the same family have been grouped with the same color.

The recombinant lines with *Ty-1*, which have only 3 MB of *S. chilense*, clearly outnumber the non-recombinant lines, which have 33 MB of *S. chilense*. It is outstanding the increase in the yield of the majority of new recombinant lines, which duplicate that of non-recombinants. This result indicates that the fragment of 30 MB of *S. chilense* that has been eliminated in the recombinant lines contains alleles of genes responsible for the decrease in fruit weight.

Table 3: *Productive traits results obtained with the different recombinant and non recombinant lines in the region of chromosome 6 that contains the* Ty-1 *gene.*

Family	Line	*Ty*-1 genotypeand size (MB)	ToMV-TSWV	Total yield (kg/plant)		Fruit number per plant		Average fruit weight (g/fruit)	
P100	19	R (3)	R-R	4.044 C	+60.0%	94..2 C	+51..3%	43.6 AB	+19.5%
P100	27	R (3)	R-R	3.122 B	+48.2%	85.6 B	+46.4%	36.6 A	+4.1%

Family	Line	*Ty*-1 genotypeand size (MB)	ToMV-TSWV	Total yield (kg/plant)		Fruit number per plant		Average fruit weight (g/fruit)	
P100	30	R (3)	R-R	1.984 A	+18.5%	41.1 A	-10.5%	47.3 B	+25.8%
P100	33	R (35)	R-R	1.617 A		45.9 A		35.1 A	
M972	226	S (0)	R-S	3,685 C		21,3 BC		201,0 E	
M322	64	R (35)	S-S	1,996 B	-48.6%	22.7 CD	-20.6%	90.0 BC	-33.5%
M322	94	S (2)	S-S	3.882 C		28.6 D		135.4 D	
M677	162	R (35)	S-S	0.978 A	-62.5%	14.1 A	-45.3%	69.7 A	-31.5%
M677	182	R (35)	S-S	1.094 A	-58.1%	14.9 B	-42.2%	72.7 AB	-28.5%
M677	190	R (3)	S-S	2.526 B		25.4 CD		100.2 C	
M677	192	R (3)	S-S	2.693 B		26.2 CD		103.2 C	
M902	214	R (35)	S-S	0.658 A	-70.7%	7.1 A	-54.8%	127.5 B	-20.6%
M902	215	R (3)	S-S	2.248 B		15.7 B		160.6 C	
M972	226	S (0)	R-S	3.685 C		21.3 C		201.0 D	
M972	245	S (30)	R-S	3.342 C	-9.3%	35.0 D	+39.1%	95.6 A	-52.4%
M972	249	S (30)	R-S	3.508 C	-4.8%	36.3 D	+41.3%	97.1 A	-51.7%

Values in a column which are followed by a different letter indicate those mean values are significantly different according to the Newman-Keuls's multiple range test ($P< 0.05$).

Table 4 contains the productive traits results obtained with the different recombinant and non-recombinant lines in the region of chromosome 9 that contains the *Tm-2a gene*, also grouping the lines of the same family with the same color.

Table 4: *Productive traits obtained with the different recombinant and non-recombinant lines in the region of chromosome 9 that contains the Tm2-agene.*

Family	Line	*Tm-2a* genotype and size (MB)	TYLCV-TSWV	Total yield (kg/plant)		Fruit number per plant		Average fruit weight (g/fruit)	
M422	411	R (45)	R-R	2.366B	-46.8%	28.4BC	-28.8%	83.6 B	-26.3%
M422	416	R (45)	S-R	4.446 D		39.9E		113.5D	
M422	395	S (0)	S-R	3.102C	-30.2%	26.81 B	-32.8%	116.8D	+2.8%
M422	412	R (45)	R-S	3.159C		34.4CDE		92.2BC	
M422	374	S (0)	R-S	1.315 A	-58.4%	20.1 A	-41.6%	66.7 A	-27.7%
M947	442	R (55)	R-S	3.529C		36.5 DE		97.2 C	
M947	466	S (5)	R-S	2.553B	-27.7%	30.7BCD	-15.9%	83.2B	-14.4%

Values in a column followed by a different letter indicate that those mean values are significantly different according to the Newman-Keuls's multiple range test ($P< 0.05$).

Quality traits

For soluble solids content and acidity (*Table 5*) the differences between the recombinant and non-recombinant lines in *Ty-1* gene region were lower than for the productive traits. Maximum variations were about 15%. For the lines recombinant in *Tm-2a* gene region the differences were minor than for *Ty-1* region, suggesting a minor effect of the first region for these quality traits.

Table 5: *Quality traits results obtained with the different recombinant and non-recombinant lines in the region of chromosome 6 that contains the* Ty-1 *gene.*

Family	Line	Ty-1 genotype and size (MB)	ToMV-TSWV	Soluble solid content (ºBrix)		Acidity (%)	
P100	19	R (3)	R-R	5.25 B	-6.9%	0.29 A	-9.4%
P100	27	R (3)	R-R	4.98 A	-11.7%	0.30 A	-6.2%
P100	30	R (3)	R-R	5.94 D	+5.1%	0.38 C	+15.8%
P100	33	R (35)	R-R	5.64 C		0.32 B	
M972	A (226)	S (0)	R-S	4.50 A		0.40 CD	
M322	B (64)	R (35)	S-S	4.92 CD	+3.7%	0.37 BC	-9.8%
M322	C (94)	S (2)	S-S	4.74 BC		0.41 D	
M677	D (162)	R (35)	S-S	4.97 D	+6.7%	0.35 AB	0
M677	D (182)	R (35)	S-S	4.89 CD	+7.1%	0.33 A	0
M677	E (190)	R (3)	S-S	4.60 AB		0.34 A	
M677	F (192)	R (3)	S-S	4.58 AB		0.34 A	
M902	214	R (35)	S-S	4.7 A	+4.3%	0.35 A	
M902	215	R (3)	S-S	4.5 A		0.36 AB	
M972	226	S (0)	R-S	4.5 A		0.40 BC	
M972	245	S (30)	R-S	4,6 A	+2,2%	0,37 AB	-7,5%
M972	249	S (30)	R-S	4,6 A	+2,2%	0,34 A	-15,0%

Values in a column followed by a different letter indicate that those mean values are significantly different according to the Newman-Keuls's multiple range test ($P< 0.05$).

Table 6: *Productive traits results obtained with the different recombinant and non-recombinant lines in the region of chromosome 9 that contains the* Tm-2a *gene.*

Family	Line	*Tm-2a* genotype and size (MB)	TYLCV-TSWV	Soluble solid content (ºBrix)		Acidity (%)	
M422	411	R (45)	R-R	4.73 AB		0.30 B	
M422	416	R (45)	S-R	4.51 A		0.39 C	
M422	395	S (0)	S-R	4.82 B	6.4%	0.41 D	4.9%
M422	412	R (45)	R-S	5.11 C		0.28 A	
M422	374	S (0)	R-S	5.57 D	8.3%	0.31 B	9.7%
M947	442	R (55)	R-S	4.92 BC		0.30 B	
M947	466	S (5)	R-S	4.70 AB	-4.5%	0.28 A	-6.6%

Values in a column followed by a different letter indicate that those mean values are significantly different according to the Newman-Keuls's multiple range test ($P< 0.05$).

Sensory analysis

The sensory analysis performed with the panel of trained tasters found significant differences between the genotypes for four of the characters evaluated (*Tables 8 and 9*). For the panelists, significant differences were found for nine of the evaluated characters, which probably reflects the effect of the taster. The interaction genotype x panelist was significant in 2 of the evaluated parameters (intensity of fruity aroma and sweetness), which allows the use the multiple range test.

No significant differences were found between the genotypes studied for green aroma intensity, fruity aroma intensity, olive oil aroma intensity, tomato taste intensity and skin perception. For the global aroma intensity, sweetness, acidity and mealiness, significant differences were found among the genotypes (*Table 9*). However, the differences between recombinants and non-recombinants were lower than for productive traits. For example, for global aroma intensity, no significant differences were found between the recombinant and non-recombinant lines within the different families (*Table 9*). Significant differences were found between line 226 and lines 64, 94 and 182. For sweetness, significant differences were found between lines 64 and 94, in favor of line 64, which is non-recombinant and has 33 MB of *S. chilense*, while line 94 only has 2 MB (*Table 9*). However, analytically, no significant differences were found for the content of soluble solids between these two lines (*Table 6*). For acidity, the tasting panel found no differences between lines 64 and 94. However, analytically, significant differences were found, in favor of line 94, which only has 2 MB of *S. chilense*, while the line 64 has 33 MB (*Table 6*). For the mealiness, the tasting panel has not found differences between the recombinant and non-recombinant lines of the same family.

Table 10 shows the average scores of the attributes studied by each taster. The large differences among some evaluators revealed the difficulty of the sensory analysis.

Table 8: ANOVA significance of the of the attributes studied for the genotype, panelist and the genotypexpanelist

	Global aroma intensity	Green-viney aroma intensity	Fruity aroma intensity	Earthy aroma intensity	Olive oil aroma intensity	Sweetness	Acidity	Tomato taste intensity	Skin perception	Mealiness
Genotype	0,0005	0,830	0,713	No detect.	0,123	$2,56.e^{-09}$	$3,9.\ e^{-07}$	0,064	0,0506	0,0003
Panelist	$8,5.\ e^{-12}$	$<2.\ e^{-16}$	$1,61.\ e^{-11}$	-	$<2.e^{-16}$	$2,0\ .e^{-05}$	$8,4.e^{-11}$	$5,04.e^{5}$	$1,1.\ 2.e^{-06}$	$7,2.\ .e^{-11}$
Genotype x Taster	0,9213	0,128	0,073	-	0,014	0,04	0,069	0,457	0,572	0,091

Table 9: Mean values of the attributes studied for each genotype. The values followed by the same letter are not significantly different according to the Tuckey test (p≤0.05). LSD: Least Significant Difference.

Family	Line	Ty-1 genotype and size	ToMV-TSWV	Global aroma intensity	Green-viney aroma intensity	Fruity aroma intensity	Olive oil aroma intensity	Sweetness	Acidity	Tomato taste intensity	Skin perception	Mealiness
M972	A (226)	S (0)	R-S	6.4 b	1.4 a	4.4 a	2.9 a	3.6 c	7.0 a	5,3 a	5,9 a	3,3 b
M322	B (64)	R (35)	S-S	7.2 a	1.2 a	4.6 a	3.8 a	5.6 a	5.4 bc	6.3 a	7,3 a	5,6 a
M322	C (94)	S (2)	S-S	7.4 a	1.2 a	4.7 a	4.2 a	4.4 bc	5.8 b	5,7 a	6,4 a	5,0 a
M677	D (182)	R (35)	S-S	7.5 a	1.2 a	4.4 a	4.1 a	5.9 a	4.5 c	6,2 a	7,0 a	4,7 ab
M677	E (190)	R (3)	S-S	6.9 ab	1.1 a	5.1 a	3.8 a	5.7 a	5.5 bc	6,2 a	6,5 a	4,6 ab
M677	F (192)	R (3)	S-S	7.1 ab	1.5 a	4.9 a	3.5 a	5.4 ab	5.2 bc	6,3 a	7,1 a	5,5 a
LSD				*0,77*	*0,92*	*1,55*	*1.51*	*1.07*	*1.10*	*1.19*	*1.38*	*1.53*

Table 10: Averages of the tasters for each of the attributes studied. The values followed by the same letter are not significantly different according to the Tuckey test (p≤0.05). LSD: Least Significant Difference.

Panelist	Global aroma intensity	Green-viney aroma intensity	Fruity aroma intensity	Olive oil aroma intensity	Sweetness	Acidity	Tomato taste intensity	Skin perception	Mealiness
Sabaté	7.9 a	1.5 bc	5.3 ab	7.2 a	4.5 bc	7.3 a	6.7 ab	7.2 ab	3.2 cd
Rivera	7.8 ab	0.3 c	6.8 a	1.2 f	5.7 ab	4.3 de	6.8 ab	7.7 a	7.3 a
Amargant	7.5 ab	0.9 c	3.8 bc	6.0 ab	5.7 ab	6.6 ab	6.9 a	7.8 a	2.0 d
López	7.4 abc	3.6 a	3.6 bc	3.7 cde	5.2 abc	6.2 abc	5.9 abc	7.0 ab	6.8 a
Casañas	7.2 abc	0.2 c	7.0 a	2.7 def	5.1 abc	4.9 cde	4.9 c	4.8 c	4.4 bc
Rull	6.9 bc	0.2 c	4.3 bc	2.0 ef	3.9 c	4.4 de	5.4 bc	6.4 abc	4.5 bc
Romero	6.6 cd	2.9 a	3.0 c	4.6 bc	4.7 abc	5.6 bcd	5.8 abc	6.8 ab	4.5 bc
Simó	5.6 de	2.5 ab	2.8 c	4.0 cd	3.9 c	4.1 e	4.8 c	5.9 bc	5.9 ab
Sans	5.5 e	0.5 c	3.6 bc	2.5 def	6.0 a	5.2 cde	5.4 bc	5.8 bc	5.6ab
LSD	*0.97*	*1.3*	*1.9*	*1.9*	*1.3*	*1.4*	*1.4*	*1.7*	*1.9*

4. Deviations

No major deviations were detected in our workplan, only that no simultaneously recombinant plant in the regions containing *Ty-1* and *Tm-2a* was found. To obtain it, crosses have been made between the simple recombinants and further developments are in process to obtain it by the end of 2018. The reason underlying the absence of double recombinants is probably the suppression of recombination in the regions containing the resistances genes. Verlaan et al. (2011) reported the occurrence of two chromosomal inversions between the *S. chilense* LA1969 accession (the donor specie of the *Ty-1* gene) and *S. lycopersicum.* Fulton et al. (1997) reported an intrachromosomal translocation of TG9 marker, in chromosome 9 of *S. peruvianum*, where *Tm-2a* gene is located. This deviation had not a major impact on other tasks.

5. Conclusions

One 'De la pera' and two 'Muchamiel' recombinant lines in the region containing *Ty-1* with the resistance gene and one 'Muchamiel' recombinant line in the region containing *Tm-2a* with the resistance gene were obtained. Recombinant lines in *Ty-1* gene region have only 3 MB of the 33 MB *S. chilense* chromosome left, indicating that 30 MB has been eliminated. The recombinant line in *Tm-2a* still has approximately 45 MB of the initial 60 MB chromosome wild type introgression.

The new recombinant lines with *Ty-1* (containing only 3 MB of *S. chilense*) are clearly an improvement over the original lines (containing 33 MB of *S. chilense*) for the productive traits studied, in terms of number of fruits per plant, average fruit weight and yield. On the other hand, effects soluble solid content and acidity were not as pronounced while the sensory traits were negligible. For *Tm-2a*, no important differences in those traits were found between recombinant and non-recombinant genotypes.

In conclusion, new tomato breeding lines derived from two types of traditional tomato varieties have been obtained carrying smaller introgressions to increase disease resilience while they eliminate previous negative consequences of linkage drag. The most import effect was due to the reduction of *Ty-1* introgression which resulted in the elimination of undesirable effects on tomato yield due to linked genes included in the original introgression. The new breeding lines are resistant to *Ty-1* with no negative impact in tomato yield even when the virus is not infecting the plant. The cultivation of these new lines are therefore recommended regardless the incidence of infection as they make possible a high stable yield with or without the incidence of the virus.

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