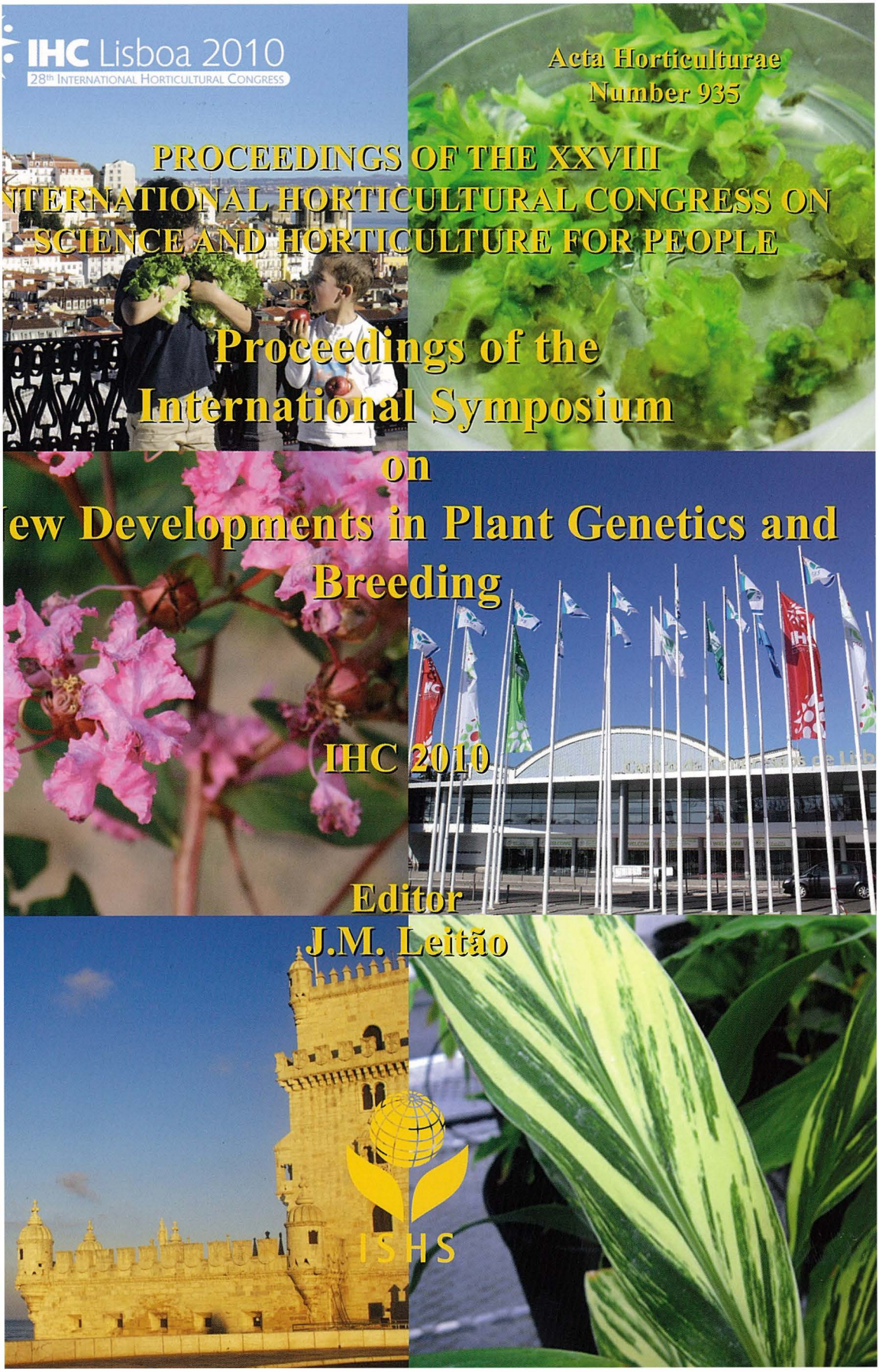
IHC Lisboa 2010
28th International Horticultural Congress
Acta Horticulturae
Number 935
PROCEEDINGS OF THE XXVIII
NTERNATIONAL HORTICULTURAL CONGRESS ON
SCIENCE AND HORTICULTURE FOR PEOPLE
Proceedings of the
International Symposium
on
ew Developments in Plant Genetics and
Breeding
IHC 2010
Editor
J.M. Leitão
ISHS

ISSN 0567-7572
ISBN 978 90 6605 398 4, Acta Horticulturae n°. 935
Price for non-members ofISHS: € 65,-
Published by ISHS, June 2012

Executive Director ofISHS: J. Van Assche
Technical Processing: M.L.M. Testor

ISHS Secretariat, PO Box 500, 3001 Leuven 1, Belgium

Printed by Drukkerij Station Drukwerk, PO Box 3099, 2220 CB Katwijk, The Netherlands



Photographs on the front cover:

| | |
|---|---|
| 1 | 2 |
| 3 | 4 |
| 5 | 6 |

1. Lisbon and the IHC2010.
2. Somatic embryogenesis, in vitro genetic manipulations, and plant regeneration in ornamental ginger *(Hedychium* J. Koenig) for the induction of polyploidy and mutagenesis. Photograph by courtesy of Hamidou F. Sakhanokho.
3. *Lagerstroemia indica* 'Supersonic'. Photograph by courtesy of Ping Song.
4. Lisbon and the IHC2010.
5. Lisbon and the IHC2010.
6. Mutagenic effect of sodium azide on ornamental ginger: Variegated plant obtained by treating *Hedychium muluense* x *Hedychium* 'White Starburst' embryogenic callus with 20 mM $NaN_3$ for 6 hours. Photograph. by courtesy of Hamidou F. Sakhanokho.

# Introgressing Resistance Genes into Traditional Tomato Cultivars: Effects on Yield and Quality

F. Rubio, S. García-Martínez, A. Alonso, A. Grau, M. Valero and J.J. Ruiz
Department of Applied Biology
Miguel Hemández University
Ctra. Beniel Km 3,2, 03312, Orihuela, Alicante
Spain

**Keywords:** *Tm-2a, Sw-5, Ty-1,* ToMV *(Tomato mosaic virus),* TSWV *(Tomato spotted wilt virus),* TYLCV *(Tomato yellow lea/curl virus),* CAPS markers, linkage drag

**Abstract**

**We have developed advanced breeding lines of sorne traditional cultivars, with introgressed genetic resistances to ToMV, TSWV and TYLCV (genes *Tm-2a, Sw-5* and *Ty-1).* In order to evaluate possible effects of the different introgressions in these local landraces, eight breeding lines of 'De la Pera' cultivar corresponding with ali the homozygote combinations for the resistance genes, were grown in an experimental open-field. About 10-15 plants of each line were evaluated for agronomic traits (commercial and non commercial fruit yield, number of inflorescences, number of fruits per inflorescence and mean fruit weight) and fruit quality traits (soluble solids content and titratable acidity). Three CAPS markers (To-3, Aps-F2 and CT220) linked to the resistance genes were used to confirm the genotypes of the lines. Lines homozygous for ToMV resistance gene showed higher values than ToMV susceptible lines for three yield traits, whereas susceptible lines showed higher values for soluble solids concentration and titratable acidity. Lines homozygous for the TSWV resistance gene showed a small decrease of fruit weight with respect to the susceptible lines. Significant differences between TYLCV-resistant and susceptible lines were found for eight of the nine traits. Most notably, resistant lines were less productive than susceptible lines and had lower soluble solids content and titratable acidity. These results indicate that the introgression of *Tm-2a, Sw-5* and *Ty-1* genes affects agronomic and quality traits, probably due to other genes introduced along with the resistance gene during backcrossing (linkage drag).**

## INTRODUCTION

'Muchamiel', 'De la Pera', 'Valenciano', 'Morunos', and 'Flor de Baladre' are traditional tomato cultivar types, which are very popular in south eastem Spain for their organoleptic fruit quality, and are still being cultivated by local farmers in small orchards. However, these landraces are severely endangered with the risk of extinction, because these cultivars are highly susceptible to several viruses, such as those caused by the ToMV, TSWV and TYLCV (Picó et al., 2002). Although the presence of the viruses in tomato fields vary from one year to another, their incidence strongly decrease the benefits obtained by farmers, and even make non-viable in many areas the cultivation of landraces. The abandonment of these traditional cultivars would lead to an irreversible loss of genetic diversity. Commercial hybrid cultivars with genetic resistances to the viruses have been developed, but these resistance genes have not been introgressed into local cultivars, since they represent only a small seed-market share.

In 1998 we started a breeding program for the simultaneous introduction of three dominant genes *(Tm-2a, Sw-5,* and *Ty-1)* that confer resistance to the three most relevant viruses in south-eastem Spain (ToMV, TSWV and TYLCV, respectively) into 'Muchamiel' and 'De la Pera' traditional cultivars using marker assisted backcrossing. The genes *Tm-2a* and *Sw-5* come from the wild tomato *Solanum peruvianum* L., and *Ty-1* originated in the accession LA1969 of another wild tomato species, *Solanum chilense* (Dunal) Reiche. As a preliminary result of the breeding program, we have obtained

Proc. XXVIII'h IHC - IS on New Developments
in Plant Genetics and Breeding
Ed.: J.M. Leitao
Acta Hort. 935, ISHS 2012

promlsing pre-breeding materials, which have to be further adapted to the specific agroclimatic conditions of different localities (Ruiz and García-Martínez, 2009).

However, there have been published reports indicating the negative effect of the resistance genes introduction, due to the introgressed genes and/or to the linkage drag (Brown, 2002). Tanksley et al. (1998) observed slight reductions in yield and fruit quality in processing tomatoes with ToMV resistance. Most recently, Lewis et al. (2007) reported yield and quality reduction in tobacco plants containing the *N* gene (coming from the wild *Nicotiana glutinosa* L.), that confers resistance to TMV, suggesting the role of linkage drag. Studying two tomato breeding lines segregating for *Ty-1* gene, Alonso et al. (2008) found that the introgression of the *Ty-1* gene was unfavorable for several productive and quality characters.

The objective of this work was to evaluate the effect of the introgressed genetic resistances to ToMV, TSWV and TYLCV (genes *Tm-2a, Sw-5* and *Ty-1)* in advanced breeding lines of 'De la Pera' type.

## MATERIALAND METHODS

A 'De la Pera' breeding line with the three resistance genes *(Tm-2a, Sw-5* and *Ty-1)* in a heterozygote was developed through nine backcross generations. By selfing this breeding line, we obtained eight lines corresponding with all the homozygote combinations for the three resistance genes, which were grown in an experimental open-field of the Miguel Hemández University in Orihuela, between March and August of 2009. The line with the combination: RR for *Tm-2a,* RR for *Ty-1* and ss for *Sw-5* (which RR is the resistance homozygote and ss is the susceptible homozygote), dueto problems with seed stokes and seed germination, was not evaluated. Among 1Oto 15 plants of each genotype were grown vertically with single steam, following the standard cultural practices for tomato crop in the SE of Spain. The genotype of each line was confirmed using three CAPS markers (To-3, Aps-F2 and CT220) linked to the resistance genes, routinely used in the breeding program in course (Ruiz and García-Martínez, 2009).

Total inflorescences and inflorescences with fruits per plant were determined. Fruits were individually harvested at commercial ripening state weekly, during 10 weeks. Each fruit was weighted, considering as commercial fruits only those weighting more than 50 g. A fruits sample was frozen and stored for analytical determinations (total soluble solids content (TSS) and titratable acidity (TA)). After the fruits had been juiced, TSS was estimated with an Atago PR-100 digital refractometer, expressing the results as ºBrix. With the same juice, TA was measured by titration with 0.1 N NaOH until pH 8.1, expressing the results as g of citric acid per 100 g.

A multifactor ANOVA was performed for each trait, with the genotype (RR or ss) for each of the three virus resistance genes serving as independent variables, using Statgraphics Plus.

## RESULTS AND DISCUSSION

Statistical analysis revealed significant differences associated with the presence of virus resistance for all the agronomic and quality traits (Table 1).

### Effects of Introgressed ToMV Resistance

Multifactor ANOVA revealed that the presence of the ToMV resistance was associated with significant differences *($P<0.05$* or $P<0.001$) for 3 out of 7 agronomic traits and for the two quality traits (Table 1). Total fruit yield, commercial fruit yield and total fruit number per plant yield differences showed a similar pattem: resistant lines had higher values than susceptible lines. This result is in disagreement with that obtained by Tanksley et al. (1998) in processing tomato. These authors reported that the ToMV resistance line showed lower yield than the susceptible line. Soluble solid content and titratable acidity were adversely affected in the resistant lines. ToMV resistant lines had significantly lower values than susceptible lines (Table 1). Thus, the gene *Tm-2a,* or any of the *peruvianum* genes on the introgressed region, may be responsible for these

decreases in the resistant lines. Tanksley et al. (1998) didn't found significant effect for the ToMV resistance in these quality traits in processing tomatoes. Backcross is the solution to remove part of the *L. peruvianum* chromosome fragment by meiotic recombination (Young and Tanksley, 1989). However, the *Tm-2a* gene is located near the centromere of chromosome 9, a region greatly suppressed in meiotic recombination (Ganal et al., 1989). As a result, the introgressed segment containing the *Tm-2a* gene corresponds to millions of base pairs of *peruvianum* DNA (and probably many genes) but experiences almost no genetic recombination in current breeding populations (Ganal et al., 1989).

**Effects of Introgressed TSWV Resistance**

For TSWV resistance, multifactor ANOVA revealed only significant differences *(P<0.05* or P<0.01) for 3 out of 7 agronomic traits (Table 1). Total inflorescences and inflorescences with fruits per plant showed increased values in the resistant lines, while the susceptible lines reached the highest values for mean fruit weight. Unlike the other two resistances introduced, significant differences in quality traits were not detected (Table 1).

**Effects of Introgressed TYLCV Resistance**

Multifactor ANOVA revealed that the presence of the TYLCV resistance was associated with significant differences (P<0.001) for 6 out of 7 agronomic traits and for the two quality traits (Table 1).

The agronomic traits with significant differences (number of inflorescences, inflorescences with fruits, fruit weight, commercial fruit weight, fruit number and mean fruit weight) and the quality traits (soluble solids content and titratable acidity) showed the same pattem: susceptible lines had higher values than resistant lines. These results are in agreement with those obtained by Alonso et al. (2008), who found that the introgression of the *Ty-1* gene (coming from the wild S. *chilense* (Dunal) Reiche) had an adverse effect for severa! productive and quality characters. *Ty-1* gene, originated from accession LA1969 of S. *chilense,* was mapped between TG97 and TG297 markers in the short arm of chromosome 6, near the centromere (Zamir et al., 1994). Severe recombination suppression has been observed in this region in crosses involving cultivated tomato and wild relatives, probably due to a chromosomal segment inversion (Messeguer et al., 1991; Kaloshian et al., 1998; Seah et al., 2004). Suppressed recombination increases the potential for linkage drag that reduces the efficiency of backcrossing in plant breeding programs.

## CONCLUSIONS

These results indicate that the introgression of *Tm-2a, Sw-5* and *Ty-1* genes into traditional tomato cultivars affected agronomic and quality traits, probably due to other genes introduced along with the resistance genes during backcrossing (linkage drag). These results would suppose a difficulty in the developing of homozygous resistant cultivars.

## ACKNOWLEDGEMENTS

The authors would like to thank J.M. Sánchez and D. Navarro for assisting in analysis.

## Literature Cited

Alonso, A., García-Martínez, S., Arroyo, A., García-Gusano, M., Grau, A., Giménez-Ros, M., Romano, M.E., Valero, M. and Ruiz, J.J. 2008. Efecto de la introducción de resistencia a TYLCV (gen *Ty-1)* en caracteres productivos y de calidad en tomate. Actas de Horticultura 51: 173-174.

Brown, J.K.M. 2002. Yield penalties of disease resistance in crops. Curr. Opin. Plant. Biol. 5:339-344.

Ganal, M.W., Young, N.D. and Tanksley, S.D. 1989. Pulsed field gel electrophoresis and physical mapping of large DNA fragments in the *Tm-2a,* a region of chromosome 9 in tomato. Mol. Genet. 215:395-400.

Kaloshian, l., Yaghoobi, J., Liharska, T., Hontelez, J., Hanson, D., Hogan, P., Jesse, T., Wijbrandi, J., Simons, G., Vos, P., Zabel, P. and Williamson, V.M. 1998. Genetic and physical localization of the root-knot nematode resistance locus *Mi* in tomato. Mol. Gen. Genet. 257:376-385.

Lewis, R.S., Linger, L.R., Wolff, M.F. and Wemsman, E.A. 2007. The negative influence of N-mediated TMV resistance on yield in tobacco: linkage drag versus pleiotropy. Theor. Appl. Genet. 115: 169-178.

Messeguer, R., Ganal, M.W., de Vicente, M.C., Young, N.D., Bolkan, H. and Tanksley, S.D. 1991. High resolution RFLP map around the root-knot nematode resistance gene *(Mi)* in tomato. Theor. Appl. Genet. 82:529-536.

Picó, B., Herraiz, J., Ruiz, J.J. and Nuez, F. 2002. Widening the genetic basis of virus resistance in tomato. Scientia Horticulturae 94(1-2):73-89.

Ruiz, J.J. and García-Martínez, S. 2009. Tomato varieties 'Muchamiel' and 'De la Pera' from the southeast of Spain: Genetic improvement to promote on-farm conservation. In: M. Vetelainen, V. Negri and N. Maxted. European landrace: on-farm conservation, management and use. Biodiversity Technical Bulletin 15: 171-176.

Seah, S., Yaghoobi, J., Rossi, M., Gleason, C.A. and Williamson, V.M. 2004. The nematode-resistance gene, *Mi-1,* is associated with an inverted chromosomal segment in susceptible compared to resistant tomato. Theor. Appl. Genet. 108:1635-1642.

Tanksley, S.D., Bemachi, D., Beckbunn, T., Emmatty, D., Eshed, Y., Inai, S., Lopez, J., Petiard, V., Sayama, H., Uhlig, J. and Zamir, D. 1998. Yield and quality evaluations on a pair of processing tomato lines nearly isogenic for the *Tm2*$^{a}$ gene for resistance to the tobacco mosaic virus. Euphytica 99:77-83.

Young, N.D. and Tanksley, S.D. 1989. RFLP analysis of the size of chromosomal segments retained around the *Tm-2* locus oftomato during backcross breeding. Theor. Appl. Genet. 77:353-359.

Zamir, D., Ekstein-Michelson, l., Zakay, Y., Navot, N., Zeidan, M., Sarfatti, M., Eshed, Y., Harel, E., Pleben, T., Van-Oss, H., Jedar, N., Rabinowitch, H.D. and Czosnek, H. 1994. Mapping and introgression of a Tomato yellow leaf curl virus tolerance gene, *Ty-1.* Theor. Appl. Genet. 88:141-146.

## Tables

Table 1. Effects of the introgressed virus resistance genes on yield and quality parameters of 'De la Pera' breeding lines, where RR is the resistance homozygote and ss is the susceptible homozygote.

| | | Fruit weight (g/plant) | Commercial fruit weight (g/plant) | Non commercial fruit weight (g/plant) | Number of Inflorescences per plant | Number of Inflorescences with fruits per plant | Number of fruits per plant | Mean fruit weight | Soluble solids content (ºBrix) | Titratable acidity (g/100g citric acid) |
|---|---|---|---|---|---|---|---|---|---|---|
| Df | | 48 | 48 | 48 | 48 | 48 | 48 | 48 | 48 | 48 |
| ToMV resistance | | *** | *** | ns | ns | ns | * | ns | *** | *** |
| | RR | 1930 b | 1809 b | 119.9 a | 5.41 a | 3.98 a | 26.1 b | 67.8 a | 3.91 a | 0.22 a |
| | ss | 1501 a | 1377 a | 124.8 a | 5.65 a | 4.45 a | 22.2 a | 65.3 a | 4.67 b | 0.25 b |
| TSWV resistance | | ns | ns | ns | ** | * | ns | ** | ns | ns |
| | RR | 2832 a | 1531 a | 126.6 a | 6.08 b | 4.71 b | 24.2 a | 64.6 a | 4.29 a | 0.23 a |
| | ss | 3006 a | 1655 a | 118.1 a | 4.99 a | 3.72 a | 24.1 a | 68.6 a | 4.29 a | 0.24 a |
| TYLCV resistance | | *** | *** | ns | *** | ••• | ••• | •• | •• | •• |
| | RR | 1065 a | 940 a | 124.1 a | 4.75 a | 3.39 a | 17.7 a | 56.1 a | 4.11 a | 0.20 a |
| | ss | 2367 b | 2246 b | 120.6 a | 6.31 b | 5.03 b | 30.6 b | 77.1 b | 4.48 b | 0.28 b |
| ToMV x TSWV interacction | | ns | ns | ns | ns | • | ns | ns | •• | •• |
| ToMV x TYLCV interacction | | ns | ns | ns | * | * | • | ns | ns | •• |
| TSWV x TYLCV interacction | | ns | ns | ns | ••• | •• | •• | ns | •• | •• |

*, **, *** Significantly different at P<0.05, 0.01 and 0.001, respectively. ns: not significant at P>0.05. Means within a column followed by the same letter are not different at P<0.05 (Newman-Keuls test).