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ARTICLE

Pyramiding disease resistance in tomato by duplex PCR targeting resistance genes and exploiting gene linkage

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Abstract: *Tomato is the second most important vegetable crop in the world after potato. Its production is hindered by fungal and viral diseases. Control of these diseases requires a significant investment that represents up to 40% of the production costs. Marker-assisted gene pyramiding is a powerful breeding tool which allow the rapid constitution of breeding lines with broad spectrum of resistance against diseases. Nevertheless, the accuracy and rapidity of introgression of genes of interest are some of the most critical barriers to successful application. In this paper, we use a simple and rapid method that combines duplex PCR for two important virus resistance genes (i.e., Sw-5 and Tm-2²) and uses the linkage between genes Tm-2² and Forl to speed the introgression of these resistance genes into a "Pomodoro di Sorrento" tomato line. The method was used through six generations of backcrossing followed by three generations of selfing and resulted in a homozygous resistant line.*

Keywords: *Multiple disease resistance, marked assisted selection, TSWV, tobamoviruses, FORL*

INTRODUCTION

Tomato (*Solanum lycopersicum* L., formerly *Lycopersicon esculentum* Mill.) is the second most important vegetable crop after potato in the world. It is estimated that over 5 million hectares of tomatoes are grown annually worldwide, producing more than 180 million tons (FAO 2021). Because of its wide use and nutritional value, there is a high and increasing demand for cultivars suited to both the fresh and processed markets. This also applies to some old tomato landraces, such as the "Pomodoro di Sorrento" (also called "Sorrentino" or "Rosa di Sorrento"), which is grown mainly on the Sorrento coast and is appreciated for its organoleptic characteristics and for which demand is increasing, even by tourists. Despite its outstanding organoleptic and nutritional qualities, the cultivar "Pomodoro di Sorrento" and tomato production in general can be affected by a number of pests and pathogens, including viruses, nematodes, bacteria, and fungi, which can lead to low yields and inferior quality.

Fusarium wilt (FW) (caused by *Fusarium oxysporum* Schlectend.) is a major disease in tomato. There are two distinct forms of the pathogen, *F. oxysporum* f. sp. *lycopersici* (FOL) W. C. Snyder & H. N. Hans, which causes vascular wilt, and *F. oxysporum* f. sp. *radicis-lycopersici* (FORL) W. R. Jarvis and Shoemaker, which causes crown and root rot (FCR). Both of these pathogens are soil borne and are present everywhere tomato is cultivated (Agrios 2005). On the other hand, diseases caused by viruses are an important limiting factor, and tomatoes can

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be affected by more than 136 different viruses (Hanssen et al. 2010). In recent years, several old tomato viruses (e.g., ToMV) have re-emerged since heirloom cultivars lacking genetic resistance have returned to popular interest because of their unique morphological (size and shape of the fruits), organoleptic (sweet or salty), and health-promoting (lycopene content) properties (Hanssen et al. 2010). Other important tomato viruses have emerged because of the natural selection of resistance breaking (RB) strains able to overcome resistance genes due to mutations in the elicitor viral protein, as in the case of the tomato spotted wilt virus-*Sw-5* gene interaction. In the case of the tomato yellow leaf curl virus-*Ty-1* interaction, disease severity has increased as recombinant viruses have been selected (Batuman et al. 2017, Urbino et al. 2020). Nevertheless, RB strains able to overcome the *Sw-5b* gene are not so widespread, and in some countries, they have a rather limited geographical distribution (Turini 2018). Moreover, reports of RB strains are often false, as most of these strains do not carry mutations that overcome resistance; rather, they are common strains of the virus that, at high temperatures, are able to invade the resistant plant because of a malfunction of the hypersensitivity reaction (Chung et al. 2018), the efficiency of which is temperature-dependent (Turini 2018). In some cases, symptoms caused by other tomato viruses, without an accurate diagnosis, can be confused with those of TSWV (Gebre-Selassie et al. 2002, Parrella 2020). Therefore, the *Sw-5* gene may still be effective in controlling TSWV infection and is therefore used in the breeding of new tomato cultivars.

Selected genes provide resistance to several isolates of the virus and even other viruses of the same genus. For example, the *Sw-5b* gene not only controls TSWV isolates from many geographic areas but also five other phylogenetically related members of the so-called "American" evolutionary clade: the orthotospoviruses groundnut ringspot virus (GRSV), tomato chlorotic spot virus (TCSV), , and chrysanthemum stem necrosis virus (CSNV) (Boiteux and Giordano 1993, Dianese et al. 2011), infecting tomato, and two additional orthotospoviruses, impatiens necrotic spot virus (INSV) and alstroemeria necrotic streak virus (ANSV) (Oliver and Whitfield 2016, Leastro et al. 2017, Oliveira et al. 2017). This broad-spectrum resistance is quite an exception for a dominant NB-LRR type of resistance gene, in contrast to the *Tsw* resistance gene in pepper (*Capsicum chinense* L.) which is effective in the control only of the TSWV isolates (Boiteux and de Avila 1994). Similarly, the *Tm-2*^{2} gene is effective in controlling many ToMV isolates and TMV (Sui et al. 2017). These characteristics allow the creation of new tomato cultivars carrying a broad spectrum of resistance. In addition, in tomato, the *Frl* gene, which confers partial resistance to FORL, maps to the long arm of chromosome 9 and is linked to the Tm-2 locus (Vakalounakis et al. 1997). Taking advantage of the linkage of Frl and Tm-2 and by developing a single tube, functional marker-based duplex PCR assay for unambiguous simultaneous detection of *Tm-2*^{2} and *Sw-5b* genes, we describe a simple method that allows pyramiding of three resistance genes in elite tomato germplasm through marker-assisted backcrossing. We demonstrated this approach to create a line of "Pomodoro di Sorrento" tomato with multiple disease resistances since this cultivar has been severely affected in recent years by multiple diseases, often caused simultaneously by both viruses and fungi (personal observation; Figure 1).

Figure 1. Viral symptoms on the Sorrento GP tomato landrace: A) mosaic, leaf area reduction and leaf wrinkling caused by tomato mosaic virus (ToMV); B) chlorotic spots and ring spots on the fruit caused by tomato spotted wilt virus (TSWV); C) chlorotic ringspots and uneven ripening of fruit caused by mixed infection of tomato mosaic virus and tomato spotted wilt virus.

MATERIAL AND METHODS

Genetic material

The Sorrento GP line of "Pomodoro di Sorrento" tomato was obtained from a local farmer who cultivated it in greenhouses in the Sorrento Peninsula and had been saving its seeds for more than 20 years. "Mospomorist" was the

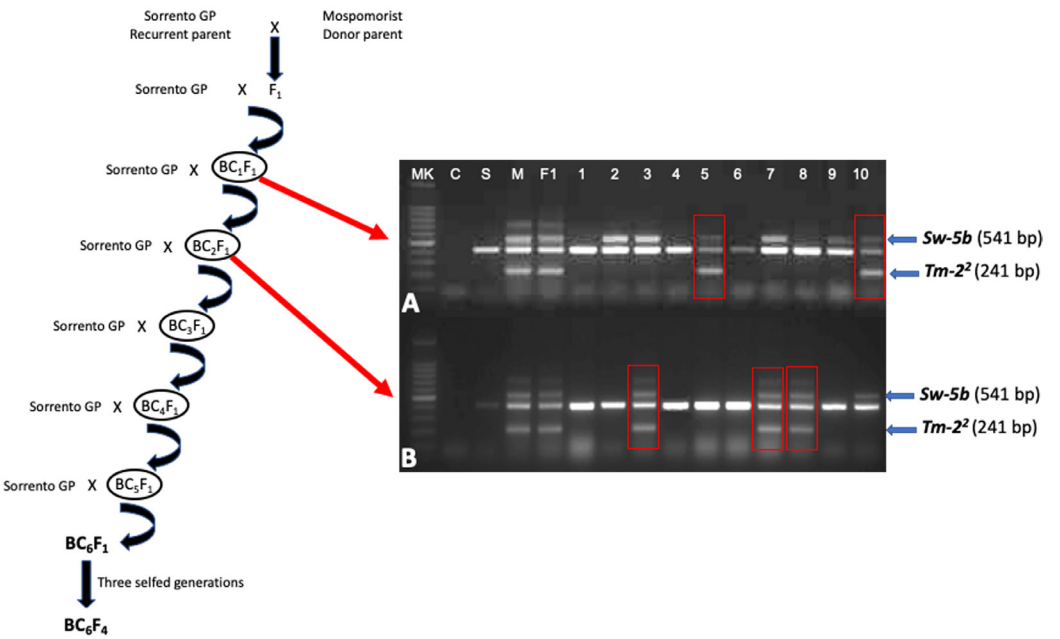

Figure 2. Breeding scheme adopted in the present work (at left), starting with a cross between Sorrento GP (recurrent parent) and Mospomorist (donor parent), continuing with six generations of backcrossing, and then three generations of selfing. On the right, the two agarose gels after electrophoresis show the products of duplex PCR and markers linked to the Sw-5 and Tm-2 resistant loci. Backcrossing was continued using the plants shown in red rectangles, which carry markers for both resistance alleles. MK, 100 bp ladder (Promega, USA); C, control without DNA; S, susceptible parent (Sorrento GP); M, resistant parent (Mospomorist); F1, F_1 hybrid (Sorrentino x Mospomorist); 1-10, ten plants of the BC_1F_1 generation (gel A) or BC_2F_1 generation (gel B).

donor of the resistance genes *Sw-5b*, *Tm-2²* and *Frl* (Laterrot 1996). The two parents and the generations of backcrossing and selfing were grown in an insect-proof greenhouse (22-26 °C) in natural lighting conditions and transplanted at one true leaf tage in 30 cm diameter pots containing commercial propagation substrate. The plants were fertilized weekly with a complete nutritive complex during the watering shift.

Crosses scheme

The F_1 generation was obtained between the recurrent parent Sorrento GP and the donor parent Mospomorist (Figure 2). Starting in BC_1F_1 and continuing in the subsequent BC_nF_1 generations, 10 plants were genotyped using molecular markers linked to the genes of interest. From these, plants carrying resistant alleles at the Sw-5 and Tm-2 resistance loci based on their marker genotypes (i.e., the *Sw-5b* and *Tm-2²* genes, respectively) that were phenotypically similar to the recurrent parents were selected as the parents for the next backcross, and the procedures lasted until the BC_6F_1 generation (Figure 2). The selected BC_6F_1 plants were self-pollinated to produce $BC_6F_{2,}$ and plants from this generation were selected based on phenotypic similarity to Sorrento GP and for homozygosity at the marker genotypes. The F_3 and F_4 plants derived from BC_6F_2 were selected with molecular markers and phenotypic selection. Finally, the stable line was evaluated for resistance to TSWV, ToMV, and FORL.

DNA extraction

Genomic DNA was extracted from 50 mg of fully expanded apical leaves of 2- to 3-week-old seedlings using an E.Z.N.A.Ò Tissue DNA kit (Omega Biotek, Inc., Norcross, GA, USA) following the manufacturer's instructions. Quantification of DNA was performed with a Qubit 4 Fluorometer (Thermo Fisher Scientific Inc., USA).

Amplification of target genes through duplex and uniplex PCR

Initially, a PCR annealing temperature gradient was performed to find the best conditions for screening the plants.

Based on these results, all subsequent duplex PCRs were performed using 50 ng template DNA, 12.5 µL of DreamTaq Green PCR Master Mix 2x (ThermoFisher Scientific, USA), 6.25 pmol of TM2-748F and TM2-1256R primers (Lanfermeijer et al. 2005), and 25 pmol of Sw5-f2 and Sw5-r2 primers (Shi et al. 2011) in 25 µL reactions with a thermal profile of 94 °C for 1 min (initial denaturation), followed by 35 cycles of denaturation at 94 °C for 45 s, annealing at 60 °C for 45 s, extension at 72 °C for 1 min and a final extension of 5 min at 72 °C. The PCR products were separated by electrophoresis on 1.2% agarose gels (Promega, USA), stained with ethidium bromide and visualized in a gel documentation system (ChemiDoc™, Bio–Rad, USA). In the generations of selfing following the sixth backcross, amplification with primers Sw5-f2 and Sw5-r2 produced a 574 bp fragment in resistant homozygotes, a 464 bp fragment in susceptible homozygotes and both fragments in heterozygotes (Dianese et al. 2010). To confirm the linkage between the Tm-2 and Frl loci and homozygosity at both loci, the codominant $SCAR_{Frl}$ marker for locus C2_At4g28660 (ch09:61774146–61775809) was used to detect the *Frl* gene in the generations of selfing (Mutlu et al. 2015).

Evaluation of disease resistance

Twenty plants each of the "Mospomorist" (resistant parent), "Sorrento GP" (susceptible parent) and BC_6F_4 generations were used in each independent test of resistance. In addition, mock-inoculated control plants of the susceptible parent were also used in each test.

Two isolates of TSWV, designated Arc-1 and LYE08, isolated from *Arctotis x hybrida* L. (Family *Asteraceae*) and tomato, respectively (Parrella et al. 2013, Zaccardelli et al. 2008), were used for two independent resistance tests. The virus isolates were multiplied in *Nicotiana glutinosa* plants for only one round per test. Tomato seedlings were transplanted at first true leaf stage to 10 cm diameter pots with commercial seeding/propagation substrate and watered weekly with a balanced nutritive solution. All plants were grown in an insect-proof greenhouse (22-26 °C and 70-80% humidity) under natural lighting. The inoculum was prepared 15 days post-inoculation (DPI) by grinding 1 g of apical *N. glutinosa* leaves in 4 ml of 0.03 M Na_2HPO_4 buffer with 0.2% sodium diethyldithiocarbamate. Before inoculation, 75 mg mL^{-1} of carborundum 600 mesh and activated charcoal were added to the leaf extract (Marrou 1967). The slurry was used to inoculate mechanically cotyledons and the first six true leaves of 16-day-old tomato seedlings (Shi et al. 2020). Symptoms were observed from one to 30 DPI. Susceptible plants showed necrotic local lesions on inoculated leaves, followed by systemic tip necrosis and often death of the plants within 30 DPI. At 15 DPI, plants were checked for TSWV infection using a commercial Double Antibody Sandwich-Enzyme Linked Immunosorbent Assay (DAS-ELISA) (Agdia Inc, USA), which includes a TSWV-specific monoclonal antibody, following the method described by Clark and Adams (1977). Plant extracts were prepared by grinding 1 g of upper non-inoculated leaves in 4 mL of inoculation buffer with a leaf grinder. Plates were read with a Tirtertek Multiskan Plus Photometer at 405 nm 1 h after addition of the substrate. The threshold value adopted was two time the absorbance value of the mean of the healthy controls, thus samples were considered as positive when their absorbance was above the threshold value.

The conditions used for the ToMV test were essentially the same as those for the TSWV test. The TMj isolate, belonging to pathotype 2 of ToMV, was used for the screening of resistance (Marchoux et al. 2008), but in this case, the virus was multiplied on *N. benthamiana* plants. In addition to visual screening, samples from upper noninoculated leaves were evaluated at 15 and 30 DPI using a commercial DAS-ELISA kit for tobamoviruses (Bioreba AG, Switzerland),

The Fusarium crown rot (FCR) isolate used in this study, SM16, originated in a tomato field in Campania (southern Italy). This isolate was stored in 50% glycerol at -80 °C and used throughout this study. The methods for inoculum preparation, inoculation and disease screening were as described in Devran et al. (2018) with minor modifications. Briefly, the fungus was grown in potato-dextrose broth on a rotary shaker for 7 days at room temperature. Then, the broth culture was filtered through two layers of cheesecloth, and the suspension was centrifuged at 4000 rpm for 10 min. Conidia were resuspended in sterile water, and the concentration was adjusted to 10^7 spores mL^{-1}. Seedlings of the resistant donor parent Mospomorist, susceptible recurrent parent Sorrento GP, and homozygous BC_6F_4 generation were grown in sterile peat moss. The roots of seedlings, at the stage of one-two true leaves, were washed to remove the substrate and dipped in the suspension of spores. Inoculated seedlings were then transplanted to a sterilized mixture and kept in a greenhouse at 22±2 °C with 13 h photoperiods and observed for symptom development for 30 DPI. Control plants consisted in seedlings treated with sterile dH_20. After the incubation period, plants were scored as resistant or susceptible based on wilting and the presence of the FCR typical lesions on roots and crowns.

Table 1. Response of the tomato genotypes Mospomorist, Sorrento GP and the homozygous BC_6F_4 line obtained in the resistance tests against TSWV, with isolates Arc-1 and LYE08; ToMV, with isolate TMj; FCR (Fusarium crown rot), with isolate SM16, in therms of the number of symptomatic plants in the set of 20 of each genotype in each test. All inoculated plants were checked for symptom development at 15 and 30 DPI. Plants inoculated with TSWV and ToMV were also checked for virus presence using DAS-ELISA in the upper noninoculated leaves at 15 and 30 DPI. The DAS-ELISA results were the same at 15 and 30 DPI for all the plants inoculated with TSWV and ToMV. In the case of the susceptible Sorrento GP genotype inoculated with both TSWV isolates, almost all the plants died within 20-25 DPI. I: inoculated plants; N: noninoculated plants

Tomato genotypes/lines	TSWV				ToMV		FCR	
	Arc-1		LYE08		TMj		SM16	
	I	N	I	N	I	N	I	N
Mospomorist	0	0	0	0	0	0	0	0
Sorrento GP	20	0	20	0	20	0	20	0
BC_6F_4	0	0	0	0	0	0	0	0

Table 2. Comparison of some morphological traits between the Sorrento GP selection of "Pomodoro di Sorrento" and the homozygous BC_6F_4 lines

Genotypes	IT[a]	GS	FLS	FSC	FC	PUF	FLC	FW	PT
Sorrento GP	3	3	3	2	4	3	4	3	3
BC_6F_4	3	3	3	2	4	3	4	3	3

[a] Scale used for each descriptor: IT: Inflorescence type (1, simple; 2, double; 3, compound); GS: Green shoulder (1, absent; 4, very strong); FSL: Shape in longitudinal section (1, flattened; 2, oblate; 3, circular; 4, cordate; 5, obcordate); FSC: Shape in cross section (1, circular; 2, angular; 3, irregular); FC: Fruit color (3, orange; 4, pink; 5, red); PUF: Puffiness (1, no puffy; 4, very puffy); FLC: Fruit flesh color (3, orange; 4, pink; 5, red); FW: Fruit weight=1 (55.5 - 117.5 g); 2 (117.6 - 179.6 g); 3 (179.7 - 241.7 g); 4 (241.8 - 303.8 g); 5 (303.9 - 365.9 g); PT: Thickness of flesh =1 (4.75 - 5.70 mm); 2 (5.71 - 6.66 mm); 3 (6.67- 7.62 mm); 4 (7.63 - 8.58 mm); 5 (8.59 - 9.54 mm).

Preliminary morphological assessment

With the aim of verifying whether during the introgression of the resistance genes, the principal morpho-agronomic features of the "Sorrento GP" had been maintained in the BC_6F_4 line, we compared the two tomato lines as described by Parisi et al. (2016). In particular, nine traits were scored and compared on a single plant basis (Table 2).

RESULTS AND DISCUSSION

Duplex-PCR based marker system for the simultaneous detection of *Sw-5b* and *Tm-2²* in backcross generations

Combining the functional markers specific for *Sw-5b* and *Tm-2²* genes, a duplex PCR assay was proposed. The marker system was able to clearly distinguish the resistant parent with both resistance genes from the susceptible parent (Figure 2). This marker system was applied to detect the genes through the generations of backcrossing and selfing, starting from the BC_1F_1 to BC_6F_1 generations. At least one plant carrying both genes was identified by the marker system among the 10 plants screened in each backcross generation (Figure 2). Thus, the duplex-PCR-based marker system for simultaneous detection of *Sw-5b* and *Tm-2²* can be used in breeding programs that target introgression of both genes by screening a small number of plants in each segregating backcross generation.

Selection in selfed generations from advanced backcrosses

Selection in generations after the sixth backcross identified a single plant based on the simultaneous detection of markers for the *Sw-5b* and *Tm-2²* genes. In the BC_6F_2 generation, three plants were identified as homozygous for the *Sw-5b* gene (Figure 3B), while perfect cosegregation was observed between the Tm-2 and Frl loci (Figure 3A and 3C). Since the three *Sw-5b* homozygous plants found in the BC_6F_2 generation were not fixed at the Frl locus, the BC_6F_3 generation was produced for each of the three selected plants. Finally, approximately 70% of the plants in the BC_6F_3 generation were homozygous dominant at the Sw-5, Tm-2 and Forl loci and thus fixed in the BC_6F_4 generation (Figure 4).

Performance of the selected progeny for disease resistance

The results of the three tests are summarized in Table 1. For TSWV, all plants of the Sorrento GP showed symptoms at 7-10 DPI when inoculated with each isolate in separate tests. The presence of the virus in infected tissues was confirmed

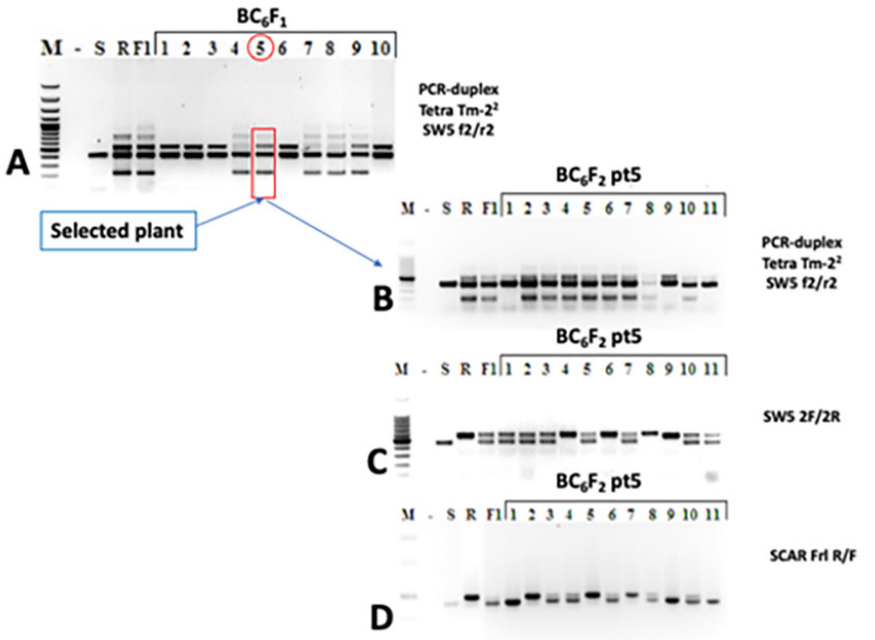

Figure 3. Example of genetic selection in the self-fertilization generations, performed with the duplex-PCR method proposed in the present work, after six backcross generations. The duplex PCR identified only one plant (boxed in red) out of 10 in the BC_6F_1 generation (A) and six plants out of 10 in the BC_6F_2 offspring from self-pollination of the selected BC_6F_1 plant (B) with markers for resistance at both the *Tm-2²* and *Sw-5b* genes. Then, the homozygosity of individual plants was evaluated separately using codominant markers (primer pair Sw5-2F and Sw5-2R) for *Tm-2²* (C) and $SCAR_{Frl}$ primers for a marker linked to *Frl* (D). The primers are indicated to the right of each corresponding gel. M, molecular ladder; -, negative control (no DNA); S, susceptible parent (Sorrento GP); R, resistant parent (Mospomorist); F_1, first generation of crossing between Sorrento GP and Mospomorist;1-10, individual plants of the BC_6F_1 of BC_6F_2 generations. The primers used are reported on the right of each corresponding gel.

at 15 DPI by ELISA. The average optical density (O.D.) at 405 nm for the Arc-1 isolate was 1.8±0.5 compared to 0.08±0.02 for the healthy noninoculated plants and 1.6±0.8 for the LYE08 isolate compared to 0.07±0.02 for the healthy control. Most of the plants of the susceptible control died within 20-25 DPI, and thus, ELISA was only performed at 15 DPI. Plants of the resistant parent Mospomorist and the BC_6F_4 generation were resistant, as indicated by only a few necrotic lesions on the inoculated leaves and no virus detected at 15 and 30 DPI in the upper noninoculated leaves (average of the O.D. in the Mospomorist and BC_6F_4 generations was 0.09±0.01 and 0.07±0.02, respectively). The same results were obtained for the test of resistance against ToMV. Mospomorist and the BC_6F_4 generation were resistant. No symptoms were observed in the inoculated or the upper non-inoculated leaves. Furthermore, the virus was not detected in their upper non-inoculated leaves (average of the O.D. in Mospomorist and BC_6F_4 were 0.09±0.01 and 0.07±0.02, respectively). In "Sorrento GP" and healthy controls, O.D. values were 2.0±0.06 and 0.07±0.01, respectively.

Finally, the FCR resistance test gave clear results. Within 30 DPI, all plants of the susceptible parent wilted and showed browning of the roots and crown, while the resistant parent and the homozygous resistant BC_6F_4 line showed no symptoms.

Morphological assessment

The comparison of morphological traits between the Sorrento GP and BC_6F_4 tomato lines is reported in Table 2. The two tomato genotypes reached exactly the same score for each of the fruit traits evaluated. These preliminary results indicate that the selection scheme adopted (assisted by molecular markers) was able to maintain the main morpho-agronomic features of the fruit of the recurrent parental. Future investigations will more precisely investigate the organoleptic characteristics and will be the subject of future investigations.

After yield, disease resistance and quality are the main focuses of tomato breeders throughout the world since they are of worldwide importance (Melomey et al. 2019). Among tospoviruses, TSWV is one of the deadliest viruses, with a host range of more than 1000 plant species belonging to more than 85 families, including tomato, bean, lettuce, groundnut, pepper, potato and tobacco, among others (Gupta et al. 2018, Parrella et al. 2003). ToMV is one of the most devastating tomato viruses, especially for crops grown in protected environments. The possibility of transmission by infected seeds and the speed of diffusion inside greenhouses, often unknowingly facilitated by agricultural operators through the use of contaminated tools and the ubiquitous status of the virus, make ToMV one of the most feared tomato viruses, and resistant cultivars are essential for greenhouse production. FORL is a disease of worldwide economic importance in commercial tomato. It is a necrotrophic pathogen causing severe losses in greenhouse, field and hydroponic cultures (McGovern 2015). Although various strategies have been employed to control these pathogens, the use of resistant cultivars is the most acceptable and economic approach, in line with the current trends in agriculture that require low environmental impact farming systems (Szczechura et al. 2013).

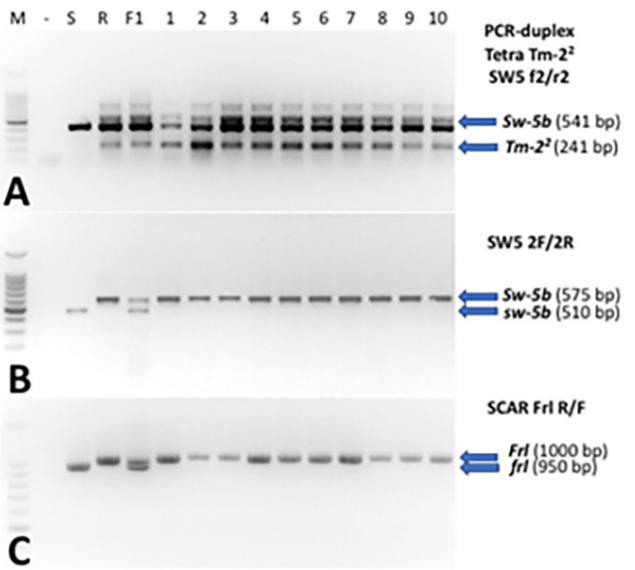

Figure 4. Plants of the BC_6F_4 generation were checked using the duplex-PCR method (A), following the same approach shown in Figure 3. Homozygosity at both marker loci was achieved in this generation, as shown on two agarose gels after electrophoresis, *Sw-5b* (B) and *Forl* (C). M, molecular ladder; -, negative control (no DNA); S, susceptible parent (Sorrento GP); R, resistant parent (Mospomorist); F_1, first generation of crossing between Sorrento GP and Mospomorist;1-10, individual plants of the BC_6F_4 generation.

Gene pyramiding obtained by adding of several resistance genes in a single cultivar conferring resistance to different pathogens is an effective strategy to reduce yield losses caused by pathogens. In particular, gene pyramiding through marker-assisted selection (MAS) and other techniques has enabled the constitution of durable resistant/tolerant lines with high precision in the shortest period of time for agricultural sustainability (Dormatey et al. 2020). Nevertheless, the principal issue in MAS is the appropriate selection of the target gene, since its introgression depends on the precise cosegregation between the target gene and the marker. According to the results of inoculation and resistance tests, the accuracy of the simultaneous selection of three resistance genes using duplex PCR targeting two broad resistance genes combined with linkage to a third resistance gene was 100%, indicating that the chosen strategy was effective for R gene pyramiding and the development of advanced lines simultaneously resistant to hortotospoviruses (TSWV, TCSV, GRSV and CSNV), tobamoviruses (ToMV and TMV) and FORL. This approach does not use a large number of plants. Compared to other methods for self-pollinated crops, the backcross method uses relatively few plants. Furthermore, the outcome is predictable. The breeder will recover the recurrent parent plus the added R genes. Further evaluation ensures that there were no unintended changes (i.e., linkage drag).

Broad-spectrum resistance gene pyramiding helps the development of cultivars with broad-spectrum resistance to different pathogens as well as races of the same pathogen. The *Sw-5b* gene controls resistance to five different tomato-infecting tospoviruses thanks to a conserved 21-amino acid viral effector epitope among the five controlled tospoviruses (Leastro et al. 2017, Zhu et al. 2017, Oliveira et al. 2018). Although *Sw-5b* has been overcome by RB strains of TSWV, these appear to be less fit than wild-type strains. The emergence of RB isolates of TSWV in many locations worldwide is most likely the result of the continuous, widespread use of tomato cultivars with the *Sw-5b* gene (Batuman et al. 2017). Several researchers have noted that the incidence of TSWV RB isolates declines over time in a region when tomato cultivars carrying the *Sw-5b* gene are not grown consistently (Gordillo et al. 2008, Turina et al. 2016).

Although new sources of resistance to RB strains have been recently reported in some wild tomato accessions (Kabaş et al. 2021), at this time, cultivars carrying these resistance genes are not yet available, and thus, it is advisable

to continue to wisely use cultivars with the *Sw-5b* gene. The agronomic strategy of using these resistant cultivars only seasonally or not continuously has proven to be an effective strategy to reduce the incidence of RB strains in the field and maximize the efficiency of the *Sw-5b* gene in the control of wild-type TSWV strains (Gordillo et al. 2008).

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