agriculture

MDPI

Article

Effect of Introgression of *Ty-1* and *ty-5* Genes on Productivity, Quality, and Antioxidant Compounds in De la Pera Tomato Breeding Lines

José Ángel Cabrera, Pedro Carbonell, Juan Francisco Salinas, Adrian Grau, Aranzazu Alonso, Francisca Hernández, Juan José Ruiz and Santiago García-Martínez *

Instituto de Investigación e Innovación Agroalimentaria y Agroambiental (CIAGRO-UMH), Miguel Hernández University, Ctra. de Beniel km 3.2, 03312 Orihuela, Spain; j.cabrera@umh.es (J.Á.C.); pcarbonell@umh.es (P.C.); juan.salinas@goumh.es (J.F.S.); agrau@umh.es (A.G.); aalonso@umh.es (A.A.); francisca.hernandez@umh.es (F.H.); juanj.ruiz@umh.es (J.J.R.)

* Correspondence: sgarcia@umh.es; Tel.: +34-96-674-9632

Abstract: Tomato (*Solanum lycopersicum* L.) is a crop that is affected by more than a hundred viral species. De la pera is a local varietal type of tomato that is very popular in southeastern Spain. However, it is highly susceptible to several viruses, such as *Tomato yellow leaf curl virus* (TYLCV), which is considered one of the most important diseases of tomato crops and is a limiting factor for production in both outdoor and protected crops, making it difficult to eradicate. This study shows the effect of gene introgression on the performance of traditional lines of De la pera by combining two genes that offer tolerance to TYLCV, *Ty-1* and *ty-5*, on some yield and quality traits and on the antioxidant capacity of tomato fruits. Two pear tomato breeding families, UMH175 and UMH220, were evaluated. Four lines from each of the families with all homozygous combinations of the *Ty-1* and *ty-5* genes were studied. The results showed that the introgression of the *ty-5* allele produced a slight negative effect on yield, mean fruit weight, total soluble solids, and titratable acidity, in contrast to *Ty-1*, which produced a large negative effect. None of the introgressions showed a negative effect on the antioxidant compounds. *ty-5* is a promising gene for use in breeding programs.

Keywords: *Begomovirus*; viral diseases; gene pyramiding; *Ty* genes; molecular marker; total phenolic content; antioxidant activity

Citation: Cabrera, J.Á.; Carbonell, P.; Salinas, J.F.; Grau, A.; Alonso, A.; Hernández, F.; Ruiz, J.J.; García-Martínez, S. Effect of Introgression of *Ty-1* and *ty-5* Genes on Productivity, Quality, and Antioxidant Compounds in De la Pera Tomato Breeding Lines. *Agriculture* **2024**, *14*, 1192. https://doi.org/10.3390/agriculture14071192

Academic Editor: Thomas Thomidis

Received: 1 May 2024
Revised: 16 July 2024
Accepted: 17 July 2024
Published: 19 July 2024

1. Introduction

Tomato (*Solanum lycopersicum* L.) is one of the most consumed and economically valuable crops worldwide. It is affected by more than a hundred viral species according to the International Committee on Taxonomy of Viruses (ICTV); the main ones are included in the genera *Begomovirus*, *Orthotospovirus*, *Tobamovirus*, *Potyvirus*, and *Crinivirus* [1]. *Tomato yellow leaf curl virus* (TYLCV) is considered one of the most significant diseases affecting tomato crops in many tropical and subtropical regions worldwide. It belongs to the family *Geminiviridae*, genus *Begomovirus*, whose genome is composed of a circular single-stranded DNA molecule [2]. Symptoms include dwarfism, upward leaf puckering, vein clearing, and excessive branching and stunting, all of which are associated with mild to severe mosaic symptoms and partial or total plant sterility [3]. The disease is transmitted via the tobacco whitefly, *Bemisia tabaci* Gennadius [4], with high population outbreaks often associated with high disease incidence. Depending on the severity of the infections, yield loss can reach 100% [5]. In Spain, the disease was first detected in 1992 in greenhouses in Murcia [6] and Almería [7], attributable to a Sardinian species (*Tomato yellow leaf curl Sardinia virus*, TYLCSV). Five years later, following on from some more severe episodes, the presence of the species *Tomato yellow leaf curl virus* was detected [8]. The occurrence of TYLCVD is highly dependent on chemical control aimed at reducing whitefly populations;

yet, due to the development of resistance in vectors and the loss of natural enemies, it is difficult to eliminate the disease among crops [5]. Therefore, an increasingly demanding international market and inexorable respect for the environment render genetic resistance the best strategy to control viral diseases.

The introduction of TYLCV-resistant alleles into cultivated tomatoes from wild species began in 1974 [9] and was successfully completed by several researchers [10–17]. To date, six TYLCV resistance loci have been identified (Table 1). Zamir et al. [16] found *Ty-1* on chromosome six of *S. chilense* accession LA1969. *Ty-2* was later mapped to chromosome 11 [17–19]. *Ty-3* was located on chromosome six of *S. chilense* accessions LA1932 and LA2779 [20] near the *Ty-1* locus, suggesting a genetic link between *Ty-1* and *Ty-3* [21]. In 2013, Verlaan et al. [22] accurately mapped *Ty-1* and *Ty-3*, concluding that the *Ty-3* assigned region of approximately 71 kb overlapped with the *Ty-1*-containing region. The *Ty-4* gene was also mapped in *S. chilense* accession LA1932 but on chromosome three [23]. Later, in another study designed to map the loci controlling TYLCV resistance in TY172, they identified a recessive QTL called *Ty-5* near the *SlNAC1* marker on chromosome four [2]. The University of Florida's tomato breeding program developed numerous breeding lines with *Begomovirus* resistance derived from Tyking, a hybrid bred by Royal Sluis (Enkhuizen, The Netherlands). Molecular-marker-assisted analysis confirmed that the TYLCV resistance offered by Tyking was not controlled by the *Ty-1*, *Ty-2*, *Ty-3*, and *Ty-4* genes, and several of these lines were consequently tested with the *Ty-5* CAPS marker, *SlNAC1*. The results showed that the Tyking-derived allele was recessive, so the authors suggested that the TY172-derived locus be renamed *ty-5* to reflect the recessive gene action. Tyking-associated resistance likely corresponds to that of TY172, although allelism of the genes remains to be demonstrated [24]. In 2015, Lapidot et al. [25] delimited the *ty-5* locus in a single gene encoding the tomato homolog of the messenger RNA surveillance factor Pelo. Finally, by analyzing a Fla.8638B X Fla.7987 F2 population, the effect of a *Begomovirus* resistance gene on chromosome ten, named *Ty-6*, was confirmed [26].

Table 1. TYLCV resistance loci identified in wild species.

Gen	Accession	Species	Chromosome	Reference
Ty-1	LA1969	*S. chilense*	6	[16,22]
Ty-2	B6013	*S. habrochaites*	11	[11,17,19]
Ty-3	LA1932, LA2779	*S. chilense*	6	[20–22]
Ty-4	LA1932	*S. chilense*	3	[23]
ty-5	TY172	*S. peruvianum*	4	[2,10,24,25]
Ty-6	LA1938	*S. chilense*	10	[26]

The genes described above offer partial resistance to the virus. With molecular-marker-assisted pyramiding, it is possible to obtain plant materials that offer more effective and longer-lasting resistance over time, facilitating the management of the cultivation of traditional and commercial varieties, both outdoors and in greenhouses. Using a combination of genes, this strategy provides higher levels of resistance, resulting in the virus encountering more barriers to overcome this resistance and infecting the plant. Several studies have evaluated different gene combinations to determine their behavior against TYLCV [27–30], but a priori, none of the lines carrying the *Ty-1* and *ty-5* genes, at least initially, have been studied. It is important to evaluate this *Ty-1*/*ty-5* combination to ascertain the response of these tolerant genes.

Introgression of the *Ty-1* gene produces a negative effect on productive and quality traits depending on the growing conditions and genetic background [31]. This is because recombination does not occur in a fragment of approximately 35.5 megabases due to two chromosomal mutations produced on chromosome 6 of the source of resistance, *Solanum chilense* accession LA1969. It is thus difficult to eliminate the genome of the wild

species during backcrossing [32]. The search for recombinants is an alternative method for eliminating part of the genome of the wild species, so another line of work associated with this study was carried out. To search for recombinant individuals, several molecular markers close to the *Ty-1* gene were designed in order to identify individuals that contain *Ty-1* and do not contain some of the designed markers. This translates into a deletion of part of the *S. chilense* genome, decreasing the negative effect of introgression and offering resistance. All markers are based on SNPs from Illumina's Tomato Infinium Array and were tuned for visualization with the high-resolution melting technique [33]. Promising studies have been conducted with the same traditional pear lines (UMH175 and UMH220), as well as others of the Muchamiel varietal type, albeit recombinant (data to be published). These lines carry the *Ty-1* and *ty-5* alleles but have lost part of the introgressed fragment of the wild species linked to *Ty-1*. The hope is that in future trials, these traditional varietal types will maintain acceptable levels of production without compromising their distinctive organoleptic and morphological qualities.

The aim of this study is to demonstrate the effect of gene introgression on the performance of traditional lines of the De la pera varietal type. It combines two genes offering tolerance to TYLCV, *Ty-1*, and *ty-5*, and examines their impact on various yield and quality traits, as well as on the antioxidant capacity of tomato fruits.

2. Materials and Methods

Due to the organoleptic quality of its fruit, De la pera is a very popular local variety of tomato in the Vega Baja del Segura region in southeastern Spain. The fruits have a juicy and firm texture, a high proportion of seeds and mucilage, and an intense flavor. Their weight ranges from 75 to 125 g, while their shape varies from elongated oval to bell-shaped, with dark green shoulders and no ribs [34]. However, like most tomato landraces, De la pera cultivars are highly susceptible to several viruses, including *Tomato yellow leaf curl virus* (TYLCV) and *Tomato leaf curl virus* (ToLCV) (genus *Begomovirus*), *Tomato spotted wilt virus* (TSWV) (genus *Orthotospovirus*), *Tobacco mosaic virus* (TMV), *Tomato mosaic virus* (ToMV) and *Tomato Brown rugose fruit virus* (ToBRFV) (genus *Tobamovirus*), *Potato virus Y* (PVY) and *Chilli veinal mottle virus* (ChiVMV) (genus *Potyvirus*), and *Tomato chlorosis virus* (ToCV) (genus *Crinivirus*), among others [1].

2.1. Plant Material

Two pear tomato breeding families, UMH175 and UMH220, were evaluated. Four lines from each of the families with all homozygous combinations of the *Ty-1* and *ty-5* genes [RR rr (*Ty-1*/*ty-5*), ss SS (ss SS), ss rr (ss/*ty-5*), and RR SS (*Ty-1*/ss)] were studied. These lines also contain the *Tm-2*^{a} and *Sw-5* alleles in the homozygous state.

2.1.1. Genealogy

In 1998, the CIAGRO-UMH breeding group started a breeding program of traditional varieties in the area with the aim of introducing resistance to the three main viruses affecting tomato cultivation (ToMV, TSWV, and TYLCV) and obtaining pure lines, either for use by farmers to obtain seeds across each crop cycle or as parental donor lines for the breeding program.

De la Pera UMH1203

UMH1203 is obtained by crossing a local P21 De la Pera line with the commercial cultivar Anastasia-F1 (Seminis Vegetable Seeds, Saint Louis, MO, USA), followed by six generations of backcrossing with the De la Pera line. After five additional generations of self-fertilization and selection, pure UMH 1203, homozygous for the three introgressed virus resistance genes, was selected from a single BC6S5 family, whose seed was multiplied by self-pollination [34]. Anastasia was used as a donor parent for the *Tm-2*^{a}, *Sw-5*, and *Ty-1* genes [34,35], which confer resistance to ToMV, TSWV, and TYLCV, respectively. P21 pear

was used as a recurrent parent previously selected for fruit morphology, high yield, and uniformity [34] from a collection of several local accessions donated by farmers in the region.

De la Pera UMH1406

In the BC6 stage of UMH1203, simultaneous with the self-fertilization, backcrossing was continued for two more generations. From the BC8 result, UMH1406 was selected, and two self-fertilizations were carried out, with UMH1406 being a BC8S2 line.

De la Pera UMH175 and UMH220

Due to the threat posed by TYLCV, since there are no materials that offer complete resistance to this viral disease, the *ty-5* gene was introgressed in order to complement the resistance of *Ty-1*. UMH1406, a carrier of *Tm-2*^{a}, *Sw-5*, and *Ty-1*, was crossed with the TX 468-RG breeding line, a donor of *ty-5*, on loan from Dr Rafael Fernández Muñoz of the Institute of Subtropical and Mediterranean Horticulture "La Mayora" (UMA-CSIC). Finally, after five generations of backcrossing with UMH1406 and two self-fertilizations, the UMH 175 and 220 lines with different combinations of the *Ty-1* and *ty-5* genes were selected. In each generation of the entire process, molecular-marker-assisted selection was used to select plants carrying the resistance genes. In addition, during each generation of backcrossing, high selection pressure was applied for pear characteristics (bell shape, green shoulder, tolerance to Blossom-end rot, BER) and good agronomic performance [36].

2.2. Field Test

De la pera lines UMH175 and UMH220 were grown in a net greenhouse at the CIAGRO-UMH facilities at the Escuela Politécnica Superior de Orihuela (Alicante, Spain), with four randomly distributed replicates of between six and seven plants of each of the four genotypes and for each of the two families (see Supplementary Materials). The first study was conducted during the spring–summer cycle in 2022 and replicated in 2023 (Table 2). The plants were grown to one stem with a planting frame of 1.0 m between rows and 0.40 m between plants, under management and fertilization conditions typical of the area (Table 2), providing inorganic fertilizers in the irrigation water. An organic amendment was made to improve the physical, chemical, and biological properties of the soil, incorporating 2.5 kg/m^2 of commercial sheep manure pellets with an organic matter content of 65% of the dry weight. The irrigation water was taken from the Segura River with a conductivity of approximately 1.6 dS/m. Crop water requirements were calculated using the FAO Penman–Monteith model. The reference evapotranspiration (ET_o) was obtained from the La Basca station located at Los Alamos in the municipality of Beniel (Spain, Lat: 38°2′4.33″, Lon: 0°59′58.72″; X: 675540, Y:4211532) belonging to the Agricultural Information System Network of the Region of Murcia. In 2022, symptoms of *Fusarium oxysporum* were detected in the final stage of the crop. In 2023, the presence of *Phytophthora infestans* was detected during the initial stage of the crop and *Fusarium oxysporum* during the final stage. No plants were found to be infected by ToMV, TSWV, or TYLCV in either year.

Table 2. Cultivation and fertilization dates for 2022 and 2023.

Dates	**2022**	**2023**
Sowing	11 February	7 February
Planting	29 April *	4 April
Beginning of harvest	13 July	10 July
End of harvest	03 August	26 July
Phase	Fertilizer units	Overall Fertilization
1 Vegetative development	1 N–2 P_2O_5–1 K_2O–1 CaO	375 N–225 P_2O_5–550 K_2O–190 CaO
2 Flowering and fruit development	1 N–1 P_2O_5–1 K_2O–1 CaO	
3 Ripening of the fruit	1 N–0.3 P_2O_5–2 K_2O–1 CaO	

* In 2022, the transplant was delayed due to rainfall.

2.3. Parameters Assessed

2.3.1. Yield and Average Fruit Weight

Production is counted per plant and expressed in kg/plant. Average fruit weight is expressed in g/fruit.

2.3.2. Total Soluble Solids and Titratable Acidity

The fruits used in the analysis of total soluble solids (TSS) and titratable acidity (TA) were selected per replicate and in a homogeneous ripening stage one week after observing the color change of the fruits. Four to five fruits per replicate and genotype were selected from each of the lines. Both TSS and TA analyses were obtained in duplicate. Results are expressed as °Brix and % citric acid/100 g fresh tissue for TSS and TA, respectively. The content of total soluble solids was determined using an Atago PR-100 digital refractometer (Atago, Bellevue, WA, USA) at 20 °C. Titratable acidity was analyzed using a CRISON pHmatic 23 (Crison, Barcelona, Spain) with 0.01 mol/L of NaOH and is expressed as % citric acid.

2.3.3. Total Antioxidant Activity and Total Phenolic Compounds

The fruits used in the analysis of total antioxidant activity (TAA) and total phenols (TPCs) were selected per replicate and in a homogeneous ripening stage one week after observing the color change of the fruits. One sample per replicate was taken, consisting of pieces of 10–15 fruits. After cutting, the fruits were immediately frozen in liquid nitrogen and stored at −80 °C. Prior to analysis, all samples were freeze-dried to remove the water content for 72 h, yielding 6.8% dry matter. With 0.5 g of powdered sample, the measurement procedure for the two parameters, TAA and TPCs, was carried out.

Several methods should be used to determine the total antioxidant activity to better contrast the results obtained since each of them is based on a different determination route and can give different results from each other [37,38]. The direct determination methods used were DPPH (described by Brand-Williams et al. [39], with a modification in the reaction time) and ABTS (according to Re et al. [40]). For the determination of antioxidant activity, a methanolic extract was prepared from each sample to be analyzed. The freeze-dried fruits (0.5 g) were mixed with 5 mL of MeOH/water (80:20, v/v) + 1% HCl, sonicated at 20 °C for 15 min, and left for 24 h at 4 °C. The extract was then returned to the sample and mixed with 5 mL of MeOH/water (80:20, v/v) + 1% HCl. The extract was then sonicated again for 15 min and centrifuged at 15,000 rpm for 10 min. Calibration curves, in the range 0.01–5.00 mmol Trolox/kg, were used for the quantification of the three antioxidant activity methods and showed good linearity ($r^2 \geq 0.998$). The analyses were carried out in four replicates, and the results are expressed in mmol Trolox/kg dry matter.

Total phenols were determined using the Folin–Ciocalteu colorimetric method described by Singleton et al. [41], with some modifications. To a sample of the prepared extract (100 µL), 200 µL (1/10) of Folin–Ciocalteu reagent and 2 mL of distilled water were added and incubated for 3 min at room temperature. Then, 1 mL of sodium carbonate (20%, w/v) was added and incubated again for 1 h. Calibration curves, with a concentration range between 0 and 0.25 g GAE/L, were used for TPC quantification and showed good linearity ($r^2 \geq 0.996$). All determinations were performed in quadruplicate, and the results are expressed as milligrams of gallic acid equivalent per 100 g of dry matter sample (mg gallic acid eq/100 g dry matter).

Analyses were performed using a UV-vis spectrophotometer (Termospectromic Helios Gamma UVG 1002 E, Cambridge, UK). The absorbance was read at 765 nm for the quantification of total phenolic compounds and at 515 and 734 nm for the quantification of total antioxidant capacity by the DPPH and ABTS methods, respectively.

2.4. Statistical Analysis

Analyses of variance were performed according to the one-factor generalized linear model, with genotypes as a factor. Fisher's Least Significant Difference (LSD) procedure

was used for the discrimination of means with a confidence level of 95%. Statgraphics and Excel were used to interpret the results.

3. Results

The analysis of variance showed statistically significant differences in yield, mean weight, total soluble solids, and total acidity for each of the years. In terms of total antioxidant activity, differences were only found in 2022 in the DPPH method. In the case of total phenols, no differences were found in any of the years (Table 3 and Figures 1–3).

Table 3. *p*-values of the one-factor GML analyses of variance, with genotypes as a factor. Significance levels: ns ($p > 0.05$), * ($p < 0.05$), and *** ($p < 0.001$). The mean and groups obtained by Fisher's LSD tests are included (values followed by the same letter are not significantly different at 5% level by Fisher). Units are in kg/plant for yield, g/fruit for mean weight, °Brix for total soluble solids, and % citric acid/100 g fresh tissue for titratable acidity. ABTS and DPPH are obtained in mmol Trolox/kg dry matter. Total polyphenol is in mg eq gallic acid/100 g dry matter.

Year		Yield	Fruit Weight	TSS	TA	TAA		TPCs
						ABTS	DPPH	
				GML Test				
2022	*p*-value	***	***	***	***	ns	*	ns
2023	*p*-value	***	***	*	***	ns	ns	ns
				Fisher's Multiple Range Test				
2022	**Genotype**							
	175 RR rr	0.80 a	37.69 abc	6.05 d	0.37 b	21.70	10.46 a	860.4
	175 ss SS	2.05 c	45.06 de	5.92 cd	0.43 d	20.60	14.72 abc	814.1
	175 ss rr	1.36 b	33.50 a	5.73 bc	0.45 e	20.30	10.71 a	817.8
	175 RR SS	1.20 ab	36.50 ab	5.74 bc	0.32 a	19.70	13.73 ab	836.8
	220 RR rr	1.48 b	39.81 bcd	5.59 ab	0.30 a	22.60	12.63 ab	835.3
	220 ss SS	2.40 c	50.76 f	5.47 a	0.40 c	19.10	14.75 abc	807.2
	220 ss rr	2.02 c	42.85 cde	5.77 bc	0.40 c	21.40	15.91 bc	812.2
	220 RR SS	2.09 c	45.56 ef	5.47 a	0.31 a	20.30	18.27 c	850.0
2023	**Genotype**							
	175 RR rr	1.43 a	46.45 ab	5.09 abc	0.32 b	18.75	13.90	827.3
	175 ss SS	2.46 b	57.13 c	5.16 bc	0.40 d	17.10	15.30	769.2
	175 ss rr	1.63 a	48.08 ab	5.13 bc	0.29 a	20.18	11.64	821.6
	175 RR SS	1.53 a	43.59 a	4.99 ab	0.29 a	19.60	12.30	822.4
	220 RR rr	1.65 a	44.38 a	5.01 ab	0.28 a	20.70	12.10	841.1
	220 ss SS	3.08 c	59.00 c	5.11 bc	0.36 c	19.30	15.10	823.2
	220 ss rr	2.58 b	50.90 b	5.23 c	0.36 c	21.5	14.70	819.5
	220 RR SS	1.82 a	47.81 ab	4.90 a	0.30 a	20.10	11.50	796.4

3.1. Yield and Average Fruit Weight

The behavior of the yield curves and average fruit weight (Figure 1a,b) were similar in the two years, both for the UMH175 and UMH220 families. In the two parameters studied, the ssSS genotype was the most productive in each of the years and lines. In production, the rest of the genotypes of the UMH175 family, containing *Ty-1*, *ty-5*, or both, were included in the same group in the two years, although the results of the genotype that only contains *ty-5* (ssrr) were slightly higher. The same was true for the average fruit weight, with the exception of the ssrr genotype, where the results were lower in 2022. In the case of the UMH220 family, for the production in 2022 and 2023, there were differences between those that carried *Ty-1* (RRrr and RRSS) and those that did not (ssSS and ssrr), differentiating the genotype RRSS corresponding to the year 2022 that was grouped with the genotypes ssSS and ssrr. Comparing the genotypes without *Ty-1* (ssSS and ssrr), the former was more productive because it did not include any introgression. In mean fruit weight, the trend of the genotypes was similar to that of production. In this context, we consider that the trend of the means of the genotypes studied in these two productive traits, in each of the years

and lines, was slightly higher in the genotypes that did not contain *Ty-1* (ssSS and ssrr), although not in all cases.

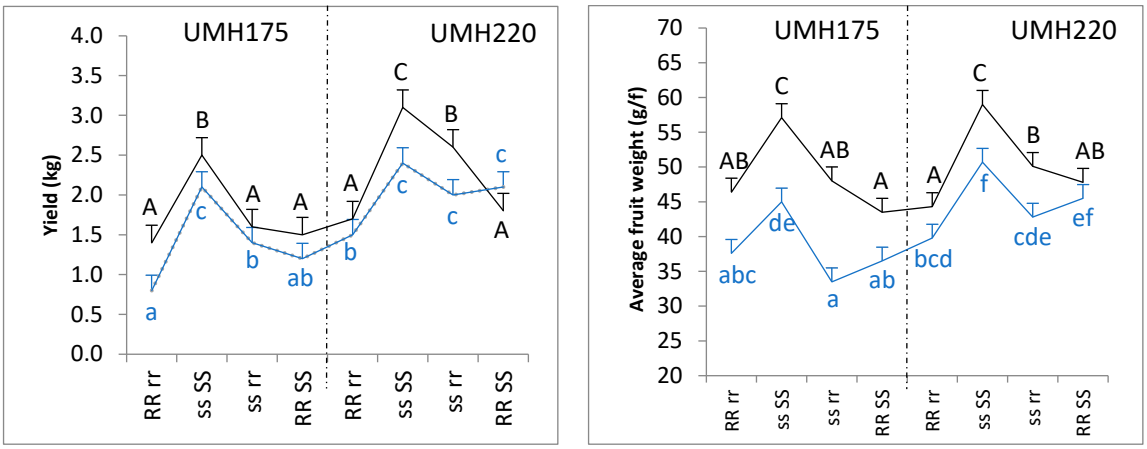

Figure 1. Discrimination of means according to Fisher's LSD test for yield (**a**) and average fruit weight (**b**) in the genotypes [RRrr (*Ty-1*/*ty-5*), ssSS (ssSS), ssrr (ss/*ty-5*), and RRSS (*Ty-1*/SS)] of the two families. The blue curve corresponds to 2022 (with lowercase letters), and the black curve to 2023 (with uppercase letters). Dashed lines separate the two families.

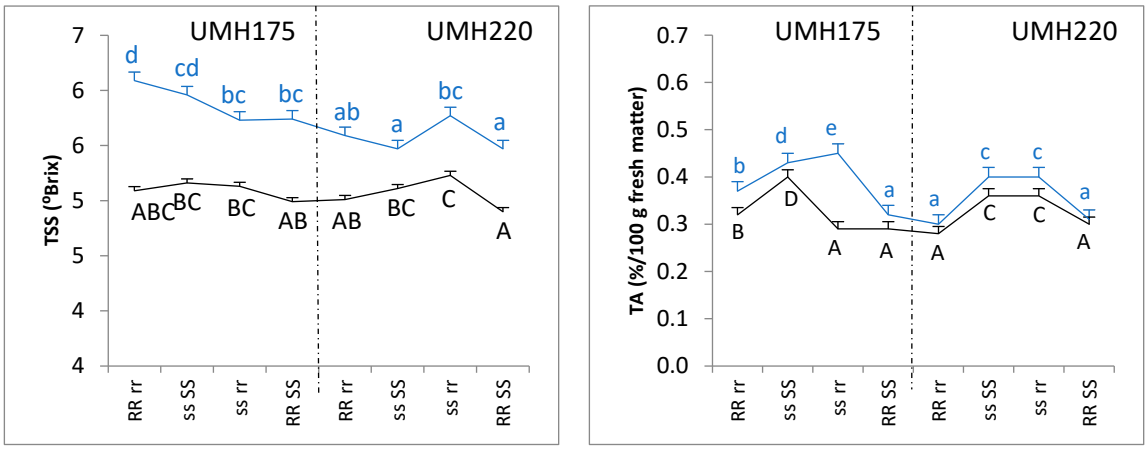

Figure 2. Discrimination of means according to the Fisher LSD test for total soluble solids (**a**) and titratable acidity (**b**) in the genotypes [RRrr (*Ty-1*/*ty-5*), ssSS (ssSS), ssrr (ss/*ty-5*), and RRSS (*Ty-1*/SS)] of the two families. The blue curve corresponds to 2022 (with lowercase letters), and the black curve to 2023 (with uppercase letters). Dashed lines separate the two families.

3.2. Total Soluble Solids and Titratable Acidity

In TSS (Figure 2a), the genotypes did not follow the established pattern in the productive parameters. The curves obtained in the two years were similar, except for genotype RRrr of line 175, which differed between the years, though the difference between the values with the rest of the genotypes was approximately 1 °Brix. Contrary to what occurred in TSS, the results obtained in TA (Figure 2b) showed the same pattern as in the productive traits, although in a clearer way. The genotypes of line 220 that did not contain *Ty-1* (ssSS and ssrr) were statistically different from the genotypes that did contain this allele (RRrr and RRSS), both in 2022 and 2023. The behavior of the genotypes in line 175 was the same in 2022. In 2023, the genotype containing only *ty-5* (ssrr) was included in the same group as the genotypes with *Ty-1* (RRrr and RRSS).

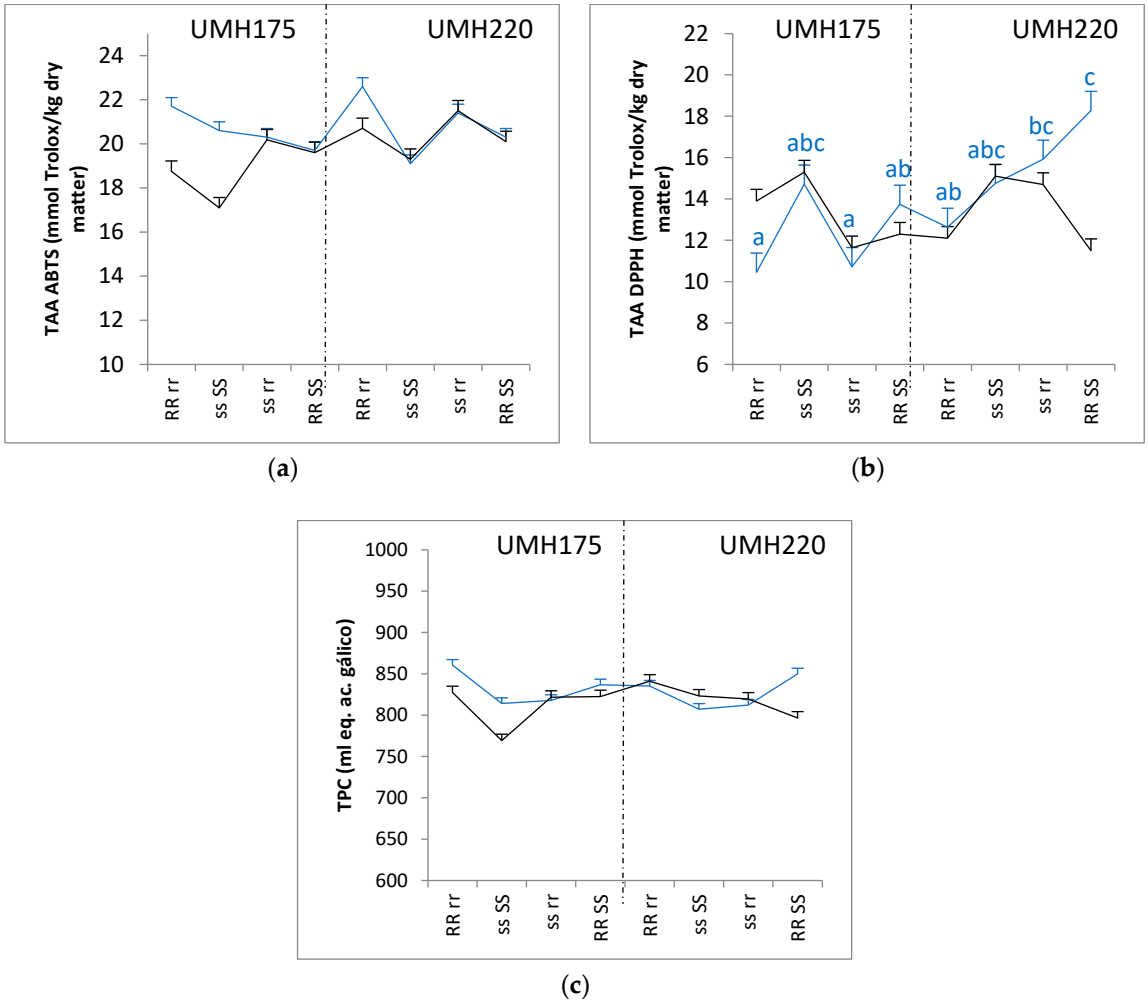

Figure 3. Discrimination of means according to the Fisher LSD test for total antioxidant activity (**a**,**b**) and total phenolic compounds (**c**) in the genotypes [RRrr (*Ty-1*/*ty-5*), ssSS (ssSS), ssrr (ss/*ty-5*), and RRSS (*Ty-1*/SS)] of the two families. The blue curve corresponds to 2022 (with lowercase letters), and the black curve to 2023. Dashed lines separate the two families.

3.3. Total Antioxidant Activity and Total Phenolic Compounds

TAA was analyzed using the ABTS and DPPH methods (Figure 3a,b). In the ABTS method, there were no differences in either of the two years. In the DPPH method, differences were present in 2022 between genotype 220 ssrr and genotypes RRrr and ssrr of line 175. In addition, there were differences between genotype RRSS of line 220 and genotype RRrr of the same line and all genotypes of line 175. In the case of total phenols (Figure 3c), it was observed that there were no differences between genotypes in each of the years.

4. Discussion

Several published papers have highlighted the negative effect of introducing resistance genes due to the genes themselves and/or the linkage load. Tanksley et al. [42] observed slight reductions in the yield and quality of tomatoes for processing with ToMV resistance. Lewis et al. [43] found a reduction in yield and quality in tobacco plants containing the N gene (from the wild species *Nicotiana glutinosa* L.), which confers resistance to TMV. Brouwer and St Clair [44] found that the chromosome fragment of the *S. hirsutum* species conferring resistance to *Phytophthora infestans* contained deleterious alleles in agronomically important traits. Verlaan et al. [32] showed that on a large part of chromosome six of *S. chilense* (where the *Ty-1* gene, which confers resistance to TYLCV, is located), recombination with cultivated tomato is very low due to two chromosomal rearrangements that occurred in

S. chilense. This would make it difficult to eliminate the chromosome of the wild species during backcrossing. To quantify the effect of the introduction of ToMV, TSWV, and TYLCV resistance genes on the yield and quality traits in Muchamiel and De la pera tomatoes, two sets of near-quasi-isogenic lines NIL containing all homozygous combinations for the three resistance genes were developed and evaluated for 3 years. The introduction of genetic resistance to TYLCV was found to produce a decrease in the main productive traits (yield, average weight) ranging from 10% to 50%, depending on the growing conditions and genetic background [31]. In this study, we report that the genotypes evaluated show that BC5S2 containing *Ty-1*, regardless of whether it contains *ty-5* or not (RRrr and RRSS), produces a negative effect on the production parameters analyzed, yield, and average fruit weight. The highest yields are found in the genotypes susceptible to both genes (ssSS), and in the absence of introgression, there is no negative effect. The BC5S2 of the UMH220 line containing only *ty-5* (ssrr) produces a slight negative effect since the results are more similar to those obtained by the susceptible genotype (ssSS). In the opposite case, the BC5S2 ssrr of the UMH175 line, the results are more similar to those obtained by the genotypes containing *Ty-1* (RRrr and RRSS). In a preliminary study carried out during the spring cycle of 2021 with the same lines, 175 and 220, although with a smaller number of plants, we concluded that the introgression of the *ty-5* gene did not produce a negative effect on production in the lines studied, unlike the *Ty-1* gene [45]. In this case, the results of the UMH175 line differ from those obtained in the preliminary study of 2021, although the results obtained by the UMH220 line are similar. In reference to the quality parameters, *Ty-1* introgression also has a negative effect on AT, while *ty-5* introgression does not, although this is clearer in the UMH220 line, in contrast to TSS, where introgressions are not decisive in any case. These results are similar to those obtained by Rubio et al. [31], who, in addition to yield, studied these two parameters, where the effect of *Ty-1* introgression was not decisive for TSS but was for TA. For total antioxidant activity, both for the ABTS and DPPH methods and for total phenols, the mean values obtained (taking into account the 6.8% dry matter obtained) are similar to those shown by Lipan et al. in 2021 [46]. These authors studied a cherry tomato variety by applying deficit irrigation (RDI), where a saving of 53% of water with the RDI of tomatoes presented, in general, a greater weight, size, TSS, sugars, antioxidant activity, lycopene, β-Carotene, and redder color with a more intense tomato flavor. We demonstrate that neither *Ty-1* nor *ty-5* introgression is a determinant. Although differences can be observed between some genotypes in 2022 for the DPPH method, when comparing the genotypes of the UMH175 family with each other, there are no differences, while when comparing those of the UMH220 family, the differences are only observed between the RRrr and RRSS genotypes.

5. Conclusions

Based on the results obtained in this study and taking into account the behavior of the genotypes of preliminary studies, we conclude that the introgression of the *ty-5* allele produces a slightly negative effect on yield, average fruit weight, total soluble solids, and total acidity. This is in contrast to *Ty-1*, which produces a large negative effect. In terms of total antioxidant activity and total phenols, the introgression of both the *Ty-1* and *ty-5* genes in the De la pera breeding lines studied does not produce a negative effect, as all the genotypes behave in a similar way to the susceptible genotype (ssSS) for both genes. *ty-5* is a promising gene for use in breeding programs, as it shows only a slight negative effect in introgression, as well as offering a high level of resistance, further accentuated in combination with *Ty-1* (data to be published). The data obtained from other preliminary studies (data to be published) with the same lines and combinations of *Ty-1* and *ty-5*, but recombinant to *Ty-1*, also showed good resistance behavior, so the results are promising.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/agriculture14071192/s1, Supplementary Figure S1. Greenhouse located at the Escuela Politécnica Superior de Orihuela (EPSO-UMH), Orihuela, Spain (38.06782;

−0.98232). Figure S2. Initial crop development in 2022. Figure S3. Advanced crop development in 2022. Figure S4. Plants at an advanced stage of cultivation in 2023.

Author Contributions: Conceptualization, J.Á.C., J.J.R. and S.G.-M.; methodology, J.Á.C., P.C., J.F.S., A.G., F.H. and A.A.; software, J.Á.C.; formal analysis, J.Á.C. and F.H.; data curation, J.Á.C. and S.G.-M.; writing—original draft preparation, J.Á.C.; writing—review and editing, J.Á.C., J.J.R. and S.G.-M. All authors have read and agreed to the published version of the manuscript.

Funding: J.Á.C. has the FPU18/01399 contract of the "Programa estatal de promoción del talento y su empleabilidad, Subprograma de Formación de Profesorado Universitario (FPU)", of the Ministry of Universities of Spain.

Institutional Review Board Statement: Not applicable.

Data Availability Statement: Data are available upon request from the authors.

Acknowledgments: The authors thank Javier Vives Solbes for his technical support in the laboratory.

Conflicts of Interest: The authors declare no conflicts of interest.

References

1. Shahriari, Z.; Su, X.; Zheng, K.; Zhang, Z. Advances and Prospects of Virus-Resistant Breeding in Tomatoes. *Int. J. Mol. Sci.* **2023**, *24*, 15448. [CrossRef]
2. Anbinder, I.; Reuveni, M.; Azari, R.; Paran, I.; Nahon, S.; Shlomo, H.; Chen, L.; Lapidot, M.; Levin, I. Molecular dissection of *Tomato leaf curl virus* resistance in tomato line TY172 derived from *Solanum peruvianum*. *Theor. Appl. Genet.* **2009**, *119*, 519–530. [CrossRef] [PubMed]
3. Vasudeva, R.S.; Sam Raj, J. A leaf curl disease of tomato. *Phytopatology* **1948**, *38*, 364–369.
4. Cohen, S.; Harpaz, I. Periodic, rather than continual adquisition of new tomato virus by its vector, the tobacco whitefly (*Bemisia tabaci* Gennadius). *Entomol. Exp. Appl.* **1964**, *7*, 155–166. [CrossRef]
5. Picó, B.; Díez, M.J.; Nuez, F. Viral diseases causing the greatest economic losses to the tomato crop. II. The Tomato yellow leaf curl virus—A review. *Sci. Hortic.* **1996**, *67*, 151–196. [CrossRef]
6. Moriones, E.; Arnó, J.; Accotto, G.P.; Noris, E.; Cavallarin, L. First report of Tomato yellow leaf curl virus in Spain. *Plant Dis.* **1993**, *77*, 953. [CrossRef]
7. Reina, J.; Jiménez, J.; Bejarano, E.R.; Guerra, J.M.; Cuadrado, I.M.; García, C. El virus del rizado amarillo del tomate (TYLCV). *Hortofruticultura* **1994**, *6*, 36–40.
8. Navas-Castillo, J.; Sanchez-Campos, S.; Díaz, J.A.; Sáez-Alonso, E.; Moriones, E. First report of Tomato yellow leaf curl virus-Is in Spain: Coexistence of two different geminiviruses in the same epidemic outbreak. *Plant Dis.* **1997**, *81*, 1461. [CrossRef]
9. Pilowsky, M.; Cohen, S. Tolerance to Tomato yellow leaf curl virus derived from *L. peruvianum*. *Plant Dis.* **1990**, *74*, 248–250. [CrossRef]
10. Friedmann, M.; Lapidot, M.; Cohen, S.; Pilowsky, M. A novel source of resistance to Tomato yellow leaf curl virus exhibiting a symptomless reaction to viral infection. *J. Am. Soc. Hortic. Sci.* **1998**, *123*, 1004–1007. [CrossRef]
11. Kalloo, G.; Banerjee, M.K. Transfer of Tomato leaf curl virus resistance from *Lycopersicon hirsutum* f. *glabratum* to *L. esculentum*. *Plant Breed.* **1990**, *105*, 156–159. [CrossRef]
12. Kasrawi, M.A.; Suwwan, M.A.; Mansour, A. Sources of resistance to Tomato-yellow-leaf-curl-virus (TYLCV) in *Lycopersicon* species. *Euphytica* **1988**, *37*, 61–64. [CrossRef]
13. Laterrot, H. Resistance genitors to Tomato yellow leaf curl virus (TYLCV). *Tomato Leaf Curl. Nswl.* **1992**, *1*, 2–4.
14. Scott, J.W.; Stevens, M.R.; Barten, J.H.M.; Thome, C.R.; Polston, J.E.; Schuster, D.J.; Serra, C.A. Introgression of resistance to whitefly-transmitted geminiviruses from *Lycopersicon chilense* to tomato. In *Bemisia: 1995 Taxonomy, Biology, Damate, Control and Management*; Gerling, D., Mayer, R.T., Eds.; Intercept Ltd.: Andover, UK, 1995; pp. 357–367.
15. Vidavsky, F.; Czosnek, H. Tomato breeding lines resistant and tolerant to tomato yellow leaf curl virus issued from *Lycopersicon hirsutum*. *Phytopathology* **1998**, *88*, 910–914. [CrossRef] [PubMed]
16. Zamir, D.; Michelson, I.E.; Zakay, Y.; Navot, N.; Zeidan, M.; Sarfatti, M.; Eshed, Y.; Harel, E.; Pleban, T.; van Oss, H.; et al. Mapping and introgression of a tomato yellow leaf curl virus tolerance gene, *Ty-1*. *Theor. Appl. Genet.* **1994**, *88*, 141–146. [CrossRef] [PubMed]
17. Hanson, P.M.; Bernacchi, D.; Green, S.; Tanksley, S.D.; Muniyappa, V.; Padmaja, A.S.; Chen, H.; Kuo, G.; Fang, D.; Chen, J. Mapping a Wild Tomato Introgression Asociated with Tomato Yellow Leaf Curl Virus Resistance in a Cultivated Tomato Line. *J. Am. Soc. Hortic. Sci.* **2000**, *125*, 15–20. [CrossRef]
18. Dueñas, F.; Martínez, Y.; Álvarez, M.; Moya, C.; Peteira, B.; Arias, Y.; Diez, M.J.; Hanson, P.; Shagarodsky, T. Caracterización agromorfológica y evaluación de la resistencia al TYLCV en nuevos genotipos de tomate (*Solanum lycopersicum* L.) como apoyo al programa de mejoramiento genético de la hortaliza para la enfermedad. *Cultiv. Trop.* **2008**, *29*, 53–60.
19. Hanson, P.M.; Green, S.K.; Kuo, G. *Ty-2*, a gene on chromosome 11 conditioning geminivirus resistance in tomato. In *Report of the Tomato Genetics Cooperative*; Scott, J.W., Ed.; University of Florida: Wimauma, FL, USA, 2006; Volume 56, pp. 17–18.

20. Ji, Y.; Scott, J.W.; Hanson, P.; Graham, E.; Maxwell, D.P. Sources of resistance, inheritance, and location of genetic loci conferring resistance to members of the tomatoinfecting begomoviruses. In *Tomato Yellow Leaf Curl Virus Disease: Management, Molecular Biology, Breeding for Resistance*; Henryk, C., Ed.; Springer: Dordrecht, The Netherlands, 2007; pp. 343–362.
21. Ji, Y.; Schuster, D.J.; Scott, J.W. *Ty-3*, a begomovirus resistance locus near the *Tomato yellow leaf curl virus* resistance locus *Ty-1* on chromosome 6 of tomato. *Mol. Breed.* **2007**, *20*, 271–284. [CrossRef]
22. Verlaan, M.G.; Hutton, S.F.; Ibrahem, R.M.; Kormelink, R.; Visser, R.G.; Scott, J.W.; Edwards, J.D.; Bai, Y. The Tomato Yellow Leaf Curl Virus Resistance Genes *Ty-1* and *Ty-3* are Allelic and Code for DFDGD-Class RNA-Dependent RNA Polymerases. *PLoS Genet.* **2013**, *9*, e1003399. [CrossRef]
23. Ji, Y.; Scott, J.W.; Schuster, D.J. Toward Fine Mapping of the Tomato Yellow Leaf Curl Virus Resistance Gene *Ty-2* on Chromosome 11 of Tomato. *HortScience* **2009**, *44*, 614–618. [CrossRef]
24. Hutton, S.F.; Scott, J.W.; Schuster, D.J. Recessive Resistance to *Tomato yellow leaf curl virus* from the Tomato Cultivar Tyking Is Located in the Same Region as *Ty-5* on Chromosome 4. *HortScience* **2012**, *47*, 324–327. [CrossRef]
25. Lapidot, M.; Karniel, U.; Gelbart, D.; Fogel, D.; Evenor, D.; Kutsher, Y.; Makhbash, Z.; Nahon, S.; Shlomo, H.; Chen, L.; et al. A Novel Route Controlling Begomovirus Resistance by the Messenger RNA Surveillance Factor Pelota. *PLoS Genet.* **2015**, *11*, e1005538. [CrossRef] [PubMed]
26. Hutton, S.F.; Scott, J.W. *Ty-6*, a major begomovirus resistance gene located on chromosome 10. In *Report of the Tomato Genetics Cooperative*; Scott, J.W., Ed.; University of Florida: Wimauma, FL, USA, 2014; Volume 64, pp. 14–18.
27. Scott, J.W.; Hutton, S.F.; Freeman, J.H. Fla. 8638B and Fla. 8624 Tomato Breeding Lines with Begomovirus Resistance Genes *ty-5* Plus *Ty-6* and *Ty-6*, respectively. *HortScience* **2015**, *50*, 1405–1407. [CrossRef]
28. Prasanna, H.C.; Sinha, D.P.; Rai, G.K.; Krishna, R.; Kashyap, S.P.; Singh, N.K.; Singh, M.; Malathi, V.G. Pyramiding *Ty-2* and *Ty-3* genes for resistance to monopartite and bipartite tomato leaf curl viruses of India. *Plant Pathol.* **2014**, *64*, 256–264. [CrossRef]
29. Elbaz, M.; Hanson, P.; Fgaier, S.; Laarif, A. Evaluation of tomato entries with different combinations of resistance genes to tomato yellow leaf curl disease in Tunisia. *Plant Breed.* **2016**, *135*, 525–530. [CrossRef]
30. Al-Shihi, A.A.; Peter, H.; Al-Sadi, A.M.; Al-Yahyai, R.A.; Briddon, R.W.; Deadman, M.; Shahid, M.S. Evaluation of tomato inbred lines for resistance to the tomato yellow leaf curl disease complex in Oman. *Crop Prot.* **2018**, *110*, 91–98. [CrossRef]
31. Rubio, F.; Alonso, A.; García-Martínez, S.; Ruiz, J.J. Introgresion of virus-resistance genes into traditional tomato varieties (*Solanum lycopersicum* L.): Effects on yield and quality. *Sci. Hortic.* **2016**, *198*, 183–190. [CrossRef]
32. Verlaan, M.G.; Szinay, D.; Hutton, S.F.; de Jong, H.; Visser, R.G.F.; Scott, J.W.; Bai, Y. Chromosomal rearrangements between tomato and *Solanum chilense* hamper mapping and breeding of the TYLCV resistance gene *Ty-1*. *Plant J.* **2011**, *68*, 1093–1103. [CrossRef] [PubMed]
33. Cabrera, J.A.; Carbonell, P.; Salinas, J.F.; Alonso, A.; Grau, A.; Ruiz, J.J.; García-Martínez, S. Diseño de marcadores moleculares ligados al gen *Ty-1* para la búsqueda de individuos recombinantes que hayan perdido parte de la carga de ligamiento asociada al gen *Ty-1*. In *Libro de Resúmenes del III Congreso Universitario en Innovación y Sostenibilidad Agroalimentaria*; LIMENCOP: Orihuela, Spain, 2022.
34. García-Martínez, S.; Grau, A.; Alonso, A.; Rubio, F.; Valero, M.; Ruiz, J. UMH 1203, a Multiple Virus-resistant Fresh-market Tomato Breeding Line for Open-field Conditions. *HortScience* **2012**, *47*, 124–125. [CrossRef]
35. Perez de Castro, A.; Blanca, J.M.; Díez, M.J.; Nuez Viñals, F. Identification of a CAPS marker tightly linked to the Tomato yellow leaf curl disease resistance gene *Ty-1* in tomato. *Eur. J. Plant Pathol.* **2007**, *117*, 347–356. [CrossRef]
36. García-Martínez, S.; Sánchez, C.; Castelló, J.; Grau, A.; Valero, M.; Ferrández, A.; Ruiz, J.J. Empleo de marcadores moleculares para la introducción múltiple de genes de resistencia a virosis (ToMV, TSWV y TYLCV) en variedades tradicionales de tomate alicantinas. *Agrícola Vergel.* **2003**, *255*, 140–143.
37. Robles-Sánchez, R.M.; Rojas-Graü, M.A.; Odriozola-Serrano, I.; González-Aguilar, G.A.; Martín-Belloso, O. Effect of minimal processing on bioactive compounds and antioxidant activity of fresh-cut 'Kent'mango (*Mangifera indica* L.). *Postharvest Biol. Technol.* **2009**, *51*, 384–390. [CrossRef]
38. Tabart, J.; Kevers, C.; Pincemail, J.; Defraigne, J.O.; Dommes, J. Comparative antioxidant capacities of phenolic compounds measured by various tests. *Food Chem.* **2009**, *113*, 1226–1233. [CrossRef]
39. Brand-Williams, W.; Cuvelier, M.E.; Berset, C. Use of a free radical method to evaluate antioxidant activity. *LWT Food Sci. Technol.* **1995**, *28*, 25–30. [CrossRef]
40. Re, R.; Pellegrini, N.; Proteggente, A.; Pannala, A.; Yang, M.; Rice-Evans, C. Antioxidant activity applying an improved ABTS radical cation decolorization assay. *Free Radic. Biol. Med.* **1999**, *26*, 1231–1237. [CrossRef] [PubMed]
41. Singleton, V.L.; Orthofer, R.; Lamuela-Raventos, R.M. Analysis of total phenols and other oxidation substrates and antioxidants by means of folin-ciocalteu reagent. *Methods Enzymol.* **1999**, *299*, 152–178. [CrossRef]
42. Tanksley, S.D.; Bernachi, D.; Emmatty, D.; Eshed, Y.; Inai, S.; Lopez, J.; Petiard, V.; Sayama, H.; Uhlig, J.; Zamir, D. Yield and quality evaluationson a pair of processing tomato lines nearly isogenic for the *Tm2a* gene forresistance to the tobacco mosaic virus. *Euphytica* **1998**, *99*, 77–83. [CrossRef]
43. Lewis, R.S.; Linger, L.R.; Wolff, M.F.; Wernsman, E.A. The negative influence of N-mediated TMV resistance on yield in tobacco: Linkage drag versus pleiotropy. *Theor. Appl. Genet.* **2007**, *115*, 169–178. [CrossRef]
44. Brouwer, D.J.; St Clair, D.A. 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*, 628–638. [CrossRef]

45. Cabrera, J.A.; Carbonell, P.; Salinas, J.F.; Alonso, A.; Grau, A.; García-Martínez, S.; Pérez de Castro, A.; Díez, M.J.; Ruiz, J.J. Estudio preliminar de la resistencia al virus del rizado amarillo del tomate en líneas de mejora de tomate De la pera. In *Libro de Resúmenes del IX Congreso Ibérico y XVII Congreso Nacional de Ciencias Hortícolas*; SECH: Mérida, Spain, 2023.
46. Lipan, L.; Issa-Issa, H.; Moriana, A.; Zurita, N.M.; Galindo, A.; Martín-Palomo, M.J.; Andreu, L.; Carbonell-Barrachina, Á.A.; Hernández, F.; Corell, M. Scheduling Regulated Deficit Irrigation with Leaf Water Potential of Cherry Tomato in Greenhouse and its Effect on Fruit Quality. *Agriculture* **2021**, *11*, 669. [CrossRef]