diversity

MDPI

Review

Twenty Years of Tomato Breeding at EPSO-UMH: Transfer Resistance from Wild Types to Local Landraces—From the First Molecular Markers to Genotyping by Sequencing (GBS)

Pedro Carbonell, Aranzazu Alonso, Adrián Grau, Juan Francisco Salinas, Santiago García-Martínez and Juan José Ruiz *

Department of Applied Biology, Miguel Hernández University, Carretera Beniel km 3,2, 03312 Orihuela, Alicante, Spain; pcarbonell@umh.es (P.C.); aalonso@umh.es (A.A.); agrau@umh.es (A.G.); salinas.kiter.92@gmail.com (J.F.S.); sgarcia@umh.es (S.G.-M.)

* Correspondence: juanj.ruiz@umh.es; Tel.: +34-096-674-9615

Received: 8 November 2017; Accepted: 25 February 2018; Published: 27 February 2018

Abstract: In 1998, the plant breeding team at the School of Engineering of Orihuela (EPSO), part of the Miguel Hernández University (UMH) in Elche, commenced a tomato breeding program. Marker-assisted selection and backcrossing were used to simultaneously introduce three genes (*Tm-2a*, *Ty-1*, and *Sw-5*) that confer resistance to relevant viruses, such as tomato mosaic virus (ToMV), tomato yellow curl virus (TYLCV), and tomato spotted wilt virus (TSWV), to traditional varieties of local tomatoes, specifically the "Muchamiel" and the "De la pera" types. After each backcross, cleaved amplified polymorphic sequence (CAPS) molecular markers were used to select the plants with the resistance genes of interest. A previously described marker was used for TSWV, and new markers were designed for ToMV, and TYLCV using available sequences in the National Center for Biotechnology Information (NCBI) database. In parallel to the breeding program, several molecular markers—Sequence Related Amplified Polymorphism (SRAP), Simple Sequence Repeats (SSRs), Amplified Fragment Length Polymorphisms (AFLPs), Single Nucleotide Polymorphisms (SNPs), and $(GATA)_4$ probes—were used to study genetic variability, and to identify a collection of Spanish and Italian traditional tomato varieties. The results showed a limited genetic variability among cultivated tomato varieties. The breeding lines Muchamiel UMH 1200, and De la pera 1203 (both with homozygous resistance to the three viruses) were the first new varieties that were obtained. They were included in the Register of Protected Plant Varieties in 2013. Lines without a resistance to TYLCV were also developed, and protected in 2017. We have begun to use SNP massive genotyping for studies of genetic association, and for selecting plants with the *Ty-1* gene with less linkage drag. Molecular markers have been extremely useful in identifying the different steps of the tomato breeding program at EPSO-UMH.

Keywords: Muchamiel; De la pera; ToMV; TYLCV; TSWV

1. Introduction

Over the years, traditional agriculture has produced an enormous range of local plant varieties that have been adapted to specific environmental conditions and for local uses and preferences. With no knowledge of genetics or statistics, farmers have created new varieties by saving the seeds from the best plants for the following year. These plants have been selected for their organoleptic qualities (including flavor, aroma and texture), their adaptation to the local environment (resistance to frost and drought, proper fruit set, etc.), and their suitability for different local uses (such as fresh consumption, canning, and drying). In southeastern Spain, there are a number of local tomato varieties, including

the "Muchamiel" from Alicante, the "Tres cascos" from Elche, the "De la pera" from the Vega Baja del Segura region of Alicante, the "Valenciano" from Valencia, the "Flor de Baladre" from Murcia, and the "Morunos" from different regions. The Muchamiel and the De la pera, which have traditionally been cultivated in the province of Alicante, are particularly esteemed for their exceptional organoleptic quality. In local markets, they can be sold at a price that is six times higher than that of hybrid varieties [1].

Intensive farming, however, requires varieties that are consistently and highly productive in different systems and cycles, that are exceptionally uniform in size, shape and color, and that show a certain level of resistance to the different infestations, diseases and disorders that can affect the crop. Traditional varieties tend to fall short in at least one of these categories.

In fact, many traditional tomato varieties in southeastern Spain are endangered because of their high susceptibility to the three main viruses affecting tomatoes in the area: tomato mosaic virus (ToMV), tomato yellow leaf curl virus (TYLCV), and tomato spotted wilt virus (TSWV) [2]. Because of these viruses, in addition to other factors, traditional tomatoes are less attractive to farmers than modern hybrids, which are resistant to these viruses and are also far more productive. Yet, giving up on the traditional varieties could result in an irreversible loss of vital genetic variability [3]. For economic reasons, improving upon varieties with a limited market share is not a priority for seed companies and therefore should be undertaken by public institutions [4].

2. Genetic Variability Studies in Tomato

Morphological traits (such as the shape, size, and color of the different parts of the plant), and agronomical traits (such as yield, the ability to set in adverse conditions, and resistance to different biotic and abiotic stresses) are often very useful to distinguish traditional and commercial tomato varieties, even by visual inspection. However, there are other traits that can only be detected by using analytical methods. This is the case for the levels of different key components (such as sugars, acidity, or microelements), and the volatile compounds responsible for aroma. To study these traits, which are subtler than the more obvious differences, it is often necessary to use a specific methodology. Despite the fact that these subtle differences (such as fruit composition) are more difficult to see, they are, nevertheless, equally as important as the more obvious differences.

We already know that cultivated tomatoes have a narrow genetic base [5]. This narrow genetic base is probably due to the bottleneck effect that occurred during tomato domestication [5]. Based on estimates made with DNA markers, the relative species of wild tomatoes are much more variable than their cultivated counterpart at the whole genome level. It is estimated that the genomes of tomato cultivars contain <5% of the genetic variation of their wild counterparts [5]. In other words, cultivated tomatoes vary tremendously in their fruit size, shape, color, firmness, soluble solids, volatile aroma content, and acidity, but have little genetic variation elsewhere in their genome [6,7]. In one study, notable differences in parameters, such as flavor, texture and micronutrient content, between the traditional Muchamiel and De la pera varieties, and a modern F1 hybrid, were found [8]. Four years later, different aromatic profiles in Muchamiel accessions, De la pera accessions, and a F1 hybrid, were also found [9].

3. Molecular Markers in Variability Studies

Despite these previous findings and observations, it had not been possible to distinguish the phenotypic variation among the characterized accessions at the genetic level. For this reason, in parallel to the breeding program, our group began to study variability using different molecular markers, which would make it possible to distinguish between the different traditional varieties and to coherently organize them according to genotype. Our group believed this would be an informative and exceptionally powerful approach in the breeding program. Tomato was one of the first crops in which molecular markers were used to study genetic variability. In the genetic variability studies of tomato, a number of DNA markers, such as SSR [10,11] and AFLP [12], were successfully used. SSR [13,14] and $(GATA)_4$ probes [15,16] were also successfully used for variety identification.

Our group has performed a range of variability studies, mostly using accessions from closely related traditional European tomato varieties. In these studies, we have been able to confirm the efficacy of certain molecular markers in differentiating between varieties, and in grouping similar varieties together.

The results of the different marker analysis studies that we have carried out are detailed below, and are summarized in Table 1.

Table 1. Markers used in the genetic variability studies.

Marker	Number	Number of Bands	% of Polymorphic Bands	Usability
SRAP	26	384	60	Distinguish between cultivar types, wild relatives and 14 of 16 traditional cultivars studied
SSR	10 + 9	77	98	Distinguish between cultivar types, wild relatives and 23 of 34 traditional cultivars studied
AFLP	7	470	40	Distinguish between cultivar types, wild relatives and 24 of 31 traditional cultivars studied
$(GATA)_4$	N.A.	30	100	Distinguish between cultivar types, Spanish traditional cultivars and 4 of 10 Italian traditional cultivars studied
SNP	41	N.A.	76	Distinguish traditional cultivars from modern cultivars, hybrids and wild relatives

N.A.: Not applicable.

3.1. Sequence Related Amplified Polymorphism (SRAP)

The SRAP technique is based on an amplified polymerase chain reaction (PCR), and it is designed to detect polymorphisms in the DNA sequence [17].

In 2005, we carried out a variability study using SRAP and single sequence repeat (SSR) markers in traditional Spanish varieties, including the Muchamiel, the De la pera, the Moruno, commercial hybrids, and wild tomato species [18]. A total of 26 not specific for tomato primers were used. These primers made it possible to identify polymorphisms between the main varieties, and to distinguish between cultivar types and the wild relatives under study (De la pera, Muchamiel, Moruno, hybrids, *S. pimpinellifolium*, *S. chilense* and *S. peruvianum*). It was even possible to differentiate between the different accessions within each group. Although the SRAP markers are dominant and not very polymorphic, they have significant coverage throughout the genome and produce a more in-depth variability study than the SSRs used in the same analysis. SRAP, for example, made it possible to distinguish between the Mexican variety of Zapotec tomato and the Spanish varieties, which SSRs were unable to do.

3.2. Simple Sequence Repeat (SSR)

SSR markers, or microsatellites, are short tandem repeats in DNA sequences, mostly between two and four base pairs (bp) [19]. These markers are highly polymorphic because the repeated sequences vary significantly between the different genotypes.

In the study mentioned above [18], our group used 10 previously selected microsatellites for tomato. With only three SSRs, we were able to differentiate between the three main types of cultivated tomatoes under study (Muchamiel, De la pera and Moruno). Nevertheless, despite using 10 SSRs, we were unable to differentiate between the different cultivars within each varietal type, which could be done using SRAP. SSR markers are highly polymorphic, but they are concentrated in very specific loci within the genome, and they have very little genomic coverage. As a result, we were unable to find polymorphisms between highly similar accessions.

In a second study, we used up to 19 microsatellites selected for tomato, but we were unable to distinguish between all of the cultivars evaluated, even though there were clear phenotypical

differences. Nevertheless, only four SSRs were necessary to differentiate between the three main varietal groups (Muchamiel, De la pera, and Moruno) [20].

The results of both studies demonstrate the minimal genetic variability among cultivated tomato varieties.

3.3. Amplified Fragment Length Polymorphisms (AFLPs)

AFLPs are molecular markers used to detect polymorphisms in the DNA sequence. After the restriction enzyme digestion of DNA, certain fragments are selected for amplification [21].

In a variability study conducted by our group [20], the obtained results were compared using 19 microsatellites and seven combinations of AFLP markers. The AFLPs made it possible to distinguish between the main varietal groups, although out of the 43 accessions studied, there were seven that could not be identified with certainty, and they were all traditional Spanish varieties. Using the SSR analysis, 11 accessions could not be differentiated, and, once again, they were all Spanish varieties. Curiously, the seven accessions that could not be identified using AFPLs were different from the 11 in the SSR analysis. This means that all of the cultivars used in the study could be identified using a combination of both markers.

Once again, the results showed the limited genetic variability among cultivated tomato varieties.

3.4. $(GATA)_4$ Probes

Labelled $(GATA)_4$ probes are molecular markers based on the hybridization of a short sequence (16 nucleotides, with the GATA motif repeated four times), which hybridizes when it finds a complementary sequence [22]. The $(GATA)_4$ technique was developed in 1995, and it has been used to successfully distinguish between varietal types, and also between different accessions [15,16,23].

In 2013, researchers at UMH and the *Università degli Studi di Napoli Federico II* (Naples, Italy) performed a variability study of traditional Spanish and Italian varieties, including accessions from the Muchamiel, and the De la pera type tomatoes, and the Italian varieties San Marzano, and Sorrento [24]. The $(GATA)_4$ probes could clearly detect differences between all of the Spanish accessions studied, but not the Italian accessions. Furthermore, the researchers found polymorphisms between different plants within a single accession in 14 of the 26 accessions studied (in a previous study, SSRs were only able to detect polymorphisms in two out of the 16 accessions studied). This level of polymorphism is not unusual, given that small-scale farmers have not traditionally considered crop uniformity as an essential factor in their selection process.

The results of this study confirmed that $(GATA)_4$ probes are a powerful tool for studying variability between closely related tomato accessions. The markers are able to distinguish between plants at a much deeper level than SSRs, SRAPs, and AFLPs.

3.5. Single Nucleotide Polymorphism (SNP)

A SNP is a variation in a single nucleotide in the DNA sequence [25]. SNPs are the most abundant polymorphisms in the genome, making them highly useful in both genetic variability and phylogenetic studies.

Our group studied 41 SNPs, obtained from expressed sequence tags (ESTs), in several different tomato varieties, including traditional varieties (mostly Spanish), commercial hybrids, and wild tomato varieties [26]. These markers were selected because they were readily detected on standard agarose or polyacrylamide gels, which was the equipment available in our laboratory at that time. This study found that it was not possible to differentiate between all of the traditional cultivars, although the researchers were able to distinguish between wild accessions and hybrid varieties.

The level of polymorphism found in this SNP study was therefore lower than the levels found in previous studies on the Muchamiel, De la pera, and Moruno varieties using SSRs, AFLPs, and SRAPs. This was perhaps due to the fact that the SNPs were obtained from ESTs, which are coding regions of DNA that show fewer mutations than non-coding regions and where it is typical to find more

variability. Perhaps it would have been more interesting to study SNPs that are outside functional genes, which are highly polymorphic regions [27].

4. Traditional Tomato Variety Breeding Program

In 1998, the plant breeding team at the School of Engineering of Orihuela (EPSO), part of the Miguel Hernández University (UMH) in Elche, commenced a breeding program using marker-assisted backcrossing in order to simultaneously introduce genes that confer resistance to the three most relevant viruses found in traditional local tomato varieties, specifically in the Muchamiel and the De la pera types. We have used the genes *Tm-2a*, and *Sw-5*, which come from the wild tomato *Solanum peruvianum* L. [28,29] to confer resistance to ToMV, and TSWW, respectively, and the gene *Ty-1*, which confers resistance to TYLCV and originated in another wild tomato series, the *Solanum chilense* (Dunal) Reiche accession LA1969 [30].

We have performed the following steps in our program: agronomic characterization of the traditional varieties and of the sources of resistance; performance of crossings; performance of backcrossings; fixation of the resistance genes; selection of the best lines; and application for registration in the Spanish Register of Protected Plant Varieties.

As mentioned above, the first step in the breeding program was to characterize and select the most suitable plant material. Since the program aims to help preserve the genetic variability of certain traditional tomato varieties in southeastern Spain, it was essential to fully understand the plant material before proceeding.

5. Molecular Markers in Resistance Gene Introgression

The first step in our breeding program was to characterize different accessions of interest in order to select those that met the minimum quality standards. Based on these characterizations, we selected M18 Muchamiel accession, and P21 De la pera accession to be used as traditional parentals in the program. The next step was to manually cross these accessions with the F1 hybrid Anastasia (Seminis Vegetable Seeds), which is a source of resistance that contains the genes *Tm-2a*, *Sw-5*, and *Ty-1*.

The descendants obtained from the traditional varieties were repeatedly backcrossed with the initial parents in order to recover as much of the genome as possible. After each backcross, cleaved amplified polymorphic sequence (CAPS) molecular markers were used to select the plants with the resistance genes of interest. To do this, it was necessary to use specific markers that would efficiently recognize the presence or absence of these genes.

CAPS markers are short DNA fragments amplified by PCR and later digested by restriction enzymes, which produces a pattern of bands that makes it possible to distinguish between homozygous and heterozygous individuals. The CAPS markers used thus far in the breeding program are detailed below.

High selection pressure for desirable traditional cultivar characteristics (such as shape, and organoleptic quality) and good agronomic behavior (proper fruit set, sufficient uniformity among fruits, and yields) was applied during the backcrossing process. Only the best plants (between one and three per progeny) were selected for further backcrossing.

5.1. CAPS Linked to the Sw-5 Gene

The CAPS markers we used to select individuals resistant to TSWV have been successful thus far in the breeding program [31].

5.2. CAPS Linked to the Ty-1 Gene

The ApsF-2 marker was developed specifically for this breeding program from the isoenzyme marker Aps [32]. In earlier studies and in this breeding program, ApsF-2 has proven to be a suitable marker for confirming TYLCV tolerance.

5.3. CAPS Linked to the Tm-2^a Gene

Initially, we tried to use two previously described CAPS markers [33,34]. After determining that these markers did not work correctly with our material, we designed new CAPS markers, using the allele sequences Tm-2^a (AF536201) and tm-2 (AF536199), which were entered into the National Center for Biotechnology Information (NCBI) database [35].

5.4. Other Examples of Tomato Breeding Programs with MAS (Marker-Assisted Selection)

Tomato was among the first crop species for which genetic markers were suggested as indirect selection criteria for breeding purposes and for which molecular markers and maps were developed [36,37]. Private companies do not disclose the markers that they use. However, there seems to be a considerable use of markers for various purposes, including testing hybrid purity, screening breeding populations for disease resistance, and marker assisted backcross breeding [38]. The use of MAS in tomato breeding in public research institutions is well documented. The breeding programs that use MAS and send their results to the Register, are summarized in Table 2.

Table 2. Public research institutions with tomato breeding programs, coordinators and objectives.

Breeding Program	Coordinators	Traits
University of Florida (USA)	J.W. Scott and S.F. Hutton	*Fusarium oxysporum f.* sp. *lycopersici*, *Verticillium dahliae*, *Stemphyllium solani*, TSWV, TYLCV
University of North Carolina (USA)	R.G. Gardner	*Fusarium oxysporum f.* sp. *lycopersici*, *Verticillium dahliae*, *Alternaria solani*, TSWV
United States Department of Agriculture (ARS)	J.R. Stommel	Beta carotene content
Ohio State University (USA)	M. Francis	*Xanthomonas euvesicatoria*

6. Lines Obtained in the EPSO-UMH Breeding Program

When we consider that the characteristics of the traditional variety have been recovered (after five to eight backcross generations, depending on the line) and the pure-breeding lines have been obtained, selecting the plants that show homozygous resistance due to introgressed genes is necessary. These plants were selected using the three CAPS markers and the progenies were obtained by self-pollination during the last backcross generation.

In 2013, the EPSO-UMH breeding program obtained its first plant variety certificates for the lines UMH 1200 (a Muchamiel-type tomato), and UMH 1203 (De la pera), which both show homozygous resistance to the three viruses of interest (Table 3). The morphological characteristics and quality of these lines are similar to those found in the traditional varieties from which they are derived. Nevertheless, homozygous resistance to TYLCV considerably reduces production (up to 40%), particularly in TYLCV-free conditions, which are possible in greenhouse cultivation [39,40]. This decrease in production is due to the introgressed genes themselves and/or to the linkage drag associated with the resistance genes, particularly the *Ty-1* gene. This problem has been previously described in tomatoes for industrial use [41], in tobacco [42], and in tomatoes for fresh consumption [43].

Table 3. Breeding lines registered in the Register of Protected Plant Varieties, with their genotypes for the three virus resistance genes. RR: resistant homozygote, Rs: resistant heterozygote, ss: sensitive homozygote.

Varietal Type	Line	Resistance	Sent to Registry	Title Obtained
		ToMV-TYLCV-TSWV		
Muchamiel	UMH 1200	RR-RR-RR	2011	2013
Muchamiel	UMH 1139	RR-ss-RR	2013	2017
Muchamiel	UMH 1101xIF	Rs-Rs-Rs	2014	2017
De la pera	UMH 1203	RR-RR-RR	2011	2013
De la pera	UMH 1422	RR-ss-ss	2013	2017
De la pera	UMH 1415	RR-ss-RR	2013	2017
De la pera	UMH 1353	RR-ss-RR	2013	2017
De la pera	UMH 1354	RR-ss-RR	2013	2017

In an attempt to avoid these problems, we have developed lines that are not resistant to TYLCV. Within the Muchamiel type, we have developed the lines UMH 1093, UMH 1127, and UMH 1139, which all have homozygous resistance to ToMV, and TSWV. UMH 1139 obtained a plant variety certificate in 2017. Within the De la pera type, we have developed the line UMH 1422 (with homozygous resistance to ToMV), and the lines UMH 1415, UMH 1353, and UMH 1354 (which all have homozygous resistance to ToMV and TSWV). All of these De la pera lines obtained plant variety certificates in 2017. The morphological characteristics and quality of these lines are similar to those found in the traditional varieties they are derived from, and there is no drop in production like that which occurs in UMH 1200, and in UMH 1203. These lines are therefore of great interest for cultivation purposes in TYLCV-free conditions, which can be achieved in a greenhouse with sufficient control measures, particularly in the spring season, when TYLCV occurs less frequently [44–46].

All of these improved lines possess homozygous resistance genes, and therefore, they can be cultivated by farmers year after year using a rigorous selection process.

In order to grow tomatoes outdoors in an environment with a high incidence rate of TYLCV, it was decided to develop hybrids with a heterozygous resistance to the three main viruses. Previous studies have shown that introducing resistance to TYLCV heterozygously has a reduced negative effect on yield and other traits [47]. The hybrid UMH 1101xIF, a Muchamiel-type tomato, was the first hybrid obtained in our breeding program, and more hybrids (from both Muchamiel and De la pera types) are currently being developed and will be ready to be presented in two years.

7. Massive Genotyping

In the last few years, the genetics team at UMH has performed numerous massive sequencing analyses using the Illumina platform. These analyses have made it possible to sequence a large number of traditional tomato varieties and accessions. In the most notable of these analyses, we use the 8K SolCap Illumina Infinium SNP chip, described as a part of the Solanaceae Coordinated Agricultural Project (SolCAP: http://solcap.msu.edu/) [48]. This chip is made up of 8784 SNPs distributed across 12 chromosomes, and it has been used for different purposes, both in the study of genetic variability (genetic association) and in the breeding program (reduction of linkage drag).

7.1. Genetic Association

As part of a collaboration with the University of Naples Federico II (Prof Rosa Rao), massive sequencing was performed on 42 traditional Spanish and Italian tomato varieties, and on three accessions of *Solanum lycopersicum* var. *cerasiforme*, and *Solanum pimpinellifolium*. The analyzed group of traditional varieties includes Muchamiel, De la pera, Valenciano, Morunos, Raf, Flor de Baladre, San Marzano, Sorrento, Costoluto, and Vesuvio. The genotypes obtained with the 8K SolCap chip will be used for future genetic association studies, taking advantage of the phenotyping performed in the group in order to study the variability among the traditional cultivars in greater depth.

7.2. Obtaining Plants with the Ty-1 Gene with Less Linkage Drag

Despite the breeding program's success, homozygous alleles for resistance, especially for the *Ty-1* gene, have been found to negatively affect both production levels and other parameters of agronomic quality [43]. This work does not clarify whether the negative effects on production and quality are directly due to the *Ty-1* gene, or whether they are caused by genes associated with the resistance gene. We do know that in the introgression of genes from wild species, large segments of chromosomes surrounding the resistant gene remain in the resulting line, even after many backcrosses [49]. In fact, it has been reported that two chromosomal inversions in *S. chilense* LA1969 (the *Ty-1* gene donor) give rise to a recombination suppression in the region of chromosome six, where the *Ty-1* gene is found in cultivated tomatoes [50].

Studying plants with a distinct number of backcrosses confirms these results. Despite backcrossing up to 10 times, a large fragment of chromosome six, in which the gene *Ty-1* is found, does not recombine. By selecting plants with TYLCV resistance, this fragment barely changes as it passes from generation to generation. Furthermore, unpublished results recently obtained by the UMH team, in collaboration with groups from the Institute of Plant Molecular and Cellular Biology (IBMCP) in Valencia, also show the negative effect of introgression on volatile compounds and other metabolites. All together, these results seem to indicate that it is the presence of DNA fragments associated with the *Ty-1* gene that is responsible for changes to the aromatic characteristics of the UMH tomato varieties. It is not clear whether this effect can be attributed to the *Ty-1* gene itself, or to other genes around it. Plants where the resistance gene is present but linkage drag was reduced by recombination may help in understanding this aspect.

8. Future Work

In the future, the main goal of the UMH breeding program is to introduce a resistance without altering the quality and productivity of the new lines of traditional tomato varieties. The *Ty-5* gene, for example, from the commercial hybrid Tyking, has shown good behavior against various mono- and bipartite begomoviruses, including TYLCV [51,52]. Using massive genotyping, next-generation sequencing (NGS) technologies, and bioinformatics, our group is currently collaborating with other groups from the Institute of Plant Molecular and Cellular Biology (IBMCP) in the search for descendants of UMH breeding program plants that self-fertilize and have been able to recombine in the zones adjacent to the *Ty-1* gene. This approach will allow us to obtain plants with different configurations in chromosome six and later study the phenotypic characteristics of these plants in the field. This process will be extremely useful in identifying the genes responsible for the most important productivity and quality parameters in tomato.

Acknowledgments: This work was partially supported by the Spanish MICINN through projects AGL2002-03329, AGL2005-03946, AGL2008-03822, AGL2011-26957 and the European Union (ACIF/2016/212). We thank Ansley Evans for the language review. We want to thank the anonymous reviewers for their suggestions.

Author Contributions: All the authors contributed to the work of the manuscript. P.C, S.G.-M. and J.J.R. conceived and wrote the paper.

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

References

1. Ruíz, J.J.; García-Martínez, S. Tomato varieties 'Muchamiel' and 'De la Pera' form the southeast of Spain: Genetic improvement to promote on-farm conservation. In *European Landrace: On-Farm Conservation, Management and Use. Biodiversity Technical Bulletin No 15*; Vetelainen, M., Negri, V., Maxted, N., Eds.; Bioversity International: Rome, Italy, 2009; pp. 171–176.
2. Picó, B.; Herraiz, J.; Ruiz, J.J.; Nuez, F. Widening the genetic basis of virus resistance in tomato. *Sci. Hortic.* **2002**, *94*, 73–89. [CrossRef]

3. Nuez, F.; Roselló, S.; Picó, B. La conservación y recuperación de nuestro patrimonio hortícola. Mejorar para conservar. *Agrícola Vergel* **1998**, *194*, 74–80.
4. Nuez, F.; Ruiz, J.J. *La Biodiversidad Agrícola Valenciana: Estrategias Para su Conservación y Utilización*; Universitat Politècnica de València: Valencia, Spain, 1999.
5. Miller, J.C.; Tanksley, S.D. RFLP analysis of phylogenetic relationships and genetic variation in the genus Lycopersicon. *Theor. Appl. Genet.* **1990**, *80*, 437–448. [CrossRef] [PubMed]
6. Tanksley, S.D. The Genetic, Developmental, and Molecular Bases of Fruit Size and Shape Variation in Tomato. *Plant Cell* **2004**, *16*, S181–S189. [CrossRef] [PubMed]
7. Ruiz, J.J.; Martínez, N.; Valero, M.; García-Martínez, S.; Moral, R.; Serrano, M. Micronutrient composition and quality characteristics of traditional tomato cultivars in the south-east of Spain. *Commun. Soil Sci. Plant Anal.* **2005**, *36*, 649–660. [CrossRef]
8. Ruiz, J.J.; Alonso, A.; García-Martínez, S.; Valero, M.; Blasco, P.; Ruiz-Bevia, F. Quantitative analisys of flavour volatiles detects differences among closely related traditional cultivars of tomato. *J. Sci. Food Agric.* **2005**, *85*, 54–60. [CrossRef]
9. Alonso, A.; García-Aliaga, R.; García-Martínez, S.; Ruiz, J.J.; Carbonell-Barrachina, A.A. Characterization of Spanish tomatoes using aroma composition anddiscriminant analisys. *Food Sci. Technol. Int.* **2009**, *15*, 47–55. [CrossRef]
10. Smulders, M.J.M.; Bredemeijer, G.; Rus-Kortekaas, W.; Arens, P.; Vosman, B. Use of short microsatellites from database sequences to generate polymorphisms among Lycopersiconesculentum cultivars and accessions of other Lycopersicon species. *Theor. Appl. Genet.* **1997**, *97*, 264–272. [CrossRef]
11. Alvarez, A.E.; Van de Wiwl, C.C.M.; Smulders, M.J.M.; Vosman, B. Use of microsatellites to evaluate genetic diversity and species relationships in the genus Lycopersicon. *Theor. Appl. Genet.* **2001**, *103*, 1283–1292. [CrossRef]
12. Park, Y.H.; West, M.A.L.; St. Clair, D.A. Evaluation of AFLPs for germplasm fingerprinting and assessment of genetic diversity in cultivars of tomato (*Lycopersicon esculentum* L.). *Genome* **2004**, *47*, 510–518. [CrossRef] [PubMed]
13. Bredemeijer, G.M.M.; Cooke, R.J.; Ganal, M.W.; Peeters, R.; Isaac, P.; Noordijk, Y.; Rendell, S.; Jackson, J.; Röder, M.S.; Wndehake, K.; et al. Construction and testing of a microsatellite database containing more than 500 tomato varieties. *Theor. Appl. Genet.* **2002**, *105*, 1019–1026. [PubMed]
14. He, C.; Poysa, V.; Yu, K. Development and characterization of simple sequence repeat (SSR) markers and their use in determining relationships among *Lycopersicon esculentum* cultivars. *Theor. Appl. Genet.* **2003**, *106*, 363–373. [CrossRef] [PubMed]
15. Andreakis, N.; Giordano, I.; Pentangelo, A.; Fogliano, V.; Graziani, G.; Monti, L.M.; Rao, R. DNA fingerprinting and quality traits of Corbarino Cherry-like tomato landraces. *J. Agric. Food Chem.* **2004**, *52*, 3366–3371. [CrossRef] [PubMed]
16. Rao, R.; Corrado, G.; Bianchi, M.; Di Mauro, A. (GATA)4 DNA fingerprinting identifies morphologically characterized San Marzano tomato plants. *Plant Breed.* **2006**, *125*, 173–176. [CrossRef]
17. Li, G.; Quiros, C.F. Sequence-related amplified polymorphism (SRAP), a new marker system based on a simple PCR reaction: Its application to mapping and gene tagging in Brassica. *Theor. Appl. Genet.* **2001**, *103*, 455–461. [CrossRef]
18. Ruiz, J.J.; García-Martínez, S.; Picó, B.; Gao, M.; Quiros, C.F. Genetic variability and relationship of closely related Spanish traditional cultivars of tomato as detected by SRAP and SSR markers. *J. Am. Soc. Hortic. Sci.* **2005**, *130*, 88–94.
19. Tautz, D. Hypervariability of simple sequences as a general source for polymorphic DNA markers. *Nucleic Acids Res.* **1989**, *17*, 6463–6471. [CrossRef] [PubMed]
20. García-Martínez, S.; Andreani, L.; García-Gusano, M.; Geuna, F.; Ruiz, J.J. Evaluation of amplified fragment length polymorfism and simple sequence repeats for tomato germplasm fingerprinting: Utility for grouping closely related traditional cultivars. *Genome* **2006**, *49*, 648–656. [CrossRef] [PubMed]
21. Vos, P.; Hogers, R.; Bleecker, M.; Reijans, M.; van de Lee, T.; Hornes, M. AFLP: A new technique for DNA fingerprinting. *Nucleic Acid Res.* **1995**, *23*, 4407–4414. [CrossRef] [PubMed]
22. Kaemmer, D.; Weising, K.; Bayermann, B.; Borner, T.; Epplen, J.T.; Kahl, G. Oligonucleotide fingerprinting of tomatoDNA. *Plant Breed.* **1995**, *114*, 12–17. [CrossRef]

23. Caramante, M.; Rao, R.; Monti, L.M.; Corrado, G. Discrimination of San Marzano accessions: A comparison of minisatellite, CAPS and SSR markers in relation to morphological traits. *Sci. Hortic.* **2009**, *120*, 560–564. [CrossRef]
24. García-Martínez, S.; Corrado, G.; Ruiz, J.J.; Rao, R. Diversity and structure of a sample of traditional Italian and Spanish tomato accesions. *Genet. Resour. Crop Evol.* **2013**, *60*, 789–798. [CrossRef]
25. Wang, D.G.; Fan, J.B.; Siao, C.J.; Berno, A.; Young, P.; Sapolsky, R.; Ghandour, G.; Perkins, N.; Winchester, E.; Spencer, J.; et al. Large-scale identification, mapping, and genotyping of single-nucleotide polymorphisms in the human genome. *Science* **1998**, *280*, 1077–1082. [CrossRef] [PubMed]
26. García-Gusano, M. Empleo de Marcadores Moleculares Para el Estudio de la Variabilidad Intraespecífica en Tomate (*Solanum lycopersicum* L.). Ph.D. Thesis, Miguel Hernandez University, Elche, Spain, 2007.
27. Le Corre, V.; Kremer, A. Genetic variability al neutral markers, quantitative trait loci and trait in a subdivided population under selection. *Genetics* **2003**, *164*, 1205–1219. [PubMed]
28. Alexander, L.J. Transfer of a dominant type of resistanceto the four known Ohio pathogenic strains of tobacco mosaicvirus (TMV), from *Lycopersicon peruvianum* to *L. esculentum*. *Phytopathology* **1963**, *53*, 869.
29. Stevens, M.R.; Scott, S.J.; Gergerich, R.C. Inheritance of a gene for resistance totomato spotted wilt virus (TSWV) from *Lycopersicon peruvianum* Mill. *Euphytica* **1992**, *59*, 9–17.
30. Zamir, D.; Ekstein-Michelson, I.; Zakay, Y.; Navot, N.; Zeidan, M.; Safatti, M.; Eshed, Y.; Harel, E.; Pleban, T.; van-Oss, H.; et al. Mapping and introgression of a Tomato yellow leaf curl virus tolerante gene, TY-1. *Theor. Appl. Genet.* **1994**, *88*, 141–146. [CrossRef] [PubMed]
31. Folkertsma, R.T.; Spassova, M.I.; Prins, M.; Stevens, M.R.; Hille, J.; Goldbach, R.W. Construction of a bacterial artificial chromosome (BAC) library of *Lycopersicon esculentum* cv. Stevens and its application to physically map the *Sw-5* locus. *Mol. Breed.* **1999**, *5*, 197–207. [CrossRef]
32. Rick, C.M.; Fobes, J.A. Association of an allozyme with nematodes resistance. *Tomato Genet. Coop. Rep.* **1974**, *24*, 25.
33. Dax, E.; Livneh, O.; Aliskevicius, E.; Edelbaum, O.; Kedar, N.; Gavish, N.; Milo, J.; Geffen, F.; Blumenthal, A.; Rabinowich, H.D.; et al. A SCAR marker linked to the ToMV resistance gene, *Tm-22*, in tomato. *Euphytica* **1998**, *101*, 73–77. [CrossRef]
34. Omori, T.; Murata, M.; Motoyoshi, F. Molecular characterization of the SCAR markers tightly linked to the Tm-2 locus of the genus Lycopersicon. *Theor. Appl. Genet.* **2000**, *101*, 64–69.
35. Lanfermeijer, F.C.; Dijkhuis, J.; Sturre, M.J.G.; de Haan, P.; Hille, J. Cloning and characterization of the durable tomato mosaic virus resistance gene Tm-22 from *Lycopersicon esculentum*. *Plant Mol. Biol.* **2003**, *52*, 1037–1049. [CrossRef] [PubMed]
36. Tanksley, S.D. Molecular markers in plant breeding. *Plant Mol. Biol. Rep.* **1983**, *1*, 3–8. [CrossRef]
37. Foolad, M. Genome mapping and molecular breeding of tomato. *Int. J. Plant Genom.* **2007**, *52*, 64358. [CrossRef] [PubMed]
38. Foolad, M.R.; Panthee, D.R. Marker-Assisted Selection in Tomato Breeding. *Crit. Rev. Plant Sci.* **2012**, *31*, 93–123. [CrossRef]
39. García-Martínez, S.; Grau, A.; Alonso, A.; Rubio, F.; Valero, M.; Ruiz, J.J. UMH 1200, a breeding line within the Muchamiel tomato type resistant to three viruses. *HortScience* **2011**, *46*, 1054–1055.
40. García-Martínez, S.; Grau, A.; Alonso, A.; Rubio, F.; Valero, M.; Ruiz, J.J. UMH 1203, a multiple virus-resistant fresh-market toamto breeding line for open-field conditions. *HortScience* **2012**, *47*, 124–125.
41. Tanksley, S.D.; Bernachi, D.; BeckBunn, T.; Emmatty, D.; Eshed, Y.; Inai, S. Yield and quality evaluations on a pair of processing tomato lines nearly isogenic for the Tm2a gene for resistance to the Tobacco mosaic virus. *Euphytica* **1998**, *99*, 77–83. [CrossRef]
42. 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 pleitropy. *Theor. Appl. Genet.* **2007**, *115*, 169–178. [CrossRef] [PubMed]
43. Rubio, F.; Alonso, A.; García-Martínez, S.; Ruiz, J.J. Introgression of virus-resistance genes into traditional Spanish tomato cultivars (*Solanum lycopersicum* L.): Effects on yield and quality. *Sci. Hortic.* **2016**, *198*, 183–190. [CrossRef]
44. García-Martínez, S.; Grau, A.; Alonso, A.; Rubio, F.; Valero, M.; Ruiz, J.J. UMH 1422 and UMH 1415: Two fresh-market tomato breeding lines resistant to Tomato Mosaic Virus and Tomato Spotted Wilt Virus. *HortScience* **2014**, *49*, 1465–1466.

45. García-Martínez, S.; Grau, A.; Alonso, A.; Rubio, F.; Carbonell, P.; Ruiz, J.J. UMH 916, UMH 972, UMH 1093, UMH 1127 and UMH 1139: Four fresh-market breeding lines resistant to viruses within the Muchamiel tomato type. *HortScience* **2015**, *50*, 927–929.
46. García-Martínez, S.; Grau, A.; Alonso, A.; Rubio, F.; Carbonell, P.; Ruiz, J.J. New breeding lines resistant to Tomato Mosaic Virus and Tomato Spotted Wilt Virus within the 'De la Pera' tomato type: UMH 1353 and UMH 1354. *HortScience* **2016**, *51*, 456–458.
47. Alonso, A.; García-Martínez, S.; Arroyo, A.; García-Gusano, M.; Grau, A.; Giménez-Ros, M.; Romano, M.E.; Valero, M.; Ruiz, J.J. Efecto de la introducción de resistencia genética a TYLCV (gen Ty-1) en caracteresproductivos y de calidad en tomate. *Actas Hortic.* **2008**, *51*, 175–176.
48. Sim, S.C.; Durstewitz, G.; Plieske, J.; Wieseke, R.; Ganal, M.W.; Van Deynze, A.; Hamilton, J.P.; Buell, C.R.; Causse, M.; Wijeratne, S.; et al. Development of a large SNP genotyping array and generation of high-density genetic maps in tomato. *PLoS ONE* **2012**, *7*, e40563. [CrossRef] [PubMed]
49. Young, N.D.; Tanksley, S.D. RFLP analisys of the size of chromosomal segments retained around the TM-2 locus of tomato during backcross breeding. *Theor. Appl. Genet.* **1989**, *77*, 353–359. [CrossRef] [PubMed]
50. Verlaan, M.G.; Szinay, D.; Hutton, S.F.; de Jong, H.; Kormelink, R.; Visser, G.F. 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]
51. 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.
52. Pereira-Carvalho, R.C.; Díaz-Pendón, J.A.; Fonseca, M.E.N.; Boiteux, L.S.; Fernández-Muñoz, R.; Moriones, E.; Resende, R.O. Recessive Resistance Derived from Tomato cv. Tyking-Limits Drastically the Spread of Tomato Yellow Leaf Curl Virus. *Viruses* **2015**, *7*, 2518–2533. [CrossRef] [PubMed]