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The Presence of Genes for Resistance against *Verticillium dahliae* in Italian Tomato Landraces

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Summary

A sample of about 169 tomato landraces of varying fruit type and of diverse provenance was tested for reaction to infection with *Verticillium dahliae*, the causative pathogen of Verticillium wilt. Some individuals of the 'Sorrentino' landrace were as resistant as 'Florida Ve', whereas certain populations of the 'Ligure', rather known as 'Cuor di bue di Albenga', type included the extremes of susceptibility and resistance. Resistant selections from each landrace were allowed to self fertilize to generate breeding lines. A PCR analysis, based on a CAPS marker targeted to the *Ve2* allele, was conducted on 'Sorrentino', 'Locale di Albenga 2', 'Locale di Albenga 3', 'Locale di Carmagnola', 'Locale di Cuneo', and 'Eraldo4', and the resulting genotype was fully consistent with reaction to infection with *V. dahliae* strain V7. Variation in the level of resistance among *Ve2* carriers suggested that other genes are also involved in modulating the response to infection. Markers for resistant alleles at *Ve* should prove useful in marker assisted breeding for wilt resistance in tomato. Homozygous resistant selections were made from landrace materials, which retained the landrace fruit type.

Key words. assisted breeding – CAPS marker – selection – *Solanum lycopersicon* – wilt – *Ve1-Ve2*

Introduction

Tomato (*Solanum lycopersicon* Mill.) is the main vegetable crop grown in Italy, and a wide range of landraces differing in fruit shape, colour, size, taste, and end use are currently in production. Some of these are increasing in economic importance, while others occupy a minor niche in the market. Verticillium wilt, caused by the fungus *Verticillium dahliae* Kleb., affects many crop species, both in the field and under glass. Disease control can be obtained by soil disinfestation through steaming or fumigation, but these techniques are expensive, and generate a biological vacuum in the soil. Disease control can also be achieved by soil solarization (Tamietti and Garibaldi 1982; Tjamos and Paplomatas 1988; Tamietti and Valentino 2001), a technique which, however, requires a relatively long treatment time and careful post-treatment tillage to avoid reintroduction of inoculum into the soil space occupied by the plant roots. Resistance genes against Verticillium wilt have been identified, including *Ve* from 'Peru Wild', *Lycopersicum esculentum* Utah accession number 665 (see Schaible et al. 1951) and introgressed in large fruited tomatoes which gradually replaced the susceptible varieties in the main growing areas. *Ve* locus has two closely linked inversely orientated genes, *Ve1* and *Ve2*, that provide resistance against the race 1 strains of *V. dahlie* and *V. albo-atrum* (Kawchuk et al. 2001) in potato, whereas in tomato it has been proved that the only *Ve1* gene is effective (Fradin et al. 2009). Strains of *V. dahliae*, designed as race 2, able to cause disease in the wilt-resistant cultivars were firstly reported in Ohio (Alexander 1962) and after in several other Countries (Pegg and Brady 2002). Nowadays race 2 is the dominant race in some regions of North America (Dobinson et al. 1996), whereas in others it seems to be present in spots only (Cirulli 1969; Tjamos 1980; Matta and Garibalbi 1984; Daami-Remadi et al. 2006). Actually almost all current commercial hybrids carry a resistant allele at *Ve*, which confers a good level of resistance against the disease caused by race 1. Most landraces and old varieties are susceptible, however, in some infested soils disease incidence and severity are rather low, suggesting the presence of suppressive properties of the soil (Davis et al. 2004; Tjamos et al. 2004) and/or genetic resistance in landraces.

Modern horticulture seeks to produce high quality vegetables avoiding too heavy a reliance on chemical control of pests, weeds and plant pathogens. The transfer of disease resistance genes to landraces, well accepted for their fruit quality (Michelmore 2003), would increase the value of these landraces, allowing them to continue to be competitive with commercial hybrids. In this study, we show that some Italian tomato landraces have genetic resistance or tolerance against Verticillium wilt, and we

have characterized the genetic basis of this resistance with molecular markers associated with *Ve*.

Material and Methods

Germplasm

The set of Italian table tomato landraces included accessions conserved in the CRA–ORA (Council for Research and Experimentation in Agriculture, Research Unit for Vegetable Crops) collection, and accessions collected from farms. Large fruited types were represented by 'Ligure', rather known as 'Cuor di bue di Albenga' characterised by red, pear-shaped and slightly ribbed fruits (twenty-five accessions), 'Pera d'Abruzzo' (thirty-seven), 'Rosa di Sorrento' (pink, globose/oblong slightly ribbed fruits) (seven), 'Rosa di Rotonda' (twenty), 'Rosso di Rotonda' (ten), 'Scatolone di Bolsena' (one), 'Pantano Romano' (two), 'Cuor di Bue' (two), 'Costoluto Fiorentino' (two), 'Locale di Belmonte' (four); small fruited ones by 'Nocerino da Serbo' (two), 'Principe Borghese' (one), 'Pizzutello' (26), 'Fiaschetti' (nine); and elongated fruited types by 'Lungo Abruzzese' (one), 'San Marzano' (five), other elongated (two); long self life fruited 13 accession from Sicily, Campania, Calabria. All the accessions were collected during the past 15 years in different Italian regions and locations, from farmers which provided on their own to select lines *via* empiric selection. Breeding lines selected from the original cross between the landrace 'Locale di Sorrento' (susceptible) and the "elite" breeding line 'L17/94' (resistant), such as 'Rosa F2 195' and 'Rosa F2 197', were considered part of the 'Rosa di Sorrento' group. A map of the regional origin of the accessions used in this study is reported in Fig. 1.

Artificial inoculation

Tomato accessions were firstly screened for their agronomical characters to select those worthy to be further improved, also by introgressing genetic resistances effective in controlling soil-borne pathogens. The best accessions within each landraces, and those collected in *Verticillium* infested fields were tested for the resistance to *V. dahliae*. Germplasm screens were carried out in 4 years, as summarized in Table 1.

A high virulent monoconidial *V. dahliae* race 1 strain (*Vd* V7) from eggplant was used for screening the germplasm set. In one additional assay, *V. dahliae* race 2 from tomato was used. *Verticillium* strains were cultured for eight days at 25 °C in malt extract broth on a rotary shaker (150 rpm). The cultures were filtered through a sterile cheesecloth and successively centrifuged (2.160 g, 10 min) to isolate the conidia. Conidia were resuspended in sterile distilled water to a concentration of 2 x 10^6 ml^{-1}. Tomato seedlings were grown in a steam sterilized 1:1 field soil:commercial compost mixture under a 14 h photoperiod, with a day temperature of 25 °C, and a night temperature of 20 °C. Thirty-day old plants (20 per accession) were inoculated by immersing the roots for 20 min in the conidial suspension; inoculated plants were potted in the same type of soil and exposed to a 24 °C day and 18 °C night regime under a 14 h photoperiod. The varieties 'Locale di Belmonte' and 'Florida Ve' were used as susceptible and resistant controls, respectively. After 60 d, disease severity on leaves (chlorosis, wilting, and necrosis) and stem (dwarfing, adventitious roots) was assessed following a modified method of DIMOND et al. (1952). In brief, an arbitrary disease index was scored on a scale ranging from 0 (healthy plant) to 5 (unproductive plant, defoliation higher than 76 %) through the following intermediate levels: 1 = asymmetry and/or chlorosis on 1–3 leaves, 2 = chlorosis on 50 % of the leaves, 3 = dwarfing, emerging adventitious roots, 50 % defoliation, 4 = dwarfing, developed adventitious roots, 51–75 % defoliation, and than transformed in percentage; a cross section was cut just above the first true leaf to expose the xylem and the percentage browning of the xylem was estimated. The mean of the two disease indexes was computed obtaining a synthetic disease severity index per plant, which was transformed to angular values (arcsine square root x $180/\pi$) and subjected to an analysis of variance. Means were compared using Tukey's test in the Statistica software package (1993). All plants with a symptom were used to compute the disease incidence (100 x diseased/inoculated plants).

Resistant or tolerant plants were allowed to produce a new shoot from the first node, planted in the field and allowed to self pollinate; progenies were evaluated for their behaviour against the pathogen following the same protocol.

PCR amplification of Ve gene fragments and marker selection

The sequences AF272367 and AF365929 were used to design three pairs of primers targeting *Ve1* and *Ve2*. The resulting amplicons partially overlap one another (Ve1_1, Ve1_2, Ve1_3 for *Ve1*; Ve2_1, Ve2_2 and Ve2_3

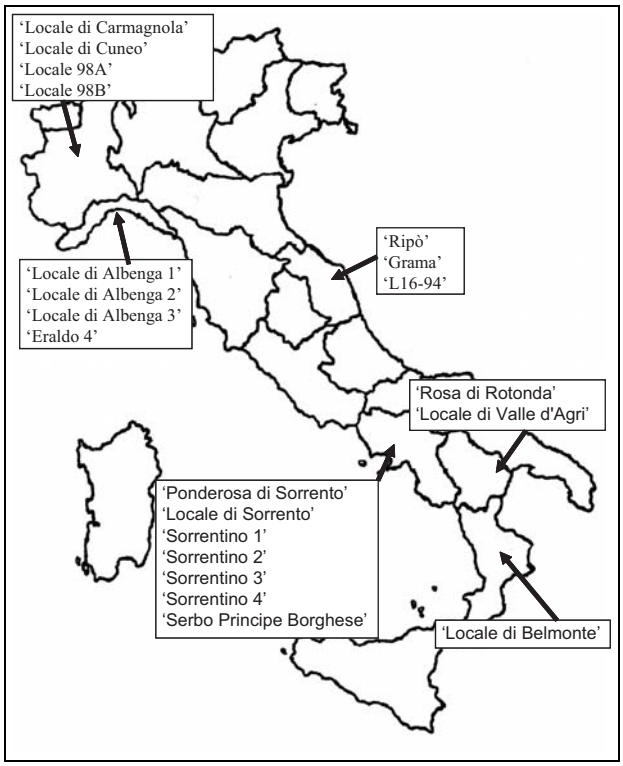


Fig. 1. Places of origin of the local cultivars used in this study.

Table 1. *Verticillium dahliae* races used to screen the resistance of tomato germplasm, accessions of the tested tomato local cultivar and their progenies, and timing of the screenings.

Verticillium-dahliae	Tested germplasm[1]			
	1st year	2nd year	3rd year	4th year
Race 1	'R Rot'	'R Rot sel1/02'	'Sorr 1 sel1/03'	'Rosa F2 197'
	'Loc 98 A'	'Prin Borghese'	'Sorr 1 sel2/03'	'Rosa F2 195'
	'Loc Agri'	'Loc Agri sel1/02'	'Sorr 1 sel3/03'	'Loc CN sel1.7.1/03'
	'Ripò'	'Sorr 2'	'Sorr 1 sel4/03'	'Loc CN sel1.7.2/03'
	'Grama'	'Sorr 3'	'Sorr 1 sel5/03'	'Loc CN sel1.7.3/03'
	'Loc 98 B'	'Sorr 4'	'Sorr 1 sel6/03'	'Loc CN sel1.7.5/03'
	'Sorr 1'	'Sorr 1 sel1/02'	'Sorr 3 sel1/03'	'Loc Carm sel8.4/02'
	'Loc Sorrento'	'Sorr 1sel2/02'	'Sorr 3 sel2/03'	'Loc Carm sel8.5/02'
	'Loc Albenga 1'	'Sorr 1 sel3/02'	'Sorr 3 sel3/03'	'Loc Alb 3 sel6.3/02'
	'Loc Albenga 2'	'Sorr 1 sel4/02'	'Sorr 4 sel1/03'	'Loc Alb 3 sel6.6/02'
	'Loc Carmagnola'	'Sorr 1sel5/02'	'Sorr 4 sel2/03'	
	'Loc Albenga 3'	'Sorr 1sel6/02'	'Sorr 4 sel3/03'	
	'Pond Sorrento'	'Loc CN sel1/02'		
	'Loc CN'	'Eraldo 4 sel1/02'		
	'L 16-94'			
	'Eraldo 4'			
Race 2		'Sorr 1 sel1/02'		
		'Florida Ve'		
		'Loc Belmonte'		

[1]'Locale di Belmonte' and 'Florida Ve' were always used as susceptible and resistant standard with *V. dahliae* race 1.
R Rot = 'Rosa di Rotonda'; Sorr= 'Sorrentino'; Loc Sorrento= 'Locale di Sorrento'; Loc Agri= 'Locale di Valle d'Agri'; Loc Alb= 'Locale di Albenga'; Loc Carm= 'Locale di Carmagnola'; Loc CN= 'Locale di Cuneo'; Pond Sor= 'Poderosa di Sorrento'; Loc 98A= 'Locale 98A'; Loc 98B= 'Locale 98B'; Loc Belmonte= 'Locale di Belmonte'; 'Prin Borghese' = 'Serbo Principe Borghese'.

for *Ve2*). Amplicons from template of 'Mogeor' (a *Verticillium* resistant line provided by Tomato Genetics Resource Center, University of California, Davis), 'L98A' (a local susceptible cultivar) and 'ISPORT L.13/3.3' (a resistant selection from the local cultivar 'Rosa di Sorrento') were sequenced to identify informative markers for genotyping. These were Ve1_2 for *Ve1* and Ve2_3 for *Ve2*. A *Ve*2 CAPS marker (VE2LeOF/R) was used for subsequent marker assisted selection. Relevant PCR conditions and primer sequences were as reported by Acciarri et al. (2007).

Results

Germplasm screen

On the whole of the trials, *Vd* V7 strain infected 100 % of 'Locale di Belmonte' plants with a level of severity ranging from 39–75 %. On 'Florida Ve' it caused very little wilt symptoms on 5–80 % of plants, depending on the experiments, mainly as a xylem discolouration with a 4–10 % severity. Among the landraces tested in the first year, seven proved to be highly susceptible to wilt (56–76 % severity), seven were moderately susceptible (39–50 %) and two were rather tolerant (27–33 %), while 'Sorrentino 1' was as resistant as 'Florida Ve' (Table 2). A sample of resistant 'Sorrentino 1' and only slightly symptomatic individuals belonging to 4 landraces of the 'Ligure' type ('Locale di Albenga 3', 'Locale di Carmagnola', 'Eraldo 4' and 'Locale di Cuneo') were allowed to self fertilize to allow a second round of selection. In the second year, six more landraces ('Locale di Belmonte', 'Serbo Principe Borghese', 'Sorrentino 2', 'Sorrentino 3', 'Sorrentino 4'), six 'Sorrentino 1' selections and one selection from each of 'Rosa di Rotonda', 'Locale di Valle d'Agri', 'Eraldo 4' and 'Locale di Cuneo' were screened. 'Serbo Principe Borghese' proved highly susceptible, 'Eraldo 4 sel1/02', 'Locale di Cuneo sel1/02', 'Sorrentino 3', 'Sorrentino 4' and four out of six of the 'Sorrentino 1' selections were as resistant as 'Florida Ve'. The remaining lines showed an intermediate level of resistance (Table 3). All the 'Sorrentino' selections, except for '1.4/03', remained highly resistant in the third generation (Fig. 2).

'Locale di Belmonte', 'Sorrentino 1 sel1/02' and 'Florida Ve' were all equally susceptible to *Vd* race 2 (Table 4).

Single plant selection among the tolerant landraces did not result in the identification of any lines with a statistically different improved level of resistance, anyway two selections of 'Locale di Albenga 3' ('6.6' and '6.3') were highly resistant, whereas 'Locale di Carmagnola 8.4' and 'Locale di Cuneo 7.2' had a disease severity of about twice that of the 'Locale di Albenga 3' selections (Fig. 3).

Table 2. Disease severity and disease incidence shown by several landraces and 'Florida Ve' tomato plants 60 days after root-dip inoculation in $2x10^6$ conidia ml^{-1} of *Verticillium dahliae* race 1 in the first year (2002).

Tomato landrace	Verticillium wilt	
	Severity	Incidence
'Locale di Belmonte'	75.8 a*	100.0
'Rosa di Rotonda'	68.2 ab	72.2
'Locale 98 A'	64.6 abc	100.0
'Locale di Valle d'Agri'	62.2 a–d	100.0
'Ripò'	61.8 a–e	100.0
'Grama'	56.6 a–f	100.0
'Locale 98 B'	56.0 b–f	100.0
'Locale di Albenga 1'	50.0 c–f	100.0
'Locale di Albenga 2'	46.2 d–f	100.0
'Locale di Carmagnola'	45.6 d–f	100.0
'Locale di Albenga 3'	45.0 d–f	100.0
'Ponderosa di Sorrento'	43.6 efg	100.0
'Locale di Sorrento'	43.6 efg	100.0
'Locale di Cuneo'	39.0 fg	100.0
'L 16-94'	32.6 fgh	94.1
'Eraldo 4'	27.2 gh	100.0
'Sorrentino 1'	3.6 i	3.7
'Florida Ve'	4.4 i	5.5

Values followed by a common letter are not statistically different following the Tuckey's test (P < 0.04).

Detection of Ve genes using molecular markers

*Hin*cII restriction of the amplified *Ve* gene region generated a polymorphic profile in comparisons between wilt resistant and susceptible genotypes (Fig. 4). The latter profile consisted of an undigested 1029 bp fragment, while that of genotypes carrying *Ve2* consisted of a 428 bp and a 601 bp fragments. On the basis of the CAPS assay, the selfed-lines from 'Sorrentino' obtained in 2003: 'Sorrentino1/03', '3/03', '5/03' and '6/03' (lanes 10, 12, 14, 15 in Fig. 4) were homozygous *Ve2*, while 'Sorrentino 2/03' (lane 11) was heterozygous. Lines 'Rosa F2 195' and 'Rosa F2 197' (lanes 16 and 17), both progeny of a hybrid between 'Locale di Sorrento' (susceptible) x 'L17/94' (resistant), segregated for *Ve2*. Among the other local cultivars, the five lines belonging to the 'Ligure' types ('Locale di Albenga 2', 'Locale di Albenga 3', 'Locale di Carmagnola sel8.4', 'Locale di Cuneo sel1', and 'Eraldo 4 sel1',), obtained in 2002 by self-pollination, were homozygous *Ve2*, and the remainder homozygous for the susceptible allele *ve*. These data are consistent with the outcome of the inoculation tests (Table 5).

Discussion

The evaluation of tomato landraces identified variation with respect to response to Verticillium wilt infection. Some 'Sorrentino' selections, which are of the 'Rosa di Sorrento' type proved to be as resistant as the control cultivar 'Florida Ve'. Some of the 'Ligure' type landraces (such as 'Eraldo 4', the three accessions of 'Locale di Albenga', 'Locale di Cuneo' and 'Locale di Carmagnola')

Table 3. Disease severity and disease incidence shown in two independent trials by some tomato landraces, and breeding lines selected in the first year, 60 days after root-dip inoculation in 2×10^6 conidia ml^{-1} of *Verticillium dahliae* race 1 (second experimental year).

Tomato line	Trial 1		Trial 2	
	Verticillium wilt		Verticillium wilt	
	Severity	Incidence	Severity	Incidence
'Locale di Belmonte'	62.5 a	100.00	48.75 a	100.00
'Serbo Principe Borghese'	53.5 a	100.00	–	–
'Eraldo 4 sel1/02'	8.1 cd	68.42	–	–
'Locale di Cuneo sel1/02'	9.8 c	52.63	–	–
'Sorrentino 2'	27.0 b	95.00	–	–
'Sorrentino 3'	7.7 cd	45.71	–	–
'Sorrentino 4'	6.2 cd	50.00		
'Rosa di Rotonda sel1/02'	16.2 b	88.89	–	–
'Locale Valle d'Agri sel1/02'	24.4 b	100.00	–	–
'Sorrentino 1 sel1/02'	14.4 bc	72.22	12.78 bc	83.33
'Sorrentino 1 sel2/02'	0.7 d	6.25	4.29 cd	42.86
'Sorrentino 1 sel3/02'	2.2 cd	18.75	14.50 bc	85.00
'Sorrentino 1 sel4/02'	–	–	13.04 bc	95.65
'Sorrentino 1 sel5/02'	1.0 d	10.00	16.67 b	85.71
'Sorrentino 1 sel6/02'	7.5 cd	50.00	0.19 d	9.52
'Florida Ve'	4.0 cd	50.00	4.40 cd	70.00

Values in a column followed by a common letter are not statistically different following the Tuckey's test (P < 0.014 trial 1; P = 0.0015 trial 2).

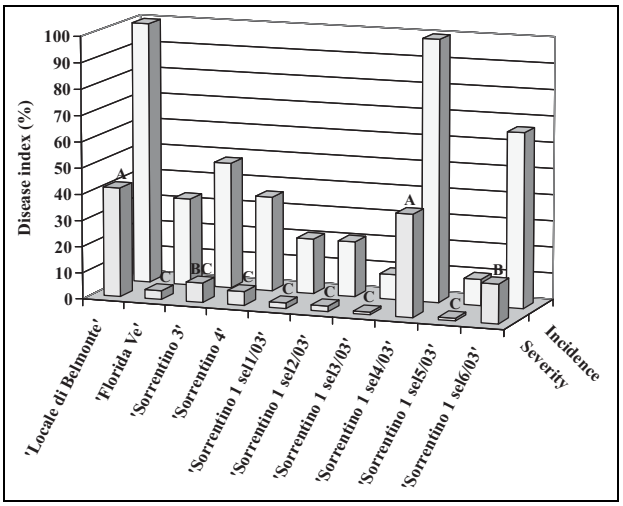


Fig. 2. Disease severity and disease incidence shown by two accessions of 'Sorrentino' collected in farms and some lines of 'Sorrentino' selected in the 2nd year of screening, 60 days after root-dip inoculation in 2 x 10^6 conidia ml^{-1} of *Verticillium dahliae* race 1. 'Florida Ve' and 'Locale di Belmonte' are the resistant and susceptible controls (4th experimental year). The values followed by a same letter are not statistically different following the Tuckey's test ($P < 0.0006$).

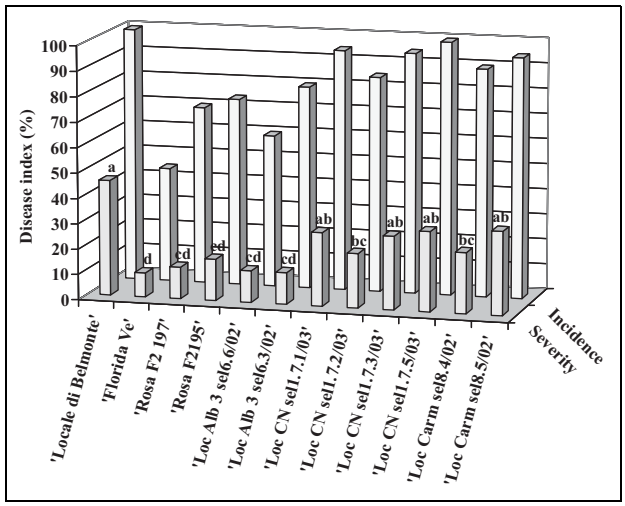


Fig. 3. Disease severity and disease incidence shown by two breeding line of 'Rosa', two selections of 'Locale di Albenga 3' and 'Locale di Carmagnola', and four selections of 'Locale di Cuneo', 60 days after root-dip inoculation in 2 x 10^6 conidia ml^{-1} of *Verticillium dahliae* race 1 in the 4th experimental year. 'Florida Ve' and 'Locale di Belmonte' are the resistant and susceptible controls. The values followed by a same letter are not statistically different following the Tuckey's test ($P < 0.018$).

segregated very susceptible and quite resistant plants as did the landrace 'Rosa di Rotonda'. By selfing plants showing little or no xylem discolouration in response to wilt infection, it was possible to identify homozygous resistant progeny. The genotyping analysis based on markers for *Ve* demonstrated the involvement of this gene in the resistance of 'Sorrentino', and a co-dominant CAPS marker enabled heterozygotes to be distinguished from homozygotes. The association between the response to infection and the marker assay showed that these markers can perform satisfactorily in a marker assisted programme targeting resistance against *Vd*.

Due to the variable pathogenicity shown by different isolates of *Vd* on different hosts (Matta et al. 1980), in preliminary experiments (not reported) we tested some *Vd* strains from different host, including tomato, for their virulence on tomato known to be susceptible to Verticillium wilt, and V7 appeared to be the most performing. In the screening tests V7 constantly caused some symptoms in some individuals of 'Florida Ve' as well as other Ve/Ve breeding lines, suggesting an incomplete resistance conferred by *Ve* against *Vd* race 1. The effectiveness of *Ve* genes against *Vd* race 1 is conflicting: actually Matta et al. (1980) concluded for a the complete effectiveness of *Ve*, whereas Dixon and Pegg (1969) observed a slight colonization of the *Ve* resistant plants, varying in non-isogenic lines and particularly with the virulence of the isolate. Our results agree with them of Dixon and Pegg

Table 4. Disease severity and disease incidence shown by the breeding line 'Sorrentino selection 1/02', 'Florida Ve' and 'Locale di Belmonte', 60 days after root-dip inoculation in 2 x 10^6 conidia ml^{-1} of *Verticillium dahliae* race 2.

Tomato line	Verticillium wilt	
	Severity	Incidence
'Sorrentino 1 sel1/02'	37.00	100
'Florida Ve'	40.00	100
'Locale di Belmonte'	38.57	100

No statistical differences were detected following the Tuckey's test.

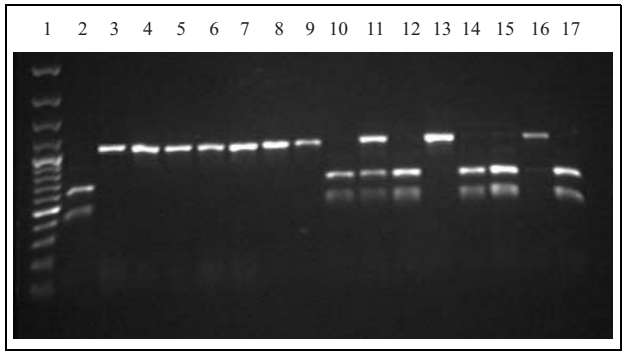


Fig. 4. PCR amplification of *Ve2* gene by CAPS marker carried out on different tomato genotypes. Lane 1: ladder; 2: 'Florida Ve'; 3: 'Marzano'; 4: 'Locale di Belmonte'; 5: 'Ponderosa di Sorrento'; 6: 'Locale di Sorrento'; 7: 'Rosa di Rotonda'; 8: 'Locale di Valle d'Agri'; 9: 'L.16-94'; 10: 'Sorrentino 1 sel1/03'; 11: 'Sorrentino 1 sel2/03'; 12: 'Sorrentino 1 sel3/03'; 13: 'Sorrentino 1 sel4/03'; 14: 'Sorrentino 1 sel5/03'; 15: 'Sorrentino 1 sel6/03'; 16: 'Rosa F2 195'; 17: 'Rosa F2 197'. The presence of *Ve2* gene is shown by 2 fragments of 428 and 601 bp; the *ve* gene by an undigested amplicon of 1029 bp.

Table 5. Genetic profile and resistance to *Verticillium dahliae* race 1 shown by different local varieties of table tomato.

Accession	Resistance[a]	Genetic profile[b]	Accession	Resistance[a]	Genetic profile
'Florida Ve'	0.51	*Ve/Ve*	'Serbo Principe Borghese'	53.5	*ve/ve*
'Locale di Albenga 3 sel6.6/02'	7.50	*Ve/Ve*	'Grama'	61.8	*ve/ve*
'Locale di Carmagnola sel8.4'	21.60	*Ve/Ve*	'Ripò'	61.8	*ve/ve*
'Locale Cuneo sel1/02'	5.15	*Ve/Ve*	'Locale 98 A'	64.6	*ve/ve*
'Eraldo 4 sel1/02'	5.54	*Ve/Ve*	'Locale 98 B'	56.0	*ve/ve*
'Rosa F2 197'	8.75	*Ve/Ve*	'Locale di Albenga 1'	41.0	*ve/ve*
'Sorrentino 1 sel5/03'	0.10	*Ve/Ve*	'Locale di Belmonte'	62.5	*ve/ve*
'Sorrentino 1 sel6/03'	10.00	*Ve/Ve*	'Ponderosa di Sorrento'	43.6	*ve/ve*
'Sorrentino 1 sel1/03'	0.44	*Ve/Ve*	'Locale di Sorrento'	43.6	*ve/ve*
'Sorrentino 1 sel2/03'	0.44	*Ve/Ve*	'Rosa di Rotonda'	68.2	*ve/ve*
'Sorrentino 1 sel3/03'	0.09	*Ve/Ve*	'Locale Valle d'Agri'	62.2	*ve/ve*
			'L.16-94'	30.7	*ve/ve*
			'Sorrentino 1 sel4/03'	39.0	*ve/ve*
			'Rosa F2 195'	11.9	*ve/ve*

[a]The resistance was assessed by recording the disease incidence and grading the disease severity both on leaves and stems, and the xylem discoloration 60 days post-inoculation on root-dip inoculated plants. The Infection coefficient is a synthetic disease index = (disease severity * disease incidence)/100.
[b]The genetic profile was determined by CAPS markers designed on the *Ve2* gene.

(1969); likely these discrepancies may be explained by the fact that *Vd* is a low specialized fungus with a broad host range, which population consists of a sum of biotypes with a variable pathogenicity (Matta et al. 1980; Zeise and Tiedemann 2002; Vallad et al. 2004; Usami et al. 2007). *Vd* strain V7 produced varying degrees of disease severity in landraces typed having the same allelic constitution at *Ve*. Thus, 'Florida Ve', selections of 'Rosa F2', 'Sorrentino', and 'Locale di Albenga 3' were apparently more resistant than 'Locale di Cuneo' or 'Locale di Carmagnola' selections. It is likely that additional genes modulate the response to *V. dahliae* race 1 in tomato as suggested by Okie and Gardner (1982) and shown in eggplant (Sunseri et al. 2003). The presence of minor genes is probably the basis for the wilt tolerance of 'Locale di Valle d'Agri', which lacks both *Ve1* and *Ve2*. More recently, Fradin et al. (2009) demonstrated *via* Virus-Induced Gene Silencing that many other factors are involved in Ve-signaling cascade and that their inhibition leads to a modulation of *Ve*1-mediated Verticillium resistance; and Ellendorff et al. (2009) showed RNA silencing is required for genome stability and plant protection against Verticillium wilt in *Arabidopsis*. Therefore, future experiments will be addressed to investigate the possibility that RNA silencing or/and sequence polimorphisms in the components of *Ve*-signaling cascade could be responsible of the differences in the level of Verticillium resistance of the cultivars used in this study.

As expected, the resistance to Verticillium wilt conferred by the *Ve* genes, to 'Florida Ve' and 'Sorrentino', was ineffective against *Vd* race 2, as reported elsewhere (Alexander 1962; Cirulli 1969; Tjamos 1980; Okie and Gardner 1982).

The presence of *Ve* alleles in Italian landraces must reflect farmer selection, since the wilt fungus is endemic in all Italian tomato growing areas. As the known source of these resistance alleles is South-American (Schaible et al. 1951), the inference is that the *Ve* gene my have been introgressed into several local Italian landraces by spontaneous or unintentional hand made crosses grown under infection pressure, however the fact that 'Sorrentino' is grown in domestic gardens around Sorrento (Naples), and that 'Ligure' type landraces are characterised by the presence of the *ovate* mutation (Sabatini et al. 2008) suggest that hybridization was a rare event. So we can suppose that some among the tomato genotypes introduced in the past into Italy were caring *Ve* alleles that were conserved along the time by some landraces selected under disease pressure: both 'Sorrentino' and 'Locale di Albenga' may be an example.

In conclusion, it is apparent that the selection within landraces has given rise to breeding material which has desirable properties, reduced defects along with a stable and satisfactory level of resistance to Verticillium wilt caused by *Vd* race 1.

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