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Phenotypic and molecular diversity in a collection of 'Pomodoro di Sorrento' Italian tomato landrace

Mario Parisi[a,*], Riccardo Aversano[b], Giulia Graziani[b,1], Valentino Ruggieri[b], Veronica Senape[c], Loredana Sigillo[c], Amalia Barone[b]

[a] Consiglio per la ricerca in agricoltura e l'analisi dell'economia agraria, Centro di ricerca per l'Orticoltura, Via Cavalleggeri, 25-84098, Pontecagnano Italy
[b] Department of Agricultural Sciences, University of Naples 'Federico II', Via Università, 100-80055, Portici, Italy
[c] Consiglio per la ricerca in agricoltura e l'analisi dell'economia agraria, Centro di sperimentazione e certificazione delle sementi, S.S. 18 km 77.700, 84091, Battipaglia, Italy

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ABSTRACT

The 'Pomodoro di Sorrento', originating from Southern Italy, represents one of the most important and remunerative Italian tomato landraces for "fresh market". Despite its outstanding organoleptic and nutritional qualities scanty information still exists on the structural variation of this traditional tomato. Here we report the evaluation of ten accessions of the 'Pomodoro di Sorrento' tomato at phenotypic and molecular levels in order to provide a basis to unequivocally distinguish them from those belonging to the other most famous similar landraces grown in Italy. Morphological characterization based on 13 descriptors highlighted the presence of a significant level of variation for puffiness, green color and green shoulder of unripe fruits, allowing distinguishing two 'Sorrento' morphotypes. Genome-wide SNP genotyping was carried out using the ILLUMINA Infinium tomato array and revealed a genetically well defined structure of the 'Sorrento' landrace with respect to the other multilocular landraces most widespread in Italy. In particular, 1450 SNPs resulted polymorphic among 20 tested genotypes, and one clearly distinguishes the 'Sorrento' from other similar landraces. Finally, resistance assay to *Verticillium* wilt allowed the identification of promising 'Sorrento' accessions. Interestingly two markers localized into the Ve2 gene showed a strong association with the resistance/susceptibility trait. Taken as whole, our results provide a description of 'Pomodoro di Sorrento' landrace diversity, which provide useful information for its utilization in breeding programs as well as for its direct use in quality markets.

1. Introduction

The cultivated tomato (*Solanum lycopersicum* L.) was introduced in Italy in the early 16th century, founding in this country an important secondary center for diversification, which resulted in a wide array of variations especially for the traits determining fruit shapes (e.g., flat, round, long, heart) and quality traits (Mazzucato et al., 2010). Such phenotypic variation has given rise to a range of landraces, many of them are still commonly cultivated and are found at local market for fresh consumption ("salad tomatoes"). Several traditional tomatoes are currently cultivated in Italy, such as 'Pera Ligure o di Albenga', 'Pera d'Abruzzo', 'Canestrino di Lucca', 'Pomodoro di Belmonte', 'Scatolone di Bolsena', 'Costoluto rosso di Rotonda', 'Costoluto fiorentino', 'Pantano romanesco', 'Cuore di Bue', 'Pomodoro di Sorrento', 'Pomodoro piatto di Cambiano' (Mazzucato et al., 1998, 2008, 2010; Acciarri et al., 2007; Parisi et al., 2008).

The 'Pomodoro di Sorrento' tomato (also called 'Sorrentino' or 'Rosa di Sorrento') comes from the Southern Italy. Its cultivation began in early 1900s near Sorrento, where some local ship-owners introduced the seed brought from South America (Parisi et al., 2008). Since then, this landrace spread throughout the surrounding region, especially as a protected cultivation. Similar to 'Cuore di Bue' and 'Belmonte' landraces, the 'Pomodoro di Sorrento' ripe fruits are pink, due to the *colorless epidermids* (*y*) mutation (Mazzucato et al., 2010). Furthermore, 'Pomodoro di Sorrento' fruits are fairly homogeneous in their size and usually grouped in clusters of 3–5 per truss; in cross section they show a different grade of puffiness and very fleshy locules with a low number of seeds (Parisi et al., 2008). Moreover, the 'Pomodoro di Sorrento' landrace shows outstanding organoleptic and nutritional qualities (Sinesio et al., 2007; Ercolano et al., 2008; Lisanti et al., 2008) that are greatly

* Corresponding author.
E-mail address: mario.parisi@crea.gov.it (M. Parisi).
[1] Present address: Department of Pharmacy, University of Naples 'Federico II', Via Domenico Montesano, 49-80131 Napoli, Italy.

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appreciated by consumers, who often are willing to pay a premium than commercial modern varieties. The promotion of traditional tomatoes that combines good organoleptic qualities and functional compounds would reinforce their quality niches and consolidating their premium prices (Cortés-Olmos et al., 2014).

To clearly distinguish this "high-valuable" 'Sorrento' from other similar tomatoes, a protection system based on the use of molecular markers would be beneficial. The current availability of cost-effective and fast genotyping assays has made the genetic analysis more feasible and informative. In particular, the single nucleotide polymorphisms (SNPs) are the markers of choice for genome-wide genetic analyses. For tomato, a high-density SNP array was recently built (Sim et al., 2012) in the framework of the The Solanaceae Coordinated Agricultural Project (SolCAP). The array consists of 7720 SNPs, identified by the re-sequencing of six tomato accessions (Hamilton et al., 2012). It was recently used for genome-wide association analyses (Ruggieri et al., 2014; Sauvage et al., 2014), for phylogenetic survey of different *S. lycopersicum* populations (Blanca et al., 2012; Sim et al., 2012) and for functional studies (Hirakawa et al., 2013). Given that, the SolCAP represents an efficient tool for exploring natural genetic diversity within the 'Sorrento' landrace.

In the present work we aimed to provide the first wide description of 'Sorrento' landrace diversity. An exhaustive survey at morphological and molecular level was carried out to provide a structure of variation of 'Sorrento' accessions useful to unequivocally distinguish them from tomatoes belonging to the other most famous similar landraces grown in Italy. In addition, the 'Sorrento' accessions were also characterized for resistance to one soil-borne pathogen causing extensive damage to tomato cultivation. The data obtained would be of great interest for the valorization of this landrace, and for its utilization in breeding programmers or direct use in markets of high quality products.

2. Material and methods

2.1. Plant materials

Ten tomato accessions representative of the 'Sorrento' landrace (PS01, PS04, PS05, PS06, PS07, PS08, PS09, PS10, PS11 and PS13) were compared to ten Italian landraces showing similar fruit morphology. These genotypes were 'Belmonte', 'Canestrino di Lucca', 'Costoluto Fiorentino', 'Costoluto Rosso di Rotonda', 'Cuore di Bue', 'Pantano romanesco', 'Parmitanella', 'Pera d'Abruzzo', 'Pera d'Albenga', 'Scatolone di Bolsena' and hereafter coded as: BELM, CANL, FIOR, ROTN, CBUE, PANT, PARM, PABR, PALB, BOLS, respectively (Table 1).

All genetic materials were provided by local farmers o by seed companies and had undergone two cycles of self-pollination before being used. A trial was set up under greenhouse with the above-described seed stocks at the experimental farm of 'CREA-Centro di ricerca per l'Orticoltura', Battipaglia (40°37′00″N; 14°58′00″E; 65 m a.s.l.; South Italy). The accessions were grown at a density of 3.7 plants m^{-2}, according to a randomized block design with three replicates and 10 plants per elementary experimental unit.

Before transplanting 50, 100 and 100 kg ha^{-1} of N (as ammonium sulfate), P (as mineral perphosphate), and K (as potassium sulfate) respectively, were distributed. Starting from full-blossom, 75 kg ha^{-1} of N were further applied by five fertigation applications. Crop protection against attacks of *Tuta absoluta* (Meyrick), *Trialeurodes vaporariorum* (Westwood), *Frankliniella occidentalis* (Pergande) and *Tetranychus urticae* (Koch) were ensured by applications of commercial pesticides containing spinosad and spiromesifen as active ingredients. Moreover to control some phytopatogenic fungi, such as *Alternaria alternata* [(Fr.) Keissl.], *Phytophthora infestans* [(Mont.) de Bary], *Botrytis cinerea* (Pers.) and *Leveillula taurica* [(Lév.) G. Arnaud], treatments based on boscalid + pyraclostrobin, mandipropamid, copper oxychloride and wettable sulfur were adopted. The cultivation techniques included stakes as support and galvanized wires and weed control was obtained using black plastic mulch film.

2.2. Morphological analysis

To assess peculiar features of 'Sorrento' tomato in respect to similar Italian landraces and to estimate morphological variability at intra-landrace level, 13 traits (one for inflorescence and 12 for fruit) were scored or measured, on single plant basis. Inflorescence type (IT) was evaluated on the 2nd truss (1, simple; 2, double; 3, compound). Mean fruit weight (FW) (grams) and external/internal fruit features were assessed on 10 representative fruits. The external

Table 1
List of 'Sorrento' tomato accessions and similar landraces assessed for phenotypic and molecular traits.

Accession Code	Landrace	Origin	
		Source	Site
PS01	'Sorrento'	CREA-ORT[a]	Pontecagnano (SA)
PS04	'Sorrento'	CREA-ORT[a]	Piano di Sorrento (NA)
PS05	'Sorrento'	La Semiorto[c]	Piano di Sorrento (NA)
PS06	'Sorrento'	CREA-ORT[a]	Piano di Sorrento (NA)
PS07	'Sorrento'	CREA-ORT[a]	Massa Lubrense (NA)
PS08	'Sorrento'	CREA-ORT[a]	Massa Lubrense (NA)
PS09	'Sorrento'	CREA-ORT[a]	Pagani (SA)
PS10	'Sorrento'	CREA-ORT[a]	Vico Equense (NA)
PS11	'Sorrento'	CREA-ORT[a]	Piano di Sorrento (NA)
PS13	'Sorrento'	CREA-ORT[a]	Piano di Sorrento (NA)
BELM	'Belmonte'	CREA-ORT[a]	Amantea (CS)
CANL	'Canestrino di Lucca'	CREA-ORT[a]	Lucca (LU)
FIOR	'Costoluto Fiorentino'	CREA-ORA[b]	Firenze (FI)
ROTN	'Costoluto rosso di Rotonda'	CREA-ORT[b]	Rotonda (PZ)
CBUE	'Cuore di Bue'	Sativa[c]	Cesena (FC)
PANT	'Pantano romanesco'	CREA-ORT[a]	Albano Laziale (RM)
PARM	'Parmitanella'	CREA-ORT[a]	Pontecagnano (SA)
PABR	'Pera d'Abruzzo'	Semencoop[c]	Cesena (FC)
PALB	'Pera di Albenga'	Semencoop[c]	Cesena (FC)
BOLS	'Scatolone di Bolsena'	CREA-ORA[b]	Bolsena (VT)

[a] CREA-ORT [(Consiglio per la ricerca in agricoltura e l'analisi dell'economica agraria, Centro di ricerca per l'Orticoltura (Pontecagnano – Italy)].
[b] CREA-ORA [(Consiglio per la ricerca in agricoltura e l'analisi dell'economica agraria, Unità di ricerca per l'Orticoltura (Monsampolo del Tronto – Italy)].
[c] Seed Company.

traits analyzed were: green shoulder (GS) (1, absent; 4, very strong); intensity of green color excluding shoulder (GCF) (1, very light; 5, dark); fruit shape in longitudinal section (FSL) (1, flattened; 2, oblate; 3, circular; 4, cordate; 5, obcordate); fruit shape cross section (FSC) (1, circular; 2, angular; 3, irregular); ribbing at peduncle end (RIB) (3, weak; 5, medium; 7, strong; 9, very strong); stem-end shape (SES) (3, weak; 5, medium; 7, strong); blossom-end shape (BES) (1, indented; 2, indented to flat; 3, flat; 4, elongated); fruit color (FC) (3, orange; 4, pink; 5, red). Internal fruit traits detected were: puffiness (PUF) (1, no puffy; 4, very puffy); fruit flesh color (FLC) (3, orange; 4, pink; 5, red); pericarp thickness (PT) (mm). FW, FSL, FSC, RIB, SES, BES, FC, FLC, PUF and PT were assessed on full-ripe fruits, while GS and GCF on unripe fruits.

2.3. Molecular analysis

Genome-wide SNP genotyping was performed using the ILLUMINA Infinium array built in the framework of the Solanaceae Coordinated Agricultural Project (SolCAP) from NIFA/USDA. The SolCAP tomato panel includes 7720 markers constructed on EST-SNPs (eSNPs) deriving from six tomato genome sequences (Sim et al., 2012). The probe sequences and SNP information are available at The Solanaceae Coordinated Agricultural Project web site (http://solcap.msu.edu) and the map of the SNPs on the current tomato genome release (SL2.50) is available on the SGN database (http://solgenomics.net/). For each accession, genomic DNA was extracted from leaf tissue using the DNeasy Plant Mini kit (QIAGEN, Valencia, CA) according to the manufacturer's recommendations and then evaluated for quality and concentration by the Nanodrop instrument (Thermo Fisher Scientific, Wilmington, USA). Genotyping was conducted at the Genomix4Life S.r.l (http://www.genomix4life.com) and the data obtained were analyzed using the Genotyping Module of the GenomeStudio software version 1.7.4 (Illumina Inc., San Diego, CA, USA). In order to perform molecular analyses, the set of SNPs was filtered considering a threshold of MAF >10% by using TASSEL program (Bradbury et al., 2007). A neighbor-joining tree was generated using PAST program (Hammer et al., 2001) with a bootstrap of 1000. A Bayesian population classification was also carried out using STRUCTURE 2.3.3 (Pritchard et al., 2000). STRUCTURE runs were carried out with a length of burn-in and MCMC (Markov chain Monte Carlo) of 500,000 each. Twelve independent runs were conducted allowing K (number of populations) varying from 2 to 14. Optimal K was inferred by using Structure Harvester program (Earl and von Holdt, 2012) based on the Evanno method (Evanno et al., 2005).

2.4. Resistance assay

Resistance to *Verticillium dahliae* (Cooke) Wint. race 0 was evaluated by performing *in vivo* tests (CPVO protocol TP/44/3) (European Union - Community Plant Variety Office, 2007) on 20 plants for each accession. Roots of plantlets with expanded cotyledons were cut and soaked in a conidia suspension at a concentration of 1×10^6 conidia/ml. Typical symptoms of *Verticillium* wilt were recorded during four weeks after inoculation. Results were expressed as number of diseased plants on the number of inoculated plants. A sample was considered susceptible when it showed more than the 20% of diseased plants. Resistant tests were carried out in climatic chamber, at temperature of $23 \pm 1\,^{\circ}C$, 12 h light, by using the pathogenic strain (code Vert5) of *V. dahliae* race 0 from 'CREA-Centro di sperimentazione e certificazione delle sementi' collection. The variety 'Gianna' was used as reference susceptible control.

2.5. Statistical analysis

To test for correlations between genetic and morphological data, a Mantel test was performed (Mantel, 1967) using the Pearson's r-value. Hierarchical clustering analysis (HCA) was performed using Pairwise Manhattan Distance to systematically analyze and visualize the full set of morphological data. XLStat v.2013 (Addinsoft, New York, NY) was used for statistical analyses, except for correlation analysis, which was carried out using R version 3.2.1 (2015-06-18).

3. Results

3.1. Morphological assessment

The fruit morphological traits are summarized in Supplementary Table 1. Within the 'Sorrento' collection five accessions (PS01, PS04, PS08, PS11 and PS13) had fruits weighting from 179.9 to 241.7 g, four accessions (PS06, PS07, PS09 and PS10) from 241.8 to 303.8 g, PS05 had the biggest berries (333.1 g). FW of PARM, FIOR, CBUE landraces was smaller than those of 'Sorrento' accessions (Fig. 1A). Regarding the PT, the ten 'Sorrento' accessions studied showed values ranging from 5.71 mm to 7.62 mm. CBUE and PARM differed from the overall 'Sorrento' accessions for the presence of a thin pericarp, on the contrary CANL and PALB tomato had thicker pericarp (Fig. 1B).

Within 'Sorrento' collection, GS was strong (PS01, PS08, PS09, PS11, PS13) or very strong (PS04, PS05, PS06, PS07, PS10). The CANL, FIOR, PANT, PARM, BOLS landraces differed from the 'Sorrento' accessions for their weak-colored green shoulder, while this trait was absent for PALB landrace (Fig. 1C). As for FSL, all 'Sorrento' accessions showed rounded fruits, which discriminated them from the ten similar landraces included in our work (Fig. 1D). Unlike 'Sorrento' genotypes, BOLS, PANT, CANL, PARM, PALB, PABR and ROTN fruits displayed a strong or very strong degree of ribbing at peduncle end (Fig. 1E). Finally, puffiness (PUF) of 'Sorrento' accessions differed from FIOR, ROTN, CBUE, PANT, PARM (no puffy) and from BOLS (very puffy) (Fig. 1F). SES shows moderate variability among the 20 genotypes studied and may be useful to distinguish all the 'Sorrento' accessions from seven similar landraces (CBUE, BOLS, FIOR, PABR, PANT, PARM and ROTN) (Fig. 1G). Finally FC was pink for BELM, CBUE and for all 'Sorrento' tomatoes, while the fruits of other landraces were red or orange (BOLS). Regarding IT, GCF, FLC and BES, a low variability was observed for these traits. Therefore they failed to describe the diversity within the 'Sorrento' collection and to well distinguish them from similar landraces.

Grouping of genotypes based on the 13 qualitative morphological traits allowed to divide them into two main clusters (M1 and M2) (Fig. 2, Supplementary Table 1).

The first group (M1) comprised mostly the Sorrento-like Italian landraces, except for BELM and CBUE, which clustered in the M2 along with all true 'Sorrento' accessions. Overall, the landraces belonging to the M1 group showed flattened, oblate or obcordate fruit shape in longitudinal section, irregular FSC and orange/red external fruit color. Two landraces (PALB and CANL) with obcordate fruits exhibiting low puffiness and highest thickness of pericarp slightly diverged from the other M1 genotypes. On the other hand, the second major group included landraces with circular or cordate (heart-shaped) fruits showing circular or angular shape in cross section, externally pink-colored as well as the fruit flesh. Within the M2 group, an intra-landrace variability was observed for the 'Sorrento' tomatoes which allowed to further divide them into two sub-clusters: the first grouping accessions characterized by very strong green shoulder (PS04, PS05, PS06, PS07 and PS10), highest values of GCF, weak degree of puffiness and ribbing at peduncle end, while the other sub-group clustered accessions with puffy fruits

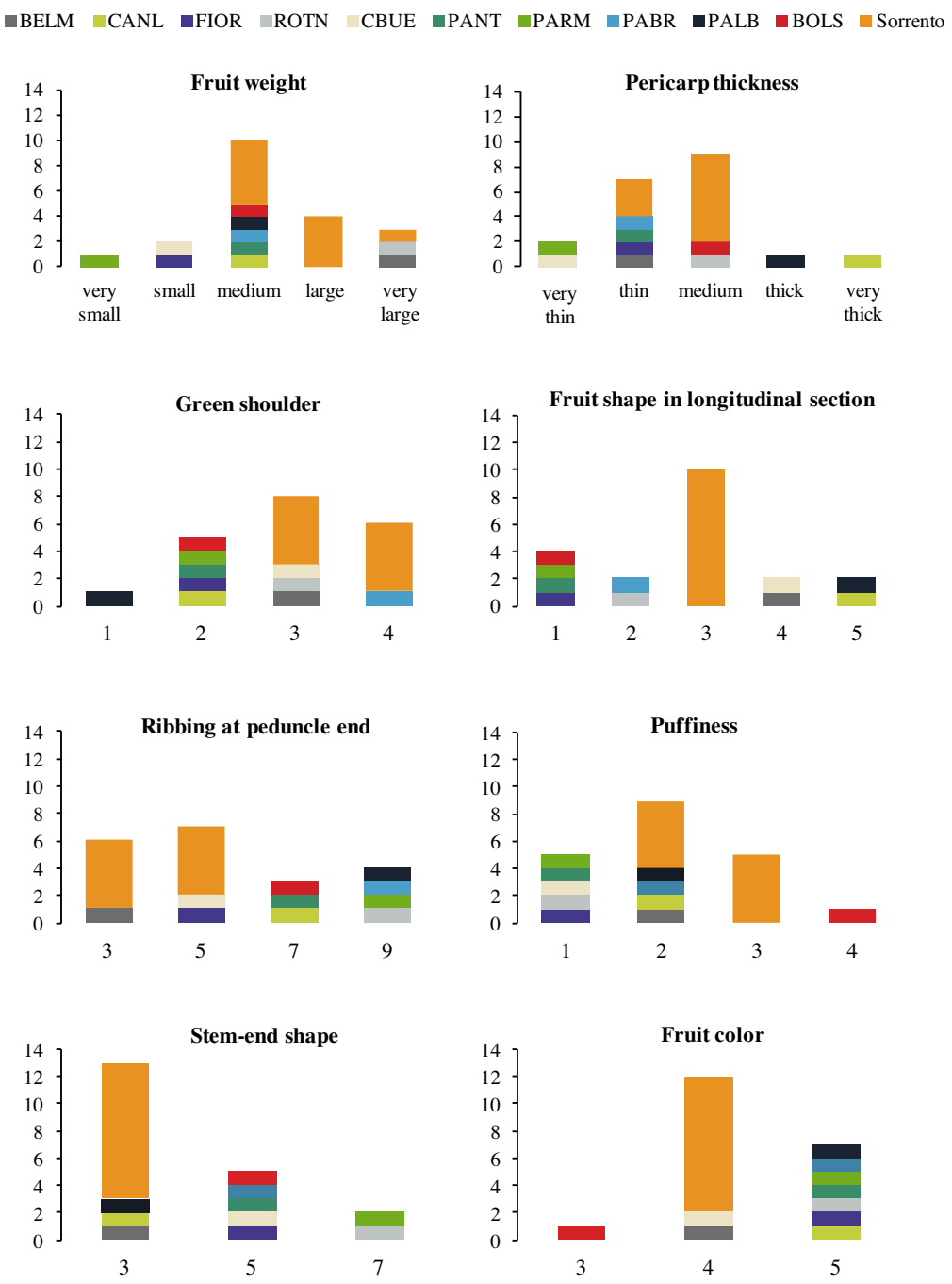

Fig. 1. Morphological assessment. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)
Number of accessions of the 'Sorrento' tomato (n = 10, orange bar) and other similar landraces (n = bar of each of them) falling into different classes for eight morphological descriptors. X-axes report class limits as dimension. Ordinate axes report absolute frequencies. Fruit weight: very small (55.5–117.5 g), small (117.6–179.6 g), medium (179.7–241.7 g), large (241.8–303.8 g), very large (303.9–365.9 g); Pericarp thickness: very thin (4.75–5.70 mm), thin (5.71–6.66 mm), medium (6.67–7.62 mm), thick (7.63–8.58 mm), very thick (8.59–9.54 mm); Green shoulder: from 1 (absent) to 4 (very strong); Fruit shape in longitudinal section; 1 (flattened), 2 (oblate), 3 (circular), 4 (cordate), 5, (obcordate); Ribbing at peduncle end: from 3 (absent) to 9 (very strong); Puffiness: from 1 (no puffy) to 4 (very puffy); Stem-end shape: 3 (weak), 5 (medium), 7 (strong); Fruit color: 3 (orange), 4 (pink), 5 (red).

showing medium values of GS and GCF (PS01, PS08, PS09, PS11 and PS13) (Fig. 3).

The Spearman rank correlation coefficients calculated between pairs of variables revealed how some were rather independent, whereas a group of traits clustered together because of a reciprocal tight correlation (Fig. 4). For example the high positive correlation found between RIB-SES, RIB-FC, FSC-FC, FSC-RIB, FLC-FSC, FLC-RIB, whereas the negative correlation between PUF-FC, PUF-SES and FSL-SES reflected the tendency for flattened/oblate fruits to have a strong ribbing and depression at peduncle end, irregular shape in cross section, external and internal red color and lowest values of puffiness. Moreover the fruits showing a little green shoulder seems to have high degree of ribbing, irregular shape in cross section and red-colored flesh (negative correlation GS-RIB, GS-FSC, GS-FLC). These features clearly identified the following landraces: ROTN, PARM, PANT, PABR, FIOR and BOLS. However, the latter landrace stands out a little from the previous group due to the remarkable puffiness and the orange color of the ripe fruits as above

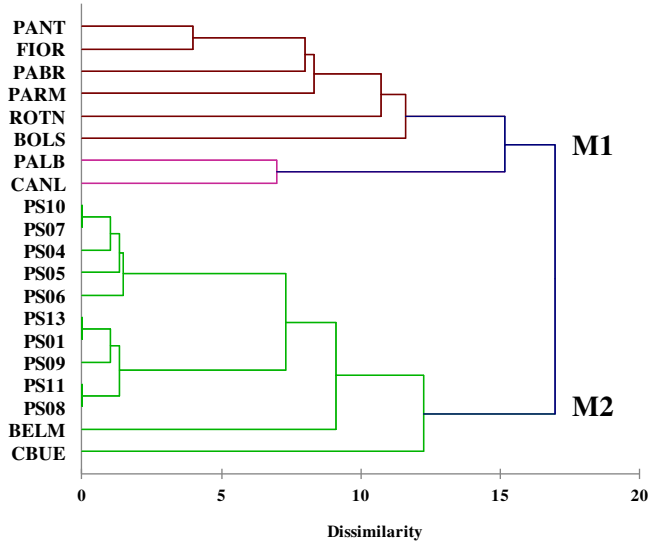

Fig. 2. Cluster analysis.
The dendrogram was built using 13 morphological traits evaluated on ten 'Sorrento' accessions and ten similar landraces.

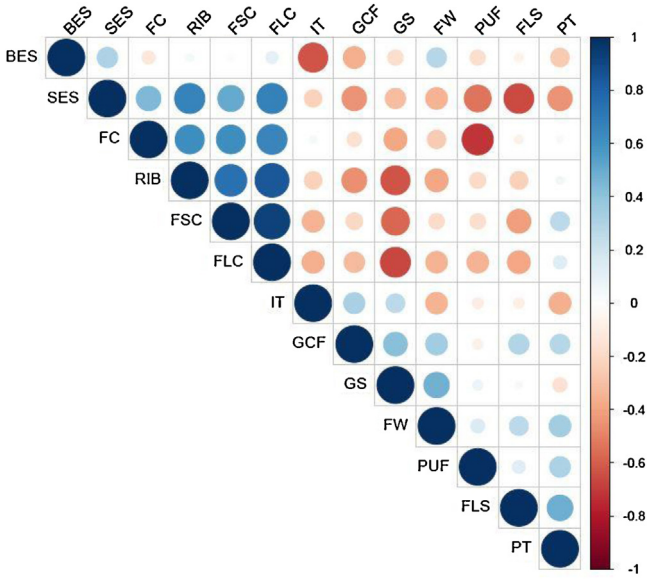

Fig. 4. Correlogram calculated using 13 morphological variables. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)
Positive correlations are displayed in blue and negative correlations in red color. Color intensity and the size of the circle are proportional to the correlation coefficients. In the right side of the correlogram, the legend color shows the correlation coefficients and the corresponding colors. IT (Inflorescence type), GS (Green shoulder), GCF (Intensity of green color excluding shoulder), FLS (Shape in longitudinal section), FSC (Shape in cross section), RIB (Ribbing at peduncle end), SES (Stem-end shape), BES (Blossom-end shape), FC (Fruit color), PUF (Puffiness), FLC (Fruit flesh color), FW (Fruit weight), PT (Thickness of pericarp).

mentioned. Most of genotypes showing compound inflorescences produced fruits with indented to flat blossom-end shape (negative correlation between IT-BES).

3.2. Genotyping

Out of 7720 scorable SNPs defined by the Infinium assay, 7639 (98.7%) were successfully genotyped and 1450 (19%) resulted polymorphic among the 20 tested lines with a MAF >10% (Supplementary Table 2). On average, around 120 SNPs for chromosome were surveyed. The highest number was scored for chromosome 11 (n = 258) and the lowest for chromosome 7 (n = 62).

According to LD decay values reported in Ruggieri et al. (2014), we selected a subset of 364 potentially unlinked SNPs for inferring population structure. The model used indicated K = 2 as the best number of sub-populations, providing support for the existence of two distinct clusters in our population. STRUCTURE results and Delta K plot are graphed in Fig. 5 and Supplementary Fig. 1, respectively. The genetic distances between all the lines were calculated based on the genotyping data of the informative SNP loci with a MAF >10%, and a dendrogram was then constructed (Fig. 5).

The two main clusters in the dendrogram corroborated the structure subdivisions. In particular the first cluster included nine out 10 'Sorrento' accessions, besides three similar landraces (CANL, PALB and PABR). By contrast, the second cluster included 8 genotypes, and only one (PS09) of them belonged to the 'Sorrento' group. Notably, PS10 and PS11, as well as genotypes PS04 and PS06, resulted highly similar at molecular level (99.5% and 99.4%, respectively). The 'Sorrento' accessions showed prevalently one ancestor (red bar), even though in three cases a reduced influence of the second ancestor (blue bar) was revealed, or a 50% admixture, as in case of PS09. Among the other Italian landraces, four of them (BOLS, FIOR, PART, PARM) showed only one ancestor, while the remaining six exhibited an admixture of two ancestors. Interestingly, the classification of the tomato landraces obtained with SNP data was complementary to that one drawn using morphological data, as revealed by the highly significant correlation between the two independent cluster analyses (r = 0.611, P = 0.0001, data not shown).

Fig. 3. Representative of 'Sorrento' morphotypes compared to other pink-colored genotypes. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)
Puffy/round-shaped fruits with medium shoulder and light green color at unripe stage (PS01); round-shaped fruits showing weak puffiness and dark shoulder, medium green color (PS04). 'Cuore di Bue' (CBUE) and 'Belmonte' (BELM) fruits are reported for comparison. For each accession, the corresponding code is reported at bottom. Bar is 1 cm.

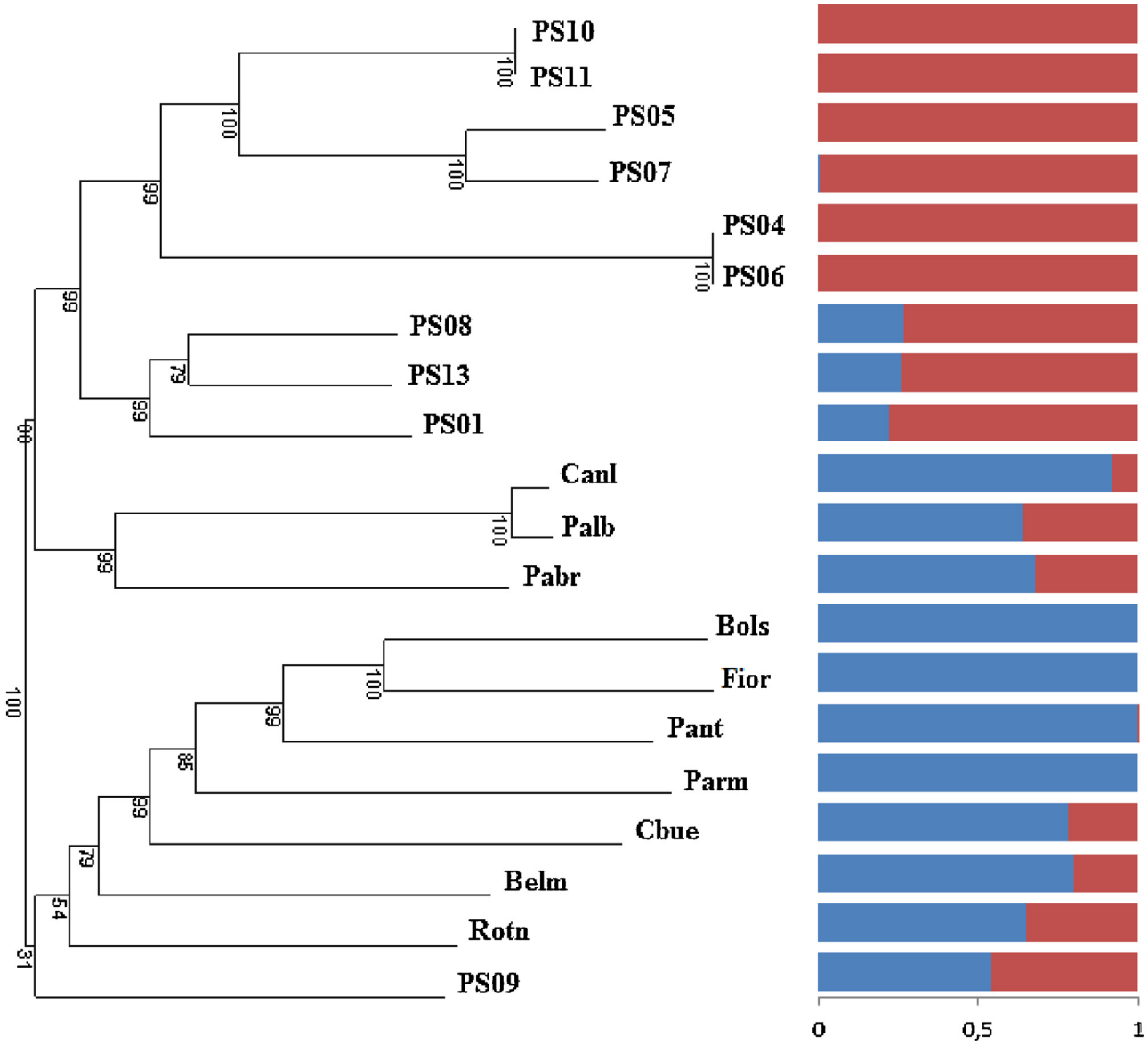

Fig. 5. Molecular screening of 20 genotypes by 1450 polymorphic SNPs.
Neighbour joining analysis was used to build the tree. Numbers at the nodes are bootstrap values for 1000 re-samplings. Structure for each genotype was also showed on the right side.

In order to enable the clear identification of 'Sorrento' tomato, a survey was carried out on the entire SNP collection to select markers that could distinguish this landrace unequivocally. A unique SNP (solcap_snp_sl_4963) was found to be specific for all 'Sorrento' accessions, except PS09. In particular, the homozygous locus GG (Supplementary Table 2) was detected in nine accessions representative of 'Sorrento' landrace, while the other genotypes showed the homozygous locus AA. The solcap_snp_sl_4963 falls in the 3′UTR of the gene Solyc01g068200.2 annotated as "Abscisic acid insensitive 8 homologue" mapping on chromosome 1.

3.3. *Resistance assay to* Verticillium dahliae

The resistance assay showed that four 'Sorrento' accessions (PS04, PS05, PS06 and PS07) were resistant to *V. dahliae* race 0, while the other six ones were susceptible (PS01, PS08, PS09, PS10, PS11 and PS13) (Supplementary Fig. 2). In order to identify putative markers associated with resistance to *V. dahliae,* SNPs within the genes *Ve1* (Solyc09g005090) and *Ve2* (Solyc09g005080) conferring resistance against this pathogen in tomato (Fradin et al., 2009) were sought. Survey on the SolCAP array highlighted six markers in the gene *Ve2* and none in *Ve1* (Fig. 6).

Only four markers in the *Ve2* gene (solcap_snp_sl_17547; solcap_snp_sl_17546; solcap_snp_sl_17545; solcap_snp_sl_17544) resulted polymorphic. Solcap_snp_sl_17545 and solcap_snp_sl_17544 showed a heterozygous condition for the minor and the major allele, respectively. Since these two markers are probably still segregating in the collection, albeit co-segregating with the others, we decided to focus only on the two homozygous markers solcap_snp_sl_17547 and solcap_snp_sl_17546.

Fig. 6 also shows the haplotype combinations of the markers aforementioned, and the resistance or susceptibility of the genotypes tested. Being the markers physically linked, their alleles co-segregated. Indeed only two haplotype combinations were detected. The first combination, solcap_snp_sl_17547(A) – solcap_snp_sl_17546(G), included all the six susceptible genotypes while the second, solcap_snp_sl_17547(C) – solcap_snp_sl_17546(A), included all the four resistant genotypes. This result suggested a strong association of these two markers with the resistance to *V. dahliae* race 0 in some of the 'Sorrento' acces-

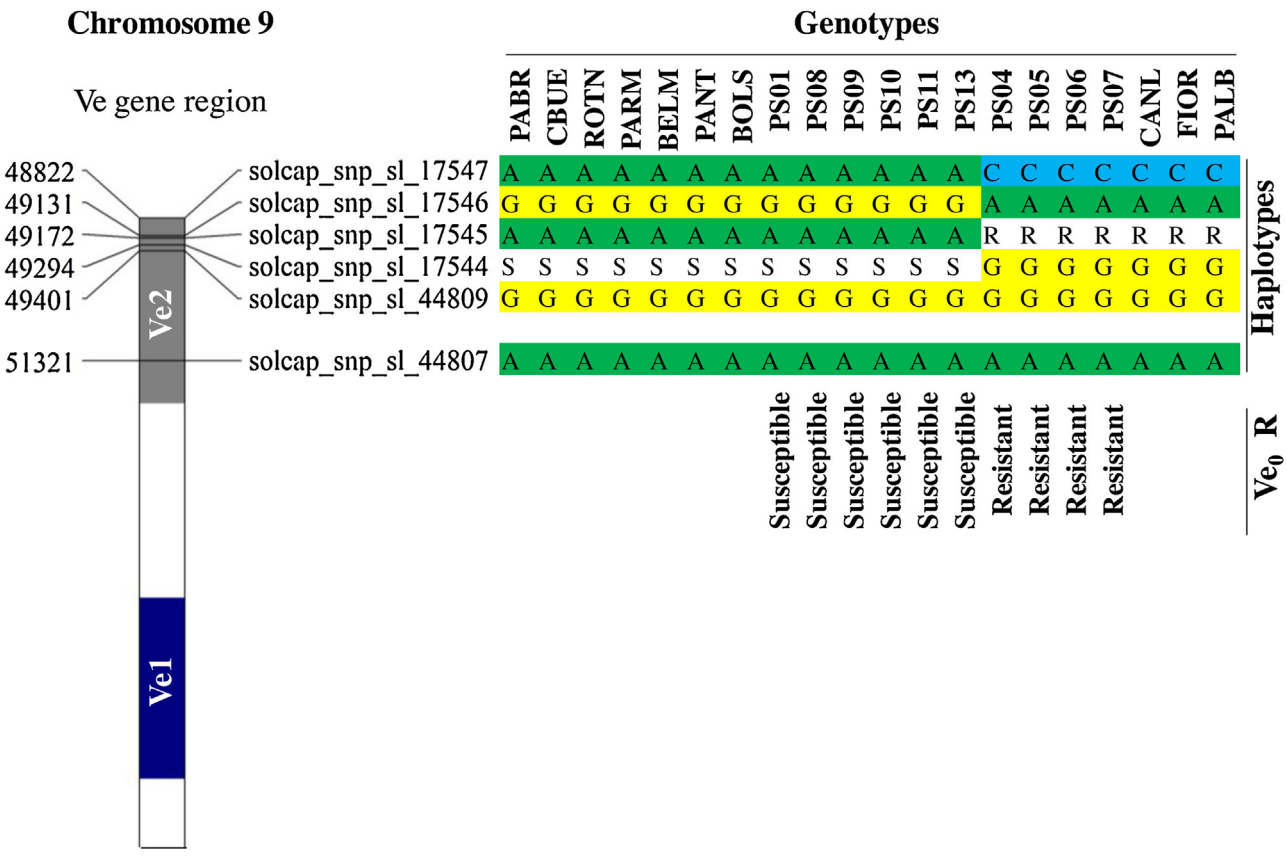

Fig. 6. SolCap Markers in the *Ve1* and *Ve2* genes on chromosome 9.
For each marker, the position in bp (on left side of the chromosome) and SolCap ID (on right side) are reported. Haplotype combinations of markers surveyed in the Ve2 gene and indication related to resistance/susceptibility to *Verticillium dahliae* race 0 (Ve_0) for 'Sorrento' genotypes is also reported. Letters of the haplotypes are referred to IUPAC nucleotide code.

sions, which could be considered efficient markers for screening genotypes for resistance to this soil-borne pathogen.

4. Discussion

The increasing demand for high-quality and high-status products has boosted in Europe the market for value-added products that carry a strong identification with a particular geographic region (McCluskey and Loureiro, 2003). Unfortunately, in Italy a great number of landraces have been dropped from the National Register, due to lack of commercial interest from major seed companies (Mazzucato et al., 2010). The only two tomato landraces that have been accepted into alternative variety protection systems are 'San Marzano' and 'Vesuviano' labeled as 'Pomodoro S. Marzano dell'Agro Sarnese-Nocerino' and 'Pomodorino del Piennolo del Vesuvio' (Protected Designation of Origin – PDO and Protected Geographical Indication – PGI, respectively). These two landraces have their own peculiar organoleptic and quality characteristics, as well as the 'Sorrento' tomato (Sinesio et al., 2007; Lisanti et al., 2008). Therefore, the same variety protection is desirable for the latter that, at this purpose, should be clearly distinguishable from other landraces characterized by multilocular fruits.

By combining a wide morphological and a high-throughput genotypic characterization, our study evidenced that is possible to clearly distinguish the 'Sorrento' landrace form other "salad" local varieties mostly commercialized on our national market of fresh tomatoes. Indeed, the morphological traits analyzed in our study might be used to discriminate 'Sorrento' tomato from other similar Italian landraces. For example, the round-shaped 'Abruzzese' or 'Pera d'Abruzzo' tomato differs from all 'Sorrento' accessions for its internal/external red-colored fruits showing high degree of ribbing at peduncle end. Furthermore 'Sorrento' landrace was clearly distinguished from other obcordate (CANL and PALB), flattened (BOLS, PARM, PANT and FIOR) and oblate (ROTN) tomatoes, while grouped with BELM and CBUE landraces, which show heart-pinked fruits and are sometimes wrongly referred to 'Sorrento'. In particular, BELM differs from 'Sorrento' for its elongated blossom-end shape and very big fruit weighting up to 1000 g. Circular shape in cross section, no puffiness and very thin pericarp discriminate CBUE tomato from all 'Sorrento' accessions.

In our study we exploited the high-throughput SolCAP platform to integrate morphological analyses in the clear differentiation between 'Sorrento' tomato and other multilocular Italian landraces. Up till now, highly informative genotyping systems have been investigated on large tomato germplasm collections aimed at studying population structure, tomato evolutionary history and the genetic architecture of agronomic traits (Ranc et al., 2008; Blanca et al., 2012; Shirasawa et al., 2013; Lin et al., 2014), but few regarded the molecular variability at intra-specific level (Andreakis et al., 2004; Rao et al., 2006; Mazzucato et al., 2010; Cebolla-Cornejo et al., 2013). Even though the morphological dendrogram clustered all the 'Sorrento' accessions in the same group together with CBUE and BELM, the high-throughput SNP analysis evidenced a strong clustering of 'Sorrento' accessions but with an outgroup position of PS09 accession. This is in line with the unique SNP (solcap_snp_sl_4963) that distinguished all 'Sorrento' types from the other genotypes, but not PS09, even when compared with a wider collection of around 120 landraces (Sacco et al., 2015). The different origin site of PS09, coming from Pagani (SA), might explain a diversified evolution of this accession from the others originating in the Sorrento penin-

sula. Moreover, only six 'Sorrento' accessions showed to share the same ancestor, while other four derived from two ancestors, as also happened for CANL, PALB, PABR, CBUE, BELM, ROTN and the outstanding PS09. The accessions showing one ancestor (PS10, PS11, PS05, PS07, PS04 and PS06) were probably grown in conditions of considerable sexual isolation. This hypothesis might be reasonable considering that the collecting area corresponds to a mountain geographical region (up to 400 mt a.s.l.), with marginal farming unsuitable for cultivation of landraces requiring high-fertility soils typical of Agro Sarnese-Nocerino valley.

When only the 'Sorrento' group was considered, the results of the present study highlighted an appreciable variability for fruit weight, puffiness, green color and green shoulder of unripe fruits, as it was also observed by Cortés-Olmos et al. (2015), when analyzed the variability of various Spanish landraces. In our work, according to fruit morphology it was possible to distinguish two 'Sorrento' morphotypes: puffy fruits with medium shoulder and light green color at unripe stage (PS01, PS08, PS09; PS11 and PS13) and fruits showing weak puffiness and dark shoulder, medium green color excluding the shoulder (PS04, PS05, PS06, PS07 and PS10). Different morphotypes have been already reported by Mazzucato et al. (2010) and Andreakis et al. (2004) for two other Italian tomato landraces ('A pera Abruzzese' and 'Corbarino'). Previous studies evaluated the genetic diversity and structure in collections of tomato landraces that also included 'Sorrento' accessions. García-Martínez et al. (2013) in a collection of 26 cultivated accessions belonging to four tomato types, 'Muchamiel' and 'De la Pera' from Spain, 'San Marzano' and 'Sorrento' from Italy, showed that the five 'Sorrento' accessions mostly grouped together, albeit the same genotypes were characterized by some degree of possible admixture with one from 'De la Pera' or the 'San Marzano' group. Corrado et al. (2014) characterized the population structure of 75 Italian landraces (mainly collected in the South) using a custom-made Illumina SNP-panel. The results highlighted that the landraces had a genetic structure mainly related to the fruit type, and that seven out of eight 'Sorrento' accessions were included in a cluster grouping with few other oxheart-shaped genotypes. Unfortunately, these studies only analyzed the landraces at the DNA level without combining molecular data with morphological ones.

To better select the most suitable 'Sorrento' accessions to be submitted to variety protection systems we carried out a resistance test to *Verticillium* wilt. In a previous study, Acciarri et al. (2010) tested a collection of 169 tomato landraces of different fruit type and provenience for reaction to infection with *V. dahliae*: some individuals of the 'Sorrentino' landrace were as resistant as 'Florida Ve' control cultivar. Similarly in our study some 'Sorrento' accessions exhibited resistance to this pathogen, thus confirming that it is possible to select accessions resistant to *V. dahliae* race 0 that are promising for breeding purposes. We also detected two polymorphic SNPs in the gene Ve2, mapping on chromosome 9, that might be suitable in the future to carry out a marker-assisted selection of resistant genotypes.

5. Conclusions

Our data showed that the 'Sorrento' tomato is well distinct from other multilocular landraces most widespread in Italy. Even the two pink-colored traditional tomatoes 'Cuore di Bue' and 'Belmonte', often wrongly referred to 'Sorrento', can be clearly distinguished from the latter landrace.

Thanks to the large genotyping performed, we identified one SNP marker that clearly distinguishes the 'Sorrento' from other similar landraces. The possibility of using a single SNP marker to discriminate 'Sorrento' accessions from similar/confounding genotypes would be an efficient and cost-effective way to characterize this landrace. This will be a crucial step to defend the quality markets associated with this landrace and to control the commercial fraud associated with it.

Resistance assay for *Verticillium* wilt allowed for the identification of promising 'Sorrento' accessions and also for the identification of two SNP markers suitable for genotyping for the susceptibility to this soil-borne pathogen.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.scienta.2016.02.038.

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