



Genetic diversity and distinctiveness in tomato (*Solanum lycopersicum* L.) landraces: The Italian case study of 'A pera Abruzzese'

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ABSTRACT

To assess the intra-population genetic variability and draw criteria for distinctiveness, 25 accessions belonging to the Italian tomato landrace 'A pera Abruzzese' were analyzed with morphological and molecular descriptors and compared with the similar landraces 'Canestrino di Lucca' and 'Cuore di bue di Albenga'. Whereas intra-accession variation for qualitative morphological descriptors was low, inter-accession variability was high. Accessions from the 'Abruzzese' landrace could be separated into three groups according to the fruit shape, i.e. flat, round and obovoid. This variability was studied in detail by digital analysis of fruit sections using the software programme "Tomato Analyzer". Four descriptors were effective in distinguishing the 'Abruzzese' round-fruited types from the 'Canestrino' and 'Albenga' controls. The molecular analysis, based on 11 polymorphic microsatellite markers, differentiated the 'Abruzzese' from the 'Canestrino' type, but was not able to separate the former from the 'Albenga' type. Although morphological and molecular descriptions did not correlate, the data presented provide a basis for distinguishing those 'Abruzzese' accessions that are unequivocally distinct from similar landraces cultivated in different regions. In addition to the specific objectives, the research represents a case study for the description of the variability that is often found within tomato landraces and an approach to identify (groups of) accessions that are eligible for distinction, protection and commercial exploitation.

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1. Introduction

The tomato (*Solanum lycopersicum* L.) was first introduced into Europe from Central and Southern America at the beginning of the 16th century and cultivated as an ornamental. In the 17th century the species gained popularity as an edible product and its cultivation spread rapidly throughout the Old World. This introduction resulted in a genetic bottleneck, narrowing the genetic diversity of the cultivated germplasm in Europe (Rick, 1976). The genetic heritage of the tomato was further eroded by the development of vintage and modern cultivars, when much of the original diversity within the cultivated *S. lycopersicum* was lost (Williams and St. Clair, 1993).

Despite the significant loss of genetic diversity and in stark contrast to the invariably round-shaped fruit of its wild relatives, the cultivated tomato displays a large diversity of fruit morphology. It is now accepted that this morphology is based on the allelic variation of a relatively small number of genes that recombined after domestication and during the early cultivation of the species

(Tanksley, 2004). In Europe, the tomato has been most successful in the Mediterranean countries, including Spain and Italy (Soressi, 1969; García-Martínez et al., 2006). In these countries, *S. lycopersicum* found a secondary centre for diversification (Bailey et al., 1960) which resulted in a wide array of variations including round, obovoid, long, heart, rectangular and even bell pepper-shaped fruits. This variation has given rise to a range of landraces that have been cultivated for centuries and many of these are still commonly found at local markets (Soressi, 1969; García-Martínez et al., 2006).

Among the tomato landraces destined for fresh consumption, the so called 'salad tomatoes', those with large isodiametric fruits are widely grown in both Spain and Italy (García-Martínez et al., 2006; Acciarri et al., 2007; Mazzucato et al., 2008). In many of these types the uneven development of the equatorial diameter at the blossom or stem end causes the fruit to develop a typical pear (obovoid) or heart shape and this distinctive feature is reflected in the name given to the particular landrace.

The landrace under study, known as 'A pera Abruzzese', is widely cultivated in Central Italy in the Northern part of the Abruzzo region, along the Adriatic sea. This landrace, which has a local niche market where it is appreciated for its outstanding organoleptic qualities, commonly includes both plants that produce spherical and obovoid-shaped fruit (Sabatini et al., 2006). Morphologically

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very similar to the 'A pera Abruzzese' type are the so called 'Cuore di bue di Albenga' (from Liguria; the name recalls an heart but the fruit shape is actually obovoid) and the 'Canestrino di Lucca' (from Tuscany) landraces. Whereas the above mentioned landraces have pear-shaped fruits, the 'Cuore di bue' tomato (different from 'Cuore di bue di Albenga') differs because of the wider extension of the stem end diameter compared to the blossom end, resulting in a characteristic heart shape. This tomato, which appears to have originated in Sicily, differs from the pear-shaped tomatoes in that its fruit is pink, due to the *colorless epidermis* (*y*) mutation. Finally, another round, multilocular tomato, known as 'Pomodoro di Sorrento', is cultivated in the Campania region. Like the 'Cuore di bue', the 'Sorrento' landrace also expresses the *y* phenotype giving the fruit a typical pinkish tone (Parisi et al., 2008).

Although these typologies are popular in the marketplace for their organoleptic qualities and their characteristic traditional shapes, the intra-landrace levels of genetic variability as well as their genetic relationships have been never studied in detail, nor have they ever been described according to clear and distinctive criteria. In this study, we set out to examine the genetic variability present within the 'Abruzzese' landrace, with the aim of establishing the extent and nature of intra-landrace variability with special reference to fruit size and shape. With this information we aim at defining the criteria that will allow us to distinguish unequivocally 'Abruzzese' accessions from those belonging to other landraces with similar fruit attributes.

2. Materials and methods

2.1. Plant material and morphological analysis

Twenty-five tomato accessions representative of the 'A pera Abruzzese' landrace (hereafter referred to as 'Abruzzese') have been collected from farmers in the Abruzzo and in the nearby Marche region (Table 1). Two accessions belonging to 'Canestrino di Lucca' (hereafter referred to as 'Canestrino') and three to 'Cuore di bue di Albenga' (hereafter referred to as 'Albenga') were used for comparison. The accession codes can be explained briefly as follows; the 'Abruzzese' types were given the symbol AB followed by two letters referring to the category of fruit shape (FL, flat; RD, round; OB, obovoid) and a progressive number. 'Canestrino' and 'Albenga' types were given the codes CAN and ALB respectively followed by a numeral (Table 1). The seeds for all the accessions were multiplied at the Research Unit for Horticultural Crops, Agricultural Research Council, Monsampolo del Tronto (Ascoli Piceno), Italy. A first field trial was established with the 25 'Abruzzese' accessions in 2007, in the summer growing season. The accessions were grown at a density of 2.2 plants m⁻², according to a randomised block design with three replicates and 12 plants per elementary experimental unit. Plants were grown following standard agronomic practices. At maturity, the number of commercial fruits, the mean fruit weight and the estimated potential production were recorded or calculated, after pooling the harvest of the first five trusses.

A second field trial, including the control accessions, was established in 2008 using the same experimental design and agronomic practices. On a single plant basis, the following traits were scored or measured: flowering date (days from sowing to the first open flower), inflorescence type (scored on the 2nd truss; 1, simple; 2, double; 3, compound), number of flowers per inflorescence (counted on the 2nd truss), style exertion (scored on the 2nd truss; 1, inserted; 4, very exerted), green shoulder (scored on a representative mature green fruit; 1, absent; 7, very strong), fruit shape (scored on representative red ripe fruits; 1, flat; 2, round; 3, obovoid). Mean fruit weight was calculated as the mean of six fruits per plant and the potential productivity, expressed in t ha⁻¹,

was estimated on the basis of the individual production and the standard plant density in open field production of fresh tomatoes.

With the exception of fruit shape that represented a purely categorical variable, differences in mean values between accessions were estimated through an analysis of variance adopting the General Linear Model (GLM) using the SAS software (SAS Institute Inc., 2002). Differences in the mean number of fruits per plant and fruit weight as estimated in two different cultivation years were estimated according to the GLM for combined experiments (McIntosh, 1983).

2.2. Morphometric analysis of fruit shape

To estimate fruit shape in more detail, the longitudinal sections of the fruits [two fruits per inflorescence (2nd and 4th inflorescence) and three representative plants per accession and per replicate] were scanned into digital images and subjected to morphometric analysis by the Tomato Analyzer ver 1.2 Software (Brewer et al., 2006; Gonzalo et al., 2009). The following descriptors, measured by the software, were taken into consideration: fruit section area (*ar*), fruit maximum width (*fdl*), fruit maximum height (*fl*), fruit shape index I° (*fsi*), fruit shape triangle (*tri*), fruit shape elliptic (*ell*), fruit shape circular (*cir*), proximal angle macro (*pan*), distal angle macro (*dan*), eccentricity (*ecc*), internal fruit shape index (*int*), degree of lobedness (*lob*) and pericarp thickness (*per*). All parameters are described in detail by Brewer et al. (2006) and Gonzalo et al. (2009). For these parameters, differences between accessions and floral trusses and the respective interactions were estimated through an analysis of variance according to the GLM model using SAS software (SAS Institute Inc., 2002). A similar analysis was carried out after grouping the 'Abruzzese' accessions into three groups according to their fruit shape scores (flat, round and obovoid) and leaving the accessions of 'Canestrino' and 'Albenga' in two separate groups.

Although some variables, such as *fsi* and *int*, showed highly significant 'Genotype × inflorescence' interaction, examination of the data revealed that this significance was due to the behaviour of a few genotypes for each trait. Therefore, allowance was made for these interactions and, after standardisation, the arithmetic means over inflorescences were used to perform an Agglomerative Hierarchical Clustering (AHC) procedure with the XLSTAT 7.5.2 Package (<http://www.xlstat.com/>). Clustering was based on the Manhattan distance and the average linkage chosen as a fusion criterion.

2.3. Molecular analysis

For the molecular analysis, DNA from control accessions of 'Cuore di bue' (oxheart type), 'San Marzano' (long type), 'Spagnolella' and 'Marmande' (flat types) were also included. Seed stocks of the controls were available in the germplasm collection held by the authors (<http://www.unitus.it/tomato/>).

DNA was extracted from one plant per accession using the method of Doyle and Doyle (1987) and analyzed with 14 SSR markers that were selected from previous analyses for being highly polymorphic in Italian landraces (Mazzucato et al., 2008). The loci scored were LEMDDNa, LE20592, LEACS4A, LESSRPSPGb, LEEF1Aa (Smulders et al., 1997), TMS42 (Areshchenkova, 2000), TMS52, TMS58, TMS60, TMS63, EST253712 (Areshchenkova and Ganai, 2002), Tom47–48, Tom59–60 and Tom236–237 (Suliman-Pollatschek et al., 2002). Details of the marker dataset are given in Table 2. PCR reactions and detection of the amplification products were carried out as described by Mazzucato et al. (2008), with the difference that the amplification products were separated on 6% (w/v) denaturing polyacrylamide (1:19 bis:acrylamide) gels and visualised by silver staining (Bassam et al., 1991).

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