Biochemical Systematics and Ecology 60 (2015) 215–224

Contents lists available at ScienceDirect

Biochemical Systematics and Ecology

journal homepage: www.elsevier.com/locate/biochemsyseco

Cuticular wax variation in the tomato (*Solanum lycopersicum* L.), related wild species and their interspecific hybrids

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ARTICLE INFO

Article history:
Received 23 January 2015
Accepted 24 April 2015
Available online

Keywords:
Tomato
Solanum lycopersicum
Solanum pennellii
Interspecific hybrids
Plant cuticular waxes
Chemotaxonomy
Abiotic stress

ABSTRACT

The tomato (*Solanum lycopersicum* L.) is one of the world's most important vegetable crop species. Among the many tomato accessions available, only a few are tolerant to abiotic stresses, which are responsible for the majority of the crop losses worldwide. Wild tomato species are then secondary gene pool in the breeding of more resistant tomato cultivars. In the current study, the composition of leaf cuticular waxes from fourteen tomato accessions, including *S. lycopersicum*, *Solanum pennellii*, *Solanum pimpinellifolium*, and their interspecific hybrids was studied in order to select the most adequate chemotaxonomic markers. Total cuticular wax load of *S. pennellii* plants was much higher than in the other plant species. Hydrocarbons were usually the most abundant wax components, followed by minor quantities of triterpenes and other compounds. Interspecific hybrids showed intermediate wax characteristics. The amount and composition of surface waxes were not correlated with the abiotic stress tolerance in *S. lycopersicum*. The composition of the hydrocarbon fraction was the least variable both within a single accession and between all the plants studied. Based on the results, cuticular hydrocarbons are proposed as potential chemotaxonomic markers in the classification of tomato and related species.

1. Introduction

The tomato (*Solanum lycopersicum* L., formerly *Lycopersicon esculentum* Mill.) is one of the most important vegetables worldwide. Its total world production has grown from 119.5 to 164.0 million tonnes during the past decade (FAO, 2015; see Table S1 in supplementary content for detailed values). The number of tomato accessions is relatively high: currently, the Tomato Genetic Resource Center database shows 2282 records for a "*Lycopersicon esculentum*" query and an additional 334 records for a "*Lycopersicon esculentum* var. *cerasiforme*" (wild and weedy accessions) query (TGRC, 2014). While 123 of these accessions display a wide array of disease resistance, only ten accessions are described as stress-tolerant: these include both

Abbreviations: ACL, average chain length; DW, dry weight; GC-FID, gas chromatography with flame ionization detector; GC–MS, gas chromatography–mass spectrometry; PCA, principal component analysis; RSD, relative standard deviation; SD, standard deviation.

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http://dx.doi.org/10.1016/j.bse.2015.04.030
0305-1978/

wild and cultivated accessions, displaying mainly heat and salt tolerance. Among wild tomato relatives, *S. pennellii* Cor. was in the past reported as a possible source of salinity tolerance (Bolarín et al., 1991; Cuartero and Fernández-Muñoz, 1999; Frary et al., 2010). Because of the presence of glucose and sucrose esters produced by glandular trichomes, *S. pennellii* also displays a wide resistance to insect feeding (Mutschler et al., 1996). The TGRC database shows 45 records for a "*S. pennellii*" query; among these accessions, only three are described as "stress tolerant" (TGRC, 2014). Strong salinity tolerance was also reported for *Solanum pimpinellifolium* L. (Bolarín et al., 1991; Cuartero et al., 1992), which is – together with *Solanum cheesmaniae* (L. Riley) Fosberg – probably the most closely related to the common tomato (Spooner et al., 2005). Thus, wild relatives might be useful in breeding of new, potentially more stress-tolerant or insect-resistant cultivars of the tomato.

Abiotic stresses, mainly drought and salinity, are already responsible for the majority of the crop losses worldwide (Vinocur and Altman, 2005). Drought, salinity and heat are factors leading to secondary osmotic stress in plants. One of the barriers protecting terrestrial plants from the excessive water loss, is the thin layer of extracellular cuticular waxes covering the above-ground parts of the plant (Riederer and Schreiber, 2001; Shepherd and Griffiths, 2006). Plants subjected to a water deficit reduce transpiration by closing their stomata. The layer of cuticular waxes reduces non-stomatal transpiration, which occurs over the whole leaf area. The composition and morphology of cuticular waxes appear to be more important in protection against water loss than cuticle thickness and total wax load (Riederer and Schreiber, 2001; Oliveira et al., 2003). However, plants, including the tomato, often react to water stress by increasing the total wax load and the amount of long-chain aliphatic compounds in the waxes (Xu et al., 1995; Shepherd and Griffiths, 2006). Hydrocarbons, which are the most effective at reducing the rate of cuticular transpiration (Oliveira et al., 2003), have already been reported as being the main components of tomato leaf cuticular waxes (Smith et al., 1996; Vogg et al., 2004). To our knowledge, composition of surface lipids from *S. pennellii* and *S. pimpinellifolium* leaves remains unknown, except sugar esters, which were already described not only in *S. pennellii*, but also in *S. lycopersicum* × *S. pennellii* hybrids and introgression lines (Goffreda et al., 1989; Mutschler et al., 1996; Schilmiller et al., 2012).

Cuticular hydrocarbons have been used several times in the past as chemotaxonomic markers in the Solanaceae family (Zygadlo et al., 1994; Haliński et al., 2011; Silva et al., 2012). To our knowledge, however, there are no studies concerning the chemotaxonomy of the tomato and related species that are based on the composition of surface waxes. The selection of hydrocarbons was preceded only in some cases by the analysis of within-accession variation in the composition of the fraction. One possible measure of this variation for aliphatic compounds is the average chain length (ACL) (Sonibare et al., 2005). Among the aliphatic compounds detected in leaf cuticular waxes of *Solanum melongena* and its relatives, hydrocarbons displayed the lowest within-accession variation in ACLs (Haliński et al., 2011).

Knowledge of the cuticular wax composition of tomato leaves and abiotic stress tolerance mechanisms in this important vegetable crop is still minimal. Hence, the main objective of the current study was to determine the composition of leaf cuticular waxes of tomato accessions, as well as of its wild relatives *S. pennellii* and *S. pimpinellifolium*, and of interspecific hybrids between *S. lycopersicum* and these two species. Further evaluation of variation in the wax composition within a single accession, between accessions and between species could help to answer several important questions: (i) Is it possible to differentiate between *S. lycopersicum*, *S. pennellii* and *S. pimpinellifolium* plants, as well as between their interspecific hybrids, on the basis of a cuticular wax analysis? (ii) Are there any differences in the wax composition of stress-tolerant and stress-susceptible plants? And (iii) Which wax components would be the most appropriate for the chemotaxonomic analysis of the tomato and related species?

2. Materials and methods

2.1. Chemicals

The internal standards used in the study (*n*-eicosane, *n*-docosane, ethyl nonadecanoate, heneicosanol and 19-methylarachidic acid; purity 96–99%) and the derivatization agent *N*,*O*-bis(trimethylsilyl)trifluoroacetamide (BSTFA) + 1% trimethylchlorosilane (TMCS) were purchased from Sigma–Aldrich Poland (Poznań, Poland). Silica gel for flash chromatography (Davisil 60, particle size 0.040–0.063 mm) was obtained from Grace Davison Discovery Science (Deerfield, IL, USA). All the other chemicals were analytical grade and were obtained from POCh S.A. (Gliwice, Poland).

2.2. Plant material

A total of fourteen tomato accessions, including *S. lycopersicum* L., *S. pennellii* Cor., *S. pimpinellifolium* L., as well as interspecific hybrids between *S. lycopersicum* and the two latter species, were obtained from the Tomato Genetics Resource Center, University of California (Davis, USA), and Centre for Genetic Resources, The Netherlands (Wageningen, the Netherlands). A detailed list of plant accessions is given in Table 1. Some of the accessions selected for the study display tolerance or resistance to a number of environmental factors, including salt, heat, waterlogging and diseases. The plants were grown from seed in an unheated greenhouse. All the plants were irrigated three times a week (ca. 400 mL per plant); no supplementary lighting was provided. Plant material was sampled from mature plants during flowering and transferred to the laboratory. The leaves from accession LA2131 were collected separately from each of three plants (ca. 45 g per plant). Material from all the remaining accessions was collected from three plants per accession and pooled. A representative sample of leaves (ca. 65 g per accession) was then used for the extraction of cuticular waxes.