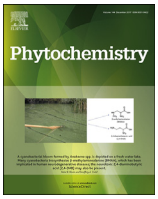
Phytochemistry 144 (2017) 87–97

Contents lists available at ScienceDirect

Phytochemistry

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



Multivariate analysis as a key tool in chemotaxonomy of brinjal eggplant, African eggplants and wild related species

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ARTICLE INFO

Article history:
Received 13 May 2017
Received in revised form
10 August 2017
Accepted 5 September 2017

Keywords:
Solanum melongena
Solanum macrocarpon
Solanum aethiopicum
Solanaceae
Brinjal eggplant
African eggplant
Chemotaxonomy
Multivariate analysis
Cuticular hydrocarbons

ABSTRACT

The brinjal eggplant (*Solanum melongena* L.) is an important vegetable species worldwide, while African eggplants (*S. aethiopicum* L., *S. macrocarpon* L.) are indigenous vegetable species of local significance. Taxonomy of eggplants and their wild relatives is complicated and still unclear. Hence, the objective of the study was to clarify taxonomic position of cultivars and landraces of brinjal, its wild relatives and African eggplant species and their wild ancestors using chemotaxonomic markers and multivariate analysis techniques for data processing, with special attention paid to the recognition of markers characteristic for each group of the plants.

The total of 34 accessions belonging to 9 species from genus *Solanum* L. were used in the study. Chemotaxonomic analysis was based on the profiles of cuticular *n*-alkanes and methylalkanes, obtained using gas chromatography-mass spectrometry and gas chromatography with flame ionization detector. Standard hierarchical cluster analysis (HCA) and principal component analysis (PCA) were used for the classification, while the latter and two-way HCA allowed to identify markers responsible for the clustering of the species. Cultivars, landraces and wild forms of *S. melongena* were practically identical in terms of their taxonomic position. The results confirmed high and statistically significant distinctiveness of all African eggplant species from the brinjal eggplant. The latter was characterized mostly by abundant long chain hydrocarbons in the range of 34–37 carbon atoms. The differences between both African eggplant species were, however, also statistically significant; *S. aethiopicum* displayed the highest contribution of 2-methylalkanes to the total cuticular hydrocarbons, while *S. macrocarpon* was characterized by elevated *n*-alkanes in the range of 25–32 carbon atoms. Wild ancestors of both African eggplant species were identical with their cultivated relatives. Concluding, high usefulness of the chemotaxonomic approach in classification of this important group of plants was confirmed.

1. Introduction

The brinjal eggplant (*Solanum melongena* L.) is an important vegetable species, grown widely for its edible fruits. While the brinjal itself is susceptible to insect herbivores and pathogens, it is more or less associated with a large number of poorly recognized wild species of much higher resistance (Collonnier et al., 2001). However, the precise taxonomic and phylogenetic relationships between *S. melongena* and its wild relatives have yet to be clarified. Many of the wild species were described basing solely on their morphology, even in the most recent reports (e.g. Knapp et al., 2013). Authors of this study have clearly shown the difficulties in the clear identification of the plant accessions, which is even more complicated because of a great morphological diversity and the use of many synonymic names in the literature. The unequivocal identification of plant accessions and clarification of their taxonomic status are required for a possible future use in plant breeding.

The second group of economically important eggplants is formed by plant species referred to as the 'African eggplant', which usually includes the Gboma eggplant (*S. macrocarpon* L.) and scarlet eggplant (*S. aethiopicum* L.) together with their wild ancestors. This group of plants cultivated for edible leaves and fruits is among relatively new African species used in agriculture (Lester and Daunay, 2001). They belong to indigenous vegetable crops, which are grown and consumed in Africa as valuable sources of nutrients and a diversification of the diet (Uusiku et al., 2010). These plants

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http://dx.doi.org/10.1016/j.phytochem.2017.09.001
0031-9422/

are well adapted to the local climate, including high temperatures and seasonal water deprivation. Their exact taxonomic position within subgenus *Leptostemonum* (Dunal) Bitter is still discussed, and it is not fully clear if their great morphological diversity is followed by the similar genetic variability.

There is a need of a greater understanding of the interrelationships between the above-mentioned species in order to maximize their potential use in agriculture, including utilization as a secondary gene pool in the brinjal breeding. Moreover, uncertainty arose about the true taxonomic position of some plants previously described as *S. incanum* (e.g. Haliński et al., 2011; Meyer et al., 2012; Samuels, 2013b). Finally, as the recent taxonomic position of the *S. aethiopicum* – *S. anguivi* and *S. macrocarpon* – *S. dasyphyllum* complexes remains poorly recognized, there is a need to address this issue. Hence, the aim of the current study was to provide an inexpensive alternative tool for the classification of this difficult group of plant species, based on the cuticular chemotaxonomic markers. Some detailed objectives of the research were: (i) to clarify the taxonomic position of cultivars, landraces and wild/feral forms of brinjal in relation to wild allied species; (ii) to determine the taxonomic position of African eggplant species, including *S. aethiopicum*, *S. macrocarpon* and their wild ancestors; and (iii) to recognize the importance of certain markers in classification of these plant species for the needs of the future studies.

The brinjal eggplant was domesticated in South or South-East Asia (Meyer et al., 2012; Samuels, 2013a) where wild, semi-cultivated or feral forms (sometimes known as *S. cumingii* Dunal, *S. insanum* L., etc.) are still found (Lester and Daunay, 2001; Lester and Hasan, 1991). Mace et al. (1999) suggested that wild brinjal forms should be classified under *S. melongena*, and Samuels (2013a) affirmed this by designating cultivated forms as *S. melongena* subsp. *melongena*, and other forms as *S. melongena* subsp. *cumingii* (Dunal) J. Samuels. Recently, Meyer et al. (2012) suggested that the latter (as "*S. undatum* Lam.") is a possible brinjal progenitor. However, the exact origin of cultivated brinjal remains unclear (Daunay and Hazra, 2012). Also, while morphological diversity in cultivated *S. melongena* is relatively high (Kumar et al., 2008), genetic diversity in the common hybrid cultivars is low (Ali et al., 2011). It is not fully recognized if landraces and wild/feral forms also display low genetic variability.

The taxonomic position of African eggplants was recently revisited. It was well established for the long time that scarlet eggplant was placed in the *Oliganthes* (Dunal) Bitter section, together with its wild ancestor (*S. anguivi* Lam.) (Lester and Daunay, 2001). It was also shown basing on the analysis of RAPD markers that the separation of both species is very weak (Stedje and Bukenya-Ziraba, 2003). Four groups of the *S. aethiopicum* plants, varying widely in terms of their morphological characteristics, were described: Gilo, Shum and Kumba groups are grown for their edible fruits or leaves, while Aculeatum group (so-called *S. integrifolium*) possibly originated in Europe as the result of interspecific hybridization. The Gboma eggplant and its wild ancestor (*S. dasyphyllum* Schum. and Thonn.), on the other hand, were usually placed in the *Melongena* section, together with the brinjal eggplant. This was somehow supported by the analysis of chloroplast DNA restriction site variation (Olmstead and Palmer, 1997). While wild *S. dasyphyllum* plants are common in all non-arid parts of Africa, cultivated forms of *S. macrocarpon* are less widespread and are grown in West and Central Africa (Bukenya and Bonsu, 2004). Like in the case of *S. aethiopicum* – *S. anguivi*, it was also suggested that *S. macrocarpon* and *S. dasyphyllum* form a single species (Bukenya and Carasco, 1994). Several studies suggested that *S. macrocarpon* should be excluded from section *Melongena* (Isshiki et al., 2008; Karihaloo et al., 2002). According to Furini and Wunder (2004), it should be placed in section *Oliganthes* together with *S. aethiopicum*. More recently, however, it was suggested that all the African eggplant species could be placed in a complex '*Anguivi* grade', which is much more variable than the uniform *Melongena*/Eggplant clade and consists of numerous poorly resolved species of much less clear taxonomic position, native to Africa, the Near East and Asia (Aubriot et al., 2016; Vorontsova et al., 2013).

The usefulness of cuticular hydrocarbons in the chemotaxonomy of species belonging to *Solanum* subgenus *Leptostemonum* (to which brinjal belongs) has already been demonstrated (Da Silva et al., 2012; Zygadlo et al., 1994). Furthermore, the usefulness of *n*-alkane profiles to the biosystematics of the brinjal eggplant and allied species has recently been reported (Haliński et al., 2011). Therefore, there is a possibility to create an inexpensive method of plant classification, that would be complementary to the already used approaches based on morphological characteristics and molecular markers. Moreover, the identification of chemotaxonomic markers, that are characteristic for a given groups of plants, could be used in the future to build a predictive model, that would define the taxonomic position of the certain accession without analysing a large dataset, and would be of great value for the fast and accurate identification of unknown plant accessions.

2. Results

The total of 36 plant accessions were used in the current study (see Table 1). In the previous report (Haliński et al., 2011) we have presented the classification of brinjal and its relatives based exclusively on the cuticular *n*-alkane profile. Because the identification of some of the accessions has changed since then, and in the current study the set of accessions examined is much larger and includes a number of *S. macrocarpon* – *S. dasyphyllum* and *S. aethiopicum* – *S. anguivi* accessions, we decided to update the previous classification. However, when the profile of *n*-alkanes in the range of 16–39 carbon atoms was used, the classification obtained was clearly incorrect (please see Table S1 in Supplementary material for the data, and Figs. S1 and S2 for the results of HCA and PCA, respectively). The results of HCA did not allow to separate some of the *S. aethiopicum* (Ae3, Ae4) and *S. macrocarpon* (M3) accession from the outgroup (Fig. S1). Moreover, one of the *S. campylacanthum* subsp. *campylacanthum* accessions (I1a) was separated from all the other plant species, probably because of the much higher content of C_{38} and C_{39} *n*-alkanes (see Table S1). The results of PCA allowed to address this issue (Fig. S2); however, only *S. melongena* (E1-E15) and closely related species (I1a-b, I2, I4) were clearly separated because of the abundant C_{33}-C_{39} *n*-alkanes. The separation of *S. macrocarpon* – *S. dasyphyllum* and *S. aethiopicum* – *S. anguivi* complexes was, however, still only partial. This was probably due to the fact, that in the whole dataset there was a large number of accessions that did not produce detectable amounts of C_{16}-C_{24} and C_{38}-C_{39} *n*-alkanes. In order to remove zero values from the dataset, that could originate from the limitations of the GC-FID technique in terms of its limit of quantification for the compounds studied, these values were replaced with the half the amount of the least abundant compound that was detected. This resulted in an artificially increased content of these *n*-alkanes in several accessions. To address this issue, in the next step the dataset was reduced to only those compounds that were detected in at least 25% of the accessions, i.e. to *n*-alkanes in range of 25–37 carbon atoms. While this operation resulted in a much clearer classification, the use of *n*-alkanes only still did not allow to differentiate between plants from *S. macrocarpon* – *S. dasyphyllum* and *S. aethiopicum* – *S. anguivi* complexes (please see Table S2 in Supplementary material for the data, and Fig. S3 for the results of HCA). Therefore, the chemical composition of the whole cuticular hydrocarbon fraction was used for the classification of the species studied.

Similarly to the approach described above, compounds that were detected only in a minority (less than 25%) of the accessions were