ESL 1: Intraspecific taxonomy of plant genetic resources – Important for differentiation of medicinal and aromatic plants?

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Abstract

Taxonomy of plant genetic resources is an important input in characterising and evaluating cultivated plants and essential for identification and documentation of the diversity of genebank collections. In former times taxonomical determination was based only on morphological characters. Nowadays, new molecular and chemical methods and techniques are available for providing additional information. As examples, investigations of parsley (*Petroselinum crispum* [Mill.] Nyman, Apiaceae) and opium poppy (*Papaver somniferum* L., Papaveraceae) collections of the German genebank are demonstrated. In addition to morphological description, the molecular distance and the phylogenetic relationship of the accessions were performed with molecular marker analysis. Essential oil compound and content for parsley and the content of the five main alkaloids (morphine, codeine, thebaine, noscapine, papaverine) for opium poppy were measured with GC (gas chromatography) and HPLC (high pressure liquid chromatography), respectively. For parsley the results of the three methods support the existing taxonomy partly, a separation of root and leaf parsley was confirmed. However, the taxonomy of opium poppy should be revised because molecular and chemical data do not verify the morphological results. But nevertheless taxonomy of cultivated plants is an important tool to describe the variability of plant genetic resources.

Keywords: Parsley, plant genetic resources, opium poppy, taxonomy

Introduction

Taxonomy of plant genetic resources is an important input in characterising and evaluating cultivated plants. Especially, for large genebank collections it is necessary to know inter- and intraspecific taxonomy to describe the genebank's material. The German *ex situ* genebank is one of the ten largest genebanks worldwide. Nearly 150,000 accessions out of more than 3,000 species and 780 genera are maintained and reproduced at the Leibniz Institute of Plant Genetics and Crop Plant Research in Gatersleben (Börner, 2006). For such a large collection taxonomy is essential for identification and documentation the wide range of diversity in the assortment. It is a great source to describe the often enormous variability by various methods and techniques (Hanelt, 1988). In former times taxonomical determination was based only on morphological characters. Nowadays, new molecular and chemical methods and techniques are available for providing additional information. The aim of this work was to study two examples, parsley and opium poppy, with the intention of a clear intraspecific taxonomy with the help of molecular markers and chemical compounds. For both species complex morphological descriptions and intraspecific taxonomy containing subspecies, convarieties, botanical varieties and forms are available (Danert, 1958; 1959; Hammer, 1981; Hanelt & Hammer, 1987). But the question is if these new methods support or even improve the existing intraspecific taxonomy or if a revision is necessary.

Material and methods

Two crops with a known high intraspecific variability, parsley and opium poppy, were selected. The parsley collection contains 220 accessions including both morphological types, leaf parsley and root parsley, and on the other hand with modern and old cultivars as well as landraces. For the standardisation of the morphological characterisation a descriptor was applied with 15 morphological (growth type, leaf type, root type, etc.) traits (Lohwasser, 2009). For the molecular studies 88 RAPD- (Random Amplified Polymorphic DNA), 53 SRAP- (Sequence-Related Amplified Polymor-

phism) and 65 AFLP- (Amplified Fragment Length Polymorphism) markers were used. From the polymorphic bands of these 206 markers a binary matrix was compiled and a tree structure based on Nei & Li distances developed using the programme PAUP*4.0b10 (SWOFFORD, 2002). Essential oil contents were measured and the compositions of the oil were analysed by gas chromatography (LOHWASSER et al., 2010).

From the large opium poppy collection of the genebank 300 accessions were selected. Again modern cultivars, old cultivars and landraces were chosen and described morphologically based on a descriptor (DITTBRENNER et al., 2008). The AFLP fingerprint technique was used to produce a binary matrix out of 300 polymorphic markers from which a neighbor joining tree based on Nei & Li distances was generated (DITTBRENNER, 2009; DITTBRENNER et al., 2008) with the programme PAUP. *Papaver glaucum* Boiss. & Hausskn. was used for a clear separation within the opium poppy. For the phytochemical studies the content of the five main alkaloids morphine, codeine, thebaine, noscapine, and papaverine was measured with HPLC (high pressure liquid chromatography) based on a method described by DITTBRENNER (2009) and DITTBRENNER et al. (2009).

Results

As examples of the use of morphological, molecular and phytochemical data in order to verify existing classifications, investigations of parsley and opium poppy collections of the German genebank are demonstrated.

For parsley the morphological description has resulted in curled leaf, smooth leaf and root parsleys. These types can be separated quite well into two convarieties one for leaf parsleys and one for root parsleys. Accessions with a remarkable long petiole (Italian parsley) as discriminated by DANERT (1959) could not be identified definitely. The molecular studies show also two clusters, one for the leaf parsleys and a second one for the root parsleys together with some leaf parsleys (DECLERCQ, 2009). The morphological and molecular data fit very well with the targeted analysis of the essential oil content and compounds. High concentration of two monoterpenes, myrcene and β-phellandrene, can be correlated with root parsley and leaf parsley, respectively. For the volatile compounds two groups could be defined, one for all leaf parsleys without any difference of the leaf type and one for the cluster with the root parsleys (DECLERCQ, 2009). But a clear separation of the varieties and forms was possible neither with morphological traits nor with molecular or phytochemical data.

The intraspecific taxonomy of the opium poppy is based on a few morphological characters like setose buds, capsule dehiscence, shape of the stigmatic lobes and colour of flower and seeds. However, the classification is difficult because of different characters on one plant or due to the presence of variation within the accession. To summarize the results of the analysis of the morphological data, only a clear separation of the subspecies *setigerum* (DC.) Corb. by bud hairiness is possible. Both other subspecies and all varieties could not be determined definitely. The molecular analysis shows also only a clear separation of the subsp. *setigerum* but no further intraspecific structure within the opium poppies (fig. 1) which supports the morphological analysis. In addition, the analyses of the five main alkaloids present different compounds and contents of the accessions which do not fit with morphological and/or molecular results (DITTBRENNER et al., 2009). In conclusion, there is no clear intraspecific taxonomy of opium poppy in the range of the convarieties and varieties available neither by morphological characters nor by molecular or phytochemical data (LOHWASSER et al., 2010).

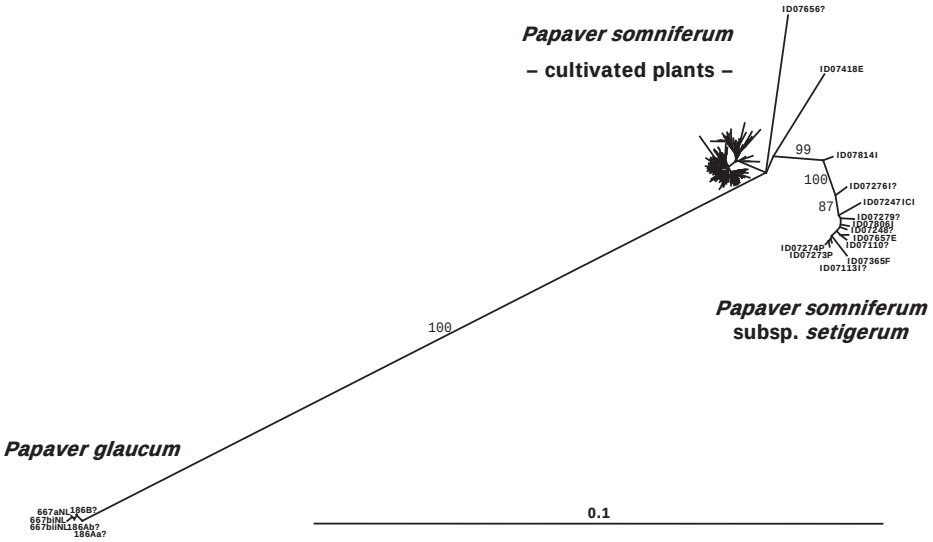

Figure 1: Neighbor joining tree based on Nei & Li distances from AFLP analysis

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