Taxonomy of Plant Genetic Resources – Use of Morphological, Molecular and Phytochemical Data in Order to Verify Existing Classifications

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Summary

Taxonomy of plant genetic resources is an important input in characterising and evaluating cultivated plants and it is 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 of the interaction of morphological, molecular and phytochemical data, investigations of a parsley (*Petroselinum crispum* [Mill.] Nyman, Apiaceae) and an opium poppy (*Papaver somniferum* L., Papaveraceae) collection of the German genebank are demonstrated. 220 parsley and 300 opium poppy accessions were cultivated and described morphologically. In addition, 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.

Key words

genebank, parsley, plant genetic resources, opium poppy, taxonomy

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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) (Tab. 1, Tab. 2). 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 Polymorphism) 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 a parsley and an opium poppy collection 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

Table 1. Taxonomic classification of parsley (Danert, 1959)

Species	Convariety	Variety	Form
Petroselinum crispum (Mill.) Nyman	*crispum*	*silvestre* (Alef.) Danert	
		vulgare (Nois.) Danert	*vulgare*
			angustifolium (Hayne) Danert
			variegatum (Nois.) Danert
		crispum	*crispum*
			hispanicum (Alef.) Danert
			tenuisectum (Thell.) Danert
		neapolitanicum Danert	
	radicosum (Alef.) Danert	*radicosum*	*radicosum*
			breve (Alef.) Danert
		erfurtense Danert	

Table 2. Taxonomic classification of opium poppy (Danert, 1958; Hammer, 1981; Hanelt & Hammer, 1987)

Species	Subspecies	Convariety	Variety
Papaver somniferum L.	*setigerum* (DC.) Corb.		
	somniferum	*somniferum*	13 varieties
		alefeldii K. Hammer	13 varieties
	songaricum Basil.	*orientale* Danert	13 varieties
		rothmaleri K. Hammer	13 varieties

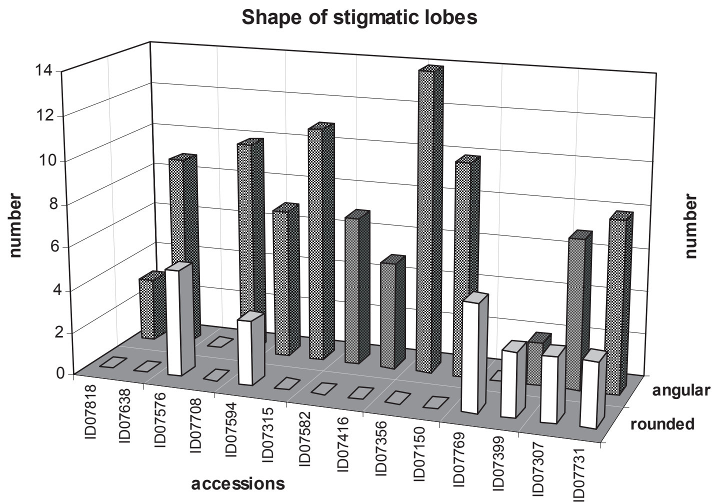

Figure 1.
Distribution of the shape of the stigmatic lobes within 14 accessions

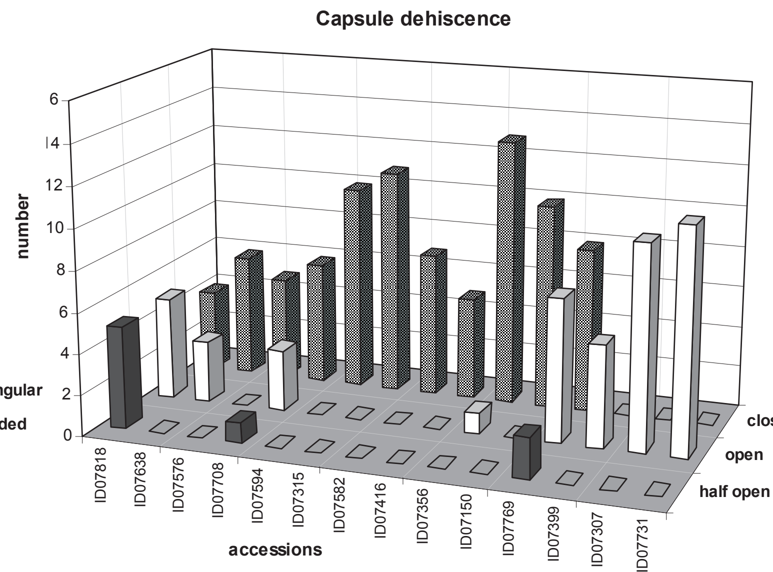

Figure 2.
Distribution of the capsule dehiscence within 14 accessions

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. As examples of the variability the results of the shape of the stigmatic lobes (Fig. 1), rounded or angular and of the capsule dehiscence (Fig. 2), totally closed (indehiscent), half open or totally opened (dehiscent), are given.

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. 3) that supports the morphological analysis.

In addition, the analyses of the five main alkaloids present different compounds and contents of the accessions that do not fit with morphological and/or molecular results (Dittbrenner et

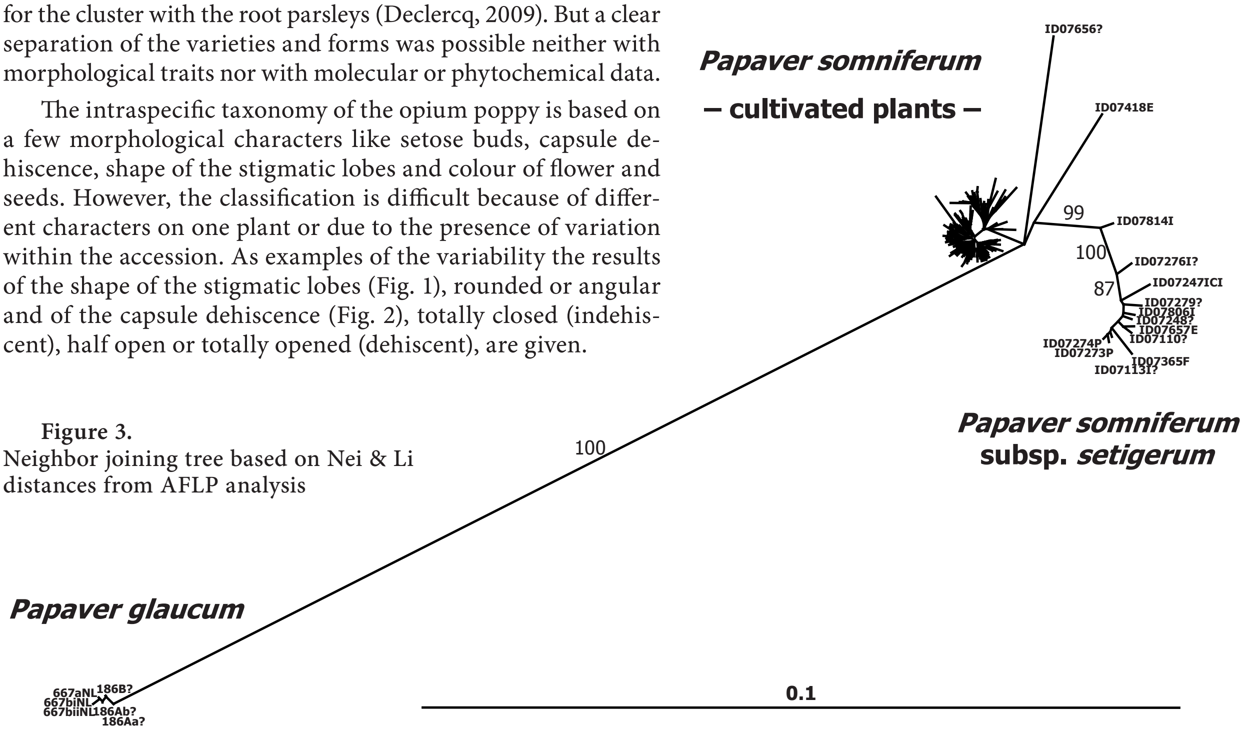

Figure 3.
Neighbor joining tree based on Nei & Li distances from AFLP analysis

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.

Discussion

Accessions of both species were described morphologically. In addition, molecular and phytochemical data were generated in order to combine and to compare the data with respect to intraspecific taxonomy.

For the botanical taxonomy of parsley the grouping of the leaf and the root parsleys fit very well together with the molecular and the phytochemical data. The convarieties *crispum* (leaf types) and *radicosum* (root types) can be well separated. But some problems are still present. Italian parsley could not be discriminated from other smooth leaf types within classifications based on neither morphological, molecular nor phytochemical data. And the morphological character of the long petiole is influenced by environmental conditions. In addition, some of the described botanical varieties (Danert, 1959) could not be found. Also the forms are not clearly detectable (Lohwasser et al., 2010). This means for the main groups an interaction of morphological, molecular and phytochemical data could be presented but the taxonomy of the lower levels needs further studies. Studies in another Apiaceae species show similar results. Combinations of morphological and volatile oil data of coriander (*Coriandrum sativum* L.) demonstrate the support of the intraspecific taxonomy but not always a clear separation (Diederichsen & Hammer, 2003). In conclusion, the intraspecific taxonomy of parsley must be revised based on the new knowledge of the molecular and phytochemical data.

For the botanical taxonomy of opium poppy only two subspecies, subsp. *setigerum* and *somniferum*, can be determined, which is also confirmed in other studies (Kadereit, 1986). All other varieties could not be separated clearly. The morphological, molecular and phytochemical data do not correspond; moreover the morphological characters vary within accessions or e.g. the seed colour even in one plant. Already in former descriptions of opium poppy varieties variability of the capsule dehiscence is mentioned (Basilevskaya, 1928; Vesselovskaya, 1933). At present an intraspecific taxonomy of opium poppy is not available. Maybe, in further studies with a higher number of poppy accessions it will be possible to determine a new intraspecific system.

Conclusion

In order to study the use of morphological, molecular and phytochemical data with respect to existing intraspecific taxonomy two collections, parsley and opium poppy, were studied. 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 poppy should be revised because molecular and chemical data do not correspond with the morphological results. But nevertheless taxonomy of cultivated plants is an important tool to describe the variability of plant genetic resources.

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