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Comparison of three *Polygonatum* species from Poland based on DNA markers

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Four categories of DNA markers were used to determine genetic similarity of three species of *Polygonatum*, i.e. *P. multiflorum*, *P. odoratum* and *P. verticillatum*, occurring in Poland. Four populations per species, represented by ten plants per population, were collected in north-eastern Poland. In this study 111 RAPD and 35 semi-random ISJ markers were detected. Moreover the application of primers complementary to bacterial sequences *IS6110* and *katG* gene enabled the detection of 9 and 34 markers, respectively. Each of the four marker categories gave species-specific bands. The highest conservativeness was observed for sequences of the *katG* gene. The degree of genetic similarity between *P. multiflorum* and *P. odoratum* was 0.57, and between these taxa and *P. verticillatum* 0.40 and 0.37, respectively. Most of the primers applied in the study enabled molecular species identification, and three of them turned out to be section-specific markers. The taxa showed no considerable intraspecific diversity, and the populations of *P. verticillatum* were almost identical. The results are consistent with the commonly accepted division of the genus *Polygonatum* into sections *Polygonatum* and *Verticillata*.

Key words: DNA markers, genetic similarity, *Polygonatum*

Introduction

In taxonomy, morphological markers constitute the main criteria of species distinction. By using molecular techniques we have the possibility to validate the taxonomic classification, because the species-specific morphological differences should correspond to certain differences on the molecular level. Hamrick and Godt (1990), in their summary of over 20 years of electrophoretic analyses of enzymes, concluded that the index of genetic similarity characterizing good biological species should be lower than 0.6. Such species have reproductively isolated genetic pools and typically show the presence of many specific markers allowing their easy identification.

However, "botanical" species do not always turn out to be good "biological" species. Two species of grass, *Lolium perenne* and *L. multiflorum* may serve as an example since they

do not have enzymatic and DNA markers and have the genetic similarity of 0.96 (Polok 2005). The enzymatic markers, despite numerous examples of their application for taxonomic purposes, seem to be inferior to DNA markers, which can be generated in any number and can represent different genomic sequences. For example, species-specific enzymatic markers have not been found in Scots pine, *Pinus sylvestris* or dwarf pine, *P. mugo*. However, these pines have different DNA markers of both unique and RAPD-type nonspecific sequences (Zieliński & Polok 2005). A sibling species, enzymatically undistinguishable from the typical form of the liverwort *Pellia endiviifolia*, has been identified similarly (Polok *et al*. 2005b).

The usefulness of different DNA sequences of nuclear and organellar genomes has been tested in molecular studies for taxonomic purposes. The bacterial sequences present in the eukaryotic genome characterized by high amplification specificity have been also used recently. The sequences complementary to the bacterial gene *katG* coding catalase-peroxidase enzyme and to the bacterial insertion sequence *IS6110* belong to this category. Employing these sequences enabled development of many markers identifying each of the 16 analyzed crop cereals and grass species (except for *Lolium perenne* and *L. multiflorum*), selected species of the genus *Pinus*, as well as liverwort species (Chmiel & Polok 2005, Zieliński & Polok 2005).

The purpose of this study was to test whether the two bacterial sequences described above could be used to identify selected species of dicotyledon plants. The effectiveness of the above markers in species identification was tested on three species of *Polygonatum* found in Poland: *P. multiflorum*, *P. odoratum* and *P. verticillatum*. These species constitute a good model because they are easy to distinguish morphologically and their taxonomic status raises no doubts. The species belong to two sections, *Polygonatum* and *Verticillata*, therefore the bacterial sequences can be used in distinguishing both species and sections. The bacterial sequences *katG* and *IS6110* were assessed in the context of widely used markers RAPD and semi-specific markers ISJ (Intron Splice Junction).

Species of the genus *Polygonatum* have not been studied with DNA markers yet. This study also gives the possibility to determine, for the first time, the levels of their intra- and inter-specific variation.

Material and methods

Material

Polygonatum belongs to the Convallariaceae and has a total of 57 species. Its major distribution centers are in East Asia, mainly China and Japan, where 40 species are found (Tamura 1997). Apart from this area, *Polygonatum* occurs in the moderate climate zone of North America and Europe.

Morphometric and cytological analyses performed so far (Therman 1957, Nowakowska and Żeglicka 1972, Abramova 1975, Tamura 1993) on *Polygonatum* enabled two sections to be distinguished: *Polygonatum* and *Verticillata*. Section *Polygonatum* is characterized by leaves arranged alternately on the stem, thick filaments, perforated pollen grains and chromosome number $x = 9$, 10 or 11. Their karyotypes contain mostly metacentric and submetacentric chromosomes.

The section *Verticillata* is characterized by verticillate leaves, thin filaments, reticulate pollen grains and chromosome number $x = 14$ or 15. Karyotypes contain mostly long subtelocentric and short metacentric chromosomes (Tamura 1993).

Additional information was provided by Tamura (1997), who investigated the chloroplast DNA to estimate the phylogenetic relationships between 23 species of *Polygonatum*. The phylogenetic tree created on the basis of these data confirmed the earlier division of the genus into two sections, as well as the classification of particular taxa into these sections, based mostly on cytological and morphological analyses.

In Poland *Polygonatum* is represented by three species, i.e. *P. multiflorum* and *P. odoratum* of the section *Polygonatum*, and *P. verticillatum* of the section *Verticillata*. *Polygonatum multiflorum* and *P. odoratum* can be found in all parts of Poland, but in different habitats. The former is reported mainly from dry-ground forests, and the latter from pine and mixed coniferous forests, and oak forests (Matuszkiewicz 2001). *Polygo-*

natum verticillatum, considered a montane species (Zając 1996), has a disjunctive distribution in Poland. It can be found in both mountain areas and lowlands of northern Poland. In the mountains it usually occurs in forest communities of the class *Vaccinio–Piceetea* and grass communities of the alliance *Calamagrostion*. In the lowlands it is most common in shaded forests of the alliances *Carpinion* and *Fagion*.

The studied material comprised four population samples of each species, *P. odoratum*, *P. multiflorum* and *P. verticillatum*, collected in northeastern Poland. The localities are shown in Table 1. Each sample consisted of ten plants randomly collected from the population. The leaves from each plant were cleaned and then stored at –20 °C.

DNA extraction

In total 1 g of leaves was taken from ten individuals of each population.

DNA was isolated by the modified CTAB procedure. The liquid nitrogen-ground leaves were thoroughly mixed with 3 ml preheated CTAB isolation buffer (2% CTAB, 100 mM Tris-HCl, pH 8.0, 20 mM EDTA, 1.4 M NaCl and 2% β-mercaptoethanol) and incubated at 55 °C for 1 h. After three chloroform extractions, the DNA was precipitated and dissolved in sterile, deionized H_2O. The purity of DNA samples was assessed spectrophotometrically and it reached 90%–95%. The DNA content of the samples was from 50.4 μg to 506.1 μg.

RAPD markers

RAPD markers are universally applied to determine genetic diversity within plant and animal populations (Williams 1990, Stenoien 1999, Polok *et al*. 2005b). In contrast to specific markers, RAPD markers are randomly distributed in the genome, thus permitting an objective assessment of genetic polymorphism of a given species, without the need to know the functions and structure of the amplified sequence, which significantly reduces the costs of analysis. The sequences of RAPD primers used for DNA amplification in this study are given in Table 2.

The PCR reaction was conducted in a volume of 20 μl containing 1 μl PCR buffer (400 mM $(NH_4)_2SO_4$ and 1 M Tris-HCl, pH 9 at 25 °C), 2 mM $MgCl_2$, Enhancer containing betaine, 200 μM each dATP, dGTP, dCTP, dTTP, 0.3 μM primer, 1 unit of Tfl polymerase (Epicentre Technology) and 80 ng of template DNA. The reaction was carried out at 94 °C for 3 min. followed by 45 cycles at 94 °C for 1 min., 36 °C for 1 min., and 72 °C for 2.5 min., with a final extension step of 72 °C for 5 min.

ISJ markers

Semi-specific ISJ markers are based on sequences that are common in plants and partly complementary to the sequences at the intron–exon splice junction (Rafalski 1997). The sequences of ISJ primers used for DNA amplification in this study are given in Table 2.

Table 1. List of analyzed *Polygonatum* populations from Poland.

Species	Locality	Symbol	Habitat	Population size (*N*)
P. odoratum	Czarny Piec	PoA	Subcontinental pine forest	30
P. odoratum	Dłużek	PoB	Subcontinental pine forest	150
P. odoratum	Kortowo Forest	PoC	Subcontinental pine forest	40
P. odoratum	Bartążek	PoD	Subcontinental pine forest	35
P. multiflorum	Welski Landsc. Park	PmA	Oak-hornbeam forest	200
P. multiflorum	Kortowo Forest	PmB	Oak-hornbeam forest	40
P. multiflorum	Dylewskie Hills	PmC	Oak-hornbeam forest	500
P. multiflorum	Kulka Nature Res.	PmD	Oak-hornbeam forest	25
P. verticillatum	Mazurian Landsc. Park	PvA	Oak-hornbeam forest	100
P. verticillatum	Iława Landsc. Park	PvB	Pine forest	50
P. verticillatum	Welski Landsc. Park	PvC	Oak-hornbeam forest	85
P. verticillatum	Reda Pieleszewo	PvD	Alluvial forest	25

The PCR reaction was conducted in a volume of 20 μl containing 1 μl PCR buffer (400 mM $(NH_4)_2SO_4$ and 1 M Tris-HCl, pH 9 at 25 °C), 2 mM $MgCl_2$, Enhancer containing betaine, 200 μM eachof dATP, dGTP, dCTP, dTTP, 0.3 μM primer, 1 unit of Tfl polymerase (Epicentre Technology) and 80 ng of template DNA. The reaction was carried out at 94 °C for 3 min. followed by 45 cycles at 94 °C for 1 min., 48 °C for 1 min., and 72 °C for 2.5 min., with a final extension step of 72 °C for 5 min.

Insertion sequence *IS6110*

The insertion sequence *IS6110* is present in the genome of *Mycobacterium tuberculosis* in the number of 1 to 19 copies. However, the location of this sequence is different among the strains, and PCR amplification enables the detection of differences in their number. This method may be employed to detect *IS6110* not only in mycobacteria, but also in other species. The sequences of *IS6110* primers are given in Table 2. The PCR reaction for this sequence was performed according to Polok *et al*. (2005b). The *IS6110* element was amplified in a volume of 20 μl containing 20 mM $(NH_4)_2SO_4$, 50 mM Tris-HCl (pH 9.0 at 25 °C), 1.5 mM $MgCl_2$, 1 × Enhancer with betaine, 200 μM each of dATP, dGTP, dCTP, dTTP, 1.0 μM of each primer, 1 unit of Tfl polymerase (Epicentre Technology) and 100 ng of template DNA. The reaction was carried out at 94 °C for 1 min. followed by 40 cycles at 94 °C for 1 min., 52 °C for 1 min., and 72 °C for 1.5 min., with a final extension step of 72 °C for 5 min.

Primers complementary to the bacterial sequences of the *katG* gene coding catalase-peroxidase

Catalase and peroxidase participate in molecular defense against reactive oxygen forms. Bacteria possess an enzyme showing the properties of catalase and peroxidase, referred to as catalase-peroxidase, which belongs to the same group as peroxidase and cytochrome c, present in plants. In all Eucaryota these enzymes possess a long homologous region, which is also present in catalase–peroxidase found in bacteria (Zamocky *et al*. 2000). The PCR reaction for three elements of the *katG* gene were carried out according to a method developed by Zieliński and Polok (2005).

Table 2. Primers used in the analysis of *Polygonatum* species.

Type of marker/equence	Abbreviation	Number of nucleotides	Primer sequence
RAPD	OPD-01	10	5´ACCGCGAGGG3´
RAPD	OPD-02	10	5´GGACCCAACC3´
RAPD	OPD-03	10	5´GTCGCCGTCA3´
RAPD	OPD-05	10	5´TGAGCGGACA3´
RAPD	OPA-01	10	5´CAGGCCCTTC3´
RAPD	OPB-05	10	5´CAGGCCCTTC3´
RAPD	OPB-10	10	5´CTGCTGGGAC3´
RAPD	OPB-13	10	5´TTCCCCCGCT3´
RAPD	OPB-14	10	5´TCCGCTCTGG3´
RAPD	OPB-20	10	5´GGACCCTTAC3´
ISJ	ISJ2	18	5´ACTTACCTGAGGCGCCAC3´
ISJ	ISJ3	10	5´TGCAGGTCAG3´
ISJ	ISJ4	18	5´GTCGGCGGACAGGTAAGT3´
IS6110	IS1	19	5´GGCTGAGGTCTCAGATCAG3´
IS6110	IS2	20	3´CAAGAACCTTTCCTACCCCA5´
katG1	katG1-1	19	5´GACTACGCCCAACAGCTCC3´
katG1	katG1-2	19	5´GCGATAACCCCGCAAGACC3´
katG3	katG3-1	18	5´AACGGCTGTCCCGTCGTG3´
katG3	katG3-2	19	5´GTCGTGGATGCGGTAGGTG3´
katG4	katG4-1	19	5´TCGACTTGACGCCCTGACG3´
katG4	katG4-2	18	5´CAGGTCCGCCCATGAGAG3´

The sequences of *katG* are given in Table 2.

The *katG* elements were amplified in a volume of 20 μl containing 20 mM $(NH_4)_2SO_4$, 50 mM Tris-HCl (pH 9.0 at 25 °C), 1.5 mM $MgCl_2$, 1 × Enhancer with betaine, 200 μM each of dATP, dGTP, dCTP, dTTP, 1.0 μM of each primer, 0.75 unit of Tfl polymerase (Epicentre Technology) and 100 ng of template DNA. The reaction was carried out at 94 °C for 1 min. followed by 30 cycles at 94 °C for 1 min., 53 °C for 1 min., and 72 °C for 1.5 min., with a final extension step of 72 °C for 5 min.

Electrophoresis

PCR samples were loaded on a 1.2% (for RAPD and ISJ markers) or 1.5% (for *IS6110* and *katG*) agarose gel containing 0.5 μg ml^{-1} ethidium bromide and separated in 1 × TBE buffer at 100 V constant power. Gels were observed in UV light, 312 nm and photographed.

Data analysis

All bands that could be reliably read were treated as single dominant loci and scored either present (1) or absent (0) across all genotypes. The degree of genetic similarity was determined with the Nei's formula (Nei & Li 1979). The dendrogram was created with UPGMA. The results were analyzed statistically in the computer program Statistica 6.0.

Results

Primer efficiency

An analysis of 12 populations of the three species of *Polygonatum* using 17 primers representing four DNA marker categories enabled 189 bands to be distinguished (Table 3). The greatest number of bands were identified in *P. verticillatum* (108), followed by *P. multiflorum* (91) and *P. odoratum* (77). The highest number, 111 different bands, were revealed by 10 RAPD primers, on average 11.1 loci per primer. 64 RAPD bands were found in *P. verticillatum*, 53 in *P.*

Table 3. Comparison of genetic variability identified on the DNA level in *Polygonatum odoratum*, *P. multiflorum* and *P. verticillatum*.

	P. odoratum				Total	*P. multiflorum*				Total	*P. verticillatum*				Total
Marker	RAPD	ISJ	*IS6110*	*katG*		RAPD	ISJ	*IS6110*	*katG*		RAPD	ISJ	*IS6110*	*katG*	
Number of bands	44	19	4	10	77	53	21	4	13	91	64	21	4	19	108
Number of polymorphic bands	12	4	1	3	20	17	8	2	2	29	14	2	1	1	18
Percentage of polymorphic bands	27.2	21.0	25.0	30.0	26.0	32.0	38.0	50.0	15.4	31.9	21.9	9.5	25.0	5.3	16.7
Number of bands specific to a species	13	5	2	0	20	17	7	2	1	27	45	7	2	11	65
Percentage of bands specific to a species	29.5	26.3	50.0	0	26.0	31.2	33.3	50.0	7.7	29.7	70.3	33.3	50.0	57.9	60.2

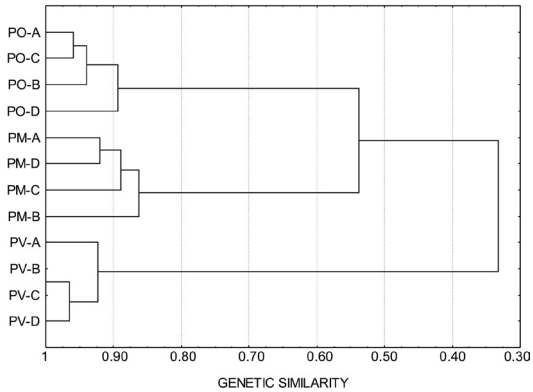

Fig. 1. UPGMA dendrogram of 12 populations of *Polygonatum* based on Nei's genetic similarity.

multiflorum and 44 in *P. odoratum*. The three ISJ primers showed similar efficiency, revealing a total of 35 different bands (11.7 loci per primer). The number of ISJ bands was similar in all species, and ranged from 21 in *P. multiflorum* and *P. verticillatum*, to 19 in *P. odoratum* (Table 3). The primers complementary to the bacterial insertion sequence *IS6110* revealed a total of 9 different bands whose number in particular taxa was equal to four (Table 3). Three pairs of primers complementary to the bacterial sequences of the *katG* gene enabled 34 different bands to be distinguished. The highest number of *katG* bands were recorded in *P. verticillatum* (19), followed by *P. multiflorum* (13) and *P. odoratum* (10) (Table 3).

DNA polymorphism in the *Polygonatum* species

The highest polymorphism was observed in the populations of *P. multiflorum* with 31.9% polymorphic loci, followed by the populations of *P. odoratum* with 26% and *P. verticillatum* with 16.7%. The RAPD markers turned out to be the most polymorphic within the species examined; 32% polymorphic RAPD bands were found in *P. multiflorum*, 27.2% in *P. odoratum*, and 21.9% in *P. verticillatum* (Table 3). Equally significant differences in polymorphism among the populations were revealed by ISJ markers. As with the RAPD markers, the highest percentage of polymorphic ISJ loci was recorded in *P. multiflorum* (38%), and the lowest in *P. veriticillatum* (9.5%). Also the insertion sequence *IS6110* was the most polymorphic in *P. multiflorum*, with 50% polymorphic bands, compared with 25% in *P. odoratum* and *P. verticillatum*. Three elements of the *katG* gene show very low intraspecific diversity. However, the correlations between polymorphism parameters and DNA marker category were not statistically significant. They revealed polymorphism in one to three bands, depending on species, thus proving their high value as species-specific markers.

Table 4. Nei's genetic similarity among investigated species on the base of various DNA markers. P.o. = *Polygonatum odoratum,* P.m. = *P. multiflorum,* P.v. = *P. verticillatum.*

Species pair	RAPD	ISJ	*katG*	*IS6110*
P.o. *vs.* P.m.	0.51	0.58	0.72	0.25
P.o. *vs.* P.v.	0.20	0.58	0.20	0.25
P.m. *vs.* P.v.	0.30	0.52	0.27	0.25

Genetic similarity on intra and interspecific levels

The level of genetic similarity between the taxa analyzed, estimated using DNA markers, indicates that they are good biological species. The In value between the closely related *P. odoratum* and *P. multiflorum* was 0.57, and between them and *P. verticillatum* 0.37 and 0.40, respectively (Table 4). Similar results were obtained when the populations were grouped according to the UPGMA method (Fig. 1) and a principal component analysis (Fig. 2).

The categories of DNA markers applied in the study differed in terms of revealing genetic similarity between the taxa examined. The ISJ markers revealed a similar degree of genetic similarity, i.e. 0.58 between *P. odoratum* and *P. multiflorum*, and respectively 0.58 and 0.52 between these two taxa and *P. verticillatum*. In RAPD markers the respective values were 0.51, 0.20 and 0.30. Among the bacterial sequences, those of the *katG* gene provided similar results as those achieved with RAPD markers, reveal-

ing remarkable similarity between *P. odoratum* and *P. multiflorum*, 0.72, and respectively 0.20 and 0.27 between them and *P. verticillatum*. The insertion sequence *IS6110* indicated the value In = 0.25 between the taxa analyzed. The results of the UPMGA grouping method for each marker category are presented in Fig. 3.

The populations showed a much higher genetic similarity at the intraspecific level than at the interspecific level (Table 5). The highest similarity was found in the populations of *P. verticillatum*. Three populations of this species were almost identical (In = 0.98–0.99), whereas the genetic similarity between them and the fourth population varied from 0.91 to 0.94. More differentiated were the populations belonging to the section *Polygonatum*. The genetic similarity between the populations of *P. multiflorum* ranged from 0.81 to 0.92, and between the populations of *P. odoratum* from 0.88 to 0.94.

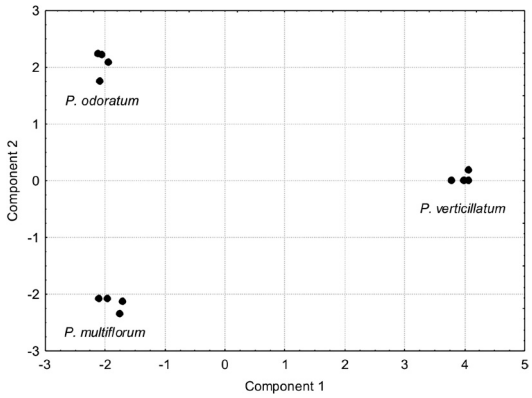

Fig. 2. Principal component analysis of 12 *Polygonatum* populations.

Species-specific and section-specific DNA markers

The four categories of DNA markers applied in the study revealed a total of 112 species-specific

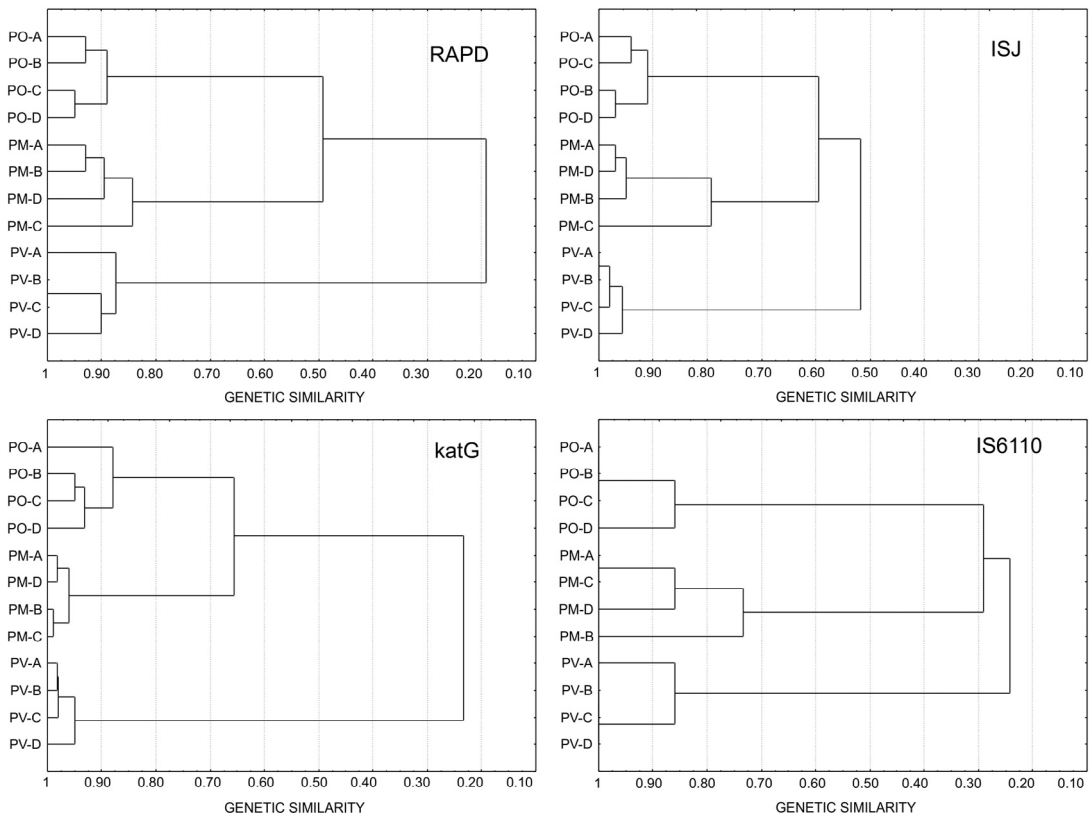

Fig. 3. UPGMA trees created for each DNA marker category.

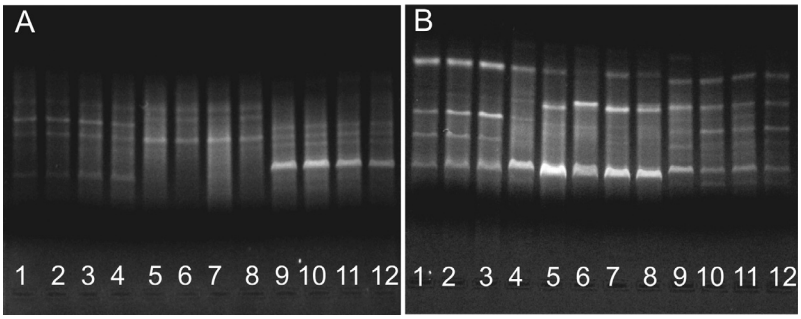

Fig. 4. DNA amplification patterns for three *Polygonatum* species (1–4: *P. odoratum*, 5–8: *P. multiflorum*, 9–12: *P. verticillatum*) with (**A**) species-specific ISJ2 and (**B**) *IS6110* primers.

bands, including 65 (60.2%) in *P. verticillatum*, 27 (29.7%) in *P. multiflorum* and 20 (26%) in *P. odoratum* (Table 3). The greatest number of species-specific markers for *P. odoratum* and *P. multiflorum* were revealed by RAPD markers (13 and 17) and ISJ markers (5 and 7), and the lowest by *katG* (0 and 1). In *P. verticillatum* the number of revealed species-specific RAPD markers was much greater (45), and the number of *katG* markers was also high (11). All markers specific to *P. verticillatum* are at the same time markers capable of differentiating between the sections *Verticillata* and *Polygonatum*. Two primers complementary to the genes coding catalase-peroxidase (*katG1* and *katG4*) and the OPD-05 primer showed no differences between *P. odoratum* and *P. multiflorum*. Similarly as species-specific markers, section-specific markers did not reveal polymorphism within the sections. Examples of amplification patterns are given in Figs. 4 and 5.

Discussion

Taxonomic species identified on the basis of morphological and anatomical characters are not always good biological species. An excellent example is the grass genus *Lolium*, in which the morphological classification of species is no longer problematic. There exist certain morphological diagnostic features helping to distinguish between *Lolium perenne* and *L. multiflorum*. However, these two seemingly good taxonomic species turned out to be one biological species (Polok 2005). Despite the application of various types of molecular markers, species-specific markers were not found for these taxa. Taking into account the high genetic similarity (0.95), these taxa cannot be considered biological species.

The species of *Polygonatum* examined in the present study, including *P. odoratum* and *P. multiflorum* showing high morphological similarity,

Table 5. Nei's (1978) genetic similarity (above the diagonal) and genetic distance (below the diagonal) for 12 populations of *Polygonatum*.

Population	PoA	PoB	PoC	PoD	PmA	PmB	PmC	PmD	PvA	PvB	PvC	PvD
PoA		0.938	0.929	0.884	0.535	0.532	0.539	0.530	0.252	0.256	0.245	0.255
PoB	0.064		0.902	0.895	0.578	0.532	0.548	0.531	0.338	0.306	0.298	0.293
PoC	0.074	0.103		0.910	0.545	0.493	0.517	0.507	0.266	0.259	0.248	0.258
PoD	0.123	0.111	0.094		0.574	0.567	0.564	0.568	0.248	0.281	0.282	0.275
PmA	0.625	0.548	0.607	0.555		0.916	0.813	0.835	0.352	0.341	0.329	0.280
PmB	0.631	0.631	0.707	0.567	0.088		0.818	0.916	0.321	0.294	0.291	0.256
PmC	0.618	0.601	0.660	0.573	0.207	0.201		0.877	0.354	0.357	0.375	0.333
PmD	0.635	0.633	0.679	0.566	0.180	0.088	0.131		0.403	0.376	0.358	0.329
PvA	1.378	1.085	1.324	1.394	1.044	1.136	1.038	0.909		0.989	0.984	0.912
PvB	1.363	1.184	1.351	1.269	1.076	1.224	1.030	0.978	0.011		0.991	0.933
PvC	1.406	1.211	1.394	1.266	1.112	1.234	0.981	1.027	0.016	0.009		0.938
PvD	1.366	1.228	1.355	1.291	1.273	1.362	1.010	1.112	0.092	0.069	0.064	

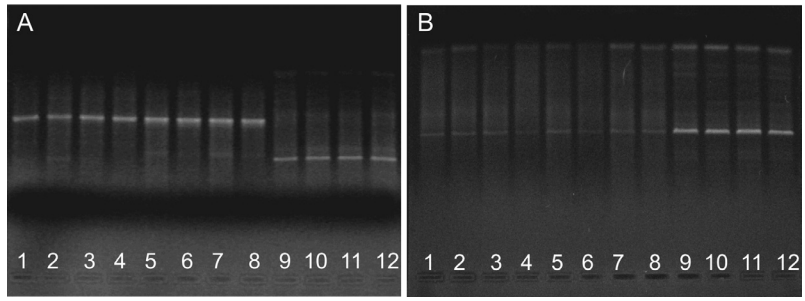

Fig. 5. DNA amplification patterns for three *Polygonatum* species (1–4: *P. odoratum*, 5–8: *P. multiflorum*, 9–12: *P. verticillatum*) with (**A**) section-specific OPD-05 and (**B**) *katG1* primers.

are good biological species. This is confirmed by a low degree of genetic similarity between them, ranging from 0.37 to 0.57, and by the high number of species-specific markers (112).

Among the 17 primers used by us, nine amplified species-specific bands were found in all types of markers. The suitability of RAPD markers, randomly amplifying the genome and widely applied in taxonomic investigations, was confirmed in the genus *Polygonatum*. Five of ten primers of this category generated about 30% species-specific bands in *P. odoratum* and *P. multiflorum*, and 70% in *P. verticillatum*. Also semi-specific ISJ markers (ISJ2, ISJ3) are species-specific, since approximately 30% of them revealed species-specific bands. Molecular identification of the taxa analyzed was also easy to perform using the insertion sequence *IS6110*, which revealed a distinct four-band pattern, with two unique bands in each species.

The highest conservativeness was observed for sequences of the *katG* gene, which differed between *P. odoratum* and *P. multiflorum* only minimally. Similarly as ISJ markers, they revealed a very low level of intraspecific polymorphism, which proves their value as section-specific markers in *Polygonatum*. The suitability of bacterial gene sequences for taxonomic identification was also confirmed in previous studies on *Pinus* (Polok & Chmiel 2005), liverworts of the genus *Pellia* (Polok *et al*. 2005b), and other taxa (Zieliński & Polok 2005).

However, many plant species show much higher values of the genetic similarity coefficient despite distinct morphological differences. This may suggest that they are still at the initial stage of divergence. Apart from the already mentioned *Lolium* species (Polok 2005), a comparable situation was encountered within the genus *Sphagnum*. The genetic similarity between species of the section *Acutifolia*, based on isoenzymatic data, was high and ranged from 0.90 to 0.50 (Cronberg 1996). The highest degree of genetic similarity (0.81) was recorded for *Sphagnum girgensohnii* and *S. fimbriatum*. An analysis of these species using RAPD and ISJ markers also indicated their high genetic similarity — 0.68 and 0.69 respectively — as well as the lack of species-specific markers (Polok *et al*. 2005a). In *Sphagnum* the division into sections based on morphological characters is not always consistent with analyses of molecular data, which was confirmed by studies on organelle DNA (Shaw 2000).

Our results based on genomic DNA positively verified the commonly accepted division of the genus *Polygonatum* into the sections *Verticillata* and *Polygonatum*, based upon morphological and karyological data (Therman 1957, Nowakowska and Żeglicka 1972, Abramova 1975, Tamura 1993), and on an analysis of chloroplast DNA (Tamura 1997). The value of the genetic similarity coefficient between species within the section *Polygonatum* was much higher (0.57) than between sections (0.37–0.40). This resulted from the presence of a greater number of section-specific markers (65) than species-specific ones (20–27). The presence of a high number of section-specific markers was affected primarily by the distinctness of *P. verticillatum*, which manifested itself by the occurrence of 60.2% of both species- and section-specific bands.

The species of *Polygonatum* examined in this study did not show high polymorphism, which may result from a high proportion of vegetative reproduction. The highest percentages of polymorphic bands were observed in *P. multiflorum* and *P. odoratum*, 31.9% and 26%, respectively, compared with 16.7% in *P. verticillatum*. The

low level of polymorphism found in *P. verticillatum* may be related to its geographical range limits. The species is Arctic-montane, and the studied populations from northeastern Poland come from the margins of its range. The populations of this species showed a much higher degree of genetic similarity, on average 0.95, as compared with that of more polymorphic populations of *P. odoratum* and *P. multiflorum* (0.92 and 0.88, respectively).

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