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Genetic variation of some botanical and cultivar varieties of *Acer* sp.

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Abstract

Genetic variation of two botanical varieties of *Acer* sp. (*Acer palmatum* var. *heptalobum*, *Acer palmatum* f. *atropurpureum*) and seven its cultivars (*Acer palmatum* 'Dissectum Atropurpureum', *Acer palmatum* 'Dissectum Ornatum', *Acer palmatum* 'Garnet', *Acer palmatum* 'Osakazuki', *Acer palmatum* 'Nicholsonii', *Acer japonicum* 'Aconitifolium' and *Acer shirasavanum* 'Aureum') from the collection of the Przelewice Dendrological Garden were investigated using inter simple sequence repeat (ISSR) markers. Thirty ISSR primers were screened and 11 were selected for their ability to produce clear and reproducible patterns of multiple bands. A total of 142 *loci* of 200 – 2750 bp were amplified, of which 84 (59.2%) were polymorphic, 8 (5.6%) monomorphic and 50 (35.2%) were accession-specific. In addition, specific ISSR *loci* were obtained for all of the nine accessions tested. A dendrogram generated using the UPGMA, based on a similarity measure of total character difference, showed that the *Acer* cultivars and varieties clustered into three main groups (a, b and c). The first group 'a' included *Acer palmatum* 'Dissectum Ornatum' and *Acer palmatum* var. *heptalobum* (52% similarity), the second one 'b' included 5 cultivars, *Acer palmatum* 'Nicholsonii', *Acer palmatum* f. *atropurpureum, Acer palmatum* 'Osakazuki', 'Garnet' and *Acer japonicum* 'Aconitifolium' (71.5% similarity) and the third one 'c' *Acer shirasavanum* 'Aureum' and *Acer palmatum* 'Dissectum Atropurpureum' (57% similarity).

Key words: *Acer* sp., polymorhism, ISSR, Dendrological Garden in Przelewice, Poland.

Introduction

The history of botanical gardens reaches three thousand years B.C. In Europe, the first scientific and didactic gardens were created in Italy, in Palermo (1309), Padwa (1545) and in Poland in Szczecin (1665) [1]. At present the Szczecin garden does not exist and its didactic, scientific and recreation role has been taken over by the Arboretum in Przelewice, which in 1976, together with the palace that it surrounds, was entered into the Register of Historical Objects of Western Pommerania [1].

The Arboretum in Przelewice was created in 1923 by Conrad von Börsig, member of the German Dendrological Society. Among much else one may find there an interesting collection of rhododendrons, azaleas, lilacs and numerous species of *Acer*, belonging to the most decorative trees and originating from Japan, Korea and East China [2]. Their taxonomy is known, but it has not been confirmed on the DNA level.

Considerable attention was paid to the problem of variability identification and preservation during conferences organised by FAO in Rome (1950, 1961, 1967, 1973, 1981). During the last – "International Board Plant Genetic Resources" seven research problems were discussed [3]. They referred to the search for variability, its identification, examining, utilisation, training, management and international co-operation. One should also mention here the Rio de Janeiro "Earth Summit" in 1992, during which several conventions were signed, creating pro-ecological societies, as an effective guarantee of nature protection and the preservation of biodiversity [1].

The dynamically developing molecular biology has created new methods for analysing genetic variability. They have been used, among much else, for evaluation of differences between species in many world collections of trees and decorative shrubs. Among others one may mention here the studies conducted by Brunner *et al.* [4] and Pandey *et al.* [5]. Brunner *et al.*[4] examined the molecular characteristic of 30 trees from the arboretum of the Swiss Federal Research Institute of Forest, while Pandey *et al.* [5] – *Acer pseudoplantanus* L. from the Forest Botanical Garden of Göttinga University.

The present studies are concordant with the FAO and UNESCO assumptions about nature protection. They were undertaken for two reasons – to popularize the practical and decorative value of the nine *Acer* sp. trees, collected in the Przelewice Arboretum and to evaluate their taxonomy on the DNA level.

Material and Methods

Material: The studies were conducted on two botanical varieties of *Acer* sp. of the Palmata series, species *Acer palmatum* (*A. palmatum* var. *heptalobum* Rehd. and *A. palmatum* f. *atropurpureum* Hogg,), 5 cultivars of the *A. palmatum* species (*A. palmatum* 'Dissectum Atropurpureum' Hogg., *A. palmatum* 'Dissectum Ornatum', *A. palmatum* 'Garnet', *A. palmatum* 'Nicholsonii' Schwer. and *A. palmatum* 'Osakazuki', known also as 'Heptolobum Osakazuki') as well as two varieties of the species *Acer japonicum* (*A. japonicum* 'Aconitifolium') and *Acer shirasavanum* (*A. shirasawanum* 'Aureum') (Photo 1). The species of the listed *Acer palmatum* forms are given after de Jong [6]. The

Photo 1. Photos of choosen genotypes represent the collection of compared accessions of *Acer*.

botanical variety *A. palmatum* var. *heptalobum* Rehd. occurs in the Przelewice Arboretum under the name *A. palmatum* 'Thunbergii' [7]. The plant material originated from the collection of the Przelewice Dendrological Garden.

Acer leaves (±100 mg) were ground in liquid nitrogen with a mortar and pestle and incubated at 37°C with protease. The protocol for DNA extraction was as described in Genomic Mini AX Plant protocol (A&A Biotechnology). The DNA was resuspended in 0.2 ml of TE buffer (10 mM Tris-HCl [pH 8.0], 1 mM EDTA). RNase A (Sigma) was added to the DNA samples to eliminate RNA by incubation at 37°C for 1 h. Next, genomic DNA was quantified (GeneQuant DNA/RNA Calculator – Pharmacia LKB). The ISSR reactions were carried out in 15 ml of buffer (1 U of Taq polymerase, 50 ng of genomic DNA template, 30 pmol of ISSR primers, 2.5 mM each dATP, dCTP, dGTP and dTTP and 1.5 ml of 10× PCR reaction buffer (500 mM KCl, 100 mM Tris-HCl [pH 8.8], 1% Triton X) and 15 mM $MgCl_2$). DNA amplifications were carried out in a Mastercycler (Eppendorf) with a preliminary step of 7 min at 94°C, followed by 35 cycles of 30 s at 94°C, 30 s at annealing temperature and 2 min at 72°C and a final step of 7 min at 72°C. The annealing temperature was usually adjusted according to the T_m of the primers being used in the reaction. Amplified products were mixed with 5 ìl of 6´ Orange Loading Dye Solution (Fermentas MBI) and were analysed on 2% agarose (Prona) in 1x TBE buffer, then stained with ethidium bromide (0.5 $mg \cdot ml^{-1}$) and photographed (Polaroid DS-34). GenRuler 200 bp DNA Ladder (Fermentas MBI) was used as a size marker (3000–200 bp). The amplification products of different primers recorded on photos were scored in a binary matrix (1 for presence and 0 for absence). From the binary matrix data the similarities were estimated by the similarity estimation method. In the TREECON for Windows software package, the UPGMA method was used to construct an unrooted tree and neighbour-joining approach was used for construction of a rooted tree [8-10]. The resulting data sets of nine *Acer* accession were bootstraped (2,000 replicates).

Results

Among the DNA ISSR primers, used for amplification, only 11 (802, 809, 810, 811, 817, 820, 821, 839, 840, 841 and 845) generated clearly visible PCR products of all the varieties of *Acer palmatum* compared (Table 1). In total, in the PCR reactions performed 142 ISSR *loci* were amplified, of which 8 (5.6%) were monomorphic, 84 (59.2%) polymorphic and 50 (35.2%) accession-specific (Table 1). In reaction with one primer a mean of 13 ISSR *loci* was amplified, the maximum being 20 with primer 840 [$(GA)_8$GT] and the minimum 6 with primer 802 [$(GA)_8$T]. The longest ISSR product (2750 bp) was generated with primer 811, the shortest (200 bp) in the reaction with primer 820.

For the *Acer* sp. varieties examined in the studies presented, a total of 433 PCR products were generated, the most (63) for *A. palmatum* var. *heptalobum*, the least (44) for cultivar *A. palmatum* 'Dissectum Ornatum'. For the remaining cultivars of the *Acer* species examined 44 to 51 ISSR products were generated.

Eight common, monomorphic ISSR *loci* were obtained in reactions with primers containing a repeated block [$(GA)_8$]: 802 (720, 610 and 540 bp) and 810 (730, 630 and 470 bp), one each 480 and 520 bp long, with primers 809 [$(GA)_8$YG] and 817 [$(AG)_8$YG], respectively (Table 2). The highest number of polymorphic ISSR *loci* was amplified using primers 840 (20) and 820 (11), the lowest (1 and 2 *loci*), with primers 802 and 810, respectively (Table 1). In reaction with one primer a mean of 8 polymorphic *loci* was amplified, visible on electrophoregrammes in the form of DNA profiles, characteristic for the given trees.

In reaction with seven primers (809, 820, 821, 839, 840, 841 and 845) DNA patterns were obtained (fingerprint) which rendered it possible to determine the genetic identity of each of the *Acer* variety examined (Table 3). However, it did not prove possible to differentiate all the *Acer* sp. genotypes examined in the reactions with four primers used (802, 810, 811 and 817). Primer 811 did not differentiate forms *A. palmatum* 'Dissectum Ornatum' and *A. palmatum* 'Nicholsonii', primer 817 did not show the differences between *A. palmatum* 'Garnet' and *A. palmatum* 'Dissectum Ornatum', primer 810 differentiated *A. palmatum* 'Dissectum Atropurpureum', *A. palmatum* 'Garnet' and *A. palmatum* 'Osakazuki'. In the case of the remaining varieties the DNA patterns obtained in reaction with this primers were the same (Table 3).

On the basis of the results obtained for each *Acer* sp. variety a series of specific ISSR *loci* was obtained. They comprise specific molecular markers, rendering it possible to differentiate the individual tree genotypes examined. In the present experiment 50 such markers were generated (Table 4). The greatest number of accession-specific ISSR *loci* (13 and 8, respectively) was amplified for *A. palmatum* var. *heptalobum* and *A. palmatum* 'Dissectum Atropurpureum'. In ISSR reactions with primers 809, 839 and 817 and 840 two specific *loci* were generated for both *A. palmatum* 'Nicholsonii' and *A. japonicum* 'Aconitifolium'. For the remaining cultivars were amplified between 6 (*A. palmatum* 'Garnet') and 4

Table 1. Characteristics of ISSR's generated for eight accession of *Acer* sp.

Primer No.	Sequence 5' – 3'	Fragment size range (bp)	Number of amplified loci: Total	Monomorphic	Polymorphic	Accessions -specific	*Acer palmatum* 'Dissectum Atropurpureum'	*Acer palmatum* 'Garnet'	*Acer shirasawanum* 'Aureum'	*Acer palmatum* 'Dissectum Ornatum'	*Acer palmatum* 'Nicholsonii'	*Acer palmatum* 'Osakazuki'	*Acer palmatum* var. *heptalobum*	*Acer japonicum* 'Aconitifolium'	*Acer palmatum* f. *atropurpureum*	Generated amplicons
802	$(GA)_8T$	720-430	6	1	3	2	4	5	4	4	4	4	4	4	4	37
809	$(GA)_8YG$	1080-270	16	10	1	5	4	6	6	5	6	5	7	5	4	48
810	$(GA)_8T$	930-470	7	2	3	2	5	4	4	4	4	5	4	4	4	38
811	$CAC(CA)_6AT$	**2750**-670	13	8	0	5	6	1	6	2	2	3	4	1	5	30
817	$(AG)_8YG$	1400-280	10	7	1	2	5	4	5	4	5	5	6	4	5	43
820	$(GA)_8YC$	1850-**200**	14	11	0	3	4	4	2	5	5	4	7	5	5	41
821	$(GA)_8YC$	1680-300	11	5	0	6	1	3	1	2	2	3	4	3	2	21
839	$(AG)_8T$	1350-400	14	8	0	6	5	1	4	4	4	3	6	2	3	32
840	$(GA)_8GT$	1500-330	20	16	0	4	7	6	8	6	6	5	9	10	7	64
841	$(GA)_8AT$	1530-420	16	9	0	7	4	3	5	4	5	3	6	5	5	40
845	$(TC)_8GT$	1750-260	15	7	0	8	4	6	0	4	2	5	6	5	7	39
		Total	**142**	**84**	**8**	**50**	49	43	45	44	45	45	63	48	51	**433**
		Mean	**13**	**8**	**1**	**5**	4	4	4	4	4	4	6	4	5	**39**

(*A. shirasawanum* 'Aureum') *loci* specific for the genotype (Table 4).

The genetic similarity between the *Acer* sp. cultivars examined, calculated on the basis of the UPGMA method, remained between 34 and 62%, the lowest being observed between *A. palmatum* 'Dissectum Ornatum' and *A. palmatum* f. *atropurpureum* (34%), while the highest between *A. palmatum* 'Nicholsonii' and *A. palmatum* f. *atropurpureum* (62%). Among the examined cultivars of *Acer palmatum* three similarity groups were separated ('a', 'b' and 'c'). Group 'a' comprised *A. palmatum* 'Dissectum Atropurpureum' and *A. palmatum* var. *heptalobum*, similar in 52%. Group 'b' comprised five accessions. In this group the most similar genetically (62%) were *A. palmatum* 'Nicholsonii' and *A. palmatum* f. *atropurpureum*. The next two cultivars *A. palmatum* 'Osakazuki' and 'Garnet' were phylogenetically similar in 52%. The last variety in group 'b' *A. japonicum* 'Aconitifolium' was phylogenetic most similar to *A. palmatum* f. *atropurpureum* (56%). Phylogenetic group 'c' comprised *A. shirasavanum* 'Aureum' and *A. palmatum* 'Dissectum Atropurpureum', similar in 57.4%.

Discussion

The cultivars of *Acer* (till now about 300 cultivars have been described) differ from each other in conformation, growth rate as well as leaf shape and colour. In the past they were often and readily grown in parks and gardens. Thus in the respective literature one may find several reports referring to their taxonomy. However, the opinions of authors as to their taxonomy differ.

De Jong [6] divided the genus *Acer* into 16 sections and some of them additionally into series. The most numerous, according to this author is section Palmata, which numbers about 50 species, divided between three series – Palmata, Sinenssia and Peminervia. In the first series De Jong included three species *A. japonicum*, *A. palmatum*, to which belong most of the cultivars examined in this work, and *A. shirasavanum*.

In turn Bugala [11] separated three *Acer* subspecies ssp. *Amoneum* Hara, ssp. *Matsumarae* Koidz. and ssp. *Palmatum*, while Gelderen [12] seven groups of cultivars of which 'Palmatum', 'Amoneum', 'Matsumarae', 'Dissectum' and 'Linearilobum' the considered to be the most popular.

Among the cultivars examined in the present study the group Palmatum, with deeply cut leaves (till ¾ of the leaf blade) comprises two cultivars 'Atropurpureum' and 'Aureum', the Amoneum group, with shallow cuts in leads (2/3 of the leaf blade), the 'Nicholsonii' cultivar, the group Dissectum, cultivars 'Dissectum Atropurpureum', 'Dissectum Ornatum' and 'Garnet'[2, 11].

In literature one may also find works on genetic variability and identification of species from the *Acer* genus. The studies conducted by Bless *et al.* [13] presented the results of identification of the *A. rubrum* species using the AFLP technique. Iwasaki *et al.* [14] studied the intraspecific cpDNA variations of many tree species, including *A. japonicum* and *A. micranthum*. Among the earlier studies are those conducted by Watmouger and Dickinson[15] on the variability of *A. pseudoplatanus* L. (Sycamore) in relation to the resistance to metals in callus cultures. The experimental material came from mature, 50 years old trees growing in seven different locations with a toxic metal content and three locations showing no toxic pollution. From the callus cultures the authors obtained 28 lines which they then tested for

Table 2. ISSR products amplified in reaction with primers with chosen sequence of repeated motif.

Sequence	Primer No.	*Loci*			
		Total	Polymorphic	Accession-specific	Monomorphic
$[(GA)_n]$	802, 809, 810, 820, 821, 840, 841	90	54	29	7
$[(AG)_n]$	817, 839	24	15	8	1
$[(CA)_n]$	811	13	8	5	0
$[(TC)_n]$	845	15	7	8	0

Table 3. Genetic diversity obtained in the ISSR reactions for *Acer* accessions.

Accession	Primer number				
	802	810	811	817	809, 820, 821, 839, 840, 841, 845
Acer palmatum 'Dissectum Atropurpureum'		+	+	+	+
Acer palmatum 'Garnet'	+	+	+		+
Acer shirasawanum 'Aureum'			+	+	+
Acer palmatum 'Dissectum Ornatum'					+
Acer palmatum 'Nicholsonii'				+	+
Acer palmatum 'Osakazuki'		+	+	+	+
Acer palmatum var. *heptalobum*			+	+	+
Acer japonicum 'Aconitifolium'			+	+	+
Acer palmatum f. *atropurpureum*			+	+	+

Table 4. Accessions-specific products revealed through ISSR fingerprinting.

Accession	Primers no. and product length in bp
Acer palmatum 'Dissectum Atropurpureum'	$810_{[920]}$; $811_{[810, 670]}$; $821_{[380]}$; $839_{[460, 400]}$; $841_{[1300]}$; $845_{[1750]}$
Acer palmatum 'Garnet'	$802_{[500, 430]}$; $809_{[770]}$; $821_{[580]}$; $839_{[430]}$; $845_{[930]}$
Acer shirasawanum 'Aureum'	$820_{[590]}$; $821_{[940]}$; $840_{[680]}$; $841_{[420]}$
Acer palmatum 'Dissectum Ornatum'	$840_{[1400]}$; $841_{[1020, 450]}$; $845_{[1010, 420]}$
Acer palmatum 'Nicholsonii'	$809_{[880]}$; $839_{[1350]}$
Acer palmatum 'Osakazuki'	$809_{[290]}$; $810_{[870]}$; $817_{[800]}$; $841_{[1160]}$; $845_{[370]}$
Acer palmatum var. *heptalobum*	$809_{[1080, 980]}$; $811_{[2750]}$; $820_{[830, 200]}$; $821_{[1680, 750]}$; $839_{[1230]}$; $840_{[940]}$; $841_{[1530, 460]}$; $845_{[670, 260]}$
Acer japonicum 'Aconitifolium'	$817_{[870]}$; $840_{[1500]}$
Acer palmatum f. *atropurpureum*	$811_{[2250, 1100]}$; $821_{[490]}$; $839_{[630]}$; $845_{[1480]}$

resistance to the content of copper, cadmium, zinc and nickel. The studies conducted by Terhui *et al.* [16] were devoted to the description of microsatellite *loci* in *Acer capillipes*. Those authors examined 14 different species from the Ikadaba National Forest in Shizuoka, Tokyo, University Forest in Chikibu, Saitama and Katashina National Forest in Gumma, Japan. Seven polymorphic *loci* were isolated and according to those authors, may be useful for the identification of the *Acer* genus.

In the present study an attempt was made at describing the genetic variability between nine species of the *Acer* genus, section Palmatum, Japonicum and Shirasavanum, using the ISSR-PCR technique. The results obtained are compliant with those of other authors who confirm the value of this method for studies on phylogenetic relations between plants [17-21]. The value of the method discussed lies principally in the amplification of a large number of polymorphic ISSR *loci*, specific for the objects examined. In the present work 142 ISSR *loci* were amplified using the method discussed. Eight (5.6%) of those were monomorphic, 84 (59.2%) polymorphic and 50 (35.2%) accession-specific. In reaction with one primer a mean of 13 *loci* was amplified, the most (20) with primer 840 $[(GA)_8GT]$, the least with primer 802 $[(GA)_8T]$. For the varieties of *Acer* sp. examined in the present study a total of 433 PCR products was generated, the most (63) for A. *palmatum* var. *heptalobum*, the least (44) for cultivar *A. palmatum* 'Dissectum Ornatum' and from 44 to 51 ISSR products for the remaining cultivars examined.

The results obtained rendered it possible to determine the genetic similarity between nine cultivars belonging to the species *A. palmatum* Thunb. (cultivars: 'Atropurpureum', 'Dissectum Atropurpureum', 'Dissectum Ornatum', 'Garnet', 'Nicholsonii', 'Osakazuki' and var. *heptalobum*) and *A. shirasavanum* 'Aureum'. This similarity ranged from 34 to 62%, what confirms that the Acer varieties examined belong to the same series – Palmata. Cultivars *A. japonicum* 'Aconitifolium' and *A. shirasavanum* 'Aureum', classified by taxonometrists as series Palmata, were in the present studies similar to other species of this series in 41-57%. In turn, the similarity of cultivar *A. palmatum* 'Dissectum Atropurpureum' to the selected from it cultivar 'Garnet' amounted to 50%. Both those varieties were classified as the same taxonomy group, but on the basis of a dendrogramme grouping the forms genetically most similar, they were ascribed to different phylogenetic groups. In the study presented the smallest similarity (34%) was observed between *A. palmatum* 'Dissectum Ornatum' and *A. palmatum* 'Atropurpureum'.

Conclusions

The DNA amplifications, conducted using the ISSR-PCR technique, rendered it possible to separate ISSR *loci*, specific for each of the *Acer* sp. objects examined. *A. palmatum* 'Dissectum Atropurpureum' differed for the other varieties examined by the presence of 8 specific ISSR *loci* on the electrophoregrammes. In *A. palmatum* 'Garnet' 6 such *loci* were identified, in *A. palmatum* 'Dissectum Ornatum', *A. palmatum* 'Osakazuki' and *A. palmatum* f. *atropurpureum* – 5 *loci*, in *A. palmatum* 'Nicholsonii' – 2 *loci*, in *A. palmatum* var. *heptalobum* – 13 *loci*, in *A. shirasavanum* 'Aureum' – 4 *loci* and in *A. japonicum* 'Aconitifolium' – 2 ISSR *loci*.

References

[1]Ciaciura, M. 2006. The significance of the creation of a botanical garden for maintaining biological diversity in Szczecin. In Tarasiuk, J. and Kepczyñski, J. (eds). A Human Being and the Natural Environment of Western Pomerania. vol. 1, Print Group, Szczecin, pp. 55–59.

[2]Banaszczak, P. 2003. It's very close to fall in love, it's only one step i.e. one maple tree. Gardens **11**:22–27.

[3]Hawkes, J. G. 1985. Plant genetic resources: The impact of the international agricultural research centers. CGIAR Study Paper No. 3, World Bank, Washington, D. C.

[4]Brunner, I., Brodbeck, S., Büchler,U. and Sperisen, C. 2001. Molecular identification of fine roots of trees from the Alps: reliable and fast DNA extraction and PCR–RFLP analyses of plastid DNA. Molecular Ecology **10**:2079–2087.ub

[5]Pandey, M., Gailing, O., Fischer, D., Hattemer, H. H. and Finkeldey, R. 2004. Characterization of microsatellite markers in sycamore (*Acer pseudoplatanus* L.). Molecular Ecology Notes **4**:253–255.

[6]Jong De, P. C. 1994. Taxonomy and reproductive biology of maples. In Van Gelderen, D. M., Jong De, P. C. and Oterdoom, H. J. (eds). Maples of the World. Timber Press, Portland, Oregon, pp. 69–104.

[7]Seneta, W. 1991. Deciduous Trees and Shrubs. A-B. PWN, Warszawa.

[8]Nei, M. and Li, W.H. 1979. Mathematical model for studying genetic variation in terms of restriction endonucleases. Proc. Natl. Acad. Sci. USA **76**:5269-5273.

[9]Van de Peer, Y. and De Wachter, R. 1994. TREECON for Windows: A software package for the construction and drawing of evolutionary trees for the Microsoft Windows environment. Comput. Appl. Biosci. **10**:569–570.

[10]Felsenstein, J. 1985. Confidence limits on phylogenesis: An approach using the bootstrap. Evolution **39**:783-791.

[11]Bugala, W. 2000. Trees and Shrubs. PWRiL.

[12]Gelderen, D. M. van., De Jong, P. C. and Oterdoom, H. J. 1994. Maples of the World. Timber Press, Portland, Oregon.

[13]Bless, C., Palmeter, H. and Wallace, M.M. 2006. Identification of *Acer rubrum* using amplified fragment length polymorphism. Journal of Forensic Sciences **51**(1):31-38.

[14]Iwasaki, T., Aoki, K., Seo, A. and Murakami, N. 2006. Intraspecific sequence variation of chloroplast DNA among the component species of deciduous broad-leaved forests in Japan. Journal of Plant Research **119**(5):539-552

[15]Watmough Shaun, A. and Dickinson, N. M. 1996. Variability of metal resistance in *Acer pseudoplatanus* L. (sycamore) callus tissue of different origins. Environmental and Experimental Botany **36**(3):293–302.

[16]Terhui, H., Lian, C. L., Saito, Y. and Ide, Y. 2006. Development of microsatellite markers in *Acer capillipes.* Molecular Ecology Notes **6**:77–79.

[17]Fang, D. Q., Roose, M. L., Krueger, R. R. and Federicé, C. T. 1997. Fingerprinting trifoliate orange germplasm accessions with isozymes, RFLPs and inter–simple sequence repeat markers. Theor. Appl. Genet. **95**:211- 219.

[18]Blair, M. W., Panaud, O. and Mc Couch, S. R. 1999. Inter–simple sequence repeat (ISSR) amplification for analysis of microsatellite motif frequency and fingerprinting in rice (*Oryza sativa* L.). Theor. Appl. Genet. **98**:780–792.

[19]Joshi, S. P., Gupta, V. S., Aggarwal, R. K., Ranjekar, P. K. and Brar, D. S. 2000. Genetic diversity and phylogenetic relationship as revealed by inter simple sequence repeat (ISSR) polymorphism in the genus *Oryza*. Theor. Appl. Genet. **100**:1311–1320.

[20]Fernández, M. E., Figueiras, A. M. and Benito, C. 2002. The use of ISSR and RAPD markers for detecting DNA polymorphism, genotype identification and genetic diversity among barley cultivars with known origin. Theor. Appl. Genet. **104**:845–851.

[21]Rzepka-Plevneš, D., Smolik, M. and Tanska, K. 2006. Polymorphism of microsatellite sequences in morphologically and phenologically different genotypes of *Syringa* sp. Dendrobiology **56**:61-67.