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GENETIC DIVERSITY OF IMPORTANT HORTICULTURAL CACTI SPECIES FROM THE GENUS *Astrophytum* AND *Frailea* ESTABLISHED USING ISSR AND SCOT MARKERS

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ABSTRACT

Background. The *Astrophytum* and *Frailea* genus are very important cacti for horticultural production and collectors over the world. The problem is their similarity at the seedling or cutting stage. Additionally many of cactus species are endangered in natural habitats and require protection. The aim of the study was to develop a reliable system of molecular markers based on multiple loci in the genomes of the cacti species.
Material and methods. The seeds of all cacti species (or a shoot fragment of *A. caput-medusae)* were surface sterilized and then grown under *in vitro* conditions to obtain sterile seedlings for DNA isolation. The six species of cacti of the genus *Astrophytum* (*A. asterias*, *A. capricorne*, *A. caput-medusae*, *A. coahuilense*, *A. myriostigma*, *A. ornatum*) and two selected species of the genus *Frailea* (*F. castanea* syn. *A. asterioides* and *F. schilinzkyana* syn. *A. schlinzkyanum*) were analyzed with the use of molecular markers: Inter-Simple Sequence Repeat (ISSR) and Start Codon Targeted Polymorphism (SCoT).
Results. A higher average polymorphism accounting for 78.95% was found for SCoT than for the ISSR (61.84%). However, specific bands dominated in the ISSR marker constituting 38.16%, while SCoT accounted for 17.02% species representing the genus *Astrophytum* and two species of the *Frailea*. Combined ISSR-SCoT cluster analysis showed that studied species were separated into two clusters: (i) *F. castanea* and (ii) large cluster including *Astrophytum* species and *F. schilinzkyana*. Moreover, a close phylogenetic relationship was found between *A. capricorne* and *A. coahuilense*.
Conclusion. We have developed a highly useful system of molecular ISSR and SCoT markers based on multiple loci in the genomes of the cacti species investigated to study the genetic identity of the species in genetic resources storage, plant breeding, horticultural production (*in vivo* and *in vitro*), and other purposes.

Key words: Cactaceae, molecular markers, *in vitro* germination, species identification

INTRODUCTION

Cactaceae (Juss.) is a family of stem succulents, which includes over 2,000 species (Pérez-Molphe-Balch *et al*., 2015). Cacti are known for the wealth of shapes and colors. For that reason they are grown primarily as

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ornamental and collector plants around the world. However, their ingredients are also used in food, cosmetics and pharmaceutical industries. For that reason the species of the Cactaceae family could be used for the development and formulation of new anticancer drugs, antioxidants, antimicrobial, or insecticidal agents (Santos-Díaz *et al*., 2019).

At natural sites, cacti occur mainly in the subtropical and tropical zones of North and South America. Many species are currently threatened with extinction in the natural environment due to over-collecting for horticultural purposes, and for that reason they are protected by the CITES (Convention on International Trade in Endangered Species of Wild Fauna and Flora). For example, *Astrophytum asterias* (Zucc.) Lem. under natural conditions occurs only in a few locations in Mexico and Texas. It is highly valued as an ornamental plant and desired by cactus growers and collectors (Martínez-Ávalos *et al*., 2007). It has been protected since 1993 (Fish, 2003).

Cacti reproduce primarily generatively through seeds or vegetatively by axillary shoots. In horticultural production, cacti are additionally propagated by grafting on rootstocks, especially used in no-chlorophyll forms. Traditional *in vivo* cacti propagation methods, however, are relatively inefficient due to a slow plant growth and a poor seed formation. Cacti micropropagation facilitates a much faster growth and a higher production efficiency. At *in vitro* conditions, morphogenetic processes in those plants are several times faster than *in vivo*, mainly due to the addition of growth regulators and sucrose to the medium (Pérez-Molphe-Balch *et al*., 2002). For that reason, the micropropagation technique is a very important tool for cacti regeneration and conservation and for horticultural production (Lema-Ruminska and Kulus, 2014; Pérez-Molphe-Balch *et al*., 2015). The first stage of plant micropropagation is obtaining a stable, pathogen-free culture. So far, there are no reports on the germination of seeds of the genera *Astrophytum* and *Frailea*, and the publications on the germination of other cactus species of horticultural importance in *in vitro* cultures are few (de Medeiros *et al*., 2006; dos Reis *et al*., 2012; Pedraza-Santos *et al*., 2015; Cortes-Olmos *et al*., 2018).

Genus *Astrophytum* Lem. includes species with a similar morphology. Throughout the 20th century, the number of species classified as the genus *Astrophytum* varied, and ranged from 4 to 7 (Sadovsky *et al*., 1979; Bravo-Hollis and Sánchez-Mejorada, 1991; Anderson, 2001). The species *A. coahuilense* was often considered earlier as *A. myriostigma*. An attempt was made by Hunt *et al*. (2006) who systematized the species questioned: *A. caput-medusae* and *A. coahuilense* (Moeller) Kaysser. The first one is considered to be a species very different from the others in terms of morphology (Hunt *et al*., 2006). It has even been described before as a separate genus of *Digitostigma caput-medusae* Velasco & Nevárez. However, the classification was based on morphological features regarding the growth form, root system structure and areoles (Velazco *et al*., 2002). According to the currently adopted Reveal system, the genus *Astrophytum* consists of 6 species: *A. asterias*, *A. capricorne*, *A. caput-medusae*, *A. coahuilense*, *A. myriostigma and A. ornatum* (Reveal, 1999).

The genus *Frailea* Br et R. is represented by a group of 12–15 species of cacti (from small spherical to short-columned), morphologically similar to those belonging to the genus *Astrophytum*, however, originating from South America and classified as a separate genus (Bárcenas *et al*., 2011). *Frailea castanea* (syn. *Astrophytum asterioides*) and *F. schilinzkyana* (syn. *Astrophytum schlinzkyanum*) are very similar (e.g., they are characterized by a perfect symmetry and white flakes) to the genus *Astrophytum*, which is also reflected in the synonymous Latin names of those species. However, there is a lack of research into the molecular characteristics of both groups to clarify the genetic differences and similarities (Metzing and Thiede, 2001). Earlier studies of the genus *Astrophytum* focused only on an incomplete number of species currently qualified as the genus *Astrophytum*, e.g., 4 species *(A. asterias, A. capricorne, A. myriostigma and A. ornatum*), and they were performed using one type of RAPD (Random Amplified Polymorphic DNA) marker (Das, 2008), or were based mainly on morphological and chloroplast markers (Vargas-Luna *et al*., 2018). In contrast, phylogenetic studies in the *Cactaceae* family carried out by Bárcenas *et al*. (2011) were based on the single gene region *trnK-matK* of chloroplast genome. And, as the authors suggest, they may provide a lower resolution and support than other studies employing multiple gene regions. To date, however, there is

a lack of an effective method of identifying all the species belonging to *Astrophytum* and *Frailea* genus.

The ISSR technique, among others, was previously used in the analysis of polymorphism in *Hordeum vulgare* L. (Fernandes *et al*., 2002), in the analysis of genetic variability of *Oryza granulata* (Qian *et al*., 2001), *Larix gmelinii* (Zhang *et al*., 2013), or with *Echinacea purpurea* (Lema-Ruminska *et al*., 2019). SCoT markers are based on short conserved regions surrounding the translation start codon (ATG) in the plant genome and were first described by Collard and Mackill (Collard and Mackill, 2009). SCoT markers were used, among others, to assess genetic variation and relationships in cultivated peanut (*Arachis hypogaea* L.) (Xiong *et al*., 2011), genetic diversity in wild and domesticated populations of *Boehmeria nivea* L. (Satya *et al*., 2015) in *Dactylis glomerata* (Zeng *et al*., 2015), in *Phoenix dactylifera* cultivars (Al-Qurainy *et al*., 2015), in *Vicia sativa* (Xutian *et al*., 2017) or wild *Salvia* accessions (Etminan *et al*., 2018). Among the various marker systems, the SCoT marker gains an advantage due to its higher polymorphism and better detection ability over other DNA marker systems, such as RAPD or ISSR (Satya *et al*., 2015, Zeng *et al*., 2015). What more, the SCoT marker system does not require genome sequence information (Shangguo *et al*., 2018). The study of cultivar or species identity and its molecular identification is known for many plant species and with various marker systems (Lema-Ruminska *et al*., 2004; Jędrzejczyk and Rewers, 2018).

The aim of our research was to develop a reliable system of molecular ISSR and SCoT markers based on multiple loci in the genomes of the cacti species, which would be useful for identification and study the genetic variation of the species in genetic resources storage, plant breeding, horticultural production (*in vivo* and *in vitro*), and other purposes. A special advantage of the molecular marker systems is their abundance, robustness, reproducibility and codominant nature. Our research covers the entire currently accepted genus *Astrophytum* (*A. asterias, A. caprocorne, A. caput-medusae, A. coahuilense, A. myriostigma, A. ornatum*) and selected similar species from the genus *Frailea* (*F. castanea* (syn. *Astrophytum asterioides*) and *F. schilinzkyana* (syn. *Astrophytum schlinzkyanum*).

MATERIAL AND METHODS

The plant material originated from mother plants of eight species of cacti representing the genus *Astrophytum* and *Frailea: A. asterias* (Zucc.) Lem., *A. capricorne* (Dietr.) Br. & R., *A. caput-medusae* Valezco & Nevares D. Hunt, *A. coahuilense* Kays., *A. myriostigma* Lem., *A. ornatum* (D.C.) Web., *F. castanea* Backeb. in Backeb. & F.M. Knuth (syn. *Astrophytum asterioides* Werderm. (Halda & Malina) and *F. schilinzkyana* (syn. *Astrophytum schilinzkyanum* F. Haage ex K. Schum.) (Fig. 1). The plant material was acquired from commercial collection of Piotr Licznerski (Osielsko near Bydgoszcz, Poland).

The 10 seeds of each species of cacti were subjected to a surface disinfection process in accordance with the procedure described by Lema-Rumińska and Pietrzykowski (2019). The material was dried on sterile filter paper and 2–3 seeds were placed in 350 ml of jars on the MS medium (Murashige and Skoog, 1962) solidified with agar (8 $g \cdot dm^{-3}$), pH set at 5.8 before autoclaving. *In vitro* cultures were maintained in a growth room at 24 ± 2°C, at a 16-hour photoperiod (Philips TLD 54/34 W, 38.1 $\mu mol \cdot m^{-2} \cdot s^{-1}$) for 8 weeks.

The amount of obtained sterile seedlings was sufficient for DNA isolation for most of the species (Fig. 2). However, seeds of *A. caput-medusae* did not germinate. Therefore, for this species shoot fragments were used for micropropagation.

Total genomic DNA was isolated from 100 mg of fresh tissues of 2–3 seedlings (without roots) from *in vitro* cultures (exceptionally a shoot fragment of *A. caput-medusae*, which did not produce seedlings) using a Genomic Mini AX Plant Kit (A&A Biotechnology, Gdynia, Poland). The DNA isolated was stored in TE buffer (10 mM TRIS, 1 mM EDTA, pH = 8) at -20°C until the analysis. The DNA concentration was established by the Quantus™ Fluorometer (Promega, Medison, USA). DNA analyzes were performed two times with four different ISSR primers and six SCoT primers (Genomed, Poland), with the sequence given in Table 1.

Fig. 1. Mother plants of the genus *Astrophytum* (a – *A. asterias*, b – *A. capricorne*, c – *A. caput-medusae*, d – *A. coahuilense*, e – *A. myriostigma*, f – *A. ornatum*) and two selected species of the genus *Frailea* (g – *F. castanea* syn. *A. asterioides* and h – *F. schilinzkyana* syn. *A. schlinzkyanum*) (bar = 1 cm)

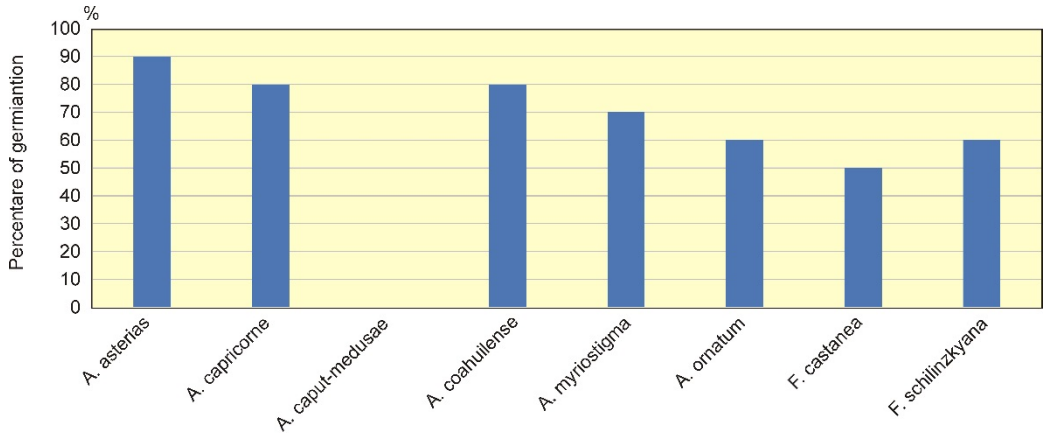

Fig. 2. The percentage of sterile seedlings obtained as a result of the surface disinfection process

Table 1. Sequences of the ISSR and SCoT primers used in PCR reactions

Primer ISSR	Sequences 5'→ 3'	Primer SCoT	Sequences 5'→ 3'
ISSR1	GAGGGTGGAGGATCT	SCoT3	CAA CAA TGG CTA CCA CCG
ISSR2	CGAGAGAGAGAGAGAGA	SCoT4	CAA CAA TGG CTA CCA CCT
ISSR4	GACAGACAGACAGACA	SCoT8	CAA CAA TGG CTA CCA CGT
		SCoT12	ACG ACA TGG CGA CCA ACG
ISSR5	CAGAGAGAGAGAGAGAG	SCoT13	ACG ACA TGG CGA CCA TCG
		SCoT25	ACC ATG GCT ACC ACC GTC

The reaction mix contained: 1 µM single primer; 0.05 $U \cdot \mu l^{-1}$ Taq DNA polymerase, 0.8 $ng \cdot \mu l^{-1}$ template DNA, 1 mM dNTP Solution Mix, 2 mM $MgCl_2$ in reaction Buffer, and sterile double-distilled water (free of nucleases) filled to reach the volume of (25 µl) (2×PCR Master Mix Plus kit, A&A Biotechnology, Gdynia, Poland). PCR reactions were performed two times in a BioRad C1000 Touch™ thermal cycler (Bio-Rad Laboratories, CA, USA), programmed as follows: 4 min initial denaturation at 94°C, 45 cycles of 1 min at 94°C for denaturation, 1 min at 53/50°C for annealing (for ISSR/SCoT respectively), and 2 min at 72°C for DNA extension. The last cycle was followed by the final extension step of 4 min at 72°C. The amplified DNA fragments were separated, detected, and recorded according to the procedure described by Lema-Rumińska *et al*. (2019). The gels were then analyzed with Image Lab 4.1 software (Bio-Rad Laboratories, Hercules CA, USA).

Statistical analyses

ISSR and SCoT loci indicated for each genotype were calculated using a binary system, where the presence of the band is marked (1) and the absence (0). The cluster analysis was performed using the Unweighted Pair Group Method (UPGMA) applying Statistica 13.1 software with hierarchical agglomerative grouping (StatSoft, Kraków, Poland).

RESULTS AND DISCUSSION

The ISSR primers used in the PCR reactions showed a high polymorphism within the genotypes tested (Table 2). A total of 76 bands were recorded for ISSR markers, 47 of which were polymorphic (61.84%) and 29 – specific (38.16%). However, no monomorphic bands were found. The highest degree of polymorphism showed the ISSR1 starter (80.77%), while the lowest – ISSR5 starter (30.77%). As for the ISSR marker, a total of 162 products in the 199-2354 bp range were produced for the reactions using the four primers. The largest amount of products was identified using the ISSR1 primer (Fig. 3) within the 209–1722 bp length. This primer generated the most polymorphic bands 21 (80.77%) and 5 specific bands (19.23%) (for *A. capricorne* at 593 bp, *A. caput-medusae* at a height of 389 bp, *F. castanea* 1160 bp, *F. schilinzkyana* 1206 bp and 1242 bp). The smallest in reaction with the ISSR5 primer in the 407–1235 bp the lenght range. Each of the ISSR primers used allowed a full molecular identification of the cactus genotypes.

Table 2. Characterization of PCR products produced using individual ISSR primers

Primer	Number of generated products	*Loci*				Polymorphism, %
		polymorphic	monomorphic	specific	total	
ISSR1	66	21	0	5	26	80.77
ISSR2	41	11	0	8	19	57.89
ISSR4	31	11	0	7	18	61.11
ISSR5	24	4	0	9	13	30.77
Total	162	47	0	29	76	NA
Share *loci*, %	NA*	61.84	0.00	38.16	NA	NA

*NA – not applicable

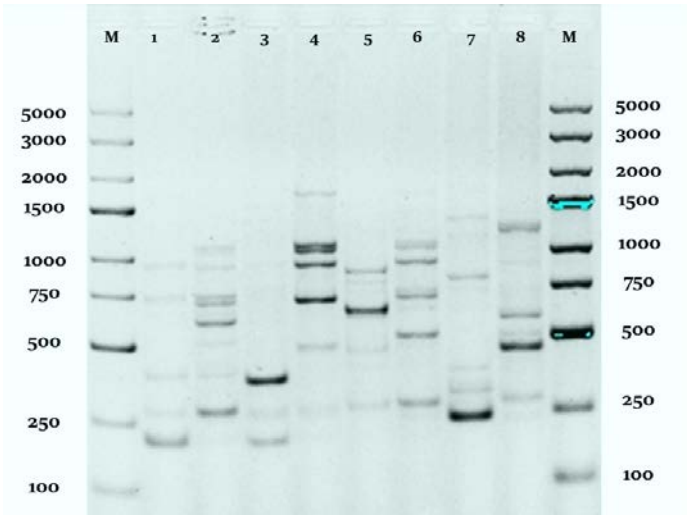

Fig. 3. ISSR amplification products using the ISSR1 primer (M – marker, 1 – *A. asterias*, 2 – *A. capricorne*, 3 – A. *caput-medusae*, 4 – *A. coahuilense*, 5 – *A. myriostigma*, 6 – *A. ornatum*, 7 – *F. castanea* (syn. *A. asterioides*), 8 – *F. schilinzkyana* (syn. *A. schlinzkyanum*)

Fernández *et al*. (2002) proved that the ISSR marker is definitely more polymorphic than RAPD in the studies on *Hordeum vulgare* L. Only one primer (RAPD S10) managed to distinguish all the barley cultivars tested. However, as for the ISSR markers, as many as four out of 10 primers facilitated distinguishing all tested genotypes. The obtained results show that despite the use of fewer ISSR primers, a greater number of polymorphic products were identified. Qian *et al*. (2001), while analyzing variability in wild rice (*Oryza granulata*), reported 30.7% (RAPD markers) and 46% (ISSR) of polymorphic products. Similarly, Zhang *et al*. (2013) showed a greater efficiency of ISSR markers in *Larix gmelinii*, the ISSR marker produced 98.83% polymorphic loci.

As a result of using of six SCoT primers, a total of 465 products were identified in the range of 177–3166 bp (Table 3). The highest number of the products was reported for the SCoT12 (Fig. 4) – 101 bands, in the lenght range 177–2118 bp, whereas the lowest number of products was shown by the primer SCoT8; 52 products were identified in the lenght range 425-2024 bp. As a result of the PCR reaction using those primers, 141 bands were produced, as many as 114 of which showed polymorphism (78.95%). The highest percentage of polymorphism was found in the SCoT13 primer (88.00%), while the lowest in the SCoT4 primer (53.33%). Like the ISSR marker, the SCoT marker allows a full identification of the species of the genus *Astrophytum* and *Frailea* tested by means of the so-called DNA-fingerprinting. Of various marker systems, the SCoT marker gains an advantage over other DNA marker systems, such as RAPD or ISSR. It shows a higher polymorphism and offers a higher detectibility (Satya *et al*., 2015; Zeng *et al*., 2016). Like the ISSR marker, the SCoT marker does not require sequence information (Shangguo *et al*., 2018). Our research of selected species of cactus of the *Astorphytum* and *Frailea* genera proves that the SCoT marker offers a higher average polymorphism share (78.95%) than the ISSR marker (61.84%). In addition, the ISSR marker is also useful in identifying the cactus species studied due to a high proportion of specific bands constituting 38.16%. Our research results allow an early identification (i.e. at every stage of development, including seeds and seedlings) and molecular identity testing for the protection of genetic resources and in horticultural production.

The first molecular studies on phylogenetic relationships in 4 species of cactus from the genus *Astrophytum* (*A. asterias*, *A. capricorne*, *A. myriostigma and A. ornatum*) with RAPD marker were made by Das (2008). The author found the maximum polymorphism (59.34%) between *A. asterias* and *A. myriostigma*, while the minimum polymorphism (50.58%) in *A. capricorne* and *A. ornatum*. The study reported by Das (2008) also indicates a close phylogenetic relationship between *A. asterias* and *A. myriostigma*, which represented one cluster, as compared with *A. capricorne* and *A. ornatum*, which constituted the second cluster.

Table 3. Characterization of PCR products produced using individual SCoT primers

Primer	Number of generated products	*Loci*				Polymorphism, %
		polymorphic	monomorphic	specific	total	
SCoT3	89	23	1	3	27	85.19
SCoT4	86	22	0	4	26	84.62
SCoT8	52	8	1	6	15	53.33
SCoT12	101	24	0	5	29	82.76
SCoT13	78	22	1	2	25	88.00
SCoT25	59	15	0	4	19	78.95
Total	465	114	3	24	141	NA
Share *loci*, %	NA*	80.85	2.13	17.02	NA	NA

*NA – not applicable

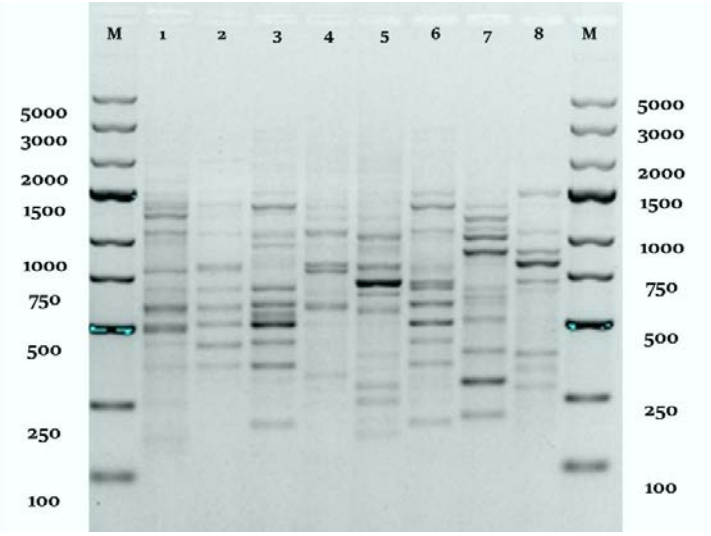

Fig. 4. SCoT amplification products using the SCoT12 primer (M – marker, 1 – *A. asterias*, 2 – *A. capricorne*, 3 – A. *caput-medusae*, 4 – *A. coahuilense*, 5 – *A. myriostigma*, 6 – *A. ornatum*, 7 – *F. castanea* (syn. *A. asterioides*), 8 – *F. schilinzkyana* (syn. *A. schlinzkyanum*)

A cluster analysis performed with the ISSR marker for the species of the genus *Astrophytum* and *Frailea* in our study showed that *F. castanea* (syn. *A. asterioides*) is the most taxonomically distant species from the others; it is a separate cluster. Other species of the genus *Astrophytum* and *F. schilinzkyana* (syn. *A. schlinzkyanum*) were classified as the second cluster. The closest phylogenetic relations within the species using the ISSR marker were shown between *A. asterias* and *A. caput-medusae* and between *A. capricorne* and *A. coahuilense* (Fig. 5a).

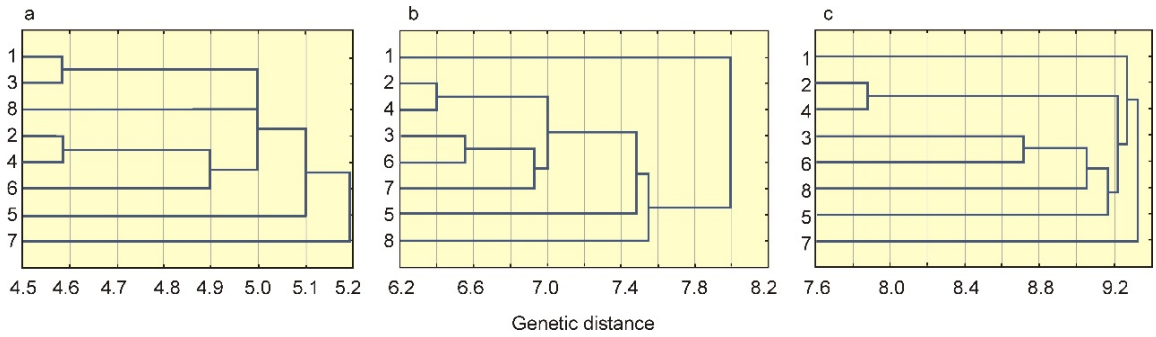

Fig. 5. Dendrograms based on the estimation of the genetic distance coefficient and UPGMA clustering presenting the relationships between genotypes of different cacti species from the genus *Astrophytum* and *Frailea* (1 – *A. asterias*, 2 – *A. capricorne*, 3 – A. *caput-medusae*, 4 – *A. coahuilense*, 5 – *A. myriostigma*, 6 – *A. ornatum*, 7 – *F. castanea* (syn. *A. asterioides*), 8 – *F. schilinzkyana* (syn. *A. schlinzkyanum*), revealed with the ISSR (a), SCoT (b) and ISSR-SCoT (c) analysis. The scale shows a real genetic distance value

Vargas-Luna *et al*. (2018) reported different results in the study of 5 species of the genus *Astrophytum* based on the analysis of 42 morphological features and four chloroplast markers and two nuclear genes. Vargas-Luna *et al*. (2018) included the fifth species (as compared to the study reported by Das in 2008),

A. caput-medusae, which was a separate cluster, as compared with the other species of the genus *Astrophytum*. There were also different relationships between the other species that formed two two-species subclusters: *A. asterias* from *A. capricorne* or *A. myriostigma* and *A. ornatum*. Our research using ISSR marker for 6 species of the genus *Astrophytum* and 2 species of the genus *Frailea* identified close phylogenetic relationships within the species between *A. asterias* and *A. caput-medusae* and between *A. capricorne* and *A. coahuilense*, whereas *F. castanea* (syn. *A. asterioides*) turned out to be the species most taxonomically distant from the others, constituting a separate cluster.

A cluster analysis made using the SCoT marker showed slightly different clusters than for the ISSR since *A. asterias* forms a separate cluster here, as compared to the other species of the genus *Astrophytum* and *Frailea*. A cluster analysis for the SCoT marker showed, like the ISSR marker, a close relationship between *A. capricorne* and *A. coahuilense*, while *A. caput-medusae* shares a small genetic distance with *A. ornatum*. The species *F. schilinzkyana* (syn. *A. schlinzkyanum*) is here a separate cluster from the other species representing *Astrophytum* and *F. castanea* (syn. *A. asterioides*). The latter species forms a subcluster together with *A. caput-medusae* and *A. ornatum* as well as *A. capricorne* and *A. coahuilense* (Fig. 5b). A joint cluster analysis for two types of markers (ISSR and SCoT) showed that the species tested were separated into two separate clusters: *F. castanea* (syn. *A. asterioides*) constituted a separate cluster from the large cluster covering the remaining *Astrophytum* and *F. schilinzkyana* (syn *A. schlinzkyanum*). The smallest genetic distance was found between *A. capricorne* and *A. coahuilense*. A significant genetic similarity was found between *A. caput-medusae* and *A. ornatum*. In contrast, *A. asterias* forms a separate sub-cluster here in relation to the other species of the genus *Astrophytum* and *F. schilinzkyana* (syn. *A. schlinzkyanum*), which additionally shares the smallest genetic distance with *A. caput-medusae* and *A. ornatum* (Fig. 5c).

Our research, performed using both ISSR and SCoT markers and a combined ISSR-SCoT analysis, showed a close phylogenetic relationship between *A. capricorne* and *A. coahuilense*. Unfortunately, the latter species was not considered in the study (Das, 2008; Bárcenas *et al*., 2011; Vázquez-Sánchez *et al*., 2013; Vargas-Luna *et al*., 2018). Phylogenetic studies in the Cactaceae family reported by Bárcenas *et al*. (2011) showed *Astrophytum* as a monophyletic group, however, those studies were based on the single gene region *trnK-matK* of chloroplast genome, therefore they can provide less information and support than the other studies employing multiple gene regions. This group includes the species *A. caput-medusae* closely related to *A. asterias* and *A. capricorne*, which in the studies (Vázquez-Sánchez *et al*., 2013) was referred to as *Digitostigma caput-medusae* and which formed a separate cluster from *Astrophutum*. According to the classification (Vázquez-Lobo *et al*., 2015), *A. caput-medusae* forms together with *A. asterias*, *A. capricorne* and *A. coahuilense* a separate large cluster separate from *A. myriostigma* and *A. orantum*, grouped in another cluster.

CONCLUSIONS

This is the first, reliable molecular markers system based on multiple loci in the genomes of the cacti species representing the genus *Astrophytum* and two species of the *Frailea*. A higher polymorphism rate (>78%) was found with the SCoT technique, but specific bands dominated in the ISSR marker. A close molecular relation between species of the genus *Astrophytum* (*A. asterias*, *A. caprocorne*, *A. caput-medusae*, *A. coahuilense*, *A. myriostigma*, *A. ornatum*) and selected similar species classified as the genus *Frailea* (*F. castanea* syn. *Astrophytum asterioides* and *F. schilinzkyana* syn. *Astrophytum schlinzkyanum*) was identified. These data can be used to distinguish all of the tested here genotypes representing the genus *Astrophytum* and *Frailea*.

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RÓŻNORODNOŚĆ GENETYCZNA WAŻNYCH GATUNKÓW OGRODNICZYCH Z RODZAJU ASTROPHYTUM I FRAILEA, USTANOWIONA Z WYKORZYSTANIEM MARKERÓW ISSR I SCOT

Streszczenie

Rodzaje *Astrophytum* i *Frailea* są bardzo ważne dla produkcji ogrodniczej i kolekcjonerów kaktusów na całym świecie. Wiele gatunków kaktusów jest zagrożonych wyginięciem w środowisku naturalnym. Ze względu na ochronę gatunków konieczne jest opracowanie skutecznych i obiektywnych metod ich identyfikacji na poziomie molekularnym. Nasiona wszystkich gatunków kaktusów (lub fragment pędu *A. caput-medusae*) wysterylizowano powierzchniowo, a następnie uprawiano w warunkach *in vitro* w celu uzyskania sterylnych siewek do izolacji DNA. Badaniom poddano sześć obecnie akceptowanych gatunków kaktusów z rodzaju *Astrophytum* (*A. asterias*, *A. capricorne*, *A. caput-medusae*, *A. coahuilense*, *A. myriostigma*, *A. ornatum*) oraz dwa wybrane gatunki z rodzaju *Frailea* (*F. castanea syn. A. asterioides i F. schilinzkyana syn. A. schlinzkyanum*) z wykorzystaniem markerów molekularnych: ISSR i SCoT. Wykazano wyższy średni polimorfizm stanowiący 78,95% dla SCoT niż dla ISSR (61,84%). Prążki specyficzne dominowały w markerze ISSR, stanowiąc 38,16%, podczas gdy SCoT stanowiły 17,02%.

Połączona analiza skupień ISSR-SCoT wykazała, że badane gatunki podzielono na dwa skupienia: *F. castanea* i duże skupienie obejmujące gatunki *Astrophytum* i *F. schilinzkyana*. Stwierdzono ponadto ścisły związek filogenetyczny między *A. capricorne* i *A. coahuilense*. Opracowano wysoce użyteczny system molekularnych markerów ISSR i SCoT, oparty na wielu loci genów w genomach badanych gatunków kaktusów w celu identyfikacji i badania tożsamości genetycznej gatunku w przechowywaniu zasobów genetycznych, hodowli roślin, produkcji ogrodniczej (*in vivo* i *in vitro*).

Słowa kluczowe: Cactaceae, identyfikacja gatunkowa, kiełkowanie *in vitro*, markery molekularne