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RAPD AND ISSR POLYMORPHISMS IN SELECTED GENOTYPES OF *LYCIUM* SP.

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Abstract. Due to the high value and economic importance of the plant *Lycium* (goji), its genome has been intensively studied in multidisciplinary research. In the present study, the structure and genetic relationships of 14 selected *Lycium* genotypes from different origins are presented. By using 18 random amplified polymorphic DNA (RAPD) decamers and 15 inter-simple sequence repeat (ISSR) primers, 200 and 183 loci were amplified, respectively. Among the amplified loci, 45.5–49.2% were polymorphic, and 6.5–7.6% were genotype-specific. Cluster and STRUCTURE analyses performed for RAPD and ISSR revealed the genetic relationships among the genotypes. The highly significant and positive value of the Mantel's correlation coefficient calculated for the Jaccard similarity matrices of RAPD and ISSR confirmed the suitability of using both these methods separately in this type of study. The significant values of F_{ST} statistics obtained in AMOVA for 'among' and 'within' group analysis confirmed the diversity of genotypes not only between the designated groups but also within them. This diversity provides opportunities to select interesting genotypes and conduct further studies on identifying markers for marker-assisted selection.

Key words: *Lycium* sp. L., diversity, RAPD, ISSR, STRUCTURE, MDS, PCoA.

INTRODUCTION

Thornberry (*Lycium* sp.), also known as scarlet, goji berry, or goji, belongs to the *Solanaceae* family. In this plant family, the genus *Lycium* alone includes approximately 100 species. *Lycium* is widespread globally, especially in temperate zones (Ruyu et al. 2021). More than 30 species of *Lycium* are used as food and as raw materials for medicines (Ruyu et al. 2018, 2021). Fruits of *Lycium* are 1–2 cm long, egg-shaped, and reddish-orange and yellow in some cultivars (Amagase i Farnsworth 2011). They contain zinc, iron, calcium, and phosphorus, and they are rich in flavonoids and carotenoids (Yin and Dang 2008; Wang et al. 2010; Qiu et al. 2014). Large amounts of polysaccharides, antioxidant compounds, and hydroxycinnamic acid amides (HCAAs) are found in *Lycium* fruits (Dahech et al. 2013). These compounds exert anti-inflammatory (Wang et al. 2017), neuroprotective effects (Xing et al. 2016), protect the liver (Liu et al. 2015) and body from fatigue (Reeve et al. 2010). They also show anticancer, antiaging (Ye et al. 2015), and free radical scavenging effects (Mocan et al. 2014).

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China is the largest producer of *Lycium barbarum* (Ruyu et al. 2018). Gong et al. (2019) noted that the increase in the area of goji cultivation for consumption should be closely associated with the need for breeding activity aimed at obtaining new cultivars, including those for the pharmaceutical industry (Nan et al. 2017; Gong et al. 2022). According to Nan et al. (2017), this requirement can be met using marker-assisted selection (MAS) methods. The authors suggest combining existing techniques and selection criteria, selecting genotypes with favorable traits, and focusing research on the most effective identification of potentially useful markers for various traits by using biotechnology tools (Kosińska-Cagnazzo et al. 2017; Rubiales et al. 2021). This can be achieved when genotypes with extremely favorable traits are included in the analysis. On the basis of these previous observations, it is possible to (1) create segregating populations, (2) select desirable, extremely different, genotypes, and (3) with a redundant genetic background, select sequences (markers) associated with the trait(s) (Rehman et al. 2020; Ruyu et al. 2021).

Such markers can be identified using high-tech but simple, economical, and effective molecular biology techniques such as simple sequence repeat (SSR, Kwon et al. 2009), sequence-related amplified polymorphism (SRAP, Liu et al. 2012), amplified fragment length polymorphism (AFLP, Wang et al. 2015), random amplified polymorphic DNA (RAPD) and inter-simple sequence repeat (ISSR). These techniques are based on the principle of 'search' by fingerprinting and subsequent identification of differences at various locations in the genomes. The capabilities and limitations of these techniques are outlined in the published papers of Williams et al. (1990) and Ziętkiewicz et al. (1994), respectively. Moreover, several studies conducted worldwide have reported the possibility of using these techniques not only for fingerprinting but also for MAS (Sze et al. 2008) or genetic map construction (Gong et al. 2019).

The present study aimed to determine genetic similarity and genetic relationship among *Lycium* genotypes by using RAPD and ISSR.

MATERIALS AND METHODS

Twelve *L. barbarum* L. genotypes of different origins and two *Lycium chinene* Mill. genotypes were included in the present study. *L. barbarum* accessions numbered 1–5 were five individual plants (clones) of 'No. 1' cultivar. This selection of plants was based on the fact that in recent years, the plants labeled (1–3) were infected by powdery mildew, while those labeled 4 and 5 were not infected. *L. barbarum* cultivars 'New Big', 'Big Berry', 'Big Lifeberry', 'Korean Big,' 'Sweet Berry', and 'Amber Sweet Goji' were also included in the study. These genotypes were obtained from the collection of the Orchard Research Station of the Department of Horticulture at WPUT in Ostoja (Szczecin, Poland). One genotype was obtained from the urban green area (*L. barbarum* GA) of Szczecin city, while two genotypes were obtained from the Tissue Culture Laboratory of the Department of Genetics, Plant Breeding and Biotechnology of WPUT in Szczecin (*L. chinense* 1 and 2). The seedlings were regenerated from seeds taken from dried goji berry fruits of an unknown cultivar of *L. chinense* Mill. The fruits were purchased from China. Various combinations of these genotypes have already been investigated in several studies. The obtained results are presented in the published papers of Kruczek et al. (2017, 2020a, 2020b, 2021), Kruczek and Ochmian (2016), and Krupa-Małkiewicz et al. (2018).

DNA preparation. Genomic DNA was isolated from the leaves of the *Lycium* plants by using the DNeasy Plant Mini Kit (Qiagen) in accordance with the manufacturer's protocol. DNA quantity and quality were determined spectrophotometrically (Epoch, BioTek, USA). Amplifications were performed in a Mastercycler 5333 thermal cycler (Eppendorf, Germany).

RAPD and ISSR. RAPD amplifications were performed according to the method of Williams et al. (1990), while ISSR was performed following the method of Ziętkiewicz et al. (1994). RAPD and ISSR primers (#9) were designed at the University of British Columbia (Canada) and

synthesized at Sigma-Aldrich (Germany). The amplification for both techniques was performed using Dream*Taq* polymerase (Thermo Fisher Scientific) in a 20 µL reaction volume. The final concentrations of the individual components were as follows: 1× PCR buffer with $(NH_4)_2SO_4$ (750 mM Tris-HCl pH 8.3, 200 mM $(NH_4)_2SO_4$), 2 mM $MgCl_2$, 0.2 mM dNTPs, 0.25 µM primer, 40 ng DNA, and 1U Dream*Taq* polymerase. The thermal profiles for RAPD and ISSR were as reported by Williams et al. (1990) and Ziętkiewicz et al. (1994), respectively. In the ISSR thermal profile, the annealing temperature for individual primers was lowered by 2°C below their T_m.

Electrophoresis. The amplified products were mixed with the loading buffer and applied to a 1.5% agarose gel. Product separation was performed using Sub-Cell GT electrophoresis cell (BioRad) in the presence of mass standards: GeneRuler 100 bp Plus DNA Ladder (Thermo Scientific) and Nova 100 bp DNA Ladder (Novazym). The products were separated at 90 V for 120 min. The gels were stained with ethidium bromide, visualized under UV light, and photographed (GeneSnap-G-Box, Syngene).

Data analysis. Electrophoregrams were scanned and processed using Diversity one version 1.3 software (Pharmacia LKB). Genetic profiles of *Lycium* sp. were developed using a binary system. The presence and absence of a band at a given locus were marked by (1) and (0), respectively. The number and nature of amplified loci were determined for each genotype studied. Genetic similarity matrices between the pairs of the studied goji genotypes were calculated using Jaccard's algorithm (PhylTool, Buntjer 2001). The genetic distance dendrogram was constructed using the UPGMA clustering algorithm, and its reliability was tested using the bootstrap method with 2000 pseudoreplications (TREECON, Van der Peer i De Wachter 1994). Correlations between the RAPD and ISSR genetic similarity matrices were determined using the Mantel test (Manly 1997).

Structure analysis. The structure of the admixture groups and individuals was analyzed, and appropriate assignment of individuals to *Lycium* groups was performed using the Bayesian clustering software STRUCTURE v.2.3.4 (Pritchard et al. 2000), STRUCTURE HARVESTER (Earl and von Holdt 2012), CLUMPP (Jakobsson and Rosenberg 2007) and DISTRUCT (Rosenberg 2004). All simulations were performed using a High-Performance Computing (HPC) cluster (University Information Technology Center) at the West Pomeranian University of Technology in Szczecin (Poland). HPC characteristics and STRUCTURE analysis parameters are presented by Smolik et al. (2022). Hierarchical analysis of molecular variance (AMOVA) was conducted using Arlequin 3.5.2.2 (Excoffier and Lischer 2010). Significance levels for variance component estimates were determined using a 10100 permutation approach. Multidimensional scaling (MDS) and principal component analysis (PCoA) plots were generated using R software (R Core Team 2017) and packages: SMACOF (de Leeuw and Mair 2009), ape (Paradis and Schliep 2019), and MultBiplotR (Villardón 2010).

RESULTS

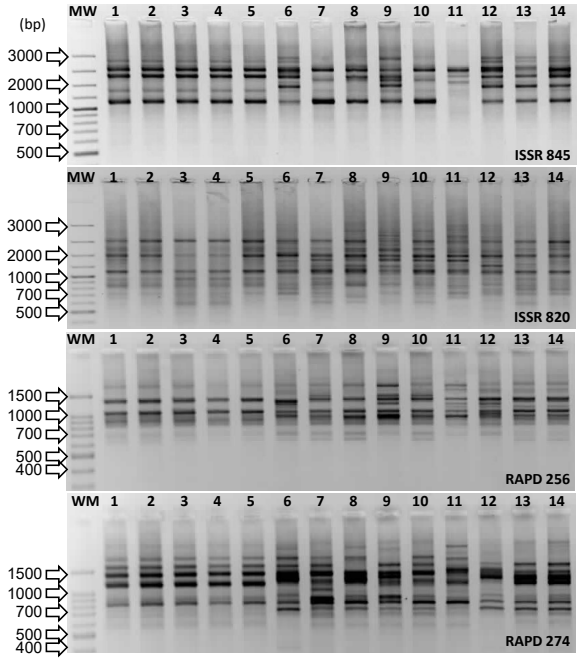
RAPD. The analysis of the RAPD and ISSR fingerprints in the 14 selected *Lycium* genotypes revealed high genetic diversity (Table 1, Fig. 1).

Based on reactions with 18 RAPD primers, 200 loci were amplified; of these 200 loci, 96 (48%) and 91 (45.5%) were described as mono- and polymorphic, and 13 (6.5%) as genotype-specific (Table 1). On average, 11 loci (108 amplicons) were amplified in the reaction with one RAPD primer. Most loci were amplified in the reaction with primer 274 (16), and 14 loci each were amplified with primers 270 and 256. Three genotype-specific RAPD products were obtained in reactions with primers 270 and 278 (Table 1). The length of the fragments generated ranged from 2800 to 320 bp. Slight variations were observed in the genetic profiles of clones 1–5 of the cultivar 'No.1'. Only in reactions with five decamers (209, 232, 241, 256, 257) were 1 to 3 polymorphic products identified for them (data not shown).

ISSR amplifications were performed using 15 primers (Table 1, Fig. 1). A total of 183 loci were amplified; of these loci, 79 (43.2%) and 90 (49.2%) were described as mono- and polymorphic and 14 (7.6%) as genotype-specific (Table 1). An average of 12 loci (114 amplicons) were amplified in the reaction with a single primer. The highest number of ISSR products (20) was obtained in reactions with primers 820 and 840, while the least numbers (4 and 8 each) were obtained with primers 873 and 844 and 852. The highest number, i.e., 3 genotype-specific loci each, was found in reactions with primers 840 and pr11. The length of the amplified ISSRs ranged from 4210 (815) to 320 bp (pr11).

Table 1. Characteristics of loci amplified using RAPD and ISSR techniques

	Primers and number of amplified loci	Amplicon length range (bp)	Amplified loci number and mean	Total amplicons number and mean	Loci		
					monomorphic	polymorphic	genotype--specific
RAPD	077 (8), 203 (11), 208 (9), 209 (11), 213 (10), 215 (12), 232 (11), 241 (12), 256 (14), 257 (12), 262 (10), 265 (10), 266 (11), 268 (9), 269 (10), 270 (14), 274 (16), 278 (10)	2800 – 320	200 (11)	1935 (108)	96 (48%)	91 (45,5%)	13 (6,5%)
ISSR	811 (15), 813 (10), 815 (10), 820 (20), 824 (9), 829 (13), 840 (20), 844 (10), 845 (8), 845 (18), 852 (8), 855 (10), 873 (4), pr11 (17), pr17 (11)	4120 – 320	183 (12)	1704 (114)	79 (43.2%)	90 (49.2)	14 (7.6)



MW – GeneRuler 100 bp DNA Ladder; WM – Nova 100 bp DNA Ladder, Molecular Weights, 1 – *L. barbarum* No.1 1, 2 – *L. b.* No.1 2, 3 – *L. b.* No.1 3, 4 – *L. b.* No.1 4, 5 – *L. b.* No.1 5, 6 – *L. b.* 'New Big', 7 – *L. b.* 'Big Berry', 8 – *L. b.* 'Big Lifeberry', 9 – *L. b.* 'Korean Big', 10 – *L. b.* 'Sweet Berry', 11 – *L. b.* 'Amber Sweet Goji', 12 – *L. b.* GA, 13 – *L. chinense* 1, 14 – *L. chinense* 2

Fig. 1. Electrophoregrams of selected fingerprints generated using RAPD and ISSR techniques for studied *Lycium* accessions

The ISSR genetic profiles of individual clones 1–5 of the cultivar 'No.1' showed polymorphic products after reactions with primers 820 (4), 824 (1), 840 (1), 845 (4), and pr17 (3) (data not shown).

Genetic similarity. Similarity coefficients for *Lycium* genotypes are presented in RAPD and ISSR matrices (Table 2).

Table 2. Similarity matrix values among *Lycium* sp. genotypes based on RAPD and ISSR analysis

		ISRR													
		L. barbarum 'No.1' 1	*L. barbarum* 'No.1' 2	*L. barbarum* 'No.1' 3	*L. barbarum* 'No.1' 4	*L. barbarum* 'No.1' 5	*L. barbarum* 'New Big'	*L. barbarum* 'Big Berry'	*L. barbarum* 'Big Lifeberry'	*L. barbarum* 'Korean Big'	*L. barbarum* 'Sweet Berry'	*L. barbarum* 'Amber Sweet Goji'	*L. barbarum* GA	*L. chinense* 1	*L. chinense* 2
RAPID	*L. barbarum* 'No. 1' 1	**1.00**	0.99	0.93	0.91	0.94	0.68	0.72	0.68	0.70	0.70	0.66	0.66	0.54	0.74
	L. barbarum 'No. 1' 2	1.00	**1.00**	0.92	0.91	0.94	0.69	0.72	0.68	0.70	0.70	0.67	0.66	0.54	0.73
	L. barbarum 'No. 1' 3	0.99	0.99	**1.00**	0.98	0.92	0.67	0.73	0.67	0.71	0.71	0.65	0.68	0.52	0.74
	L. barbarum 'No. 1' 4	0.95	0.95	0.96	**1.00**	0.92	0.68	0.74	0.67	0.71	0.72	0.65	0.69	0.53	0.74
	L. *barbarum* 'No. 1' 5	0.93	0.93	0.94	0.98	**1.00**	0.66	0.75	0.67	0.72	0.73	0.66	0.66	0.52	0.74
	L. barbarum 'New Big'	0.74	0.74	0.73	0.74	0.74	**1.00**	0.66	0.65	0.67	0.63	0.72	0.69	0.59	0.81
	L. barbarum 'Big Berry'	0.81	0.81	0.80	0.81	0.82	0.74	**1.00**	0.77	0.77	0.86	0.70	0.70	0.54	0.71
	L. barbarum 'Big Lifeberry'	0.76	0.76	0.75	0.75	0.76	0.77	0.89	**1.00**	0.73	0.80	0.66	0.72	0.55	0.71
	L. barbarum 'Korean Big'	0.73	0.73	0.73	0.73	0.74	0.70	0.78	0.76	**1.00**	0.79	0.76	0.74	0.59	0.74
	L. barbarum 'Sweet Berry'	0.75	0.75	0.76	0.77	0.78	0.71	0.87	0.84	0.77	**1.00**	0.69	0.74	0.54	0.71
	L. barbarum 'Amber Sweet Goji'	0.65	0.65	0.64	0.64	0.65	0.71	0.67	0.71	0.68	0.69	**1.00**	0.75	0.59	0.71
	L. barbarum GA	0.65	0.65	0.65	0.67	0.68	0.80	0.68	0.74	0.70	0.72	0.73	**1.00**	0.60	0.75
	L. chinense 1	0.53	0.53	0.52	0.51	0.51	0.61	0.57	0.58	0.55	0.58	0.64	0.61	**1.00**	0.52
	L. chinense 2	0.72	0.72	0.72	0.73	0.72	0.81	0.75	0.77	0.70	0.77	0.78	0.80	0.59	**1.00**

For RAPD, the highest similarity was found between genotypes 1 and 2 of 'No. 1'. The lowest similarity was noted between genotypes 4 and 5 of 'No.1' and *L. chinense* 1 (Table 2). Based on the analysis of the topology of the genetic distance dendrogram, the genotypes were divided into 3 groups (Fig. 2a). Group A included clones 1–5 of 'No. 1'; group B included *L. barbarum* cultivars 'Sweet Berry', 'Big Berry', 'Big Life Berry', and 'Korean Berry'; and group C included the other cultivars, namely, 'Amber Sweet Goji', 'New Big', *L. barbarum* GA, and *L. chinense* 1 and 2. Similar results were obtained for ISSR (Fig. 2a). The highest similarity was noted between clones 1 and 2 of the cultivar 'No.1', and the lowest similarity was observed between clone 3 of the cultivar 'No.1' and *L. chinense* 1 (Table 2). Three groups were distinguished based on the analysis of the ISSR dendrogram. Group A included genotypes 1–5 of cultivar 'No.1'; group B included cultivars 'Big Berry', 'Sweet Berry', 'Korean Big', and 'Big Life Berry'; and group C included 'Amber Sweet Goji', *L. barbarum* GA, 'New Big', and *L. chinense* 1 and 2 (Fig. 2). The Mantel test showed a highly significant and positive correlation (r) for the RAPD and ISSR similarity matrices ($r = 0.925$, $P < 0.0001$).

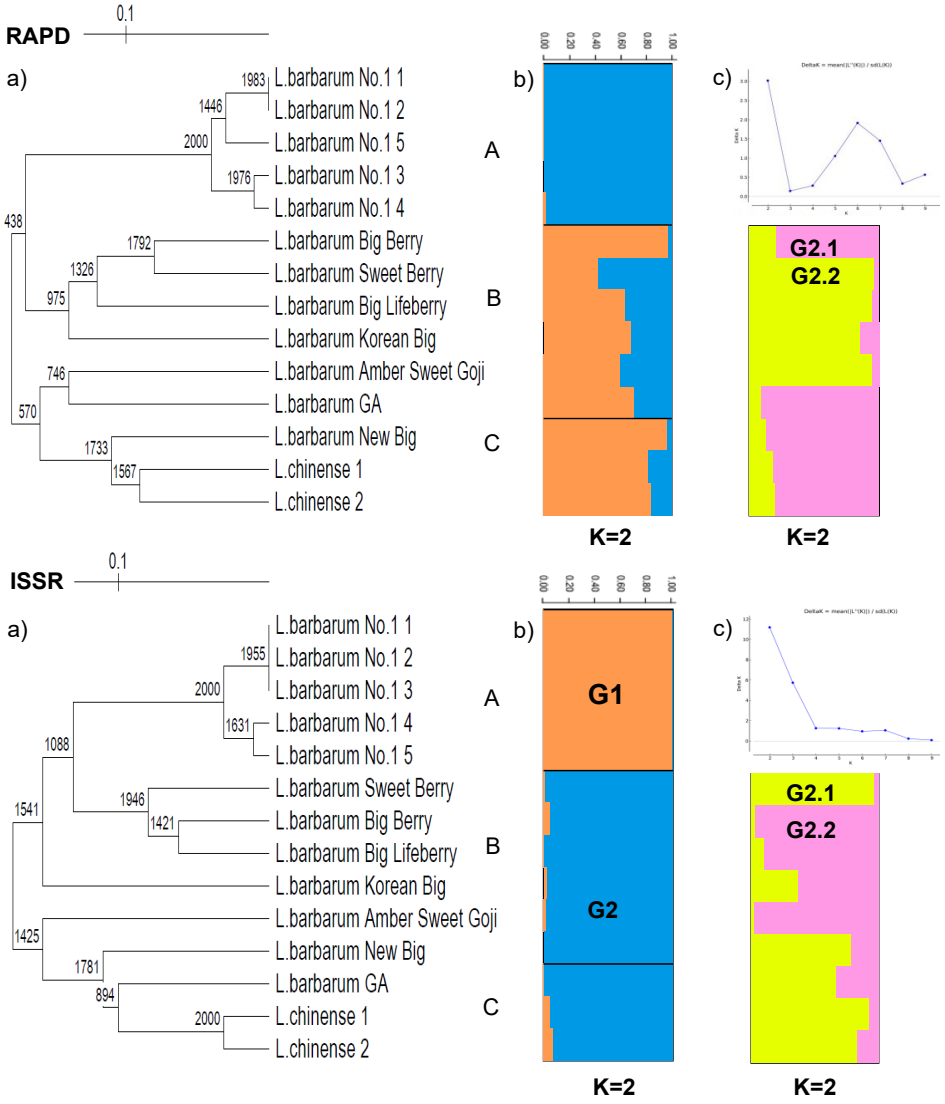


Fig. 2. STRUCTURE analysis results. (a) Letters A–C denote phylogenetic groups; (b) STRUCTURE bar charts with groups G1 and 2. Each genotype is represented by a single vertical line assigned into different K colored segments with lengths proportional to each of the K inferred clusters; (c) delta*K* reposition for Bayesian simulation involving 9 genotypes and STRUCTURE bar charts with groups G2.1 and G2.2

Bayes-based clustering for binary datasets (RAPD and ISSR). By using the Bayesian algorithm in STRUCTURE to simulate group partitioning of the 14 genotypes, it was observed that in both RAPD and ISSR analyses, the *Lycium* genotypes tended to split into two groups (G1 and G2) (Fig. 2b). The highest peak and Δ*K* value were found for *K* = 2 (data not shown). In both RAPD and ISSR, 5 genotypes (36%) belonging to the cultivar 'No. 1' were present in the G1 group, and the remaining 9 (64%) genotypes were present in the G2 group (Fig. 2b). Additional analyses were conducted to extract discrete groups, and a set of G2 genotypes were subjected to Bayesian simulations for RAPD and ISSR. Both simulations showed the division of the G2 group into two subgroups of G2.1 and G2.2. For RAPD G2.1 included *L. barbarum* 'Big Berry', GA, 'New Big', *L. chinense* 1 and 2 (Fig. 2c), G2.2 included 'Sweet Berry', 'Big Lifeberry', 'Korean Big', and 'Amber Sweet Goji', for ISSR G2.1 included *L. barbarum* 'Sweet Berry', GA, 'New Big', *L. chinense* 1 and 2 (Fig. 2c), G2.2 included 'Big Berry', 'Big Lifeberry', 'Korean Big' and 'Amber Sweet Goji'.

AMOVA. AMOVA was conducted to evaluate the split made after RAPD and ISSR matrix analyses using STRUCTURE. Both intra- and inter-group variabilities were significant ($F_{ST\text{-}RAPD}$ = 0.37583; $F_{ST\text{-}ISSR}$ = 0.39683; P (rand > obs.value) = 0.000) for both datasets (RAPD and ISSR) (Table 3). Variability among the groups ranged from 38% (RAPD) to 40% (ISSR), while variability within the groups ranged from 60% (ISSR) to 62% (RAPD) (Table 3).

Table 3. Summary of molecular variance (AMOVA) analysis among and within *Lycium* groups for RAPD and ISSR markers

Markers	Source of variation d.f.	Among populations 1	Within populations 12	Total 13
RAPD	Sum of squares	72.611	178.889	251.500
	Variance components	8.976 Va	14.907 Vb	23.883
	Percentage of variation	37.58	62.42	
	F_{ST} = 0.37583, P(rand ≥ obs. value) = 0.0000			
ISSR	Sum of squares	74.297	170.489	244.786
	Variance components	9.347 Va	14.207 Vb	23.555
	Percentage of variation	39.68	60.32	
	F_{ST} = 0.39683, P(rand ≥ obs. value) = 0.0000			

Significance tests (1023 permutations).

MDS and PCoA. The results of both MDS and PCoA analyses are presented as 2D plots for both RAPD and ISSR. These plots were generated assuming the division of the test material, as in STRUCTURE, into two groups (Fig. 3). For both RAPD and ISSR, the distinctiveness of 5 clones of the 'No.1' cultivar (group A) was confirmed. Moreover, both RAPD and ISSR revealed discrete subgroups within Group B, including those with *L. barbarum* GA and *L. chinense* 1 and 2 genotypes (Figs. 2c, 3). For RAPD markers, PCoA showed that the first two principal components explained 53.3% and 18.7% of the total variance, respectively, while for ISSR, the first two principal components explained 39.8% and 30.7% of the total variance, respectively (Fig. 3).

DISCUSSION

According to Ruyu et al. (2021), the defined biological origin of *Lycium* genotypes is essential for their use in food and for their safe and effective use in herbal medicines and products derived from them. Specialized techniques have been developed for the quality control of goji (Yao et al. 2018). These include a combination of a multidisciplinary approach involving chemical analysis, DNA barcoding, and value chain analysis (Yao et al. 2018).

Previous studies have shown that related species of *Lycium*, including *L. barbarum* and *L. chinense*, can be characterized by both similarity (Liu et al. 2020) and diversity based on important, pharmaceutically relevant chemical profiles. In some cases, for pharmaceutical use, they can be used interchangeably (Garnatje et al. 2017) in others they cannot (Yao et al. 2018). The current study on the profiling of various *Lycium* genotypes can serve as a source of information, for example, for pharmacists by relating the biochemical profiles to the genetic profiles of the selected genotypes and for breeders when selecting components for crossbreeding for various purposes (Liu et al. 2020). In the present study, the use of RAPD and ISSR revealed the following findings. There was no variability among the five individual clones of the cultivar 'No.1' that differed in resistance to powdery mildew. The association between several identified polymorphic products of both RAPD and ISSR and resistance to powdery mildew would require separate studies. Genetic variation was observed between the six cultivated *L. barbarum* cultivars, *L. barbarum* GA, and *L.*

chinense 1 and 2. By using 18 RAPD and 15 ISSR primers, 200 and 183 loci were amplified, respectively, with lengths ranging from 2800 to 320 bp for RAPD and from 4120 to 320 bp for ISSR. On average, 11 RAPD loci and 12 ISSR loci were amplified in the reaction with one primer.

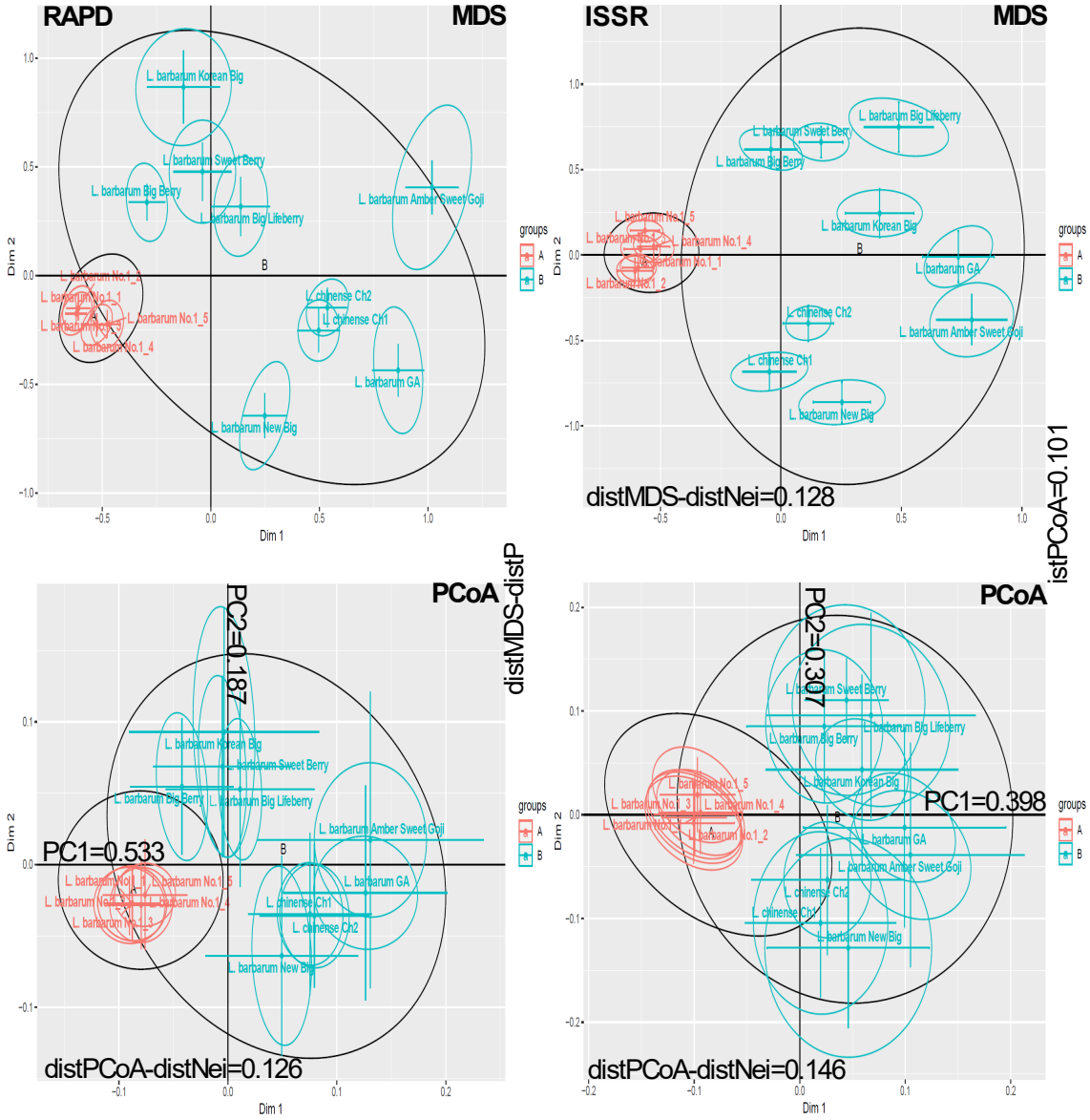


Fig. 3. 2D plots of MDSs and PCoAs performed for RAPD and ISSR datasets. The first and second principal coordinates account has been presented above main axex on PCoAs plots. As presented in Smolik et al. (2022) paper, error bars correspond to the square root of the stress statistics. The bootstrap shows the sensitivity of the spatial configuration of the genotypes to the missing random loci in the dataset (500 alternative coordinates of the spatial configuration). The ellipses shown around the genotype (point) indicate the covariance of 500 coordinates, based on the assumption that the resulting alternative coordinates of the point configurations follow a 2D normal distribution. The covariances of the alternative coordinates belonging to the groups designated by STRUCTURE 2.3.4 are represented by black ellipses

Among the amplified RAPDs, 45.5% and 48% were described as polymorphic and monomorphic, respectively, while for ISSRs, 49.2% and 43.2% were described as polymorphic and

monomorphic, respectively. The characteristics of the methods viewed in light of the selected results presented above are in line with those reported in the literature.

Zhang et al. (2001) used RAPD to distinguish eight genotypes of *L. barbarum*, including five species, two varieties and one cultivated variety, from other closely related species of the same genus. Using ten primers distinctive fingerprints corresponding to different *Lycium* species were obtained. Analysis revealed that the genetic variability was higher within than among species. Ahn et al. (2004) used RAPD to search for somaclonal variants among 40 regenerants of *L. chinense* Mill. RAPD analysis with 15 different oligomers was performed to examine the somaclonal variants. No differences in fingerprints were found after amplifications implying no DNA changes during differentiation into shoots. Liu et al. (2020) studied 16 *Lycium* genotypes from different regions of China by using 10 ISSR oligonucleotides. The authors amplified 956 bands with lengths ranging from 200 to 2000 bp. One primer used in this study amplified an average of 6 products. Among these products, 88.3% were described as polymorphic. Jung et al. (2021) used ISSR to assess the stability of regenerated under in vitro conditions plantlets. A total of ten primers used produced identical 110 scorable bands, ranging from 100 to 1000 bp, with an average of 10.5 bands per one primer. Bands were monomorphic. The fingerprints of in vitro regenerated and donor plants were similar.

The genetic similarity coefficients obtained for the studied *Lycium* genotypes using RAPD and ISSR were similar. Moreover, the high significant value of the $r_{RAPD+ISSR}$ correlation coefficient ($r = 0.925$; calculated by the Mantel test) confirmed the fact that both RAPD and ISSR described in a similar manner the genetic relationships between the studied genotypes. The most genetically distant genotypes in the cultivars were ‘New Big’ and ‘Sweet Berry’. The similarity between them was 0.71 and 0.63 for RAPD and ISSR, respectively. The highest similarity was assigned to the pairs of ‘New Big’ and *L. chinense* 1 and 2.

Based on the Bayesian algorithm, the *Lycium* genotypes were divided into two groups (G1 and G2). For both RAPD and ISSR, clones of the cultivar ‘No. 1’ were assigned to the G1 group, and the remaining genotypes were assigned to the G2 group. AMOVA for RAPD and ISSR highlighted significant variations for ‘among group’ and ‘within group’, with ‘within group’ variation showing a significantly higher value. This finding suggests the presence of a large genetic variation among the genotypes assigned to the groups; this, according to Coulon et al. (2008), provides the possibility of differentiation of hidden subpopulations/subgroups, which these authors call discrete groups. Based on the findings of Coulon et al. (2008) and Smolik et al. (2022), additional analyses were conducted to extract discrete groups, and a set of G2 genotypes were subjected to Bayesian simulations for RAPD and ISSR. Both simulations showed the division of the G2 group into two subgroups of G2.1 and G2.2.

The groups and discrete groups are clearly visible in the MDS plots for RAPD and ISSR. This is because the MDS algorithm found such 2D configurations of points (genotypes) for which the Euclidean distances are closest to the distances represented in the genetic distance matrix. Moreover, the PCoA algorithm found and rotated the coordinate system to correspond to the principal components; thus, the PCoA images represent the coordinates of each genotype on the subspace including the most important principal components.

Regarding the multidisciplinary approach to assess goji quality, as proposed by Xing et al. (2016), and Yao et al. (2018), two studies of Kruczek et al. (2020a, 2020b) should be noted. In the first study involving ‘No.1’ and ‘New Big’ cultivars, the authors showed that ‘New Big’ had a higher macronutrient content in its leaves than ‘No.1’ and a higher micronutrient content in its fruits. The study also indicated that the leaves had a significantly higher content of selected polyphenols than the fruits (Kruczek et al. 2020a). In the second study, the authors evaluated seven goji cultivars (‘No. 1’, ‘New Big’, ‘Sweet Berry’, ‘Big Berry’, ‘Big Lifeberry’, ‘Korean Big’ and ‘Amber Sweet Goji’) for physicochemical parameters, antidiabetic and antioxidant activity,

and polyphenol content (Kruczek et al. 2020b). The studied parameters were analyzed for the range of variation and significance of the average values. A large range of variation was observed within the studied group of cultivars. Thus, the results of genotype variation could be used for pharmaceutical research in developing herbal medicine and in the breeding as components for developing crossbreeds.

CONCLUSIONS

The genome of *Lycium* sp. has been studied intensively because of its application in pharmaceutical and food industries. Previous studies have developed procedures for multidisciplinary profiling of genotypes/seeds of *Lycium* sp., which could enable the precise quantification of their usefulness. The need to develop MAS-assisted breeding work has been demonstrated. By using RAPD and ISSR, genetic relationships among 14 genotypes of *Lycium* sp. were described. Little variation was observed between the clones of the cultivar 'No.1', while a high genetic variation was noted between the other nine genotypes in this cultivar and the *L. chinense* genotypes obtained from China. The analysis of RAPD and ISSR fingerprints of each of the 14 genotypes studied showed their diversity and tendency to form discrete groups and clusters. Thus, the biodiversity found in the present study can be used in selection breeding materials and identifying markers for marker assisted selection.

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POLIMORFIZM RAPD ORAZ ISSR U WYBRANYCH GENOTYPÓW *LYCIUM* SP.

Streszczenie. Ze względu na wartość i znaczenie gospodarcze genom *Lycium* jest obiektem multidyscyplinarnych badań. W niniejszej pracy przedstawiono strukturę i relacje genetyczne między 14 wybranymi obiektami goji o różnym pochodzeniu. Używając 18 dekamerów RAPD oraz 15 starterów ISSR, amplifikowano odpowiednio 200 i 183 loci. Wśród amplifikowanych 45,5–49,2% loci opisano jako polimorficzne, 6,5–7,6% jako genotypowo specyficzne. W analizach klastrowych i STRUCTURE przeprowadzonych dla RAPD i ISSR opisano relacje genetyczne między genotypami *Lycium* sp. Wysoce istotna i dodatnia wartość współczynnika *r* obliczonego testem Mantela dla macierzy podobieństwa Jaccarda RAPD oraz ISSR potwierdziła przydatność każdej metody z osobna do wykorzystania w tego typu badaniach. Otrzymane w AMOVA istotne wartości statystyk F_{ST} dla „pomiędzy" i „wewnątrz" grup potwierdziły zróżnicowanie genotypów nie tylko między wyznaczonymi grupami, ale szczególnie w ich obrębie. Zróżnicowanie to umożliwia wybór ciekawych genotypów, a także prowadzenie prac nad identyfikacją markerów dla selekcji wspomaganej markerami.

Słowa kluczowe: *Lycium* sp. L., różnorodność, RAPD, ISSR, STRUCTURE, MDS, PCoA.