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RAPD MARKERS ARE EFFECTIVE TOOL FOR THE DIFFERENTIATION OF COMMON AND TARTARY BUCKWHEAT GENOTYPES

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ABSTRACT

Common and tartary buckwheat are important cultivated species of the genus *Fagopyrum*. Genetic polymorphism of thirty-five genotypes of common buckwheat (*Fagopyrum esculentum* Moench) and tartary buckwheat (*Fagopyrum tataricum* Gaertn.) was analyzed using 10 random amplified polymorphic DNA (RAPD) primers. A total of 119 DNA fragments were amplified using ten RAPD primers with an average of 11.9 fragments per primer. The number of amplified fragments ranged from 6 (OPB-08) to 16 (SIGMA-D-01). PIC values ranged from 0.782 (OPC-08) to 0.919 (SIGMA-D-01) with an average of 0.871 per primer. The marker index (10.364) and diversity detecting index (2.961) were high and presented the utility of used marker technique. To evaluate the genetic relationships among buckwheat genotypes a phylogenetic tree based on UPGMA algorithm was constructed. The genotypes of common and tartary buckwheat were separated independently into cluster I and II. Cluster I separated 14 genotypes of tartary buckwheat into two subclusters (Ia, Ib). Genotype 903016, which originated in Pakistan, was separated individually into the subcluster Ia. Cluster II included genotypes of common buckwheat and was further subdivided into subcluster IIa and IIb. Two genotypes of subcluster IIb (Bamby and Hruszowska) were genetically the closest. Bamby and Hruszowska reflected the maximum similarity value (0.605) according to Jaccard's coefficient of similarity. Based on the presented data it is suggested that the RAPD technique is suitable for differentiation among genotypes of common and tartary buckwheat. RAPD primers have proven to be reliable and useful method for studying genetic variability of buckwheat genotypes.

Keywords: Random Amplified Polymorphic DNA, polymorphism, *Fagopyrum esculentum* Moench, *Fagopyrum tataricum* Gaertn., dendrogram, PCoA

INTRODUCTION

As the world population is expected to grow rapidly, studies dealing with the food crisis have become a hot topic of research **(Usman *et al.*, 2022)**. It is necessary to look for alternative types of crops with high productivity and a more complete nutritional profile compare to traditional cereals. Pseudocereals are highly rated attractive crops due to their nutritional and health-promoting properties **(Ciudad-Mulero *et al.*, 2019; Graziano *et al.*, 2022)**. The pseudocereals contain important level of total fibre, minerals such as K, Mg, Ca, P and unsaturated fatty acids **(Elsohaimy *et al.*, 2015)**. The most analyzed and widely cultivated pseudocereals are quinoa (*Chenopodium quinoa*), amaranth (*Amaranthus* spp.) and buckwheat (*Fagopyrum* spp.). Buckwheat belongs to the family *Polygonaceae* and the genus *Fagopyrum* **(Yasui *et al.*, 2016)**. Buckwheat species, *Fagopyrum tataricum* Gaertn. and *Fagopyrum esculentum* Moench are dicotyledonous diploid crops (2n = 2x = 16) **(Yasui *et al.*, 2016)**, are grown in China, India, and some European countries **(Zhu, 2021)**. Buckwheats (*Fagopyrum tataricum* and *Fagopyrum esculentum*) cultivation, is more widespread than quinoa or amaranth cultivation. Buckwheat, in general, has been cultivated for centuries in many countries, both for its grains and leaves **(Singh *et al.*, 2020)**. Common buckwheat and tartary buckwheat domestication is located in the western Yunnan and Sichuan regions of China **(Mushtaq *et al.*, 2018; Hunt *et al.*, 2017)**. *F. esculentum* is widespread in the temperate zones of the Northern hemisphere **(Ohnishi, 1998)**. *F. tataricum* is mostly cultivated at high altitudes in the Himalayan region **(Ohnishi, 2000)**. **Campbell (1997)** stated, that in terms of ecophysiology, *F. tataricum* has a higher frost tolerance than *F. esculentum*. Common and tartary buckwheat are widely recognized as a future crop with multiple uses, from restoring soil productivity to nutritional properties and supporting the livelihood security of the world's population **(Babu *et al.*, 2018)**. Buckwheats (*Fagopyrum tataricum* and *Fagopyrum esculentum*) can be used for the production of functional foods with a high content of starch and flavonoids and a low glycemic and insulin index **(Stokić *et al.*, 2015; Gao *et al.*, 2016)**. In 2021, Russia, China, and Ukraine were the leading producers of buckwheat worldwide. Among the European Union states, Belarus belongs to the top ten countries with a production of 26,269 tons of buckwheat for year 2021 **(FAOstat, 2023).** Buckwheat is a minor crop in Slovakia. The development and use of molecular markers have significantly dealt with various aspects of molecular genetics, which forms the basis for the study of DNA polymorphisms **(Cullis, 2002)**. The Random Amplified Polymorphic DNA (RAPD) technique based on polymerase chain reaction has been one of the most commonly used molecular techniques for DNA marker development in recent decades **(Al-Samarai and Al-Kazaz, 2015)**. RAPD technology provides rapid and efficient screening for DNA polymorphism. The main advantage of RAPD is that it does not require any prior DNA sequencing. The origin of buckwheat has been studied using random amplified polymorphic DNA (RAPD) markers **(Murai and Ohnishi 1996)**. Genetic distance among parental lines of common buckwheat was successfully calculated based on the RAPD markers and sequence tagged site (STS) by **Li *et al.* (2021)**. Similarly, STS and RAPD markers were used to carry out genetic mapping of hybrids between variety of common buckwheat (*F. esculentum* Moench) and wild accession (*F. esculentum* var. *homotropicum*) by **Pan and Chen, (2010)**. The diffusion routes of *F. esculentum* were investigated using phylogenetic trees based on the variability of RAPD markers **(Murai and Ohnishi, 1996)**. The RAPD technique was used to analyze the genetic diversity of wild *Fagopyrum cymosum* Meisn **(Bimb *et al.*, 2001)**, to detect the geographic origins of tartary buckwheat **(Sharma and Jana, 2002)**. RAPD analysis is widely used for various purposes such as genetic diversity analysis of agriculturally important crops **(Al-Samarai and Al-Kazaz, 2015)**. For example, evaluation of molecular diversity using RAPD markers was used for differentiation of ricin **(Vivodík *et al.*, 2015)**, triticale **(Vyhnánek and Bednář, 2006)**, for maize cultivars **(Balážová *et al.*, 2016)**. RAPD method was also used in the study of stability in explant cultures for the preservation of genotypes of broccoli **(Pavlović *et al., 2024*)**. Another marker technique used for analyses of buckwheat was AFLP (the amplified fragment length polymorphism) marker system. Using the AFLP fingerprinting technique, **Gupta *et al.* (2012)** studied 195 accessions of tartary buckwheat for rutin content variations. **Yasui *et al.* (2004)** created the first high-density buckwheat genetic map of *F. esculentum* and *F. homotropicum* using AFLP markers spanning the whole genome. Combinations of 20 primers generated 669 bands. Of the 669 bands, 462 were polymorphic. Twenty AFLP markers were used to analyze the genetic diversity of 165 accessions of tartary buckwheat. A previous study showed that geographical distribution is correlated with the genetic relationship of tartary buckwheat **(Hou *et al.*, 2009)**. The AFLP method is a simplified analysis of the genetics and breeding of buckwheat. AFLP and RAPD markers need to be converted to Sequence Characterized Amplified Regions (SCAR) markers because of low reliability. Therefore, another technique, which

do not need to be converted to the SCAR, were applied **(Yasui, 2020)**. Higher reproducibility, high polymorphism information content, and stable co-dominance are important attributes of the simple sequence repeat (SSR) marker system **(Mukhtar *et al*., 2021)**. Although the cost of SSR marker development is high, SSR markers have been developed for common and tartary buckwheat **(Iwata, 2001; Hou *et al*., 2016)**. The SSR marker system was initially described by **Iwata *et al*. (2005)**. Authors analyzed genetic diversity among Japanese common buckwheat cultivars using 5 SSR markers **(Iwata *et al.*, 2005)**. Low level of gene flow between wild and cultivated common buckwheat was demonstrated using SSR markers **(Konishi and Ohnishi, 2006)**. Research on genetic diversity in the genus *Fagopyrum* was also supported by **Ma *et al*. (2009)** through the development of 136 SSR markers. Expressed sequence tag (EST) markers, which are important for gene mapping and marker-assisted selection (MAS) **(Liu *et al*., 1999)**, together with SSR markers, represent significant advances in breeding and population genetics studies in buckwheat **(Yasui, 2020)**. Based on EST sequences, 170 primers were designed by **Hara *et al.* (2011)**. In the last decade, RAPD technique was replaced also by Start codon targeted (SCoT) technique, mainly because it is simple, novel, cost-effective, easy reproducible technique. SCoT markers have been applied for detection of diversity, sex determination, analyses of genetic relationships in many important crops but also in underutilized species, such as buckwheat **(Rai, 2023)**. **Balážová *et al*. (2018)** proved SCoT markers as highly informative and effective tool for analyzation of genetic variation among 17 buckwheat varieties using 7 SCoT markers.

The aim of our study was to analyze genetic variability among twenty-one genotypes of *F. tataricum* Gaertn. and fourteen genotypes of *F. esculentum* Moench genotypes. Ten RAPD primers were suitable for differentiation among the genotypes and at the same moment to evaluate the effectiveness of the RAPD markers used for the separation of common and tartary buckwheat from each other.

MATERIAL AND METHODS

Material

Thirty-five genotypes of common and tartary buckwheat were used for the genetic diversity analysis (Table 1). Twenty-one genotypes of *F. esculentum* Moench obtained from the Gene Bank in Piešťany (The Slovak Republic) and 14 genotypes of F. *tataricum* Gaertn. obtained from the Gene Bank in Prague (The Czech Republic) were used in our study.

DNA isolation

Genomic DNA was isolated from the fresh seedlings of buckwheat (10 days old) using the GeneJet™ Kit (Thermo Scientific, USA). The concentration and quality of the DNA was assessed using a Biodrop (Biochrom, Ltd, Cambridge, UK).

RAPD analysis

Ten RAPD primers, chosen based on the literature, were applied for the genetic variability analysis of buckwheat genotypes (see Table 2, for the primer sequence). Amplification reactions were performed in a total volume of 25 µL of the reaction mixture contained 100 ng of DNA, 12.5 µl of Master Mix (Promega, Madison, WI, USA) and 10 pmol of primer. PCR reactions were run in a TProfessional Basic Thermocycler (Biometra, Göttingen**,** Germany) programmed for 45 cycles of 1 min. each at 93.5 ° C, 2 min. at 36 ° C, 3 min. at 72 ° C for PCR. The final incubation temperature was 72 ° C for 7 min. PCRs was performed as described by **Bimb *et al*. (2001)**.

Electrophoresis of DNA

The amplified fragments of DNA were separated on 1.5% agarose gels in 1x TBE buffer (Tris-borate-EDTA) at 100 V for 1 h. Agarose gels were stained with ethidium bromide and photographed under UV light using UVP photoDoc-t® (Ultra-Violet Products Ltd., UK). The size of the amplified fragments was determined by comparison with the standard-length marker Quick-Load® Purple 2-Log DNA ladder (New England Biolabs, Inc).

Data analysis

A binary matrix was prepared from electrophoreograms based on the presence (1) and absence (0) of RAPD bands for each genotype. Binary data were evaluated with statistical software using the UPGMA construction method based on Jaccard's coefficient of similarity **(Garcia-Vallvé *et al*., 1999, http://genomes.urv.cat/UPGMA/)**.

Table 1 List of analyzed *Fagopyrum* genotypes

Number	Accename	Genus	Species	Origin	Names of cultivars in PCoA plot
1	**Aiva**	*Fagopyrum*	*esculentum* Moench	LVA	LVA1
2	**Ballada**	*Fagopyrum*	*esculentum* Moench	RUS	RUS2
3	**Bamby**	*Fagopyrum*	*esculentum* Moench	AUT	AUT3
4	**Bogatyr**	*Fagopyrum*	*esculentum* Moench	RUS	RUS4
5	**Darina**	*Fagopyrum*	*esculentum* Moench	SVN	SVN5
6	**Darja**	*Fagopyrum*	*esculentum* Moench	SVN	SVN6
7	**Emka**	*Fagopyrum*	*esculentum* Moench	POL	POL7
8	**Amurskaja**	*Fagopyrum*	*esculentum* Moench	RUS	UNK8
9	**Kazanska**	*Fagopyrum*	*esculentum* Moench	RUS	UNK9
10	**Hruszowska**	*Fagopyrum*	*esculentum* Moench	POL	POL10
11	**Kasho-2**	*Fagopyrum*	*esculentum* Moench	JPN	UNK11
12	**Kora**	*Fagopyrum*	*esculentum* Moench	POL	POL12
13	**La Harpe**	*Fagopyrum*	*esculentum* Moench	FRA	UNK13
14	**Pulawska**	*Fagopyrum*	*esculentum* Moench	POL	POL14
15	**Pyra**	*Fagopyrum*	*esculentum* Moench	CZE	CZE15
16	**Rana 60**	*Fagopyrum*	*esculentum* Moench	SVN	SVN16
17	**Siva**	*Fagopyrum*	*esculentum* Moench	SVN	SVN17
18	**St Jacut**	*Fagopyrum*	*esculentum* Moench	FRA	FRA18
19	**Spacinska 1**	*Fagopyrum*	*esculentum* Moench	SVK	SVK19
20	**Tohno Zairai**	*Fagopyrum*	*esculentum* Moench	CAN	UNK20
21	**Winsor Royal**	*Fagopyrum*	*esculentum* Moench	USA	UNK21
22	**PI 481644**	*Fagopyrum*	*tataricum* Gaertn.	BTN	BTN22
23	**PI 481671**	*Fagopyrum*	*tataricum* Gaertn.	BTN	BTN23
24	**903016**	*Fagopyrum*	*tataricum* Gaertn.	PAK	PAK24
25	**PI 451723**	*Fagopyrum*	*tataricum* Gaertn.	MEX	MEX25
26	**PI 476852**	*Fagopyrum*	*tataricum* Gaertn.	USA	USA26
27	**Weswod Ican**	*Fagopyrum*	*tataricum* Gaertn.	UNK	UNK27
28	**290**	*Fagopyrum*	*tataricum* Gaertn.	BTN	BTN28
29	**PI 427239**	*Fagopyrum*	*tataricum* Gaertn.	NEP	NEP29
30	**PI 481661**	*Fagopyrum*	*tataricum* Gaertn.	BTN	BTN30
31	**Jianzui**	*Fagopyrum*	*tataricum* Gaertn.	CHN	CHN31
32	**Liuqiao-3**	*Fagopyrum*	*tataricum* Gaertn.	CHN	CHN32
33	**Zhaoqiao-1**	*Fagopyrum*	*tataricum* Gaertn.	CHN	CHN33
34	**Jinqiao-2**	*Fagopyrum*	*tataricum* Gaertn.	CHN	CHN34
35	**Sarasin a Ployes**	*Fagopyrum*	*tataricum* Gaertn.	USA	USA35

Legend: SVN –Slovenia, SVK –Slovakia, POL -Poland, CZE – Czech Republic, AUT –Austria, BTN –Bhutan, CHN -China, RUS –Russia, LVA –Latvia, FRA -France, NEP –Nepal, USA –United States of America, PAK –Pakistan, MEX -Mexico, JPN –Japan, UNK - Unknown

Dendrogram was constructed using the iTOL program version 6.7.3, which is available online **(Letunic and Bork, 2021, https://itol.embl.de/)**. Principal Coordinate Analysis (PCoA) plot was constructed using the free statistical program R project version 4.0.5. To determine the polymorphism of common and tartary buckwheat genotypes and used RAPD markers, PIC values (polymorphic information content) were calculated for each RAPD marker **(Weber, 1990)** according to the formula:

$$PIC = 1 - \left(\sum_{i=1}^{n} p_i^2 \right) - \sum_{i=1}^{n-1} \sum_{j=i+1}^{n} 2 p_i^2 \cdot p_j^2$$

The utility and efficiency of the marker technique were evaluated by marker index (MI) and diversity detecting index (DDI) **(Myśków *et al*., 2010)**.

MI = PPPFG * PIC
DDI = PIC * PPL/PAG

PPPFG – average number of polymorphic fragments per genotype
PPL – number of polymorphic loci
PAG – number of analyzed genotypes
PIC – polymorphic information content

RESULTS AND DISCUSSION

Results

The RAPD markers are PCR based and widely used due to their simple experimental methodology and appropriateness for genetic screening of intraspecific and interspecific diversity **(Younis *et al*., 2020)**.
The RAPD amplification was primary done using 25 random primers of which only 10 were scorable and could be used to detect polymorphism and for the differentiation of common and tartary buckwheat genotypes (Table 1).
The number of bands amplified by each primer varied from 6 (OPB-08) to 16 (SIGMA-D-01) with an average of 11.9 fragments per genotype (Table 2). Of the 119 DNA fragments generated all 119 were evaluated as polymorphic. RAPD profiles of buckwheat genotypes (OPD-08 marker) are shown in the figure 1 and figure 2. The size of the PCR products ranged from 200 bp (OPA-02, OPE-01) to 3000 bp (SIGMA-D-01) (Table 2).
The polymorphic information content (PIC) values ranged from 0.782 (OPC-08) to 0.919 (SIGMA-D-01) with an average of 0.871 per primer. PIC values indicated a high level of polymorphism in the chosen RAPD primers. The utility of the marker technique was evaluated using marker index (MI) and diversity detecting index (DDI). The MI index for chosen RAPD markers was 10.364 and the DDI value was calculated 2.961. It presents high polymorphism and good possibility to differentiate chosen genotypes, respectively (Table 2).
Based on obtained data from the molecular analysis the UPGMA dendrogram was constructed. Two main clusters (I and II) were formed in the dendrogram (Figure 3). All fourteen genotypes of tartary buckwheat were grouped in cluster I. Cluster I was further subdivided into subclusters Ia and Ib. Genotype 24 (903016) was separated in the subcluster Ia. Remaining tartary buckwheat genotypes (13) were grouped in the subcluster Ib which was further subdivided to subclusters. Weswod Ican (27) and Zhaoqiao-1 (33) according to Jaccard' s coefficient achieved the maximum distance value (0.830) and revealed as the furthest genotypes within the species *F. tataricum*.
Twenty-one genotypes of common buckwheat were separated into cluster II. Cluster II was subdivided into two subclusters (IIa, and IIb) (Figure 3). Two genotypes, Bamby (3) originated in Austria and genotype Hruszowska (10) originated in Poland, were grouped together into cluster IIb. They were evaluated as genetically the closest. Genotypes 3 and 10 also showed the maximum similarity value (0.605) according to Jaccard's coefficient of similarity. On the other hand, genetically the furthest were genotypes Aiva (1) and Rana 60 (16), which achieved the highest genetic distance value (0.891) based on Jaccard. Genotype Aiva (1) originating from Latvia and Darja (6) originating from Slovenia were clustered separately into subcluster IIa. The results of clustering of the common and tartary buckwheat genotypes in the dendrogram were comparable using the PCoA plot analysis. *Fagopyrum esculentum* and *Fagopyrum tataricum* genotypes were separated into two main groups in the constructed PCoA plot (Figure 4). We can conclude that the *Fagopyrum tataricum* genotypes (Table 1) were grouped in the red circle in the 1st and 4th quadrant and separated from the *Fagopyrum esculentum* genotypes (Table 1) included in the blue circle mainly in the 2nd and 3rd quadrant.

Table 2 Statistical characteristics of the RAPD markers used in the genotypes of *Fagopyrum* spp.

Primer	Sequence (5´3´)	PIC	No. of polymorphic bands	% of polymorphism	MI	DDI	Molecular weight range (bp)
OPA-02	TGCCGAGCTG	0.910	15	100 %			200-2000
OPA-05	AGGGGTCTTG	0.897	15	100 %			400-2000
OPA-13	CAGCACCCAC	0.884	13	100 %			400-2000
OPB-08	GTCCACACGG	0.808	6	100 %			300-2000
OPC-08	TGGACCGGTG	0.782	7	100 %			400-1000
OPD-07	TTGGCACGGG	0.839	11	100 %			300-1000
OPD-08	GTGTGCCCCA	0.908	13	100 %			300-2000
OPE-01	CCCAAGGTCC	0.872	11	100 %			400-2500
OPE-07	AGATGCAGCC	0.889	12	100 %			200-2500
SIGMA-D-01	AAACGCCGCC	0.919	16	100 %			300-3000
Total			**119**	**100 %**	**10.364**	**2.961**	**200-3000**
Average		**0.871**	**11.9**				

Legend: PIC- polymorphic information content, MI- marker index, DDI- diversity detecting index

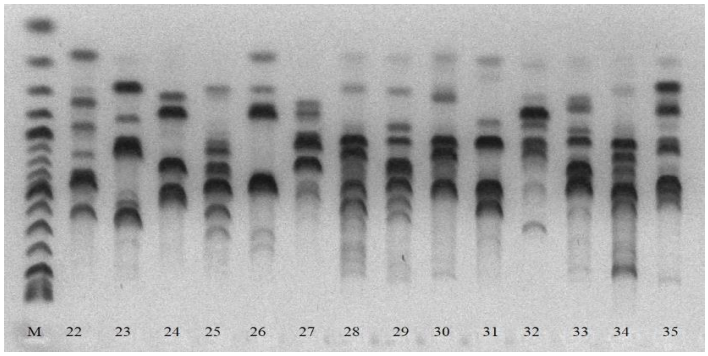

Figure 1 RAPD profiles of *F. tataricum* genotypes (OPD-08 marker)
Legend: M is Quick-Load® Purple 2-Log DNA ladder, 22-35 are analyzed genotypes of tartary buckwheat (Table 1)

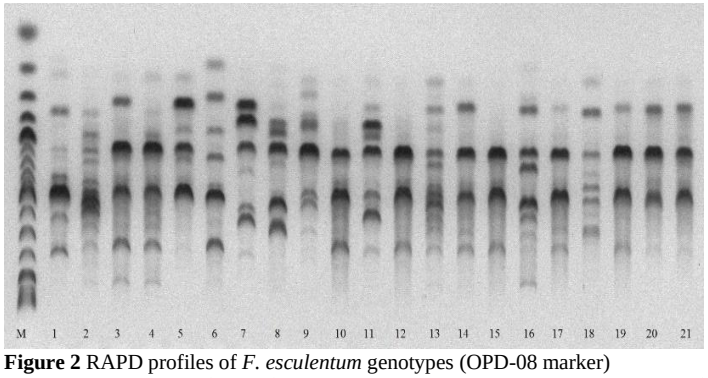

Figure 2 RAPD profiles of *F. esculentum* genotypes (OPD-08 marker)
Legend: M is Quick-Load® Purple 2-Log DNA ladder, 1-21 are analyzed genotypes of common buckwheat (Table 1)

Discussion

Important properties of plants are effectively detected by molecular markers, through which plant breeding has become more successful and practical **(Luthar *et al*., 2021)**. Random amplified polymorphic DNA (RAPD) markers are efficient in detecting polymorphisms at the DNA level **(Yin *et al*., 2022)**.

The RAPD technique is simple, not expensive, and not labour intensive. However, reactions conditions must be optimized to obtain reproducible results **(Luthar *et al*., 2021)**. RAPD markers are used in phylogenetic studies of common buckwheat, despite their lack of reproducibility **(Yasui, 2020)**.

Genetic diversity of twenty-one genotypes of *Fagopyrum esculentum* Moench and 14 genotypes of *Fagopyrum tataricum* Gaertn. was evaluated using 10 RAPD markers and a total of 119 fragments with an average of 11.9 fragments per genotype were obtained in our study. All detected fragments were polymorphic. Similarly, **Bimb *et al*. (2001)** used RAPD markers to assess genetic diversity of Nepalese populations of *Fagopyrum cymosum* Meisn. (wild buckwheat), respectively. Totally 105 DNA bands were detected. The number of DNA fragments varied from 9 to 12. A total of 46 polymorphic bands were used for the statistical analyses. **Pan and Chen (2010)** used 19 RAPD primers for genetic mapping of common buckwheat. The RAPD primers produced 269 DNA fragments with an average number of 14.16 per primer. Out of total 269 DNA bands, 189 fragments were polymorphic (70.26 %). On the other hand, a lower level of polymorphism, in comparison with our results (100 %), was achieved by **Tsuji and Ohnishi (1998)** who studied phylogenetic relationships among landraces and natural populations of tartary buckwheat. **Tsuji and Ohnishi (1998)** used 40 RAPD primers. Among the 149 DNA bands detected in cultivated landraces, only twenty-four DNA were polymorphic (16.1%) compared to wild species of tartary buckwheat where out of 197 DNA fragments 105 fragments were polymorphic (53.3%). **Sharma and Jana (2002)** analyzed the genetic diversity among 52 landraces and cultivars of tartary buckwheat and one accession of its wild ancestor using RAPD primers. Out of 240 obtained fragments 87 bands were polymorphic (36.25 %.) The authors recorded the usability of the RAPD marker technique for characterization of buckwheat accessions **(Sharma and Jana 2002; Luthar *et al*., 2021)**. **Rout and Chrungo (2007)** applied RAPD primers to study the genetic variations and phylogenetic relationships between accessions of Himalayan buckwheat (*F. esculentum, F. tataricum, F. cymosum*). They considered the RAPD marker system able to distinguish between accessions from the same as well as different species of the genus. The study of genetic diversity and relationships among wild and cultivated accessions of tartary buckwheat revealed by RAPD markers proved the usefulness of RAPD markers for assessing of genetic variation in *F. tataricum* germplasm collections **(Kump and Javornik, 2002)**.

The sizes of the amplicons in our study ranged from 200 bp to 3kb. Comparable results were obtained in other studies [**(Murai and Ohnishi (1996)**; **Tsuji and Ohnishi (1998); Sharma and Jana (2002)]**. In contrast, **Pan and Chen (2010)** detected molecular weights of DNA fragments in the range of 300-1500 bp.

The UPGMA dendrogram, constructed based on the binary matrix divided the analyzed genotypes into two main clusters. Cluster I included all genotypes of tartary buckwheat. Common buckwheat genotypes were grouped together into cluster II. Based on the obtained data we concluded that the applied RAPD markers were able to differentiate between our selected genotypes of tartary and common buckwheat at the interspecific and intraspecific diversity level. The results may be relevant to breeders for crossbreeding.

Sharma and Jana (2002) used UPGMA-based pairwise Jaccard's coefficient of similarity to analyze the relationships between genetically diverse accessions of tartary buckwheat. **Tsuji and Ohnishi (1998)** studied the relationships among 29 cultivated landraces, 17 wild subspecies and one weedy type of tartary buckwheat. Phylogenetic tree was constructed using the neighbor-joining method based on the genetic distance matrix. Similarly, the constructed dendrogram based on the RAPD method was shown to be a useful tool for differentiating among populations of tartary buckwheat.

UPGMA trees based on RAPD markers in the study by **Murai and Ohnishi (1996)** clearly grouped and show that the wild ancestor populations are distantly related to the cultivated land races of *Fagopyrum esculnetum* Moench. Phylogenetic trees were constructed using the UPGMA and neighbor-joining method **(Murai and Ohnishi, 1996)**. Hierarchical cluster analysis using the UPGMA algorithm based on RAPD markers has also been used for studies of other important crops such as *Glycine max* L. **(Vivodík *et al*., 2022)**, *Zea mays* L. **(Balážová *et al*., 2016; Rahardja *et al*., 2022)**, *Phaseolus vulgaris* L. **(Hündürel *et al*., 2023; Hromadová *et al*., 2023)**, *Solanum tuberosum* L. **(Islam *et al*., 2022),** *Oryza sativa* L**. (Settu *et al*., 2021)**, *Brassica* species **(Raza *et al*., 2020)**.

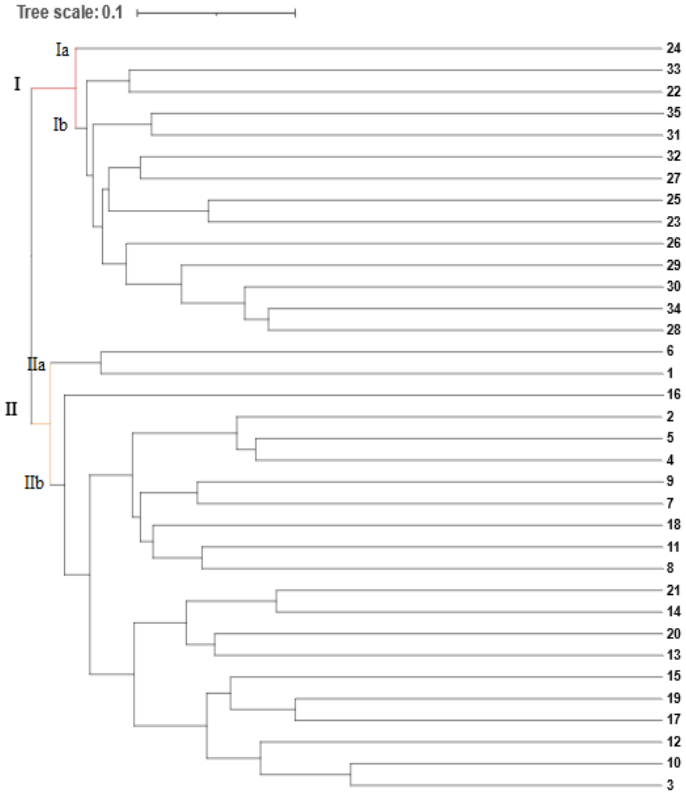

Figure 3 Dendrogram of 35 genotypes of common and tartary buckwheat based on 10 RAPD markers

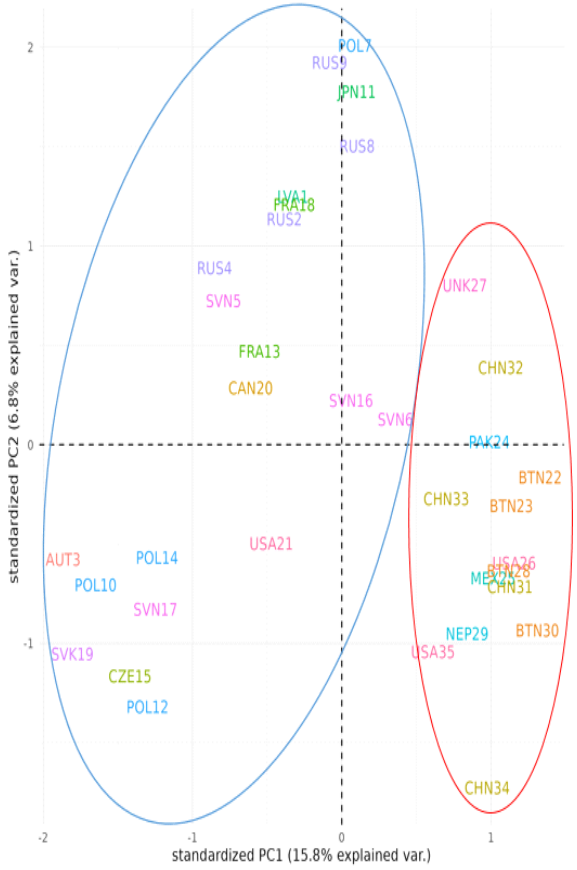

Figure 1 PCoA plots of 35 genotypes of common and tartary buckwheat based on 10 RAPD markers. The red circle contains genotypes of *Fagopyrum tataricum* (Table 1), the red circle groups genotypes of *Fagopyrum esculentum* (Table 1).

PIC values were calculated based on the obtained data. PIC values ranged from 0.782 (OPC-08) to 0.919 (SIGMA-D-01). Similarly, **Vivodík *et al*. (2015)** who analyzed a set of 111 ricin genotypes using 13 RAPD primers calculated PIC values, which ranged from 0.491 (OPE-07) to 0.898 (SIGMA-D-01). Comparable results also observed **Balážová *et al*. (2016)** who evaluated molecular diversity of central European maize cultivars, **Kuťka Hlozáková *et al*. (2016)** studied genetic polymorphism in European wheat genotypes using RAPD markers, **Petrovičová *et al*. (2015)** assessed RAPD polymorphism in rye. In contrast, lower values of PIC ranged from 0.31 (OPA-1) to 0.47 (OPA-6) were calculated by **Mallikarjuna *et al*. (2022)** who used 15 RAPD markers for the analysis of molecular diversity of black gram (*Vigna mungo*). Efficacy of RAPD markers was evaluated using the MI index in addition to the PIC values. Higher MI value indicates that marker technique could detect the differences among genotypes. The MI index for chosen RAPD markers was 10.364. Lower values of marker index were obtained respectively for rye (***Myśków et al*., 2010**), common bean (***Sakhravi et al*., 2023**; ***Hromadová et al*., 2023**) and wheat **(*Khaled et al*., 2015)**.

CONCLUSION

The evaluated data showed that the RAPD maker technique is an effective tool for the detection of polymorphism. Detected number of polymorphic fragments indicates the high genetic diversity among the studied buckwheat genotypes.
Chosen RAPD primers revealed the high level of polymorphism by achieving the PIC values higher than 0.6. The constructed dendrogram separated common and tartary buckwheat genotypes from each other to two main clusters which was confirmed using PCoA analysis. Our study demonstrates the suitability of RAPD primers for the differentiation among two species, common and tartary buckwheat, as well as the usefulness for differentiation within the species.

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