

GENETIC VARIABILITY OF *FAGOPYRUM* SP. GENOTYPES DETERMINED BY GENE-TARGETED MARKERS

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ABSTRACT

Fagopyrum represents an economically and nutritionally important crop which belongs to the group of pseudocereals. Buckwheat is referred to be a functional crop with a wide range of use around the world. Grains of buckwheat are nutritionally significant. They are characterised by a high content of starch, proteins, flavonoids and fibre. SCoT technique is used for the detection of plant genes polymorphism, for the evaluation of genetic diversity and for mapping the functional regions of the genome. The aim of the work was to perform molecular analyses of 21 genotypes of *Fagopyrum esculentum* Moench and 14 genotypes of *Fagopyrum tataricum* Gaertn. using ten gene-targeted SCoT markers. The seedlings of buckwheat were used for DNA isolation and subsequently for the amplification of the DNA using a polymerase chain reaction. The number of amplified fragments ranged from 12 (SCoT 60, SCoT 13) to 27 (SCoT 12). The total number of fragments was 176 of which 162 were polymorphic with an average of polymorphic fragments 16.2. The percentage of polymorphism ranged from 58.33 % (SCoT 60) to 100 % (SCoT 12, SCoT 13, SCoT 29, SCoT 30) with an average of 90.29 %. Polymorphic information content (PIC) values characterize the polymorphism of used SCoT markers. The PIC values ranged from 0.578 (SCoT 60) to 0.932 (SCoT 36) with an average of 0.859. The genetic diversity of buckwheat was determined by hierarchical cluster analysis using the UPGMA algorithm in the created dendrogram. Genotypes of buckwheat were divided into two main clusters. Two genotypes of common buckwheat (Siva, Špačinska I) from Slovenia and the Slovak Republic, respectively, as well as another two tartary buckwheat genotypes (Tohno Zairai, Winsor Royal) from unknown regions, respectively, were genetically the closest. The PCoA plot confirmed the separation of tartary buckwheat from common buckwheat resulting in the constructed dendrogram. According to our results, we can consider the SCoT technique appropriate for differentiation of *Fagopyrum esculentum* Moench and *Fagopyrum tataricum* Gaertn. genotypes leading to genotype identification and utilization in the breeding process to improve the buckwheat genetic material.

Keywords: *Fagopyrum esculentum* Moench, *Fagopyrum tataricum* Gaertn., polymorphism, SCoT markers, dendrogram

INTRODUCTION

Fagopyrum tataricum Gaertn. and *Fagopyrum esculentum* Moench belong to the family of Polygonaceae and the genus *Fagopyrum* (Kim and Hwang 2020) which includes 27 species (Tang et al., 2016). *Fagopyrum tataricum* Gaertn. and *Fagopyrum esculentum* Moench are diploid species ($2n = 2x = 16$) cultivated for food and feed purposes (Zhang et al., 2017). In general, buckwheat is consumed worldwide (Kim and Hwang 2020) and is an important crop whose seeds are used for consumption as well as potential functional food, mainly due to high-quality protein, rich phenolic compounds and well-balanced composition of the amino acids and minerals (Jing et al., 2016). The basic chemical composition of *Fagopyrum tataricum* Gaertn. is very similar to *Fagopyrum esculentum* Moench (Rysová, 2018). Buckwheat seeds contain flavonoids such as rutin and quercetin (Zhu, 2016). Rutin is the most important antioxidant considered as the best health-promoting flavonoid (Zhou et al., 2016). The amount of rutin in seeds and sprouts of *Fagopyrum tataricum* Gaertn. is 47 times higher than in seeds and sprouts of *Fagopyrum esculentum* Moench (Lee et al., 2016). *Fagopyrum tataricum* Gaertn. is more tolerant to adverse environmental conditions, especially low temperatures (Betekhtin et al., 2018) mainly due to the higher content of polyphenol components (Lee et al., 2016). Because of their agronomic importance in Asia, Eastern Europe, the USA, Brazil, India, and France (Betekhtin et al., 2018), numerous genetic studies and breeding practices have been carried out to improve existing buckwheat varieties and create new ones. One goal is to obtain plants that combine the useful properties of *Fagopyrum esculentum* Moench and *Fagopyrum tataricum* Gaertn. Currently, Russia, China and Kazakhstan are the world's largest buckwheat growers (Singh et al., 2020). *Fagopyrum esculentum* is consumed as food and has a medicinal rate. It is a source of bioactive nutrients and can be used for the treatment and prevention of many ailments. Traditionally, it is used to treat hypertension, diabetes, constipation, and cancer (Panihar et al., 2020). *Fagopyrum tataricum* Gaertn. is less used for consumption. In some parts of Asia and Eastern Europe, it can be used as food or as a source of medicinal substances in pharmacy and traditional medicine. However, due to its bitter taste, it is mainly used for feeding purposes (Rysová, 2018).

In molecular research of plants DNA markers are very often used. DNA markers have proven to be a useful technique for the detection of genetic variability in the genus *Fagopyrum* mainly the PCR (Polymerase Chain Reaction) based techniques. Sharma and Jana (2002) used the RAPD (Random Amplified Polymorphic DNA) technique to study relationships in *Fagopyrum* species. Park et al. (2006) and Saunders Bulan et al. (2017) used the AFLP (Amplified Fragment Length Polymorphism) technique to detect genetic diversity among wild-growing tartary buckwheat varieties and tartary buckwheat (*Fagopyrum tataricum* Gaertn.) diversity in its center of origin in China, respectively. Hou et al. (2009) studied 165 buckwheat populations using twenty pairs of AFLP primers. Li et al. (2009) employed the SRAP (Sequence-related amplified polymorphism) marker to analyze genetic diversity. The ISSR (Inter Simple Sequence Repeat) technique was used to detect the genetic diversity of 15 genetic resources of buckwheat germplasm (Kishore et al., 2013). Sabreena et al. (2021) compared the ISSR and SSR (Simple Sequence Repeat) techniques in detecting of the genetic diversity of 63 tartary buckwheat genotypes. A relatively new PCR technique, the SCoT (Start Codon-Targeted) technique, was used by Balážová et al. (2018), who analyzed the genetic variability of 17 buckwheat genotypes using 7 SCoT markers. Polymorphism based on the short conserved region in genes of plants surrounding the AGT translation is known as start codon targeted polymorphism (Collard and Mackill, 2009). The SCoT markers are used as a credible technique (Dilipan et al., 2020). There are highly reproducible and polymorphic. The method is based on the short conserved region flanking the ATG start codon in plant genes that is more advantageous compare to other multilocus techniques such as ISSR, AFLP or RAPD. Validation of this method was done in rice by using genotypes of the genetically diverse set (Khan and Dhawan, 2016). Genetic studies of buckwheat are also nowadays limited due to insufficient genetic resources (Liu et al., 2022). The SCoT technique was applied in many crops such as wheat (Etminan et al., 2016; Ghobadi et al., 2021; Nosair, 2020), barley (Habiba et al., 2021), maize (Vivodík et al., 2017, Sadek and Ibrahim, 2018), rye (Petrovičová et al., 2017), castor (Vivodík et al., 2018) and many other crops. The aim of our study was to evaluate genetic diversity within the set of 35 common and tartary buckwheat genotypes originating from different countries using 10 SCoT markers, and to testify the usefulness of these markers in terms of

differentiation and characterization of *Fagopyrum* genotypes. The obtained results may be useful in the genomic mapping and breeding process to improve buckwheat genotypes with required agronomic important traits leading to managing the genetic resources.

MATERIAL AND METHODS

Plant material

Twenty-one genotypes of *Fagopyrum esculentum* Moench and fourteen genotypes of *Fagopyrum tataricum* Gaertn. obtained from the Gene Bank of the Research Institute of Plant Production in Piešťany, Slovak Republic and Prague, Czech Republic were used in our work (Table 1). Genotypes of buckwheat originate in 15 different countries (Table 1).

DNA Isolation

The genomic DNA of the buckwheat was extracted from 7-10 days old seedlings according to the protocol GeneJET™ (Thermo Scientific, USA). The quality and quantity of isolated DNA was checked by Biodrop (Biochrom, Ltd, United Kingdom).

PCR analysis

Isolated DNA was subsequently used to amplify DNA fragments by using PCR reaction according to the literature (Collard and Mackill, 2009; Luo et al., 2010). Ten SCoT primers were chosen for our analysis (Table 2). PCR was done in total

volume of 15 µl of the reaction mix in programmed TProfessional Basic Thermocycler (Biometra, Germany). Initial denaturation was at 94 °C for 3 min, subsequently 35 cycles starting at 94 °C for 1 min, continuing at 50 °C for 1 min and 72 °C for 2 min. The program was followed by a final temperature of 72 °C for 5 min.

Electrophoresis of DNA

Amplified fragments were separated on 1.5 % agarose gels in 1× TBE (Tris-borate-EDTA) buffer. The gels were stained with ethidium bromide and documented using the gel documentation system UVP PhotoDoc-t® (Ultra-Violet Products Ltd., United Kingdom). The size of amplified fragments was determined by comparing them with the standard length marker Quick-Load® Purple 2-Log DNA ladder (New England Biolabs, Inc).

Statistical analyses

The SCoT bands were evaluated as present (one) or absent (zero) and binary matrix was prepared. A dendrogram based on hierarchical cluster analysis using the unweighted pair group method with arithmetic average (UPGMA) with the SPSS® professional statistics version 17 software package (IBM®, USA) was constructed. PCoA (Principal Coordinate Analysis) plot was constructed using the free statistical program R project version 4.0.5.

Based on the number of fragments of each genotype and their frequency the polymorphic information content (PIC) according to Weber (1990) was calculated to evaluate the polymorphism of used SCoT markers.

Table 1 List of analyzed genotypes of buckwheat

	Genotype	Taxon	Country of origin	Names of cultivars in PCoA plot
1	Aiva	<i>F.esculentum</i> Moench	LVA	LVA1
2	Ballada	<i>F.esculentum</i> Moench	RUS	RUS2
3	Bamby	<i>F.esculentum</i> Moench	AUT	AUT3
4	Bogatyr	<i>F.esculentum</i> Moench	RUS	RUS4
5	Darina	<i>F.esculentum</i> Moench	SVN	SVN5
6	Darja	<i>F.esculentum</i> Moench	SVN	SVN6
7	Emka	<i>F.esculentum</i> Moench	POL	POL7
8	FAG 29/79 (Amurskaja)	<i>F.esculentum</i> Moench	RUS	RUS8
9	FAG 38/82 (Kazanska)	<i>F.esculentum</i> Moench	RUS	RUS9
10	Hruszowska	<i>F.esculentum</i> Moench	POL	POL10
11	KASHO-2	<i>F.esculentum</i> Moench	JPN	JPN11
12	Kora	<i>F.esculentum</i> Moench	POL	POL12
13	La Harpe	<i>F.esculentum</i> Moench	-	UNK13
14	Pulawska	<i>F.esculentum</i> Moench	POL	POL14
15	Pyra	<i>F.esculentum</i> Moench	CZE	CZE15
16	Rana 60	<i>F.esculentum</i> Moench	SVN	SVN16
17	Siva	<i>F.esculentum</i> Moench	SVN	SVN17
18	St Jacut	<i>F.esculentum</i> Moench	FRA	FRA18
19	Špačinská 1	<i>F.esculentum</i> Moench	SVK	SVK19
20	Tohno Zairai	<i>F.esculentum</i> Moench	-	UNK20
21	Winsor Royal	<i>F.esculentum</i> Moench	-	UNK21
22	PI 481644	<i>F. tataricum</i> Gaertn.	BTN	BTN22
23	PI 481671	<i>F. tataricum</i> Gaertn.	BTN	BTN23
25	903016	<i>F. tataricum</i> Gaertn.	PAK	PAK25
27	PI 451723	<i>F. tataricum</i> Gaertn.	MEX	MEX27
28	PI 476852	<i>F. tataricum</i> Gaertn.	USA	USA28
29	Weswod Ican	<i>F. tataricum</i> Gaertn.	-	UNK29
30	290	<i>F. tataricum</i> Gaertn.	BTN	BTN30
31	PI 427239	<i>F. tataricum</i> Gaertn.	NEP	NEP31
32	PI 481661	<i>F. tataricum</i> Gaertn.	BTN	BTN32
33	Jianzui	<i>F. tataricum</i> Gaertn.	CHN	CHN33
34	Liugiao-3	<i>F. tataricum</i> Gaertn.	CHN	CHN34
35	Zhaogiao-1	<i>F. tataricum</i> Gaertn.	CHN	CHN35
36	Jinqiao-2	<i>F. tataricum</i> Gaertn.	CHN	CHN36
37	Sarasin a Ployes	<i>F. tataricum</i> Gaertn.	USA	USA37

Note: SVN – Slovenia, SVK – Slovakia, POL – Poland, CZE – The 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

Table 2 List of used SCoT markers (Collard and Mackill, 2009; *Luo et al., 2010)

SCoT primer	Sequences of primers (5'-3')	Ta (50 ° C)
SCoT 12	ACGACATGGCGACCAACG	50 ° C
SCoT 13	ACGACATGGCGACCATCG	50 ° C
SCoT 14	ACGACATGGCGACCACGC	50 ° C
SCoT 18	ACCATGGCTACCACCGCC	50 ° C
SCoT 26	ACCATGGCTACCACCGTC	50 ° C
SCoT 28	CCATGGCTACCACCGCCA	50 ° C
SCoT 29	CCATGGCTACCACCGGCC	50 ° C
SCoT 30	CCATGGCTACCACCGGCG	50 ° C
SCoT 36	GCAACAATGGCTACCACC	50 ° C
SCoT 60*	ACAATGGCTACCACCACA	50 ° C

Note: Ta – annealing temperature

RESULTS AND DISCUSSION

Molecular markers are neutral, effective, not specified in particular tissues, highly informative and are not affected by the factors of the environment (Kumar et al., 2014; Uchoi et al., 2017).

The SCoT markers, as well as other molecular markers, have become an important technique for detection of genetic polymorphisms for the genus *Fagopyrum* as well as for many agriculturally important crops. In our study ten SCoT primers (Table 2) for analyses of 21 genotypes of *Fagopyrum esculentum* Moench and 14 genotypes of *Fagopyrum tataricum* Gaertn. (Table 1) were used. Ten SCoT markers produced 176 DNA bands with an average of 17.6 fragments per primer. Out of 176 DNA fragments 162 fragments (92.05 %) were polymorphic with an average of 16.2 fragments per primer. The number of amplified fragments ranged from 12 (SCoT 60) to 27 (SCoT 12). The percentage of polymorphism ranged from 58.33 % (SCoT 60) to 100 % (SCoT 12, SCoT 13, SCoT 29, SCoT 30). Electrophoreogram of the SCoT 29 marker is shown in the Figure 1a, and Figure 1b. The average percentage of polymorphism was 90.29 % (Table 3).

Balážová et al. (2018) analyzed 17 common buckwheat genotypes using 7 SCoT markers. Of the total number 52 fragments detected, 38 were polymorphic, with the average number of polymorphic fragments 5.43 per primer. SCoT26 and SCoT29 markers produced the highest number of polymorphic fragments. The highest number of DNA bands was also produced by SCoT 29 in our analyses (Table 3). The SCoT29 marker also achieved the highest percentage of polymorphism (87.5 %).

SCoT markers are relatively new marker technique and have become important functional markers which have been used often in genetic diversity and phylogenetic studies of several plants (Amom et al., 2020). Many authors studied different crops using SCoT markers and achieved comparable results. Khodae et al. (2021) analysed the genetic diversity of Iranian cultivars of *Aegilops triuncialis* by using SCoT technique. A total of 162 DNA fragments were amplified by using 14 SCoT primers. Most of them were polymorphic (90.74%). The average number of fragments was 10.5 per primer. Habiba et al. (2021) applied 10 SCoT primers for detection of the molecular variability of barley lines (*Hordeum vulgare* L.) The polymorphism ranged from 66.67% to 100%. Ghobadi et al. (2021) used 15 SCoT markers to analyse the molecular diversity of *Triticum aestivum* L. and the two species *Aegilops crassa* and *Aegilops cylindrica*, which are considered to be wild wheat precursors. Fifteen SCoT primers produced 262 polymorphic fragments. The number of polymorphic bands ranged from 14 to 23. Ghobadi et al. (2021) concluded that the SCoT technique is very useful for assessing genetic diversity in wild relatives of wheat species. Similarly high levels of polymorphism (over 90

%) were obtained by SCoT method in different types of unusual plants which would be marked as functional plants for example cowpea known as black-eyed pea (*Vigna unguiculata*) (Igwe et al., 2017), camellia (*Camellia oleifera*) (Xiao et al., 2020).

On the other hand, lower polymorphism was reported in the study of Lema-Rumińska et al. (2018). They used 9 SCoT markers and 9 RAPD markers in the analyses of Polish *Chenopodium quinoa* Willd lines. The highest number of fragments was analyzed for SCoT 3 (17 fragments in the Titicaca line) and SCoT 33 (12 fragments in the Faro line). The polymorphism demonstrated by the SCoT technique was 61% for the Faro group and 80 % for the Titicaca group. The study showed that the SCoT technique is more informative than the RAPD technique, as demonstrated by higher number of amplified bands (Lema-Rumińska et al., 2018). Thirty-seven SCoT markers were utilized for differentiation of 56 Tunisian castor genotypes by Vivodík et al. (2018). Altogether 230 polymorphic bands with an average of 6.22 polymorphic fragment per primer were amplified. The average percentage of polymorphic bands was 85.2 % that was comparable with our results. Genetic diversity of the genus *Fagopyrum* has been studied using different molecular markers. The ISSR and SSR techniques were used by Sabreena et al. (2021) who detected the polymorphism of buckwheat germplasm and determined the genetic diversity of 63 buckwheat genotypes using 7 ISSR markers and 7 SSR markers. Sabreena et al. (2021) using seven ISSR and seven SSR primer pairs amplified 55 and 32 polymorphic fragments, respectively. ISSR had an average of 7.85 polymorphic bands per assay unit, whereas SSR had an average of 4.57 that is much lower compared to our analysis. Sabreena et al. (2021) have shown that both marker systems are highly effective in detection of polymorphism of buckwheat germplasm. Dar et al. (2021) analysed 42 accessions of four buckwheat species using 12 ISSR markers. The amplification of primers generated 102 identifiable bands, of which 85 (83.33%) were polymorphic with an average of 7.08 polymorphic bands. The average number of polymorphic bands was much lower compared to our analysis but was similar with Sabreena et al. (2021). Gupta et al. (2012) used the AFLP fingerprinting to analyse tartary buckwheat accessions. Hou et al. (2015), Bashir et al. (2021) and Song et al. (2022) used SSR markers to analyse genetic diversity of buckwheat genotypes. Bashir et al. (2021) utilized 15 SSRs to study the polymorphism among 52 genotypes of *Fagopyrum esculentum* Moench. Out of 15 SSRs, 14 were found polymorphic in *Fagopyrum esculentum* Moench genotypes. The total number of alleles identified was 143 in which most of the alleles were polymorphic with the average number of 9 alleles per primer.

The usefulness of the molecular marker for the detection of polymorphism and the ability to distinguish between different individuals is characterized by the polymorphic information content (PIC) which takes into account also the frequency of present DNA fragments. The PIC values ranged from 0.578 (SCoT 60) to 0.932 (SCoT 36) with an average of 0.859. The PIC values were higher than 0.8 (Table 3) in 9 SCoT markers used, which indicates high polymorphism of used SCoT markers and we can consider them appropriate for molecular analyses of used common and tartary buckwheat genotypes. Less appropriate was SCoT 60 marker, whose PIC value was 0.578. Balážová et al. (2018) in analysis of 17 common buckwheat genotypes using 7 SCoT markers detected the average PIC value of 0.729. The average value of PIC (0.729) was a bit lower compared to our average value of PIC (0.859) that could be caused using of more variable plant material. Sabreena et al. (2021) using 7 ISSR markers and 7 SSR markers in study of 63 buckwheat genotypes detected lower average PIC value (0.36 for ISSR markers, and 0.43 for SSR markers).

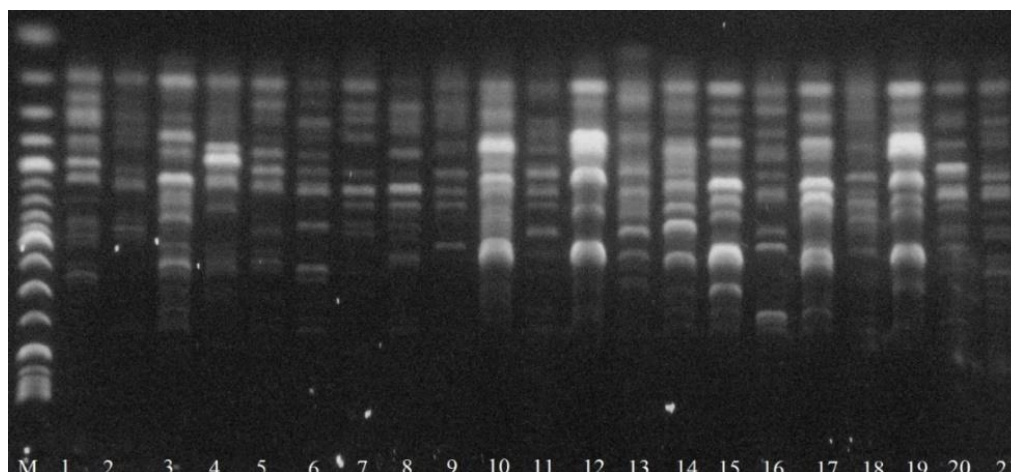


Figure 1a Electrophoreogram of the SCoT 29 marker of *Fagopyrum esculentum* Moench. Note: M is Quick-Load® Purple 2-Log DNA ladder. Lanes 1 – 21 are genotypes of *Fagopyrum esculentum* Moench (Table 1).

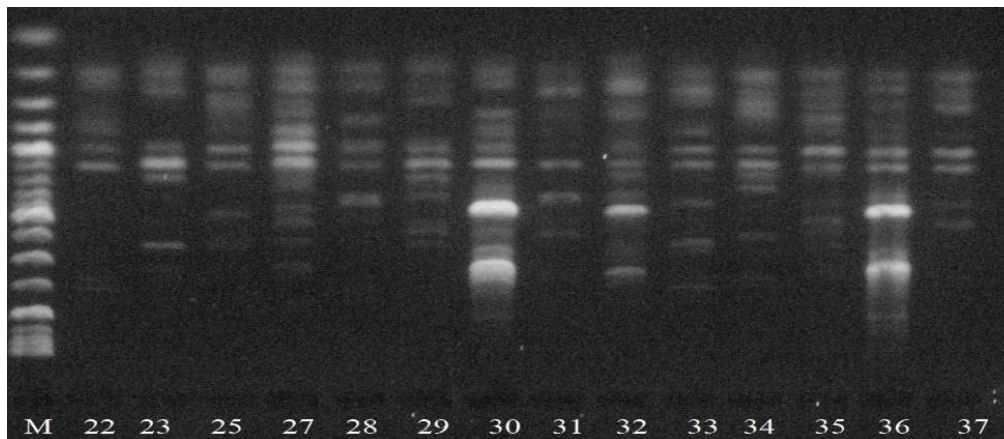


Figure 1b Electrophoreogram of the SCoT 29 marker of *Fagopyrum tataricum* Gaertn. genotypes. Note: M is Quick-Load® Purple 2-Log DNA ladder. Lanes 22 - 37 represent genotypes of *Fagopyrum tataricum* Gaertn. (Table 1). (Siva, Špačinská I, Kora, Hruzsowska, Pyra, Bamby)

Using binary matrix, the dendrogram by hierarchical cluster analysis using the UPGMA algorithm was constructed (Figure 3). The genotypes of buckwheat were divided into two main clusters (I, II) in the dendrogram. Twenty-seven genotypes were separated in the subcluster I and eight genotypes in the subcluster II. All genotypes of *Fagopyrum esculentum* Moench were grouped in subcluster Ia and IIa. Red arrow indicates all *Fagopyrum esculentum* Moench genotypes with two genotypes of *Fagopyrum tataricum* Gaertn. (Jinqiao-2, 290) included in the subcluster IIb. On the other hand, twelve (blue arrow) genotypes of *Fagopyrum tataricum* Gaertn. separated in the clusters Ib and Ic and two genotypes (Jinqiao-2, 290) of tartary buckwheat were separated in the subcluster IIb. Two genotypes Siva and Špačinská I, originated from Slovenia and the Slovak Republic, respectively, were genetically the closest and grouped in the subcluster IIa (marked with yellow in the Figure 3).

Results of SCoT markers were also used to construct PCoA plot (Figure 4) which showed 3 clusters of genotypes of the *Fagopyrum esculentum* Moench and *Fagopyrum tataricum* Gaertn. Genotypes of common buckwheat (1-21 in Table 1) were mostly grouped together (red circles), as well as the genotypes of tartary buckwheat (22-37 in Table 1, blue circle) respectively. Genotypes originated in the central Europe (Table 1) separated together, as well as genotypes of America and Asia (Table 1), respectively. The genotype of *Fagopyrum tataricum* Gaertn. JINQIAO-2 (CHN36) separated little from other tartary buckwheat genotypes. Common buckwheat genotypes could be divided into two groups located at the bottom and on the right side but separated clearly from tartary buckwheat genotypes. The genotype 290 (BTN30) from Bhutan and the genotype JINQIAO-2 (CHN36) from China were separated from the others. This is comparable with the results in the constructed dendrogram (Figure 3) where genotypes 290 and JINQIAO-2 were grouped in the subcluster IIb.

Table 3 Results of statistical characteristics of the SCoT markers used

SCoT marker	NAB	NPB	PPB (%)	PIC
SCoT 12	27	27	100.00	0.924
SCoT 13	12	12	100.00	0.815
SCoT 14	16	13	81.25	0.837
SCoT 18	15	14	93.33	0.899
SCoT 26	16	13	81.25	0.887
SCoT 28	13	12	92.31	0.890
SCoT 29	23	23	100.00	0.929
SCoT 30	20	20	100.00	0.897
SCoT 36	22	21	95.45	0.932
SCoT 60	12	7	58.33	0.578
TOTAL	176	162		
AVERAGE	17.6	16.2	90.29	0.859

Note: NBA – number of all bands, NPB – number of polymorphic bands, PPB – a percentage of polymorphic bands, PIC – polymorphic information content.

Hierarchical cluster analysis based on UPGMA algorithm in the constructed dendrogram and also PCoA plot confirmed the separation of tartary and common buckwheat genotypes from each other using SCoT analysis. **Dar et al. (2021)** obtained similar results where 42 accessions of four buckwheat species were divided into three major groups in the constructed dendrograms prepared based on UPGMA and PCoA. **Hou et al. (2015)** used SSR markers to analyze the genetic diversity of tartary buckwheat genotypes and based on the UPGMA algorithm divided the cultivars of tartary buckwheat into two groups. They concluded that the SSR analysis contributed to identifying and utilizing germplasm resources for improving tartary buckwheat breeding. **Gupta et al. (2012)** used AFLP fingerprinting of tartary buckwheat accessions to display rutin content variation. They constructed a dendrogram, where buckwheat cultivars were grouped into two subclusters according to the rutin content. They expected that the results of AFLP fingerprints associated with high rutin content accessions of *F. tataricum* Gaertn.

can be helpful for the evaluation, conservation and genetic improvement of buckwheat.

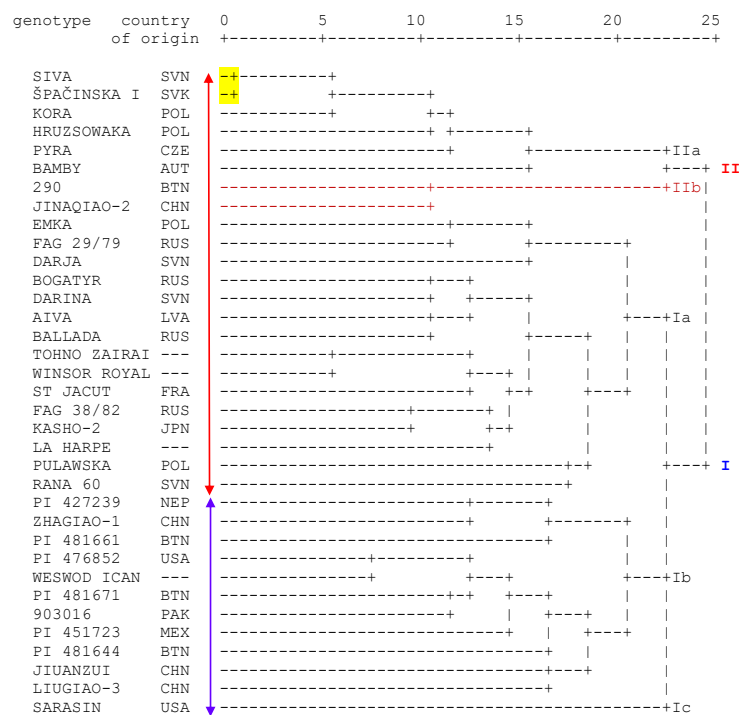


Figure 3 Dendrogram of 35 common and tartary buckwheat genotypes based on 10 SCoT markers.

Note: 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

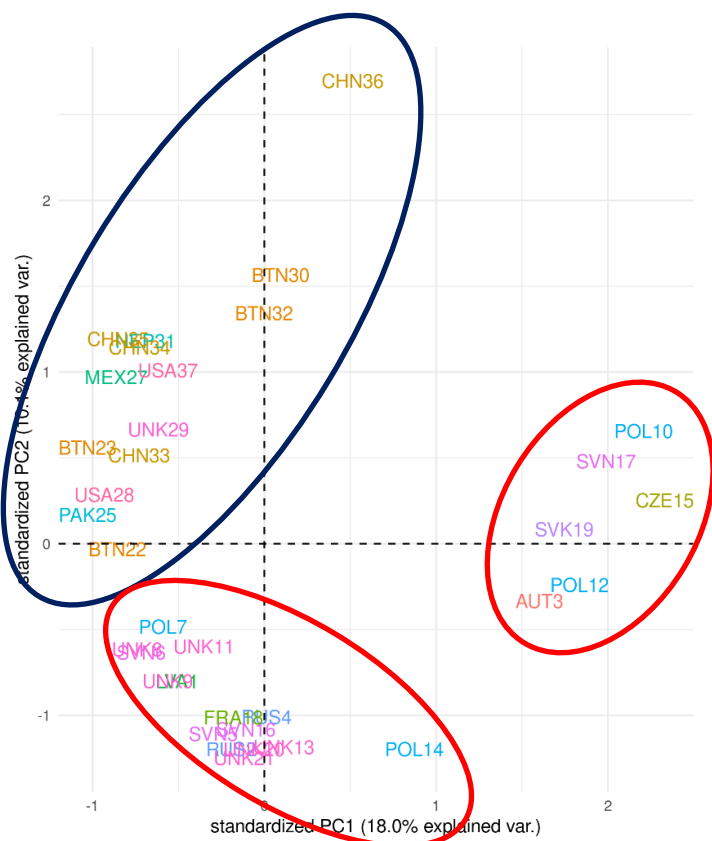


Figure 4 PCoA plot of 35 buckwheat genotypes based on 10 SCoT markers.

CONCLUSION

Based on our results we can consider the SCoT markers appropriate for the molecular analyses of common and tartary buckwheat genotypes. The average value of PIC for used SCoT markers was higher than 0.8 in 90 % of used SCoT markers that means sufficient polymorphism was detected in the chosen common and tartary buckwheat genotypes. In the UPGMA dendrogram 35 buckwheat genotypes were divided into two main clusters (I, II). It was possible to distinguish all analyzed genotypes of buckwheat in the constructed dendrogram based on 10 SCoT markers. SCoT markers are a powerful tool for assessing the genetic diversity in buckwheat cultivars. Based on the results obtained, SCoT markers showed sufficient polymorphism between the analyzed genotypes of common and tartary buckwheat genotypes, so the technique is suitable for identification and differentiation of genotypes of buckwheat. SCoT markers reveal to be suitable for application in the process of breeding and detecting new genotypes containing important genes.

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REFERENCES

Amom, T., Tikendra, L., Apana, N., Goutam, M., Sonia, P., Koijam, A. S., Potshangbam, A. M., Rahaman, H., Nongdam, P. (2020). Efficiency of RAPD, ISSR, iPBS, SCoT and phytochemical markers in the genetic relationship study of five native and economical important bamboos of North-East India. *Phytochemistry*, 174, 112330. <https://doi.org/10.1016/j.phytochem.2020.112330>

Eram Bashir, Reetika Mahajan, Rakeeb Ahmad Mir, W. A. Dar, Sajad Majeed Zargar. (2021). Unravelling the genetic variability and population structure of buckwheat (*Fagopyrum* spp.): a collection of north western Himalayas. *Nucleus*, 64, 93–101. <https://doi.org/10.1007/s13237-020-00319-y>

Balážová, Ž., Gálová, Z., Vivodík, M., Chňápek, M., & Hornýák Gregáňová, R. (2018). Molecular analysis of buckwheat using gene specific markers. *Potravinárstvo*, 12(1). <https://doi.org/10.5219/954>

Betekhtin, A., Rybicka, A., Wolna, J., & Hasterok, R. (2018). Chromosomes and chromosome studies in buckwheat. *Buckwheat Germplasm in the World*, 37–44. <https://doi.org/10.1016/b978-0-12-811006-5.00004-5>

Collard, B. C. Y., & Mackill, D. J. (2008). Start Codon Targeted (SCoT) Polymorphism: A simple, novel DNA marker technique for generating gene-

targeted markers in plants. *Plant Molecular Biology Reporter*, 27(1), 86–93. <https://doi.org/10.1007/s11105-008-0060-5>

Dar, A. F., Tahir, I., Qari, S. H., Abulfaraj, A. A., Aljabri, M., Alharby, H. F., Hakeem, K. R., Rehman, R. U. (2021). Molecular characterization and population genetic structure of *Fagopyrum* species cultivated in himalayan regions. *Sustainability*, 13, 12165. <https://doi.org/10.3390/su132112165>

Dilipan, E., Ramachandran, M., & Arulbalachandran, D. (2020). Population genetics and gene flow of the seagrass, *Syringodium isoetifolium* based on Start codon targeted (SCoT) marker from Palk Bay and Chilika Lake, India. *Meta Gene*, 26, 100774. <https://doi.org/10.1016/j.mgene.2020.100774>

Etminan, A., Pour-Aboughadareh, A., Mohammadi, R., Ahmadi-Rad, A., Noori, A., Mahdavian, Z., & Moradi, Z. (2016). Applicability of start codon targeted (SCoT) and inter-simple sequence repeat (ISSR) markers for genetic diversity analysis in durum wheat genotypes. *Biotechnology & Biotechnological Equipment*, 30(6), 1075–1081. <https://doi.org/10.1080/13102818.2016.1228478>

Ghobadi, G., Etminan, A., Mehrabi, A. M., & Shoohtari, L. (2021). Molecular diversity analysis in hexaploid wheat (*Triticum aestivum* L.) and two Aegilops species (*Aegilops crassa* and *Aegilops cylindrica*) using CBBDP and SCoT markers. *Journal of Genetic Engineering and Biotechnology*, 19(1). <https://doi.org/10.1186/s43141-021-00157-8>

Gupta, N., Sharma, S. K., Rana, J. C., & Chauhan, R. S. (2012). AFLP fingerprinting of tartary buckwheat accessions (*Fagopyrum tataricum*) displaying rutin content variation. *Fitoterapia*, 83(6), 1131–1137. <https://doi.org/10.1016/j.fitote.2012.04.015>

Habiba, R., Bashasha, J., Haffez, S. H., & Abo Leilah, A. A. A. (2021). Assessment of genetic diversity using SCoT markers and some morphological traits in ten lines of barley (*Hordeum vulgare* L.). *Assiut Journal of Agricultural Sciences*, 52(4), 53–65. <https://doi.org/10.21608/ajas.2022.111745.1077>

Hou, S., Sun, Z., Linghu, B., Xu, D., Wu, B., Zhang, B., Wang, X., Han, Y., Zhang, L., Qiao, Z., & Li, H. Y. (2015). Genetic Diversity of Buckwheat Cultivars (*Fagopyrum tataricum* Gaertn.) Assessed with SSR markers developed from genome survey sequences. *Plant Molecular Biology Reporter*, 34(1), 233–241. <https://doi.org/10.1007/s11105-015-0907-5>

Hou, Y., Zhang, Z., Wu, B., & Li, Y. (2009). Genetic diversity in tartary buckwheat revealed by AFLP analysis. *Scientia Agricultura Sinica*, 42(12), 4166–4174. <https://www.cabdirect.org/cabdirect/abstract/20103048470>

Igwe, D. O., Afiukwa, C. A., Ubi, B. E., Ogbu, K. I., Ojuederie, O. B., & Ude, G. N. (2017). Assessment of genetic diversity in *Vigna unguiculata* L. (Walp) accessions using inter-simple sequence repeat (ISSR) and start codon targeted (SCoT) polymorphic markers. *BMC Genetics*, 18(1). <https://doi.org/10.1186/s12863-017-0567-6>

Jing, R., Li, H.-Q., Hu, C.-L., Jiang, Y.-P., Qin, L.-P., & Zheng, C.-J. (2016). Phytochemical and Pharmacological Profiles of Three *Fagopyrum* Buckwheats. *International Journal of Molecular Sciences*, 17(4), 589. <https://doi.org/10.3390/ijms17040589>

Khan, N., & Dhawan, S. (2016). Role of Molecular Markers in Assessing Genetic Diversity in *Mentha*: A Review. *Scientific Journal of Genetics and Gene Therapy*, 2(1), 022–026. <https://doi.org/10.17352/sjgg.000011>

Khodaei, L., Azizinezhad, R., Etminan, A. R., & Khosroshahi, M. (2021). Assessment of genetic diversity among Iranian *Aegilops triuncialis* accessions using ISSR, SCoT, and CBBDP markers. *Journal of Genetic Engineering and Biotechnology*, 19(1). <https://doi.org/10.1186/s43141-020-00107-w>

Kim, J., & Hwang, K. T. (2020). Fagopyrins in different parts of common buckwheat (*Fagopyrum esculentum*) and tartary buckwheat (*F. tataricum*) during growth. *Journal of Food Composition and Analysis*, 86, 103354. <https://doi.org/10.1016/j.jfca.2019.103354>

Kishore, G., Pandey, A., Dobhal, R., & Gupta, S. (2013). Population Genetic Study of *Fagopyrum tataricum* from Western Himalaya Using ISSR Markers. *Biochemical Genetics*, 51(9–10), 750–765. <https://doi.org/10.1007/s10528-013-9604-y>

Kumar, A., Mishra, P., Singh, S. C., & Sundaresan, V. (2014). Efficiency of ISSR and RAPD markers in genetic divergence analysis and conservation management of *Justicia adhatoda* L., a medicinal plant. *Plant Systematics and Evolution*, 300(6), 1409–1420. <https://doi.org/10.1007/s00606-013-0970-z>

Lee, D.-G., Woo, S. H., & Choi, J.-S. (2016). Biochemical Properties of Common and Tartary Buckwheat: Centered with Buckwheat Proteomics. *Molecular Breeding and Nutritional Aspects of Buckwheat*, 239–259. <https://doi.org/10.1016/b978-0-12-803692-1.00019-5>

Lema-Rumińska, J., Miler, N., & Gęsiński, K. (2018). Identification of New Polish Lines of *Chenopodium Quinoa* (Willd.) by Spectral Analysis of Pigments and a Confirmation of Genetic Stability With SCoT and RAPD Markers. *Acta Scientiarum Polonorum Hortorum Cultus*, 17(1), 75–86. <https://doi.org/10.24326/asphc.2018.1.7>

Li, Y., Fan, X., Shi, T., Zhang, Q., Zhang, Z. (2009). SRAP Marker Reveals Genetic Diversity in Tartary Buckwheat in China. *Front. Agric. China*, 3, 383–387. <https://doi.org/10.1007/s11703-009-0071-5>

Liu Y, Fang X, Tang T, Wang Y, Wu Y, Luo J, Wu H, Wang Y, Zhang J, Ruan R, Zhou M, Zhang K, Yi Z. (2022). Inflorescence Transcriptome Sequencing and Development of New EST-SSR Markers in Common Buckwheat (*Fagopyrum esculentum*). *Plants*, 11(6):742. <https://doi.org/10.3390/plants11060742>

- Luo, C., He, X.-H., Chen, H., Ou, S.-J., & Gao, M.-P. (2010). Analysis of diversity and relationships among mango cultivars using Start Codon Targeted (SCoT) markers. *Biochemical Systematics and Ecology*, 38(6), 1176–1184. <https://doi.org/10.1016/j.bse.2010.11.004>
- Nosair, H. (2020). Genetic diversity studies on seven Egyptian wheat (*Triticum aestivum*) cultivars using SCoT and ISSR polymorphism markers. *Taeckholmia*, 0(0), 0–0. <https://doi.org/10.21608/taec.2020.39905.1025>
- Panihar, N., Vasudeva, N., Sharma, S., & Jangir, B. L. (2020). Exploring the Pharmacognostic Features, Anti-oxidant and Lipid Lowering Potential of *Fagopyrum esculentum* Moench. Seed. *Current Traditional Medicine*, 6(2), 155–169. <https://doi.org/10.2174/2215083805666190807105935>
- Park, B. J., Lee, J., Shin, J. H., Soo, K. N., Ho, P. Ch. (2006). Genetic Diversity of Cultivated and Wild Tartary Buckwheat (*Fagopyrum tataricum* Gaert.) Accessions by AFLP Analyses. *Genes Genomics*, 28(2), 91–98. <https://www.kci.go.kr/kciportal/ci/sereArticleSearch/ciSereArtiView.kci?sereArticleSearchBean.artid=ART001112462>
- Petrovičová, L., Balážová, Ž., Vivodík, M., & Gálová, Z. (2017). Detection genetic variability of *Secale cereale* L. by scot markers. *Potravinárstvo*, 11(1), 197–202. <https://doi.org/10.5219/726>
- Rayan, W. A., & Osman, S. A. (2019). Phylogenetic relationships of some Egyptian soybean cultivars (*Glycine max* L.) using SCoT marker and protein pattern. *Bulletin of the National Research Centre*, 43(1). <https://doi.org/10.1186/s42269-019-0197-4>
- Rysová, J. (2018). Nutriční hodnota a využití pohanky. *Výživa a potravin*, 4, 86–89. https://www.vupp.cz/wpcontent/uploads/2018/09/Nutriční_hodnota_a_vyuziti_pohanky_Rysova.J..pdf
- Sabreena, Nazir, M., Mahajan, R., Hashim, M. J., Iqbal, J., Alyemeni, M. N., Ganai, B. A., Zargar, S. M. (2021). Deciphering allelic variability and population structure in buckwheat: An analogy between the efficiency of ISSR and SSR markers. *Saudi Journal of Biological Sciences*, 28(11), 6050–6056. <https://doi.org/10.1016/j.sjbs.2021.07.061>
- Sadek, M. S. E., & Ibrahim, S. D. (2018). Genetic relationships among maize inbred lines as revealed by start codon targeted (SCoT) analysis. *Journal of Innovations in Pharmaceutical and Biological Sciences*, 5(1), 103–107. <https://jipbs.com/index.php/journal/article/view/297>
- Saunders Bulan, M., Wu, J.C., Emshwiller, E., Berres, M.E., Posner, J.L., Peng, D.Y., Wang, X.H., Li, J.F., Stoltenberg, D.E., Zhang, Y.P. (2017). Social and environmental influences on tartary buckwheat (*Fagopyrum tataricum* Gaertn.) varietal diversity in Yunnan, China. *Genet. Resour. Crop Evol.* 64, 113–125. <https://doi.org/10.1007/s10722-015-0337-0>
- Singh, M., Malhotra, N., & Sharma, K. (2020). Buckwheat (*Fagopyrum* sp.) genetic resources: What can they contribute towards nutritional security of changing world? *Genetic Resources and Crop Evolution*, 67(7), 1639–1658. <https://doi.org/10.1007/s10722-020-00961-0>
- Sharma, R., Jana, S. 2002. Species relationships in *Fagopyrum* revealed by PCR based DNA fingerprinting. *Theor Appl Genet.* vol. 105, p. 306–312. <https://doi.org/10.1007/s00122-002-0938-9>
- Song, Y. Cheng, Z. Dong, Y., Liu, D., Bai, K., Jarvis, D., Feng, J., Long, C. (2022). Diversity of Tartary Buckwheat (*Fagopyrum tataricum*) Landraces from Liangshan, Southwest China: Evidence from Morphology and SSR Markers. *Agronomy*, 12, 1022. <https://doi.org/10.3390/agronomy12051022>
- Tang, Y., Ding, M.-Q., Tang, Y.-X., Wu, Y.-M., Shao, J.-R., & Zhou, M.-L. (2016). Germplasm Resources of Buckwheat in China. *Molecular Breeding and Nutritional Aspects of Buckwheat*, 13–20. <https://doi.org/10.1016/b978-0-12-803692-1.00002-x>
- Uchoi, A. , Malik, S. K. , Choudhary, R. , Kumar, S. , Pal, D. , Rohini, M. R. , Chaudhury, R. (2017). Molecular markers in assessing genetic variation of Indian citron (*Citrus medica* L.) cultivars collected from different parts of India. *Indian Journal of Biotechnology*, 16 (3), 346–356. <http://nopr.niscair.res.in/handle/123456789/43336>
- Vivodík, M., Balážová, Ž., Gálová, Z., & Petrovičová, L. (2017). Genetic Diversity Analysis of Maize (*Zea mays* L.) Using SCoT Markers. *Journal of Microbiology, Biotechnology and Food Sciences*, 6(5), 1170–1173. <https://doi.org/10.15414/jmbfs.2017.6.5.1170-1173>
- Vivodík, M., Saadaoui, E., Balážová, Ž., Gálová, Z., & Petrovičová, L. (2018). Genetic diversity and population structure in tunisian castor genotypes (*Ricinus communis* L.) detected using scot markers. *Potravinárstvo*, 12 (1). <https://doi.org/10.5219/873>
- Weber, J. L. (1990). Informativeness of human (dC-dA)_n · (dG-dT)_n polymorphisms. *Genomics*, 7(4), 524–530. [https://doi.org/10.1016/0888-7543\(90\)90195-z](https://doi.org/10.1016/0888-7543(90)90195-z)
- Xiao, P., Liu, H., Wang, D., Tang, W., Yang, H., Wang, C., ... Tian, Y. (2020). Assessment of genetic diversity in *Camellia oleifera* Abel. accessions using inter-simple sequence repeat (ISSR) and start codon targeted (SCoT) polymorphic markers. *Genetic Resources and Crop Evolution*, 67(5), 1115–1124. <https://doi.org/10.1007/s10722-020-00924-5>
- Zhang, L., Li, X., Ma, B., Gao, Q., Du, H., Han, Y., Li, Y., Cao, Y., Qi, M., Zhu, Y., Lu, H., Ma, M., Liu, L., Zhou, J., Nan, Ch., Qin, Y., Wang, J., Cui, L., Liu, H., Liang, Ch., Qiao, Z. (2017). The Tartary Buckwheat Genome Provides Insights into Rutin Biosynthesis and Abiotic Stress Tolerance. *Molecular Plant*, 10(9), 1224–1237. <https://doi.org/10.1016/j.molp.2017.08.013>
- Zhou, M., Woo, S.H., Wieslander, G., Kreft, I., Chrungoo, N. (2016). *Molecular Breeding and Nutritional Aspects of Buckwheat*. Academic press: Cambridge. <https://doi.org/10.1016/c2015-0-00352-5>
- Zhu, F. (2016). Chemical composition and health effects of Tartary buckwheat. *Food Chemistry*, 203, 231–245. <https://doi.org/10.1016/j.foodchem.2016.02.050>