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RESEARCH ARTICLE

Genetic diversity of maize resources revealed by different molecular markers

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Abstract Maize (*Zea mays* L.) is the third most important cereal crop in the world because of its nutritional value and industrial benefits. Molecular markers are used mainly by the breeders to study the genetic variability of genotypes and its application in the breeding process. Two types of molecular markers, 10 random amplified polymorphic DNA (RAPD) primers and 10 start codon target (SCoT) primers, were assayed to determine the genetic diversity of 25 Slovak maize lines and 25 maize cultivars. A high level of polymorphism was found with both RAPD and SCoT markers, which was confirmed by high average polymorphism information content (PIC) values using both techniques. The efficiency of individual marker techniques in the detection of genotype diversity can be compared by calculating the marker index (MI), detecting diversity index (DDI), discriminating power, resolving power (RP) and other indices. A higher MI (11.788), DDI (2.358) and RP (53.08) value was achieved by the SCoT technique compared to the RAPD method. Three joint dendrograms and PCoA plots constructed based on RAPD, SCoT and both methods combined confirmed the unambiguous separation of maize lines and cultivars from each other. The results obtained from the RAPD and SCoT analysis can be used for the selection of potentially suitable biological sources for further marker-assisted breeding.

Keywords *Zea mays* L. · Polymorphism · SCoT · RAPD · Genetic variability · Dendrogram

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Introduction

Maize, wheat, rice, barley, and sorghum were the five most produced cereal species in 2022 (FAO 2023). Maize (*Zea mays* L.) is the third most cultivated cereal crop in the world and is believed to have spread from Mexico. Its domestication has not yet been fully clarified from a genetic point of view. Maize had the highest production (almost 1.2 billion tonnes) in 2022 and the fastest growth since 2000 (+97 percent) compared to the other top cereals, as it is widely used in sectors other than food, ranging from biofuels to animal feed (FAO 2023).

Most of the world's maize production (70%) is used as livestock feed, about 20% is processed in the food industry, and about 5% is used industrially (Orhun 2013).

In the Slovak Republic, the area sown with corn in 2023 for grain was 141,000 ha, the harvest was with a production of 1,067,000 tons. Corn was grown on 15.38% of arable land of the Slovak Republic. Maize is used mostly for ethanol production (37.2%), as fodder (35.1%) and for food purposes (26.6%). The

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U.S.A. grows corn on 85% of the grain, Brazil on 79% of the grain, and China on 43% of the grain. So, in these countries, corn is in the first place among the cultivated cereals (Moravčík 2023).

For corn growers in 2024 in the Slovak Republic was developed the breeding program by Syngenta company which using molecular genetic methods where they are looking for genes that have proven to be associated with higher drought tolerance, they have developed hybrids with high yield, excellent fiber, high starch content in the silage and resistance to cold (Syngenta 2024).

In certain parts of the world, maize is the most important cereal crop as well as a source of nutrients, so research into its genetic diversity and proposals to improve its production and quality are important (Orhun 2013).

A maize grain contains 60–80% starch, 6–22% protein, 1.5–15% fat, 2–11% cellulose, and 1.5–4% ash. The maize storage proteins can also serve as genetic markers for the identification of genotypes and determination of their homogeneity since they are genetically determined by certain loci and are polymorphic (Gregová and Hauptvogel 2015).

With its size of 2.5 billion nucleotides, the maize genome is average-sized, and is comparable to the human genome. Maize has primarily 10 pairs of chromosomes ($2n = 20$) of variable length and composition. The maize genome research has revealed a considerable genetic diversity characterized by a large number of polymorphic sites in the DNA. Great variation in the maize genome size even within the same species has been observed (Huang et al. 2016).

The genetic polymorphism of maize is of great importance from the point of view of genome mapping, as it is possible to distinguish individual cultivars and optimally direct their use. Several DNA markers based on the polymerase chain reaction (PCR), such as random amplified polymorphic DNA (RAPD) (Berhitu et al. 2019; Al-Obaidi et al. 2018; Vargas et al. 2018; Vivodík et al. 2017b; Balážová et al. 2016; Mukharib et al. 2010; Sun et al. 2001); amplified fragment length polymorphism (AFLP) (Roy and Kim 2016; Losa et al 2011); inter simple sequence repeat (ISSR) (Soliman et al. 2021; Muhammad et al. 2017; Carvalho et al. 2002); simple sequence repeat (SSR) (Belalia et al. 2019; Vivodík et al. 2017a; Salami et al. 2016; Kumar et al. 2012; Sharma et al. 2010; Kostova et al. 2006); start codon target (SCoT) polymorphism (Al-Tamimi 2020; Sadek and Ibrahim 2018; Vivodík et al. 2016) and many other methods, were used in the research of genetic diversity of maize. The PCR-based markers show higher polymorphism and require a lower DNA concentration compared to the hybridization-based markers. Other advantages of the DNA markers include the distribution of polymorphic markers throughout the genome, high sensitivity of the PCR reaction (automation, shorter analysis time, unambiguousness and accuracy of the method) and lower financial costs (Adhikari et al. 2017; Kumar et al. 2009).

The genetic diversity of maize was also detected by RAPD markers. This technique was described in 1990 and was considered one of the first techniques for analyzing the genome of plant species (Sarwat et al. 2012). The RAPD markers have their advantages and disadvantages but have been used extensively to study the maize genome. They are not locus-specific, they bind to the complementary sites randomly throughout the genome, and they are dominant and serve for genome mapping. A single RAPD primer allows the amplification of a high number of fragments. The main advantages of RAPD marker are the possibility to detect polymorphism of DNA bands in large quantities, there are consistent and not influenced by the environment (Lubis and Butarbutar 2022; Kumar et al. 2009). With its help, it is possible to map the monogenic and polygenic traits in a small amount of DNA (Al-Badeiry et al. 2013). Sun et al. (2001) studied the genetic relationships among 37 maize hybrids using the RAPD technique and microsatellites. Mukharib et al. (2010) used the RAPD technique to assess the genetic diversity in a selected group of inbred maize lines. Abdellatif and Khidr (2010) analyzed the genetic diversity of 4 new crosses and 5 new inbred maize lines using the RAPD molecular markers, the ISSR technique and biochemical markers. Al-Badeiry et al. (2013) studied the genetic diversity and relationships among the maize cultivars and recommended the RAPD markers for mapping the monogenic and polygenic traits of maize while working with small amounts of DNA. Mrutu (2015) used the RAPD markers to assess the genetic purity of the UH6303 maize hybrid originating from Tanzania. The parental lines of 47 maize genotypes were used for the analysis. The RAPD markers were effective in identifying the hybrids due to the high degree

of polymorphism (100%). Balážová et al. (2016) used 13 RAPD markers as a marker system to assess the genetic diversity in a set of 40 maize genotypes originated from Central European countries and Russia and to determine the polymorphism. The Start codon targeted (SCoT) markers, which advantages are the simplicity, detection of high polymorphism and well reproducibility, were used by Sadek and Ibrahim (2018) and Vivodík et al. (2016). Vivodík et al. (2016) detected the genetic diversity of 40 old maize from Eastern European countries and Russia using 20 SCoT markers. They proved the effectiveness of employing SCoT markers in analysis of maize. Sadek and Ibrahim (2018) used 10 SCoT markers to analyze 8 yellow inbred maize lines. All markers showed a high degree of polymorphism except the SCoT9 marker. Research indicates that the analysis of SCoT markers is an effective method in the evaluation of genetic relationships between the maize genotypes.

The detection of genetic diversity with the help of different types of DNA markers is used in the breeding of new cultivars of agricultural and food-production plants. Marker-assisted breeding (MAS) significantly shortens the breeding time and enables the selection of suitable parent cultivars for breeding in terms of their subsequent processing direction (Baran et al. 2023; Luqman et al. 2023).

The objective of this study is to (1) reveal the RAPD-based polymorphism in the cultivars and lines of maize germplasm, (2) reveal the SCoT-based genetic variability in the germplasm of the cultivars and lines of maize, and (3) compare the effectiveness of random RAPD markers and gene-specific SCoT markers, which can be used to assess the genetic diversity of cultivars and lines of maize (*Zea mays* L.) and at the same moment show the applicability of both DNA methods which are very time and finance consuming.

Materials and methods

Plant material

The seeds of 25 cultivars of maize (*Zea mays* L.) obtained from the Gene Bank at the Research Institute of Plant Production (RIPP) Prague—Ruzyně in the Czech Republic, and the seeds of 25 lines of maize from the breeding company Zeainvent Trnava s.r.o. in the Slovak Republic, were analyzed (Tables 1 and 2).

Table 1 List of maize cultivars (Gene Bank RIPP Praha—Ruzyně, Czech Republic)

Cultivar number	Cultivar name	Country of origin
1	Black Mexican	USA
2	Black Sugar	USA
3	Howling Mob	USA
4	Rostrata	USA
5	Whiple´S Early White	USA
6	Early King	USA
7	Miniature	USA
8	Fore Most Coss F1	USA
9	Trucker´S Favourite White	USA
10	Ioana	USA
11	Stowell´S F1	USA
12	Illinois Hulless	USA
13	The Burpee	USA
14	Golden Cross Bantam (Early)	USA
15	Early Evergreen	USA
16	Carmel Cross	USA
17	Golden Harvest	USA
18	Spring Gold	USA
19	Fore Most Extra Early (Ee1) F1	USA
20	Golden Beauty F1	USA
21	Extra Early Golden Bantam	USA
22	Barbecue	USA
23	North Star	USA
24	Queen Anna	USA
25	Wonderful	USA

DNA extraction

The total genomic DNA was isolated from the 14-days-old seedlings using the GeneJET Plant Genomic DNA Purification Mini Kit (Thermo Scientific, USA) according to the manufacturer's instructions (GeneJET Plant Genomic DNA Purification Mini Kit. User guide). The maize genotypes were grown in a growth chamber on humus soil. The concentrations of isolated DNA were estimated by Biodrop (Biochrom, Ltd, Cambridge, United Kingdom) and the final concentration of DNA was adjusted to 50 ng/µl. All DNA samples were stored at − 20 °C.

Table 2 List of maize lines (Zeainvent Trnava s.r.o., Slovak Republic)

Line number	Sample designation	Background
1	1	Iodent
2	2	Lancaster
3	12	Lancaster, Flint
4	13	Lancaster, CM7
5	14	Iodent, Lancaster. SSS, DE811
6	15	Iodent, Lancaster
7	16	Iodent, Unknown, DE811
8	17	SSS
9	18	SSS, Iodent
10	19	Iodent, F2
11	20	Iodent, F2
12	21	Iodent, SSS, Lancaster
13	22	Iodent, SSS, Lancaster
14	23	SSS, Iodent
15	24	SSS, Iodent
16	25	Flint, F2, Co255.Unknown
17	26	Lancaster, Iodent
18	27	Unknown Flint
19	28	Unknown Flint
20	29	SSS, Lancaster, Reid, Unknown
21	30	SSS, Flint
22	31	Flint
23	32	Iodent, F2
24	33	Unknown Flint
25	34	Unknown Flint

RAPD markers

The amplification of RAPD fragments was performed according to Gajera et al. (2010) using decamer arbitrary primers (Tables 3 and 4). 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. DNA amplification was performed in a thermocycler (Biometra, Göttingen, Germany) programmed as follows: initial DNA denaturation at 94 °C for 5 min, followed by 42 cycles of denaturation at 94 °C for 1 min, primer annealing at 38 °C for 1 min, synthesis of the new DNA strand at 72 °C for 1 min and the final step at 72 °C for 5 min. The amplified DNA products were separated by horizontal gel electrophoresis in 1.5% agarose gels in 1×TBE buffer containing 0, 5 µg/ml ethidium bromide at a constant voltage of 100 V for approximately 1 h. The evaluation of gels stained with ethidium bromide was performed under a UV lamp using the UVP PhotoDoc-It® system. 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).

SCoT markers

A total of 10 SCoT primers developed by Collard and Mackill (2009) were selected for the present study (Tables 5 and 6). Each 15-µl amplification reaction consisted of 1.5 µl (100 ng) of template DNA, 7.5 µl of Master Mix (Promega, Madison, WI, USA), 1.5 µl of 10 pmol primer, and 4.5 µl of distilled water. Amplification was performed in a programmable thermocycler (Biometra, Göttingen, Germany) using the following program: 94 °C for 3 min; 35 cycles of 94 °C for 1 min, 50 °C for 1 min, and 72 °C for 2 min; final extension at 72 °C for 5 min. The amplified products were separated in 1.5% agarose gels in 1×TBE buffer. The gels were stained with ethidium bromide and documented using the UVP PhotoDoc-t® camera system for gel documentation. 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).

Data analysis

Amplified fragments of the RAPD and SCoT markers were scored as present (1) or absent (0) in the agarose gels. The binary matrices were used to compute pairwise similarity based on the Jaccard coefficient for the preparation of the similarity matrices.The dendrograms were constructed based on hierarchical cluster analysis using the Unweighted Pair Group Method implementing arithmetic averages (UPGMA) in the iTOL program, which is available online (Letunic and Bork 2019; https://itol.embl.de/upload.cgi). The joint dendrogram of maize cultivars and lines was constructed based on joint RAPD and SCoT matrices. The polymorphic information content (PIC) was used for the assessment of polymorphism between the maize genotypes and usability of the RAPD and SCoT markers. The PIC values were calculated for

Table 3 Statistical characteristics of the random amplified polymorphic DNA (RAPD) markers used in the maize lines

Primer	Primer sequence (5′-3′)	Number of fragments	Number of polymorphic fragments	Percentage of polymorphism (%)	Fragment size (bp)	PIC
OPA02	TGCCGAGCTG	9	5	55.56	400–2000	0.870
OPA03	AGTCAGCCAC	10	8	80	500–2000	0.884
OPA13	CAGCACCCAC	5	3	60	500–1200	0.781
OPB08	GTCCACACGG	7	7	100	800–2000	0.795
OPD02	GGACCCAACC	10	8	80	500–1500	0.827
OPD08	GTGTGCCCCA	5	4	80	500–2000	0.772
OPD13	GGGGTGACGA	9	9	100	300–1500	0.866
OPE07	AGATGCAGCC	8	2	25	300–1500	0.871
OPF14	TGCTGCAGGT	8	7	87.5	400–2000	0.822
SIGMA-D-01	AAACGCCGCC	8	8	100	500–2000	0.799
Total		79	61	–	–	–
Average		7.9	6.1	76.81	–	0.829
Total bp range		–	–	–	300–2000	–

bp number of base pairs, *PIC* polymorphic information content

Table 4 Statistical characteristics of the random amplified polymorphic DNA (RAPD) markers used in the maize cultivars

Primer	Primer sequence (5′-3′)	Number of fragments		Percentage of polymorphism (%)	Fragment size (bp)	PIC
		Total	Polymorphic			
OPA02	TGCCGAGCTG	5	3	60	400–2000	0.660
OPA03	AGTCAGCCAC	7	5	71.43	500–1500	0.825
OPA13	CAGCACCCAC	4	2	50	500–1200	0.641
OPB08	GTCCACACGG	5	5	100	1000–2000	0.724
OPD02	GGACCCAACC	7	6	85.71	500–1500	0.778
OPD08	GTGTGCCCCA	3	2	66.67	500–2000	0.525
OPD13	GGGGTGACGA	5	5	100	400–1500	0.784
OPE07	AGATGCAGCC	7	5	71.43	400–1500	0.849
OPF14	TGCTGCAGGT	12	11	91.67	300–2000	0.882
SIGMA-D-01	AAACGCCGCC	6	5	83.33	500–1500	0.784
Total		61	49	–	–	–
Average		6.1	4.9	78.02	–	0.745
Total bp range		–	–	–	300–2000	–

bp Number of base pairs, *PIC* Polymorphic information content

each RAPD and SCoT primer according to the formula (Weber 1990):

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

Pij is the frequency of the ith pattern revealed by the jth primer summed across all patterns revealed by the primers:

The PCoA (Principal Coordinate Analysis) plots were constructed using the free statistical software R Project, version 4.0.5. to visualize the pattern of genetic dissimilarities within and between sub-groups.

The efficiency of individual marker techniques (RAPD and SCoT) can be compared by calculating the marker index (MI) and detecting diversity index (DDI). The marker index (MI) is used to describe the overall

Table 5 Statistical characteristics of the start codon target (SCoT) markers used in the maize lines

Primer	Primer sequence (5′-3′)	Number of fragments	Number of polymorphic fragments	Percentage of polymorphism (%)	Fragment size (bp)	PIC
SCoT6	CAACAATGGCTACCACGC	12	10	83.33	400–3000	0.893
SCoT8	CAACAATGGCTACCACGT	9	8	88.89	300–2000	0.861
SCoT12	ACGACATGGCGACCAACG	9	9	100	200–1200	0.831
SCoT23	CACCATGGCTACCACCAG	10	8	80	400–1500	0.888
SCoT28	CCATGGCTACCACCGCCA	10	6	60	300–2000	0.860
SCoT29	CCATGGCTACCACCGGCC	9	6	66.67	200–2000	0.862
SCoT30	CCATGGCTACCACCGGCG	10	6	60	150–2000	0.869
SCoT54	ACAATGGCTACCACCAGC	6	5	83.33	400–2000	0.766
SCoT62	ACCATGGCTACCACGGAG	7	7	100	400–2000	0.798
SCoT63	ACCATGGCTACCACGGGC	8	6	75	600–2000	0.829
Total		90	71	–	–	
Average		9	7.1	79.72	–	0.846
Total bp range		–	–	–	150–3000	

bp Number of base pairs, *PIC* Polymorphic information content

Table 6 Statistical characteristics of the start codon target (SCoT) markers used in the maize cultivars

Primer	Primer sequence (5´-3´)	Number of fragments	Number of polymorphic fragments	Percentage of polymorphism (%)	Fragment size (bp)	PIC
SCoT6	CAACAATGGCTACCACGC	9	8	88.89	600–5000	0.870
SCoT8	CAACAATGGCTACCACGT	10	9	90	300–2000	0.888
SCoT12	ACGACATGGCGACCAACG	7	5	71.43	200–1000	0.813
SCoT23	CACCATGGCTACCACCAG	8	6	75	400–1500	0.840
SCoT28	CCATGGCTACCACCGCCA	12	8	66.67	250–1500	0.892
SCoT29	CCATGGCTACCACCGGCC	9	7	77.78	200–2000	0.850
SCoT30	CCATGGCTACCACCGGCG	7	6	85.71	150–2000	0.835
SCoT54	ACAATGGCTACCACCAGC	6	4	66.67	400–2000	0.715
SCoT62	ACCATGGCTACCACGGAG	9	8	88.89	600–2000	0.812
SCoT63	ACCATGGCTACCACGGGC	10	8	80	600–2000	0.867
Total		87	69	–	–	–
Average		8.7	6.9	79.10	–	0.838
Total bp range		–	–	–	150–5000	–

bp Number of base pairs, *PIC* Polymorphic information content

ability of a marker system to detect polymorphism. The diversity detecting index (DDI) is used to estimate the required number of marker loci (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.

Evaluation of the RAPD and SCoT markers potential to estimate genetic variability was performed using the heterozygosity index (H), efective multiplex ratio (E), discriminating power (D),

arithmetic mean of H (H.av), and resolving power (RP) (Amiryousef et al. 2018).

Results

Genetic variability based on RAPD markers

The RAPD method is based on the amplification of the non-coding DNA sequence by annealing a random primer. Ten decameric primers (Table 2) designed by Gajera et al. (2010) were used in the PCR, and the PCR products were visualized on 1, 5% agarose gels (Figs. 1 and 2).

The primers in a set of 25 Slovak lines from Zeainvent Trnava s.r.o. (Table 2) amplified a total of 79 DNA bands (Table 3), of which 61 (77%) were polymorphic and 18 (23%) were monomorphic (Fig. 1). The number of fragments produced by one primer ranged from 5 (OPA13) to 10 (OPA03 and OPD02), with an average of 7.9 fragments per primer. The percentage of polymorphism ranged from 25% (OPE07) to 100% (OPB08, OPD13, SIGMA-D-01). An average number of polymorphic fragments per primer was 6.1. The size of amplified fragments varied from 300 to 2000 bp. To evaluate the maize polymorphism in a set of maize lines, PIC values were calculated, which provide an estimate of the RAPD primer's differential power. The PIC values ranged from 0.772 (OPD08) to 0.884 (OPA03), with an average value of 0.829. It can be concluded that all the primers used were sufficiently polymorphic because all PIC values were higher than 0.7 and can be recommended for the detection of genetic diversity maize lines.

Within the collection of 25 cultivars from the Gene Bank in Prague (Table 1) originating from the USA, ten RAPD primers amplified a total of 61 different DNA fragments (Table 4), of which 49 (77%) were polymorphic and 12 (23%) monomorphic (Fig. 2). The number of fragments produced by one primer varied from 3 (OPD08) to 12 (OPF14), with an average of 6.1 fragments per primer. An average number of polymorphic fragments per primer was 4.9. The percentage of polymorphism ranged from 50% (OPA13) to 100% (OPB08, OPD13). The size range of the PCR products varied from 300 to 2000 bp. The PIC values ranged from 0.525 (OPD08) to 0.882 (OPF14), with an average value of 0.745. Nine out of ten RAPD primers used had the PIC value higher than 0.6, therefore 90% of the primers used were sufficiently polymorphic and are suitable for studying the genetic diversity of maize cultivars. The OPD08

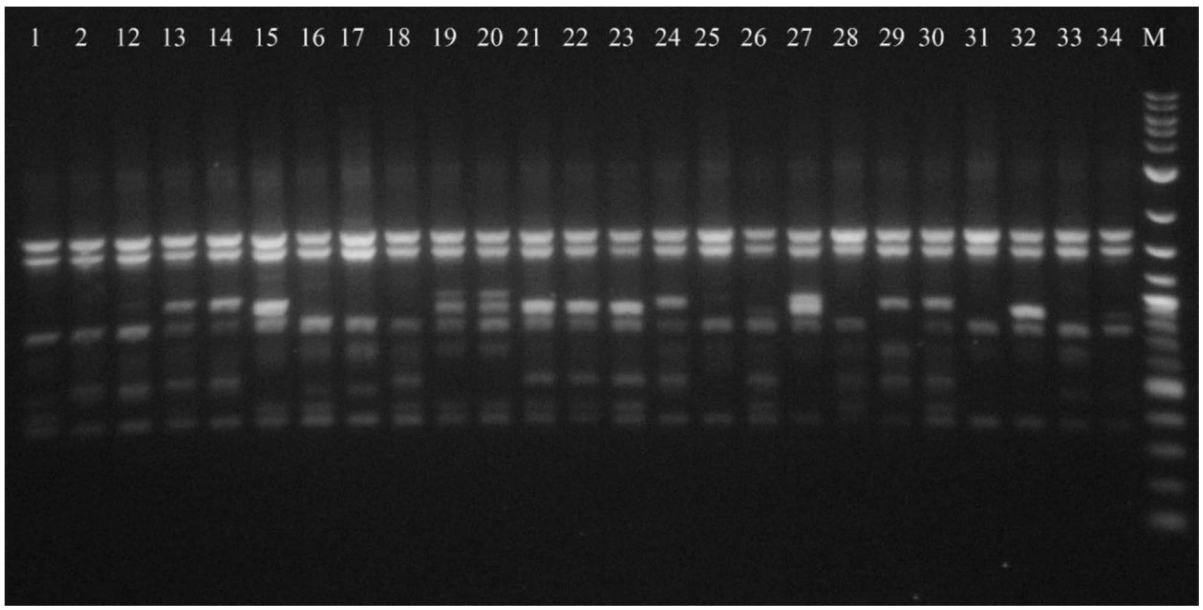

Fig. 1 Electrophoretic profiles of 25 maize lines obtained using the OPA02 primer

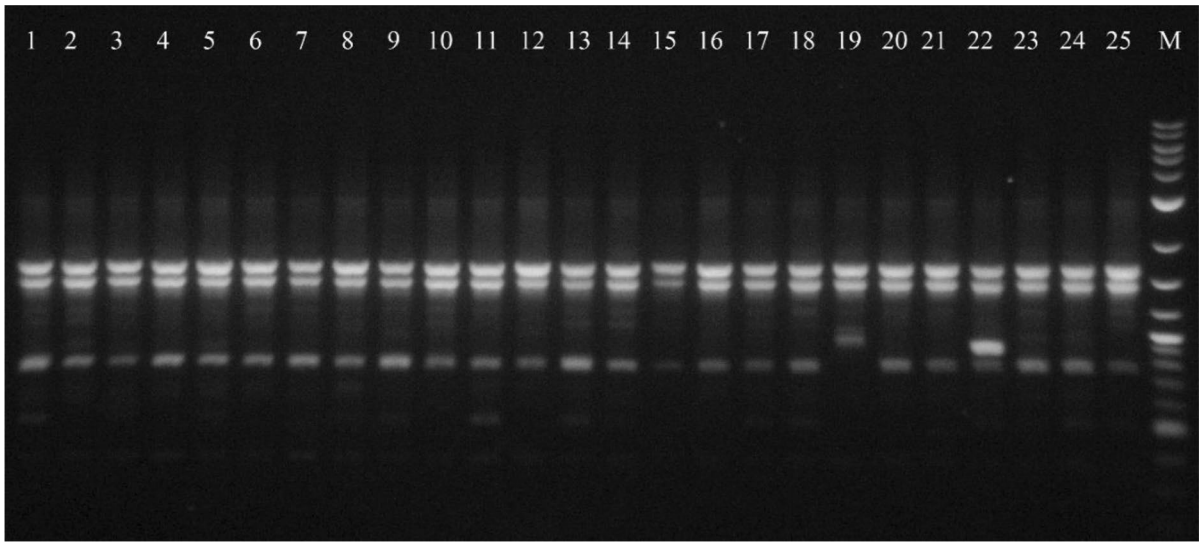

Fig. 2 Electrophoretic profiles of the maize cultivars obtained using the OPA02 primer

primer had the lowest PIC value (0.525) and is moderately polymorphic.

The UPGMA dendrogram based on the similarity matrix obtained by the RAPD method was constructed. The analyzed lines and cultivars (Fig. 3) divided in two main clusters (I and II). Cluster I contained all the maize lines (1 to 34) and cluster II included all the maize cultivars (1a to 25a), which confirms the effectiveness of the RAPD method.

Cluster I was further subdivided into two subclusters, Ia containing two maize lines (19, 20), and Ib including 23 maize lines. Maize lines 19 and 20 grouped in cluster Ia were genetically the closest lines in terms of polymorphism using the RAPD markers Their genetic distance reached the highest value (0.978) and they contain the same genetic background (Iodent, F2). Lines 22 and 21 with the same genetic background (Iodent, SSS and Lancaster) were genetically less similar (0.875). The genetic background of Lines 23 and 21 (0.868) consists of two identical parents: SSS and Iodent. Line 21 also contains Lancaster in the pedigree. Lines 25 (Flint, F2 and Co255. unknown genetic background) and 23 (SSS and Iodent genetic background) were found to be genetically most distant, therefore these new breeding lines turn out to be a potentially suitable biological source for further breeding, as there is a possibility to obtain new cultivars with improved properties.

In subcluster II, the cultivars were divided into two subclusters (IIa, IIb) in the dendrogram (Fig. 3). The Extra Early Golden Bantam (21a) cultivar included in the subcluster IIa separated from 24 other maize cultivars grouped in IIb. With a genetic distance of 0.878, Black Mexican (1a) and Black Sugar (2a) were genetically most similar in terms of the RAPD polymorphism. Based on the obtained results, the Fore Most Extra Early (ee1) F1 (19a) and Rostrata (4a) cultivars were genetically most distant, therefore these cultivars are the most suitable samples for marker assisted breeding.

Genetic variability based on SCoT markers

The SCoT technique is based on the short conserved region flanking the ATG start codon in the plant genes (Gajera et al. 2010). Ten primers designed by Collard and Mackill (2009) were used in the PCR. The amplified DNA fragments were visualized on 1,5% agarose gels (Figs. 4 and 5).

The primers in a set of 25 Slovak lines from Zeainvent Trnava s.r.o. (Table 2) amplified a total of 90 DNA bands (Table 5), of which 71 (79%) were polymorphic. The number of fragments produced by one primer ranged from 6 (SCoT54) to 12 (SCoT6), with an average of 9 fragments per primer (Fig. 4). An average number of polymorphic fragments per primer was 7.1. The percentage of polymorphism varied from

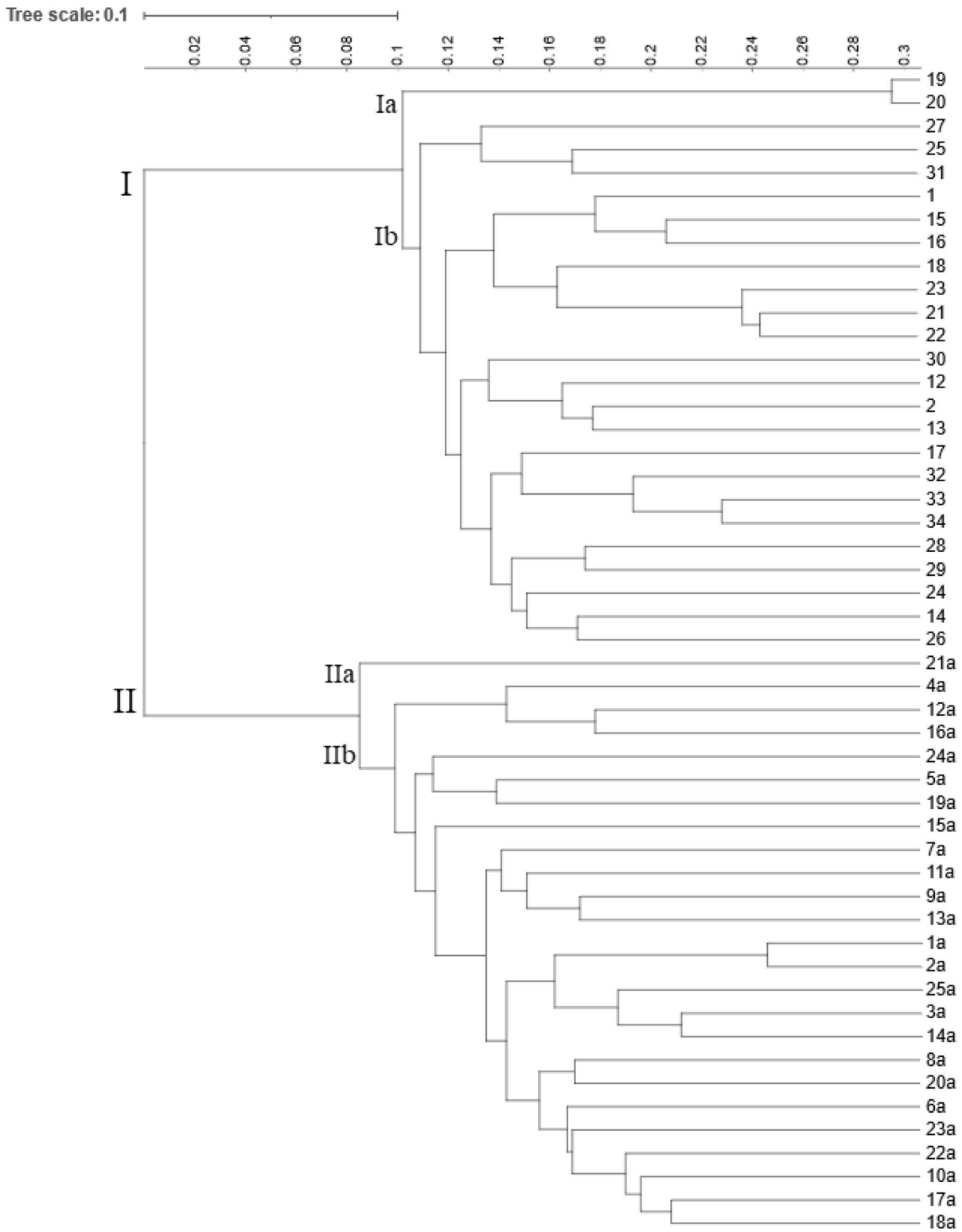

Fig. 3 Dendrogram of 25 maize lines (cluster I) and 25 cultivars (cluster II) constructed using 10 random amplified polymorphic DNA (RAPD) markers

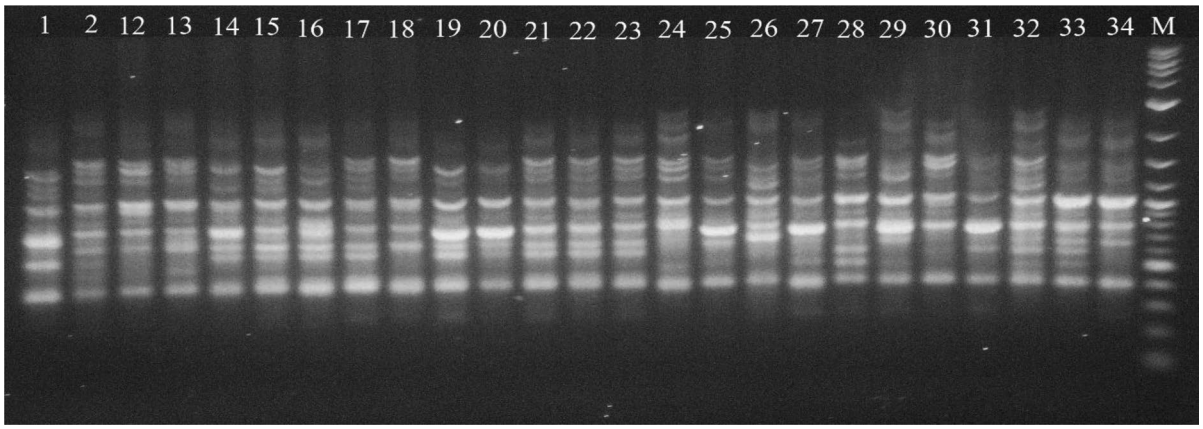

Fig. 4 Electrophoretic profiles of select maize lines obtained using the SCoT23 primer

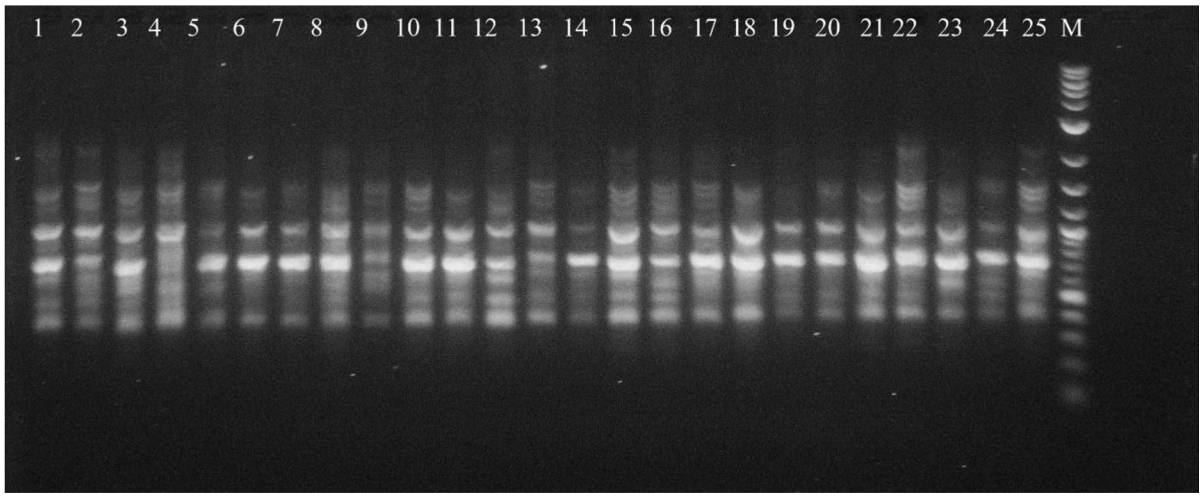

Fig. 5 Electrophoretic profiles of the maize cultivars obtained using the SCoT23 primer

60% (SCoT28, SCoT30) to 100% (SCoT12, SCoT62). The fragment size ranged from 150 to 3000 bp. The PIC values ranged from 0.766 (SCoT54) to 0.893 (SCoT6), with an average value of 0.846. Since all the PIC values in each primer used were higher than 0.7, we can conclude that all the primers used were sufficiently polymorphic and they are suitable for the detection of genetic diversity in maize lines.

The primers in a set of 25 maize cultivars from the Gene Bank in Prague (Table 1), originating from the USA, amplified a total of 87 different DNA fragments (Table 6), of which 69 (79%) were polymorphic and 18 (21%) monomorphic (Fig. 5). The number of fragments obtained by one primer ranged from 6 (SCoT54) to 12 (SCoT28) with an average value of 8.7 fragments per primer. An average number of polymorphic fragments per primer was 6.9. The percentage of polymorphism ranged from 66.67% (SCoT28, SCoT54) to 90% (SCoT8). The fragment size ranged from 150 to 5000 bp. The PIC value ranged from 0.715 (SCoT54) to 0.892 (SCoT28) with an average of 0.838. The PIC values obtained for the SCoT markers used were higher than 0.7, it can be concluded that the primers used are sufficiently polymorphic for the genetic research of maize cultivars.

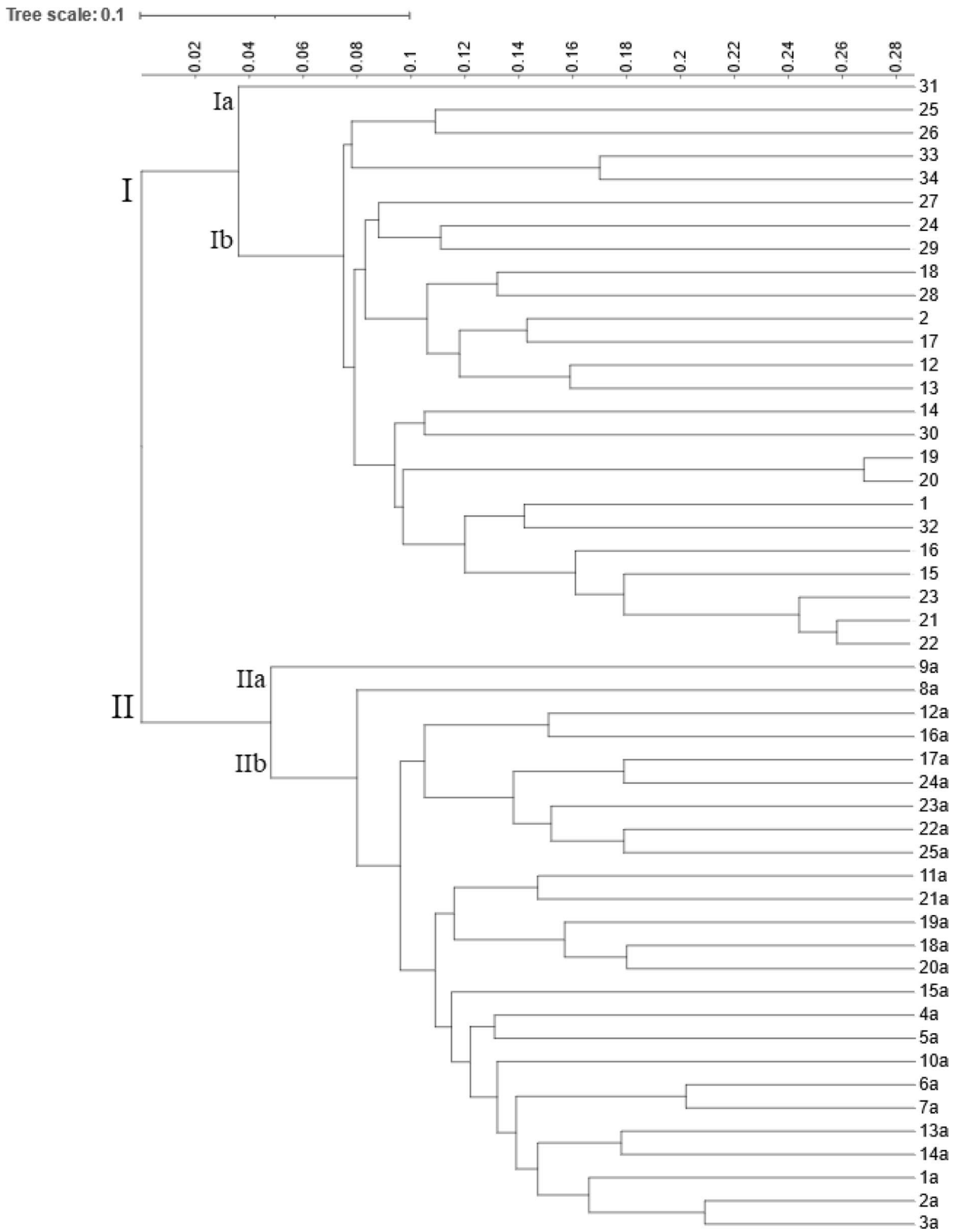

Fig. 6 Joint dendrogram of 25 maize lines (claster I) and 25 cultivars (claster II) constructed using the start codon target (SCoT) markers

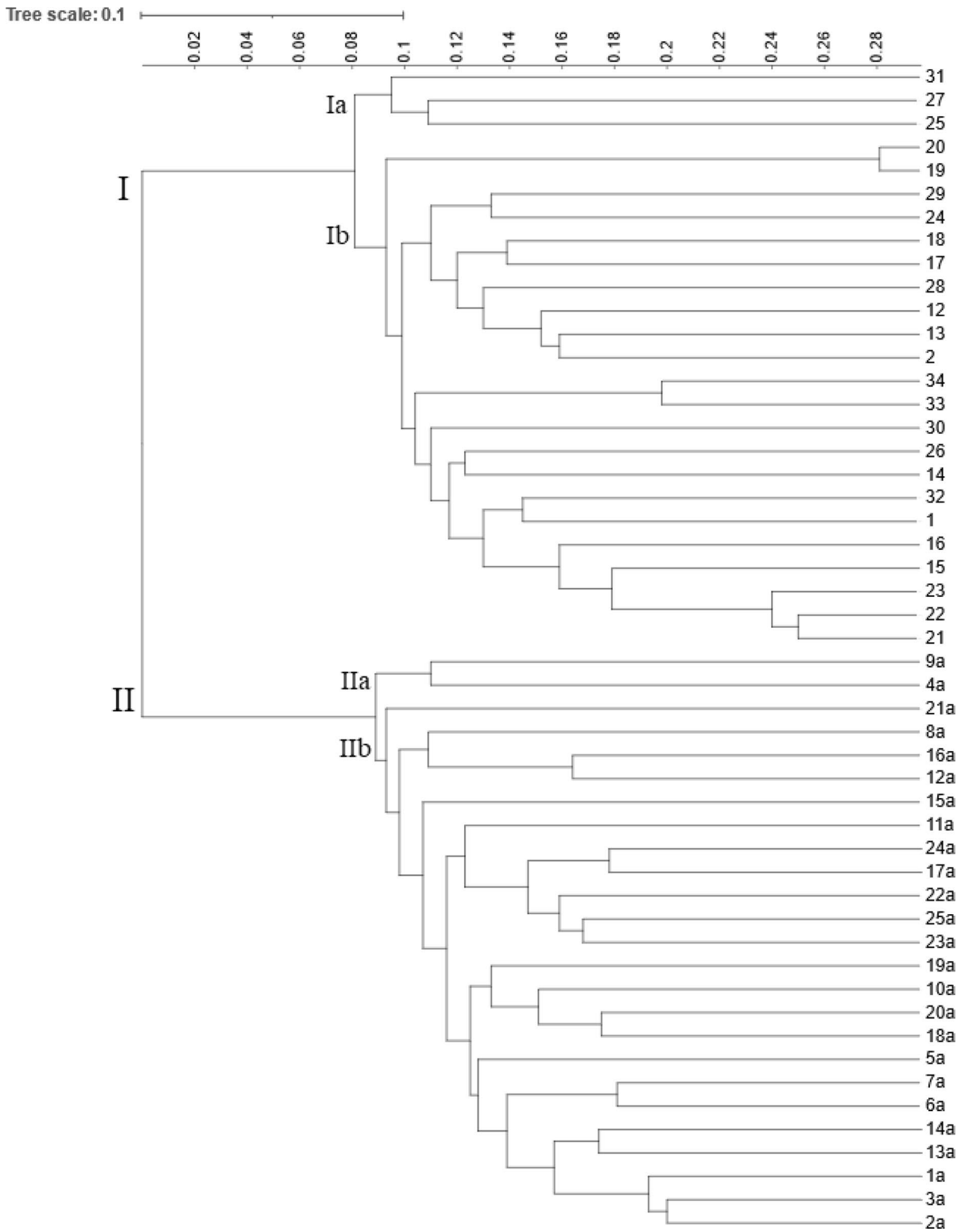

Fig. 7 Joint dendrogram of 25 maize lines (cluster I) and 25 cultivars (cluster II) constructed using the random amplified polymorphic DNA (RAPD) and start codon target (SCoT) markers

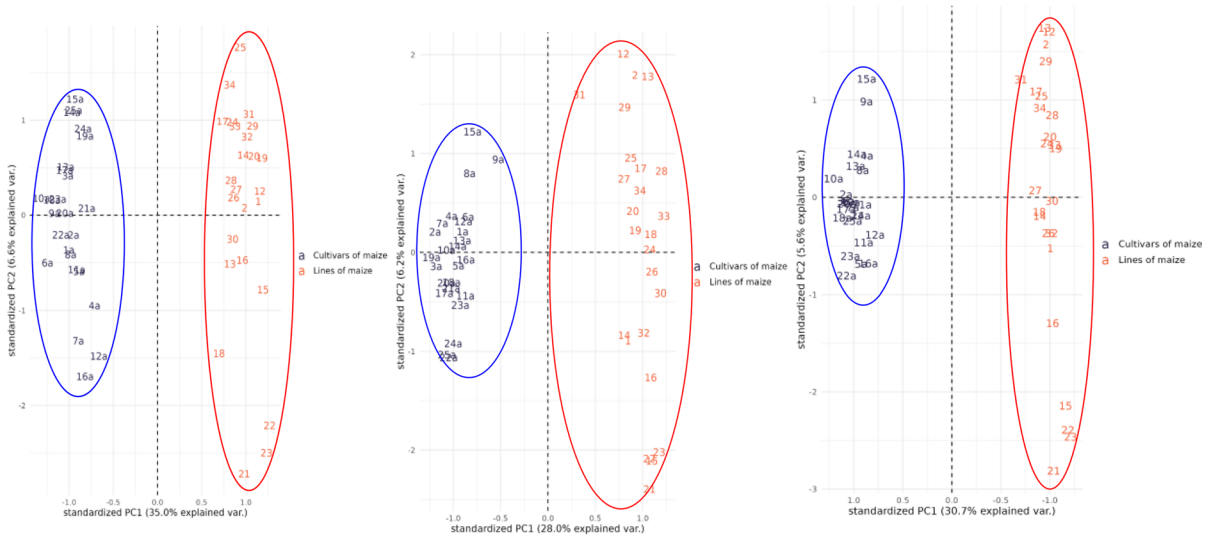

Fig. 8 PCoA plots of 25 maize cultivars and 25 maize lines based on the random amplified polymorphic DNA (RAPD) markers (**a**), start codon target (SCoT) markers (**b**) and RAPD + SCoT markers (**c**) (the maize cultivars are in the blue circle and marked in blue, the maize lines are in the red circle and marked in red)

Table 7 Characteristics of random amplified polymorphic DNA (RAPD) and start codon target (SCoT) marker systems to assess the genetic similarity of the analysed maize lines and cultivars

Indicator	Type of marker	
	RAPD	SCoT
Number of analysed genotypes	50	50
Number of primers	10	10
Total number of polymorphic fragments	110	140
Number of polymorphic loci	110	140
Average number of polymorphic fragments per primer	11	14
Polymorphic information content	0.787	0.842
Polymorphic information content (range)	0.525–0.884	0.715–0.893
Marker index	8.657	11.788
Diversity detecting index	1.731	2.358
Heterozygosity index	0.496	0.484
Effective multiplex ratio	43	52.9
Arithmetic mean of H	0.0001	0.0001
Discriminating power	0.703	0.654
Resolving power	44.64	53.08

The effectiveness of the SCoT method for the differentiation of maize genotypes analyzed in the study is shown in the joint dendrogram for lines and cultivars (Fig. 6), in which two main clusters (I and II) were formed. Cluster I contained all the lines (1 to 34) and cluster II all the cultivars (1a to 25a), which shows the effectiveness of the primers used in the detection of genetic diversity of maize genotypes.

The lines located in cluster I were divided into two subclusters in the dendrogram (Fig. 6). Line 31 with the Flint genetic background separated from the other lines in subcluster Ia. Lines 19 and 20 (genetic distance 0.964), located in the subcluster Ib, with the same genetic background (Iodent and F2) were the two genetically most similar lines in terms of the SCoT polymorphism. Similarly, Lines 21 and 22

(genetic distance 0.945) with the same genetic background (Iodent, SSS and Lancaster) were grouped together, as was the case with Lines 22 and 23 (genetic distance 0.907) in which the genetic background consisted of two identical parents (SSS and Iodent). Lines 18 (the genetic background consisting of SSS and Iodent) and 31 (the genetic background constisting of Flint) were the two genetically most distant lines (genetic distance 0.597), and can be recommended for breeding.

The maize cultivars grouped in cluster II were divided into two subclusters (IIa, IIb) in the dendrogram (Fig. 6). In cluster IIa, two cultivars the Trucker's Favorite White (9a) cultivar and cultivar Rostrata (4a) separated from the remaining maize cultivars. The Black Sugar (2a) and Howling Mob (3a) (0.844) cultivars were the genetically most similar cultivars in terms of the SCoT polymorphism, followed by Early King (6a) and Miniature (7a) (0.831). According to the distance matrix based on the Jaccard coefficient, Trucker's Favorite White (9a) and Wonderful (25a) were the genetically most distant cultivars, and can be recommended for breeding with the aim to obtain new cultivars with improved properties.

A joint dendrogram (Fig. 7) of the maize lines and cultivars was constructed based on binary data of both methods, i.e. RAPD and SCoT, combined. The results were compared with the dendrogram of maize lines and cultivars prepared separately by the RAPD and SCoT markers. The joint dendrogram of both methods confirmed the separation of the maize lines from maize cultivars which separated into two main clusters (I, II). In the similarity matrix computed with the Jaccard coefficient, lines 19 and 20 (0.97) were identified as the genetically closest genotypes of maize with the same genetic background, Iodent and F2, respectivaly. In the RAPD dendrogram, the genetic distance was higher (0.978), and therefore closer compared to the SCoT (0.964) and joint results of RAPD and SCoT (0.97). The maize line 17 and cultivar 9a (Trucker's Favorite White) with a genetic distance of 0.671 were the most distant genotypes.

Three-dimensional plot based on the PCoA analysis

The results of the molecular analysis of maize lines and cultivars using the RAPD and SCoT markers and the joint RAPD and SCoT binary matrix were also used to draw a PCoA plot (Fig. 8), which shows the clustering of maize lines and cultivars. The hierarchical cluster analysis based on the UPGMA algorithm in the constructed dendrogram, and likewise in the PCoA plot, confirmed a clear differentiation of the maize lines and cultivars from each other using the RAPD and SCoT method and both methods combined. We can conclude that the maize lines (Table 2) were grouped in the red circle in the 1st and 4th quadrant and separated from the maize cultivars (Table 1) included in the blue circle in the 2nd and 3rd quadrant based on the RAPD and SCoT analysis, and also based on the joint results from both methods.

Comparing the efficiency of RAPD and SCoT marker systems

To compare the efficiency of the DNA marker systems; i.e. RAPD and SCoT, in the detection of genetic variability of two sets of maize genotypes, two parameters were calculated: the marker index (MI), diversity detecting index (DDI), heterozygosity index (H), efective multiplex ratio (E), discriminating power (D), arithmetic mean of H (H.av), and resolving power (RP) (Table 7). Higher MI (11.788), DDI (2.358) and RP (53.08) value was achieved by the SCoT technique. Based on the experimental results achieved, it can be concluded that the SCoT markers are a more suitable technique for the detection of DNA polymorphism in maize compared to the RAPD markers. Slightly higher values were achieved heterozygosity index and discriminating power by the RAPD technique so the RAPD technique has slighty higher probability to detect that an individual is heterozygous for the locus in the population. Discriminating power presents the probability that two randomly chosen individuals have different patterns, and thus are distinguishable from one another.

Discussion

Molecular markers are widely used for evaluating plant germplasm and genetic diversity for assisting genetic polymorphism, germplasm characterization, genetic distance as well as in marker-assisted selection (Meng et al. 2018). In the present study, RAPD primers in the set of Slovak lines amplified a total of 79 DNA bands, of which 77% were polymorphic. An average PIC value was 0.829. In a set of 25 maize

cultivars, the primers amplified 61 DNA fragments, of which 77% were polymorphic. An average PIC value was 0.745.

The results of RAPD analysis correspond to the research of Vivodík et al. who used 5 RAPD primers to detect the genetic diversity in 20 maize genotypes and obtained 33 DNA fragments of different sizes ranging from 250 to 2000 bp. The individual primers amplified from 5 to 10 fragments, with an average value of 6.6 fragments per primer. The average PIC value was 0.781. The average PIC value in the analyzed maize lines was 0.829 and 0.745 in the cultivars, with an average of 0.787. Comparable results were presented by Balážová et al. (2016) who evaluated a set of 40 maize genotypes using 13 RAPD primers. They obtained 92 fragments, the number of fragments per primer varied from 5 to 10 and the size of DNA fragments from 100 to 2500 bp. On average, 7.08 polymorphic fragments were amplified per primer. The PIC value reached an average of 0.801. The results of Berhitu et al. (2019) confirmed that the RAPD technique is also suitable to characterize the maize genome. Only two maize genotypes were compared. The DNA profiles obtained using 3 RAPD primers consisted of 22 bands and up to 91% of them were polymorphic. The size of the amplified fragments ranged from 263 to 1200 bp. Vivodík et al. (2017b) evaluated 20 maize genotypes using 5 RAPD primers, amplifying an average of 7 fragments per primer (ranging from 5 to 8 and size from 150 to 2500 bp). The average PIC value was 0.799, which is consistent with our results. [43] studied 30 genotypes with 5 RAPD primers and detected 32 fragments, of which 26 were polymorphic. They constructed a dendrogram in which 3 main clusters emerged.

On the other hand, the authors Radwan et al. (2021), Al-Obaidi et al. (2018), Ristic et al. (2013) and Sharawy et al. (2011) detected a higher number of DNA fragments per primer and a higher percentage of polymorphism. Radwan et al. (2021) examined the genetic diversity of 16 Zea mexicana populations. 14 RADP primers amplified 141 DNA fragments (average of 10.1 bands per primer) out of which, 102 (72.3%) were polymorphic. PIC value was higher (in average 3,04) in comparison to our results. Al-Obaidi et al. (2018) studied genetic variation among 30 maize inbred lines and its relationship with hybrid Vigor by RADP markers. The genotypes were divided into three main groups according to the nearest neighbor method and RAPD technique showed 97% of the polymorphisms. Ristic et al. (2013) analyzed 21 maize genotypes with 7 RAPD primers and obtained an average of 10.8 bands per primer. Sharawy et al. (2011) obtained a higher level of polymorphism (84.44%) studying maize inbred lines using RAPD markers.

In our study, SCoT primers in the set of Slovak lines amplified a total of 90 DNA bands, of which 79% were polymorphic. An average PIC value was 0.846. In a set of 25 cultivars, the primers amplified 87 DNA fragments, of which 79% were polymorphic. An average PIC value was lower compare to maize lines, 0.838. Similar research using the SCoT technique was conducted by Vivodík et al. (2016) who studied 40 maize genotypes using 20 SCoT primers that produced a total of 114 fragments, of which 86 (76.43%) were polymorphic. The average number of polymorphic fragments per one primer was 4.3 and the average PIC value was 0.739. The dendrogram constructed by the UPGMA method divided the genotypes into two main clusters, which were further differentiated into subclusters. Sadek and Ibrahim (2018) analyzed 8 maize lines using 10 SCoT markers. These primers produced a total of 136 fragments, of which 74 (54%) were polymorphic with an average of 7.4 polymorphic fragments per primer and the number of amplified fragments ranged from 4 to 13, which is consistent with our results. The dendrogram of eight maize lines based on the SCoT markers using the UPGMA contained two main clusters. In another study, Vivodík et al. (2016) studied 20 maize genotypes using 5 SCoT markers. The primers produced a total of 29 DNA fragments, of which 22 (78%) were polymorphic with an average of 4.4 polymorphic fragments per primer, and the number of amplified fragments ranged from 4 to 7. The PIC ranged from 0.652 to 0.816, with a mean of 0.738. A dendrogram was constructed using the UPGMA algorithm in which the maize genotypes were divided into two main clusters, which also corresponds to our results. The analysis by Al-Tamimi (2020), who evaluated 10 maize genotypes using 11 SCoT markers, aimed to clarify the diversity between the individual genotypes. The primers produced a total of 627 fragments, of which 56 were polymorphic with an average of 5.09 fragments per primer.

The use of a suitable molecular technique and the number of markers in the genetic analyzes of plants applied to the given genome is very important for a reliable detection of their genetic diversity. We compared the RAPD and SCoT markers used for the analysis of genetic variability of the maize genome in two sets of maize genotypes (Table 7). The efficiency of individual marker techniques to detect the genotype diversity can be compared by calculating the marker index (MI), detecting diversity index (DDI), heterozygosity index (H), efective multiplex ratio (E), discriminating power (D), arithmetic mean of H (H.av), and resolving power (RP). The informativeness of the primer combinations can be ascertained by the PIC, MI, E, D and RP values because the values indicate the discriminatory power of a marker system by taking into consideration the number of alleles at a locus and the relative frequencies of these fragments. Higher value of MI (11.788), DDI (2.358) and RP (53.08) in our analyzes was achieved by the SCoT marker technique. Balážová (2016) determined lower values of MI and DDI using the RAPD technique (MI = 5.66; DI = 1.84) and SCoT (MI = 4.21; DDI = 2.11) compared to our results, however, she considered RAPD to be the most effective technique for determining maize diversity. Radwan et al. (2021) compared the efficiency of RAPD and AFLP markers in the analysis of the *Zea mexicana* genome and found that the marker index (MI) of RAPD (1.1) was significantly lower compared to AFLP (11.21). Other authors used MI and DDI to evaluate the polymorphism and diversity analyzes in other cereals. In the study of genetic diversity of rye, Hou et al. (2005) used the RAPD technique in their calculations, and their MI value was lower compared to our analyzes (1.16). Using the RAPD technique, they detected a lower DDI value (0.46) than us. Based on their results, they concluded that the low DDI values do not sufficiently estimate the number of marker loci for a proper assessment of genetic diversity. On the contrary, Hou et al. (2005) detected a DDI of 1.0 in the RAPD analyzes of barley, which is a lower value than in our RAPD analyzes of maize.

In current research, the UPGMA dendrograms and PCoA plots confirmed a clear separation of the maize lines and cultivars from each other using the RAPD, SCoT and joint results of both methods. The maize cultivars and maize lines were further subdivided into subclusters. The clustering of maize lines reflected the origin of the lines used. The maize lines with the genetic background containing Lancaster (Table 7) may have high yield potential (genotype 2–6, 13–14, 17, 20). On the other hand, the lines with the genetic background containing Flint (genotypes 16, 18–19, 21–22, 24–25) will be characterized by a higher glassiness of the endosperm, which can be used in the food industry for the preparation of flours from which various bakery products are made. The lines with genetic background containing Iodent are characterized by a floury type of endosperm (genotypes 1, 5–7, 9–15, 17, 23), which can be used in the starch industry. The Stiff Stalk Synthetic (SSS) background is a guarantee of a firm stalk (genotypes 17–18, 21–24, 29–30) of the plant (Mansfield and Mumm 2014; Brekke et al. 2011). Al-Tamimi (2020), who evaluated 10 maize genotypes using 11 SCoT markers, showed that the changes in genetic distance between the genotypes were correlated with their different geographic origins and, in the constructed UPGMA dendrogram, the genotypes with the closest related ancestry were grouped in the same cluster. Vargas et al. (2018) investigated the genetic variability among 32 maize genotypes from different regions using the RAPD markers. The dendrogram divided the genotypes into four clusters that were related to the origin of the genotypes as well as to the grain phenotypic traits. The UPGMA dendrograms and PCoA plots confirmed the grouping of the cultivars according to the place of origin in the study of oat cultivars using the SCoT and ISSR markers (Cieplak et al. 2021).

It follows from the above that to detect the genetic diversity of individual plant species, it is always necessary to choose the type of molecular technique that reveals the polymorphism at the DNA level most effectively. Based on our results, it can be concluded that both gene-specific SCoT markers and random RAPD markers are suitable for the detection of genetic diversity of maize. Both techniques show sufficient DNA polymorphism, but SCoT markers proved to be more efficient compared to RAPD markers. However, we recommend to breeders to apply both types of DNA markers in the breeding process in the selection of genotypes with the desired characteristics due to their simplicity, low price and the possibility of testing a large number of samples in a relatively short time.

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Data availability The data supporting the findings of this study are available from the corresponding author (Želmíra Balážová) upon reasonable request.

Declarations

Conflict of interest The authors declare no conflict of interest.

References

Abdellatif KF, Khidr YA (2010) Genetic diversity of new maize hybrids based on SSR markers as compared with other molecularand biochemical markers. J Crop Sci Biotechnol 13:139–145. https://doi.org/10.1007/s12892-010-0068-x

Adhikari S, Saha S, Biswas A, Rana TS, Kumar Bandyopadhyay T, Ghosh P (2017) Application of molecular markers in plant genome analysis: a review. Nucleus 60:283–297. https://doi.org/10.1007/s13237-017-0214-7

Al-Badeiry NAH, Merza TKM, Al-Saadi AH (2013) Assessment of genetic diversity and relationships among maize (*Zea mays* L.) varieties in Iraq using random amplified polymorphic DNA (RAPD) markers. J Life Sci 7:1260–1271

Al-Obaidi SSM, Al-Ubaidi MOG, Gen FO (2018) Molecular evaluation of genetic variation among some maize *zea mays* L. inbred lines and its relationship with hybrid vigor. Anbar J Agric Sci 16:860–875

Al-Tamimi AJ (2020) Genetic variation among *Zea mays* genotypes using start codon targeted marker polymorphism. SABRAO J Breed Genet 52(1):1–6

Amiryousef A, Hyvonen J, Poczai P (2018) iMEC: online marker efciency calculator. Appl Plant Sci 6(6):e1159. https://doi.org/10.1002/aps3.1159

Balážová Ž, Vivodík M, Gálová Z (2016) Evaluation of molecular diversity of central European maize cultivars. Emir J Food Agric 28:93–98. https://doi.org/10.9755/ejfa.2015-05-204

Balážová Ž (2016) Molecular characterization of selected cereal species in terms of genetic diversity. In: Habilitation thesis. Nitra, faculty of biotechnology and food sciences, Slovak University of agriculture in Nitra Slovakia 126

Baran N, Shimira F, Nadeem MA et al (2023) Exploring the genetic diversity and population structure of upland cotton germplasm by iPBS-retrotransposons markers. Mol Biol Rep 50:4799–4811. https://doi.org/10.1007/s11033-023-08399-0

Belalia N, Lupini A, Djemel A, Morsli A, Mauceri A, Lotti C, Khelifi-Slaoui M, Khellfi L, Sunseri F (2019) Analysis of genetic diversity and population structure in Saharan maize (*Zea mays* L.) populations using phenotypic traits and SSR markers. Genet Resour Crop Evol 66:243–257. https://doi.org/10.1007/s10722-018-0709-3

Berhitu EC, Hiariej A, Seumahu CA (2019) Genetic characterization of maize kisar var kuning genjah and maize var bisi-II-hibrida based on molekular RAPD marker. Agrotech J 4:91–98. https://doi.org/10.31327/atj.v4i2.1127

Brekke B, Edwards J, Knapp A (2011) Selection and adaptation to high plant density in the Iowa stiff stalk synthetic maize (Zea mays L.) Population. Crop Sci 51:1965–1972. https://doi.org/10.2135/cropsci2010.09.0562

Carvalho VP, Ruas PM, Ruas CF, Ferreira JM, Moreira RMP (2002) Assessment of genetic diversity in maize (*Zea mays* L.) landraces using inter simple sequence repeat (ISSR) markers. Crop Breed Appl Biotechnol 2:557–568. https://doi.org/10.12702/1984-7033.v02n04a09

Cieplak M, Okon S, Werwinska K (2021) Genetic similarity of *Avena sativa* L. varieties as an example of a narrow genetic pool of contemporary cereal species. Plants 10(7):1424. https://doi.org/10.3390/plants10071424

Collard BC, Mackill DJ (2009) Start codon targeted (SCoT) polymorphism: a simple, novel dna marker technique for generating gene-targeted markers in plants. Plant Mol Biol Rep 27:86–93. https://doi.org/10.1007/s11105-008-0060-5

FAO. Agricultural production statistics 2000–2022. Available online: https://www.fao.org/3/cc9205en/cc9205en.pdf

Gajera BB, Kumara N, Singha AS, Punvara BS, Ravikirana R, Subhasha N, Jadejab GC (2010) Assessment of genetic diversity in castor (*Ricinus communis* L.) using RAPD and ISSR markers. Crops Prod 32:491–498. https://doi.org/10.1016/j.indcrop.2010.06.021

GeneJET Plant Genomic DNA Purification Mini Kit. User guide. Available online: https://www.thermofisher.com/document-connect/document-connect.html?url=https://assets.thermofisher.com/TFS-Assets%2FLSG%2Fmanuals%2FMAN0016131_GeneJET_Plant_Genomic_DNA_Purification_Mini_Kit_UG.pdf

Gregová E, Hauptvogel P (2015) Analýza zeínov v kukurici siatej a kukurici cukrovej elektroforetickými metódami / Analysis of zeins in maize and sweetcorn by electrophoretic methods. Available online: https://www.vurv.sk/fileadmin/VURV/Viac/PUblikacna_cinnost_VURV/Analyza_zeinov_kukurici_siatej_a_kukurici_cukrovej_elektroforetickymi_metodami_-_Gregova.pdf. Accessed 6 July 2023

Hou YC, Yan ZH, Wei YM, Zheng YL (2005) Genetic diversity in barley from west China based on RAPD and ISSR analysis. Barley Genet Newsl 35:9–22

Huang W, Du Y, Zhao X, Weiwei J (2016) B chromosome contains active genes and impacts the transcription of a chromosomes in maize (*Zea mays* L.). BMC Plant Biol 16:88. https://doi.org/10.1186/s12870-016-0775-7

Kostova A, Todorovska E, Christov N, Sevov V, Atanassov AI (2006) Molecular characterization of Bulgarian maize germplasm collection via SSR markers. Biotechnol Biotechnol Equip 20:29–36. https://doi.org/10.1080/13102818.2006.10817339

Kumar P, Gupta VK, Misra AK, Modi DR, Pandey BK (2009) Potencial of molecular markers in plant biotechnology. Plant Omies J 2:141–162

Kumar A, Rakshit A, Mangilipelli KN, Varalaxmi Y, Vijayalakshmi T, Vanaja MJ, Yadav KS, Venkateswarlu B, Maheswari M (2012) Genetic diversity of maize genotypes on the basis of morpho-physiological and simple sequence repeat (SSR) markers. Afr J Biotechnol 11:16468–16477

Letunic I, Bork P (2019) Interactive tree of life (iTOL) v4: recent updates and new developments. Nucleic Acids Res 47:256–259. https://doi.org/10.1093/nar/gkz239

Losa A, Hartings H, Verderio A, Motto M (2011) Assessment of genetic diversity and relationships among maize inbred genotypes developed in Italy. Maydica 56(1):95–104

Lubis K, Butarbutar LKS (2022) Genetic diversity analysis of several maize lines (Zea mays L.) developed in acid soil using molecular markers RAPD. In: IOP Conferences series: earth environment science 977:012041. Doi: https://doi.org/10.1088/1755-1315/977/1/012041.

Luqman T, Qamar Zu, Tabasum A et al (2023) Genetic characterization of coarse and basmati rice (*Oryza sativa* L.) through microsatellite markers and morpho-agronomic traits. Genet Resour Crop Evol 70:2307–2320. https://doi.org/10.1007/s10722-023-01620-w

Mansfield DB, Mumm HR (2014) Survey of plant density tolerance in U.S. Maize Germplasm Crop Sci 54:157–173. https://doi.org/10.2135/cropsci2013.04.0252

Meng Z, Song F, Liu T (2018) Genetic diversity and genetic structure analysis of maize (*Zea mays*) landraces in Tibet. Int J Agri Biol 20:791–798

Moravčík (2023) Facts and some thoughts about growing corn for grain in 2023/ Skutočnosti a niekoľko myšlienok o pestovaní kukurice na zrno v roku 2023.Association of Corn Growers and Processors in Slovakia/Zväz pestovateľov a spracovateľov kukurice Slovenska. Growing corn in 2023/Pestovanie kukurice v roku 2023. Available online: http://www.zpsk.sk/files/images/article/kukurica_2023_2.pdf

Mrutu AB (2015) Assessment of seed genetic purity of hybrid maize variety UH6303 in southern highlands of Tanzania by random amplified polymorphic DNA (RAPD) markers1. Afr J Agric Res 10:2911–2918. http://www.academicjournals.org/AJAR

Muhammad RW, Qayyum A, Ahmad QM, Hamza A, Yousaf M, Ahmad B, Younas M, Malik W, Liaqat S, Noor E (2017) Characterization of maize genotypes for genetic diversity on the basis of inter simple sequence repeats. Genet Mol Res 16:1–9. https://doi.org/10.4238/gmr16019438

Mukharib DS, Patil VC, Biradar DP, Salimath PM, Chimmad VP (2010) Assessment of molecular diversity in selected maize inbreds. Karnataka J Agric Sci 23(3):409–412

Myśków B, Milczarski P, Masojś P (2010) Comparison of RAPD, ISSR and SSR markers in assessing genetic diversity among rye (*Secale cereale* L.) inbred lines. Plant Breed Seed Sci 62:107–115. https://doi.org/10.2478/v10129-011-0009-y

Orhun GE (2013) Maize for life. Int J Food Sci Nutr Eng 3:13–16

Radwan KH, Abdelfattah GA, Badawi MA, Zayed EM, Tarrd MM, El-Baghdady MMS, Abd El-Maksoud RM (2021) Genetic variations in some Egyptian *Zea mexicana* genotypes based on RAPD and AFLP markers. J Biosci Appl Res 7(2):77–92. https://doi.org/10.21608/jbaar.2021.177577

Ristic D, Vojka B, Violeta A, Snežana MD, Dragana I (2013) Genetic diversity in maize dent landraces assessed by morphological and molecular markers. Genetika 45:811–824. https://doi.org/10.2298/GENSR1303811R

Roy NS, Kim NS (2016) Genetic diversity analysis of maize lines using AFLP and TE-based molecular marker systems. Gen Genom 38:1005–1012

Sadek MSE, Ibrahim SD (2018) Genetic relationships among maize inbred lines as revealed by start codon targeted (SCoT) analysis. J Innov Pharm Biol Sci 5(1):103–107

Salami HA, Ch SK, Padonou W, Aly D, Yallou Ch, Adjanohoun A, Kotchoni S, Moussa LB (2016) Genetic diversity of maize accessions (*Zea mays* L.) cultivated from benin using microsatellites markers. Am J Mol Biol 6:12–24. https://doi.org/10.4236/ajmb.2016.61002

Sarwat M, Nabi G, Das S, Shankar P (2012) Molecular markers in medicinal plant biotechnology: past and present. Crit Rev Biotechnol 32:74–92. https://doi.org/10.3109/07388551.2011.551872

Sharawy WM, Ibrahim HIM, EL-Fiky ZA (2011) Phylogenetic relationships among teosinte, maize and its hybrids. Egy J Gen Cyt 40(1):129–144

Sharma L, Prasanna MB, Ramesh B (2010) Analysis of phenotypic and microsattelite-based diversity of maize landraces in India, especially from the North East Himalayan region. Genetica 138:619–631. https://doi.org/10.1007/s10709-010-9436-1

Soliman SRE, El-Shazly HH, Börner A, Badr A (2021) Genetic diversity of a global collection of maize genetic resources in relation to their subspecies assignments,

geographic origin, and drought tolerance. Breed Sci 71(3):313–325. https://doi.org/10.1270/jsbbs.20142

Sun GL, William M, Liu J, Kasha KJ, Pauls KP (2001) Microsatellite and RAPD polymorphisms in Ontario corn hybrids are related to the commercial sources and maturity ratings. Mol Breed 1(7):13–24

Syngenta Slovensko/Slovakia. Kukurica/Corn 2024. Available online: https://www.syngenta.sk/sites/g/files/kgtney1546/files/media/document/2023/10/13/09_23_katalog_kukurica_2023_syngenta_web.pdf

Vargas AY, Marquetti G, Cabrara M, Granda FL, Oramas DBP (2018) Genetic diversity of maize (*Zea mays* L.) from pinar del río and guantánamo by using of RAPD. Revista Protección Vegetal 33:2224

Vivodík M, Gálová Z, Balážová Ž, Petrovičová L (2016) Start codon targeted (SCoT) polymorphism reveals genetic diversity in european old maize (*Zea mays* L.) Genotypes. Potravinárstvo 10:563–569. https://doi.org/10.5219/660

Vivodík M, Gálová Z, Balážová Ž, Petrovičová L (2017a) Genetic variation of European maize genotypes (*Zea mays* L.) detected using SSR markers. Potravinárstvo Slovak J Food Sci 11:126–213. https://doi.org/10.5219/697

Vivodík M, Petrovičová L, Balážová Ž, Gálová Z (2017b) Assessment of RAPD polymorphism in maize (*Zea mays* L.) genotypes. Agrobiodiversity for Improving Nutrition, Health and Life Quality. https://doi.org/10.15414/agrobiodiversity.2017.2585-8246.514-518.

Weber JL (1990) Informativeness of human (dC-dA)n x (dG-dT)n polymorphism. Genomics 7:524–530. https://doi.org/10.1016/0888-7543(90)90195-z