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USE OF EBAP MARKERS FOR IDENTIFICATION AND DIFFERENTIATION OF OLD MAIZE GENOTYPES

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ABSTRACT

Knowledge of the genetic diversity of corn is important in the corn breeding process. This study was conducted to determine genetic diversity among 40 old maize genotypes using 36 EBAP primers and to verify the usability of these primers for the study of maize polymorphism. All 36 EBAP primers have amplified products, with the highest number of fragments provided by primer EBAP18 (14) and the lowest number of fragments provided by primers EBAP5, EBAP11, EBAP12 and EBAP 31. The total average number of fragments per primer was 6.75. The number of polymorphic fragments using 36 EBAP primers ranged from 13 (EBAP18) to 1 (EBAP3, EBAP5, EBAP12, EBAP20, EBAP23, EBAP28, EBAP31 and EBAP33) with an appropriate number of 4.36 polymorphic fragments per primer. The percentage of polymorphism of the analyzed fragments ranged from 100% (EBAP4, EBAP 27 and EBAP36) to 16.67% (EBAP3 and EBAP28) with an average value of 57.94%. For each primer was calculated the values of index: TNB, NPB, PPB, Na, Ne, H, I, PI, Rp, PIC, MI. By using 36 EBAP primers, we managed to divide the 40 old analyzed maize genotypes into 2 main clusters (cluster 1 and cluster 2), while 2 genotypes from Poland (Przebedowska Biala and Przebedowska Burskynowa) were separated separately in the obtained dendrogram. Based on the obtained results, we confirmed the usability of EBAP primers for the study of polymorphism of old maize genotypes.

Keywords: EBAP primers, old maize, polymorphism, genetic diversity, breeding

INTRODUCTION

Maize (*Zea mays* L.) is one of the important economic and staple food crops and an energy plant among cereals that is cultivated globally for fulfilling the requirements of human beings **(Kamara *et al.*, 2020)**. It is a vital source of the income-overwhelming population. With a high yield potential, it has become a model crop among cereals and is therefore called the queen of cereal crops **(Stanley *et al.*, 2020)**. In addition, it is utilized as an industrial resource for the production of starch, pharmaceuticals, alcoholic beverages, oil, cosmetics, and textiles **(Kumar *et al.*, 2019)**. The production of high-yielding maize cultivars has always been the primary objective of breeding. For increasing the production of maize, several varieties, including sweet corn, popcorn, and high-quality protein corn, are being developed globally **(Wani *et al.*, 2022)**. In spite of huge work on developed varieties of maize, its yields are below their potential because of abiotic and biotic stresses, indicating the need to assessthe genetic diversity. Knowledge of genetic diversity in maize crop, especially of germplasm and inbred lines, have significantly impacted crop improvement **(Antony *et al.*, 2021)**.
Genetic diversity can be assessed by common morphological traits or molecular markers. Molecular markers have become the study of choice for the plant genetic diversity. Over the last three decades, the advent of molecular markers has revolutionized the entire scenario of plant and animal sciences. Molecular markers offer several advantages over traditional morphological traits, as molecular markers are not environmentally influenced **Nepolean *et al.*, (2013)**. The following types of DNA markers have been successfully used to study genetic diversity of maize: RAPD (Random Amplified Polymorphic DNA) **(Javed *et al.*, 2021)**, which they analyzed 20 maize genotypes representing popcorn, white corn, sweet corn and yellow corn, through morphological characteristics and RAPD, ISSR (Inter Simple Sequence Repeats) **(Dar *et al.*, 2018),** which they study 10 cultivars of maize collected from 10 different sites (five from each site) in district Rajouri of Jammu and Kashmir State**,** SSR (Simple Sequence Repeat) **(Bocianowski *et al.*, 2021)**, which they used 30 SSR markers to study the polymorphism of 13 maize varieties, AFLP (Amplified Length Fragment Polymorphism) **(Roy *et al.*, 2016),** which study polymorphism seventy-eight corn accessions or hybrid varieties using AFLP markers, SCoT (Start Codon-Targeted) **(Rizk *et al.*, 2024)** analyzed twelve Egyptian *Zea mays* L. hybrids using 12 SCoT markers and SNP (Single Nucleotide Polymorphism) **(Madankar *et al.*, 2024)** focused on the genetic diversity, population structure and clustering of 56 Indian maize inbreds using 1166 informative SNP markers..

The single EBAP (Exon based amplified polymorphism primer) is designed based on the GC bases which are rich in exon region of genes in eukaryote genome. The single primer acts as both, upstream and downstream primers and plays the same role as the single primer of RAPD, ISSR, SCoT, and CDDP. The main difference is that the single primer of EBAP can probably simultaneously anchor two sites which are not far apart in exon region of genes in eukaryote genome. Sometimes the two binding sites are located in the same exon, sometimes located in two different exons spanning introns **(Xiong *et al.*, 2022)**. The resulting amplified products probably include the exons region and even the adjacent introns region. To determine whether the amplified products include the exons region or introns region or not, the next step is to clone and sequence the amplified products. Although most of molecular markers produced by EBAP technique are dominant (polymorphisms caused by point mutations at single primer binding sites), perhaps very few codominant molecular markers caused by insertion/deletion and intron length variation between single primer binding sites may also be produced. In addition, EBAP also can be used to display differentially expressed genes in plants **(Xiong *et al.*, 2022)**. Currently, only **Boutsika *et al.*, (2024)** used the technique of EBAP primers, who analyzed 12 genotypes of *Rosa canina* L. using ISSR, SCoT and EBAP primers. Actual, various techniques for studying DNA polymorphism and PCR reaction have been used by many authors around the world: **Sevindik *et al.*, (2022); Sevindik & Delibay, (2022); Beranová *et al.*, (2022); Žiarovská *et al.*, (2022); Golian *et al.*, (2022); Žiarovská and Urbanová, (2022); Sevindik *et al.*, (2023a); Sevindik *et al.*, (2023b); Farkasová *et al.*, (2023); Tahir *et al.*, (2023); Vivodík *et al.*, (2023); Ruiz-Chután *et al.*, (2024); Čišecká et al., (2024).** The aim of the work was to analyze a set of 40 old maize genotypes using 36 EBAP primers and to verify the usability of these primers for the study of maize polymorphism. The obtained results can be further used in corn breeding and for improving the quality of corn grain.

MATERIAL AND METHODS

Maize genotypes (40) were obtained from the Gene Bank VURV Praha-Ruzyne (Czech Republic) and from the Gene Bank in Piešťany, the Slovak Republic (Table 1). Genomic DNA of maize genoypes was extracted from leaves of 14-day old plantlets with GeneJET Plant Genomic DNA Purification Mini Kit according to the manufacturer's instructions. DNA was isolated from leaves of one seed of each analyzed line. Concentrations of isolated DNA were estimated using UV-VIS spectrophotometer and the final concentration of DNA was adjusted to 50 ng/µl. All the DNA samples were stored at − 20 °C.

Table 1 List of 40 analyzed genotypes of maize. We got such an origin of genotypes and with such a designation from the Gene Bank VURV Praha-Ruzyne (Czech Republic) and from the Gene Bank in Piešťany, the Slovak Republic.

Genotypes	Country of origin	Year of registration
1. Feheres Sarga Filleres	Hungary	1965
2. Mindszentpusztai Feher	Hungary	1964
3. Zakarpatskaja	Union of Soviet Socialist Republics	1964
4. Przebedowska Burskynowa	Poland	1964
5. Krasnodarskaja	Union of Soviet Socialist Republics	1964
6. Mesterhazy Sarga Simaszemu	Hungary	1964
7. Slovenska biela perlova	Czechoslovakia	1964
8. Zuta Brzica	Yugoslavia	1975
9. Zloty Zar	Poland	1964
10. Slovenska Florentinka	Czechoslovakia	1964
11. Juhoslavska	Yugoslavia	1964
12. Kostycevskaja	Union of Soviet Socialist Republics	1964
13. Mindszentpusztai Sarga Lofogu	Hungary	1964
14. Stodnova	Czechoslovakia	1964
15. Slovenska žltá	Slovak Republic	1964
16. Slovenska krajová velkozrná	Slovak Republic	1964
17. Partizanka	Union of Soviet Socialist Republics	1964
18. Voroneskaja	Union of Soviet Socialist Republics	1964
19. Kocovska Skora	Slovak Republic	1964
20. Milada	Czechoslovakia	1964
21. Moldavskaja	Union of Soviet Socialist Republics	1964
22. Bučiansky Konský Zub	Slovak Republic	1964
23. Hodoninský konský zub žltý	Czechoslovakia	1964
24. M Silokukurica	Hungary	1964
25. Valticka	Czechoslovakia	1964
26. Przebedowska Biala	Poland	1964
27. Toschevska	Slovak Republic	1964
28. Šamorinsky konský zub	Hungary	1964
29. Wielkopolanka	Poland	1964
30. Czechnicka	Poland	1964
31. Manalta	Czechoslovakia	1964
32. Zlota gorecka	Poland	1964
33. Celchovicka ADQ	Czechoslovakia	1964
34. Belaja mestnaja	Union of Soviet Socialist Republics	1964
35. Bučanská žltá	Slovak Republic	1964
36. Iregszemeseil 2 hetes	Hungary	1964
37. Dnepropetrovskaja	Union of Soviet Socialist Republics	1964
38. Bezuncukskaja	Union of Soviet Socialist Republics	1964
39. Mikulická	Czechoslovakia	1964
40. Aranyozon sarga lofogu	Hungary	1964

The EBAP amplification **(Xiong *et al*., 2022)** (Table 2) reaction was carried out in a 20 µl volume containing 200 µM of each dNTP, 1.5 mM $MgCl_2$, 1 U Taq DNA polymerase, 10 pmol single primer, 1xPCR buffer, and 50 ng DNA. The EBAP-PCR amplification was performed as follows: initial denaturation at 94 °C for 4 min, followed by 35 cycles of denaturation at 94 °C for 30 s, annealing at 50 °C for 1 min, and extension at 72 °C for 1 min 30 s, with a final extension at 72 °C for 10 min **(Xiong *et al*., 2022)**. After amplification, 6 µl loading buffer was added to the PCR products, then 5 µl PCR products were separated by 1.5% agarose gel electrophoresis with ethidium bromide (1 x TAE buffer) and photographed on the UV gel imaging system.

Table 2 List of 36 EBAP primers used for analysis of 40 old maize genotypes (**Xiong *et al.*, 2022)**

1.	EBAP1	GAATTCCGGCGGCGATA	19.	EBAP19	GAATTCCGGCGGCGACG
2.	EBAP2	GAATTCCGGCGGCGATC	20.	EBAP20	GAATTCCGGCGGCGAGC
3.	EBAP3	GAATTCCGGCGGCGATG	21.	EBAP21	GAATTCCGGCGGCGAGT
4.	EBAP4	GAATTCCGGCGGCGAAC	22.	EBAP22	GAATTCCGGCGGCGAAT
5.	EBAP5	GAATTCCGGCGGCGTGC	23.	EBAP23	GAATTCCGGCGGCGATT
6.	EBAP6	GAATTCCGGCGGCGTAC	24.	EBAP24	GAATTCGGCGGCGGACT
7.	EBAP7	GAATTCCGGCGGCGTCG	25.	EBAP25	GAATTCGGCGGCGGTAG
8.	EBAP8	GAATTCCGGCGGCGTGA	26.	EBAP26	GAATTCGGCGGCGGTGA
9.	EBAP9	GAATTCCGCCGCCGATA	27.	EBAP27	ATATATCGGCGGCGACT
10.	EBAP10	GAATTCGGCGGCGGATC	28.	EBAP28	ATATATGGCGGCGGTCG
11.	EBAP11	ATCGATCGGCGGCGATC	29.	EBAP29	AGTACTCGGCGGCGATA
12.	EBAP12	ATATATCGGCGGCGATA	30.	EBAP30	AGTACTCGGCGGCGTAC
13.	EBAP13	ATATATCGGCGGCGATC	31.	EBAP31	TGATCACGGCGGCGATA
14.	EBAP14	ATATATCGGCGGCGATG	32.	EBAP32	TGATCACGGCGGCGTAC
15.	EBAP15	ATATATCGGCGGCGTCG	33.	EBAP33	AATATTCGGCGGCGATA
16.	EBAP16	GAATTCCGGCGGCGACT	34.	EBAP34	AATATTCGGCGGCGTAC
17.	EBAP17	GAATTCCGGCGGCGAAA	35.	EBAP35	TTTAAACGGCGGCGATA
18.	EBAP18	GAATTCCGGCGGCGTTT	36.	EBAP36	TTTAAACGGCGGCGTAC

EBAP is regarded as a multiloci dominant molecular marker technique **(Xiong *et al*., 2022)**. Only the clearly distinguishable bands were counted with band marked as 1 and no band marked as 0, thus forming a binary data matrix. According to the binary data matrix, total number of bands (TNB), number of polymorphic bands (NPB) and percentage of polymorphic bands (PPB) were calculated. Genetic similarity was calculated by the formula GSij = 2Nij/(Ni + Nj), where in Nij was the number of common amplified bands between accessions i and j, and Ni and Nj were the number of amplified bands in accessions i and j respectively **(Raina *et al.*, 2001)**. Genetic distance was calculated by the formula GDij = 1-GSij **(Raina *et al.*, 2001)**. Polymorphic index (PI) was calculated by the previous reported method **(Raina *et al.*, 2001)**. Observed number of alleles (Na), effective number of alleles (Ne), Nei's gene diversity (H) and Shannon's information index (I) were

calculated using POPGENE32. Resolving power was calculated by the formula $Rp=PI_b$, where $I_b = 1-(2x|0.5-pl)$, where p is the portion of the samples containing the observed band **(Amiryousef *et al.*, 2018)**. Effective multiplex ratio (EMR), that is, the average number of polymorphic bands is equal to the number of polymorphic bands generated by the amplification of all polymorphic primers divided by the number of used polymorphic primers **(Powell *et al.*, 1996)**. Polymorphism information content (PIC) was calculated by the formula $PIC = 1-PP_{ij}^2$, where Pij represents the frequency of the i-th site that appears in the j-th gene **(Powell *et al.*, 1996)**. Marker index (MI), a parameter that can reflect the overall efficiency of a molecular marker technique or single primer, was calculated by multiplying the effective multiple ratio by the average polymorphism information content or the polymorphism information content of single primer **(Powell *et al.*, 1996)**.

RESULTS AND DISCUSSION

To verify the usability of EBAP primers for the study of polymorphism of old maize genotypes, in our work we used 36 EBAP primers designed by **Xiong *et al.*, (2022)**. All 36 EBAP primers produced product, with the highest number of fragments provided by primer EBAP18 (14) and the lowest number of fragments provided by primers EBAP5, EBAP11, EBAP12 and EBAP 31 (3). In Figure 1 you can see the number and size of fragments obtained using the EBAP4 marker. The total average number of fragments per primer was 6.75. The number of polymorphic fragments using 36 EBAP primers ranged from 13 (EBAP18) to 1 (EBAP3, EBAP5, EBAP12, EBAP20, EBAP23, EBAP28, EBAP31 and EBAP33) with an appropriate number of 4.36 polymorphic fragments per primer. The percentage of polymorphism of the analyzed fragments ranged from 100% (EBAP4, EBAP 27 and EBAP36) to 16.67% (EBAP3 and EBAP28) with an average value of 57.94% (Table 3). For all used EBAP primers, the following values were also calculated: Na - Observed Number of Alleles; Ne - Effective Number of Alleles; H - Nei's Gene Diversity; I - Shannon's Information Index; PI - Polymorphic Index; Rp - Resolving Power; PIC - Polymorphism Information Content; MI - Marker Index. All obtained calculated values are in Table no. 3. High PIC values were calculated, ranging from 0.8614 (EBAP9 and EBAP17) to 0.6507 (EBAP4) with an average value of 0.7845. In general, if the PIC value is higher than 0.5, then the discriminating power of the primers for the study of polymorphism is good. We determined a PIC value higher than 0.6 for all 36 EBAP primers used, which indicates a very good discriminating ability of EBAP primers for the study of polymorphism of old maize genotypes.

By using 36 EBAP primers, we managed to divide the 40 old analyzed maize genotypes into 2 main clusters (cluster 1 and cluster 2) (Figure 2), while 2 genotypes from Poland (Przebedowska Biala and Przebedowska Burskynowa) were separated separately in the obtained dendrogram. Dendrogram results suggest that these 2 Polish genotypes may have the same genetic origin. Cluster 1 contained 3 genotypes, of which 2 were from Poland (Zlota gorecka and Wielkopolanka) and 1 genotype originated from the Union of Soviet Socialist Republics (Voroneskaja). Cluster 2 is divided into 2 subclusters (2A and 2B). Subcluster 2A contains 5 genotypes, 3 originating from the Union of Soviet Socialist Republics (Belaja mestnaja, Moldavskaja and Partizanka) and 2 maize genotypes originating from Poland (Zloty Zar and Czechnicka).

Subcluster 2B is also divided into 2 subclusters (2BA and 2BB). Subcluster 2BA contains 6 analyzed maize genotypes, of which 4 are from the Union of Soviet Socialist Republics (Dnepropetrovsk, Kostycevskaja, Zakarpatskaja and Krasnodarskaja) and 2 genotypes originating from Hungary (Šamorinsky konský zub and M Silokukurica). Subcluster 2BB is further divided into subclusters 2BBA and 2BBB. Subcluster 2BBA contains 1 genotype originating from the Union of Soviet Socialist Republics (Bezuncukskaja). The 2BBB subcluster is also divided into 2 subclusters - 2BBBA and 2BBBB. Subcluster 2BBBA contains 3 maize genotypes originating from Hungary (Aranyozon sarga lofogu, Iregszemeseil 2 hetes and Mindszentpusztai Feher). Subcluster 2BBBB contains 20 analyzed maize genotypes, of which 3 are from Hungary, 6 from Slovakia, 2 from Yugoslavia and 9 from Czechoslovakia. The results from the obtained dendrogram confirmed the usability of EBAP primers for the study of corn polymorphism and also the ability to distinguish the analyzed old genotypes of corn from each other. The newly developed EBAP primer technique has many advantages over classical techniques used to study plant polymorphism. Its main advantages include simplicity, speed of analysis, polymorphism detection and detection of a large number of fragments, only one simple primer and the need for a small number of personnel to perform the analysis. Also, the easy and simple evaluation of the results and the possibility of using it in all plant species are a great advantage over other techniques (SSR, AFLP, ISSR) for the detection of polymorphism and relatedness between genotypes. So far, only two authors have used EBAP primers to study plant polymorphism. **Xiong *et al.*, (2022),** who developed the EBAP technique, used it to study polymorphism of maize, sugarcane, potato, cassava, cabbage, and peanut. The results showed that it detected more abundant DNA polymorphisms in the first five crops, but not in the cultivated peanut. In addition, different band patterns were obtained by amplifying peanut cDNA with different single primers. For ten maize, thirty-three single EBAP primers were screened, of which eighteen single primers could successfully amplify clear and repeatable bands. A total of one hundred and thirteen bands were amplified, of which sixty-four were polymorphic bands, accounting for 56.64% of the total bands. The value of polymorphic information content (PIC) for the authors ranged from 0.5644 (EBAP4) to 0.8686 (EBAP20) with an average value of 0.8165. In our work, we calculated PIC values from 0.6488 (EBAP11) to 0.8614 (EBAP9 and EBAP17) with an average value of 0.7845. These minimum, maximum and average PIC values determined by us coincide with the values calculated by the authors in their work. These results therefore indicate a good discriminatory ability of EBAP markers and their usefulness for studying polymorphism and relatedness in different plant species.The authors also calculated the values of index: TNB, NPB, PPB, Na, Ne, H, I, PI, Rp, PIC, MI for each primer. The calculated index values were similar to the values we also obtained in our analyses. Similar results to me have **Boutsika *et al.,* (2025)** used three molecular markers namely inter simple sequence repeats (ISSRs), start codon targeted (SCoTs), and exon-based amplifed polymorphisms (EBAPs) to conduct the frst comprehensive genetic analysis of 12 *Rosa canina* genotypes. As for the EBAP markers, a total of 95 loci across 12 population samples were detected. The average number of distinct alleles (Na) was 1.821, whereas the average number of efective alleles (Ne) was 1.537. The Shannon's Information Index (I) was 0.457, suggesting a moderate to high amount of genetic variation. The diversity (h) and unbiased diversity (uh) were 0.309 and 0.342, respectively. In total, 95 bands were recorded, each with a frequency of 5% or above. Suggestively, no bands that were prevalent in 25% or less or 50% or less of the populations were observed, thus suggesting a lack of general uniformity in band frequencies. Additionally, UPGMA dendrograms based on Dice distance were constructed for each marker. The author in his work achieved a much higher Percentage of Polymorphism (%P- 82,11) than we calculated in our work (%P-57,94). This result indicates that the EBAP technique has a huge use for studying polymorphism and can be used to distinguish genotypes based on origin and has great use in plant breeding and marker-assisted selection (MAS). The results of this author also confirmed that it is a suitable technique for determining genetic relatedness and can be used in a large number of plants. **Bocianowski *et al*., (2021)** analyzed 13 hybrid maize genotypes using 30 SSR markers. They determined the PIC value as the most significant indicator of polymorphism, which ranged from 0.077 (phi041) to 0.497 (phi061) with an average value of 0.274. These minimum, maximum and average values are much lower than those determined in our work, and therefore it can be concluded that the EBAP technique is much more effective for determining genetic similarity and polymorphism than the SSR technique. Another author who analyzed the genetic biodiversity of maize was **Rizk *et al*., (2024)**. The authors used 12 SCoT markers in their work to study the polymorphism of twelve Egyptian *Zea mays* L. hybrids and calculated various indices that speak about polymorphism and the best index to determine genetic relatedness was PIC, whose values were from 0.27 (SCoT-1) to 0.37 (SCoT-4-7) with an average of 0.34. Again, these authors determined lower values using SCoT markers than we did, which again points to the great importance of EBAP markers for the study of maize polymorphism. **Madanar *et al*., (2024)** analyzed 56 Indian maize genotypes using 1166 SNP markers. They calculated PIC values from 0.09 to 0.3 which were again lower than those calculated in our work. In conclusion, we can say that the new EBAP technique is very suitable for studying polymorphism and relatedness in maize and has further great applications in plant breeding and marker-assisted selection (MAS) of plants. It is a suitable alternative technique to techniques such as RAPD, SCoT, TRAP, ISSR, SSR techniques.

Table 3 DNA polymorphisms among 40 old maize varieties revealed by 36 EBAP primers.

Primer	TNB	NPB	PPB(%)	Na	Ne	H	I	PI	Rp	PIC	MI
EBAP1	4	2	50	1.5	1.27	0.16	0.24	0.79	1.2	0.76	2.43
EBAP2	8	7	87.5	1.87	1.61	0.34	0.49	2.32	3.33	0.81	8.04
EBAP3	6	1	16.67	1.16	1.02	0.02	0.03	0.22	0.25	0.83	4.78
EBAP4	10	10	100	2	1.53	0.32	0.49	3.73	5.8	0.65	2.31
EBAP5	3	1	33.33	1.33	1.17	0.11	0.1	0.48	0.8	0.71	2.55
EBAP6	6	3	50	1.52	1.37	0.21	0.30	1.44	2.42	0.83	2.67
EBAP7	9	6	66.67	1.66	1.29	0.17	0.27	1.88	2.5	0.75	4.28
EBAP8	13	10	76.92	1.76	1.43	0.25	0.38	3.27	4.87	0.81	4.49
EBAP9	8	6	75	1.75	1.50	0.29	0.43	1.7	2.2	0.86	3.06
EBAP10	5	2	40	1.4	1.22	0.13	0.19	0.8	1.2	0.80	2.57
EBAP11	3	2	66.67	1.66	1.25	0.17	0.29	0.8	1.2	0.64	2.07
EBAP12	3	1	33.33	1.33	1.17	0.11	0.17	0.48	0.8	0.71	2.55
EBAP13	7	4	57.14	1.57	1.26	0.17	0.27	1.76	2.8	0.79	2.52
EBAP14	8	4	50	1.51	1.23	0.14	0.22	1.3	1.8	0.82	2.93
EBAP15	5	3	60	1.6	1.34	0.20	0.31	1.12	1.6	0.78	2.78
EBAP16	5	4	80	1.8	1.52	0.29	0.44	1.6	2.4	0.75	2.39
EBAP17	8	6	75	1.75	1.50	0.29	0.43	1.7	2.2	0.86	3.06
EBAP18	14	13	92.86	1.92	1.61	0.35	0.51	5.05	7.55	0.77	7.64
EBAP19	12	10	83.33	1.83	1.50	0.29	0.43	3.8	5.77	0.77	7.65
EBAP20	5	1	20	1.2	1.04	0.03	0.06	0.32	0.4	0.81	2.59
EBAP21	8	5	62.5	1.62	1.31	0.19	0.29	1.76	2.4	0.79	2.53
EBAP22	8	7	87.5	1.87	1.61	0.34	0.49	2.32	3.33	0.81	8.04
EBAP23	4	1	25	1.25	1.09	0.06	0.11	0.42	0.6	0.77	2.76
EBAP24	6	3	50	1.52	1.37	0.21	0.30	1.44	2.42	0.83	2.67
EBAP25	9	4	44.44	1.44	1.20	0.13	0.20	1.56	2.4	0.84	3.01
EBAP26	5	2	40	1.4	1.22	0.13	0.19	0.8	1.2	0.80	2.57
EBAP27	4	4	100	2	1.64	0.38	0.57	1.44	2.8	0.67	2.16
EBAP28	6	1	16.67	1.16	1.02	0.02	0.03	0.22	0.25	0.83	4.78
EBAP29	7	3	42.85	1.42	1.09	0.07	0.13	0.88	1.12	0.79	4.36
EBAP30	6	4	66.67	1.66	1.43	0.24	0.36	1.6	2.4	0.8	2.55
EBAP31	3	1	33.33	1.33	1.17	0.11	0.17	0.48	0.8	0.71	2.55
EBAP32	9	8	88.89	1.88	1.65	0.35	0.51	2.43	3.12	0.84	4.62
EBAP33	5	1	20	1.2	1.04	0.03	0.06	0.32	0.4	0.81	2.59
EBAP34	6	3	50	1.52	1.37	0.21	0.30	1.44	2.42	0.83	2.67
EBAP35	8	7	87.5	1.87	1.61	0.34	0.49	2.32	3.33	0.81	8.04
EBAP36	7	7	100	2	1.58	0.30	0.45	1.82	2.37	0.65	3.61
Mean	**6.75**	**4.36**	**57.94**	**1.59**	**1.34**	**0.21**	**0.30**	**1.55**	**2.29**	**0.78**	**3. 69**
Total	**243**	**157**									

Note: TNB - Total Number of Bands; NPB - Number of Polymorphic Bands; PPB - Percentage of Polymorphic Bands; Na - Observed Number of Alleles; Ne - Effective Number of Alleles; H - Nei's Gene Diversity; I - Shannon's Information Index; PI - Polymorphic Index; Rp - Resolving Power; PIC - Polymorphism Information Content; MI - Marker Index.

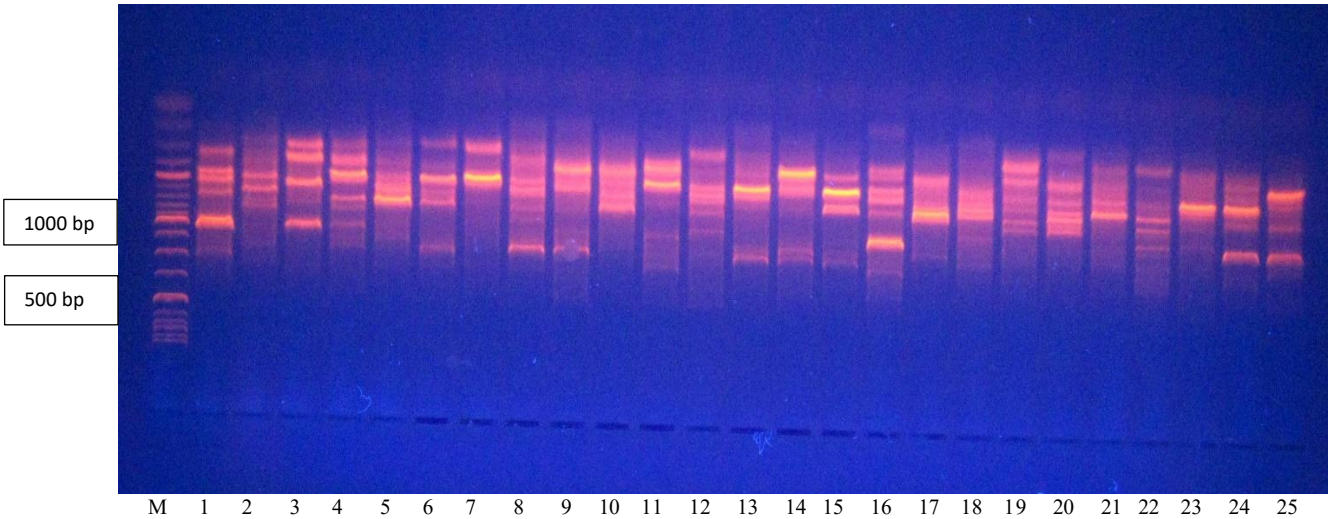

Figure 1 The agarose gel electrophoresis of 25 old maize genotypes with primer EBAP4. Numbers 1-25 are designations of genotypes from table no. 1 and M - 100 bp DNA ladder.

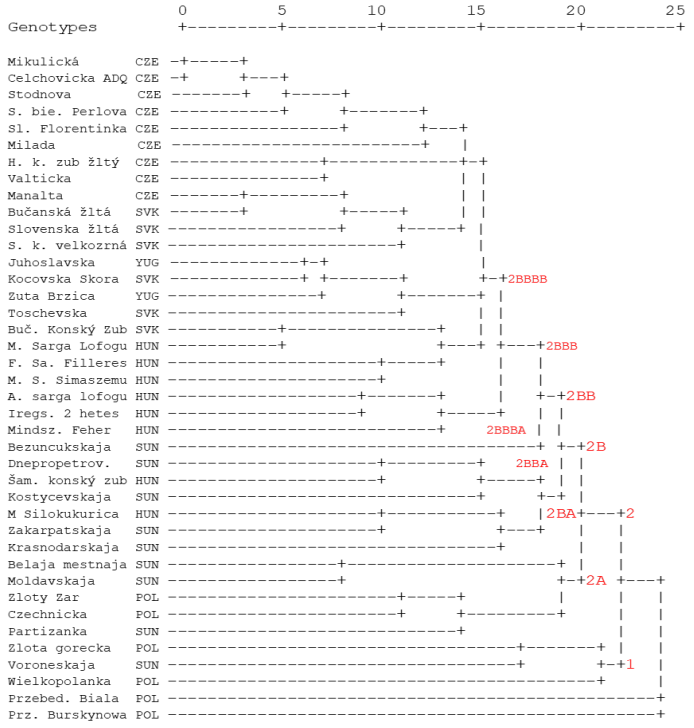

Figure 2 Dendrogram of 40 maize genotypes prepared based on 36 EBAP markers. CZE - Czechoslovakia, HUN - Hungary, POL - Poland, SUN – Union of Soviet Socialist Republics, SVK – Slovakia, YUG- Yugoslavia. We got such an origin of genotypes and with such a designation from the Gene Bank VURV Praha-Ruzyne (Czech Republic) and from the Gene Bank in Piešťany, the Slovak Republic.

CONCLUSION

The genetic diversity analysis of the old maize germplasm collection using EBAP markers has demonstrated substantial genetic variation within the species. All 36 EBAP primers produced product, with the highest number of fragments provided by primer EBAP18 (14) and the lowest number of fragments provided by primers EBAP5, EBAP11, EBAP12 and EBAP 31 (3). The total average number of fragments per primer was 6.75. The number of polymorphic fragments using 36 EBAP primers ranged from 13 (EBAP18) to 1 (EBAP3, EBAP5, EBAP12, EBAP20, EBAP23, EBAP28, EBAP31 and EBAP33) with an appropriate number of 4.36 polymorphic fragments per primer. The percentage of polymorphism of the analyzed fragments ranged from 100% (EBAP4, EBAP 27 and EBAP36) to 16.67% (EBAP3 and EBAP28) with an average value of 57.94%. We determined a PIC value higher than 0.6 for all 36 EBAP primers used, which indicates a very good discriminating ability of EBAP primers for the study of polymorphism of old maize genotypes. By using 36 EBAP primers, we managed to divide the 40 old analyzed maize genotypes into 2 main clusters (cluster 1 and cluster 2), while 2 genotypes from Poland (Przebedowska Biala and Przebedowska Burskynowa) were separated separately in the obtained dendrogram. These findings have significant implications for sustainable exploitation strategies and future breeding programs of maize. Such assets are ultimately aimed to enable future growers to optimize production and meet market demands more effectively.

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