

# Exploring chemical composition and genetic dissimilarities between maize accessions

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## Abstract

The capacity of maize (*Zea mays* L.) accessions to tolerate drastically extreme conditions in Iraq, contributes to the characterization of the genetic resources for germplasm management and the identification of the finest genotypes for genetic improvement. Therefore, breeding maize program requires knowledge of genetic variation and genetic structure. A total of 25 maize accessions from three regions (Iraq-Sulaimani, Iraq-Erbil and Iran-Sanandaj) were genotyped by chemical and phytochemical components and simple sequence repeats (SSR) markers to evaluate genetic diversity, population composition and the relationships between genetic and chemical composition dissimilarities. In terms of proximate and phytochemical parameters, the maize accessions exhibited large significant disparity, in which oil, phenol contents and 2,2-diphenyl-1-picrylhydrazyl (DPPH) characteristic appeared to be the most discriminating features of maize accessions. Altogether, 18 SSR markers produced 77 polymorphic alleles across the 25 samples, and the chosen SSR was extremely informative with polymorphic information content (PIC) varied from 0.91 (BnlG1890) to 0.37 (Umc1630 and BnlG1189), as well as gene diversity (ranging from 0.48 to 0.91, with an average of 0.75) illustrating the broad genetic variability of the accessions investigated. Molecular variance assessment (AMOVA) showed that there was only 21% genetic variation among populations. Pairwise PhiPT distance (0.10 to 0.31) stated high population distinctions among the populations investigated. In addition, the accessions from three regions were differentiated into seven clusters by both methods; clustering and population structure analysis and the accessions are not grouped in term of geographic locations. Both chemical composition and SSR markers differentiated 25 maize accessions. The results of the Mantel test exhibited a significant positive linkage between chemical components and SSR matrices. The results of this research revealed that maize accessions have a broad genetic diversity that provides a source of new and unique alleles that are helpful for maize breeding programs to address the continuing and future significant challenges and determining collections of well-known cultivars and disparities between them.

## Introduction

Maize (*Zea mays* L.) is one of the world's most diverse crops, characterized by a large level of genetic variability owing to an expanded selection mechanism before distribution to other parts of the world (Matsuo-ka et al., 2002; Whitt et al., 2002). It is annually C4 plant, cross-pollinated and has 10 chromosomes (Singh and Jauhar, 2006). In the latest years, interest in maize as a food plant has also increased in developing countries due to some factors. Maize kernels provide many macro and micronutrients needed for human metabolic requirements, and the quantity of some vital nutrients

are poorly balanced or insufficient for customers who depend on maize as a significant source of food. It is used as human food, animal feed, revenue and source of employment for an overwhelming majority of the population (Legesse et al., 2007). The composition of chemical substances in maize grain is particularly interesting because of the existence of substantial phytochemicals expressing antioxidant activity and other potential health advantages. Maize is rich in antioxidant molecules like phenols, tocopherols, carotenoids, anthocyanins and flavonoids (Nuss and Tanumihardjo, 2010). The maize breeding program in Iraq lacks distantly related

sources and has had little impact in producing high yield hybrids. Moreover, the characterization of maize germplasm in terms of molecular, chemical components and antioxidants is important with a particular focus on local germplasm to define the most appropriate raw materials to be utilized by the food industry and for sustainability.

To highlight promising combinations to be used as source materials in a study program for the use of heterosis, understanding of biological diversity and relationships between maize accessions is crucial. Although phenotypic differences are a method for assessing the effectiveness of agronomic plants while also being inexpensive and convenient, they lead to low polymorphism and are influenced by the climate, thereby determining a restricted heritability in accession collections. Other techniques, molecular markers like amplified fragment length polymorphism (Giordani et al., 2019), single nucleotide polymorphism (Dari et al., 2018) and simple sequence repeats (Aci et al., 2018; Tahir and Maeruf, 2018b), are commonly used to assess the accession relationships. In comparison with other molecular markers, microsatellite markers are the easy and the perfect choice to study the maize populations genetic structure due to their co-dominant character and elevated variability. The simple sequence repeats (SSR) loci consisting of 2 to 6 base pair tandem repeats and are regarded to be co-dominant markers, multiallelic, extremely polymorphic and randomly dispersed over the genome, are widely used to analyze maize genetic distance. The current research evaluated proximate and phytochemical components and molecular diversity in 25 maize accessions using phytochemical and SSR analysis, to assist categorize the identity of accessions gathered from distinct locations and evaluate the association between molecular and chemical composition distance.

## Materials and Methods

### Plant materials and preparation

Twenty-five accessions of maize (*Zea mays* L.) representing the three areas (Iraq-Sulaimani, Iraq-Erbil and Iran-Sanandaj) were sampled (Table 1). The soil used for the trial was ploughed. Ridges were manually produced using hoes. Three replicated randomized complete block design (RCBD) layout was used in the field of College of Agricultural Sciences, University of Sulaimani. On April 16, 2017, three seeds per hill were planted for each genotype. Thirty plants per plot with a length of 3 m were held in three rows. The range between two rows and two plants, respectively, was 0.75 m and 0.25 m. The plots were fertilized with the 20% N urea, 20%

P<sub>2</sub>O<sub>5</sub>, superphosphate and 20% potassium with 20 g m<sup>-2</sup>, respectively, as the first amount at implanting, and the second amount as urea (32 g m<sup>-2</sup>) after 45 days from germination. Self-plant crosses were produced; each cross was produced in 10 ears as well as the self-pollination for each accession was produced. The 10 ears per plot were collected at maturity for each accession.

### DNA extraction

Ten leaves from each accession were gathered and ground with liquid nitrogen. A modified cetyltrimethylammonium bromide (CTAB) method was used to extract DNA from leaves of two-week-old seedlings as described in Tahir et al. (2019).

### Proximate composition

One gram of ground seeds was used for determining the total oil content (TOC) and total carbohydrate content (TCC) by using the protocol described by Tahir and Maeruf (2018a), and Hedge and Hofreiter (1962).

### Phytochemical and antioxidant analysis

One mL of distilled water was added to 0.10g of powdered seeds. The mixture was shaken for 2 h at 20±1°C and incubated overnight for 16 h at 4° C. The total components of phenols and antioxidant capacity was determined as described by Tahir et al. (2019). The amount of phenolic, flavonoid compounds and DPPH activity in samples were displayed in the equivalent of mg gallic acid/g dry matter (DM), the equivalent of mg quercetin/g dry matter (DM), and the equivalent of µg Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid)/g dry matter (DM), respectively.

### SSR assay

The eighteen microsatellite loci variable supplied a high-quality amplification product and was used to analyze all maize samples. From the Maize GDB page ([https://www.maizegdb.org/gene\\_center/gene](https://www.maizegdb.org/gene_center/gene)), these SSR loci were chosen based on chromosome location. PCR reactions were performed in an overall quantity of 20 µL, comprising approximately 100 ng of DNA template, 0.2 µM each primer (Biolytic Lab Performance, INC), and 10 µL master mixes (Promega). The requirements for PCR were as follows: initial denaturation at 94° C for 8 min, accompanied by 36 cycles of 1 min denaturation at 95° C, 1 min annealing at 55 or 58 or 59° C, 2 min incubation at 72° C and final elongation at 72° for 7 min. The separation of the amplification products was performed with 6% polyacrylamide (Huang et al., 2018).

### Scoring and analyzing statistical data

The scorable bands were both present (1) and absent (0) coded manually. Scored data was used to calculate the similarity coefficient of Jaccard using XLSTAT 2016 software (XLSTAT version, 2016). Using the unweighted pair-group technique with arithmetic averages (UPGMA), the Jaccard coefficient was transformed into a dissimilarity matrix to generate dendrogram. The Power Marker version 3.25 software has been used to calculate polymorphism data content (PIC) and gene diversity (GD) (Tahir and Omar, 2017). GenAlEx version 6.5 software also used to assess the PhiPT distance and molecular variance between the populations and within them. A model assessment was carried out for population structure to conclude genetic composition and explain the number of sub-populations using the STRUCTURE version 2.3.4 software (Pritchard et al., 2000; Evanno et al., 2005). The models of ancestry and allele frequency implemented in this work were, respectively, admixture model and correlated allele frequencies. The number of alleged populations (K) has been set from 1 to 10 and the assessment has been repeated 6 times. The burn-in and MCMC were solved at 50,000 each for each run and placed iteration in 6. To set accessions into populations, the run with the greatest probability

was used. The Mantel test was calculated by using software XLSTAT, version 2016.

### Results and discussion

#### Accessions diversity in proximate and phytochemical components

Data of Table 2 demonstrated the chemical proximity and phytochemical investigation of 25 maize accessions and significant distinctions have been stated among different accessions. These materials varied widely by the proximate and phytochemical parameters, which ranged from: 3.33 (Cantabpis) to 7.83% (M844), 59.52 (ZP.434xB) to 66.91% (MAS-52C), 0.10 (M3007) to 0.38 mg g<sup>-1</sup> DM (MSI-4279), 0.005 (Medium-791) to 0.15 mg g<sup>-1</sup> DM (M15-H3OV), and 384.57 (M3007) to 585.95 µg g<sup>-1</sup> DM (MSI-4279) for the TOC, TCC, TPC, TFC and DPPH activity, respectively. The dissimilarities in the comparative concentration of proximate components are due to the construction of the mature kernel consisting of 80% of the endosperm portion and 10% of the dry matter segment of the germ. These variabilities indicated the presence of an interesting variant among the maize accessions for the ability to accumulate these compounds and suggested differences in genetic potential between maize accessions. In addition, the distinction in chemical composition among the twenty-five accessions affirmed that varietal differences impact the biosynthesis of the proximate constitution and phytochemical components in maize accessions. To obtain a more informative description of these materials, the discriminatory analysis was designed for the chemical and phytochemical composition parameters by using UPGMA cluster analysis based on Euclidean distance coefficients (Fig. 1). This has attracted great interest due to the wide range of genetic distances from 2.15 to 201.38, suggesting that the accessions for proximate and phytochemical constituents were genetically varied. As expected, cluster analysis shared the 25 accessions into five distinctive clades. It was found that the third clade comprised the highest number of accessions (13 accessions), while the second clade record the minimum number of accessions (1 accession). It was observed that both accessions; Medium-791 and MOH-53 created a tightly associated sub-cluster within the third cluster (with a genetic distance of 2.15), whereas MSI-4279, appeared the highest content in TPC, TFC and DPPH activity, had been located in isolated class. The DPPH activity was the distinguishing character among the clades. It's important to remember that the clusters obtained with the chemical data did not seem noticeably connected to the geographical origin of the accessions and the accessions were dispersed uniformly across all of the clusters. The iden-

**Table 1 -Code, name and origin of different maize accessions.**

Code	Name of accession	Sources
G1	MSI-4279	Iraq-Erbil
G2	ES-Solito-635	Iran-Sanandaj
G3	PR36-BO8	Iraq-Erbil
G4	ZP.434xA	Iraq-Sulaimani
G5	ZP.434xD	Iraq-Sulaimani
G6	Talar	Iraq-Sulaimani
G7	Medium-791	Iran-Sanandaj
G8	MAS-52C	Iraq-Erbil
G9	Dhqan	Iran-Sanandaj
G10	Cantabpis	Iran-Sanandaj
G11	Niarano-10501	Iraq-Erbil
G12	ZP.434xC	Iraq-Sulaimani
G13	MSIxB	Iraq-Sulaimani
G14	Btaris	Iran-Sanandaj
G15	NK COBALT-Nx 34476	Iraq-Erbil
G16	M3078xB	Iraq-Erbil
G17	M844	Iraq-Erbil
G18	MOH-53	Iraq-Erbil
G19	ZP.434xB	Iraq-Sulaimani
G20	PR-35T36	Iraq-Erbil
G21	Pio-3751	Iraq-Erbil
G22	M15-H3OV	Iraq-Erbil
G23	M3007	Iraq-Erbil
G24	ES-Solito-278	Iran-Sanandaj
G25	MSI-43100	Iraq-Erbil

tifying of favourable chemical characteristics in maize accessions might be useful for the exploiting of these traditional accessions, with the introduction of the preferred characters in an accession. However, protein, oil, and starch kernel content, as well as phytochemical features of the kernel, are significant quality attributes (Goggi et al., 2008; Moore et al., 2008). TOC in our work was higher than the results of previous studies (Thomison et al., 2003; Jaradat and Goldstein, 2013), which were between 3.80 to 4.30% and 4.60 to 5.30%, respectively and smaller than the outcome of Vancetovic et al. (2017), which was in the range of 4.00-11.00%. The average TCC found in this research, however, was smaller (74.29) than earlier reported by Siyuan et al. (2018).

population structure of collections is a significant basis for maize breeding and genetic study. The assay of SSR markers contains a lot of genetic data. SSR markers showed an elevated degree of polymorphism and general 77 alleles were discovered with an average of 4.28 alleles per locus marker (Table 3). With only eight polymorphic bands in the 25 maize accessions, Bnlgl1890 had the highest number of polymorphic alleles (8 alleles). The total length of detecting SSR fragments was between 81 and 652 base pairs. The average number of alleles discovered for SSRs with two base pair motifs was higher (5 alleles) compared to longer-motifs SSRs (4 alleles). The observations from the results of this experiment seem to show that the average number of alleles per maize accession over 18 SSR mar-

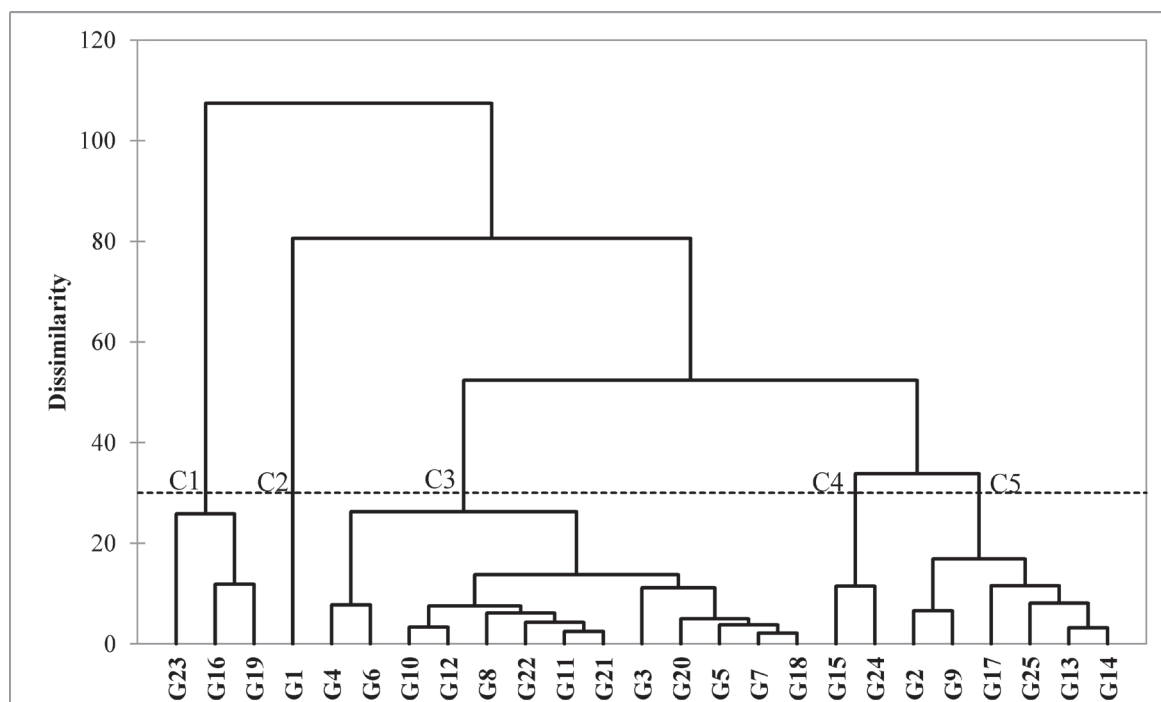
**Table 2 - Proximate contents, phytochemical and antioxidant analysis of 25 maize accessions**

Accessions	TOC (%)	TCC (%)	TPC (mg g <sup>-1</sup> DM)	TFC (mg g <sup>-1</sup> DM)	DPPH (µg g <sup>-1</sup> DM)
G1	5.50 efgh	62.33 abcd	0.38 a	0.14 a	585.95 a
G2	5.50 efgh	62.42 abcd	0.31 c	0.01 lm	472.78 ghij
G3	5.00 hi	62.42 abcd	0.31 c	0.02 lm	505.32 defg
G4	5.67 efgh	60.69 cd	0.34 b	0.06 fg	543.51 bc
G5	5.33 fg	64.34 abc	0.30 c	0.03 kl	518.05 bcdef
G6	5.83 defgh	60.94 bcd	0.36 b	0.09 e	551.27 b
G7	5.17 ghi	62.74 abcd	0.27 d	0.005 m	515.22 cdef
G8	3.83 j	66.91 a	0.26 d	0.03 jk	524.65 bcde
G9	4.33 ij	66.86 a	0.21 ef	0.11 cd	468.07 hij
G10	3.33 j	61.11 bcd	0.26 d	0.05 hi	530.31 bcde
G11	3.83 j	62.75 abcd	0.30 c	0.12 bc	525.12 bcde
G12	5.00 hi	61.91 bcd	0.27 d	0.12 bc	533.14 bcd
G13	4.33 ij	60.83 cd	0.21 ef	0.05 gh	487.87 fgh
G14	5.67 efgh	63.08 abcd	0.19 fg	0.12 b	485.10 fghi
G15	6.50 bcde	63.26 abcd	0.12 hi	0.04 hij	453.50 ij
G16	6.33 bcdef	60.60 cd	0.14 h	0.12 bc	416.16 kl
G17	7.83 a	65.60 ab	0.18 g	0.12 bc	494.10 efgh
G18	6.17 cdefg	64.01 abcd	0.30 c	0.07 f	513.81 cdef
G19	7.17 abc	59.52 d	0.18 g	0.11 bc	404.38 l
G20	7.33 ab	60.13 cd	0.21 ef	0.01 lm	518.05 bcdef
G21	5.67 efgh	61.35 bcd	0.21 ef	0.10 de	524.18 bcde
G22	7.67 a	59.76 cd	0.23 e	0.15 a	526.54 bcde
G23	6.83 abcd	62.51 abcd	0.10 i	0.03 kl	384.57 l
G24	5.83 defgh	62.28 abcd	0.18 g	0.06 fg	442.10 jk
G25	5.00 hi	64.34 abc	0.23 e	0.03 ijk	479.25 ghi
Mean	5.63	62.51	0.24	0.07	496.19
Standard deviation	1.19	2.00	0.07	0.05	47.77
Minimum	3.33	59.52	0.10	0.005	384.57
Maximum	7.83	66.91	0.38	0.15	585.95

### Molecular diversity parameters

Discovering genetic distinctions and interpreting genetic connections among accessions are of excellent significance for species protection and sustainable use of plant genetic resources. Genetic variability and

markers was 30.48, resulting in an average of 1.69 alleles per SSR marker. Moreover, the lowest number of alleles was noticed in Btaris with 22 alleles and the highest in PR36-BO8 with 40 alleles. In this investigation, the average of polymorphic allele (4.28) was significantly higher than the maize previously reported by Legesse



**Fig. 1 - Cluster analysis of 25 maize accessions initiated from different regions based on information on the proximate and phytochemical elements using Euclidean distance and UPGMA methods.**

et al. (2007) and Bantte et al. (2003) who identified 3.85 alleles with 28 SSR loci and 3.25 alleles using 3 SSR markers, respectively and slightly lower than that obtained by Warburton et al. (2002) and Patto (2004) who discovered 4.90 alleles with 85 SSR loci and 5.30 alleles with 80 SSR loci, respectively. With respect to PIC and

GD, 15 primers showed values of PIC and GD greater than 0.60. The fact that the majority of primers used in our research were therefore extremely polymorphic. The PIC ranged from 0.91 (Bnlg1890 and Phi126) to 0.37 (Umc1630 and Bnlg1189). The higher value of PIC referred to a higher degree of SSR marker polymorphi-

**Table 3 - Primers information and diversity parameters of 18 SSR markers used in this study**

Marker	Bin	Motif	No. of polymorphic bands	PIC	GD
Umc1630	1.11	ATGGG	2.00	0.37	0.48
Umc1946	2.07	GCTGCT	6.00	0.89	0.90
Bnlg1189	4.07	AG	2.00	0.37	0.48
Umc1069	8.08	GGAGA	6.00	0.82	0.83
Umc1653	6.07	GAAA	5.00	0.79	0.82
Bnlg1810	9.01	AG	4.00	0.70	0.74
Phi069	7.05	GAC	6.00	0.87	0.88
Phi126	6.00	AG	5.00	0.91	0.91
Bnlg1867	6.01	AG	6.00	0.79	0.80
Umc2013	5.07	NA	2.00	0.43	0.53
Phi037	1.08	AG	6.00	0.75	0.77
Umc1038	10.07	CT	6.00	0.88	0.89
Bnlg1194	8.02	AG	3.00	0.68	0.73
Bnlg1429	1.02	AG	3.00	0.68	0.73
Bnlg1890	4.11	AG	8.00	0.91	0.91
Bnlg1108	3.08	AG	2.00	0.62	0.69
Umc401	2.05	GGA and AG	2.00	0.58	0.62
Umc1126	2.08	AG	3.00	0.80	0.82
Mean			4.28	0.71	0.75

sm and thus assisted in the selection of the best SSR markers in the genetic divergence analysis.

In this work, the average PIC value (0.71) was high compared to other research findings reported by Enoki et al. (2002), Legesse et al. (2007), Li et al. (2014), Lopes et al. (2015) and Aci et al. (2018), who displayed 0.69, 0.58, 0.49, 0.41 and 0.62, respectively as the PIC mean value. In this study, dinucleotide SSR loci recognized the largest mean PIC value relative to tri, tetra and pentanucleotide repeats, which is also in close agreement with previous observations of maize (Senior et al., 1998; Enoki et al., 2002; Legesse et al., 2007). The maize genotypes collection also had the largest gene diversity, ranging from 0.48 (Umc1630 and BnlG1189) to 0.91 (BnlG1890 and Phi126) with an average of 0.75. Overall gene diversity (0.75) was higher than in the Algerian (0.40), Ghanaian (0.47) and Algerian Sahara (0.46) previous report (Aci et al., 2013; Oppong et al., 2014; Aci et al., 2018). It can be seen that this wide distinction between the minimum and the highest values of gene diversity means the existence of great variability between maize accessions. Based on the results presented here, it can be concluded that there is a large genetic variation among maize accessions.

#### Genetic relationship and clusters analysis disclosed by SSR data

Band results of genome-covered SSRs were collected and converted to binary datasets and used to conduct cluster analysis on maize accessions to better show genetic structure, linkage and understanding relationships between all the maize accessions acquired from different regions. It was found that the genetic distance for 25 accessions ranged from 0.32 to 0.83. Four accession pairs had dissimilarity coefficients equal to or greater than 0.80. However, the highly dissimilar accessions (0.83), between PR36-BO8 and Dhqan produced in the provinces of Iraq-Erbil and Iran-Sanandaj and between Dhqan and Pio-3751 from Iran-Sanandaj and Iraq-Erbil. This elevated dissimilarity supported the presence of a high rate of DNA divergence. Likewise, ZP.434xD and MAS-52C (0.32) were the least different accessions from Iraq. This small difference proposed that both of them could share a common parentage. All 25 accessions could be split into seven major clusters according to the dendrogram plot (Fig. 2). Cluster VII, the main and largest cluster, was made up of 13 accessions and fully occupied by the accessions of three areas, while the cluster VI included only one genotype (ZP.434xC). From the dendrogram, it is clear that the two most distinct accessions assigned to cluster III, are Dhqan and NK COBALT-Nx 34476 with a strong dissimilarity coefficient. These results demonstrated greater

reliability in the investigation of genetic relationships among tested materials using prospective SSRs. In total, 25 maize accessions from different regions had a very wide genetic base.

To explore the genetic differences among 7 populations, both AMOVA and pairwise PhiPT distance assessment were conducted (Tables 4 and 5). The outcome showed that only 21% of the overall genetic variability occurred in populations and 79% of the demographic variation was due to individual heterozygosity within each population. The PhiPT value generally was 0.21 (with a PhiPTmax of 0.66 and a PhiPT of 0.32) with a corresponding permutation p-value of 0.001. This outcome showed a very broad genetic base for the 25 corn accessions.

In addition, the pairwise PhiPT distance between 7 clades or populations ranged from 0.10 to 0.34 in pairs.

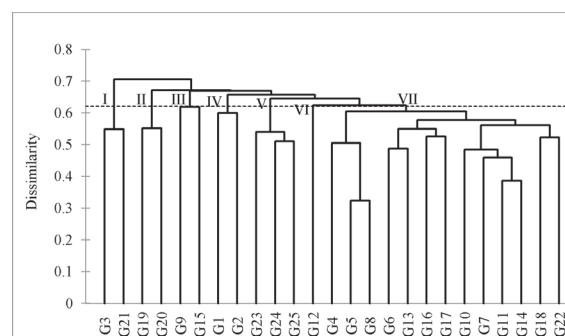


Fig. 2 - Dendrogram based on 18 SSR markers indicating diversity among 25 maize accessions

The largest rate occurred among clades 2 and 4 (0.34), while the lowest was among clades 3 and 5 (0.10). It was found that six values of the PhiPT clade were significant at  $p \leq 0.05$  including the distances between clade 1 and clade 3 (PhiPT=0.17,  $p=0.01$ ), clade 3 and clade 5 (PhiPT=0.10,  $p=0.05$ ), clade 2 and clade 3 (PhiPT=0.30,  $p=0.01$ ), clade 3 and clade 4 (PhiPT=0.15,  $p=0.01$ ), clade 3 and clade 6 (PhiPT=0.18,  $p=0.01$ ) and clade 3 and clade 7 (PhiPT=0.23,  $p=0.00$ ). This is a prevalent area of importance and research interest because the matrix of the pair clade range PhiPT also reveals the genetic difference between the 25 individuals from the distinct areas analyzed in our research.

To analyze the structure of the population of 25 maize accessions, an admixture model-based method was introduced. The studied population's optimum cluster number (K) was seven, with the biggest values of delta K (23.21). A graphical depiction of each individual's estimated involvement coefficient was shown in Fig. 3A. Each color indicates the ratio of each member to the seven populations or clusters, depicted by a vertical line. The accession with a likelihood greater than

**Table 4 -Molecular variance analysis displaying the division of genetic variation between and within the different population based on the data for clustering analysis.**

Source	df	SS	MS	Est. Var.	%	P
Among clades	6	142.99	23.83	3.52**	21%	0.001
Within clade	18	238.25	13.24	13.24**	79%	0.001
Total	24	381.24		16.76	100%	

**Table 5 -Pairwise PhiPT genetic distances (below diagonal) and probability (above diagonal) between 7 clades.**

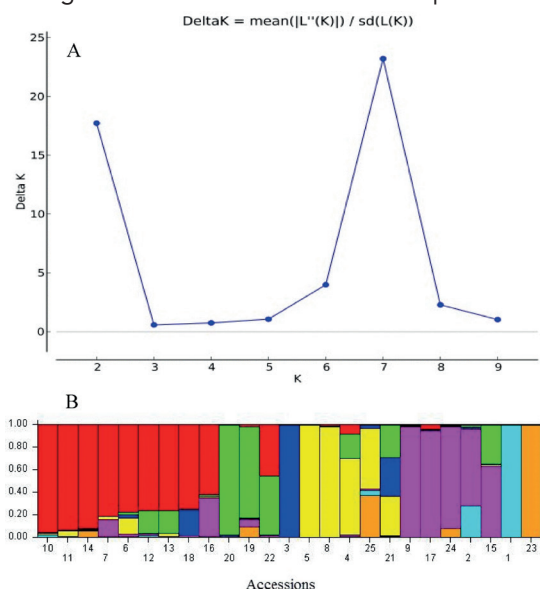
Clades	Clade-1	Clade-2	Clade-3	Clade-4	Clade-5	Clade-6	Clade-7
Clade-1	0.00	0.33	0.01	0.33	0.33	0.35	0.11
Clade-2	0.30	0.00	0.01	0.32	0.33	0.33	0.11
Clade-3	0.17*	0.30**	0.00	0.01	0.05	0.01	0.00
Clade-4	0.19	0.34	0.15*	0.00	0.33	0.35	0.11
Clade-5	0.13	0.24	0.10	0.17	0.00	0.33	0.12
Clade-6	0.21	0.29	0.18**	0.20	0.19	0.00	0.09
Clade-7	0.23	0.29	0.23**	0.24	0.15	0.30	0.00

0.75 was regarded as a pure one and an admixture smaller than 0.75. Of the 25 accessions, 18 were pure, while 7 were admixture (Fig. 3B). In this assessment, the red cluster included 8 pure accessions, the green cluster composed of two pure accessions (Talar and Medium-791) and 1 admixture (M15-H3OV), the cobalt clade consisted of one pure accession (PR36-BO8), the yellow group included 2 pure accessions (ZP.434xD and MAS-52C) and one admixture accession (ZP.434xA), the magenta class incorporated three pure accessions (Dhqan, M844 and ES-Solito-278) and two admixture accessions (ES-Solito-635 and NK COBALT-Nx 34476), the light blue cluster consisted of one pure accession

(MSI-4279) and the golden yellow comprised of one pure accession (M3007). Likewise, as the dendrogram, there is no apparent structure according to the geographic locations. The seven STRUCTURE clusters recognized were mainly consistent with the relationship pattern proposed by the dendrogram (Fig. 2). These results are consistent with earlier studies (Xia et al., 2004a; Xia et al., 2004b; Aci et al., 2018). The output of structure analysis clarified that MSI-4279 and M3007 accessions are distinct in the genome constitution through the population structure assessment.

#### **Correlation between genetic dissimilarity obtained by chemical components and SSR markers**

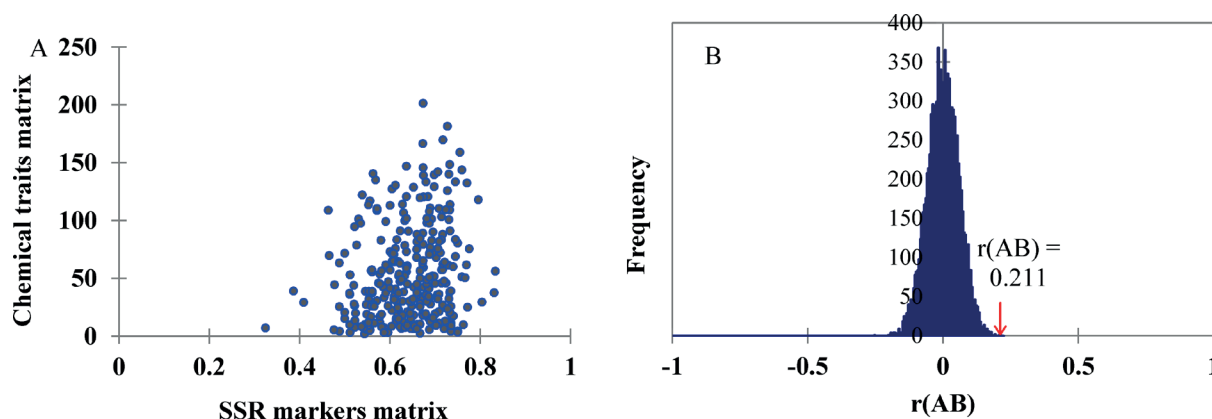
Mantel test was used to assess the relationship between the genetic dissimilarity matrices produced by chemical elements and molecular markers. The weak correlation coefficient value ( $r$ ) was 0.21 with  $p$ -value 0.0001 representing a highly significant correlation (Fig. 4). This demonstrates the possibility of composing reference collections of well-known maize accessions using the information acquired from accession genetic profiles.



**Fig. 3 - Population structure of maize accessions. A: Determining the optimal value of K through the delta K formula as designated by Evanno et al.(2005). B: Results of STRUCTURE assessment based on SSR information for 25 maize accessions, showing the accession group separation as evaluated by k-means partitioning for k =7 with distinct group colors**

#### **Conclusions**

The analyzed genotypes confirmed the excellent dietary values of the maize kernel, especially with regard to the antioxidant compound content and antioxidant capacity. The outcome of proximate and phytochemical components showed the significance of screening germplasm of maize, with particular emphasis on kernel composition. The screening of maize accessions by antioxidant compounds and capacity can be a helpful benefit in the selection of genotypes for foods with elevated antioxidant potential. The set of 18 SSR markers



**Fig. 4 - Mantel test analysis showing the relationship between chemical and SSR data matrices. A: Association between matrices of genetic dissimilarity with Mantel tests. B: p-value and  $r(AB)$  was acquired from the distribution of mantel test results.**

for molecular fingerprinting of maize accessions was effective and cost-effective. High genetic variability was identified among all accessions indicating the chance to exploit the genotypes for the creating of varieties and heterotic communities used to obtain promising inbred lines. The weak significant association between genetic distances revealed that the possibility of composing reference collections of well-known maize accessions using the information acquired from accession genetic profiles.

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