academicJournals

Vol. 12(27), pp. 4269-4276, 3 July, 2013
DOI: 10.5897/AJB12.2644
ISSN 1684-5315 ©2013 Academic Journals
http://www.academicjournals.org/AJB

African Journal of Biotechnology

*Full Length Research Paper*

# Genetic variation and relationships of *Zea mays* and *Sorghum* species using RAPD-PCR and SDS-PAGE of seed proteins

**Osman, G.[1,2*] Munshi, A.[1] Altf, F.[3] and Mutawie, H.[1]**

[1]Biology Department, Faculty of Science, Umm-Al-Qura University, Makka 673, Saudi Arabia.
[2]Agricultural Genetic Engineering Research Institute (AGERI) - (ARC), Giza, Egypt.
[3]Faculty Of Medicine, Umm-Al-Qura University, Makka 673, Saudi Arabia.

Accepted 28 June, 2013

**Genetic relationship between some species of *Zea mays* and *Sorghum* was determined using sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) of seed protein and random amplification of polymorphic DNA (RAPD-PCR) markers. According to SDS-PAGE analysis, 78 band were identified across the studied species. The number of bands varies from 17 bands in sample number 5 to 6 in sample number 6. Analysis of RAPD-PCR of DNA provided more precise information concerning relationships between *Zea mays* and *Sorghum* species than SDS-PAGE analysis. A remarkable result from this study was identifying a close relationship between *Zea mays* spp mays and *Zea mays* spp Mexicana. Further support comes from the molecular data of RAPD, which indicate that close relationship between *Sorghum valgare* and *Sorghum bicolor*.**

**Key words:** *Zea mays, Sorgum volgare pres, Punciu milia ceaum* L. protein, random amplification of polymorphic DNA.

## INTRODUCTION

Phenotypic variation is positively associated with genetic diversity, but is also dependent on environmental factors, as well as, on the interaction between genotypes and environment (Moose and Mumm, 2008). Thus, determining genetic diversity through variation between genotypes, genotype groups, or populations is essential to plant genetic breeding programs. Determining genetic diversity can be based on agronomic, morphological, biochemical, and molecular types of information, among others (Sudre et al., 2007; Goncalves et al., 2009). However, molecular markers have advantages over other kinds, where they show genetic differences on a more detailed level and without interferences from environmental factors, and where they involve techniques that provide fast results detailing genetic diversity (Goncalves et al., 2008; De Souza et al., 2008). With the beginning of studies that led to the development of polymerase chain reaction (PCR) technology (Mullis and Faloona, 1987), there were amazing advances in the refinement of techniques to obtain specific or non-specific DNA fragments, relevant mainly to research in genetic diversity.

The following techniques are those mostly used and are listed in chronological order: simple sequence repeats

*Corresponding author. E-mail:geosman@uqu.edu.sa.

or just microsatellites (SSR) (Tautz, 1989), randomly amplified polymorphic DNA (RAPD) ( Williams et al., 1990) or arbitrarily primed PCR (AP-PCR) (Welsh and McClelland, 1990), inter-simple sequence repeats (ISSR) (Zietkiewicz et al., 1994),amplified fragment length polymorphism (AFLP) (Vos et al., 1995), single nucleotide polymorphisms (SNPs) (Chen and Sullivan, 2003) and, more recently, diversity array technology (DarT) (Kilian et al., 2005). These different types of molecular markers are also different as to their potential to detect differences between individuals, their cost, facilities required, consistency and replication of results (Schlotterer, 2004; Schulman, 2007; Bernardo, 2008).

Maize (*Zea mays* L.) is one of the most diverse crop species, containing tremendous variation in morphological and physiological traits and extensive polymorphism in its DNA sequences. This exceptional diversity allows maize to be cultivated in a range of environments from temperate to tropical regions including parts of Africa, the Amazonian rainforest, Arizona deserts, the Gaspe Peninsula in Canada, and the Andes Mountains in Latin America. Internationally, maize-breeding programs have focused on the development of high-yielding cultivars that can meet the challenges of biotic and abiotic stresses and resistance against pests and diseases. Hybrid seed production and distribution in maize has been the main focus of private sectors around the world. The genus Zea has been classified into two sections (Iltis and Doebley, 1980) section Luxuriantes, which is composed of *Z. diploperennis, Z. luxurians* and *Z. perennis*, and section Zea, which contains four subspecies: *Z. mays* ssp*. mays, Z. mays* ssp*. mexicana, Z. mays* ssp*. parviglumis* and *Z. mays* ssp. huehuetenangensis (Doebley, 1990).

In genus Zea, both wild taxa have the common name “teosinte” and cultivated maize are diploid (n = 10) with the exception of tetraploid Z. perennis (n = 20). As the closest wild relative of maize, teosinte, which is indigenous to Mexico and Central America Vollbrecht and Sigmon 2005, is a potentially important resource for the study of maize genetics and evolution and for plant breeding. A new teosinte was recently discovered from Pacific Coastal Nicaragua, named *Z. nicaraguensis*. Recently-developed molecular genetic techniques have provided another opportunity to assess the degree of genetic relatedness between maize and teosinte. Genetic diversity of maize (*Z. mays* L.) plays a key role in maize breeding (William and Michael 2002). Knowledge of the amount and the distribution of genetic variation within and among maize landraces will provide a guide for predicting the degree of inheritance, variation, and level of heterosis, that are essential for maize breeding (Duan et al. 2006).

The objective of our specific study was to analyze the genetic diversity among some species of maize and sorghum, to clarify the relationships among the species. We report the results of using two molecular techniques, randomly amplified polymorphic DNA (RAPD) of total genomic DNA and sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) of seed proteins.

## MATERIALS AND METHODS

### Plant materials

Plant material used in this study consists of three subspecies of *Zea mays* and three species of broom corn (Sorghum): 1. *Zea mays* ssp. *mays*, 2. *Sorghum valgare*, 3. *Zea mays* ssp. *Mexicana,* 4. *Zea mays* spp. *Parviglumis*, 5. *Sorghum bicolor*, and 6. *Sorghum grande*. The samples were provided by the Ministry of Agriculture at Kingdom of Saudi Arabia.

### SDS-PAGE of seed protein method

To extract seed proteins, 0.5 g of mature healthy seeds were ground on liquid nitrogen in 0.2 MTris pH 8, 2% (w/v) SDS, 10% sucrose and 1% BME. Proteins were separated by SDS-PAGE according to Laemmli (1970). Gel slab was scanned using gel proanalyzer ver. 3.3 (Media Cypermetics 93 to 97). The presence or absence of each band was treated as binary character in a data matrix that is, coded 1 and 0, respectively. Data were statistically analyzed by using gel Doc 2000 Bio-Rad system.

### RAPD method

Total genomic DNA was extracted from seeds. The seeds were first ground into a fine powder in liquid nitrogen using a pestle and mortar following the steps of CTAB protocol (Porebski et al., 1997). RAPD was performed as described by Williams et al. (1990) with slight modification. PCR reactions were carried out in 25 µL volumes containing 25 ng of total genomic DNA, 10 pmol primers, 200 µM dNTP, 2mM MgCl2, 1X PCR buffer and 2 units ampliTaq polymerase (RTS *Taq*DNA polymerase). Five random oligonuclotide primers OPA-16, OPB-11, OPO-09, OPO-11 and OPZ-7 were used in the experiment (Operon technologies, Alameda, USA) (Table 1).

Amplification was performed in Perkin Elmer 9600 thermal cycler (Foster City, USA) with the following temperature profile: 94°C for 5 min followed by, 40 cycles of 94°C for 1 min, 36°C for 1 min, and extension at 72°C for 90 s. The final extension step was carried out by 72°C for 5 min.

### Data analysis

The gel profiles were visually scored by assigning a number to each distinctive band. The PCR reactions for polymorphic primers were repeated to verify reproducibility of results. The presence or absence of bands was scored as 1 or 0, respectively. Estimation of genetic similarity (GS) was calculated for all possible comparisons among the species was calculated by Jaccard‘s coefficient. An UPGMA phenogram was constructed by using the multivariate statistical Package supported by Kovach computing services. UPGMA was performed with matrix of GS estimates to measure the in formativeness of each marker.

**Table 1.** Characteristics of amplification products generated by the ten decamer arbitrary RAPD-PCR primers (Operon model).

| Number | Primer name | Sequence | Number of polymorphic fragments of DNA | Size of DNA fragments (bp) (min-max) |
|---|---|---|---|---|
| 1 | OPO-09 | 5'-TCCCACGCAA-3' | 6 | 214-1360 |
| 2 | OPO-11 | 5'- GACAGGAGGT-3' | 1 | 196 |
| 3 | OPB-11 | 5'-GTAGACCCGT-3' | 3 | 197-508 |
| 4 | OPA-16 | 5'- AGCCAGCGAA-3' | 3 | 439-518 |
| 5 | OPZ-7 | 5'- CCAGGAGGAC-3' | 3 | 190-1601 |

## RESULTS AND DISCUSSION

Scientists commonly use more than one method to consider genetic similarity. The purpose of such a procedure beside its methods comparison is to determine the number of the polymorphic amplification products. Matos et al. (2001) showed that the picture of genetic similarity can differ depending on the number of polymorphic bands generated by a given method. They said that the more polymorphic products are obtained, the smaller is the similarity between objects.

In our results, the use of RAPD gave higher similarity coefficients than SDS-PAGE did for the same data, regardless of the higher number of polymorphic products generated by SDS-PAGE. To select a method for revising the genetic similarity, when the dendrogram obtained by both methods are different, as in Vaillancourt et al. (2008), Nowosielski et al. (2002), and Potokina et al. (2000) then the more exact, more replicable and more detailed method should be chosen. In our results, a significant majority of the studied taxa were grouped in the RAPD method. The genetic relationships among corn were reported in many previous studies on the basis of the morphological, cytogentiacal, chemical and molecular data. The present work provided the molecular investigation of 3 *Zea mays* and 3 *Sorghum* corn samples by using tow genetic markers RAPD and SDS-PAGE.

### SDS-PAGE of seed protein method

Electrophoretic analysis of proteins exposed a total of 78 protein bands in the seeds of the six species under investigation. The analysis of the results reveals that some bands are characteristic and constant markers for each species and allow the unequivocal identification of their electrophoregrams. Other bands are shared by more than one species. The number of bands varies from one species to another, with the largest number (17) in *Sorghum valgare*, and the lowest number (6) in *Sorghum grande* (Figure 1). For ease of comparison, the 78 protein bands were lumped together with molecular masses and the number of bands from each molecular mass was scored for every species (Table 2). Two major clusters with about 0.50 similarity index were obtained (Figure 2). The first clade included 1, 3 and 4 and the second clade comprised 2, 5 and 6. Our SDS-PAGE of seed proteins analysis results provides useful information on relationships among closely related taxa. However, the relationships between the sections and subgenera of the six samples remain unclear from SDS-PAGE analysis. To get a clearer picture of the situation, we used the RAPD approach.

### RAPD method

Using five primers, a total of 48 bands were visualized; among these, 16 were polymorphic in at least one pair wise comparison between species. The mean number of polymorphic DNA fragments obtained per primer was 3.2. Primer OPO-09 generated the highest number of polymorphic amplification products (6 bands) with size ranging from 214 to 1360 bp (Figure 3). Relations between the studied taxa are presented in a dendrogram built on the basis of similarity indexes (Figure 4). For ease of comparison, the 48 bands were taken together and the number of bands from each size of DNA fragments (bp) was scored for every species. Two main clusters with about 0.91 similarity index were obtained (Figure 4). The first group included two subgroups with 0.38 genetic similarities: the first subgroup included sample 4, with about 0.38 similarities; the second subgroup comprised samples 1 and 3, showing 0.59 genetic similarity. The second group comprised two subgroups with 0.40 genetic similarities: the first subgroup included sample 6, with about 0.40 similarity; the second subgroup comprised samples 2 and 5, showing 0.60 genetic similarities.

### Conclusions

The analysis of variation between corns species studied

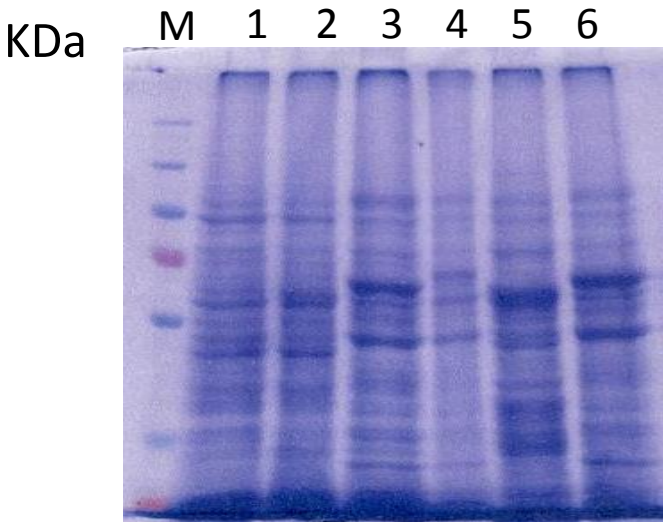


**Figure 1.** SDS-PAGE analysis of corn seed proteins numbered from 1 to 6. 1. *Zea mays* ssp. *mays*, 2. *Sorghum valgare*, 3. *Zea mays* ssp. *Mexicana,* 4. *Zea mays* spp. *Parviglumis*, 5. *Sorghum bicolor*, and 6. *Sorghum grande*.

**Table 2.** Comparative analysis of molecular weight (Mol.wt), relative front (RF) of SDS-PAGE protein profile used in the analysis of the maize.

| Band number | Relative front | Molecular weight (KDa) | Species | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | 1 | 2 | 3 | 4 | 5 | 6 |
| 1 | 0.13 | 225 | 0 | 0 | 0 | 0 | 0 | 1 |
| 2 | 0.233 | 150 | 0 | 0 | 0 | 0 | 0 | 1 |
| 3 | 0.245 | 124 | 0 | 1 | 0 | 0 | 0 | 0 |
| 4 | 0.257 | 118 | 1 | 0 | 0 | 0 | 0 | 0 |
| 5 | 0.265 | 116 | 0 | 0 | 1 | 0 | 0 | 0 |
| 6 | 0.295 | 109 | 0 | 0 | 0 | 1 | 0 | 0 |
| 7 | 0.294 | 108 | 0 | 1 | 0 | 0 | 0 | 0 |
| 8 | 0.315 | 103 | 1 | 0 | 0 | 0 | 0 | 0 |
| 9 | 0.321 | 102 | 0 | 1 | 0 | 0 | 0 | 0 |
| 10 | 0.319 | 101 | 0 | 0 | 1 | 0 | 0 | 0 |
| 11 | 0.326 | 100 | 0 | 0 | 0 | 1 | 0 | 1 |
| 12 | 0.325 | 99 | 0 | 0 | 0 | 1 | 0 | 0 |
| 13 | 0.357 | 92 | 0 | 1 | 0 | 0 | 0 | 0 |
| 14 | 0.363 | 90 | 1 | 0 | 0 | 1 | 1 | 0 |
| 15 | 0.367 | 89 | 0 | 0 | 1 | 0 | 0 | 0 |
| 16 | 0.39 | 84 | 0 | 0 | 0 | 0 | 1 | 0 |
| 17 | 0.391 | 83 | 0 | 0 | 1 | 0 | 0 | 0 |
| 18 | 0.447 | 75 | 0 | 0 | 0 | 0 | 0 | 1 |
| 19 | 0.432 | 74 | 1 | 1 | 0 | 1 | 1 | 0 |
| 20 | 0.44 | 73 | 0 | 0 | 1 | 0 | 0 | 0 |
| 21 | 0.455 | 70 | 0 | 0 | 0 | 0 | 1 | 0 |
| 22 | 0.464 | 69 | 0 | 0 | 0 | 1 | 0 | 0 |
| 23 | 0.488 | 66 | 0 | 1 | 0 | 0 | 0 | 0 |

**Table 2.** Contd.

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| 24 | 0.493 | 65 | 0 | 0 | 0 | 1 | 1 | 0 |
| 25 | 0.52 | 62 | 0 | 0 | 1 | 0 | 0 | 0 |
| 26 | 0.526 | 61 | 0 | 0 | 0 | 0 | 1 | 0 |
| 27 | 0.541 | 60 | 1 | 1 | 1 | 0 | 0 | 0 |
| 28 | 0.55 | 59 | 0 | 0 | 0 | 1 | 0 | 0 |
| 29 | 0.556 | 58 | 0 | 0 | 0 | 0 | 1 | 0 |
| 30 | 0.565 | 57 | 0 | 1 | 0 | 0 | 0 | 0 |
| 31 | 0.578 | 56 | 1 | 0 | 0 | 0 | 0 | 0 |
| 32 | 0.584 | 55 | 0 | 0 | 1 | 1 | 0 | 0 |
| 33 | 0.599 | 54 | 0 | 0 | 0 | 0 | 1 | 0 |
| 34 | 0.624 | 52 | 0 | 1 | 0 | 0 | 0 | 0 |
| 35 | 0.631 | 51 | 1 | 0 | 0 | 0 | 0 | 0 |
| 36 | 0.591 | 50 | 0 | 0 | 1 | 1 | 0 | 1 |
| 37 | 0.634 | 49 | 0 | 0 | 0 | 0 | 1 | 0 |
| 38 | 0.664 | 47 | 1 | 1 | 1 | 1 | 0 | 0 |
| 39 | 0.691 | 45 | 0 | 1 | 0 | 0 | 0 | 0 |
| 40 | 0.721 | 44 | 1 | 0 | 1 | 0 | 0 | 0 |
| 41 | 0.725 | 43 | 0 | 1 | 1 | 0 | 1 | 0 |
| 42 | 0.745 | 41 | 0 | 0 | 0 | 1 | 1 | 0 |
| 43 | 0.781 | 40 | 1 | 1 | 0 | 0 | 0 | 0 |
| 44 | 0.775 | 39 | 0 | 0 | 1 | 1 | 1 | 0 |
| 45 | 0.83 | 37 | 1 | 0 | 0 | 0 | 0 | 0 |
| 46 | 0.829 | 36 | 0 | 1 | 1 | 1 | 0 | 0 |
| 47 | 0.854 | 35 | 1 | 0 | 0 | 0 | 1 | 1 |
| 48 | 0.866 | 34 | 0 | 0 | 1 | 1 | 0 | 0 |
| 49 | 0.891 | 33 | 0 | 1 | 0 | 0 | 1 | 0 |
| 50 | 0.901 | 32 | 0 | 0 | 1 | 0 | 0 | 0 |
| | **Total** | | **12** | **13** | **16** | **14** | **17** | **6** |

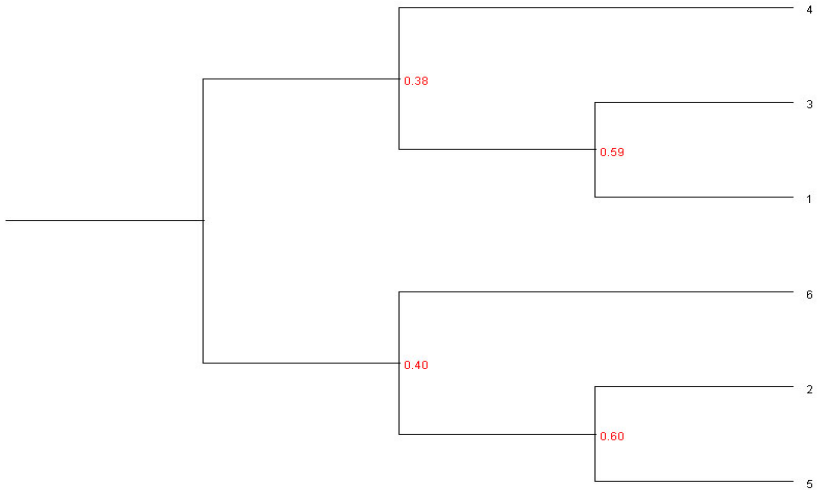


**Figure 2.** Clustering of the investigated taxa of corn based on the total seed protein profiles separated by SDS-PAGE. 1. *Zea mays* ssp. *mays*, 2. *Sorghum valgare*, 3. *Zea mays* ssp. *Mexicana,* 4. *Zea mays* spp. *Parviglumis*, 5. *Sorghum bicolor*, and 6. *Sorghum grande*.

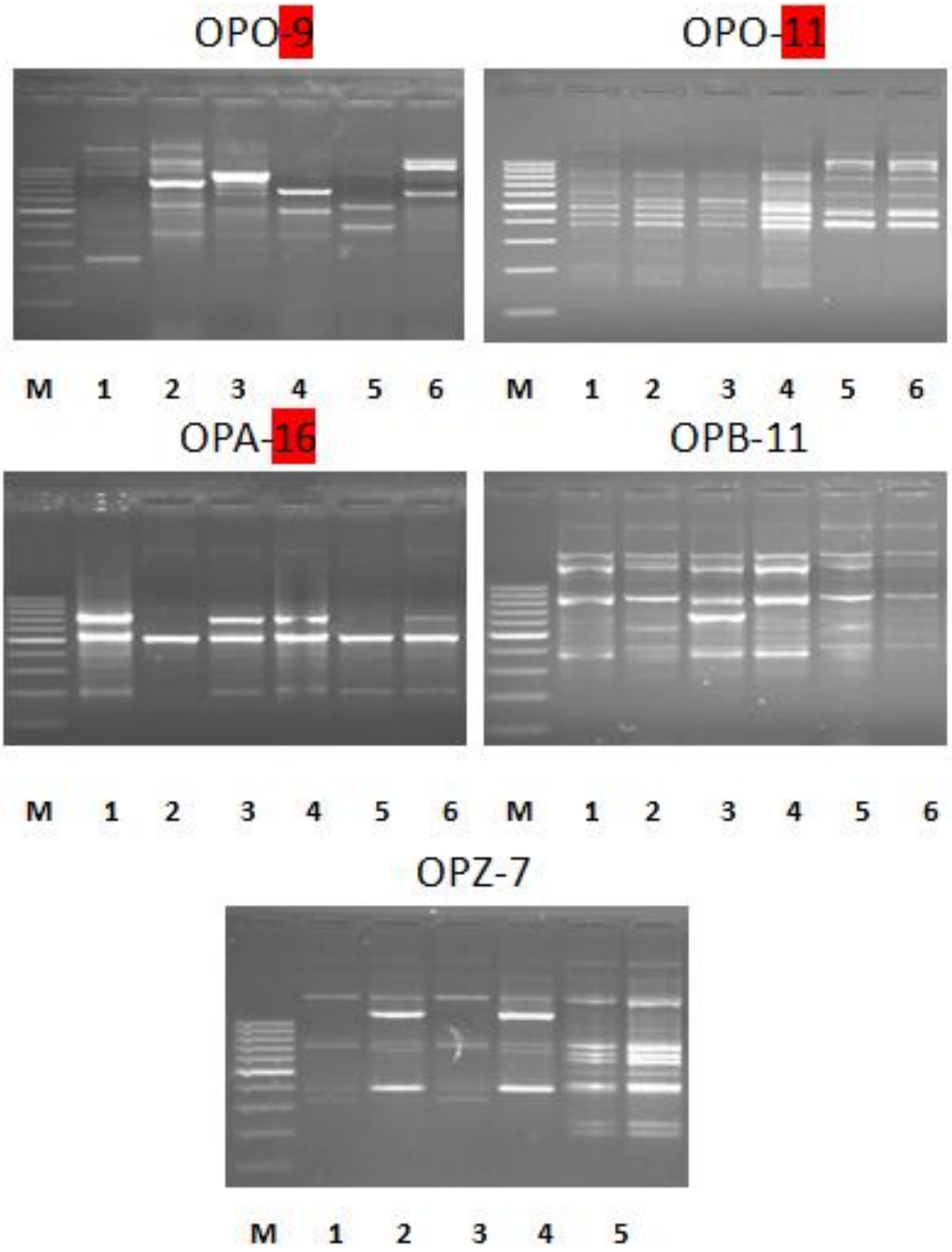


**Figure 3.** DNA polymorphism generated by five primers from the genomic DNA of the investigated species of corn. 1. *Zea mays* ssp. *mays*, 2. *Sorghum valgare*, 3. *Zea mays* ssp. *Mexicana,* 4. *Zea mays* spp. *Parviglumis*, 5. *Sorghum bicolor*, and 6. *Sorghum grande*.

showed that there are major differences in the frequencies of the electrophoretic profiles in the different species. RAPD-PCR of DNA is effective in determining relationships between sections and subgenera. We found that PCR-RAPD of DNA provided more precise information concerning relationships between corn than SDS-PAGE analysis. A remarkable result from this study was identifying a close relationship between *Zea mays* spp mays and *Zea mays* spp. Mexicana and further support comes from the molecular data of RAPD which indicate close relationship between *Sorghum valgare* and *Sorghum bicolor*.

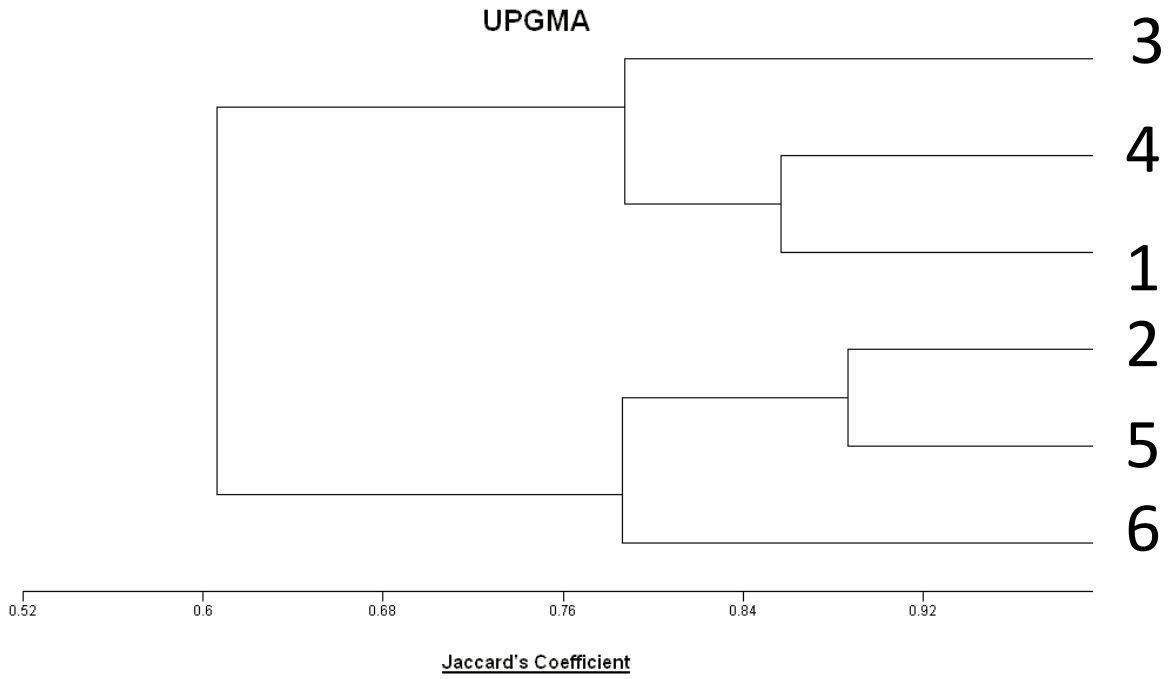


**Figure 4.** Dendogram of phylogenetic relationships between the investigated taxa of corn based on Jaccard's similarity coefficient obtained from 5 RAPD primers. 1. *Zea mays* ssp. *mays*, 2. *Sorghum valgare*, 3. *Zea mays* ssp. *Mexicana,* 4. *Zea mays* spp. *Parviglumis*, 5. *Sorghum bicolor*, and 6. *Sorghum grande*.

## REFERENCES


Chen X, Sullivan PF (2003). Single nucleotide polymorphism genotyping: biochemistry, protocol, cost and throughput. Pharmacogenomics J. (3):77-96.

De Souza SGH, Carpentieri-Pipolo V, de Fatima Ruas C, de Paula Carvalho V, Ruas PM , Gerage AC (2008). Comparative analysis of genetic diversity among the maize inbred lines (Zea mays L.) obtained by RAPD and SSR markers. Braz. Arch. Biol. Technol. (51):183-192.

Doebley J (1990). Molecular systematics of *Zea* (Gramineae). Maydica.(35):143-150.

Duan YP, Chen WG, Li MS, Li XH, Liu X (2006). The genetic diversity among 27 maize populations based on SSR data. Sci. Agric. Sinica. (39):1102-1113.

Goncalves LSA, Rodrigues R, Sudre CP, Bento CS, Moulin MM (2008). Divergencia genetica em tomate estimada por marcadores RAPD em comparacao com descritores multicategoricos. Hortic. Bras. (26):1113-1117.

Goncalves LS, Rodrigues R, do Amaral Junior AT, Karasawa M, Sudre CP (2009). Heirloom tomato gene bank: Assessing genetic divergence based on morphological, agronomic and molecular data using a Ward-modified location model. Genet. Mol. Res. (8): 364-374.

Iltis HH, Doebley JF (1980). Taxonomy of *Zea* (Gramineae). I. A subgeneric classification with key to taxa. Amer. J. Bot. (67):994-1004.

Kilian A, Huttner E, Wenzl P, Jaccoud D, Carling J( 2005). The Fast and the Cheap: SNP and DArT-based Whole Genome Profiling for Crop Improvement. Proceedings of the International Congress in the Wake of the Double Helix: From the Green Revolution to the Gene Revolution, Tuberosa, R., R.L. Phillips and M. Gale, (Eds.) Bologna. 27-(31):443-461.

Laemmli K (1970). Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature. (227):680-685.

Matos M, Pinto-Carnide O, Benito C (2001). Phylogenetic relationships among Portiguese rye based on isoenzyme, RAPD and ISSR markers. Hereditas. (134):299-336.

Moose SP, Mumm RH (2008). Molecular plant breeding as the foundation for 21st century crop improvement. Plant Physiol. (147):969-977.

Mullis KB, Faloona FA (1987). Specific synthesis of DNA in vitro via a polymerase-catalyzed chain reaction. Methods Enzymol. (155):335-350.

Nowosielski J, Podyma W, Nowosielska D (2002). Molecular research on the genetic diversity of Pollish varieties and landraces of Phaseolus coccineus L. and Phaseolus vulgaris L. using the RAPD and AFLP method. Cell Mol. Biol. Let. (7):753-762.

Potokina E, Vaughan DA, Eggi EE Tomooka N (2000). Population diversity of the Vicia sativa (Fabaceae) in the flora of the former USSR deduced from RAPD and seed protein analysis. Gen. Res. Cr. Evol. (47):171-183.

Porebski SL, Bailey G, Baum RB (1997). Modification of CTAB DNA extraction protocol for plants containing high polysaccharide and polyphenol components. Plant Mol. Biol. Rep. 15: 8-15.

Schlotterer C (2004). The evolution of molecular markers - just a matter of fashion. Nat. Rev. Genet. 5:63-69.

Schulman AH (2007). Molecular markers to assess genetic diversity. Euphytica. (158):313-321.

Sudre CP, Leonardecz E, Rodrigues R, Junior A, Maria M, Landro G (2007). Genetic resources of vegetable crops: a survey in the Brazilian germplasm collections pictured through papers published in the journals of the Brazilian Society for Horticultural Science. Hortic. Bras. (25):337-342.

Tautz D (1989). Hypervariability of simple sequences as a general

source for polymorphic DNA markers. Nucleic Acids Res. (17):6463-6471.
Vaillancourt A, Nkongolo KK, Michael P, Mehes M (2008). Identification, characterization and chromosome locations of rye and wheat specific USSR and SCAR markers useful for breeding purposes. Euphytica. (159):297-306.
Vollbrecht E, Sigmon B (2005). A mazing grass: Developmental genetics of maize domestication. Biochem. Soc. Trans. (33):1502-1506.
Vos P, Hogers R, Bleeker M, Reijans M, Lee TVD (1995). Hornes AFLP: A new technique for DNA fingerprinting. Nucleic Acids Res. (23):4407-4414.
Welsh J, McClelland M (1990). Fingerprinting genomes using PCR with arbitrary primers. Nucleic Acids Res. (18): 7213-7218.
Williams JGK, Kubelik AR, Livak KJ, Rafalski JA Tingey SV (1990). DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. Nucleic Acids Res. (18): 6531-6535.
William SK, Michael RC (2002). Essentials of genetics. Higher Education Press, Beijing. p: 600.
Zietkiewicz E, Rafalski A, Labuda D (1994). Genome fingerprinting by simple sequence repeat (SSR)-anchored polymerase chain reaction amplification. Genomics (20): 176-183.