

Full Length Research Paper

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

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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 bands 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 vulgare* and *Sorghum bicolor*.

Key words: *Zea mays*, *Sorghum vulgare 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

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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 Gaspé 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 vulgare*, 3. *Zea mays* ssp. *Mexicana*, 4. *Zea mays* ssp. *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 Cybernetics 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 μ L volumes containing 25 ng of total genomic DNA, 10 pmol primers, 200 μ M dNTP, 2mM MgCl₂, 1X PCR buffer and 2 units ampliTaq polymerase (RTS TaqDNA polymerase). Five random oligonucleotide 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 informativeness 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, cytogenetical, chemical and molecular data. The present work provided the molecular investigation of 3 *Zea mays* and 3 *Sorghum* corn samples by using two 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

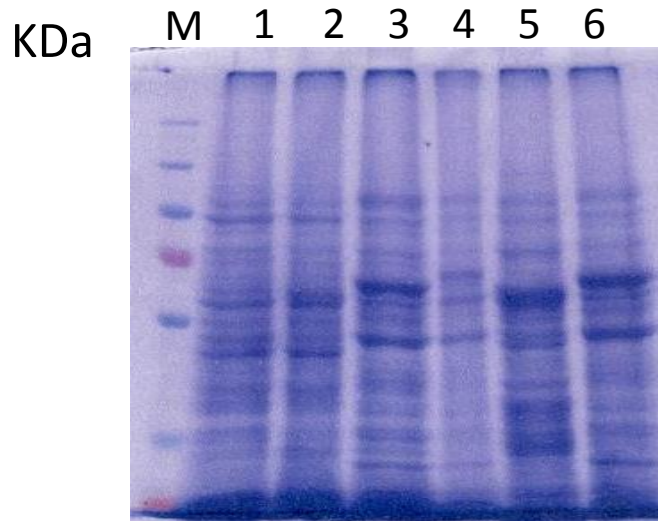


Figure 1. SDS-PAGE analysis of corn seed proteins numbered from 1 to 6. 1. *Zea mays* ssp. *mays*, 2. *Sorghum valgare*, 3. *Zea mays* ssp. *Mexicana*, 4. *Zea mays* spp. *Parviglumis*, 5. *Sorghum bicolor*, and 6. *Sorghum grande*.

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

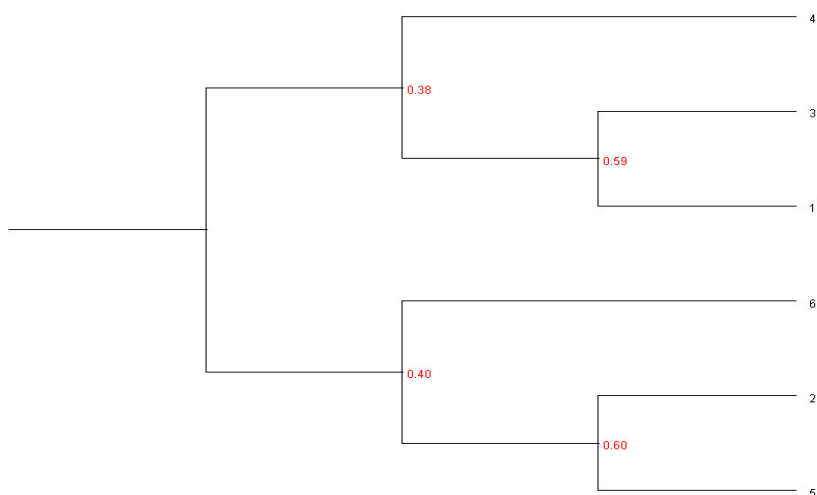


Figure 2. Clustering of the investigated taxa of corn based on the total seed protein profiles separated by SDS-PAGE. 1. *Zea mays* ssp. *mays*, 2. *Sorghum vulgare*, 3. *Zea mays* ssp. *Mexicana*, 4. *Zea mays* spp. *Parviglumis*, 5. *Sorghum bicolor*, and 6. *Sorghum grande*.

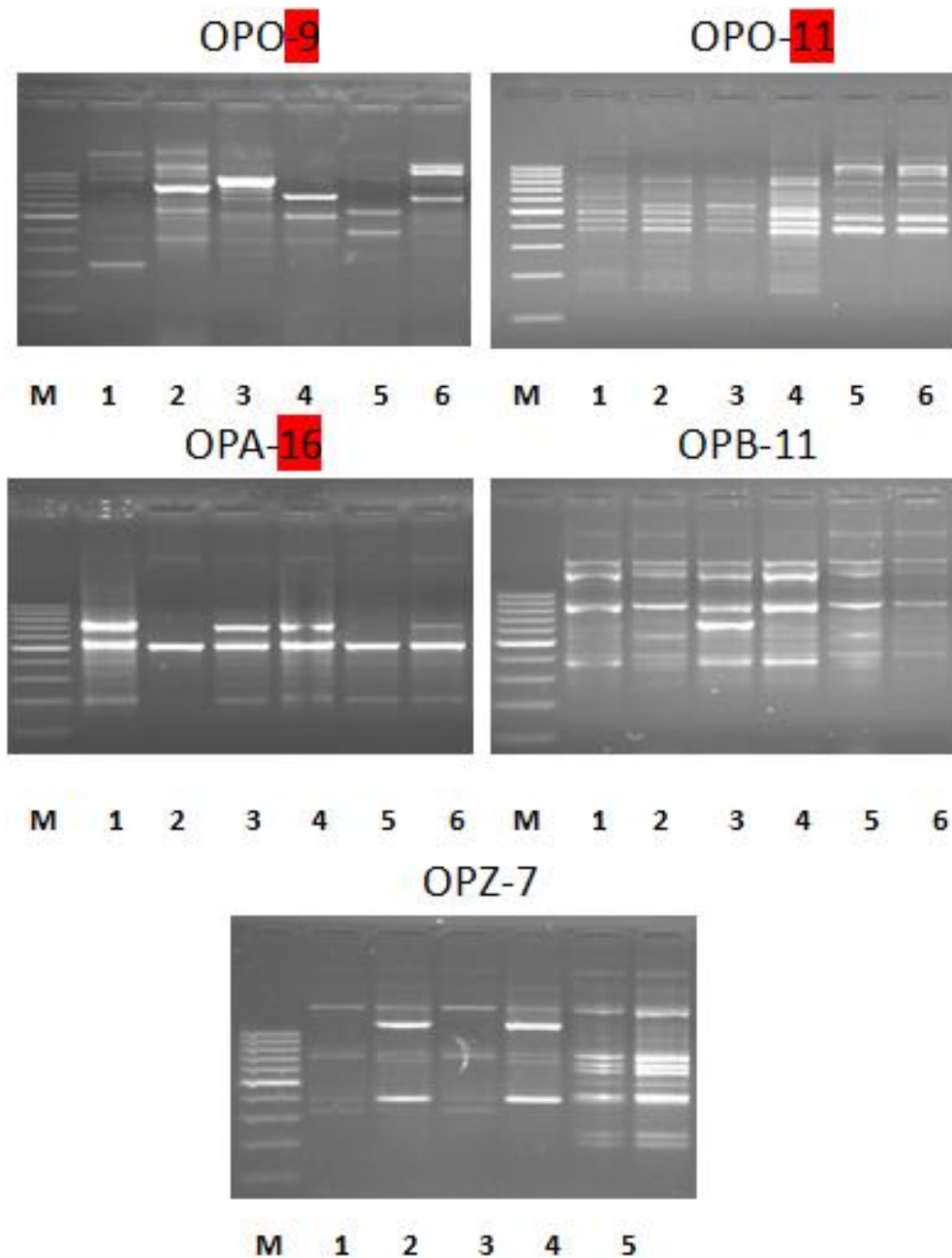


Figure 3. DNA polymorphism generated by five primers from the genomic DNA of the investigated species of corn. 1. *Zea mays* ssp. *mays*, 2. *Sorghum valgare*, 3. *Zea mays* ssp. *Mexicana*, 4. *Zea mays* ssp. *Parviglumis*, 5. *Sorghum bicolor*, and 6. *Sorghum grande*.

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*.

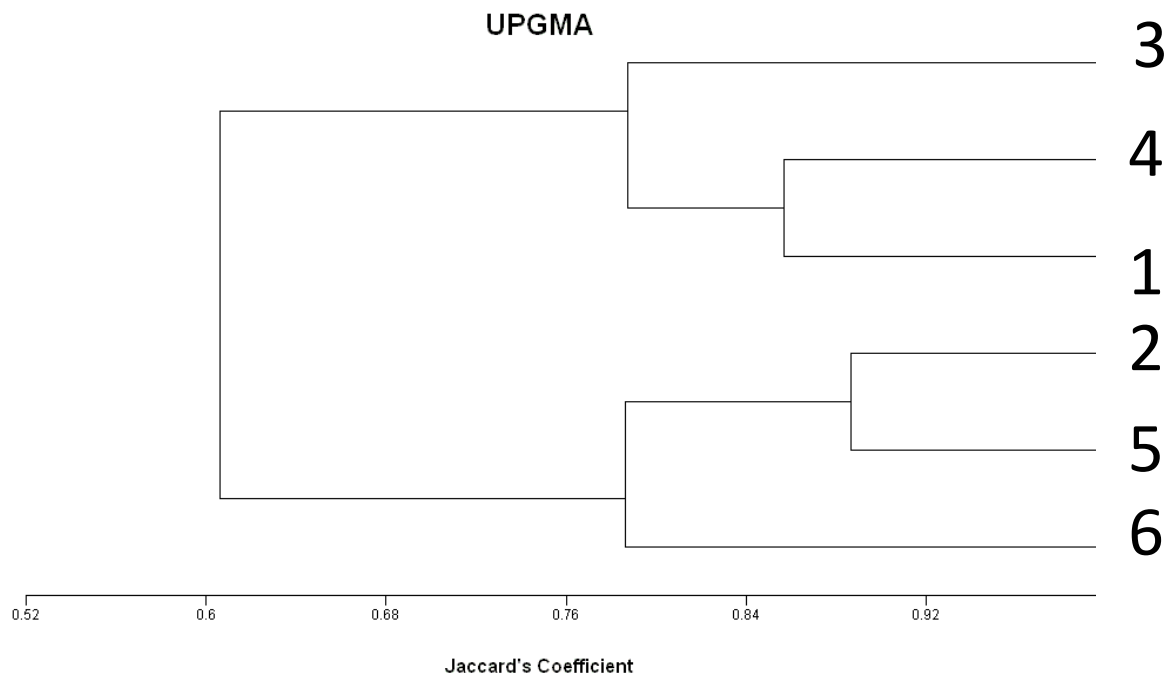


Figure 4. Dendrogram of phylogenetic relationships between the investigated taxa of corn based on Jaccard's similarity coefficient obtained from 5 RAPD primers. 1. *Zea mays* ssp. *mays*, 2. *Sorghum valgare*, 3. *Zea mays* ssp. *Mexicana*, 4. *Zea mays* spp. *Parviglumis*, 5. *Sorghum bicolor*, and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

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