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GENETIC DIVERSITY OF SIX DIFFERENT EGYPTIAN FORAGE CROPS BY MOLECULAR TOOLS

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ABSTRACT

The aim of the present study was to estimate genetic variation in grass sorghum (sorghum, Tunis grass, Sudan grass) and other forage species (ray grass, pearl millet, guar). Three molecular systems i.e. isozymes-PAGE, seed protein, SDS-PAGE and AFLP were used. The results indicated the presence of high similarity matrix among sorghum and ray grass; pearl millet and Sudan grass (100%) and low similarity matrix among guar and sorghum; guar and Tunis grass (44.4%) in peroxidase analysis, where the total bands was 21 with 100% polymorphism and without any unique bands. This data was different comparing with superoxidase dismutase, protein and AFLP analysis. On the other hand, the combined set data of three molecular system revealed that Tunis grass have similarity matrix in a value of (70.6%) with sorghum and (90.7%) with Sudan grass.

Also, Tunis grass gave similarity matrix of (89.7%), (87.9%) and (71.2%) with ray grass, pearl millet and guar, respectively. In cluster analysis with combined data set, Tunis grass was located in sub cluster 1 with sudan grass and pearl millet in the same group, and in the same cluster of ray grass. The different clusters were found between Tunis grass, sorghum and guar. Tunis grass did not give any unique bands with all analysis but sorghum, guar and ray grass gave unique bands with protein and AFLP analysis.

Keywords: Forage crops, sorghum, diversity, molecular profile, Cluster analysis

INTRODUCTION

Grass sorghums include Sudan grass and Tunis grass are annuals and grow quickly and are generally used for summer pasture. Johnson grass, a perennial grass sorghum, is considered a pest when it is out of control, however, it makes an excellent hay for cattle feed. For human consumption sorghum is used for its grain and a syrup depending on the type grown. Sorghum is considered to be a native to tropical Africa, continues to be a leading cereal grain in the most areas of the continent, and is a major staple food and fodder crop grown worldwide, with an annual average production of 61 million tones over the past decade (FAO, 2005). Moreover, according to FAO, sorghum ranks fifth in world grain production behind wheat, rice, maize, and barley.

Sorghum (*Sorghum bicolor* ssp. *bicolor*) as a traditional cultivars were classified by Harlan and De Wet (1972) into five main races (*bicolor*, *caudatum*, *durra*, *guinea*, kafir) and 10 intermediates (e.g. bicolor-caudatum, durra-kafir), mainly on the basis of spike let and grain morphology. Snowden (1936) defined 7 weedy, 13 wild, and 28 cultivated species and numerous varieties and forms from within this variability. A refinement of Snowden's

classification was developed by Jakuševskij (1969) and is still used in some parts of the world (Fritsch *et al.*, 2001). De Wet and Huckabay's (1967) classification of *S. bicolor* separated the perennial plants as "*S. bicolor* subsp. *halepense*"; from the annual plants of this complex where they were combined into *S. bicolor* subsp. *bicolor*, treating the cultivated members as *S. bicolor* var. *bicolor* and partitioning the wild and weedy relatives into three varieties, *S. bicolor* vars. "*arundinaceum*", "*aethiopicum*", and "*verticilliflorum*" *(Piper, 1915).* For the same reason as before, these varietals names were also not validly published. To these three varieties, assumed to have been established by De *Wet et al. (1970)* was added a fourth, "*S. bicolor* var. *virgatum* (Hack.) as well as "*S. virgatum* (Hack.) Stapf according to John and Jeff (2007). In Egypt, Tunis grass is a new forage crop and it was identified by the Egyptian flora and Phytotaxonomy Research Department, Agriculture Museum, Dokki, Giza, Egypt. It has a high fresh and dry yield with follow condition, cutting in 120 cm and fertilized 80.2 kg N per hectare for three cuts Abdel-Aziz and Abdel-gwad (2008).

In sorghum breeding and genomic resources are less than the other major cereals as rice, wheat, maize and barley according to economic values. However, when interest has focused on the crop due to its drought resistance and small genome size (~760 Mb) compared to close relatives maize (~2500 Mb) and sugarcane (2550 to 4200 Mb). In recent years, the potential of sorghum as a biofuel crop has led to additional investment culminating in the sequencing of the sorghum genome (Bowers *et al.*, 2007). Many molecular marker technologies have been developed and applied to studying patterns of genetic diversity in grass sorghums germplasm collections and in breeding programs (Ferreira, 2005) and Kawar *et al.* (2009). Progress in sorghum characterization of the transcriptome has been paralleled by identification of differential gene expression in response to biotic and a biotic factors, including green bug feeding Park *et al.* (2006). Pratibha Brahmi *et al.* (2004) analyzed the genetic diversity in cultivated guar using allozyme polymorphism and compare it with reported morphological diversity. As well as, Lamy *et al.* (1994) are using pearl millet molecular markers to follow the introgression of genomic segments from the wild progenitors of this crop into several populations based on crosses of wild and cultivated accessions from various parts of western and central Africa. Finally, Ruby Tiwari, *et al.* (2009) established allergenic cross reactivity between the members of the Pooids (*Lolium perenne*, *Phleum pratense*, and *Poa pratensis*) and Chloridoids (*Cynodon dactylon* and *Paspalum notatum*). In the present study, the variation of grass sorghum and other forge species were estimate by molecular tools.

MATERIALS AND METHODS

Plant Materials

This work was carried out in collaboration between the Agricultural Research Center, Field Crops Research Institute, Forage crops research department; National Research Centre, Division of Genetic Engineering and Botany Department, Faculty of Agriculture, Suez Canal University, during two

years 2009 and 2010. Seeds of different six Egyptian forage crops have been obtained from Forage Crops Research Department, Field Crops Research Institute, Agricultural Research Center, Giza, Egypt. The list of these forage crops is presented in Table I.

Table 1: The common and botanical name, Chromosome number, wild type, season and Egyptian location.

No	Forage crop		Chromosome number Somatic cells polyploidy	Wild type	Season	Egypt location
	Common name	Botanical name				
1	Sorghum	*Sorghum bicolor*	20**	imported	Summer	all
2	Sudan grass	*Sorghum vulgare var. sudanense*	20**	found	Summer	all
3	Tunis grass	*sorghum virgatum*	20**	found	Perennial	north
4	Ray grass	*Lolium multiflorum*	14*	imported	Winter	north
5	Pearl millet	*Pennisetum glaucum*	14#	imported	Summer	all
6	Guar	*Cyamopsis tetragonoloba*	14##	found	Summer	upper

****Kim *et al.*(2005), * Ahloowalia (1965),# Techio et al.(2006) ## Bewal et al.(2009)**

Isozyme Analysis

Isozymes extraction from the six cultivars by homogenizing 0.5 g fresh leaves and roots samples in 1 ml extraction buffer (10% glycerol) using a mortar and pestle. The extract was then transferred into clean eppendorf tubes and centrifuged at 10000 rpm for 5 minutes according to *Stegemann et al. (1985b).* The supernatant was transferred to new clean eppendorf tubes and kept at –20 ^{o}C until needed for electrophoretic analysis. A volume of 40 µl extract of each sample was mixed with 20 µl sucrose and 10 µl bromophenol blue, then a volume of 50 µl from this mixture was applied to each well. The run was performed at 150 volt until the bromophenol blue dye reached the separating gel and then the voltage was increased to 200 volt. Electrophoresis apparatus was placed inside a refrigerator during running duration. After electrophoresis, the gels were stained according to their enzyme systems with the appropriate substrate and chemical solutions, and then incubated at room temperature in dark for complete staining for about 1 to 2 hours. Gel was placed into this solution and 5 drops of hydrogen peroxide was added. The gel was incubated at room temperature until bands appear *(Brown, 1978)*.

Protein Analysis

Sodium dodecylsulfate polyacrylamide gel electrophoresis (SDS-PAGE) was used to study the banding patterns of the six cultivars. Protein fractionation was performed on vertical slab (16.5 cm x 18.5 cm x 0.2 cm) Hoefer E600, Amersham Pharmacia biotech. According to the method of Laemmli *(*1970*)* as modified by Studier (1973), Sample extraction buffers (Tris-HCl buffer, pH 7.5) *(*Jonathan and Weaden*,* 1990*)* and Staining solution Coomassie briliant blue-R250 staining solution was well mixed and kept at room temperature in a dark bottle.

The lower buffer tank was filled with running buffer and attached with upper buffer tank, so that the gels were completely covered. Gels were distained with 350 ml distaining solution. The distaining solution was changed

several times until the gel background was clear. The electrodes were connected to a power supply and adjusted at 100 V until the bromophenol blue dye reached the resolving gel. The voltage was Increased to 250 V until the bromophenol blue reached near the bottom of the resolving gel. Gels were photographed using a 35 mm color film (200 ASA) and scanned with Bio-Rad Video Densitometer Model 620 USA, at a wavelength of 577.

Software data analysis for Bio-Rad Model 620 densitometerand computer was used as recommended by the manufacturer. The frequency of two isozymes, protein among 6 forage crops genotypes was calculated based on the presence of band as '1' or absence of band as '0' *(Sokal and Rohlf, 1995).* The genetic associations between varieties were evaluated by calculating the Jaccard similarity coefficient for pair-wise comparisons based on proportion of shared band

Aflp Analysis

DNA preparation, used in this study are described in details in (Pažoutová *et al.*, 2000b, 2002a). AFLPs were generated as described by Zeller *et al.* (2000) and Vos *et al.* (1995). Genomic DNA (100 ng) was digested by *Eco*RI and *Mse*I and ligated to adapters, pre amplified using primers *Eco*RI-core (CTCGTAGACTGCGTACCAATTC) and *Mse*I-core (GACGATGAGTCCTGAGTAA) and amplified with final amplification primer pairs *Eco*RI + AG/*Mse*I + C (Tooley *et al.*, 2000), *Eco*RI + TT/*Mse*I + AC, and *Eco*RI + GG/*Mse*I + CT. *Eco*RI primer were used and fragments were separated in 6% polyacrylamide gel (Long Ranger FMC, USA). Polymorphic AFLP fragments were scored as binary characters for each genotype. A distance matrix of genotypes was calculated for both fingerprinting methods according to Nei and Li (1979) with 500× bootstrapping, and a dendrogram was constructed using the unweighted pair group method with arithmetic mean (UPGMA) as implemented in TREECON 1·3b (Van de Peer and De Wachter, 1997).

RESULTS AND DISCUSSION

Isozyme Analysis

Six different forage crop species were studied by Peroxidase (Pox) and Total superoxide dismutase (Sod).

Peroxidase isozymes (Pox):

The results revealed 21 bands from peroxidase isozymes without any monomorphic which made polymorphism 100 % as shown in Table 7 and Figure 1. The data in Table 2 explained the Similarity matrix among six different Egyptian forage crops which were demonstrated the value among sorghum and Tunis grass is 33.3%, the same value between ray grass and Tunis grass. Moreover, two values of: (75.0%) and (40.0%) were given among pearl millet, sorghum, Tunis grass and ray grass and also between guar and the same plants, respectively. Sudden grass gave the same similarity matrix value of pearl millet of (75.0%). Guar gave (57.1%) with peal millet and Sudden grass. The high similarity matrix value (100%) was given among ray grass and sorghum, pearl millet and Sudden grass in peroxidase

analysis. Ke ming *et al.* (1995) found that presence or disappearance of some peroxidase and esterase isozyme bands was related to wounding. Some isoperoxidase bands disappeared at the time of vascular tissue formation. Marie and Harold (1971) found that each species pattern was unique, and no single peroxidase band was common to all the species. Based on considerations of interspecific cross compatibility and chromosomal rearrangements the taxonomic division of the genus into three sections was further subdivided to give five groups. Jean *et al.* (1971) have demonstrated that peroxidase was a single polypeptide chain and that subunit association was not involved in the isoenzyme system. On the basis of tryptic peptide maps, it was apparent with the peroxidase isozymes. Isozymes within a group appeared to possess very similar primary structures. Whether more than one gene is involved in their biosynthesis cannot be ascertained at present. The catalytic properties of the isozymes followed an identical pattern.

So, the genetic variation among six species in peroxidase activity due to more than one gene is involved in their biosynthesis and chromosomal rearrangements.

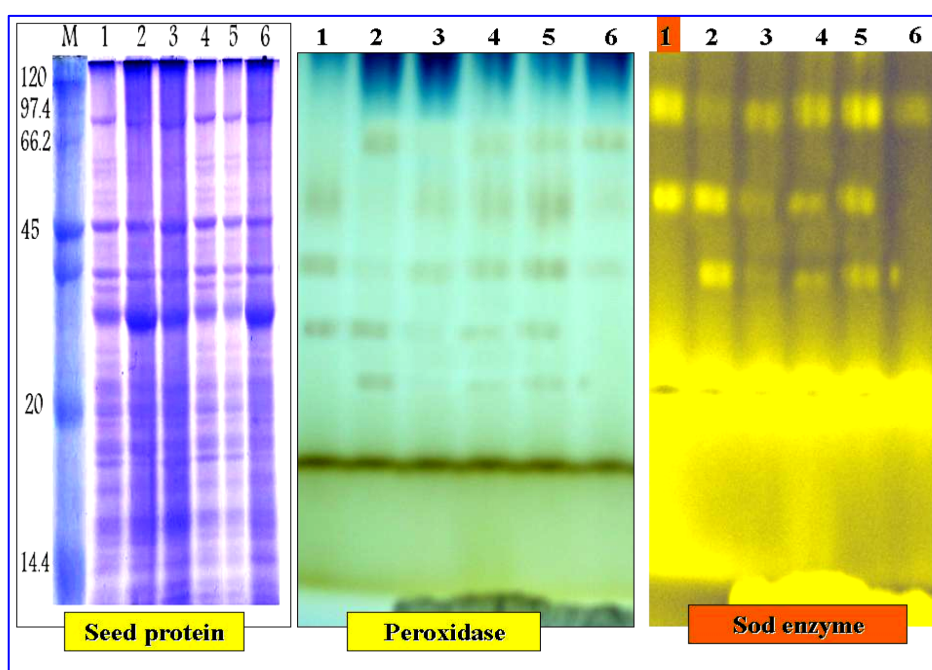

Figure 1: Isozymes (Peroxidase and Total superoxide dismutase (Sod)) and seed protein analysis of six different Egyptian forage crops (lane 1 in seed protein : M. marker; 1- 6 in seed protein , peroxidase, and Sod isozymes sorghum [Tunis grass, Ray grass, Pearl millet, Sudan grass and Guar].

Table 2: Similarity matrix among six different Egyptian forage crops were used based on peroxidase analysis.

Proximity Matrix					
Case	Matrix File Input				
	Tunis grass	Ray grass	Pearl millet	Sudan grass	Guar
Sorghum	33.3	100.0	75.0	75.0	40.0
Tunis grass		33.3	75.0	75.0	40.0
Ray grass			75.0	75.0	40.0
Pearl millet				100.0	57.1
Sudan grass					57.1

Superoxide dismutase isozyme (Sod):

The results in total superoxide dismutase (Sod) activity gave 15 bands as a total bands which were divided into 6 bands monomorphic and 9 polymorphic in 60 % polymorphism as shown in Table 7 and Figure 1. In Table 3, it revealed that guar forage crops have the same similarity matrix value (50%) with Tunis grass, ray grass, pearl millet and Sudden grass. This value is lowest value among six forage crops.

Table 3: Similarity matrix among six different Egyptian forage crops were used based on superoxide dismutase analysis.

Case	Matrix File Input				
	Tunis grass	Ray grass	Pearl millet	Sudan grass	Guar
Sorghum	80.0	80.0	80.0	80.0	66.7
Tunis grass		1.000	100.0	100.0	50.0
Ray grass			100.0	100.0	50.0
Pearl millet				100.0	50.0
Sudan grass					50.0

The highest value 100 % was repeated among Sudden grass with Tunis grass, ray grass and pearl millet; pearl millet with tunis grass and ray grass; ray grass with tunis grass. The result of Wang *et al.* (2006) showed an obvious and stable variation in the isozyme phenotypes in two different pearl oyster species. The SOD and EST isozymes from gill and MDH, ME and G6PDH from adductor muscle were species-specific. The electrophoretograms of these isozymes could be used as markers to differentiate the two pearl oysters. Li *et al.* (1995) reported that the banded characters at EST- 1, SOD-1, SOD-2, and SOD-3a loci may be used as biochemical markers to identify the *R. kamoji* chromosomes carrying these loci in a *T. aestivum* ×*R. kamoji* hybridization program. The lowest similarity with the rest of the species was in accord with the morphological studies (Khatamsaz, 1998) and other numerical taxonomic works (Sneath, and Sokal, 1973), (Pooler Simon, 1993) and (Sheidai *et al.,* 2000) So, the considerable molecular diversity which could be found among six forage crops were obvious by isozymes Sod.