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QUALITY OF THIRTEEN GRAIN SORGHUM GENOTYPES IN RELATION TO GROWTH CHARACTERS, YIELD AND YIELD COMPONENTS AND FINGERPRINTS

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ABSTRACT

Aiming to evaluate thirteen grain sorghum genotypes for seed quality traits, to characterize some of their growth characters, yield, yield components and biotechnology characters including the distinguish of their genetic fingerprint, a field trial was carried out at the experimental farm, of Agric. Res. Station, Sids, Agric. Res. Center (ARC) and the Lab. of Seed Technology Dept & Cell Research Studies Dept, in the two growing seasons of 2008 and 2009. The results showed significant variability among these genotypes in seed germination and seedling characters. Seed germination ranged between 95.7 % for genotype GZ R4 to 85.5 % for the genotype ICSB 88015. Accelerated ageing germination ranged from 81.7 % for the genotype GZ R4 to 61.3 % for genotype BTX 629. Seedling length ranged between 18.4 cm for the genotype GZ R4 to 16.0 cm for the genotype GZ R3. The electrical conductivity was high for the genotype ICSB 88015 (36.3 $\mu S\ cm^{-1}g^{-1}$) while it was low 22.0 $\mu S\ cm^{-1}g^{-1}$ for genotype GZ R4. The results revealed significant variability among the tested genotypes in days to 50 % flowering which ranged from 73.5 to 69.2 days for genotypes GZ R3 and Giza 113, respectively. Plant height ranged from 255.8 cm for (GZ R4) to 129.6 cm for (ICSB 88015). Panicle length ranged from 30.4 cm for BTX 629 to 21.5 cm for BTX 623, respectively. Grain yield/plant ranged from 93.1 for the genotype G2 R4 to 57.5 g $plant^{-1}$ for the genotype BTX 629. Genotype productivity based on grain yield arb./ $faddan^{-1}$ ranged from 21.7 for genotype GZ R1 to 15.7 arb./$faddan^{-1}$ for the genotype GZB 11. Besides, there were significant differences for their chemical composition. Significant differences were observed among the grain sorghum genotypes for crude protein that ranged from 12.0 % for genotype BTX 623 to 11.2 % for genotype GZ B11. By contrast, there were no significant differences among the grain sorghum genotypes for total carbohydrates. The highest percentage was 72.6 for GZB 16 while the lowest was for the genotype GZ R3 (71.1 %). The genotypes BTX 629 and ICSR 89025 gave the highest oil percentage (3.4 %), whereas the lowest value (3.0%) was recorded for genotype GZ R4. The SDS-PAGE for water soluble protein of the thirteen genotypes displayed a maximum of 22 bands with molecular weight ranging from 199.0 to 48.0 kDa. Twenty two polymorphic bands (100 %) were observed. Four random primers were used that generated a total of 57 bands, from which 53 (92.9%) were polymorphic. Twelve out of 57 (21.05%) bands were found to be useful as genetic-specific markers. The RAPD technique has proved its potential producing unique genetic fingerprint for each genotype. Based on biotechnology characters including distinguished genetic fingerprint and the results laboratory test and field trails some genotypes would be of great interest to plant breeder and be included in sorghum breeding program specially these of high viability value, high yielding and good storability.

Key words: *Sorghum, Seed vigor, Viability, Yield, Yield Components, Chemical Characteristics, DNA, RAPD.*

INTRODUCTION

Grain sorghum [*Sorghum bicolor* (L.) Moench] ranks the fourth among cereal crops after wheat, rice and maize in terms of acreage and production. It is grown in an area of about 0.4 million faddans in Upper Egypt. Most of this area concentrated in four Governorates, viz. Assiut, Sohag, Fayoum and Qena (Bashir *et al* 2008).

Seed quality has direct influences on the success of the crop and significantly contributes to productivity levels (Bewley and Black 1994). Seed vigor may provide a more accurate appraisal of seed quality relating to field emergence than the standard germination test that measures viability i.e. the seed's ability to germinate and develop into a normal seedling under ideal field conditions, while seed vigor measures the seed's ability to establish a normal seedling under wide range of field conditions, including adverse conditions (Herbek and Eitzer 2007). High quality seed causes rapid germination, emergence and better root and shoot growth during the early stages of development; hence a prerequisite for successful crop establishment is a seed of high quality because such seed will determine the ability to cope with suboptimal conditions (Harris *et al* 1987)

Several agronomic and physiological traits such as plant height, days to 50% heading, leaf area, number of kernels per head, kernel size and seed color affect yield quantity and quality which considers the final performance of any crop. (Quinby 1963).

Electrophoretic assays have been widely used as a rapid and accurate test to identify and characterize different crop cultivars. By the use of appropriate and refined techniques, it is now possible to actually "fingerprint" each cultivar to assess its identity and may be its agronomic properties. SDS-PAGE and RAPD analyses were successfully used for both identification and differentiation of plant cultivars. They are reliably rapid means for establishing genetic profiles and elucidation of genetic relationships within and between taxa (Aly *et al* 2000 and Hassan 2001).

DNA analysis, now a frequent courtroom evidence tool, is being as a faster way to fingerprint genotypes. The development of RAPD-PCR markers by (Williams *et al* 1990) have a number of advantages over the other DNA-based markers systems (Sane *et al* 1997). RAPD may be used for analysis of many plant samples using small quantities, pedigree analysis (Gallois *et al* 1998), molecular mapping, taxonomy and phylogeny (Wolfe and Liston 1998), identification of genotypes associated with genes of interest and genetic diversity studies (Ranade and Farooki 2002).

Therefore, the objective of this work was to evaluate seed quality by using some vigor and viability test, growth characters, yield and yield components, determine the major chemical constitutes and detect the genetic purity of thirteen genotypes from grain sorghum using (SDS-PAGE) and molecular markers (RAPD-PCR).

MATERIALS AND METHODS

This work was carried out at Sids Agric. Res. Station and laboratory of seed Technology Dept. & Cell Research Studies Dept. Field Crops Res. Institute, ARC during 2008 and 2009 seasons. The grain sorghum genotypes: ICSB 14, BTX 623, BTX 629, ICSB 88015, ICSR 89025, ICSR 91022, ICSV 273, GZ R1, GZ R3, GZ R4 GZ B16, GZB 11 and Giza 113 were used. All of them seed source and pedigree of the studied genotypes are presented in Table (1). Seeds of the different genotypes were received from Grain Sorghum Research Dept., Field crops Research Institute, Agricultural Research Center.

The experiments were carried out in a randomized complete block design (RCBD) with three replications in the two seasons. Each plot consisted of two rows 4-m long, 60 -cm apart and 20 cm between hills within a row. After full emergence, the seedlings were thinned to two plants per hill. The recommended cultural practices for sorghum production were adopted throughout the growing season.

Target traits were measured on ten guarded plants in each plot for the following traits:

a - Laboratory tests

Seed vigor and seedling characters

1- **Standard germination:** 50 pure seeds of each sorghum genotype and three replications were placed in petri dishes containing filter paper soaked with distilled water. The petri dishes were placed in an incubator at 25 ± 1°C for 4 days to first count and 10 days to final count. Normal seedlings were counted according to the international rules of ISTA (1993) and expressed as germination percentage

2- **Seed vigor index:** was calculated using the following formula (Copeland 1976):

$$\text{Seed vigor index} = \frac{\text{Number of seeds germinated (1st count)}}{\text{Number of days to first count}} + \frac{\text{Number of seeds germinated (last count)}}{\text{Number of days to final count}}$$

3- **Accelerated Ageing Germination % (A.A.G.):** the seeds were kept in an ageing chamber at 45°C and 100 % relative humidity for 3 days. After ageing, the seeds were sun dried. Seed survival percentage was determined by the standard germination test at 25 °C and the mean normal seedling percentage was calculated (AOSA 1983).

Table 1. Pedigree and source of sorghum genotypes used.

No.	Genotype	Pedigree	Source
1	ICSB 14	Exotic	ICRISAT
2	BTX- 623	Exotic	USA
3	BTX 629	Exotic	USA
4	ICSB 88015	Exotic	ICRISAT
5	ICSR 89025	Exotic	ICRISAT
6	ICSR 91022	Exotic	ICRISAT
7	ICSV 273	Exotic	ICRISAT
8	GZ R1	pop-1-1 x G113	Egypt
9	GZ R3	Pop-1-2 x G15	Egypt
10	GZ R4	Pop-1 x G113	Egypt
11	GZB 16	ICSB 37 x ICSB 1	Egypt
12	GZB 11	BTX 623 x ICSB 89039	Egypt
13	Giza 113	BTX 399 x G114	Local variety

4- **Electrical conductivity test**: the electrical conductivity of the leachate was determined according to procedures described by **AOSA (1983).** Four sub- samples of 50 seeds of each cultivar were weighed and placed into plastic cups with 250 ml of distilled water, and held at 25°C. After 24 h, the electrical conductivity of the leachate was determined using EC meter. The mean values were expressed in $\mu S\ cm^{-1}g^{-1}$ seed weight.

5- **Seedlings characters**: normal seedlings obtained from standard germination test were used for seedling evaluation according to the rules of the Association of Official Seed Analysis (AOSA 1983). Seedling shoot and root length were measured after 10 days (final count) of germination test. Twenty-five seedlings from each petri dish were randomly selected and shoot and root lengths of individual seedling were recorded. The shoot and root were also dried at 70 °C for 72 h.

6- **Seedling vigor index:** was calculated using data recorded on germination percentage and seedling growth according to International Seed Testing Association (ISTA 1985) by using the formula:

Seedling vigor index = seedling length (cm) x germination percentage
Seedling vigor index 1 = seedling dry weight (g) x germination percentage

In addition, the following growth measurements were calculated

$$\text{Seedling length rate/day "cm"} = \frac{\text{Seedling length "cm"}}{\text{No. of days at final count}}$$

$$\text{Seedling growth rate/day "mg"} = \frac{\text{Seedling dry weight "mg"}}{\text{No. of days at final count}}$$

b- Growth characters:

- Plant height (cm) was measured from soil surface to the top of the panicle.

- Days to 50% flowering.
- Leaf area (cm2), leaf length x leaf width x 0.75, using the 6 th leaf from the top.
- Length of the panicle (cm).
- Panicle cycle (cm).

c- Yield and yield components

- Panicle weight (g).
- Grain yield /plant (g).
- 1000 –grain weight (g).
- Grain yield (ard/fed).

d- Chemical composition:

Samples of about 50g of air dried ground seeds of each genotype were randomly chosen from two replications for estimating seeds chemical composition. Crude proteins, total carbohydrates and oil percentage, were determined according to the methods of (AOAC 2000).

All data were statistically analyzed as completely randomized design according to the procedures outlined by Snedcor and Cochran (1989) and the differences among genotype means were compared using the test for least significant differences (L.S.D.) at the level of 5 %. Bartelett test of homogeneity was adopted indicating no statistical evidence for heterogeneity thus; combined analysis of variance for genotypes over seasons was worked out.

e- Electrophoresis of total proteins

Soluble proteins were extracted from seeds and SDS-PAGE was conducted according to the protocol described by Laemmli (1970). After the protein has been established, it was stained, photographed and compared with the patterns of known proteins (Protein ladder).

f- RAPD-PCR Analysis

a. Genomic DNA extraction

Young and fresh leaf samples were collected separately from 10 plants for each genotype. All selected leaves were normal and free from any pathogenic symptoms. Leaf samples were saved in ice box and quickly transported to laboratory. Plant tissues were ground under liquid nitrogen to a fine powder, then bulked DNA extraction was performed using DNeasy plant Mini Kit (QIAGEN).

b. Polymerase chain reaction (PCR)

PCR amplification was performed using four random 10 mer arbitrary primers. Table (2) represented names and sequences of the four RAPD primers. All primers were used for PCR following the protocol of Williams *et al* (1990).

Table 2. Names and sequences of the four used RAPD primers.

No	Name	Sequence
1	0P- C13	5' GGGTAACGCC3'
2	0P-D02	5' AGCCAGCGAA3'
3	0P-N12	5' TGGGGGACTC3'
4	0P-E06	5' CCTTGACGCA3'

Amplification was conducted in 25 µl reaction volume containing the following reagents: 2.5 µl of dNTP's (2.5 mM), 2.5 µl Mgcl2 (2.5 mM), and 2.5 µl of 10 x buffer, 3.0 µl of primer (10 pmol), 3.0 µl of template DNA (25 ng / µl), 1 µl of Taq polymerase (1U/ µl) and 10.5 µl of sterile dd H2O. The DNA amplifications were performed in an automated thermal cycler (MJ Research, Watertown, USA) programmed as follows: Denaturation, 94 °C for 2 minutes, then for 40 cycles. Each cycle consisted of 1 minute at 94 °C, 1 minute at 37 °C, 2 minutes and 30 second at 72 °C, followed by a final extension time of 12 minutes at 72 °C and 4 °C (infinitive). Gel electrophoresis was applied according to Sambrook *et al* (1989). Amplified products were size-fractioned using ladder marker (100bp) Fermentas CO, by electrophoresis in 1.2 % agarose gels in TBE buffer at 80 V for 1 h in pharmacia submarine (20 x 20 cm). The bands were visualized by ethidium bromide under UV–transilluminator and photographed by Gel documentation 2000, Bio- Rad.

RESULTS AND DISCUSSION

Seed viability and vigor

Mean values of seed vigor and seedling characters of the thirteen grain sorghum genotypes over two seasons are presented in Table (3). There were differences in seed germination among genotypes. The genotype GZ R4 surpassed other genotypes in germination % with mean value of 95.7%, however, the lowest value was 85.5 % for ICSB 88015. All characteristic traits of seedling significantly differed viz., seedling length, seedling dry weight, seedling length rate, seedling growth rate, seedling vigor index and seedling vigor index 1 shown (Table 3). The average of seedling length ranged from18.4 cm for genotype GZ R4 to 16.0 cm for GZ R3. These results are in harmony with those obtained by Diaga and Gebisa (2003), El Hawary *et al* (2008) and El-Emam *et al* (2009). The genotype GZ R4 surpassed other genotypes and produced the highest seedling dry weight, seedling length rate seedling growth rate, seedling vigor index and seedling vigor index 1 (19.4 mg, 1.8 cm/day, 1.9 mg/day, 1762 and 1.8, respectively). While the genotype ICSB 88015 produced the lowest values

Table 3. Mean of seed vigor and other seedling characters for some grain sorghum genotypes (combined data over two seasons).

Genotype	Standard germination %	Seedling length (cm)	Seedling dry weight (mg)	Accelerated aging %	Electrical conductivity $\mu S\ cm^{-1}g^{-1}$	Seedling length rate (day/cm)	Seed vigor index	Seedling growth rate (mg/day)	Seedling vigor index	Seedling vigor index 1
ICSB 14	93.2	16.4	17.6	80.7	24.5	1.6	19.3	1.8	1525	1.6
BTX- 623	92.2	16.8	19.2	72.5	26.8	1.7	21.8	1.9	1551	1.8
BTX 629	89.7	17.8	18.0	61.3	32.9	1.8	18.8	1.8	1596	1.6
ICSB 88015	85.5	16.1	12.6	65.2	36.3	1.6	19.3	1.3	1377	1.1
ICSR 89025	86.7	17.7	17.9	65.2	36.0	1.8	19.5	1.8	1541	1.5
ICSR 91022	90.3	17.5	17.7	68.8	28.6	1.7	18.3	1.8	1578	1.6
ICSV 273	92.7	16.2	17.9	76.0	25.6	1.6	20.4	1.8	1503	1.7
GZ R1	91.8	16.4	13.5	72.2	27.2	1.6	21.0	1.3	1508	1.2
GZ R3	88.5	16.0	18.4	66.3	35.8	1.6	18.8	1.8	1419	1.6
GZ R4	95.7	18.4	19.4	81.7	22.0	1.8	22.4	1.9	1762	1.8
GZ B16	89.7	17.1	13.6	68.3	29.2	1.7	19.9	1.4	1535	1.2
GZB 11	91.3	17.9	17.8	69.5	27.4	1.8	20.8	1.8	1637	1.6
Giza 113	92.5	17.1	12.7	74.8	26.4	1.7	21.9	1.3	1583	1.2
LSD 0.05%	4.8	0.8	0.7	2.6	1.4	0.1	0.6	0.1	108.4	0.1
CV	4.5	3.9	3.7	3.2	4.3	3.9	2.7	3.7	6.0	5.8

of seedling dry weight, seedling length rate, seedling growth rate, seedling vigor index and seedling vigor index I with means (12.6 mg, 1.6 cm/day, 1.3 mg/day, 1377 and 1.1), respectively. Seed vigor index ranged from 22.4 for genotype GZ R4 to 18.3 for ICSR 91022. It was observed that grain sorghum genotypes varied significantly in the accelerated ageing germination (Table 3), which ranged from 81.7% (GZ R4) to 61.3 in (BTX 629). These results are in accordance with those obtained by El Hawary *et al* (2008) and El-Emam *et al* (2009). Electrical conductivity was much higher in genotype ICSB 88015 (36.3 $\mu S\ cm^{-1}g^{-1}$) than that of genotype GZ R4 (22.0 $\mu S\ cm^{-1}g^{-1}$) (Table 3) suggesting the presence of more deteriorated germination in the former genotype. Higher electrical conductivity of deteriorated seeds was also observed by Schuttle and Loepold (1984) in soybean.

There is a negative relationship between electrical conductivity and seed germination, which indicated that more cell leachates escaped from deteriorated seed and lowered the seed germination. The electrical conductivity test is more commonly used for determining seed vigor of crops that based on the principle that seeds which are losing vigor release materials, such as sugars or other electrolytes in solution, into the soil, which may increase the activity of soil fungi, which, in turn may infect and interfere with the development of seedling growth, especially under cold and wet conditions.

Growth characters

Results in Table (4) showed that the earliest genotypes in flowering were Giza 113, ICSB 88015 and BTX 623 which flowered at 69.2, 69.3 and 69.3 days, respectively with no significant difference with other genotypes, except GZ R3 which flowered at 75.5 days.

For plant height, genotypes GZ R4 and Giza 113 were the tallest plants and gave 255.8 and 233.7 cm, respectively. While the genotypes BTX 623, BTX 629, ICSB 88015 and GZB 11 were the shortest plants and gave 131.9, 151.6, 129.6 and 153.1 cm, respectively. Regarding leaf area, the results showed significant differences among genotypes where the leaf area ranged from 583.1 to 389.8 cm^2 for genotypes GZ R4 and ICSB 88015, respectively. The average of panicle length over the two years ranged from 30.4 to 21.5 cm for BTX 629 and BTX 623, respectively (Table 4 & Fig 1). Regarding panicle cycle, the results showed significant differences among genotypes, which ranged from 15.2 to 11.7 cm for genotypes GZ R4 and ICSB 88015, respectively. These results are in harmony with those obtained by Ali *et al* (2006) and Saba *et al* (2010).

Table 4. Mean performance of some grain sorghum genotypes for grain yield and yield components, panicle measurements and some agronomic traits (combined data over two seasons).

Genotype	Days to 50% flowering	Plant height (cm)	Leaf area	Panicle length (cm	Panicle cycle (cm)	Panicle weight (g)	1000 Kernels (g)	Grain yield /plant (g)	Grain yield (arb/fed)
ICSB 14	71.8	166.6	472.5	27.9	12.3	94.7	25.6	66.3	17.3
BTX- 623	69.3	131.9	468.9	21.5	14.2	87.7	25.5	61.4	17.0
BTX 629	70.3	151.6	477.5	30.4	13.0	82.1	27.5	57.5	15.8
ICSB 88015	69.3	129.6	389.8	23.5	11.7	85.0	27.6	59.5	18.6
ICSR 89025	72.0	165.2	459.7	25.7	13.6	89.0	24.0	62.3	17.7
ICSR 91022	72.8	196.4	495.3	25.7	14..0	88.1	25.6	61.7	16.9
ICSV 273	72.5	217.1	465.3	23.8	14.6	88.2	27.2	61.7	19.4
GZ R1	72.5	230.4	538.2	25.7	14.3	112.4	36.8	82.4	21.7
GZ R3	73.5	203.1	518.0	27.9	14.2	116.2	29.5	86.3	19.3
GZ R4	70.3	255.8	583.1	26.8	15.2	135.0	34.4	93.1	21.6
GZ B16	71.5	199.5	486.2	27.0	13.3	86.8	29.3	60.8	17.1
GZB 11	71.0	153.1	480.0	29.1	13.2	85.9	25.9	60.1	15.7
Giza 113	69.2	233.7	517.0	22.9	14.2	99.6	30.5	69.7	20.8
LSD 0.05%	4.02	24.2	83.83	3.6	2.1	11.1	1.8	7.7	2.0
CV	4.86	11.11	14.78	11.97	13.2	10.0	5.5	9.7	9.3

ardab =150 Kg

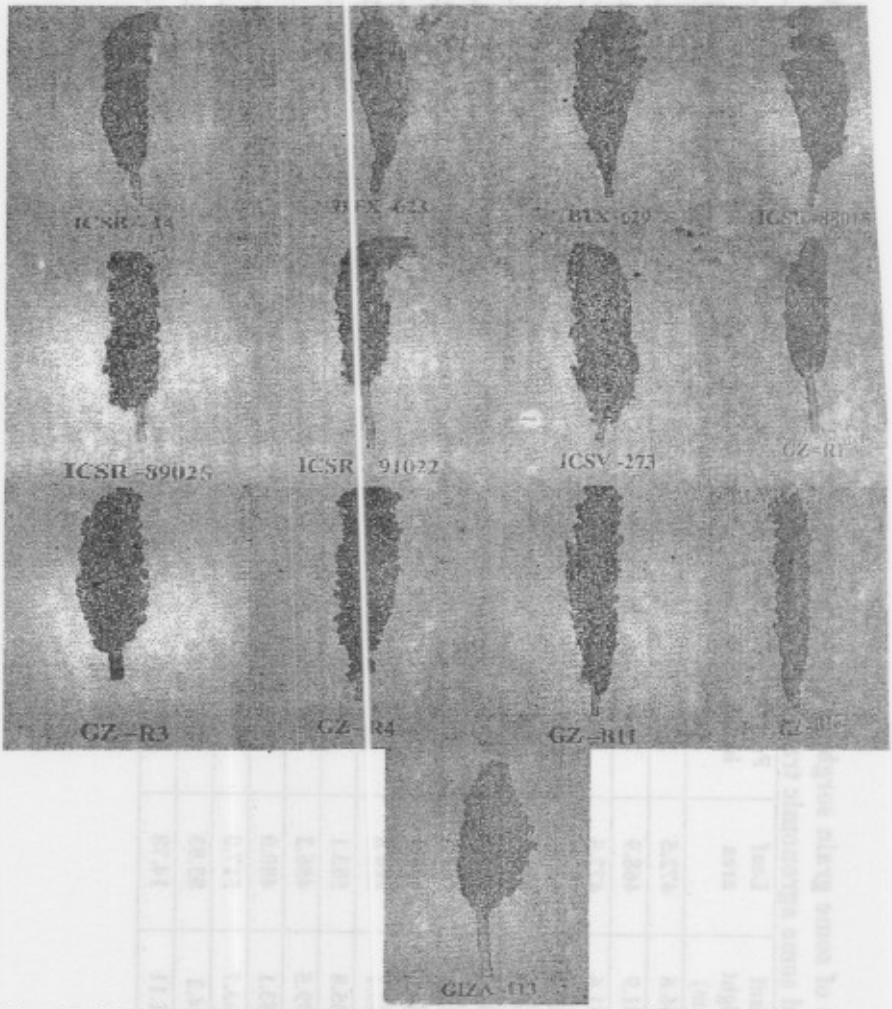

Fig. (1): Panicle shape at maturity and compactness of different grain sorghum genotypes.

Yield and yield components

Regarding panicle weight, genotypes; GZ R4, GZ R3 and GZ R1 surpassed the general mean with averages 133.0, 116.2 and 112.4 g, respectively. However, the lowest panicle weight observed for the genotype BTX 629, which gave 82.1 g (Table 4). The heaviest 1000- kernels weight was recorded for the genotypes GZ R1, GZ R4, Giza 113 and GZ R3 which gave 36.8, 34.4, 30.5 and 29.5 g, respectively. However, the lowest 1000-kernels weight observed for the genotype BTX 623, which gave 25.5 g.

Regarding grain yield plant^{-1}, wide differences were observed among genotypes, grain yield plant^{-1} ranged from 93.1 g for genotype GZ R4 to 57.5 for genotype BTX 629 (Table 4).

The grain yield arb/fed measured on plot base were 21.7, 21.6, 20.8, 19.4 and 19.3 arb/fad, for GZ R1, GZ R4, Giza 113, ICSV 273 and GZ R3, respectively. These genotypes were the most productive genotypes. These genotype performances consent with that affected by El- Nagar (1997), Ali *et al* (2006).

Relationships among some studied characters

The simple correlation coefficients among some studied characters (Table 5) indicated that high germination % was uncorrelated with panicle grain weight (r = 0.197) and panicle weight (r = 0.188), whereas, high panicle weight was correlated positively and significantly with panicle grain weight (r = 0.984 **), high panicle grain weight (plant /yield) was correlated positively and significantly with yield arb/fed (r = 0.589 **).
(n-2)= 76.

Accumulated data of agronomy and seed quality characteristics suggest that sorghum genotype GZ R4 combined high seed yield and high good seed quality and could be recommended for release to be grown in Upper Egypt.

Table 5. Simple correlation coefficients among some studied characters.

	Germination	Seed vigor index	Seedling vigor index	Days to 50 % flowering	Plant height (cm)	Leaf area	Panicle weight (g)	1000 Kernel (g)	Grain yield /plant (g)
Germination %									
Seed vigor index	0.550**								
Seedling vigor index	0.759**	0.458**							
Days to 50%flowering	0.074	0.125	0.220						
Plant height (cm)	0.108	0.146	0.303*	0.206*					
Leaf area (cm^2)	0.001	0.107	0.022	0.051	0.252*				
Panicle weight (g)	0.188	0.095	0.360*	0.162	0.525**	0.033			
1000 Kernel wt. (g)	0.039	0.144	0.208*	0.142	0.498**	0.001	0.584**		
Grain yield /plant (g)	0.197	0.111	0.391**	0.173	0.543**	0.045	0.984**	0..607**	
Grain yield (arb/fed)	0.042	0.224*	0.155*	0.260*	0.551**	0.170	0.576**	0.511**	0.589**

Chemical composition

The grain sorghum of studied genotypes was analyzed for their chemical composition. The results are shown in Table (6). Significant differences were observed among the grain sorghum genotypes for crude protein that ranged from 12.0 % for BTX 623 to 1.2 % for GZ B11. In addition, GZB 16 gave the highest total carbohydrates (72.6%), while GZ R3 gave the lowest percentage (71.1%) but with no significant difference from other genotypes. Results in Table (6) also showed that BTX 629, ICSR 89025 gave the highest oil percentage (3.4 %), whereas the lowest value (3.0%) was recorded for GZ R4. These results are in agreement with those obtained by (Mohammed *et al* 2011) who reported that the protein content was range from 12.25% to 10.70%, The values obtained for fat content were 4.24a(±0.21) and Carbohydrate 74.68%(±1.0).

Table 6. Chemical composition of some grain sorghum genotypes (combined data over two seasons).

Genotype	Protein %	Carbohydrate %	Oil %
ICSB 14	11.7	71.7	3.3
BTX 623	12.0	72.0	3.3
BTX 629	11.8	71.6	3.4
ICSB 88015	11.8	72.5	3.2
ICSR 89025	11.5	71.6	3.4
ICSR 91022	11.7	72.0	3.3
ICSV 273	11.3	72.5	3.2
GZ R1	11.6	72.6	3.2
GZ R3	11.3	71.1	3.3
GZ R4	11.8	71.9	3.0
GZ B16	11.3	72.6	3.2
GZB 11	11.2	72.4	3.3
Giza 113	11.6	72.2	3.2
LSD 0.05%	0.5	NS	0.2
CV	3.8	2.4	6.4

NS = not significant

Protein banding patterns

The electrophoretic banding patterns of proteins extracted from the grains of the thirteen sorghum genotype are shown in Table (7). The results of SDS-PAGE revealed a total number of 22 bands with molecular weights (MW) ranging from 199.0 to 48.0 KDa, which were not necessarily present in genotypes. All bands were polymorphic with 100% polymorphism. There is no resemblance between any genotype and each was characterized by a unique fingerprint. Eight positive specific molecular weights were scored

Table 7. Densitometric analysis for SDS seed storage protein (water soluble fraction) of thirteen grain sorghum genotypes.

Bands No.	MW KDa	Sorghum genotype												
		1	2	3	4	5	6	7	8	9	10	11	12	13
1	199.0	+	-	-	-	-	-	-	-	-	-	-	-	-
2	162.0	-	-	-	-	-	-	-	-	+	-	+	+	+
3	129.0	-	+	-	-	-	-	-	-	-	-	-	-	-
4	126.0	-	-	-	-	-	-	-	-	-	+	-	-	-
5	121.9	-	-	+	+	+	+	+	+	-	-	-	-	-
6	120.0	+	-	-	-	-	-	-	-	-	-	-	-	-
7	116.7	-	+	+	+	+	+	+	+	+	-	-	-	-
8	106.6	-	+	-	+	+	+	+	+	-	+	-	-	-
9	101.0	+	-	-	-	-	-	-	-	-	-	-	-	-
10	98.3	+	+	+	+	+	+	+	+	+	+	+	+	+
11	87.0	+	-	-	-	-	-	-	-	+	-	+	+	+
12	86.1	-	-	-	-	-	-	-	-	-	-	+	+	+
13	84.7	-	+	+	+	+	+	+	+	+	+	-	-	-
14	83.0	+	-	-	-	-	-	-	-	-	-	-	-	-
15	80.2	-	+	+	+	+	+	+	+	-	+	-	-	-
16	69.3	-	-	+	-	-	-	-	+	+	+	+	+	+
17	64.7	-	+	-	+	+	+	+	+	+	+	+	+	+
18	61.0	-	-	-	-	-	-	-	+	+	+	+	+	+
19	55.7	-	+	+	+	+	+	+	-	-	-	-	+	-
20	50.6	-	+	-	-	-	-	-	-	-	-	-	-	-
21	49.0	-	+	-	-	-	-	-	-	+	-	+	-	+
22	48.0	+	-	-	-	-	-	-	-	-	-	-	-	-
Total bands		6	10	7	8	8	8	8	9	9	8	8	8	8

+ = present - = absent

Sorghum genotypes: 1= ICSV 273, 2= ICSR 89025, 3= GZB 16, 4= GZ R3, 5 = GZ R4, 6= ICSB 14, 7= GZB 11, 8= GZ R1, 9 = ICSB 88015, 10= BTX 629, 11= ICSR 91022, 12= Giza 113 and 13= BTX 623

for the absence of a common band. These molecular weights were 199.0, 129.0, 126.0, 120.0, 101.0, 83.0, 50.6 and 48.0 KDa for genotypes ICSV 273, ICSR 89025, BTX 629, ICSV 273, ICSV 273, ICSV 273, ICSR 89025 and ICSV 273, respectively. One negative specific marker was observed for the presence of unique bands at 98.3 KDa for the ICSV 273 genotype. The results indicate that the thirteen genotypes could be identified by their protein banding patterns. The usefulness of seed protein variability for

discriminating among genotypes as well as for studying the genetic relationships has been reported by (Nagaraja *et al* 2000).

RAPD fingerprints

Four random primers were used to differentiate among genotypes through RAPD analysis among the thirteen grain sorghum genotypes as illustrated in Fig. 2 and Table (8). Table (8) summarizes the number of generated fragments using four RAPD primers, which produce 57 amplified fragments across the thirteen grain sorghum genotypes. The results produced scorable amplified fragments with the four primers used (Table 2).

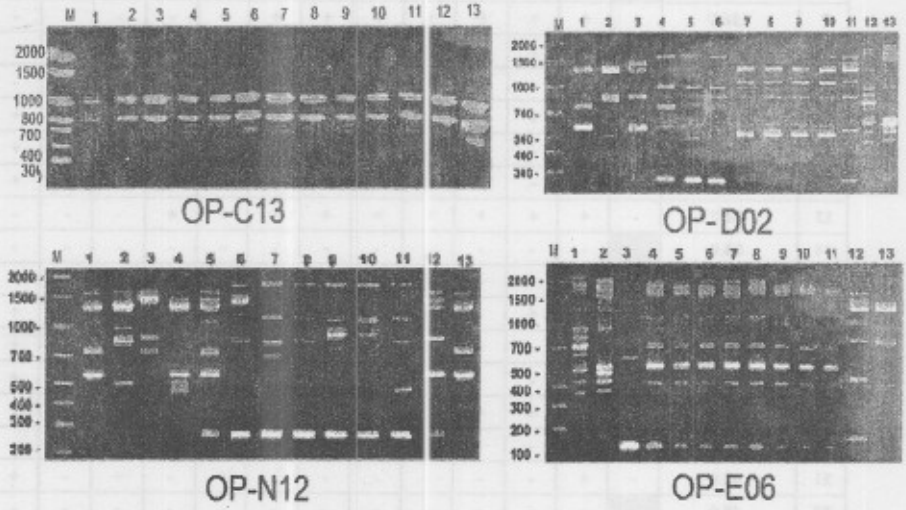

Fig. (2): RAPD fingerprinting of the thirteen sorghum genotypes using four primers OP-C13, OP-DO2, OP-N12 and OP-EO6. genotypes from 1-13 are: 1= ICSB 14; 2 = BTX 623; 3 = BTX 629; 4 = ICSB 88015; 5 = ICSR 89025; 6 = ICSR 91022; 7 = ICSV 273; 8 = GZ R1; 9 =GZ R3; 10 = GZ R4; 11 =GZ 11; 12 = GZ B 16 and 13 = Giza 113.

All primers were polymorphic and showed a high percentage of polymorphism (92.9%). The lowest number of polymorphic RAPD amplified fragments was detected by OP-C13 primer that generated only three polymorphic fragments with 50% of polymorphism, while the largest number was 20, which was generated by OP-E06 primer and gave the highest percentage of polymorphism (100%). Besides, primer OP-N12 gave 100% of polymorphism. The four primers were very informative and generated molecular markers that considered as genotype-specific markers. The largest number of specific markers was scored for genotypes; BTX 629, GZ B16 and Giza 113 (three markers), while the lowest number (one marker) was scored for genotype ICSV 14. A number of 8 positive specific

markers were scored for the presence of unique bands for a given genotype, while 4 negative specific markers were scored for the absence of a common band. In the meantime, the largest number of genotype–specific markers was generated by primers OP-D02 and OP-E06 (five markers), while the primer OP-N12 does not produce any specific markers. The primer OP-C13 generated the least number of genotype -specific markers (two specific unique bands which identified the genotype Giza 113).

Table 8. Levels of polymorphism and unique genotype-specific bands based on RAPD analysis.

Bands / Primer	Total bands	Polymorphic bands	Monomorphic bands	% Polymorphism	Unique bands	
					Genotype	MS
OP-C13	6	3	3	50	Giza 113	2200 bp
					Giza 113	2000 bp
OP-D02	19	18	1	94.7	GZB 16	1930 bp
					Giza 113	1812 bp
					GZB 16	680 bp
					BTX 623	- 590 bp
					GZB 16	360 bp
OP-N12	12	12	0	100		
OP-E06	20	20	0	100	ICSB 14	2100 bp
					BTX 623	2000 bp
					BTX 629	- 1430 bp
					BTX 629	- 1220.bp
					BTX 629	- 430 bp
Total	57	53	4	92.9		

In conclusion, our investigation revealed that RAPD-PCR analysis may be considered as a good molecular technique to obtain genotype-specific markers in sorghum. Moreover, RAPD molecular markers could be used as a tool for marker-assisted selection (MAS) in sorghum breeding programs. The results assess the potentiality of the RAPD technique for characterizing at the molecular level and for generating unique fingerprint for each genotype. These results are in agreement with those obtained by Medraoui *et al* (2007).

Tao *et al* (1993) reported that molecular markers such as RAPD was used to determine the frequency of DNA polymorphism in grain sorghum Hallden *et al* (1994) reported that RAPD markers are easier and quicker to use. These markers may be preferred in applications where the relationships between closely related breeding lines are of interest.

Azzam *et al* (2010) reported that random amplified polymorphic (RAPD)-Polymerase chain reaction (PCR), inter simple sequence repeat (ISSR)-PCR and simple sequence repeats (SSR)-PCR are considered good molecular techniques to obtain molecular markers for grain sorghum resistance and susceptibility to *Acremonium strictum* that cause acremonium

wilt disease. These techniques could be used as a tool for marker-assisted selection (MAS) in sorghum breeding programs directed to predict the resistant and susceptible genotypes to *Acremonium strictum*. Our results also agreed with those of Demeke *et al* (1997) in grain sorghum.

Genetic similarity

Similarity index among the thirteen grain sorghum genotypes based on RAPD-PCR analysis, carried out using UPGMA computer program are presented in Table (9). The highest similarity index (0.984) was recorded between GZ R3 and both of GZ R1 and GZ R4, while the lowest similarity index (0.431) was recorded between BTX 629 and both of GZB 16 and Giza 113.

Table 9. Similarity matrix among thirteen grain sorghum genotypes based on RAPD analysis.

G	1	2	3	4	5	6	7	8	9	10	11	12	13
1	1.00												
2	0.635	1.00											
3	0.453	0.538	1.00										
4	0.600	0.678	0.449	1.00									
5	0.585	0.656	0.519	0.852	1.00								
6	0.567	0.678	0.531	0.821	0.852	1.00							
7	0.594	0.667	0.566	0.767	0.800	0.83	1.00						
8	0.613	0.656	0.549	0.759	0.730	0.79	0.935	1.00					
9	0.603	0.645	0.538	0.780	0.750	0.78	0.921	[illegible]	1.00				
10	0.594	0.635	0.566	0.767	0.769	0.76	0.938	0.968	[illegible]	1.00			
11	0.613	0.689	0.510	0.724	0.762	0.72	0.871	0.867	0.885	0.871	1.00		
12	0.581	0.623	0.431	0.621	0.667	0.62	0.548	0.567	0.590	0.581	0.567	1.00	
13	0.645	0.590	0.431	0.552	0.571	0.51	0.581	0.600	0.623	0.613	0.667	0.667	1.0

G = genotypes, 1= ICSB 14, 2 = BTX 623, 3 = BTX 629, 4 = ICSB 88015, 5 = ICSR 89025, 6 = ICSR 91022, 7 = ICSV 273, 8 = GZ R1, 9 = GZ R3, 10 = GZ R4, 11= GZB 11, 12 = GZB 16 and 13=Giza 113

The dendrogram of the genetic distances between the thirteen grain sorghum genotypes based on RAPDs analysis is presented in Figure (3). The genotypes were separated into three clusters; cluster 1 included genotypes; ICSB 14, GZB. 16 and Giza 113, while the second cluster included only genotype: BTX 629 but the rest of the studied genotypes appeared in cluster 3. Cluster 1 included two subclusters, the first subcluster included ICSB 14 genotype only, while the other two genotypes are appeared in the second subcluster The third cluster included two main subclusters, the first subcluster included the genotype BTX 623 only, while the second subcluster separated into two main sub-subclusters, one of the included genotypes; ICSB 88015, ICSR 89025 and ICSR 91022, while the other included the rest of genotypes. The dendrogram as well as Table (9)

confirmed that genotypes; GZ R1, GZ R3 and GZ R4 are the most closely related genotypes.

In general, SDS-PAGE and RAPD analyses were successfully used for both identification and differentiation of plant cultivars. They are reliably rapid means for establishing genetic profiles and elucidation of genetic relationships within and between taxa (Aly *et al* 2000 and Hassan 2001).

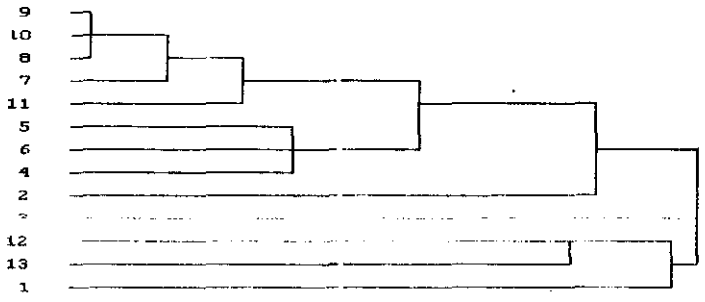

Fig. (3): Dendrogram of the genetic distances between the thirteen grain sorghum genotypes based on RAPDs analysis while, 1= ICSB 14, 2= BTX 623, 3= BTX 629, 4= ICSB 88015, 5= ICSR 89025, 6= ICSR 91022, 7= ICSV 273, 8= GZ R1, 9= GZ R3, 10= GZ R4, 11= GZB 11, 12= GZB 16 and 13=Giza 113.

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الجودة فى ثلاثة عشر تركيبا وراثيا من الذرة الرفيعة وعلاقتها بصفات النمو والمحصول ومكوناته والبصمات الوراثية

نعمت عدلي نجيب

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يعتبر تقييم صفات جودة محصول الذرة الرفيعة من أهم أهداف برامج التربية لذا تم تقييم ثلاثة عشر تركيبا وراثيا من خلال زراعة تجربتين حقليتين بمزرعة سدس بمركز البحوث الزراعية خلال موسمى 2008 و 2009 وكذلك أجريت بعض التجارب المعملية بقسم بحوث تكنولوجيا البذور وقسم بحوث دراسة الخلية بالجيزة، ولقد أظهرت النتائج وجود فروق معنوية بين التراكيب الوراثية بالنسبة لجميع الصفات تحت الدراسه.

أظهرت النتائج إن التركيب الوراثى أعطى GZ R4 أعلى نسبة إنبات (95.7 %) ، في حين أن التركيب الوراثي ICSB 88015 أظهر أدنى نسبة إنبات (85.5 ٪). وتراوحت نسب الإسراع بالشيخوخة من 81.7 ٪ في التركيب الوراثي GZ R4 الى 61.3% في التركيب الوراثي BTX 629. أما متوسط طول البادرة فقد تراوح بين 18.1 سم للتركيب الوراثى GZ - R4 الى 16.0 سم للتركيب الوراثى GZ R3 وبالنسبة لسقيمة التوصيل الكهربائي فقد تراوحت بين 36.3 ميكروسيمون/سم/جم للتركيب الوراثى ICSB 88015 الى 22.0 ميكروسيمون/سم/جم للتركيب الوراثى GZ R4. كما أظهرت النتائج إن هناك اختلاف واضح في صفات النمو بين جميع التراكيب الوراثية حيث تراوح عدد الايام حتى 50% تزهير بين 73.5 – 69.2 يوما للتراكيب الوراثية GZ R3 و جيزة 113، على التوالي ، وتراوح ارتفاع النبات بين 255.8 – 129.6سم للتراكيبGZ R4 و ICSB 88055 ، على التوالي ، وتراوح طول القنديل بين30.4 – 21.5 سم للتراكيب الوراثية , BTX 629 و BTX 623 على التوالي. وفيما يتعلق بمحصول الحبوب /نبات كانت هناك أختلافات كبيرة بين التراكيب الوراثية التي تراوحت بين 53.10 جم في التركيب الوراثي GZ R4 , 57.5 جم للتركيب الوراثيBTX 629. وفيما تعلق بمحصول الحبوب (أردب /فدان) تراوح كمية المحصول بين 21.7 للتركيب GZ R1 و 15.7 للتركيب ZB 11Gعلى التوالي. وقد تم إجراء التحليل الكيماوي للتراكيب الوراثية من الذرة الرفيعة (البروتين ، الكربوهيدرات ، الزيت). وقد أوضحت التحليلات وجود اختلاف معنوى بين التراكيب الوراثية تحت الدراسة فى نسبة البروتين التى تراوحت بين 12.0% للتركيب الوراثي BTX 623 و11.2% للتركيب الوراثيGZ B11 ، لكنها كانت غير معنوية فى نسبة الكربوهيدرات التى تراوحت بين 72.6% للتركيب الوراثي GZB 16 و 71.1% للتركيب الوراثي GZ R3 ونسبة الزيت تراوحت بين 3.4 % للتراكيب الوراثيةICSR 89025 وBTX 629 و3.0 % للتركيب الوراثي GZ R4. وتم الكشف بواسطة التاريد الكهربى للبروتينات الذائبة فى الماء بطريقة SDS - PAGE عدد الوحدات البروتينية كحد أقصى 22 حزمه وجد ان هناك اختلافا فى الوزن الجزيئي تراوح بين 199.0–48.0 كيلو دالتون. شوهدت 22 حزمة متعددة الأشكال (100 ٪) ، تم

متعددة الأشكال (١٠٠ ٪) ، تم استخلاص الحمض النووي DNA من الثلاث عشر تركيبا وراثيا تحت الدراسة واستخدمت اربعة بادئات عشوائية RAPD وذلك لاظهار البصمة الوراثية للتراكيب المختبرة . وقد اظهرت النتائج وجود ٥٧ حزمه منها ٥٣ حزمه متباينه (٩٢.٩%). وكذلك ١٢ حزمه يمكن الاستفادة منها كدلائل خاصه للتراكيب الوراثية . وقسمت الشجره التطورية التراكيب الى مجموعتين رئيسيتين .وقد أظهرت طريقة RAPD القدرة علي تحديد البصمة الوراثية من حيث عدد العلامات الجزيئية المميزة للتراكيب الوراثية المختبرة.