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Determination of Genetic Purity in Three Common Wheat (*Triticum aestivum* L.) Varieties

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Abstract: The present study aimed to verify genetic purity of three wheat varieties namely Gemmiza 10, Giza 168 and Sakha 94 using morphological, biochemical characters as well as grain yield of each variety. Two field experiments were conducted at the Experimental Farm of Tag El-Ezz. Agricultural Research Station Dakahlia Governorate, Agricultural Research Center (ARC), Egypt during the two wheat successive seasons of 2007/08 and 2008/09 were conducted. The results indicated that the Sakha 94 variety was identified by absent anthocyanin coloration of coleoptile. Absent flag leaf area anthocyanin coloration, strong culum glucosity. Very long plant height and medium coloration with phenol test 1%. The Giza 168 variety was identified by medium anthocyanin coloration of coleoptile. Medium of flag leaf area anthocyanin. Very light with phenol coloration. Gemmiza 10 variety identified by strong anthocyanin coloration of coleoptile. Very strong of flag leaf area anthocyanin. Short plant height, very dark with phenol coloration. Results indicated that the percentage of off-types increased as the farmer relied on himself for preparing seeds for crop production. The grain yield and genetic purity based on number of off-types and seed fingerprint were significantly reduced from planting farmer-saved seeds compared with basic, registered and certified seeds. It is possible to utilize farmer- saved seed for one year of certified seed provided that the farmer takes out the off-types from the part of his field he keeps for next planting. Laboratory testing including phenol test of the seed came the same conclusion.

Key words: Seed categories, SDS-PAGE, wheat, yield

INTRODUCTION

Wheat (*Triticum aestivum* L.), is the most important world's leading cereal crop. In Egypt, wheat is considered the main source for human food crops (Seasonal statistical book, Agricultural Economic Research Institute, Agricultural Research Center). The over expanding of increasing population in Egypt have under lined the importance of increasing the productivity of wheat per unit area of land since the area devoted for wheat production is limited. In Egypt, the cultivated area reached about (2.7 and 2.9 million feddan) wheat in the winter seasons of 2007 and 2008 produced an average of (18.1 and 18.2 Ardab/Feddan) of grain and the seed production averaged about 220 tonnes in the same seasons (FAO, 2008).

Improved varieties and its seed quality is the key to agriculture progress. Not enough foundation (basic) seed could be produced to sowing all the areas allocated to production

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of registered seed (Attia, 2010). The fields of seed production were contaminated with off-types and volunteer plants. Therefore the genetic composition of the variety initially developed by the breeders must be the same as marketed to the grower after several generations. It means that purity-genetic varietals must be found in every class of seed certification program which included breeder seed, basic seed, registered seed and certified seed (Selim, 2004). Traditionally, breeder seed companies and certification agencies determine genetic purity using physical traits expressed by the seed However, the success of laboratory and field inspection is limited (El-Sayed, 2005). As a result, genetic purity determinations have shifted to biochemical characterization of seed often separated on electrophoresis gels. On the other hand certified and farmer saved seeds for one year are suggested for wheat production but seed should be neglected. Cultivars characterization and verification are continuing to attract the attention of breeders, the seed industry, breeder's rights protection institution, certification agencies and seed control laboratories. The ultimate objectives are to determine the extant to which a seed sample conforms to a given cultivar and to assure the quality seed marketed to the consumer.

Farmers occasionally complain from certified seeds and they explain their failure to achieve a good field stand or spread of volunteer plant and building up of disease to planting the certified seeds. Thus, there is a need to check on the validity of farmers complaints and assure that substandard seed does not reach farmer hands. Limited information is available pertaining to the evaluation of different classes of wheat seed (Khan *et al*., 2007). Therefore, the present study was undertaken to evaluate the performance of different wheat seed categories especially farmer's seed. So, the study aimed to verify genetic purity and planting value of three wheat varieties using morphological, physiological and biochemical characters as well as grain yield of each tested variety. The overwhelming majority of producers plant certified seed the first year and then use farmer-saved seed for 1-2 years, at which time seed purity declines and then they purchase certified seed again (Stanelle *et al*., 1986). Ebrahim (1999) analyzed the protein profile of different wheat varieties grown in Egypt and found that polyacrylamide gel electrophoresis (PAGE) could be considered as a good tool for identification of wheat varieties. Selim (2000) demonstrated that seed protein profile may serve as useful tool for wheat varieties identification. The aim of this study is to verify genetic purity and planting value of three wheat varieties using morphological, physiological and biochemical characters as well as grain yield of each tested variety.

MATERIALS AND METHODS

Two field experiments were conducted at the Experimental Farm of Tag El-Ezz, Agricultural Research Station (ARC), Dakahlia Governorate, Egypt during the two successive seasons of 2007/08 and 2008/09 to inspect the verification of quality characters and genetic purity of three wheat varieties (*Triticum aestivum* L.) namely Sakha 94, Giza168 and Gemmiza 10) (Table 1). To find out a reliable morphological and biochemical characters for identifying various genotypes. Split-plot design with four replicates was used. The Agricultural practices of growing wheat were performed as recommended to growers.

Table 1: Used experimental material and their pedigree

Varieties	Pedigrees
Sakha 94	OPATA/RAYON//KAUZ.CMBW 90Y3180-OTOPM-3Y-010M-010M-010Y- 10M-015- OY-OAP-0S.
Gemmiza	10 MAYA 74'S'/ON//1160-147/3/BB/GLL/4/CHAT'S'/5/CROW'S'.CGM 5820-3GM-1GM-2GM-OGM
Giza 168	MIL/BUC//Seri CM93046-8M-0Y-0M-2Y-0B.

Table 2: Off types allowable percentage of seeds

	Breeder	Basic	Registered	Certified
Off types allowable	0.01%	0.05%	0.1%	0.2%
Percentage	1: 10.000	1: 2000	1: 1000	1: 500

- **The morphological characters:** Were deformed usually using the recommended scales as reported by IBPGR (1985), Tottman (1986) and UPOV (1990) and the international union for the protection of new varieties recognized UPOV (1994)
- **Fraction of proteins:** Protein extracts of seeds of various varieties and their seed categories were identified by SDS-PAGE according to the method of Laemmli (1970)
- **Grain coloration (+) with phenol test:** According to Banerjee and Chandre (1974)
- **Off-types percentage (%):** According to Table 2 it was estimated by off types divided by total plants of every seed categories for studied varieties, following the standard by Seed testing and Certified Administration, ministerial decree as follows:
- **Grain yield (ardab/feddan):** The data were statistically analyzed by Gomez and Gomez (1984). The treatment means were compared using the new least significant differences (N-LSD)

RESULTS AND DISCUSSION

The Morphological Characteristics

Data in Table 3 shows that the coleoptil anthocyanin pigmentation with wheat varieties of the Gemmiza 10 was strong; the Giza 168 was medium while the Sakha 94 was non-existed. Variety the Gemmiza 10 was very strong in flag leaf anthocyanin coloration while the Giza 168 was medium and the Sakha 94 was absent.

The Sakha 94 and Giza 168 was low but the Gemmiza 10 was absent. Glucosity of neck was strong in the Sakha 94 but weak in the Giza 168 and Gemmiza 10. The Sakha 94 was very long in plant length but the Giza 168 was long while the Gemmiza 10 was short. All wheat varieties in this study was white of ear except the Gemmiza 10 was colored of ear. Grain color of wheat varieties was white in the Sakha 94 and the Giza 168 but it was red in the Gemmiza 10. Grain coloration with phenol was very light in the Giza 168 and brown in the Sakha 94 but it was very dark in the Gemmiza 10. These results are in general agreed with those obtained by Gandy (1996), Abd El-Razik (2005) and Selim (2004). Regarding seasonal type in all wheat varieties in this study it was spring seasons.

In addition, the results in Table 4 and Fig. 1 shows that distinct differences in protein banding patterns of seed three wheat varieties i.e. (The Gemmiza10, the Giza 168 and the Sakha 94) and their classes (basic, registered, certified and farmer's seed), respectively characterized by proteins with molecular weight of (155.514, 121.895, 113.500, 110.384, 106.464, 94.394, 86.895, 86.211, 83.000, 75.722, 67.844, 51.273, 50.120, 48.480, 48.000, 47.420 and 46.900 KD at the Gemmiza 10), (155.514, 121.895, 113.500, 110.384, 106.464, 94.394, 86.895, 86.211, 83.000, 75.722, 67.844, 51.273, 50.120, 49.680, 49.560, 49.000, 48.680, 48.000, 47.420 and 46.900 KD at the Giza 168), (155.514, 121.895, 113.500, 110.384, 94.394, 86.895, 86.211, 83.000, 75.722, 67.844, 51.273, 50.120, 48.000, 47.420 and 46.900 KD at the Sakha 94) respectively. Similar results were obtained by Galterio *et al* (1994) and Ebrahim (1999). Except (EL-Sayed 2005) who reported to some distinguished bands in some seed types.

Grain Coloration with Phenol

The results in Table 5 and 6 revealed that using phenol solution at the concentration of 1%.As to the percentage of seed responded positively to phenol solution, the results

Table 3: Characteristics of the used wheat genotypes

Characteristics	Sign	Stage	Sakha 94	Giza 168	Gemmiza 10
Coleoptile:(+) anthocyanin coloration	Absent or very weak, medium strong and very strong	09-11 VS	Absent	Medium	Strong
Flag leaf:anthocyanin coloration	Absent or very weak, medium strong and very strong	49-51 VG	Absent	Medium	Very strong
Frequency of plant with recurved flag leaves	Absent or very low or low or medium or high or very hight	47-51 VG	Low	Low	Absent
Culm:glucosity of neck	Absent or very weak, medium strong and very strong	60-69 VG	Strong	Weak	Weak
Plant length (stem, ear, awns)	Very short,medium long and very long	75-92 M	Very long	Long	Short ,
Ear color	White and colored	90-92 VG	White	White	Colored
Grain color	White and red	92 VG	White	White	Red
Grain (+) coloration with phenol	Non or very light or medium or dark or very dark	92 VS	Medium	Very light	Very dark

Table 4: Protein fraction of three wheat varieties (Namely, the Gemmiza 10, Giza 168 and Sakha 94) by using SDS-PAGE

Protein MW	G 10 B	G 10 R	G 10 C	G 10 F	Giza 168 B	Giza 168 R	Giza 168 C	Giza 168 F	Sakha 94 B	Sakha 94 R	Sakha 94 C	Sakha 94 F
155.514	+	+	+	+	+	+	+	+		+		
121.895	+	+	+	+	+	+	+	+		+	+	
113.500	+	+	+	+	+	+	+	+	+	+	+	+
110.384	+	+	+	+	+	+	+	+	+	+	+	+
106.464				+	+							
94.394	+	+	+	+	+	+	+	+	+	+	+	+
86.895	+	+	+	+	+	+	+	+	+	+	+	+
86.211	+	+	+	+	+	+	+	+	+	+	+	+
83.000	+	+	+	+	+	+	+	+	+	+	+	+
75.722	+	+	+	+	+	+	+	+	+	+	+	+
67.844	+	+	+	+	+	+	+		+	+	+	+
61.000										+	+	+
51.273	+	+	+	+	+	+	+	+	+	+	+	+
50.120	+	+	+	+	+	+	+		+	+	+	+
49.680						+						
49.560								+			+	+
49.000							+	+	+	+	+	+
48.680							+	+	+	+	+	+
48.480	+	+	+	+								
48.000	+	+	+	+	+	+	+	+	+	+	+	+
47.420	+	+	+	+	+	+	+	+	+	+	+	+
46.900	+	+	+	+	+	+	+	+	+	+	+	+
Total bands	16	16	16	17	16	16	17	17	15	18	18	17

MW: Molecular weight, G: Gemmiza 10

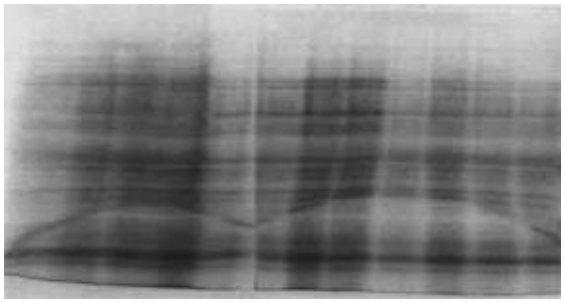

Fig. 1: The distinct differences in protein banding patterns of seed three wheat varieties. (1) Gemmiza 10 basic, (2) Gemmiza 10 reg, (3) Gammiza 10 cert, (4) Gammiza 10 FSS, (5) Giza 168 basic, (6) Giza 168 reg, (7) Giza 168 cert, (8) Giza 168 FSS, (9) Sakha 94 basic, (10) Sakha 94 reg, (11) Saksa 94 cert and (12) Sakha 94 FSS. FSS: Farmer saved seeds

Table 5: The phenol reaction of used wheat varieties and seed categories after 24 h

Varieties	Basic	Registered	Certified	Commercial
Sakha94	Brown	Brown	Brown	Brown
Giza 168	Light brown	Light brown	Light brown	Light brown
Gemmiza 10	Black	Black	Black	Black

Table 6: Averages of off type's % as affected by wheat varieties' performance and seed categories

	Phenol test (%)		Off types (%)		Grain yield (ardab/fed)	
Parameters	2008/2007	2009/2008	2007/2008	2009/2008	2008/2007	2009/2008
Varieties						
Sakha94	93	93	1.139	1.085	13.7	13.9
Giza168	89	90	1.192	1.171	14.3	14.1
Gemmiza10	91	92	1.185	1.164	14.8	14.4
F-Test	**	**	**	*	**	**
NLSD at 5%	0.6	1.1	0.1	0.1	0.3	0.1
Seed categories						
Basic seed	100	100	0		14.6	14.1
Registered	99	98	0.054	0	14.1	14.1
Certified	87	92	0.756	0.039	14.2	14.3
Farmer's seed	77	78	3.878	0.753	14.3	14.2
F-Test	**	**	**	3.767	NS	NS
NLSD at 5%	0.7	1.1	0.1	**	-	-

*Significant, **Highly significant, NS: Not significant, NLSD

indicated that it was significantly high for certified seeds as compared with farmer-saved seeds. The percentage of seed did react to phenol treatment increased as the multiplication number of farmer seeds increased, so that it ranged between (11-18 %) when the farmer seeds were used. These results are in harmony with Selim (2004). The seeds of Giza 168 variety took light brown colour which was deepened after 24 h. The same result was found in all seed categories of Giza 168 variety. All the seed categories of Giza 168 showed no reaction during 24 h observation period. However, the phenol colour reaction can be used to assist in distinguishing wheat seeds of the Gemmiza10 variety as a rapid and not expensive method. It Gemmiza10 contains tyrosinase enzyme. The results were achieved in this study are in agreement with those obtained by Jaiswal and Agrawal (1995) who stated that the use of phenol test was simple, quick and cheap laboratory method for determination of the varietal purity of samples (Gandy, 1996).

Off-Types Percentage

The results in Table 6 revealed that off-types % significantly affected by the wheat varieties in both seasons. Seeds of the Giza168 variety recorded the maximum off types %, which were 1.171 and 1.192% in the first and second seasons, respectively. Seeds of the Sakha 94 variety show the lowest off types % which were 1.085 and 1.139 in both seasons. These results are in complete agreement with those reported by Selim (2004) and Khan *et al.* (2007).

Regarding to the effect of seed categories on off types %, results in Table 6 reported that there were significant differences among basic, registered, certified and commercial seed in both seasons.

Averages of off types % of seed categories within varieties are shown in Table 7, there were no off types in basic seed of three wheat varieties. The farmer's seed of Gemmiza 10 recorded the highest off types % which were 3.94 and 3.86% in the first and second seasons. The results revealed that off types % of seed categories within varieties were lower than off types allowable %. It means that the genetic purity of these varieties are good.

Table 7: Averages of off types % as affected by the interaction between varieties performance and seed categories

	Seasons							
	2007/2008				2008/2009			
Categories and varieties	Basic	Regis.	Certif.	Farmer's	Basic	Regis.	Certif.	Farmer's
Sakha94	0.000	0.080	0.733	3.742	0.000	0.038	0.725	3.575
Giza 168	0.000	0.042	0.775	3.950	0.000	0.041	0.775	3.867
Gemmiza10	0.000	0.039	0.758	3.942	0.000	0.039	0.758	3.858
F Test.			**				*	
NLSD 5%			0.20				0.12	

NLSD: New least significant design

Table 8: Averages of grain yield (ardab/fedan) as affected by the interaction between varieties performance and seed categories

	Seasons							
	2007/2008				2008/2009			
Categories and varieties	Basic	Regis.	Certif.	Fss	Basic	Regis.	Certif.	Fss
Sakha94	13.9	13.7	13.7	13.6	14.2	14.0	13.7	13.5
Giza 168	14.6	13.8	14.3	14.6	14.0	14.0	14.2	14.0
Gemmiza10	15.6	14.6	14.6	14.4	15.1	13.9	14.9	13.9
F Test.			NS				**	
NLSD 5%			-				0.3	

Fss: Farmer-saved seeds

As known, the grain yield is ultimate output of each crop and every type of production. Data belongs to the grain yield are shown in Table 8. Analysis of the data shows that the grain yield of wheat varieties were significantly affected by different seed categories. Basic seed of the Gemmiza 10 gave maximum grain yield (15.661 and 15.105 ardab/feddan) followed by registered, certified and farmers seed category. Farmer's seed obtained from the Sakha 94 gave the minimum grain yield (13.609 and 13.584) in both seasons. Maximum grain yield of the basic seed category of the Gemmiza 10 might be due to high vigor, genetic makeup and yield potential and adaptation of the seed and environmental suitability which resulted more uniform of vigorous seedling and first development. The performance of farmer's seed was not up to the mark because of poor storage which resulted poor quality seedling. The other possible argument for less grain yield might be due to absorption of less nutrient availability and other insufficient inputs for the growth and development of the crop. These results are in line with (Chastain *et al*., 1995; Agrawal and Misra, 1996; Karababa *et al*., 2000), who observed more grain yield for the approved seed category.

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