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DESCRIPTION OF FIVE RICE GENOTYPES BASED ON SEED, SEEDLING CHARACTERS, CHEMICAL TESTS AND BIOCHEMICAL MARKERS

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ABSTRACT

Identification of rice genotypes is of prime importance to ensure quality seed, which is required for achieving global food demand .Two rice hybrids and their parental lines Hybrid Misery 1 (IR 69625A x Giza 178) and Hybrid Misry 2 (IR 69625A x Giza181) were identified on the basis of seed colour, seed size and 1000 seed weight, response to different chemicals (phenol, NaOH, GA_3 and 2,4-D) and electrophoresis of soluble seed protein (SDS-PAGE) and isozymes. All the genotypes were identified based on 1000 seed weight and seed size. Individual chemical tests were unable to distinguish all the genotypes, based on the response to GA_3 100 ppm concentration it was possible to categorize the genotypes into different groups; Low (Giza181), Medium (Hybrid Misery 2 and IR 69625A), high (Hybrid Misery 1) and very high (Giza 178). Based on the effect of 2,4-D (10 ppm) on shoot length inhibition, the genotypes were categorized into two groups; tolerant i.e (Hybrid Misery 2) and susceptible rest of the other genotypes. The genotype IR 69625A was of the highest protein content (13.24 %), while genotype Giza181 gave the lowest value (10.75 %). The carbohydrate contents of the Giza 181 genotype contained the highest value (73.32%), whereas IR 69625A genotype had the lowest content (70.14 %). However, the combination of different chemical tests were useful in identification of individual genotypes. All the genotypes were identified successfully by using electrophoresis of seed protein (SDS - PAGE) and isozmes, which could be used as a powerful tool to identify every genotype in a short period of time.

Key Words: ***Rice, Description, Seed, Seedling, GA3 test, NaOH test, SDS- PAGE, Ilsozmes***

INTRODUCTION

Rice *(Oryza sativa* L.) is the most important cereal grain of the world. It is estimated that half of the world's population depends on rice as main source of food. Cultivated area of Rice in Egypt is about 1.369 million fed in 2009, with an average dry seed production 5.518 million ton. (Agriculture Economics Bulletin of, Ministry of Agriculture, Egypt, 2009).

Egypt has the largest acreage under rice growing countries of world that ranks first in productivity, To sustain its high production and productivity, a number of high yielding varieties and hybrids have been developed and notified recently past, out of which many varieties and hybrids are now in seed production. The release of large number of rice

hybrids has increased the task as well as the responsibilities of seed technologists in order to ensure the quality of seed. Seed technologists must be well equipped to identify different varieties and hybrids, both at field and at seed level. Varietal descriptions given by the breeders most often relate to field characters and not sufficient to identify genotypes or seed lots adequately. Frequently, information is required rapidly, which can be only provided by identification at the seed or seedling level or some chemical tests, which should be rapid, reliable and reproducible.

Many researchers have used seed, seedling morphological characters and different chemical tests for varietal identification. Electrophoresis is a relatively sophisticated, reliable and reproducible technique that has been used extensively by many workers for other crop species for varietals identification (Grabe 1957, Cooke 1987, Vanangamudi *et al* 1988, Varier 1993 and Cooke 1993).

Chemical composition of seeds is basically determined by genetic factors as sources for different raw materials. Modern genotypes of rice have been developed for higher content of carbohydrates which represent significant improvement over earlier varieties. Al-Bahrany (2007) studied the chemical composition of some rice genotypes seeds. He reported that the chemical composition analysis showed that seeds contained moisture (8.83-9.20%), protein (9.24-10.68 %), oils (2.04-2.29%) and carbohydrates (75.69 -77.38%).

Biochemical markers, especially the electrophoretic profiles of isozymes and proteins, have been widely used for identification of crop varieties. Electrophoretic methods have been standardized for a large number of crops and found useful for the purpose of variety identification and characterization (Patra and Chawla 2010). SDS-PAGE of seed storage proteins showed variability and could be effectively used for identification of rice varieties (Diwakar *et al* 2009). Isozyme techniques have been used in plant genetics and breeding. As biochemical markers, isozymes can be used for germplasm classification, gene mapping, selection, monitoring genetic segregation and recombination in distant crosses, variety/hybrid purity, and determination of phylogenic relationship in plants. Once isozyme genes are mapped, they can be utilized efficiently as biochemical markers to map other genes such as morphological and physiological genes or otherwise classical linkage methods are utilized (Tanksley and Rick 1980). In rice, isohyets has provided data relevant to several lines of research, including gene mapping, gene regulation, developmental genetics and evolution (Endo and Morishima 1983).

The aim of the present study was to develop laboratory keys based on seed and seedling characters, chemical tests and biochemical markers for identification of some rice hybrids and their parental lines.

MATERIALS AND METHODS

Pure seeds of two rice hybrids, viz., Hybrid Misery 1 and Hybrid Misery 2: their parental lines (IR69625A, Giza 181 and Giza 178) were obtained from Dept. of Rice Research. This work was conducted at Giza Agric. Res. Station and lab. of seed Technology Dep., Field Crops Res. Instit., ARC during 2009 and 2010 seasons. Observations on seed size and 1000 seed weight were recorded. Four replications of 100 seeds for each genotype were used to measure test weight.

Laboratory tests: seed vigor and seedling characters.

1-**Standard germination:** fifty pure seeds of each 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 14 days. Normal seedlings were counted according to the international rules of ISTA (1993). Germination percentage was calculated using the following formula outlined by Krishnasamy and Seshu (1990):

$$\text{Germination (\%)} = \frac{\text{Number of normal seedlings} \times 100}{\text{Number of tested seed}}$$

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

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

3- **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 leachates was determined using EC meter. The mean values were expressed in $\mu S\ cm^{-1}g^{-1}$ seed weight.

4- **Seedling 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 14 days of germination test. Twenty-five seedlings from each petri dish were randomly selected and shoot and root lengths of individual seedlings were recorded. The shoot and root were also dried at 70 °C for 72 h.

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

Seedling vigor index = seedling length (cm) X germination percentage

6- **GA_3 test :** Four hundred seeds were soaked in 25, 50 and 100 ppm GA_3 for 24 hours and germinated as according to ISTA (1996). The shoot length was measured and the percentage increase in shoot length over that of control was computed and different groups were made based on their response, viz. very high (90-100%), high (75-89%) medium (50-74%) and low (<50%).

7- **2,4-D test:** Four hundred seeds were soaked in 5 and 10 ppm 2,4-D for 24 hours and germinated as per ISTA (1996). Observations were recorded on 14 days in terms of decrease in shoot length over that of control and genotypes were grouped as tolerant (<50%) and susceptible (>51%).

8- **Phenol test:** The standard phenol test for varietal purity testing as suggested by Walls (1965) was followed .Four replications of 100 seeds each were soaked in dististilled water for 24 hours. The seeds were then placed in Petri dishes containing filter paper moistened with 5 ml of 1% phenol solution and kept at room temperature (28°C) for 24 hours. After which the seeds were examined and grouped into different colour classes as no color change, light brown, brown and dark brown.

Chemical composition:

Untreated seed samples were randomly taken to determine moisture, crude protein, crude fat, total carbohydrate, crude fibers and ash according to A.O.A.C (1990).

SDS- protein electrophoresis:

Sodium dodecyl sulfate polyacrylamid gel electrophoresis (SDS-PAGE) technique was used to characterize the total protein of the different genotypes. Protein profiling was carried out according to Laemmli (1970) and modified by Studier (1973).

Isozymes electrophoresis:

Polyacrylamide gel electrophoresis (PAGE) technique was used to charactarize the isozymes profiles such as esterase (EST), peroxidase (Prx), glutamate oxaloacetate transaminase (GOT) and 6-phosphogluconate dehydrogenase (6PGD). Isozyme fractionation was performed according to Jonathon and Wendel (1990).

RESULTS AND DISCUSSION

The data for seed characteristics, presented in Table (1) showed an ample amount of variation based on 1000 seed weight; the genotypes were grouped into three different categories viz., light (< 20.0g; Giza178) medium (21- 23.0g; Hybrid misery 1, Hybrid misery 2 and IR 69625A) and heavy (>24.0g; Giza181). The data for seed germination are presented in Table (1). Three groups were made based on seed germination, namely very

Table 1. Changes in seed coat colour due to various chemicals in rice genotypes.

Genotype	GA_3 test	2,4-D test	NaOH test	Standard phenol test
Giza 178	Very high	Susceptible	LY	B
Giza 181	Low	Susceptible	LY	LB
Hybrid misery 1	High	Susceptible	LY	B
Hybrid misery 2	Medium	Tolerant	LY	B
IR 69625A	Medium	Susceptible	LY	D B

Note:- LY= Light Yellow; LB = Light Brown; B = Brown; DB =Dark Brown.

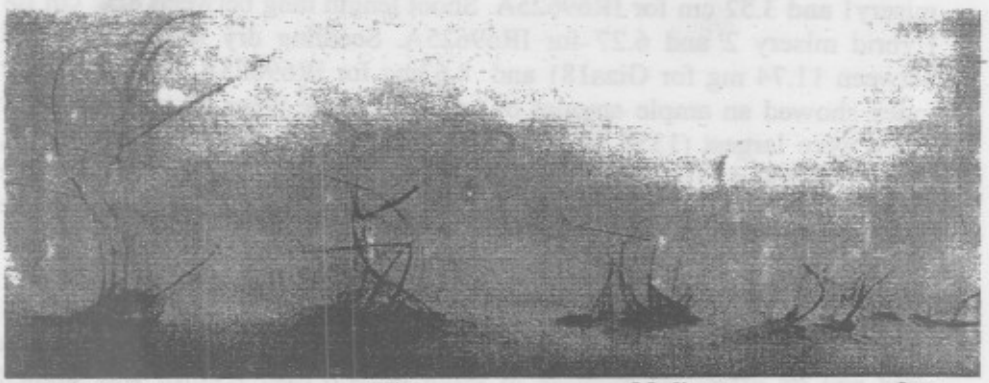
excellent (100%; IR69625A), excellent (96 - 99.5%; Giza 181, Hybrid misery 1 and Hybrid misery 2) and high (95.33 % ; Giza 178). Two types for electrical conductivity were observed in the genotypes studied, viz. low (16.86- 18.02 $\mu s\ cm^{-1}g^{-1}$; G.181 and IR 69625A) medium (21.96- 24.26($\mu s\ cm^{-1}g^{-1}$; Hybrid misery 1, Hybrid misery 2 and G.178). Three groups were made based on seed vigor index, namely the greatest (25.68; IR69625A), greater (23.70- 23.36; Hybrid misery2, Giza178 and Giza181) and great (15.82; Hybrid misery1). The seedling characters were of little differences between the genotypes. Radical length rang between 5.49 for Hybrid misery1 and 3.52 cm for IR69625A. Shoot length rang between 8.52 cm for Hybrid misery 2 and 6.27 for IR69625A. Seedling dry weight is varied between 11.74 mg for Giza181 and 8.63mg for IR69625A. Seedling vigor index showed an ample amount of variation; three types of seedling vigor index were largest (1356 and 1257; Hybrid misery1 and Hybrid misery2), larger (1194 and 1101; G.178 and G.181) and large (978.3; IR69625A). The genotypes were also grouped on the basis of the reaction of seeds to the various chemical tests (Table 2)

1-Chemical tests

GA_3 test: In the present study, GA_3 (25, 50 and 100 ppm GA_3) was used and its effect on increase in shoot growth over control was studied (Goyal and Baijal, 1980 and Bansal *et al* 1992). Based on the response to 100 ppm concentration it was possible to categorize the genotypes into different groups; Low (<40 % Giza181), Medium (50- 74% Hybrid Misery 2 and IR 69625A), high (75-89% Hybrid misery 1) and very high (> 90 Giza 178) (Fig. 1).

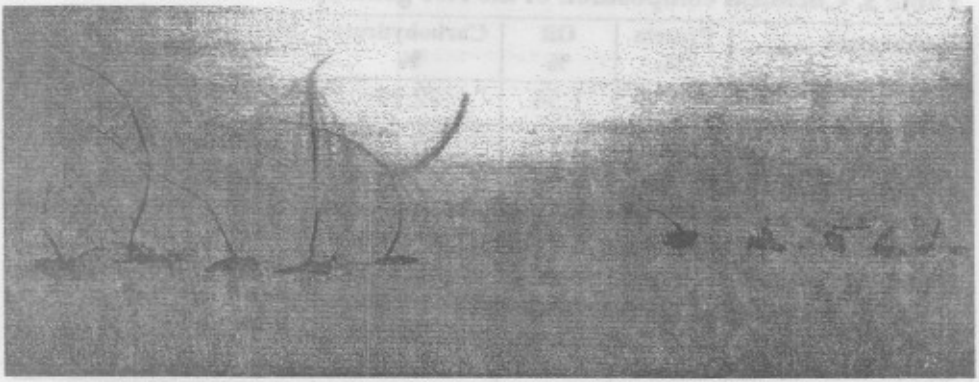
Table 2. **Means of seed vigor and seedling characters for some rice genotypes.**

Genotype	Seed vigour				Seedling vigour			
	Germination %	1000-seed weight (g)	Electrical conductivity ($\mu s^{-1} g^{-1}$)	Seed vigour index	Radical length (cm)	Shoot length (cm)	Seedling dry weight (mg)	Seedling vigour index
Giza 178	95.33	19.73	24.26	23.48	4.62	7.90	11.21	1194
Giza 181	99.33	24.58	18.02	23.36	3.45	7.63	11.74	1101
Hybrid misery 1	97.33	23.82	21.96	15.82	5.49	8.44	11.16	1356
Hybrid misery 2	96.67	22.26	22.62	23.70	4.48	8.52	9.55	1257
IR 69625A	100	21.97	16.86	25.68	3.52	6.27	8.63	978.3
L.S.D 0.05%	3.51	1.03	1.82	0.98	0.41	0.39	1.09	72.40
c.v.	1.91	2.43	4.67	2.35	5.10	2.72	5.55	3.27



Very high G. 178 | High H.M.1 | Medium H.M.2- IR69625A | Low G.181

Fig. 1. Response of rice genotypes to GA3 (100ppm)



Tolerant **Susceptible**
H.M.2 **G178, G181, H.M.1, IR69625A**

Fig. 2. Response of rice genotypes to 2, 4-D (10ppm).

2, 4-D test: Based on the effect of 2,4-D (5 and 10 ppm) on shoot length inhibition, the genotypes were categorized into two groups; tolerant i.e. < 40% reduction than control (H.M.2) and susceptible i.e. > 40% reduction (rest of the other genotypes). The difference in reduction in seedling growth among genotypes may be due to differential ethylene production after application of 2, 4-D (Sundaru *et al* 1983).

Standard Phenol test: The five genotypes were grouped depending on the intensity of staining. (IR69625A) stained dark brown, Giza 181 stained light brown, while rest of the genotypes stained brown.

NaOH test: Based on the NaOH test, all the genotypes were stained light yellow. These results are in agreement with Nethra *et al* (2006). Response of seeds to different chemicals helped in grouping the genotypes rather than clear cut differentiation from each other. No individual chemical test was efficient in distinguishing the genotypes individually; they can be used as a supplement to each other.

2-Chemical composition

The gross chemical composition analysis of five rice genotypes under study is given in Table (3).

Results in Table (3) showed the crude protein content of rice for the genotypes under investigation. Results indicated that the genotype IR 69625A was of the highest protein content (13.24 %), while genotype Giza181 gave the lowest value (10.75 %). However, protein content was 11.97, 11.24 and 11.26 % for Hybrid Misery 2, Hybrid Misery1 and Giza178 genotypes, respectively.

Table 3. Chemical composition of the rice genotypes.

Genotypes	Protein %	Oil %	Carbohydrate %	Moisture %	100 seed Size (mm)
Giza 178	11.26	1.54	72.14	10.45	90.00
Giza 181	10.75	1.19	73.32	10.65	94.00
Hybrid misery 1	11.24	1.76	71.92	10.65	94.00
Hybrid misery 2	11.97	1.46	71.83	10.65	92.00
IR 69625A	13.24	1.72	70.14	9.35	86.00
L.S.D. 0.05%	1.70	0.19	2.04	0.42	8.59
C.V.	7.74	6.45	1.51	2.18	5.00

Oil content values of genotypes under study are presented in Table (3). Results indicated that the highest value of oil content was obtained for Hybrid Misery1 (1.76%). while the lowest value was found in Giza181 (1.19 %). The mean oil content of the other genotypes ranged from 1.19 to 1.76 %

Data on the carbohydrate contents of different rice genotypes under investigation are presented in Table (3). The Giza 181 genotype contained the highest value (73.32%) whereas IR 69625A genotype had the lowest content (70.14 %). Carbohydrate content for seeds of the other genotypes ranged between the previously mentioned limits.

Moisture content ranged from 9.35 to 10.65%. The highest value was 10.65% for Hybrid Misry 2, Giza 181 while the lowest one was 9.35 %, for Hybrid Misery1 and IR 69625A genotypes. Moisture content of Giza 178 genotype was 10.45 %. Such results of moisture content are affected by many factors as location, variety, maturity and storage (Rosario *et al.* 1980) Results present in Table (3) showed the 1000 seed size of the tested rice seeds. The highest value of 1000 seed size (94.00 mm) was recorded for rice genotypes Giza 178 and Hybrid Misery1, whereas, the lowest 1000 seed size value was found for IR 69625A genotype (86.00 mm). It is generally noticed that the values of 1000 seed size in the other genotypes under study ranged between these two limits. Results of the chemical composition of rice genotypes under study are in agreement with those of Ahmed *et al* (1998), Ali *et al* (2001) and Al-Bahrany (2007).

SDS-PAGE of total proteins

Five rice genotypes were identified by SDS- polyacrylamide gel electrophoresis of total protein. The highest number of bands was 17 bands (Table 4) with molecular weights (MW mg) range from 146.75 to 44.10 KD. There were differences between these genotypes in number and intensity of the bands. They revealed variation in the number of bands

Table 4. Molecular weight (MW) of SDS-PAGE 0f total proteins.

MW(KDa)	Giza 178	Giza 181	Hybrid misery 1	Hybrid misery2	IR 69625A
146.75	-	-	-	+	-
114.65	-	+	+	+	-
104.90	-	+	-	+	-
99.79	-	-	+	+	+
95.92	-	+	+	+	-
92.69	+	+	+	+	+
88.74	-	-	-	+	-
86.53	+	+	+	+	+
84.05	+	-	+	+	+
82.44	+	+	+	+	+
74.39	+	+	+	+	+
55.15	+	+	+	+	+
50.10	+	+	+	+	+
49.03	+	+	+	+	+
47.57	+	+	+	+	+
45.87	+	+	+	+	+
44.10	+	+	+	+	+
Total	11	13	14	17	12

+= present - = absent

which ranged between 17 in Hybrid Misery2 and 11 in Giza 178. Bands at MW of 92.69, 86.53, 82.44, 74.39, 55.15, 50.10, 49.03, 47.57, 45.87 and 44.10 KDa were found in all genotypes and could be considered as common bands for these genotypes. While, there were two specific bands for Hybrid Misery2 at MW of 146.75 and 88.74 KDa. The other bands were polymorphic. These results are in agreement with Nethra *et al.* (2007). Also, Diwakar *et al.* (2009) found that SDS-PAGE of seed storage proteins and leaf proteins showed variability and could be effectively used for identification of rice varieties.

Isozyme electrophoresis

Electrophoresis of isozymes is a good technique for identification and characterization of genotypes.

Peroxidase (Prx) isozyme

The peroxidase electrophoretic isozyme exhibited a maximum of six bands, which were not necessarily present in all genotypes as shown in Table (5). The number of peroxidase bands ranged between 4 bands for Giza 178 , Giza 181 and Hybrid Misery1 and 5 bands in Hybrid Misery 2 and IR 69625A. All bands were polymorphic and revealed variation in their densities. The peroxidase produced similar number of bands but the Rf values of the bands were different. These patterns can be used for differentiation among studied genotypes.

Table 5. Rf of peroxidase isozyme (Px).

Genotypes	Prx-1	Prx-2	Prx-3	Prx-4	Prx-5	Prx-6
Giza 178	0.062	—	0.208	—	0.419	0.529
Giza 181	0.062	—	0.208	0.254	0.419	—
Hybrid Misery 1	0.062	—	0.208	—	0.419	0.529
Hybrid Misery2	0.062	0.163	0.208	0.254	0.419	—
IR 69625A	0.062	—	0.208	0.254	0.419	0.529

Esterase (Est) isozyme

Esterase isozyme Table 6 revealed high number of bands which ranged between 6 in Giza178, Giza181 and Hybrid Misery1 and 7 bands in Hybrid Misery 2 and IR 69625A. G. 178, G. 181 and Hybrid Misery1 genotypes contained 6 bands but none can distinguish between them depending on the difference in Rf value because each of them contained the same Rf values. IR 69625A has a specific band at Rf 0.896. while the bands at Rf 0.091, 0.604, 0.726, 0.787 and 0.924 were common bands.

Table 6. Rf of esterase isozyme (Est)

Genotypes	EST-1	EST-2	EST-3	EST-4	EST-5	EST-6	EST-7	EST-8
Giza 178	0.091	—	0.604	0.726	0.787	0.866	—	0.924
Giza 181	0.091	—	0.604	0.726	0.787	0.866	—	0.924
Hybrid Misery 1 1 Misery 1	0.091	—	0.604	0.726	0.787	0.866	—	0.924
Hybrid Misery2	0.091	0.134	0.604	0.726	0.787	0.866	—	0.924
IR 69625A	0.091	0.134	0.604	0.726	0.787	—	0.896	0.924

Glotamate oxaloacetate transaminase (GOT) isozyme

Results in Table (7) on the glotamate oxaloacetate transaminase (GOT) electrophoretic patterns showed no differences between the genotypes in the densities. All the genotypes had two bands with the same Rf values at 0.359 and 0.629.

Table 7. Rf of glotamate oxaloacetate transaminase isozyme (GOT).

Genotypes	GOT-1	GOT-2
Giza 178	0.359	0.629
Giza 181	0.359	0.629
Hybrid Misery1	0.359	0.629
Hybrid Misery2	0.359	0.629
IR 69625A	0.359	0.629

Phosphogluconate dehydrogenase (6PGD) isozyme

The electrophoretic patterns of 6-phosphogluconate dehydrogenase isozyme (Table 8) revealed only one band in all the genotypes at Rf 0.199. This system was insensitive to reveal the genetic variation existing between the genotypes under study.

Table 8. Rf of 6-phosphogluconate dehydrogenase isozyme (6PGD).

Genotypes	6-PGD
G. 178	0.199
G. 181	0.199
Hybrid misery1	0.199
Hybrid misery2	0.199
IR 69625A	0.199

Biochemical markers revealed only a moderate level of polymorphism. These results are in agreement with **Diwakar *et al.* (2009)** who found that peroxidase and esterase isozymes were useful for identification of varieties as well as parents and hybrids and served as marker isozymes for rice varieties.

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توصيف خمسة تراكيب وراثية من الأرز بأستخدام صفات البذرة والبادرة والاختبارات الكيميائية والبيوكيميائية

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يعتبر توصيف بعض التراكيب الوراثية ذو اهمية قصوى لضمان جودة البذور. تم تعريف اثنين من هجن من الارز وأبائها باستخدام صفات البذور (وزن ١٠٠٠ بذرة - حجم البذور)و صفات البادرات (طول الريشة و طول الجذيروالوزن الجاف للبادرات) والاستجابة للمواد الكيميائية المختلفة (الفينول هيدروكسيد الصوديوم والجوبربيك و 2- 4-D) وكذلك التفريد الكهربى لبروتينات البذرة (SDS –PAGE) والمشابهات الانزيمية. جميع التراكيب الوراثية تم تعريفها باستخدام صفات البذور وزن ال ١٠٠٠ بذرة وحجم البذرة .

الاختبارات الكيميائية منفردة لم تستطيع تمييز جميع التراكيب الوراثية ولكن استخدامها مجتمعة استطاعت تعريف هذه التراكيب الوراثية بناء على الاستجابة لتركيز ١٠٠ جزء فى المليون من حمض الجوبربينك من الممكن تصنيف التراكيب الوراثية الى مجموعات مختلفة منخفض (جيزة /١٨١) – متوسط (هجين مصرى ٢ – سلالةA ٦٩٦٢٥) – عالى (هجين مصرى ١) وعالى جدا(جيزة ١٧٨) وعلى اساس تأثير-D 4-2- تركيز ١٠ جزء فى المليون على تثبيط طول الريشة امكن التصنيف الى مجموعتين مقاوم هجين مصرى ٢ وحساس باقى التراكيب الوراثية. وتميز التركيب الوراثى A ٦٩٦٢٥ باعلى محتوى من البروتين % ١٣.٢٤ فى حين التركيب الوراثى جيزة ١٨١ اعلى فى قيمة ١٠.٧٥% . محتوى الكربوهيدرات أعلى قيمة لتركيب الوراثى جيزة ١٨١ (٧٣.٣٢%) فى حين السلالة A ٦٩٦٢٥ كان لديها اقل محتوى ٧٠.١٤(%). بينما جميع التراكيب الوراثية تم تعريفها بنجاح باستخدام التفريد الكهاربى لبروتينات البذور SDS –PAGE والمشابهات الانزيمية والتى يمكن ان تستخدم كاداة قوية لتعريف التركيب الوراثية فى وقت قصير.