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Morphological and Molecular Biochemical Identification of Some Rice (*Oryza sativa* L.) Cultivars

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Abstract: Two field experiments were carried out in Tag AL-Ezz, Agricultural Research Station Farm, ARC, Dakahlia Governorate during 2003 and 2004 seasons. The main objectives of this investigation were to study the genetic purity for four rice varieties through their seed classes and study the morphological characters of the studied cultivars at different growth stages. The main results could be summarized as follows: Giza 177 cultivar was identified by low flag leaf area, narrow angle of the flag leaf, pale straw grain colour and partly awned. Giza 178 cultivar was characterized with thin seed width, high flag leaf area narrow angle of the flag leaf, high number of tillers/hill and gold grain colour. Sakha 101 cultivar was identified by short plant height, late time of heading, large angle of the flag leaf, straw grain colour, absent awing and strong lemma and palea pubescence. Sakha 102 cultivar was identified by tall plant height, thin culm diameter, straw grain colour and partly awned. The analysis of SDS-PAGE had significant effect of studied cultivars at different classes. Planting with basic seed produced highest number of secondary branches/panicle, number of grains/panicle, panicle density, panicle weight, 1000 grain weight and grain yield (ton/fed). Planting using basic seed of Sakha 102 and Giza 178 cultivars produced tallest plants, harvest 1000 grain weight and grain yield/fed, respectively. It could be recommended that seed has been produced under a seed certification program are the best for high grain yield due their high vigor, viability and genetic purity.

Key words: Egyptian rice cultivars, morphological, molecular biochemical identification, SDS-PAGE

INTRODUCTION

Rice (*Oryza sativa* L.) is one of the most important food crops in the world. In Egypt, rice is the second export crop after cotton, moreover it is considered as one of the most important source human food and hard currency earning as an exportable crops. Seed quality of improved varieties is the key to agriculture progress. The introduction of plant breeders right has brought even more exacting requirements for cultivars and distinctness testing in seed certification. Katayama[1] stated that the extensive data on grain length, width and thickness were thought useful for variety classification and studies of differential within and among 64 rice genotypes. Katayama[2] reported that it was possible to distinguish the two types of 50 rice varieties on the basis of 6 characters studied. There were significant differences among five rice varieties in plant height, seed length, seed breadth and days to maturity[3]. Katayama[4] identified 21 strains of rice and stated that eleven out of 25 characters were highly significant. Galussi *et al.*[5] characterized 8 rice varieties in Argentina according to density of pubescence on sheath, blade first leave and density of hairiness. El-Aidy, Nadia *et al.*[6] showed a significant differences among 23 rice varieties for seed length, seed width, seed shape and flag curvature. They concluded that the japonica group ranged from 7.08 to 8.26 mm, grain shape of the most japonica varieties is bold. Twelve rice varieties showed significant differences for the qualitative and quantitative characters[7]. El Hawary *et al.*[8] characterized nine rice varieties used seed length, seed width, lemma anthocyanine and flag leaf area for verification of genetic purity. El Heissewy *et al.*[9] showed a significant differences among three rice cultivars in plant height and plant duration. Giza 176 gave the tallest plants (161 cm). Selim, Amal[10] found that the grain yield and genetic purity were significantly reduced from planting second generation of farmer saved seeds compared with certified seed.

The aim of this investigation was to identify the purity of four rice varieties i.e., Giza 177, Giza 178, Sakha 101 and Sakha 102 for studying the genetic purity of mentioned tested varieties through the following seed classes i.e. basic seed, registered seed, certified seed and commercial seed.

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MATERIALS AND METHODS

Two field experiments were carried out in Tag Al-Ezz Agricultural Research Station Farm, ARC, Dakahlia Governorate during 2003 and 2004 seasons. The experimental design was Split Plot Design ith four replicates. The main plots included the following four rice cultivars as follows:

- Giza, 177
- Giza, 178
- Sakha 101
- Sakha 102

The sub-plots were allocated to the following four seed classes:

- Basic seed
- Registered seed
- Certified seed
- Commercial seed.

The experimental plot area was 3x4 m occupied an area of 12 m^2 i.e. 1/350 fed.

Rice grains at a rate of 60 kg/fed for the nursery bed of each feddan were soaked in water for about 24 h and incubated for 20 h as recommended by rice program in Egypt. Thereafter, it were handy broadcasted with 2-3 cm of standing water in the nursery land on 18 and 24 May in the first and second seasons, respectively.

The winter crop was wheat and after harvesting the land was well prepared and Calcium Superphosphate (15.5 % P_2O_5) at a rate of 100 kg/fed was added on the dry soil before ploughing. Thirty days old seedlings were transplanted at a rate 4-5 seedlings/hill, which were sown at random with the rate of 25 hills m^{-2}, with 2-3 cm of standing water.

The morphological characters were determined using the descriptors is issued by International Union for the Protection of New Varieties of Plants[11]. Sodium Dodecyl Sulphate Polyacrylamide gel electrophoresis (SDS-PAGE) was carried out under the laboratory condition of Seed Technology Research Department, Agriculture Research Center, Giza Governorate. The extracted protein grains samples of various genotypes were identified by SDS-PAGE according to the method of Laemmeli[12].

All obtained data of quantitative characters were subjected to the statistical analysis according to the technique of Analysis Of Variance (ANOVA) of the split plot design, as described by Gomez and Gomez[13]. Combined analysis was done for the significant interaction characters. LSD method was used to test the differences between treatment means.

RESULTS AND DISCUSSION

Morphological identification:

Quantitative characters: Results in Table 1 and 2 clearly indicated that the four studied rice cultivars were significantly varied among them on seed thickness, flag leaf area, plant height, number of tillers/hill, angle of the flag leaf, culm diameter, time of heading and number of secondary branches/panicle in both seasons. Giza 177 cultivar recorded highest values of seed thickness in both seasons and gave the lowest flag leaf area, number of tillers/hill, angle of the flag leaf and number of secondary branches/panicle in both seasons. Giza 178 cultivar produced highest flag leaf area, number of tillers/hill, culm diameter and number of secondary branches/panicle in both seasons and gave the lowest seed thickness in both seasons. Planting Sakha 101 cultivar exhibited highest angle of the flag leaf and time of heading in both seasons. Sakha 101 cultivar recorded lowest plant height in both seasons. Sakha 102 cultivar recorded highest plant height in both seasons and gave the lowest column diameter in both seasons. It is worthy to mention that the differences among studied varieties are in agreement with the results previously obtained with those for seed length, seed

Table 1: Seed and plant characters of some rice cultivars at different seed classes

	Seed thickness (mm)		Flag leaf area (cm^2)		Plant height (cm)		No. of tillers/hill	
Cultivars	2003	2004	2003	2004	2003	2004	2003	2004
Giza 177	2.25	2.24	24.78	23.71	97.2	95.9	18.2	18.2
Giza 178	2.03	2.01	38.65	36.89	95.9	94.1	23.5	22.5
Sakha 101	2.17	2.17	34.03	32.07	90.7	89.9	21.3	20.1
Sakha 102	2.20	2.17	27.48	25.47	105.2	103.4	19.7	19.9
F-test	**	**	**	**	**	**	**	**
LSD 5%	0.03	0.02	0.62	0.71	2.1	1.2	1.3	1.5
LSD 1%	0.04	0.03	0.89	1.02	3.1	1.7	1.8	2.2
Seed classes								
Basic seed	2.16	2.15	31.71	30.15	96.8	95.5	20.9	20.5
Registered seed	2.17	2.15	31.36	29.83	97.2	95.8	20.7	20.0
Certified seed	2.17	2.15	31.36	29.89	97.5	95.8	20.9	20.2
Commercial seed	2.15	2.15	30.53	28.28	97.5	96.1	20.3	20.0
F-test	NS	NS	NS	NS	NS	NS	NS	NS

thickness and angle of the flag leaf[6], plant height and time of heading[7,8].

Results in Table 1 and 2 showed that seed classes had significantly effect on number of secondary branches/panicle in both seasons. Planting basic seed recorded highest number of secondary branches/panicle in both seasons. Planting commercial seed gave the lowest number of secondary branches/panicle in both seasons.

Yield and yield attributes: Results in Table 3 showed that the four studied rice cultivars were significantly varied among them on number of grains/panicle, panicle weight, 1000 grain weight and grain yield in both seasons. Giza 177 cultivar recorded the lowest number of grains/panicle in both seasons. Giza 178 cultivar produced highest number of grains/panicle and grain yield in both seasons and gave the lowest panicle weight in both seasons. Sakha 101 cultivar recorded highest panicle weight in both seasons. Sakha 102 cultivar produced highest 1000 grain weight in both seasons.

Results in Table 3 revealed that seed classes had significantly effect on number of grains/panicle, panicle density, panicle weight, 1000 grain weight and grain yield in both seasons. Planting with basic seed produced highest number of grains/panicle, panicle density, panicle weight, 1000 grain weight and grain yield/fed in both seasons. Commercial seed gave lowest number of grains/panicle, panicle weight, panicle density, 1000 grain weight and grain yield /fed in both seasons.

Results in Table 4 showed that the interaction between studied cultivars and seed classes had

Table 2: Morphological characters of some rice cultivars at different seed characters

	Angle of flag leaf		Culm diameter (mm)		Time of heading		No. of secondary branches/panicle	
Seasons	2003	2004	2003	2004	2003	2004	2003	2004
Cultivars								
Giza 177	10.4	10.7	3.96	4.02	95.1	95.1	15.3	14.4
Giza 178	13.7	13.9	4.93	5.01	97.2	96.8	31.3	30.2
Sakha 101	21.1	19.9	4.63	4.53	100.9	101.0	24.1	22.9
Sakha 102	20.5	20.6	3.58	4.54	92.4	92.4	23.9	21.9
F-test	**	**	**	**	**	**	**	**
LSD 5%	1.1	0.9	0.11	0.07	0.6	0.7	1.7	1.8
LSD 1%	1.6	1.3	0.16	0.11	0.9	1.1	2.4	2.6
Seed classes								
Basic seed	15.9	16.2	4.27	4.28	96.7	96.6	24.5	23.4
Registered seed	16.6	16.6	4.29	4.28	96.6	96.5	23.8	22.4
Certified seed	16.5	15.8	4.27	4.23	95.9	95.7	23.9	22.7
Commercial seed	16.6	16.6	4.27	4.23	96.4	96.4	22.2	20.9
F-test	NS	NS	NS	NS	NS	NS	**	**
LSD 5%	-----	-----	-----	-----	-----	-----	1.5	1.3
LSD 1%	-----	-----	-----	-----	-----	-----	1.9	1.7

** and NS: High significant and Non-significant

Table 3: Grain yield and yield attributes of some rice cultivars at different seed classes

	No. of grains/panicle		Panicle weight (g)		1000 grain weight (g)		Grain yield (ton/fed)	
Seasons	2003	2004	2003	2004	2003	2004	2003	2004
Cultivars								
Giza 177	122.9	120.0	3.42	3.31	27.23	27.09	3.678	3.691
Giza 178	146.9	141.4	3.02	2.80	20.42	20.08	4.001	4.056
Sakha 101	142.0	136.7	3.86	3.62	26.79	27.04	3.965	3.863
Sakha 102	124.2	123.6	3.81	3.56	27.63	27.52	3.825	3.813
F-test	**	**	**	**	**	**	**	**
LSD 5%	3.8	3.3	0.12	0.10	0.62	0.60	0.033	0.040
LSD 1%	5.4	4.7	0.18	0.15	0.88	0.87	0.048	0.053
Seed classes								
Basic seed	135.3	131.7	3.58	3.40	26.30	25.84	3.900	3.874
Registered seed	134.6	130.9	3.56	3.39	25.59	25.69	3.858	3.852
Certified seed	135.7	131.2	3.57	3.40	25.8	25.80	3.913	3.876
Commercial seed	130.2	128.0	3.40	3.11	24.55	24.74	3.798	3.823
F-test	**	**	**	**	**	**	**	**
LSD 5%	3.5	2.6	0.14	0.14	0.63	0.44	0.040	0.037
LSD 1%	4.8	3.5	0.19	0.19	0.84	0.59	0.054	0.049

Table 4: Number of grains/panicle, 1000 grain weight and grain yield as affected by the interaction between rice cultivars and seed classes. (Combined data in 2003 and 2004 seasons)

	Plant height (cm)				1000 grain weight (g)				Grain yield (ton/fed)			
Cultivars	Basic	Reg.	Cert.	Com.	Basic	Reg.	Cert.	Com.	Basic	Reg.	Cert.	Com.
Giza 177	97.2	97.1	95.0	96.7	27.53	27.52	27.52	26.05	3.665	3.698	3.736	3.642
Giza 178	95.7	93.8	95.5	94.7	20.87	20.16	20.83	19.13	4.133	3.963	4.043	3.972
Sakha 101	89.8	91.4	89.7	90.0	27.57	27.27	27.32	25.55	3.943	3.922	3.935	3.872
Sakha 102	101.6	103.6	106.3	105.7	27.97	27.84	27.87	27.84	3.805	3.837	3.862	3.770
F-test			**				**				**	
LSD 5%			2.5				1.06				0.077	
LSD 1%			3.3				1.42				0.103	

**: High significant and Non-significant

Table 5 : Morphological characteristics of rice UPOV Descriptor No. TG/16/4

			Rice cultivars			
Characters	Degree	Note	Giza 177	Giza 178	Sakha 101	Sakha 102
Flag leaf colour	Pale green	3				
	Green	5	5	7	5	5
	Dark green	7				
Ligule shape	Acute	1				
	Cleft	3	3	3	3	3
	Truncate	5				
Collar colour	Pale green	1				
	Green	3	1	1	1	1
	purple	5				
Grain colour	Pale straw	3				
	Straw	5	3	7	5	5
	Gold	7				
Auricle colour	Pale green	1				
	Purple	2	1	1	1	1
Awing	Absent	3				
	Partly absent	5				
	Long and pertly absent	7	5	3	3	5

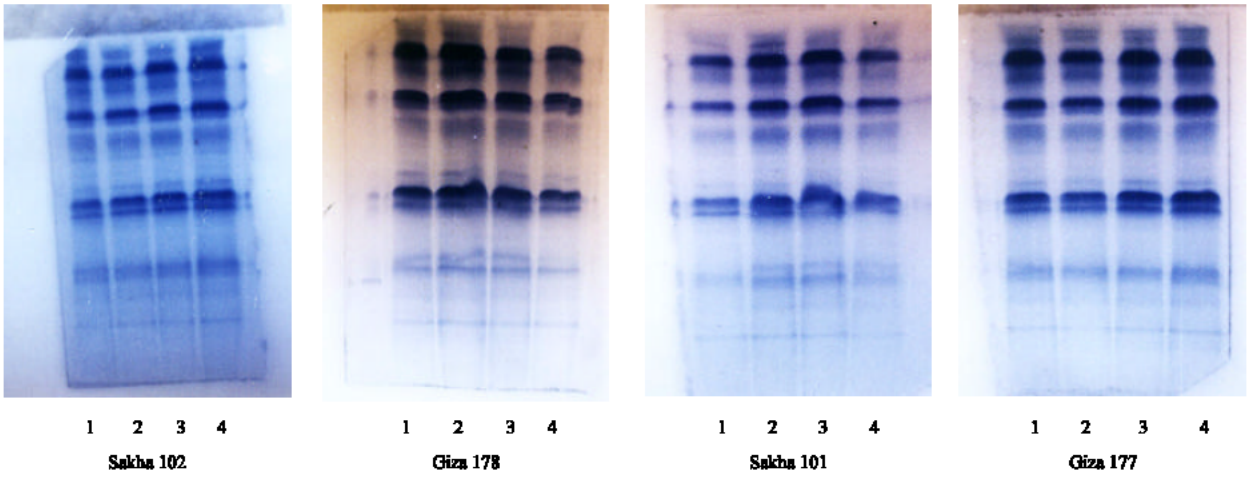

Fig. 1: Results of SD-PAGE patterns of seed protein fraction of seed classes (1-Basic, 2-Registered, 3-Certified, 4-Commercial) for studied cultivars

significantly effect on plant height, 1000 grain weight and grain yield in both seasons. Planting with basic seed of Giza 178 and Sakha 102 cultivars gave highest grain yield/fed and 1000 grain weight, respectively. Planting with commercial seed of Giza 177 variety gave the lowest grain yield. Commercial seed of Giza 178 gave the lowest 1000 grain weight. Planting certified seed of Sakha 102 gave tallest plants.

Table 6: Results of SDS-PAGE patterns of seed protein fraction of seed classes for studied cultivars

	Seed classes			
Varieties	Basic	Registered	Certified	Commercial
Giza 177	17	17	17	16
Giza 178	15	15	16	15
Sakha 101	14	14	15	12
Sakha 102	14	14	14	13

Qualitative characters: Results in Table 5 revealed that Giza 177 cultivar was identified by green flag leaf colour, cleft ligule shape, pale green collar colour, pale straw grain colour, pale green auricle colour and partly awned. Giza 178 cultivar was identified by dark green flag leaf colour, cleft ligule shape, pale green collar colour, gold grain colour, pale green auricle colour and absent awned. Sakha 101 cultivar was identified by green flag leaf colour, cleft ligule shape, pale green collar colour, straw grain colour and absent awned. Sakha 102 cultivar was identified by green flag leaf colour, cleft ligule shape, pale green collar colour, straw grain colour and partly awned. The differences among studied rice cultivars in qualitative characters are in harmony with those reported by El-Aidy Nadia *et al.*[6] and El-Hawary *et al.*[8].

Sodium Dodecyl Sulphate Polyacrylamide gel electrophoresis (SDS-PAGE) of seed protein fraction: Results in Table 6 and Fig. 1 revealed that seed classes of basic, registered, certified and commercial seed of Giza 177 cultivar had 17, 17, 17 and 16 total proteins bands, respectively. Basic, registered, certified and commercial seed of Giza 178 cultivar had 15, 15, 16 and 15 total proteins bands, respectively. Basic, registered, certified and commercial seed of Sakha 101 cultivar had 14, 14, 15 and 12 total proteins bands, respectively. Basic, registered, certified and commercial seed of Sakha 102 cultivar had 14, 14, 14 and 13 total proteins bands, respectively. The differences among seed classes of studied cultivars are in harmony with those reported by El-Aidy *et al.*[6], El-Hawary *et al.*[8] and Selim[10].

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