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Identification and characterization of some aromatic rice mutants using Amplified Fragment Length Polymorphism (AFLP) technique.

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التعرف على بعض طفرات الأرز الأروماتية باستخدام تقنية تمايز الأطوال المقطعة بعد التكبير

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1- كلية الزراعة – جامعة عين شمس – قسم الوراثة – القاهرة – مصر.

2- هيئه الطاقة الذرية – مركز البحوث النووية – قسم البحوث النباتية – أنشاص – مصر.

3- مركز البحوث والتدريب علي الأرز – سخا – مصر.

4- الوكالة الدولية للطاقة الذرية – معامل الوكالة بسيبرسدورف – وحدة تربية النبات – فيينا – النمسا.

خلاصة

[illegible] التعرف الدقيق على التراكيب الوراثية من أهم الآليات الت[illegible] أو حماية الأصناف النباتية. أجرى هذا البحث بالمزرعة التجريبية بهيئة الطاقة الذرية – أنشاص. بهدف تقييم صفات الجودة والتباينات الوراثية على المستوى الجزيئى باستخدام تقنية تمايز الأطوال المقطعة بعد التكبير (AFLP) بين ستة تراكيب وراثية وهى صنف الأرز العطري الياسمين المصري وخمسة طفرات أرز عطرية (الجيل الثالث الطفري M_3). شملت الطفرات كل من الطفرتين Egy22 و Egy24 اللتين أنتخبتا من الجيل الثانى بعد تشعيع الصنف سخا 102 بالجرعتين 200،400 جراى بأشعة جاما على التوالي والطفراتEgy32 و Egy33 و Egy34 التى أنتخبت من الجيل الثانى بعد تشعيع الصنف سخا 103 بالجرعات 200 ، 300 ، 400 جراى بأشعة جاما على التوالي. أظهرت النتائج أن الرائحة العطرية القوية وحدت فى

الطفرة Egy22 مقارنة بصنف الأرز الياسمين المصري. تم استخدام سبعة توليفات من البادئات للتقييم الوراثى على المستوى الجزيئى (AFLP) وكان حجم الشظايا (الحزم) يتراوح بين 51- 494bp. و العدد الكلى للحزم هو997 من بينها 919 متباينة (polymorphic) بنسبة 92.2%. بينما كان أعلى متوسط نسبة تشابه وراثى (89%) بين صنف الأرز ياسمين المصري والطفرة Egy32 يليها الطفرة Egy34 بنسبة (82%) وكان أقل متوسط نسبة تشابة وراثى (48%) بين صنف الأرز ياسمين المصري والطفرة Egy24. تميزت الطفرة Egy24 بأكبر عدد من الواسمات وهو 49 واسم (23موجب و 26سالب) فى حين تميزت الطفرة Egy22 بـ 33 واسم (27موجب , 6سالب) ثم الطفرة Egy33 17 واسم (13موجب ، 7 سالب) وأخيراً صنف الأرز ياسمين المصري تميز بـ 10 واسمات (6موجب ، 4سالب). وأكدت الدراسة أن تقنية الـ AFLP تمكن من تمييز التراكيب الوراثية للأرز بعدد أكبر من الواسمات الفريدة.

ABSTRACT

Accurate identifying of the genotypes is considered one of the most important mechanisms used in the recording or the protection of plant varieties. This investigation was conducted at the experimental farm belonging to the Egyptian Atomic Energy Authority, Inshas. The aim was to evaluate grain quality characteristics and molecular genetic variation using Amplified Fragment Length Polymorphism (AFLP) technique among six rice genotypes; Egyptian Jasmine aromatic rice cultivar and five aromatic rice mutants in (M_3 mutagenic generation). Two mutations (Egy22 and Egy24) were selected from irradiated Sakha102 population with 200 and 400Gy of gamma rays in the M_2 generation, respectively, and three mutations (Egy32, Egy33 and Egy34) were selected from irradiated Sakha103 population with 200, 300 and 400Gy of gamma rays in the M_2 generation, respectively. The obtained results showed that the strong aroma was obtained for mutant Egy22 as compared with Egyptian Jasmine rice cultivar (moderate aroma). Seven primer combinations were used through six rice genotypes on the molecular level using AFLP markers. The size of AFLP fragments were ranged from 51-494bp. The total number of amplified bands was 997 band among them 919 polymorphic bands representing 92.2 %. The highest similarity index (89 %) was observed between Egyptian Jasmine and Egy32 followed by (82 %) observed between Egyptian Jasmine and Egy34. On the other hand, the lowest similarity index was (48 %) between Egyptian Jasmine and Egy24. In six rice genotypes, Egy24 produced the highest number of the AFLP markers giving 49 unique markers (23 positive and 26 negative), then Egy22 showed 23 unique markers (27 positive and 6 negative) while Egy33 was characterized

by 17 unique markers (12 positive and 5 negative). At last, Egyptian Jasmine was discriminated by the lowest number of unique markers, 10 (6 positive and 4 negative). The study further confirmed that AFLP technique was able to differentiate rice genotypes by a higher number of unique markers.

INTRODUCTION

Induced mutations have played a significant role for the improvement of rice by developing a large number of semi-dwarf and high yielding varieties in many countries **[1] and [2].** Recently, International Atomic Energy Agency and Food and Agriculture Organization (IAEA / FAO) has reported that 443 rice varieties have been developed through induced mutations **[3].**

Grain quality in rice play an important role in consumer acceptability and ranks second after yield as the major breeding objective for crop improvement **[4]**. The quality in rice is considered based on milling quality, grain size, shape, and aroma, and other cooking characteristics **[5].**

The estimation of genetic diversity on the basis of morphological traits alone does not reflect the actual level of diversity among germplasm because morphological traits are the product of gene and environmental interactions **[6].** Therefore, techniques based on DNA markers along with morphology traits have been widely used to detected variation at DNA level to distinguish between closely related genotypes. The development of the Amplified fragment length polymorphism (AFLP) analysis **[7]** allowed genome characterization in several crops **[8], [9], [10], [11].**

The main objective of this study was to assess grain quality characteristic and genomic diversity using AFLP technique among five aromatic rice mutants and the Egyptian Jasmine cultivar.

MATERIALS AND METHODS

This investigation was conducted at the experimental farm belonging to the Egyptian Atomic Energy Authority, Inshas.

Materials :

Six rice genotypes i.e., cultivar Egyptian Jasmine and five aromatic rice mutants in M_3 generation; Egy22 and Egy24 selected from irradiated Sakha 102 population with 200 and 400 Gy of Gamma rays in the M_2 respectively and Egy32, Egy33 and Egy34 selected from irradiated Sakha 103 population with 200, 300 and 400 Gy of gamma ray in the M_2 generation, respectively. Egyptian

Jasmine rice cultivar was used for comparison with aromatic rice mutants because its characterized by an increase in aromatic trait.

Methods :

Grains of each selected plant were grown in May 12th as a single plant progeny to obtain M_3 plants. At harvest, thirty individual plants from each selection were used to detect the breeding value of the selected variants. In M_4 generation, six rice genotypes were grown on May 15th to evaluate grain quality characters. Randomized complete block design with three replications were used.

The grain quality parameters for the six genotypes; Egyptian Jasmine rice cultivar and the five aromatic rice mutants were measured. These parameters were; grain length, grain width, grain shape, amylose and aroma contents.

Grain length was determined as an average of 15 grain of rough rice per genotype. Amylose content % was estimated by the method outlined in **[12].** Fourty grains of each genotype were soaked in 10ml 1.7% KOH solution at room temperature in a covered glass petri dish for about one hour. The sample was scored on 1-4 scale with 1, 2, 3 and 4 corresponding to absence of aroma, slight aroma, moderate aroma, and strong aroma, respectively. The same four panels of researchers and staffs from Division of Plant Breeding, Nuclear Research Center, Egyptian Atomic Energy Authority were invited to score the aroma in each genotype **[13].**

AFLP technique:

The molecular genetics analyses were conducted at the laboratories of Plant Breeding Unit, International Atomic Energy Agency, Seibersdoorf, Vienna, Austria. Five grams of leaves were collected after growing M_3 grains for a month to perform the fingerprinting. DNA was extracted from the progeny of the selected mutants according to **[14] and [15]**. AFLP technique is based on the selective PCR amplification of restriction fragments from a total digestion of genomic DNA. The technique involves three steps, restriction of the DNA and legation of oligonucleotide adaptors, selective amplification of sets of restriction fragments and gel analysis of the amplified fragments. Table (1) shows the sequences of each primer combination for AFLP analysis.

GeneMapper Software:

The GeneMapper v4.0 Software analyzed samples were prepared using an AFLP technique that incorporates the applied biosystems fluorescent dye-labeling and detection technology and

samples run on a compatible applied biosystems electrophoresis instrument. Applied biosystem has adapted the AFLP technique for use with its fluorescent dye-labeling and detection technology. In the modified system, a 5' dye-labeled primer has been substituted for one of the selective primers used in the final amplification step .

Statistical analysis:

Data M_4 generation were subjected to proper statistical analysis of variance for randomized complete block design **[16].** The L.S.D. test was used for comparison between the means **[17]**.

Table 1. The sequences of each primer combination for AFLP analysis

1-	Primer combination E-CTG / T-GTC
Eco-CTG	5`- GACTGCGTACCAATTC**CTG** -3`
Tru91-GTC	5`- GATGAGTCCTGAGTAA**GTC** -3`
2-	Primer combination E-GAG / T-CTC
Eco-GAG	5`- GACTGCGTACCAATTC**GAG** -3`
Tru91-CTC	5`- GATGAGTCCTGAGTAA**CTC** -3`
3-	Primer combination E-GAA / T-CTT
Eco-GAA	5`- GACTGCGTACCAATTC**GAA** -3`
Tru91-CTT	5`- GATGAGTCCTGAGTAA**CTT** -3`
4-	Primer combination E-CTC / T-CTA
Eco-CTC	5`- GACTGCGTACCAATTC**CTC** -3`
Tru91-CTA	5`- GATGAGTCCTGAGTAA**CTA** -3`
5-	Primer combination E-CTA / T-CAA
Eco-CTA	5`- GACTGCGTACCAATTC**CTA** 3`
Tru91-CAA	5`- GATGAGTCCTGAGTAA**CAA** -3`
6-	Primer combination E-AGG / T-GAT
Eco-AGG	5`- GACTGCGTACCAATTC**AGG** -3`
Tru91-GAT	5`- GATGAGTCCTGAGTAA**GAT** -3`
7-	Primer combination E-AG / T-GTA
Eco-AG	5`- GACTGCGTACCAATTC**AG** -3`
Tru91-GTA	5`- GATGAGTCCTGAGTAA**GTA** -3`

RESULTS AND DISCUSSIONS

Grain quality parameters for the six genotypes; Egyptian Jasmine aromatic rice cultivar and five aromatic rice mutants are presented in Table 2. The results showed significant variation among the materials for grain quality traits.

Significant differences were observed among the Egyptian Jasmine and five aromatic rice mutants for grain shape while grain length and width were comparable. Grain shape values ranged from 3.9 in Egy33 to 3.6 in Egy34. Grain length ranged from 10.1 mm in Egy22, Egy32 and Egy33 to 9.3 in Egy34. Grain width ranged from 2.7 mm in Egy22 and Egyptian Jasmine to 2.6 in Egy33.

Amylose content ranged from 24.2% in Egy24 to 15.9% in Egy32 while Egy22 showed 16.9 %, Egyptian Jasmine gave (16.8%), Egy34 16.8% and Egy33 16.7%. Rice cultivars were grouped on the basis of their amylose content into waxy (0-2%), very low (3-9%), low (10-19%), intermediate (20-25%) and high (> 25%) **[18].** Intermediate amylose rice are preferred types in most of the rice growing areas of the world, except where low amylose Japonicas are grown **[5].**

Table 2. Grain quality traits for the six rice genotypes.

Genotypes	Grain length	Grain Width	Grain shape	Amylose content	Aroma content*
Egy22	10.1	2.7	3.9	16.9	4.0
Egy24	9.9	2.6	3.8	24.2	3.0
Egy32	10.1	2.6	3.8	15.9	3.0
Egy33	10.1	2.6	3.9	16.7	3.0
Egy34	9.3	2.6	3.6	16.8	3.0
Egyptian jasmine	9.9	2.7	3.6	16.8	3.0
LS.D 0.05	N.S	N.S	0.236	-	-

*1 = none, 2 = slight, 3 = moderate and 4 = strong.

As far as aromatic volatile components estimation, the best mutant was Egy22 scored (4) indicating strong aroma as compared with Egyptian Jasmine aromatic rice cultivar as well as the remaining four mutant genotypes which scored 3 (moderate aroma). These results agree with **[19]**, who found that six rice mutants had higher aroma level compared with the original parent Egyptian Jasmine. The Middle East consumers prefer rice with strong aroma. For consumers in Europe, trace of aroma is an objective trait. **[20].**

AFLP analysis:

Table 3 illustrates the AFLP profile of the six rice genotypes as revealed by the seven primer combinations. The total number of markers was 997 amplified bands among them 919 were polymorphic, representing 92.2% rate of polymorphism. The highest percentage of polymorphism was obtained with E-CTG/T-GTC

(100%) and E-GAG/T-CTC (100%) and the lowest percentage of polymorphism was obtained with E-CTC/T-CTA (88%) (Table 3).

The size of ALFP fragment with the different primer combinations ranged from 51 to 494bp.

Table 3. Primer combinations with polymorphism (%) obtained through AFLP technique among the six genotypes

Primer combinations	total no of amplified bands	polymorphic bands	polymorphism (%)
E-AGG /T-GAT	148	142	96%
E-CTA/T-CAA	183	165	90%
E-CTC /T-CTA	145	127	88%
E-CTG/T-GTC	106	106	100%
E-GAA / T-CTT	234	210	90%
E-GAG / T-CTC	74	74	100%
E-AG/T-GTA	107	95	89%
Total	**997**	**919**	**92.2%**

Polymorphism% = (Number of polymorphic/ total number of amplified bands) *100

Similarity indices among Egyptian Jasmine and the five aromatic rice mutants ranged from 48% to 89% (Table 4). The highest similarity index means (89%) was observed between Egyptian Jasmine and Egy32 followed by 82% between Egyptian Jasmine and Egy34. On the other hand, the lowest similarity % was found between Egyptian Jasmine and Egy24 (48%).

These results were comparable with those reported by **[8]** who detected 179 polymorphic bands (44% polymorphism), when using four combinations of AFLP primers which generated of total of 410 AFLP bands. The AFLP technology is a powerful tool for the detection of evolution in germplasm collections and in the screening of biodiversity as well as for fingerprinting **[21]**. In another study **[22]** of genetic variability and the relationships among seven Egyptian rice genotypes using eight AFLP primer combinations, the level of polymorphism was 67.9%. The highest genetic relationship was detected between Giza175 and Giza177 (83.4%) while the lowest similarity was found between Giza178 and Sakha101 (61.5 %).

Table 4. The similarity indices for Egyptian Jasmine with each one of the five genotypes with the seven primer combinations

Primer combinations	Egyptian jasmine				
	Egy22	**Egy24**	**Egy32**	**Egy33**	**Egy34**
E-AGG /T-GAT	0.65	0.47	0.85	0.54	0.66
E-CTA/T-CAA	0.56	0.49	0.93	0.48	0.87
E-CTC /T-CTA	0.63	0.60	0.89	0.68	0.79
E-CTG/T-GTC	0.76	0.37	0.90	0.55	0.77
E-GAA / T-CTT	0.70	0.41	0.95	0.46	0.84
E-GAG / T-CTC	0.38	0.31	0.78	0.48	0.94
E-AG/T-GTA	0.91	0.73	0.93	0.78	0.88
	0.65	**0.48**	**0.89**	**0.57**	**0.82**

Similarity index % = (1- polymorphism) * 100

AFLP markers

The unique AFLP markers that characterized and differentiate the six rice genotypes are listed in Table 5. In the present study, seven primer combinations were able to characterize the six rice genotypes by a total of 133 unique AFLP markers (79 positive and 54 negative). The primer combination E-GAA/T-CTT revealed the highest number of unique markers (19 positive and 14 negative markers). On the other hand E-GAG/T-CTC revealed the lowest number of unique markers (8 positive and 3 negative). Mutant Egy24 produced the highest number of unique AFLP markers giving 49 unique markers (23 positive and 26 negative). Followed by mutant Egy22 which produced 33 unique AFLP markers (27 positive and six negative), mutant Egy33 gave 17 unique markers (12 positive and 5 negative), Egy34 showed 13 unique markers (8 positive and 5 negative) and mutant Egy32 exhibited 11 unique markers (3 positive and 8 negative). Egyptian Jasmine produced the lowest number of 10 AFLP unique markers (6 positive and 4 negative). In a comparable study **[22]**, seven rice varieties with eight primer combinations showed that the primer combination E-CAC/N-CAT produced the highest number of unique markers (2 positive and 33 negative), while the primer combination E-AGT/M-CAT produced the lowest number of unique markers (one positive and one negative). Sakha101 produced the highest number of 40 unique AFLP markers (3 positive and 37 negative), while Giza 172 was characterized by the lowest number (only one unique positive marker) **[22]**.

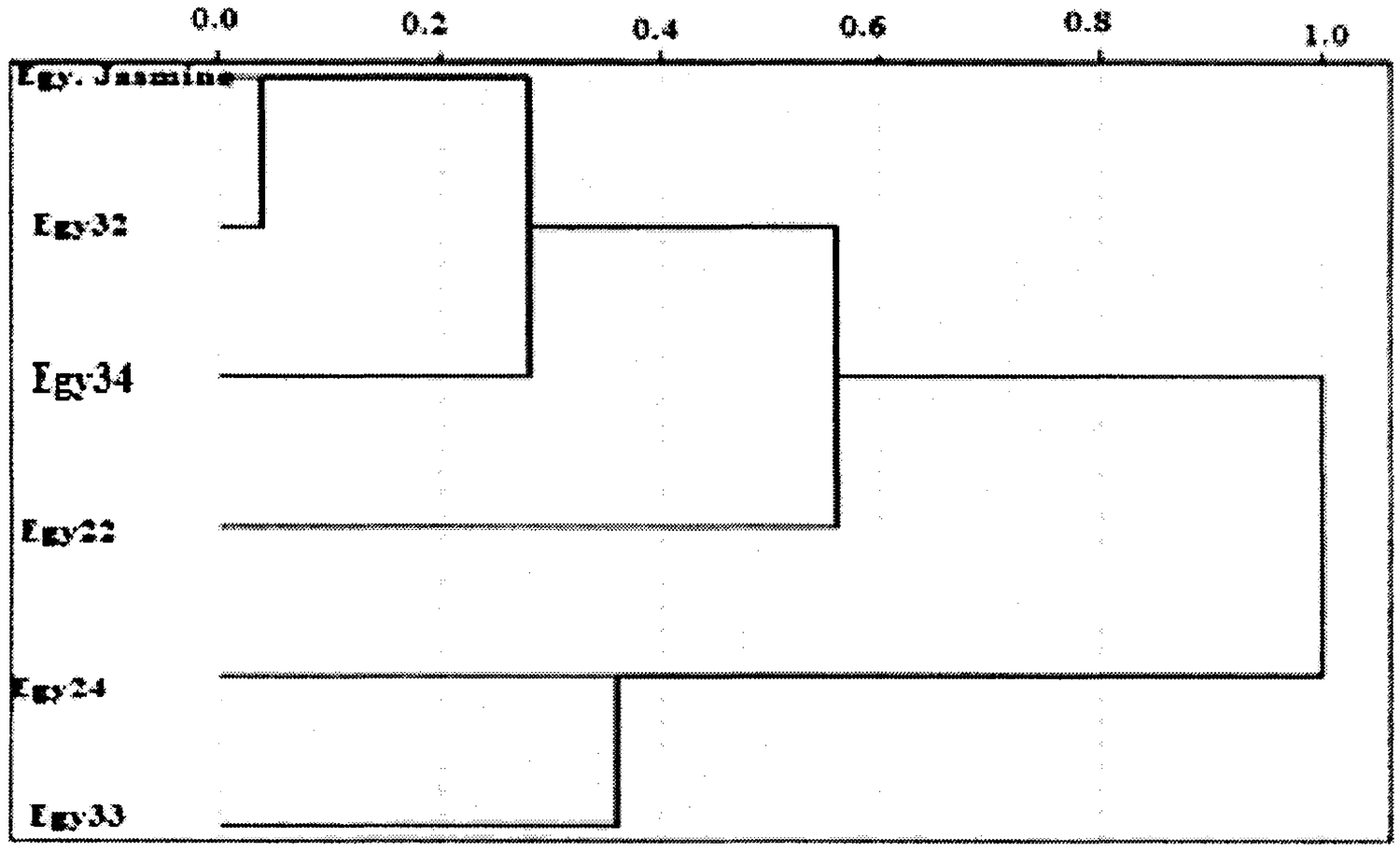
Table 5. Rice genotypes characterized by unique positive and/or negative AFLP markers.

Name	Primer combinations	unique positive markers (+)	Total Number	Unique negative markers (-)	Total Number
		Molecular Weight		Molecular Weight	
Egyptian jasmine	E-AGG /T-GAT	163, 306	2	182, 222	2
	E-CTA/T-CAA	346	1	81	1
	E-CTC /T-CTA		0	127	1
	E-CTG/T-GTC		0		0
	E-GAA / T-CTT	269, 342	2		0
	E-GAG / T-CTC		0		0
	E-AG/T-GTA	310	1		0
Egy22	E-AGG /T-GAT	55, 207, 380	3	119	1
	E-CTA/T-CAA	88, 100, 111, 181, 239, 317, 322, 323	8	156, 171, 220	3
	E-CTC /T-CTA	118, 150	2		0
	E-CTG/T-GTC	176, 180, 270, 348	4		0
	E-GAA / T-CTT	174, 175, 176	3		0
	E-GAG / T-CTC	56, 275, 276	3	57, 59	2
	E-AG/T-GTA	75, 79, 114, 180	4		0
Egy24	E-AGG /T-GAT	62, 147	2	60, 61, 167, 254, 255, 344	6
	E-CTA/T-CAA	102	1	132, 173, 202	3
	E-CTC /T-CTA	125, 137, 291, 448	4	51, 101, 323,	3
	E-CTG/T-GTC	279, 280	2	148, 192, 295	3
	E-GAA / T-CTT	131, 162, 184, 195, 226, 238, 330, 331, 440	9	163, 188, 248, 249, 292	5
	E-GAG / T-CTC	113	1	322	1
	E-AG/T-GTA	102, 163, 185, 320	4	109, 188, 195, 197, 255	5
Egy32	E-AGG /T-GAT	74	1		0
	E-CTA/T-CAA		0	97	1
	E-CTC /T-CTA	243	1	193	1
	E-CTG/T-GTC		0	65	1
	E-GAA / T-CTT		0	108, 126, 127, 277	4
	E-GAG / T-CTC	97	1		0
	E-AG/T-GTA		0	94	1
Egy33	E-AGG /T-GAT	66, 233	2		0
	E-CTA/T-CAA	103, 111, 283	3	137, 186	2
	E-CTC /T-CTA		0		0
	E-CTG/T-GTC	243, 336	2		0
	E-GAA / T-CTT	125, 311, 337	3	136, 210	2
	E-GAG / T-CTC	178, 240	2		0
	E-AG/T-GTA		0	198	1
Egy34	E-AGG /T-GAT	59, 115	2		0
	E-CTA/T-CAA		0		0
	E-CTC /T-CTA	100	1	135	1
	E-CTG/T-GTC	119, 213	2	437	1
	E-GAA / T-CTT	88, 220	2	158, 213, 321	3
	E-GAG / T-CTC	245	1		0
	E-AG/T-GTA		0		0
Total			79		54

Genetic relationship among the genotypes

A dendrogram of the six rice genotypes was constructed using unweighted pair–group arithmetic average (UPGMA) and similarity matrices computed according to Dice coefficients (SPSS) to obtain the relationships between Egyptian jasmine and their genotypes (Fig.1).

The relationships showed two main clusters; the first cluster contains Egyptian jasmine, Egy32, Egy34 and Egy22 which was further divided into three sub-clusters (Fig. 1). The second cluster



contains Egy24 and Egy33. This grouing means that Egy32 is more related to Egyptian jasmine than the other genotypes.

Figure 1. The dendrogram of the relationships of the six rice genotypes according to their similarity indices.

Conclusion

The results showed that, Mutant Egy22 had the best grain quality for strong Aroma, while Egyptian Jasmine rice cultivar showed moderate aroma record in grain quality. It is clear that AFLP technique was able to differentiate rice genotypes namely; Egy24, Egy22, Egy33, Egy34, Egy32 and Egyptian Jasmine rice cultivar by a higher number of unique markers, which revealed 49, 33, 17, 13, 11 and 10 unique markers, respectively.

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