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Full Length Research Paper

Morphological, molecular and biochemical evaluation of Egyptian jasmine rice variety and its M_5 derived mutants

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The current investigation was carried out at Sirw, northern part of Egypt, during 2007 and 2008 seasons. The present study mainly aimed to evaluate the morphological and molecular variation among Egyptian Jasmine and its 10 M5 derived mutants under saline soil conditions. Some mutant lines have the advantage of early maturation nearly one month earlier compared with the original variety Egyptian Jasmine. In addition, other derived mutants significantly surpassed the Egyptian Jasmine in terms of yield and its components. The biomass and grain yield recorded the highest value of expected genetic advance. The values of heritability were high for all yields and yield attributes. Some morphological traits were utilized in order to identify the M_5 morphology. DNA Markers namely Short Sequence Repeats (SSR) and Intron Splice Junction (ISJ) were used to reveal the molecular variations at molecular level among all entries. A significant level of polymorphism based on morphological and molecular levels was observed. The overall evaluation for the newly developed lines revealed that the best line was in Jasmine Sirw Line No. 3 and followed equally by Jasmine Sirw Line Nos. 8 and 9.

Key words: Jasmine rice, induced mutation, DNA markers.

INTRODUCTION

Rice is the primary staple food for more than two billion people in Asia, Africa and Latin America. Breeding for earliness is one of the basic objectives in breeding programs. The breeding for such trait is considered an indirect way to save water. Egyptian Jasmine is one of our local varieties that possess aroma trait, a type of rice preferred increasingly preferred by local consumers. The period from sowing date to maturity of this variety is about 155 days, which is considered a long period (National Rice Research Program Proceeding, Unpublished data).

The induced rice mutants have proved to be useful research tools in genetic and physiological assessment on yield-limiting factors in rice. Mutants have enabled us to demonstrate that critical elements for developing high yield potential varieties with semi-dwarfism, early maturity, more panicles/plant and high fertility percentage.

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Reimei rice variety was released in Japan in 1966 as a national registered variety (Futsuhara, 1968) and produced by means of radiation. By 2003, 440 mutant rice varieties had been developed, 264 of which evolved through direct use of mutants and the other 176 developed by cross breeding with induced mutants.

The success achieved with mutation breeding techniques, especially for the improvement of the major cereals of the world like rice, would indicate that it is no longer a controversial breeding method, but should be considered as an important technique to complement with conventional breeding technology.

Using mutation breeding, we successfully developed ten mutants' lines in M_5 generation that possesses desirable traits compared with their original parent.

The main objective of the current study was to evaluate these selected lines at morphological level and to estimate the molecular variation among Egyptian Jasmine and its 10 derived mutants using PCR based DNA markers, as well as to determine aroma content in those newly developed lines. The extent of variability will reflect

Table 1. DNA Primers Sequence used in the current study.

Primer	Ch. No	F-Sequence	R-Sequence
RM21*	11	ACAGTATTCCGTAGGCACGG	GCTCCATGAGGGTGGTAGAG
RM204	6	GTGACTGACTTGGTCATAGGG	GCTAGCCATGCTCTCGTACC
RM263	2	CCCAGGCTAGCTCATGAACC	GCTACGTTTGAGCTACCACG
RM218	3	TGGTCAAACCAAGGTCCTTC	GACATACATTCTACCCCCGG
RM164	5	CGTTTCTCCGAGAAAAGTCG	CAAGGTGGTCGTTGAGGC
ISJ5	-	CAG GGT CCC ACC TGC A	
ISJ9	-	AGG TGA CCG ACC TGC A	

*Source of SSR Sequences: www.gramene.org.

the powerfulness of mutation breeding in rice.

MATERIALS AND METHODS

Field evaluation

The seeds used for the present study were originally obtained from the genetic stock of the Egyptian National Rice Research Program. Two hundred pure seeds of Egyptian Jasmine with a moisture content of 12% were subjected for gamma irradiation treatments dosages of 200, 300 and 400 Gy using the Co source at the National Center for Radiation Research and Technology, Nasr City and Cairo, Egypt. The irradiated materials were grown and series of selections among the mutant population were carried out during 2002 - 2006 seasons (M_0 - M_4).

The field evaluation was carried out at the saline experimental farm of Sirw, Damietta, Egypt, during 2007 and 2008 rice growing seasons under saline soil with salinity level of 10 dsm^{-1}. Ten M_5 mutants along with their original parent (Egyptian Jasmine) were evaluated for their basic characteristics at morphological and molecular level.

The seeds were sown directly in the nursery during 2007 and 2008 seasons. After 30 days from sowing, seedlings were transplanted individually in a randomized complete block design (RCBD) with three replications. Fertilizer was added at a recommended rate and hand weeding was done when needed. During growth period, all the data concerning vegetative, yield and its component characters were recorded.

Molecular analysis

The seeds of Egyptian Jasmine and 10 derived mutants in M_5 generation were grown in the green house of RRTC for 21 days. Total genomic DNA was isolated from young leaves by using CTAB method (Murray and Thompson, 1980). The PCR for Simple Sequence Repeats (SSR) were carried out in 10 µl volume containing 1.0 µl total genomic DNA + 4.9 µl H_2O + 1.0 µl 10 × PCR buffer + 0.8 µl Mg Cl_2 + 0.4 µl dNTPs + 0.3 µl Taq DNA polymerase + 0.3 µl forward primer + 0.3 reverse primer. Amplification was performed in Berkin Elemar Gene Amp PCR system 2400 and DNA Engine Peltier Thermal Circler with following profile: 95°C for 5 min (for initial denaturation), 35 cycles with 95°C for 1 min, and 55°C for 1 min, 72°C for 2 min, and final extension 72°C for 7 min.

ISJ markers are semi-random primers. The sequences were obtained according to Polok (2007). We used 2 primers, ISJ 5 and ISJ 9. The PCR were carried out in 10 µl volume containing 1.0 µl total genomic DNA + 4.35 µl H_2O +1.0 µl 10x PCR buffer + 0.8 µl Mg Cl_2 + 0.1 µl dNTPs + 0.25 µl Taq polymerase + 2.5 µl ISJ primer. The profile for PCR reaction was 94°C for 3 min (initial denaturation), 45 cycles with 94°C for 1 min, 48°C for 1 min and 72°C for 2.30 min, and final extension temperature 72°C for 7 min. The PCR products were analyzed directly on 1.5% agarose gels in 0.5x TAE buffer, visualized by staining with ethidium bromide and trans-illumination under ultra violet light. Comparison of genotypes, based on the presence (1) or absence (0) of unique and shared polymorphic bands was used to generate similarity coefficients using statistical software package DARwin (Perrier and Jacquemoud-Collet, 2006). The sequences of used SSR and ISJ primers are shown in Table 1.

The data obtained were subjected to the statistical analysis to estimate means and standard error according to Gomez and Gomez (1984). For comparison of the mean values and variance, the analysis of variance was done for each character of each entry to estimate genotypic (Vg) and phenotypic variances (Vp), phenoltypic coefficient of variation (PCV), genotypic coefficient of variation (GCV), heritability (h^2), genetic advance (GA), expected genetic advance of main (GS%), separately as suggested by Panse and Sukhatme (1957).

Biochemical evaluation for aromatic flavor

This method is based on the amount of lipoxygenase detected in the embryo extraction of the studied lines. The detected lipoxygenase is an indicator to aroma amount. In this method linoleic acid was used as a substrate for lipoxygenase found in embryo. The enzyme was extracted in borate buffer (0.2 M, pH = 9), mixed with triton 100 to make emulsion. The reaction between linoleic acid and lipoxygenase produces 9- or 13-hydroperoxides of linoleic acids, which oxidizes the iodide ion to iodine and release iodine which combined with starch and develops a pink with faint purple color, and absorbs at 410 λ nm

Thirty embryos from each lines were separated from seeds and incubated in a test tube containing 1 ml of solution A [0.08% triton 100, 0.2 mol borate buffer pH 9 (8 ml borax + 2 ml boric acid) and 1 mM linoleic acid/ml], and kept for 10 min at room temperature. 100 ml of freshly prepared solution C (1% w/v soluble starch) was added to 100 ml of freshly prepared solution B [5 ml of saturated aqueous potassium iodide per 100 ml of 15 % (v/v) acetic acid]. After 10 min, 1 ml of solution A incubated with embryos, were added to flask containing 200 ml mixture B and C. The former mixture was incubated for 40 h at dark. Standard tube containing all solutions without embryos were prepared. After 40 h, the reaction mixture was measured at 410 nm (wave length of absorbance) against distilled water as a blank, using spectrophotometer. This method was implemented successfully by Randa (2009) to screen for the aromatic rice lines.

Table 2. Means of some morphological characters in ten mutants derived from Egyptian Jasmine as well as their parents at Sirw.

Name	Plant height / cm	Duration/ days	No. Tiller /plant	No. Panicle/ plant	Panicle length/ cm	Panicle weight/g	Plant biomass/ g	Grain yield/ plant/g	1000 Grain weight/g
Jasmine-Sirw 1	63.6	132	12	7	19.3	2.12	27.79	11.30	22.90
Jasmine-Sirw 2	97.0	145	11	8	19.7	2.47	29.56	12.40	23.30
Jasmine-Sirw 3	78.4	132	12	10.3	19.0	3.30	40.60	16.82	27.90
Jasmine-Sirw 4	78.3	132	12	10.3	19.5	3.15	40.60	16.42	24.50
Jasmine-Sirw 5	73.0	132	11	7.3	20.5	2.62	32..17	12.60	22.23
Jasmine-Sirw 6	97.0	135	12	10.3	15.5	3.02	36.25	15.63	18.22
Jasmine-Sirw 7	78.2	132	11	10.7	21.4	2.81	36.63	14.67	23.70
Jasmine-Sirw 8	65.7	130	12	7	19.5	2.53	34.74	12.36	22.65
Jasmine-Sirw 9	84.8	140	12	9	20.9	2.72	28.15	13.36	23.20
Jasmine-Sirw 10	64.8	130	9	10.4	21.3	2.81	33.48	14.45	22.17
Egyptian Jasmine	66.9	150	10	8	20.1	2.43	26.50	12.70	22.30
L.S.D 0.05	16	2.1	NS	2.20	1.8	0.55	1.98	1.7	1.8

Grain shape and quality traits

Different grain traits were studied; grain length, width and shape. Also studied are several traits that are related to grain quality; elongation, amylase content, gel consistency, hulling, milling and percentage of broken rice. The data were analyzed by GENESTAT Release 3.2 (P.C.W NT) copy right 1995, Laws Agricultural Trust (Rothamsted experimental station) statistical package software, using the ordinary analysis of variance (completely randomized complete block design) in order to test the significance of differences among the tested genotypes.

RESULTS AND DISCUSSION

Similar research was conducted by Saleem et al. (2005) who induced several mutant lines out from their aromatic rice named Basmati Rice**.**

The mean performance

The data presented in Table 2 represents the mean performance of the tested entries for agronomic performance. The results showed that all tested genotypes including Egyptian Jasmine and its new derived mutants were significantly varied in their growth duration, yield and yield components except number of tillers. Interestingly, derived mutants significantly headed earlier than Egyptian Jasmine. The days to heading recorded for the 10 derived mutants ranged from 130 days in case of Jasmine Sirw line No. 8 and 10, to 145 days in case of Jasmine Sirw line No 2. This means that these mutants have shorter growth stages and reflects the possibility of significant improvement of such important agronomic parameter using mutation breeding. On the other hand, the derived genotypes; Jasmine Sirw line No 2, 6 and 9 were significantly taller than Egyptian Jasmine. The derived mutants Jasmine Sirw line No 3, 4, 6, 7 and 10 significantly surpassed the Egyptian Jasmine in their number of panicles per plant. It is worth noting here that most of the derived mutants showed better performance than Egyptian Jasmine for, panicle weight except for Jasmine-Sirw line No 1. For plant biomass, all the derived mutant lines except Jasmine-Sirw- line No 1 performed better than the original parent. And for grain yield per plant; Jasmine-Sirw line No 3, 4, 6, 7, 10 gave higher yield than the original parent. Regarding panicle length, none of the derived mutant significantly surpassed the Egyptian Jasmine except the mutant of Jasmine-Sirw line No 6 that gave the significant shortest panicle length compared with the original parent. As for 1000 grain weight there are many derived mutants such as Jasmine-Sirw line No 3 and 4 that significantly exceeded the Egyptian Jasmine in the 1000- grain weight and the rest of them were at a par in this trait with original Egyptian Jasmine. The results clearly showed the existence of considerable amount of variation at the morphological level and demonstrate the significance of mutation breeding in enhancing genetic variability in the breeding programs. Similar results have been reported by Gomma et al. (1995a, b), Abdul-Majeed (1997) and Abdallah (2000).

The genetic parameters

Data in Table 3 illustrates the estimated genetic parameters for Egyptian Jasmine and its derived mutants. The data revealed that the expected genetic advance values were so high in all studied traits except for the number of tillers per plant which gave the lowest expected genetic advance of only 2.5%. The biomass yield had the highest value of expected genetic advance (29.65) followed by plant height (24.9) and grain yield per plant (22.0). The rest values of expected genetic advance of

Table 3. Genetic parameters of some morphological characters in ten mutants derived from Egyptian Jasmine as well as their parent at Sirw.

Genetic parameters	Plant height/ cm	Duration/ days	No. Tiller /plant	No. Panicle/ plant	Panicle length/ cm	Panicle weight/ g	Plant biomass/ g	Grain yield/ plant/g	1000 Grain weight/g
P V	203.4	44.10	0.65	2.17	2.59	0.119	24.68	3.36	5.22
G V	158.4	43.55	0.20	1.55	2.22	0.084	24.24	3.0	4.79
EV	45.00	0.51	0.46	0.62	0.36	0.035	0.44	0.35	0.42
PCV	18.99	4.90	7.12	16.90	8.19	12.60	14.91	13.2	9.93
GCV	16.76	4.80	3.90	14.78	7.60	10.59	14.78	12.48	9.51
Hb	0.63	0.98	0.18	0.56	0.74	0.55	0.96	0.81	0.84
GA	18.7	13.36	0.30	1.69	2.50	0.39	9.90	3.1	4.00
ExGA	24.90	10.0	2.6	19.38	12.74	14.16	29.65	22.0	17.37

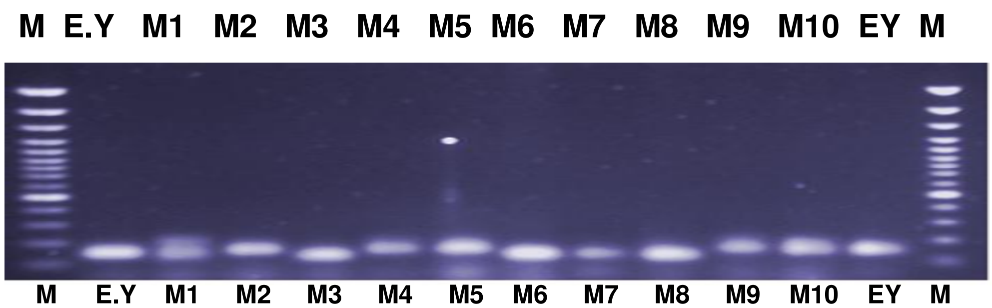

Figure 1. Polymorphism detected with RM218 primer for the tested materials.

studied traits ranged from 10 to 20 indicating that using gamma rays had high affinity to induce remarkable improvement in rice growing even under saline soil conditions. Genotypic variances (GV) ranged from medium to high among studied traits in the different mutants. The highest values of genetic variance were 158 for plant height followed by 43.55 for duration period. Genetic variances were greater than environmental variances in all of the studied traits, indicating the possibility of phenoltypic selection of these traits in the breeding program since the heritable portion of variances were high. Genotypic coefficients of variation (GCV, %) were higher in all studied traits and ranged from 3.90 up 16.76. This also reflects the possibility of efficient selection and genetic improvement of these traits using such set of genotypes.

Broad sense heritability (h^2 b) estimates were found to be moderate to high in all the studied traits except for number of tillers/plant. The estimated values of h^2 ranged from 18% in case of number of tillers/plant to 98% in case of total duration period. The results indicate also the possible improvement of these traits using selection since the phenotype could be more reliable during selection of these traits. Expected genetic advance ranged from 18.7 for plant height to 0.3 for number of tillers per plant. Similar findings as high estimates of genetic parameters for genotypes/lines derived from irradiation were found by Meheter et al. (1996), Bordoli and Talukder (1999) and Elayaraja et al. (2005). This reflects clearly the fact that irradiation not only induces higher proportion of chromosomal and physiological changes but also brings about a high frequency of gene mutations. It is evident that gene mutations using irradiation could generate a considerable amount of genetic variability and opens a new avenue for crop improvement and diversification.

Molecular diversity assessment

There was highly significant polymorphism detected among the studied genotypes. Among seven markers, one marker showed monomorphism, which is RM164 and other SSR marker RM218 showed highly polymorphic bands. In the case of RM 204 marker, Egyptian Jasmine and M10 had the same allele while other mutations had another allele (data not shown). ISJ markers were also good marker to display the high level of polymorphism. The number of SSR alleles detected ranged from one in case of RM164 to 3 in RM218, (Figure 1). On the other hand, 5-8 bands were detected using ISJ primers (Figure 2). This is because of the multiple complementary sites for ISJ primers compared with SSR, making them the

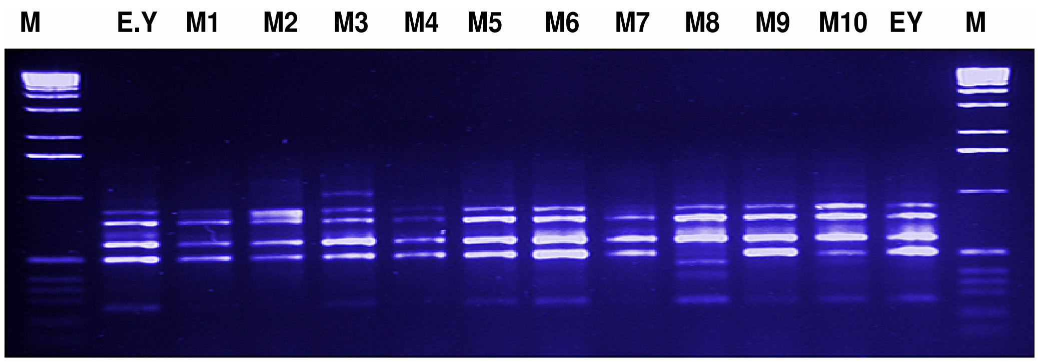

Figure 2. Polymorphism detected with ISJ 9 primer for the tested materials.

marker of choice during fingerprinting, yet it is more reliable than RAPD primers due to the higher annealing temperature and hence the reducibility issue is not in question. It is clear from the data that mutations and their original parent significantly varied at the molecular level.

Based on the polymorphism detected, dendrogram explaining the phylogenic relationships among the derived mutants and their original parent was constructed (Figure 3) using DARwin software. The tested genotypes were clustered in three main groups based on similarity percentage. The first group included Jasmine Sirw line No 7 and 9, as the most divergent group to the original parent. The second group included Jasmine Sirw line No 3, 5, 8, 4 and 10. While the third group included the Jasmine Sirw line No 1, 2 and the original parent Egyptian Jasmine. Jasmine Sirw line No 1 showed close genetic relation with the original parent at the molecular level followed by Jasmine Sirw line No 2 and Jasmine Sirw line No 6. The second group also was further spited into 3 subgroups (Figure 3). Variation within and between groups were clearly detected. This diversity demonstrates also the powerfulness of irradiation in germplasm diversification and hence the possibility of relying on this approach in enhancing the diversity of local collection.

Grain quality and aroma assessment

Grain quality parameters for the tested entries are presented in Table 4. The results showed considerable variation among the tested materials for grain quality and aroma estimates. F-test proved that they were significant differences among the tested entries. LSD also proved significant differences among the Egyptian Jasmine and its derived lines for all studied traits. Grain length ranged from 7.02 mm in Jasmine Sirw line No 2 to 8.54 in Jasmine Sirw line No 6.

Grain width ranged from 2.64 in Jasmine Sirw line No 5 and 6 to 3.34 in Jasmine Sirw line No 9. As a result, grain shape values ranged from 2.16 in Jasmine Sirw line No 9 to 3.01 in Jasmine sirw line No 4. The Egyptian consumers prefer the short and bold grains of rice; hence the Jasmine Sirw line No 2, 9 and 10 are the most desirable as far as grain shape is concerned.

Grain elongation is a quality related trait and increases in aromatic and Indica rices, the values indicates that grain length increases about 60% upon KOH treatment or cooking. The values ranged from 61.15% elongation in Jasmine Sirw line No 6 to 63.85 in case of Jasmine Sirw line No 9. Jasmine Sirw line No 9 also possesses the lowest amylase content, making it a non-sticky type of grains with amylase content of 17.82%. Jasmine Sirw line No 7 had even lower amylase (17.29%). On the other hand, half of the lines exceed the line of 20%. These lines are Jasmine Sirw line No. 3, 4, 5, 6 and 8.

Gel constancy values ranged from 80.51 in Jasmine Sirw line No. 6 to 90.58 in Jasmine Sirw line No 2. Hulling percentage ranged from 75.50% in Jasmine Sirw line No 2 to 80.77% in case of Jasmine Sirw line No 9. Milling also showed the same scenario with mean values for Jasmine Sirw line No 2 and 9 (69.07 and 72.93%, respectively).

As far as aromatic volatile components estimation, only 3 derived mutants had aroma level lower than Egyptian Jasmine. These lines are Jasmine Sirw line Nos 5, 6 and 10. Their amount of lipoxygenase detected in the embryo extraction as indicator for aroma were 0.06433, 0.01433 and 0.06033, respectively, compared with Egyptian Jasmine value (0.06500). The remaining six mutants had high aroma levels compared with the original parent Egyptian Jasmine. Their values ranged from 0.06533 in Jasmine Sirw line No 1 and 9 to 0.09500 in Jasmine Sirw line No 4.

Broken rice trait, ranged from 24.70% in Jasmine Sirw Line No. 3 to 5.33% Jasmine Sirw Line No. 2.

Conclusion

The current study showed the existence of significant amount of genetic diversity of the irradiation derived mutants as compared with their original parent; it also enhanced the genetic parameters and this diversity was further confirmed at the molecular level. The study also

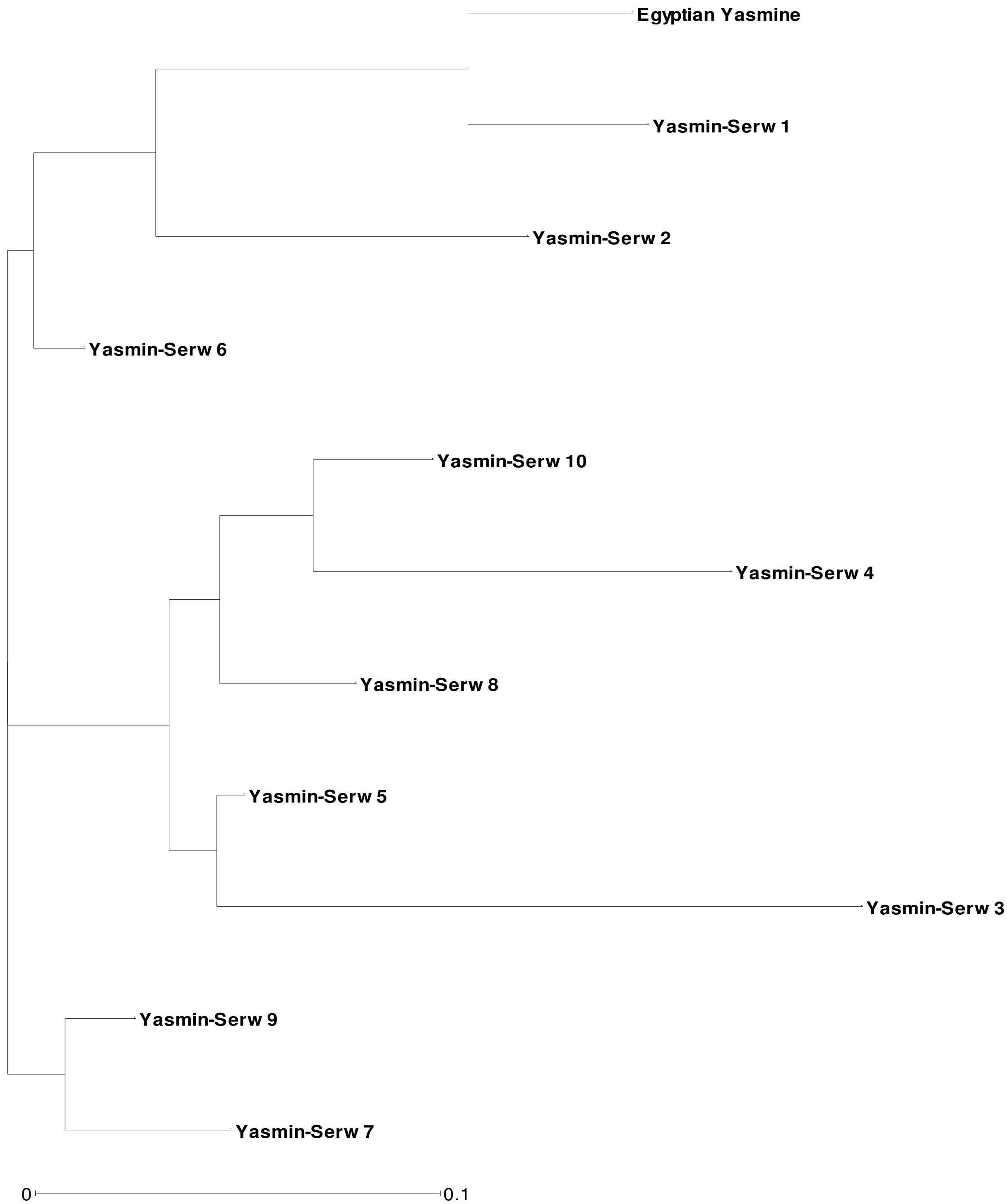

Figure 3. Dendrogram displays the molecular relationship among the tested genotypes.

demonstrated the powerfulness of ISJ markers in fingerprinting and varietal identification with still higher level of reproducibility and thus could be easily replace RAPD markers whenever the reliability and reproducibility is questioned. Quality assessment proved that many of the derived lines still possess high level of accepted grain quality and aromatic fragrance. Those derived lines will undergo the regional yield trails to pick up the most superior line over the Egyptian Jasmine with shorter duration and higher aromatic fragrance levels. The over-all evaluation for the newly developed lines revealed that the best line is Jasmine Sirw Line No. 3 and followed

Table 4. Grain quality and aroma assessment of E. Jasmine and its 10 derived mutants.

Name	Length	Width	Shape	Elongation	Amylose	Gel	Hulling	Milling	Broken rice	Aroma
Jasmine-Sirw 1	7.78	2.81	2.75	62.14	19.52	86.16	79.00	71.87	10.50	0.06533
Jasmine-Sirw 2	7.02	2.88	2.44	62.84	18.91	90.58	75.50	69.07	5.33	0.08000
Jasmine-Sirw 3	7.57	2.75	2.76	61.78	22.61	87.44	79.73	71.50	24.70	0.09000
Jasmine-Sirw 4	8.40	2.79	3.01	62.82	23.30	83.64	78.43	69.80	19.10	0.09500
Jasmine-Sirw 5	7.43	2.64	2.81	62.45	22.63	83.21	77.50	69.10	26.10	0.06433
Jasmine-Sirw 6	8.54	2.64	3.23	61.15	23.11	80.51	76.60	70.30	10.00	0.010433
Jasmine-Sirw 7	7.25	2.73	2.66	63.52	17.29	87.00	77.80	69.80	10.37	0.08700
Jasmine-Sirw 8	7.64	2.76	2.77	62.66	21.36	84.71	76.80	71.50	9.10	0.07533
Jasmine-Sirw 9	7.26	3.34	2.16	63.85	17.82	89.50	80.77	72.93	14.47	0.06533
Jasmine-Sirw 10	7.11	2.87	2.48	61.44	17.89	90.50	75.83	67.80	12.27	0.06033
Egyptian Jasmine	10.4	2.9	2.3	43	17.4	92.75	69.7	68.5	40	0.06500
F Test	**	**	**	**	**	**	**	**	**	---
L.S.D 0.05	0.0354	0.0226	0.0307	0.1589	0.3197	0.4217	0.3137	0.7313	0.7019	0.01008

equally by Jasmine Sirw Line Nos. 8 and 9. For the SSR markers, all of them showed homozygous bands indicating the genetic stability of the mutants.

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