Pak. J. Bot., 43(2): 1183-1194, 2011.

AGRONOMIC AND MOLECULAR EVALUATION OF INDUCED MUTANT RICE (*ORYZA SATIVA* L.) LINES IN EGYPT

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Abstract

The present study was conducted at the farm of the Rice Research and Training Center, Sakha, Kafr El-Sheikh, Egypt, during 2000-2007 rice sowing seasons. Five rice varieties viz., Giza 171, Giza 175, Giza 176, Giza 181 and GZ 1368 were the most widely grown Japonica and Indica types in Egypt during the last period, possesses at that time many positive agronomic characteristics including wide adaptability, high yield potential, tolerance to stresses and good eating quality. But with the passage of time it has lost its vigor. In Rice Research Program, Egypt, dry seeds of the above mentioned varieties were treated with different doses of gamma rays (100, 200, 300, 400, and 500 Gy) for raising M_1 generation. M_1 plants were established by transplanting in the year 2000 season. One hundred independent lines have been advanced to M_5 generation enabling evaluation of quantitative traits by replicated trials and promising lines were selected and tested in multi-location trials as M_6, M_7 and M_8 generations. Morphological variations at vegetative and reproductive stages including plant type and various physiological characters were observed in the five populations. The mutant lines characteristics consisted of better resistance to lodging, blast disease, high yield potential, as well as early maturity. Results from yield trials and molecular assessments indicated that the mutants differed genetically from their parents. So, these mutants could be used as a donor parents in rice breeding program and some of them can be recommended as new rice varieties suitable for rice belt in Egypt.

Abbreviations: M, mutants; Gy, Gray (SI unit of absorbed radiation dose); SSR, simple sequence repeat.

Introduction

Rice is one of the most important crops in Egypt and its production plays a significant role in the strategy to overcome food shortage and improvement of self sufficiency for local consumption and export. Introducing new varieties of rice characterized by early heading, short stature, lodging resistance, blast resistance and improved grain quality characters are main objectives for vertical increase of grain yield of rice. The total production increased during this period from 2.4 million tons (Ave. 1980-1989) to 4.64 million tons (Ave. 1990-2001). In 2005 season, the total rice production in Egypt reached 6.6 million tons with a national average of 10.00 tons/ha (Badawi, 2005). This increase in rice production is mostly due to the development and releasing of new improved varieties having many desirable characters.

Most of the rice area starting from 1990 till 1995 was planted with the traditional varieties viz., Giza171, Giza175, Giza176, Giza181, GZ1368 and IR28. These varieties have some constraints such as late maturity, lodging, susceptibility to blast and some defects in their grain quality characters. Induced mutations, have thus, played a vital role for the improvement of rice by developing a large number of semi-dwarf, earliness, tillering ability, blast resistance, low amylose content and high yielding varieties in the world (Soomro *et al*., 2006). For direct improvement of any agronomic trait, the basic requirement is the availability of adequate genetic variability. Induced mutations, with the discovery of array of radiation mutagen and improved treatments methods, offers possibility for the induction of desired changes in various attributes, which can be exploited as such or through recombination breeding (Akbar & Manzoor, 2003; Khin, 2006).

Many valuable mutant lines were induced in rice; some of them have been developed directly or indirectly into national regional new varieties. Ionizing radiation mutagenesis has been routinely used to generate genetic variability for breeding research genetic studies. More than 2200 crop varieties were released by the end of the last century using irradiation radiation mutagenesis among them 434 are rice varieties (Elayaraja, *et al*., 2005, Wu, *et al*., 2005 and Mohamed *et al*., 2006).

There are different types of ionizing radiation viz., X rays, gamma rays, protons, neutrons, alpha and beta particles. However, gamma rays are widely employed for mutation studies as they have shorter wave length and therefore, posses more energy per photon than X rays and penetrate deep into the tissue (Khin, 2006; Zhou *et al*., 2006). The idea of producing artificial mutations and utilizing them for breeding cultivars plants was initiated as early as 1901 by the induction of mutations fore factors which govern the heredity of quantitative characters as a promising tool for releasing new genotypes (Gomaa *et al*., 1995). It is an established fact that mutagen, besides causing changes in major genes, also induce mutations at loci governing the quantitative characters. Mutagen agents, including gamma rays, offered great possibilities for increasing genetic variability of quantitative traits such as yield. The morphological mutations also provided genetic marker for the development of linkage maps. However, irradiation-induced mutations have not been the mainstay of gene identification tools because the mutations are not physically tagged; requiring considerable effort to isolate the gene after a phenotype has been identified. Yet, with high through put genotyping, the efficiencies in detecting genetic polymorphism has been significantly improved (Borevitz *et al*., 2003).

Mutation breeding method by using gamma rays was started in Egypt for rice in 1960 to improve some desirable characters without disturbing the constellation of the original varieties. The present study was carried out to induce and evaluate some mutation derived promising lines that possess high yielding ability, resistant to blast and superior in grain quality characters. Morphological and DNA based molecular assessment of the mutant lines and their corresponding original parental lines have been conducted to assist in detection the level of genetic variations.

Materials and Methods

Plant material and their basic characteristics

Giza 171: A popular short grain Japonica type variety was developed from the cross Nahda and Calady 40. It was released in 1977. It is late (120 days from sowing), tall (140 cm) susceptible to blast and it has low yielding potential.

Giza 175: The origin was breeding line (1394-10) selected from the local top cross made between IRRI varieties and the local variety, Giza 14. It was registered as the new variety in 1991. It has high yield potential and other agronomic characters. Its cooking and eating quality were less acceptable to Egyptian consumers.

Giza 176: A breeding line (2175-5-6) selected from the local top cross Calrose 76/ Giza 172//GZ 242. It was a short grain Japonica type released in 1989 and was registered in 1991 as a new variety. It has yield potential, short stature, medium growth duration (145 days) but it has low hulling and milling (%) and became susceptible to blast.

Giza 181: Introduced to Egypt from IRRI through INGER program as IR1626-203 in advanced generation. It was released in 1987 and it was characterized by medium maturity (145 days), non-lodging, high yielding, resistant to blast but was not excellent in grain and cooking qualities.

GZ 1368: It was an Indica/Japonica type bred in Egypt, medium grain, high yielding, resistance to blast, medium growth duration but also was not excellent in cooking and eating characters.

Seeds of the above mentioned five rice varieties were irradiated with different doses of gamma rays (100 , 200 , 300, 400 and 500 Gy, from Co^{60} source at the National Center for Radiation Research and Technology, Nasr City, Cairo, Egypt. Two hundred and fifty seeds at 14% moisture content were used for each treatment and the same number of seeds was kept untreated as a control for each variety. Seeds of all treatments were directly sown after radiation treatment in order to raise M_1 plants. The selection of the best mutant lines from M_1 to M_5 was carried out based on individual plants. Seedlings were transplanted to the permanent field in June, and singly grown in hills. Three plots were laity-out for each radiation treatment. Each plot contained 10 rows, 5 m long with 20 cm distance between rows and hills. All plants were under keen observation from sowing till ripeness and each plant was carefully examined.

In 2001 season, M_2 plants were grown and 77 progenies were selected and harvested individually, as promising mutants. These mutants comprised 8 mutants from Giza 171, 28 mutants from Giza 175, 9 mutants from Giza 176, 7 mutants from GZ 1368-S-5 and 25 mutants from Giza 181. In 2002 season, 77 M_3 mutants were tested with their original parents in a randomized complete block design with three replications and the best plants from each mutant were selected to raise the 77-M_4 mutants. Similarly in 2003 season, the 5 parents and 77 M_4 mutants were planted in the seedbed. After 30 days, seedlings of the above populations were individually transplanted in the field in a randomized complete block design with 3 replications. All observations were recorded during the growing season till harvesting. Again in 2004 season, the same technique was used for raising M_5 generation. Seeds of the best plants were selected and individually harvested for each mutant. The manner of planting and experimental design and collection data were similar to those followed in the previous generations. It should be noticed that all cultural practices, were followed as usually done with ordinary rice culture in each growing season and weed control were applied manually and chemically.

After harvest of M_5 plants, samples of seeds of each mutant line, as well as, the parental varieties were tested in the laboratory for hulling (%), milling (%), gel consistency, gelatinization temperature and amylose content characters. After analysis of

M_5 generation, it appeared that different mutant lines were morphologically and genetically stable. From M_6 generation, those mutant lines were continuously evaluated with their respective parents in replicated yield trials at different locations and seasons from 2005 to 2007.

After analysis of M_6 generation, it appeared that different mutant lines were morphologically and genetically stable. It would be better if these mutant lines promoted to be grown under different locations as a yield trails experiments , hoping that some of these mutants lines may be surpassed the local varieties for yield and its component characters, resistant to blast as well as grain quality characters. From M_6 generation, those mutant lines were continuously evaluated in replicated yield trials at different locations and seasons from 2005 to 2007. Besides yield and its components, some agronomic characters i.e., maturity, plant stature, photoperiod sensitivity, grain quality characters i.e., milling recovery, amylose content and resistance to blast were evaluated. Based on the overall performance, five mutants were selected from the five varieties, as the best mutant lines, according to their desirable characters comparing with the others.

DNA isolation and PCR reaction: For the molecular evaluation, DNA isolation and purification from the 5 parental lines as well as the 5 derived lines was carried out using CTAB method. The DNA was quantified using gel assay method and then PCR was performed. A total of 9 SSR markers were used for the screening purpose (Table 1).

The PCR was performed in 10µl PCR volume containing 50 ng of template DNA, five pmole of each of forward and reverse primers, 0.1mM dNTP`s, 1x PCR buffer (10mM Tris, pH 8.0, 50mM KCl and 50mM ammonium sulphate), 1.8mM MgCl2 , and 0.2 units of *Taq* DNA polymerase. Initial denaturation at 94°C for 5 min was followed by 35 cycles of amplification with template denaturation at 94°C for 1 min, primer annealing at 55.7°C for 1 min and primer extension at 72°C for 2 min. After the end of the 35^{th} cycle, a final extension at 72°C for 70 min was given followed by storage at 4.0°C. The PCR products were separated using 1.5% agarose gel stained with Et Br solution (1 mg/l). The banding pattern was then scored and used to prepare the matrix. Employing the computer package DARwin software that was developed by Perrier & Jacquemoud (2006) was used to establish genetic relationship among the genotypes based on Unweighted Pair Group Method of Arithmetic Averages (UPGMA).

Statistical analysis: The analysis of variance for the randomized complete block design was done for each studied trait in the last 3 years, M_6, M_7 and M_8 generations under normal conditions. The combined analysis was calculated over the 3 years to test the interaction of the different genetic components with the three years. The homogeneity of error variance was tested as described by Bartlett (1937). The data were statistically analyzed following Burton (1952) and Chang *et al*., (1974) with parameters such as, phenotypic variance (PV), genotypic variance (GV), phenotypic coefficient of variation (PCV), genotypic coefficient of variation (GCV), heritability in broad sense(h_b) and phenotypic correlation coefficients were computed (Johanson *et al*.,1955; Lush, 1940; Burton,1951). Means of the different mutants were compared with their respective control, using the least significant differences (LSD) method.

Table 1. List of SSR markers with sequence information and number of alleles generated.

Marker	Chromosome	Primer sequence (F)	Primer sequence (R)	No. of alleles
RM289	5	TTCCATGGCACACAAGCC	CTGTGCACGAACTTCCAAAG	2
RM223	8	GAGTGAGCTTGGGCTGAAAC	GAAGGCAAGTCTTGGCACTG	1
RM148	3	ATACAACATTAGGGATGAGGCTGG	TCCTTAAAGGTGGTGCAATGCGAG	2
RM164	5	TCTTGCCCGTCACTGCAGATATCC	GCAGCCCTAATGCTACAATTCTTC	2
RM212	1	CCACTTTCAGCTACTACCAG	CACCCATTTGTCTCTCATTATG	2
RM242	9	GGCCAACGTGTGTATGTCTC	TATATGCCAAGACGGATGGG	2
RM412	6	CACTTGAGAAAGTTAGTGCAGC	CCCAAACACACCCAAATAC	2
RM108	9	TCTCTTGCGCGCACACTGGCAC	CGTGCACCACCACCACCACCAC	2
RM36	3	CAACTATGCACCATTGTCGC	GTACTCCACAAGACCGTACC	3

Table 2. Rice mutants induced from local varieties by using gamma rays.

Mutant	Parent variety	Mutagenic treatment	Improved characterizes
G.71-M_6	Giza 171	Dried seeds, 200 GY gamma rays	Higher yield, tolerant to blast, short stature and earlier in heading
G.75-M_{13}	Giza 175	Dried seeds, 300 Gy gamma rays	Higher yield and low amylos content
G.81-M_{11}	Giza 181	Dried seeds, 300 Gy gamma rays	Higher yield and good grain quality characters
G.76-M_{17}	Giza 176	Dried seeds, 200 Gy gamma rays	Higher yield, high hulling, milling % and resistance to blast
GZ1368 -M_{13}	GZ 1368	Dried seeds, 100 Gy gamma rays	Higher yield, high milling % and low Amylose content

Results and Discussion

Development of rice mutants

Giza 171- M_6: The rice mutant Giza 171-M_6 was evolved by treating the seeds of Giza 171 with gamma rays (200 Gy). The major improvement of the Giza 171-M_6 over Giza 171 were the earliness, where this mutant has 107.00 days to heading, which is 13 days earlier than the original variety Giza 171(120 days). With early maturity the mutant can be cultivated with considerably less water. The mutant, also, has reduction in plant height (95.00cm) by 40.00 cm comparing with the original variety, Giza 171(135.00 cm). Semidwarf *indica* varieties of rice (*Oryza sativa* L.) were developed by induced mutation. They generally have a yield capacity equal to that of the semidwarf varieties (T(N)1, IR 8 and others) selected from hybridization. Genetic studies showed that semidwarf genes induced in the mutants and spontaneous ones in existing varieties are at the same locus (Hu, 2004).

It also has 30 more grains/panicle (165.00 grains/panicle) comparing with the original variety Giza 171 (135.00 grains/panicle). The mutant Giza 171-M_6 possessed high yield (35.00g/plant), good grain quality characters; i.e., high milling (72.00%), low amylose content (19.00%) and good resistance to blast. It could be concluded that the mutant Giza 171-M_6 possessed good characteristics, such as short growth duration, short stature, resistance to blast, high yielding ability and high milling (%) (Table 3). This mutant could easily replace the original variety due to its desirable characteristics with still higher yield potential.

Giza 175- M_{13}: Giza 175-mutant was developed from the variety Giza 175 through gamma rays treatment (300 Gy) of dried seeds. Both yield potential and quality characteristics were significantly improved (Table 3). It has more grains/panicle (166.00 grains/panicle) than the original variety Giza 175 (155.00 grains/panicle). The yield potential is 45.00 grams/plant as compared to its parental variety 39.00 grams/plant. It has a bold grain and low amylose content (20.00%) as compared to its parent Giza 175 (26.00%). It could be concluded that Giza 175-M_{13} are good mutant with high yield and good grain quality characters.

Giza 176- M_{17}: Giza 176 is a japonica rice variety well known for Egyptian farmers as a breeding line 2175-5-6 since 1991. Giza 176 was occupying a large area annually till it becomes susceptible to blast disease. Due to its susceptibility to blast disease, its long duration (145.00 days), this variety has had limited production. Through induced mutation and selection, mutant line Giza 176- M_{17} has been developed by using 200 Gy gamma rays, (Table 3) that have improved traits in comparison to the original variety. This mutant was earlier than its original parent (105.00 days to heading), high yield potential (40.00 grams/plant), resistant to blast disease and good grain quality characters

GZ 1368 – M_{13}: GZ 1368-S-5 is an indica / japonica rice line, resistant to salinity. The mutant GZ1368-M_{13} was induced from this line (Table3) by exposing the seeds to 100 Gy gamma rays. This mutant has high yielding ability, and good grains quality characters.

Giza 181- M_{11}: It is an indicia type developed by exposing the seeds of a local rice variety Giza 181 to 300 Gy gamma rays. The parent variety has a long slender grain and medium grain yield. The mutant Giza 181-M_{11} has a medium grain and high grain yield as compared to its parent (Table 3).

Table 3. Performance of the promising mutants selected in M5 generation and evaluated in three successive generations (M6, M7 and M8) of the studied traits.

Designation	Days to heading (days)	Plant height (cm)	No. of panicles/ plant	No. of grains/ panicle	Grain yield/ plant(g)	100-grain weight (g)	Milling %	Blast reaction	A.C %
Giza 171-M_6	107	95	28	165	35	2.60	72	R	19
Giza 171	120	135	22	135	32	2.50	72	S	19.5
Giza 175-M_{13}	100	102	28	166	45	2.52	70	R	20
Giza 175	105	95	24	155	39	2.45	69	R	26
Giza176-M17	105	100	29	187	40	2.62	70	R	19
Giza 176	110	102	25	145	36	2.56	68	R	19
Giza181-M_{11}	99	100	28	165	38	2.55	70	R	20
Giza 181	100	95	20	150	36	2.50	69	R	19.5
GZ1368 –M13	109	98	23	156	43	2.58	71	R	19
GZ1368-S-5-4	101	98	21	128	35	2.50	69	R	24
IR-M25	107	90	26	165	42	2.45	69	R	21
IR 28	100	98	22	115	30	2.45	67	R	26
LSD 0.05	3.89	4.32	2.50	7.60	3.40	0.03	0.08	-	1.50

Table 4. Estimates of phenotypic correlation coefficients between grain yield and the other agronomic characters over three years and their combined data (2004, 2005 and 2006).

Characters	Days to heading (day)	Plant height (cm)	No. of panicles/ plant	No. of grains/ panicle	100-grain weight (g)	Milling %	A.C %
Grain yield/plant(g)	-0.356 ^{NS}	-0.287 ^{NS}	0.975**	0.668**	0.750**	0.475**	0.218
Days to heading(day)		-0.180	-0.265	-.0127*	-0.058	-0.118*	0.290
Plant height(cm)			-0.372	-0.198	0.016*	0.220	0.123
No.of panicles/plant				0.528**	0.492**	0.238	0.115
No.of grains/panicles					0.882**	0.318	0.210
100-grain weight(g)						0.485**	0.220
Milling (%)							0.113
Amylose content (%)							-

*, ** Significance at 0 .05 or 0.01 level of probability, respectively

^{NS} Non significance

It could be concluded that the results obtained by our mutation breeding program during the last seven (2000-2006) years, shows that this programme besides helping to generate an awareness of the potentiality of the mutation technique in creating valuable new variability, has resulted in some major achievements, as follows: (1) promising short culm mutants from all the studied varieties, (2) promising early maturity mutants from the late maturity varieties, (3) mutants possessing resistance to blast have been selected from the susceptible rice varieties such as Giza 171 and Giza 176, (4) amylose content in some of the studied varieties has been reduced and (5) promising high yielding mutants as compared to their parent varieties. Mutation breeding is one of the most effective ways of inducing genetic variable new mutant lines with desirable traits (Mei, 2007), (Mohamed, 2006).

Phenotypic correlation coefficients: The phenotypic correlation coefficients between grain yield and the seven agronomic characters for the three years and their combined data are presented in Table 4. Obviously, grain yield was positive and highly significantly correlated with number of panicles per plant, number of grains per panicles, 100- grain weight and milling percentage. On the other hand, negative and non- significant correlation coefficient values between grain yield and each of days to heading (-0.356) and plant height (-0.287) were obtained. This means that grain yield, might mainly depend on number of panicles per plant, number of grains per panicle, 100- grain weight for these promising mutants. The same results were obtained by Basak & Ganguli, (1996) by using different materials. Furthermore, number of panicles per plant was highly significant correlated with each of number of grains per panicle (0.528) and 100- grain weight (0.492). Moreover, the correlation was highly significant between number of grains per panicle and 100-grain weight (0.882). Also, 100-grain weight was positive and highly significantly correlated with milling percentage (0.485).

Variation and interaction: Results of combined analysis of the three years viz. 2005, 2006 and 2007 indicated the performance of highly significant differences between the mutant lines and their respective control, on one hand and among the lines on the other hand, for studied agronomic traits (Table 5), indicating overall wide differences among these populations and these mutant lines differ genetically. As shown in Table 5, main effect of years was not significant for all the studied characters, indicating that there is no difference of lines performances regarding the studied characters in the three years, which may be due to the occurrence of stability and these lines were not affected significantly by environmental conditions. It could be concluded that these mutation lines could be grown in any season or year.

Mean squares of the interaction between the mutant lines and years were found to be not significant for all studied characters in the three years and their combined data, if the interaction of lines was highly significant than the interaction of lines with years and therefore, the most superior lines could be recommended.

Molecular diversity assessment: Estimates of genetic variance (GV), coefficient of genotypic variability (GCV %), heritability (Hb) and genetic advance (GS) for the studied characters are given in Table 6. It was evident from the relative magnitude of genetic variance that the heritable (genetic) and non-heritable (non-genetic) component of variation, were ascertained with the help of some genetic parameters, like genetic coefficient of variation, heritability estimates, and genetic advance of selection.

Table 6 shows high genetic coefficient of variation for plant height, grain yield/plant and milling (%) (ranging 26.28 to 13.48%). However, moderate values (the range 8.40 to 11.59%) were obtained for days to heading, number of panicles/plant and amylose content and low estimates (the range 1.78 to 1.96) for 100- grain weight and number of grains/panicle. These results agreed with those obtained by Abd-Allah *et al*., (2002).

Using the genetic coefficient of variation alone, however, it is impossible to estimate the magnitude of heritable variation. The heritable portion of the variation could be found out with the help of heritability estimates and genetic gain under selection. High heritability values (Table 6) had been obtained for days to heading, plant height, grain yield/plant and amylose content (the range 0.91 to 0.98). However, moderate values were estimated for number of panicles/plant, number of grains/panicle, 100-grain weight and milling % (with the range of 0.66 to 0.87).

Johnson *et al*., (1955) reported that heritability estimates along with the genetic gain, were more valuable than the former alone in predicting the effect of selection. If heritability was mainly owing to non-additive gene effect, the expected gain would be low and if there was additive gene effect, a high genetic advance might be expected. In the present study, it is very interesting to note that characters having heritability estimates gave almost high values of genetic co- efficient of variation such as milling %. These results were similar to those obtained by Singh *et al*., (1996).

Many investigations reported that genetic coefficient of variation and high heritability, were not always associated with high genetic advance for a character. But to make effective selection, high heritability should be associated with high genetic advance. In the present study, the results showed that high genetic gain was associated with relatively high heritability value, in most of the studied characters. Therefore, individual plant selection for these characters should be effective and satisfactory for successful breeding purposes.

Molecular diversity assessment: Among the 9 tested SSR primers used, only one primer RM223 showed monomorphic pattern, while, all others showed considerable polymorphism (Table 6). The number of alleles detected per primer ranged from one in RM223 to three alleles in RM136. The rest of primers gave two alleles. The amount of polymorphism detected reflects the existence of considerable amount of molecular diversity among the tested entries. Based on the banding pattern, similarity percentage among each pair of the tested genotype was calculated and phylogenic tree expressing the genetic relationships, among the entries was constructed, using DARWIN software package (Fig.1).

The 5 mutants, as well as, their original parents were clustered into 3 main groups. The first group (A) included only the mutation derived from the Indica/ Japonica line GZ1368-S-5 (GZ1368- M_{13}). The second group (B) subdivided further to two subgroups B1 and B2. B1 group consisted of the two very late duration parents Giza 171 and the Indica rice variety Giza 181. The other sub-grouped (B2) had Giza 175, Giza 171-M_6 and Giza 175-M_{13} genotypes. The Third group (C) consisted of Giza 181- M_{11}, Giza 176, Giza 176-M_{17} and GZ1368-S-5. The clustering represent very clearly that the mutations are indeed different from their original parents. Except for GZ1368-S-5 and its derived mutation, the clustering was mostly co-leaner with pedigree and/or duration. For instance, Giza 171 and Giza 181 though have different genetic background, but both are very late maturing varieties. Giza 175 and its derived mutant clustered in the same subgroup B2. Also, Giza 176 and its derived mutant were clustered in one cluster C. As the mutations were selected over many years, the genetic variability between some parents and their derived mutations as in case of GZ1368- M_{13} and its original parent were obtained.

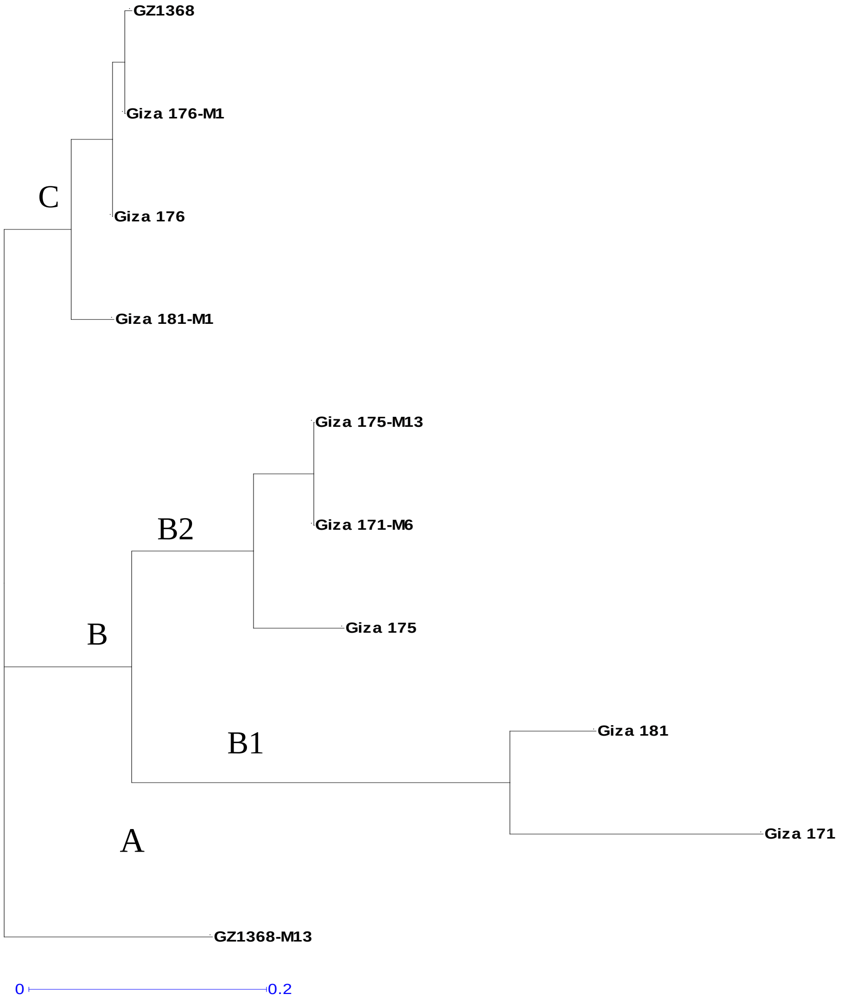

Fig. 1. Genetic similarity among five mutants and five parental lines generated by using Nei's unbiased estimates of genetic diversity. The horizontal axis is Nei's genetic distance.

The scenario is much less in case of Giza 181 and its derived mutant Giza 181- M_{11} where they clustered in two neighboring subgroups B and C. The results demonstrate the efficiency of mutation breeding on both morphological and molecular levels to develop new elite lines that possess desirable characteristics and differ significantly from their original parents. However, the lack of linear relationships between molecular data and genetic background might be due to the natural divergence between mutations and their original parents by using strong ionizing irradiation that causes point mutations, as well as, serious genetic changes that was reflected in the amount of character change between the parent and its selected mutant. The other scenario of this discrepancy in the case of GZ1368-S-5 and its mutant, could be explained as low number of specific primers, were used in the current study that may not exactly reflects the genetic background.

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(Received for publication 19 November 2009)