Australian Journal of Crop Science AJCS

AJCS 6(5):922-927 (2012) ISSN:1835-2707

Development of two high yielding mutant varieties of mustard [*Brassica juncea* (L.) Czern.] through gamma rays irradiation

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Abstract

Seeds of the well-adapted and popular mustard variety BARIsarisha-11 were irradiated with gamma ray using ^{60}Co gamma cells. Irradiated seeds were grown as M_1 during 2004-05. Selection was made from M_2 generation during 2005-06. Desirable mutants were confirmed in M_4 generation during 2007-08 and ten true breeding mutants having higher seed yield per plant with desirable morphological characters and yield attributes were selected. Selected mutants were evaluated along with the mother variety BARIsarisha-11 to select the most desirable ones considering higher seed yield and improved yield attributes under different replicated yield trials during 2008-09 to 2010-11. Results showed that two mutants, MM-10-04 and MM-08-04 selected from 700 Gy produced higher seed yield than BARIsarisha-11 in most of the trials conducted in 13 locations of Bangladesh. Mean of three years trial showed that seed yield of MM-10-04 and MM-08-04 was 2043 and 1893 kg ha^{-1}, respectively, which was 23% and 14% higher than BARIsarisha-11 (mother variety). Mutants MM-10-04 and MM-08-04 also had the higher number of siliquae plant^{-1}, 1000-seed weight and oil content than BARIsarisha-11. These two mutants also showed tolerance against *Alternaria* blight disease and lower aphid infestation. Results of the yield trials as well as screening against *Alternaria* blight disease and aphid carried out across the country indicated that MM-10-04 and MM-08-04 were suitable for widespread cultivation. Consequently, the National Seed Board of Bangladesh registered MM-10-04 and MM-08-04 in 2011 as two high yielding mustard varieties, Binasarisha-7 and Binasarisha-8, respectively for commercial cultivation.

Keywords Gamma rays; induced mutants; high yielding; *Brassica juncea*.

Abbreviations: BARI: Bangladesh Agricultural Research Institute; AYT: Advanced Yield Trial; RYT: Regional Yield Trial; DMRT: Duncan’s Multiple Range Test.

Introduction

In Bangladesh, domestic oilseed production can hardly meet 15% demand of the country. As a result substantial amount of foreign exchange is spent on its import. Hence, there is an urgent need to make efforts to increase the local oilseed production. Oleiferous *Brassica* (rapeseed and mustard) is the leading oilseed crop in Bangladesh but its national average seed yield is 902 kg ha^{-1} only (BBS, 2010). There are many factors responsible for its low yield but the most important one is the lack of high yield producing varieties. Therefore, development of high yielding varieties of oilseed seems necessary to lessen the import of imported edible oil. The application of mutation techniques has generated a vast amount of genetic variability and has played a significant role in plant breeding and genetic studies throughout the world. Induced mutations are now widely used for developing improved crop varieties and for the development of genes controlling important traits and understanding the functions and mechanisms of actions of these genes. Progress is also being made in deciphering the biological nature of DNA damage, repair and mutagenesis (Qu Liang, 2009). Mutation breeding is now one of the successful techniques to induce genetic variability in the adapted crop cultivars. Induced mutations are also widely used for promoting genetic recombination, creation of new genetic resources and breakage of unwanted linkages. As a result, mutation breeding has become an effective way for supplementing existing germplasm and improving cultivars, and has been recognized as one of the important accessories to the main stream of plant breeding (Micke, 1987). Mutation breeding throws high frequency and spectrum of mutations within a short time (Bolbhat and Dhumal, 2009). Compared to conventional breeding methods, it saves time to develop a new crop cultivar (Gaul, 1961; Manjaya, 2009).

Among the different breeding methods, induced mutation has been extensively and successfully used for genetic improvement of any yield attributes, either qualitative or quantitative in nature for rapeseed-mustard and other crops (Das et al., 2000; Javed et al., 2000; Abdullah et al., 2004; Khatri et al., 2005; Gaur et al., 2007; Naeem et al., 2009; Brave et al., 2009). It is mentionable that until year of 2000 more than 2250 mutant varieties of different crops have been released worldwide (Maluszynski et al., 2000). The commercial utilization of approximately 3000 mutant-induced and mutant-derived varieties strongly shows that mutation breeding is a useful tool for generating new germplasm for crop improvement (Ishige, 2009). Among the different mutagenic agents, irradiation has been successfully used for induced mutation breeding in various crops and ornamental plants (Song and Kang, 2003) and has proven an adept means of encouraging the expression of recessive genes and producing new genetic variations (Schum, 2003; Song and Kang, 2003). Most of the mutant varieties (89%) have been developed worldwide using physical mutagens (X-rays, gamma rays, thermal and fast neutrons), with gamma rays alone accounting for the development of 60% mutant varieties (Kharkwal et al., 2004). Under the above context, Bangladesh Institute of Nuclear Agriculture (BINA) initiated a research programme to develop mustard varieties having higher seed yield potential with higher oil content and earliness during 2004 using gamma rays treatment on the parent variety BARIsarisha-11. In this research we present the course of selection and consequently development of two high yielding mutant varieties of mustard, namely, Binasarisha-7 and Binasarisha-8 which have been registered in 2011 for commercial cultivation in Bangladesh.

Results

Means of morphological characters, yield attributes and seed yield of the mutants and mother variety of different yield trials evaluated during 2008-09 to 2010-11 (Table 1).

Advanced yield trial (AYT) during 2008-09

Results of AYT during 2008-09 with 10 M_5 mutants and mother variety BARIsarisha-11 showed that combined means over two locations exhibited significant variations for all characters except number of branches plant^{-1}. Three mutants (MM-01-04, MM-04-04 and MM-06-04) showed significant shorter maturity period (97 days) than mother variety BARIsarisha-11 (100 days). Among the 10 mutants, eight mutants produced significantly shorter plant height (124 to 138 cm) than BARIsarisha-11 (146 cm). Three mutants produced significantly higher number of seeds siliqua^{-1} and six mutants produced significantly higher seed yield than BARIsarisha-11. Mutants MM-01-04 and MM-10-04 produced the highest seed yield of 1560 kg ha^{-1} followed by MM-04-04 (1526 kg ha^{-1}) and MM-08-04 (1475 kg ha^{-1}) and it was also observed that these four mutants produced higher number of siliquae plant^{-1}.

Regional yield trial (RYT) during 2009-10

Results of combined means over three locations under RYT with six M_6 mutants during 2009-10 showed that BARIsarisha-11 required the highest maturity period (112 day) and showed non-significant variation with MM-10-04, while other five mutants required significantly shorter maturity period (108 to 109 days) than BARIsarisha-11 (112 days). Four mutants produced significantly shorter plant height (139 to 147 cm) than BARIsarisha-11 (163 cm). Mutants MM-08-04 and MM-10-04 produced significantly higher number of siliquae plant^{-1} than other mutants and parental BARIsarisha-11. MM-10-04 produced the highest seed yield of 2389 kg ha^{-1}. Other two mutants, MM-08-04 and MM-02-04 produced the seed yield of 2253 and 2229 kg ha^{-1}, respectively while BARIsarisha-11 produced 1945 kg ha^{-1} seed yield.

On-station and on-farm trials during 2010-11

Means combined over eight locations under on-station and on-farm trials with three M_7 mutants during 2010-11 showed that MM-10-04 produced the highest number of siliquae plant^{-1} (145), 1000-seed weight (3.9 g) and produced the highest seed yield (2219 kg ha^{-1}). Mutant MM-08-04 also produced the significantly higher number of siliquae plant^{-1} (139), 1000-seed weight (3.5 g) and produced significantly higher seed yield (1952 kg ha^{-1}) than BARIsarisha-11 and mutant MM-04-04. Oil content was found to be higher in the seeds of all three mutants than BARIsarisha-11.

Means over three years trial

Means over three years trial of two top yielding mutants, MM-10-04 and MM-08-04, selected from 700 Gy treatment along with BARIsarisha-11 are presented in Table 2. Means showed that BARIsarisha-11 and MM-10-04 required 106 days to maturity with higher plant height around 158 and 161 cm, respectively. Both mutants produced higher number of siliquae plant^{-1}, 1000-seed weight and produced higher seed yield 2043 and 1893 kg ha^{-1}, respectively. BARIsarisha-11 produced 1662 kg ha^{-1} seed yield. Seeds of MM-08-04 contained maximum oil (37.2%) and it was followed by MM-10-04 (36.4%). Seeds of BARIsarisha-11 contained 35.3% oil.

Reaction to Alternaria blight disease and aphid infestation

Mean severity of *Alternaria* blight and infestation of aphid were studied in different locations during 2010-11. Results of four locations showed that all the mutants (MM-04-04, MM-08-04 and MM-10-04) along with the parent variety BARIsarisha-11 are tolerant to *Alternaria* blight disease (Table 3). Among the six locations, no aphid infestation was found at Mymensingh and Faridpur. For other four locations, it was observed that the lowest percentage of plants was infested by aphid in MM-10-04 followed by MM-08-04 (Table 4). The lowest number of aphids plant^{-1} was also observed in MM-10-04 followed by MM-08-04 in all locations (Table 5).

Discussion

The reduction in plant height in oliferous *Brassica* causes an increase in grain yield because of good response to higher doses of fertilizer and tolerance to lodging under unfavourable weather conditions. Moreover, the dwarfness in plant height is associated with earliness in maturity (Olejinizac and Adamska, 1999), which is highly desirable character in crop plants including *Brassica*. Shah et al. (1990) and Javed et al. (2003) isolated short statured mutants from mutagen treated populations of rapeseed and mustard. Their results were also consistent with the present results. From the present and earlier results it is confirmed that induced mutation through gamma rays plays a significant role in the alteration of plant architecture in oilseed *Brassica*.

Breeding for high yield plants significantly depends on the generation of new genotypes with improved yield components. The most important factors responsible for the increase in the productivity of oilseed *Brassica* are the number of siliquae plant^{-1}, seeds siliqua^{-1} and seed weight. The two selected mutants, MM-08-04 and MM-10-04 produced higher number of siliquae plant^{-1} with higher 1000-seed weight, which produced a higher seed yield than the mother variety. These two mutants also had higher oil content

Table 1. Means of mutants and mother variety for morphological characters, yield attributes and seed yield of different trials conducted during 2008-09 to 2010-11.

Mutants/ mother variety	Days to maturity	Plant height (cm)	Branches plant^{-1}	Siliqua plant^{-1}	Seeds siliqua^{-1}	1000-seed wt.(g)	Seed oil content (%)	Seed yield (kg ha^{-1})
AYT during 2008-09								
MM-01-04	97	126	2.9	106	13.6			1560
MM-02-04	98	134	3.2	98	13.8			1375
MM-03-04	98	127	3.0	93	12.9			1164
MM-04-04	97	127	3.2	105	13.3			1526
MM-05-04	100	138	3.2	103	12.4			1225
MM-06-04	97	152	3.2	103	13.1			1138
MM-07-04	100	131	3.4	99	12.6			1361
MM-08-04	97	124	3.2	104	12.7			1475
MM-09-04	99	132	3.0	105	12.2			1299
MM-10-04	100	151	3.0	115	12.6			1560
BARIsarisha -11	100	146	3.3	98	12.3			1254
$LSD_{(0.05)}$	1.53	7.6	NS	9.7	0.95			87.0
RYT during 2009-10								
MM-01-04	108c	142cd	3.8NS	125c	11.9NS			2067c
MM-02-04	109bc	158b	4.2	126bc	12.1			2229b
MM-04-04	109bc	139d	3.8	134bc	11.7			2187b
MM-08-04	108c	140cd	3.6	145a	12.2			2253b
MM-09-04	109bc	147c	3.5	127bc	11.5			1959cd
MM-10-04	111a	169a	3.4	152a	11.3			2389a
BARIsarisha-11	112a	163ab	4.2	137b	11.5			1945d
On-station and on-farm trials during 2010-11								
MM-04-04	103b	141b	3.7NS	133bc	11.5NS	3.5b	35.7	1793c
MM-08-04	102b	140b	3.8	139b	11.1	3.5b	37.2	1952b
MM-10-04	106a	161a	3.5	145a	11.0	3.9a	36.4	2219a
BARIsarisha-11	106a	161a	3.8	131c	11.0	3.2c	35.3	1788c

Means followed by the same letter(s) in a column are not significantly different at $p \leq 0.05$ by DMRT; NS= not significant.

Table 2. Means (average of three years trial) of two mutants and BARIsarisha-11 for morphological characters, yield attributes and yield.

Mutants/ mother variety	Days to maturity	Plant height (cm)	Branches plant^{-1}	Siliqua plant^{-1}	Seeds siliqua^{-1}	1000-seed weight (g)	Seed oil content (%)	Seed yield (kg ha^{-1})	Yield increased over BARIsarisha-11
MM-10-04	106	161	3.33	140	11.5	3.85	36.4	2043	23%
MM-08-04	102	136	3.58	132	11.8	3.45	37.2	1893	14%
BARIsarisha-11	106	158	3.78	124	11.4	3.20	35.3	1662	-

than the mother variety. Mutants having higher seed yield over mother variety were also reported earlier in rapeseed-mustard (Rahman et al., 1992; Javed et al., 2003; Das et al., 2004; Brave et al., 2009). Mutants with higher number of siliquae plant^{-1} have also been reported in oilseed *Brassica* (Naz and Islam, 1979; Shah et al., 1990; Javed et al., 2003; Malek and Monshi, 2009) as a consequence of mutagenesis. Yadav et al. (1973) and Javed et al. (2003) demonstrated that number of seeds siliqua^{-1} and 1000-seed weight directly influenced the seed yield in rapeseed-mustard. Having a larger seed size is one of the important yield attributes in all crops. In the present study, two selected mutants, MM-10-04 and MM-08-04, exhibited higher 1000-seed weight, which indicates an increase in the size of seed as a result of induced mutation. Improvement in seed size i.e., obtaining bold-seeded mutants in oilseed *Brassica* has been achieved earlier by Shah et al. (1990). Induction of early maturity is one of the most frequent characters modified in the mutation breeding experiments in many crops including oilseed *Brassica* (Kharkwal et al., 2004). Development of several early matured mutants has been reported in oilseed *Brassica* (Rahman et al., 1992; Brave et al., 2009; Das et al., 1999, 2004; Malek and Monshi, 2009), which was found consistent with the present result. Result showed that seeds of all three mutants contained higher percentages of oil than mother variety. Improvement in seed oil content in the mutants has also been achieved earlier through induced mutations in *Brassica* oilseeds by Javed et al. (2003).

A little variation was observed in the percentage of aphid infested plants among the six locations and number of aphid plant^{-1} in four locations. These variations are normally due to the variation in environmental factors in different locations.

Materials and methods

Irradiation of seeds with gamma rays and preliminary selection

Four thousand and eight hundred seeds (1200 seeds for each of four doses) of the popular mustard variety, BARIsarisha-11 were irradiated with four different doses of gamma rays (600, 700, 800 and 900 Gy) from ^{60}Co gamma cell in 2004 to induce new genetic variability for the selection of improved mutant genotypes. The treated seeds were sown to obtain the M_1 generation along with parental control at the experimental field of BINA head quarters, Mymensingh. Seeds from five

Table 3. Severity and reaction of the mutants and BARIsarisha-11 to *Alternaria* blight disease under natural conditions at four locations.

Mutants/ mother varieties	Leaf area diseased (%)				Disease reaction
	Mymensingh	Ishurdi	Magura	Rangpur	
MM-04-04	8.8ab	6.7NS	7.6NS	9.7b	Tolerant
MM-08-04	9.7ab	7.6	7.4	9.8b	Tolerant
MM-10-04	7.5b	6.8	7.2	9.3b	Tolerant
BARIsarisha-11	11.0a	8.0	8.9	12.3a	Tolerant

Means followed by the same letter(s) in a column are not significantly different at $p \leq 0.05$ by DMRT; NS= not significant.

Table 4. Per cent aphid infested plant in mutants and mother variety at five locations.

Mutants/ mother variety	Aphid infested plant (%)			
	Magura	Rangpur	Jessore	Satkhira
MM-04-04	10.4a	1.2a	5.6a	25.9ab
MM-08-04	9.8ab	1.2a	4.1b	24.7bc
MM-10-04	8.5b	0.6b	3.0b	23.5c
BARIsarisha-11	10.8a	1.5a	6.2a	27.3a

Means followed by the same letter(s) in a column are not significantly different at $p \leq 0.05$ by DMRT.

Table 5. Population of aphid in mutants and mother variety at five locations.

Mutants/ mother variety	Average number of aphid plant^{-1}			
	Magura	Rangpur	Jessore	Satkhira
MM-04-04	8.9a	3.6b	4.5ab	20.4a
MM-08-04	5.5b	3.7b	4.1ab	19.6a
MM-10-04	3.0b	3.1b	3.5b	17.3b
BARIsarisha-11	10.3a	4.8a	5.0a	22.1a

Means followed by the same letter(s) in a column are not significantly different at $p \leq 0.05$ by DMRT.

siliquae, developed from the lower part of the main rachis of each M_1 plant, were harvested. The M_2 seeds from 2341 individual M_1 plants (745, 632, 508 and 456 plants from 600, 700, 800 and 900 Gy, respectively) were grown along with parental control in plant to row progenies with single replicate at the experimental field of BINA head quarters, Mymensingh during 2005-06. In M_2 generation, all the plants were carefully observed for morphological mutations and plants appearing different from the mother variety for one or more morphological traits were harvested separately.

From first segregating M_2 population, a total of 521 mutant variants (97, 142 157 and 125 mutant variants from 600, 700, 800 and 900 Gy, respectively) appearing different form from the mother variety for one or more morphological traits were selected and M_3 seeds from 521 individual mutant variants were collected separately. Observation trial was conducted with M_3 population to evaluate 521 individual mutant variants. For observation trial, M_3 seeds from 521 individual mutant variants were grown in plant-to-row along with mother variety with single replicate at the experimental field of BINA head quarters, Mymensingh during 2006-07. A total of 32 mutant variants (2, 13, 12 and 5 mutant variants from 600, 700, 800 and 900 Gy, respectively) were selected from M_3 population. Selection was made considering their better field performance regarding earliness and improved morphological characters as well as yield attributes compared to mother variety during 2006-07. An observation trial was conducted to evaluate the 32 M_4 mutant variants. For the observation trial, M_4 seeds of 32 mutant variants were grown following in plant-to-row progenies along with mother variety with single replicate at the experimental field BINA head quarters, Mymensingh during 2007-08 to study their breeding behavior for further selection. From M_4 population 10 true breeding mutants (four from each of 700 and 800 Gy, and two from 900 Gy) were selected considering their desirable morphological characters, yield attributes and seed yield compared to mother variety.

Conduction of different yield trials

Ten M_5 true breeding mutants were evaluated in replicated yield trials in different mustard growing areas of Bangladesh during 2008-09 to 2010-11. AYT was conducted with 10 M_5 mutants along with mother variety in two locations i. e., experimental fields at BINA sub-stations at Ishurdi and Magura during 2008-09. RYT was conducted at three locations i.e., experimental fields at BINA head quarters, Mymensingh and BINA sub-stations at Ishurdi and Magura with selected six M_6 mutants along with mother variety during 2009-10. On-station trial was conducted during 2010-11 at four locations (experimental fields of BINA head quarters' farm, Mymensingh and BINA sub-stations at Ishurdi, Rangpur and Magura) and on-farm trial at four locations (farmers' field at Satkhira, Jessore, Faridpur and Natore) with selected three M_7 mutants along with mother variety. AYT and RYT were laid out in randomized complete block design with three replicates while on-station and on-farm trials were also laid out in a randomized complete block design with four replicates. Unit plot size was maintained 15 m^2 (5 m × 3 m) for AYT, 20 m^2 (5 m × 4 m) for RYT and 25 m^2 (5 m × 5 m) for on-station and on-farm trials. The urea, triple superphosphate (TSP), muriate of potash (MP), gypsum, zinc sulphate and borax were used in the experimental field as a source of nitrogen, phosphorus, potassium, zinc, sulphur and boron at the rate of 205, 160, 80, 180, 10 and 5 kg ha^{-1}, respectively. The half amount of urea and total amount of TSP, MP, gypsum, zinc sulphate and borax were applied as basal dose during land preparation. The remaining half of urea was applied as top dress at 25 days after sowing. Seeds were sown within the second week of November of each year. Plant to plant distance was maintained 6 to 8 cm in a row and row to row distance was 25 cm. Intercultural operations were followed as and when necessary for proper growth and development of the plants.

Three M_7 mutants along with mother variety were evaluated under field conditions against *Aternaria* blight disease at four locations (experimental fields of BINA head quarters, Mymensingh and BINA sub-stations at Ishurdi, Magura and Rangpur). The experiment was laid out in a randomized complete block design with four replicates. Unit plot size was 16 m^2 (4 m × 4 m). Necessary cultural practices were done as and when necessary but no fungicides were applied.

Three M_7 mutants along with mother variety were evaluated against aphid under field conditions at six locations (experimental fields of BINA head quarters, Mymensingh, BINA sub-stations at Rangpur and Magura, and farmers' field ad Faridpur, Jessore and Satkhira) during 2010-11. The experiment was laid out in a randomized complete block design with three replicates. Unit plot size was 12 m^2 (4 m × 3 m). Necessary cultural practices were done as and when necessary but no insecticides were applied.

Data recording

Data were taken on different morphological characters and yield attributes from 10 randomly selected representative plants from each plot at maturity. Plot seed yield was taken after proper drying of seeds and converted into kg ha^{-1}. For recording *Alternaria* blight data, ten plants were randomly selected and tagged from inner six rows of each plot. Then data on disease severity of leaves were recorded. The diseased leaf area was estimated visually avoiding older and younger leaves from the top and base of 10 tagged plants. The leaves were scored as 0 - 5 scale: where 0 = no disease, 1 = 0.1 - 6.0% leaf area diseased (LAD), 2 = 6.1 - 12% LAD, 3 = 12.1 - 25% LAD, 4 = 25.1 - 50% LAD and 5 = more than 50% LAD according to Meah (1994). Data on percent of plant infested by aphids were recorded from five randomly selected rows $plot^{-1}$. Data on number of aphids $plant^{-1}$ were also recorded.

Statistical analysis

Data were analyzed statistically according to the design used, following the analysis of variance (ANOVA) technique and the mean differences were compared with DMRT at 5% level of significance using the statistical computer package programme, MSTAT-C (Russell, 1986).

Conclusion

It was concluded that the overall performance of the two selected mutants, MM-10-04 and MM-08-04, was better than the popular mother variety, BARIsarisha-11. Consequently, the National Seed Board registered MM-10-04 and MM-08-04 in 2011 as two high yielding mustard varieties, Binasarisha-7 and Binasarisha-8, respectively for commercial cultivation in Bangladesh.

Acknowledgment

The authors acknowledge the authority of Bangladesh Institute of Nuclear Agriculture, for providing financial support to carry out the experiments from the Research and Development Project.

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