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Assessment of Genetic Diversity of Rapeseed Mutants in Diverse Environments

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ABSTRACT

Seven mutants of rapeseed (RM-01-05, RM-02-05, RM-03-05, RM-04-05, RM-05-05, RM-10-05 and RM-11-05) along with mother variety Binasarisha-4 were evaluated in four different locations to evaluate their performances regarding seed yield and yield contributing characters and to select promising mutants having high seed yield with short maturity period. The experiment was laid out in a Randomized Complete Block Design (RCBD) with three replications. Results showed significant variation among the mutant lines and check variety for most of the characters in four different locations. Combined means that all mutants matured earlier and most of the mutants produced higher number of branches per plant compared to mother variety. Combined means over locations showed that three mutants namely RM-01-05, RM-04-05 and RM-10-05 produced the seed yield of 1957, 1910 and 1837 kg ha⁻¹, respectively which were significantly higher than the mother variety. These three mutants also produced higher number of siliquae per plant, seeds per siliqua and 1000-seed weight than the other mutants and mother variety and may be used for further study for achieving a rapeseed variety for the Bangladeshi farmers. In location wise performance of all the characters, Magura showed the best followed by Ishurdi than the other two locations, may be due to the environmental and soil characteristics of a particular location. Therefore, present research suggests that gamma rays irradiation can be fruitfully applied to develop mutants with higher seed yield and other improved agronomic traits in *Oleiferous brassica*.

Key words: *Brassica napus* L., rapeseed, promising mutants, evaluation

INTRODUCTION

The *Oleiferous brassica* represented by rapeseed-mustard plays an important role in vegetable oil production of the world and is the third most important oil source in the world after soybean and palm. Rapeseed-mustard is the leading oilseed crop in Bangladesh but its national average seed yield is 949 kg ha⁻¹ only (BBS., 2013). Domestic oilseed production can hardly meet 15% demand. Due to the versatile nature of this crop, its contribution to industrial, agricultural and medicinal sectors is significantly increasing. In Bangladesh, to fulfill the requirements of edible oil, there is little scope of horizontal expansion of the rapeseed cultivation in the country, attempt should be made to increase the yield per unit area within the shortest possible time.

Increased seed yield is the ultimate goal of the breeders. But seed yield itself is a product of interaction of many component traits which influence yield directly or indirectly. So, it is important to see the contribution of each of the traits in order to give more attention to those having the highest influence on yield. Induced mutations have generated a vast amount of genetic variability and are now widely used for the development of genes controlling important traits and understanding the functions and mechanisms of actions of these genes in plants (Liang, 2009). Using mutation breeding, genetic improvement of any yield attributes either qualitative or quantitative trait, has been successfully achieved in rapeseed-mustard (Seyis *et al.*, 2006; Spasibionek, 2006; Zhao *et al.*, 2009; Malek *et al.*, 2012). Furthermore, mutation breeding requires less time to develop crop cultivars as compared to the conventional breeding (Manjaya, 2009).

Availability of genetic diversity and genetic variation is the heart of any breeding program which plays a critical role in developing well-adapted and improved crop varieties. Plant breeding requires genetic variation of useful traits for crop improvement. Often, however, desired variation is lacking. The application of mutation breeding in plant improvement has been successfully used in several crops to generate new sources of genetic variations and this technique has greatly enhanced the development of new crop varieties (Shu and Lagoda, 2007). Mutation breeding is characterized by its merit, creation of new mutant characters and addition of very few traits without disturbing other characters of variety. The commercial utilization of approximately 3000 mutant induced and mutant-derived varieties strongly shows that among the different methods, mutation breeding is a useful tool for generating new germplasm for crop improvement (Ishige, 2009). Kharkwal *et al.* (2004) reported that among the mutant varieties, 89% have been developed worldwide using physical mutagens like X-rays, gamma rays, thermal and fast neutrons and with gamma rays alone accounting for the development of 60% of the mutant varieties.

This study investigated the morphological variability among 7 rapeseed mutants along with mother varieties using quantitative morphological traits including yield attributes. For an effective breeding program for crop variety development through hybridization, the analysis of genetic diversity is one of the useful tools and plays a fundamental role in identification of parents (Mazid *et al.*, 2013). As a traditional method, morphological traits are used to assess genetic divergence and classify existing germplasm materials. According to Din *et al.* (2010) scientific classification of the plant still relies on morphological traits. Moreover, this technique is easier, cost effective and easy to score and requires less time and finally it does not need any technical knowledge. Under above context, Bangladesh Institute of Nuclear Agriculture (BINA) initiated a research program to develop rapeseed mutants having higher seed yield potential during using gamma rays on mother variety Binasarisha-4 and succeeded mutants with desirable traits. The present study was carried out to evaluate the performances of the mutants for their seed yield and yield components at different rapeseed growing agro-ecological zones of Bangladesh to select the promising mutant (s).

MATERIALS AND METHODS

To create genetic variations, seeds of popular variety Binasarisha-4 were irradiated with 600, 700 and 800 Gy doses of gamma rays using Co⁶⁰ gamma cell. Treated seeds were then planted to grow M₁ generation at Bangladesh Institute of Nuclear Agriculture (BINA), Mymensingh, Bangladesh in 2005 for the selection of improved genotypes in the subsequent generations. In each of M₂, M₃, M₄, M₅ and M₆ generation, selection for the desired agronomic traits was made. From M₆

generation, seven true breeding mutants (RM-01-05, RM-02-05, RM-03-05, RM-04-05, RM-05-05, RM-10-05 and RM-11-05) were selected for further evaluation. Those seven mutants along with mother variety Binasarisha-4 were used as plant materials for evaluation in this experiment. The experiment was conducted in randomized complete block design with three replications at four locations of Bangladesh (farms of BINA head quarters, Mymensingh and BINA sub-stations at Magura, Ishurdi and Rangpur) during 2010-2011. Seeds were sown on 3 November in BINA head quarters, 24 October at Magura, 28 October at Ishurdi and 25 October at Rangpur in 2010. Unit plot size was 12 m² (4×3 m) with 25 cm row to row spacing and 6-8 cm from plant to plant within rows. Recommended production packages i.e., application of fertilizers, weeding, thinning, irrigation, application of pesticide etc. were followed to ensure normal plant growth and development of the plants in each plot. Data were taken on morphological characters such as plant height and branches/plant and yield attributes like siliquae/plant, 1000 seed weight and seeds per siliqua from 10 randomly selected representative plants from each plot at maturity. Maturity period was counted when approximately 70% siliquae of each plot turned into yellowish brown colour. Plot seed yield was taken after proper drying of seeds and converted into kg ha⁻¹. The data were then analyzed statistically according to the design used using the analysis of variance (ANOVA) technique following Gomez and Gomez (1984) and the mean values were compared by DMRT at 5% level of significance.

RESULTS AND DISCUSSION

Induction of early maturity is one of the most frequent characters modified in the mutation breeding experiments in many crops including oilseed *Brassica*. Significant differences were observed for days to maturity both in individual locations and combined over locations. Mutants RM-01-05 and RM-02-05 required shortest maturity period (87 days) than other five mutants and the mother variety had the longest maturity period of 92 days at Mymensingh. The RM-01-05 required the shortest period of 80 days to mature while mother variety Binasarisha-4 took the highest maturity period of 87 days at Magura. Mutant RM-11-05 required the shortest maturity time of 90 days while Binasarisha-4 required the longest duration 98 days at Ishurdi. At Rangpur, Binasarisha-4 also required the longest duration of 88 days to mature but both RM-10-05 and RM-11-05 required the shortest period (83 days). In combined over locations, days to maturity varied from 86 days in RM-01-05 to 91 days in Binasarisha-4 (Table 1). So, most of the mutants matured earlier than the mother variety Binasarisha-4. This is in agreement with observation of Malek *et al.* (2012) who reported that maturity time and time to flowering were closely related to yield and yield components. Positive correlation of plant height with days to maturity indicated

Table 1: Days to maturity of rapeseed mutants with check variety

Mutants/Check	Mymensingh	Magura	Ishurdi	Rangpur	Combined mean
RM-01-05	87 ^b	80 ^d	94 ^b	84 ^{cd}	86 ^d
RM-02-05	87 ^b	82 ^c	94 ^b	85 ^c	87 ^{cd}
RM-03-05	89 ^{ab}	84 ^b	96 ^{ab}	87 ^{ab}	89 ^b
RM-04-05	91 ^{ab}	82 ^c	97 ^a	86 ^{bc}	89 ^b
RM-05-05	88 ^b	84 ^b	94 ^b	84 ^{cd}	88 ^{bc}
RM-10-05	92 ^a	85 ^b	92 ^c	83 ^d	88 ^{bc}
RM-11-05	89 ^{ab}	85 ^b	90 ^d	83 ^d	87 ^{cd}
Binasarisha-4	89 ^{ab}	87 ^a	98 ^a	88 ^a	91 ^a
CV (%)	2.84	2.69	3.09	2.77	

Values having same letter (s) in a column do not differ significantly at 5% level of significance as per DMRT

that genotypes with taller plants tend to longer maturity period (Khatri *et al.*, 2005; Spasibionek, 2006). Maturity time might be changed due to the influence of environmental factors (Shah and Rahman, 2009).

A significant variation was observed on the plant height both in individual locations and combined over locations. At Mymensingh, mutant RM-02-05 produced the tallest plant (100 cm) followed by RM-05-05 (99 cm) while RM-11-05 produced the shortest plant (91 cm). Both RM-01-05 and RM-10-05 produced the tallest plant (113 cm) whereas Binasarisha-4 produced the shortest plant (104 cm) at Magura. Similarly at Ishurdi, RM-01-05 produced the tallest plant (115 cm) whereas RM-04-05 produced the shortest plant (102 cm). At Rangpur, RM-02-05 produced the tallest plant (116 cm) followed by RM-01-05, RM-10-05 and Binasarisha-4 which was 115 cm whereas RM-11-05 produced the shortest plant (104 cm). In combined over locations, mutant RM-01-05 gave the highest plant height of 110 cm followed by RM-10-05 (109 cm) while the mutant line RM-11-05 had the shortest plant height of 102 cm (Fig. 1). Plant heights may vary due to the genetic effects present among the genotypes and as well as the proper agronomic management. Medium size plants are more likely to produce greater number of branches, number of siliquae per plant and number of seeds per siliqua that leads to higher yield of rapeseed (Javed *et al.*, 2003). Seyis *et al.* (2006) showed that induced mutation through gamma rays played a significant role in the alteration of plant architecture in rapeseed-mustard. Malek *et al.* (2012) isolated short statured mutants from mutagen treated populations in rapeseed-mustard.

Number of branches per plant reflects the overall plant growth. It is the important morphological parameters contributing to yield. A significant variation was found on the number of branches per plant both in individual locations and combined over locations (Fig. 2). Mutant RM-01-05 produced the highest number of branches (2.7) followed by RM-04-05 and RM-05-05 (2.5) while Binasarisha-4 produced the lowest number (2.1) at Mymensingh. Mutant RM-10-05 produced

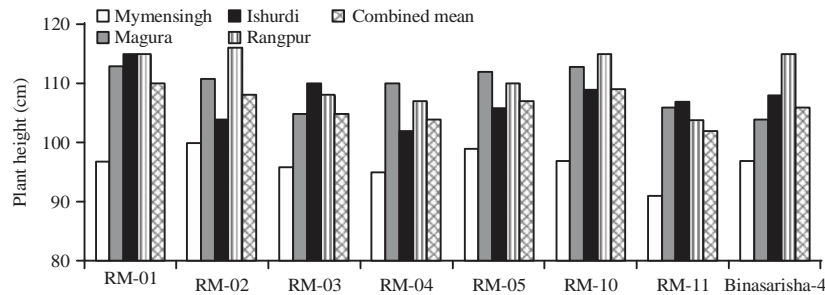


Fig. 1: Plant height of rapeseed mutants with check variety

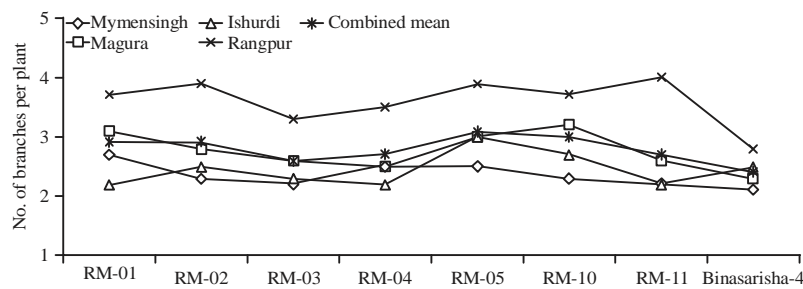


Fig. 2: Number of branches per plant of rapeseed mutants with check variety

the highest number of 3.2 branches per plant followed by RM-01-05 and RM-05-05 (3.1 and 3.0, respectively) and Binasarisha-4 produced the lowest number of 2.3 branches at Magura. At Ishurdi, branches number varied from 2.2-2.9. Mutant RM-11-05 produced the highest number of branches (4.0) followed by RM-02-05 and RM-05-05 (3.9) while Binasarisha-4 produced the lowest number (2.8). Combined means over locations showed that mutant RM-05-05 produced the highest number of branches per plant (3.1) which was closely followed by RM-10-05 (3.0) while Binasarisha-4 produced the lowest number of 2.4 branches per plant. Number of branches may vary due to the qualitative characters present in the genotypes of rapeseed. High branching quality enhances the increasing trend of siliquae per plant, seeds per siliqua and seed yield of rapeseed, which are in agreement with the findings of Khatri *et al.* (2005) and Shah and Rahman (2009).

Siliquae per plant, the most important yield attributes, was significantly different among the rapeseed mutants and check variety both in individual locations except Ishurdi and Rangpur and combined over locations. At Mymensingh RM-01-05 produced the highest number of siliquae per plant (90) whereas mother variety Binasarisha-4 produced the lowest number (61). The RM-01-05 produced the highest number of siliquae per plant (96) followed by RM-01-05 (92) while check variety Binasarisha-4 produced the lowest number (68) at Magura. Combined means showed that RM-01-05 produced the highest number of 78 siliquae per plant followed by RM-10-05 (72), RM-02-05 (71) and RM-04-05 (70) and the lowest was found in Binasarisha-4 (62) (Fig. 3). These results were consistent with the finding of Malek *et al.* (2012) who stated high yielding genotypes had greater number of siliquae per plant than low yielding ones. Higher branching pattern of a plant enhances the more siliquae per plant that ultimately influences the higher seed yield. Mutants with higher number of siliquae per plant over mother variety have also been reported in oilseed *Brassica* (Khatri *et al.*, 2005; Zhao *et al.*, 2009) as a consequence of mutagenesis.

Number of seeds per siliqua was found significant in individual locations but insignificant in combined over locations. At Mymensingh, mutant RM-11-05 produced the highest number of seeds per siliqua (33) followed by RM-05-05 and Binasarisha-4 (29) whereas RM-03-05 produced the lowest number of seeds per siliqua (25). Mutant RM-01-05 produced the highest number of seeds per siliqua (30) while RM-11-05 produced the lowest number of seeds per siliqua (24) at Magura. At Ishurdi, mutant RM-03-05 produced the highest number of seeds per siliqua (35) while both RM-01-05 and RM-05-05 produced the lowest number of seeds per siliqua (28). Mutant RM-05-05 produced the highest number of seeds per siliqua (32) whereas RM-11-05 produced the lowest number of seeds per siliqua (24) at Rangpur (Table 2). All mutants and check variety produced more or less same seeds per siliqua which was ranged from 27-29 in combined over location. Spasibionek (2006) reported similar type of results and found positive and significant correlation in number of seeds per siliqua. Seeds per siliqua depends on the size and shape of the siliqua and

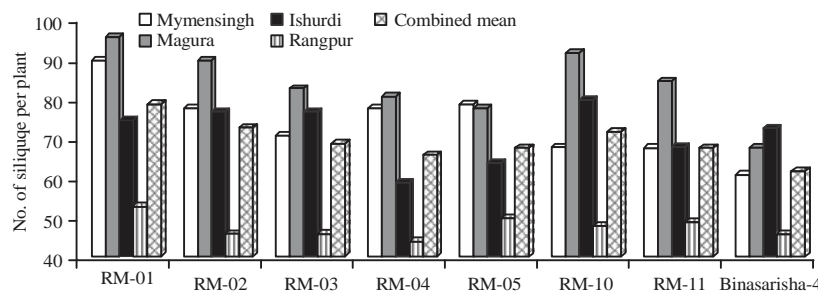


Fig. 3: Number of siliquae per plant of rapeseed mutants with check variety

Table 2: Number of seeds per siliqua of rapeseed mutants with check variety

Mutants/Check	Mymensingh	Magura	Ishurdi	Rangpur	Combined mean
RM-01-05	27 ^c	30 ^a	28 ^d	29 ^b	29
RM-02-05	27 ^c	28 ^b	31 ^b	27 ^c	28
RM-03-05	25 ^d	26 ^d	35 ^a	30 ^{ab}	29
RM-04-05	26 ^{cd}	27 ^{bc}	29 ^c	27 ^c	27
RM-05-05	29 ^b	27 ^{bc}	28 ^d	32 ^a	29
RM-10-05	27 ^c	26 ^d	30 ^{bc}	26 ^{cd}	27
RM-11-05	33 ^a	24 ^e	30 ^{bc}	24 ^d	28
Binasarisha-4	29 ^b	29 ^{ab}	30 ^{bc}	27 ^c	29
CV (%)	1.97	2.07	2.16	1.89	

Values having same letter (s) in a column do not differ significantly at 5% level of significance as per DMRT

Table 3: 1000 seed weight (g) of rapeseed mutants with check variety

Mutants/Check	Mymensingh	Magura	Ishurdi	Rangpur	Combined mean
RM-01-05	3.71 ^a	3.89 ^a	4.05 ^a	3.68 ^a	3.83 ^a
RM-02-05	3.54 ^{bc}	3.75 ^b	3.76 ^b	3.23 ^e	3.57 ^{bc}
RM-03-05	3.47 ^d	3.58 ^c	3.41 ^d	3.35 ^d	3.45 ^c
RM-04-05	3.65 ^{ab}	3.88 ^a	3.52 ^c	3.26 ^e	3.58 ^{bc}
RM-05-05	3.42 ^d	3.67 ^{bc}	3.50 ^c	3.38 ^d	3.49 ^c
RM-10-05	3.61 ^b	3.76 ^b	3.57 ^c	3.57 ^b	3.62 ^b
RM-11-05	3.43 ^d	3.62 ^c	3.53 ^c	3.48 ^{bc}	3.52 ^c
Binasarisha-4	3.32 ^e	3.40 ^d	3.38 ^d	3.25 ^e	3.34 ^d
CV (%)	3.28	3.15	3.34	3.18	

Values having same letter (s) in a column do not differ significantly at 5% level of significance as per DMRT

as well as the size of the seeds. Large sized siliqua produced the highest number of seeds that increase the yields and oil contents of rapeseed (Radoev *et al.*, 2008; Guohuai *et al.*, 2002).

The 1000 seed weight is the most important yield attributes in rapeseed. The 1000 seed weight was recorded at final harvest and the result is presented in Table 3. There was significant variation in 1000 seed weight among the mutant and check variety and calculated in the unit of gram. Mutant RM-01-05 had the highest seed weight (3.71 g) closely followed by RM-04-05 (3.65 g) which was significantly higher than the control variety Binasarisha-4 (3.32 g) at Mymensingh. At Magura, RM-01-05 had the highest seed weight (3.89 g) which is statistically similar to the mutant RM-04-05 (3.88 g) while the lowest seed weight was found in Binasarisha-4 (3.40 g). The highest seed weight was obtained from the mutant RM-01-05 (4.05 g) whereas the lowest was found in Binasarisha-4 (3.38 g) at Ishurdi. Similarly, at Rangpur, mutant RM-01-05 produced the highest seed weight (3.68 g) which was closely followed by RM-10-05 (3.57g) while Binasarisha-4 yielded the lowest seed weight (3.25 g). In combined mean, 1000-seed weight ranged from 3.34 to 3.83 g, the mutant RM-01-05 obtained the highest and the mother variety Binasarisha-4 produced the lowest seed weight. Javed *et al.* (2003) demonstrated that number of seeds per siliqua and 1000 seed weight directly influenced the seed yield in rapeseed and mustard. Improvement in seed size i.e., obtaining bold-seeded mutants has also been achieved earlier through induced mutations in oilseed *Brassica* by Malek *et al.* (2012) which confirm the present results.

Yield is a complex quantitative character governed by large number of genes and is greatly affected by environmental fluctuations. Seed yield is the most important character for considering as a promising variety of a particular crop. Mutant RM-01-05 produced the highest seed yield (2108 kg ha⁻¹) followed by RM-10-05 (1817 kg ha⁻¹) at Mymensingh. Mutant RM-04-05 produced the highest seed yield (2305 kg ha⁻¹) followed by RM-10-05 (2055 kg ha⁻¹) and RM-11-05 (2041 kg ha⁻¹) while the control variety Binasarisha-4 produced the lowest seed yield of 1766 kg ha⁻¹ at Magura. At Ishurdi, RM-01-05 produced the highest yield (2121 kg ha⁻¹) closely

Table 4: Seed yield (kg ha⁻¹) of rapeseed mutants with check variety

Mutants/Check	Mymensingh	Magura	Ishurdi	Rangpur	Combined mean
RM-01-05	2108 ^a	1958 ^{bc}	2121 ^a	1644 _c	1957 ^a
RM-02-05	1717 ^c	1930 ^{bc}	1889 ^{bc}	1599 ^c	1784 ^{bc}
RM-03-05	1783 ^{bc}	1833 ^{cd}	1800 ^c	1622 ^c	1760 ^{bc}
RM-04-05	1550 ^d	2305 ^a	1966 ^b	1822 ^a	1910 ^a
RM-05-05	1600 ^{cd}	1944 ^{bc}	1911 ^b	1489 ^d	1736 ^{cd}
RM-10-05	1817 ^b	2055 ^b	1822 ^c	1655 ^c	1837 ^b
RM-11-05	1617 ^{cd}	2041 ^b	1847 ^c	1766 ^{ab}	1818 ^b
Binasarisha-4	1733 ^{bc}	1766 ^d	1711 ^d	1533 ^c	1686 ^d
CV (%)	5.69	5.47	5.12	5.34	

Values having same letter (s) in a column do not differ significantly at 5% level of significance as per DMRT

followed by RM-04-05 (1966 kg ha⁻¹) and Binasarisha-4 produced the lowest seed yield of 1711 kg ha⁻¹. At Rangpur, mutant RM-04-05 produced the highest seed yield (1822 kg ha⁻¹) followed by RM-11-05 (1766 kg ha⁻¹). Combined means over locations showed that three mutants namely RM-01-05, RM-04-05 and RM-10-05 produced the seed yield 1957, 1910 and 1837 kg ha⁻¹, respectively which were significantly higher than the mother variety. These three mutants also produced higher number of siliquae per plant than the mother variety. The result indicated that there was a direct effect of the important yield attributes like number of siliqua per plant, seeds per siliqua and 1000-seed weight, which determine the seed yield. The results are supported with the findings of Javed *et al.* (2003), Seyis *et al.* (2006) and Zhao *et al.* (2009) who obtained considerable genotypic variability for seed yield. Rapeseed yield varies depending on the variety and agro-ecological zones with proper agronomic management practices. Mutants having higher seed yield over mother variety were also reported earlier in rapeseed-mustard (Table 4) (Radoev *et al.*, 2008; Barve *et al.*, 2009; Malek *et al.*, 2012).

CONCLUSION

In plant breeding, generation of new genotypes from the existing ones with improvement in plant traits is the main objective. The present study revealed the presence of high levels of variations for seven different morphological traits including yield attributes and seed yield among the newly developed 7 mutants along with mother genotype of rapeseed. These mutants could be served as raw materials for further genetic improvement of different characters of the rapeseed. Most of the traits showed positive correlations between each other, which will assist in the combined improvement of these traits by selecting only highly heritable and easily measurable phenotypic traits. These results also confirm that not only the geographical background but also induced mutations significantly contribute to creating genetic variations. Therefore, among the mutants and mother variety, three mutants RM-01-05, M-04-05 and RM-10-05 showed better performance for seed yield and yield attributes and hold promise to be mutant varieties. Moreover, this suggests that gamma rays irradiation can be fruitfully applied to develop mutants with higher seed yield and other improved agronomic traits in oleiferous *Brassica*. This study indicated the presence of high levels of genetic diversity among the mutants for evaluated characters.

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