Improving yield components and overall yield in M_2–M_4 phenotypic mutants, induced by EMS, of upland red rice (*Oryza sativa* L.) cv. Dawk Kha 50

Watcharin Soonsuwon[a,*], **Nucharee Chadakan**[a,b], **Ali Awais**[a,c], **Charassri Nualsri**[a]

[a] Agricultural Innovation and Management Division, Faculty of Natural Resources, Prince of Songkla University, Songkhla 90110 Thailand
[b] Department of Plant Science, Faculty of Agriculture, Rajamangala University of Technology Srivijaya, Nakorn Sri Thammarat Campus, Nakhon Si Thammarat 80240 Thailand
[c] Department of Agricultural and Environmental Sciences, Universià Degli Studi Di Milano, Milano 20133 Italy

*Corresponding author, e-mail: watcharin.s@psu.ac.th

ABSTRACT: Mutation breeding has been used to enhance qualitative and quantitative traits of rice. The objective of this study was to improve yield or yield component in mutated population of upland red rice induced by ethyl methanesulfonate (EMS). Two thousand and five hundred seeds of upland red rice cultivar Dawk Kha 50 were exposed to EMS at 1.23% based on root length of LD_{50}, to make M_1; M_2 generation was segregated for selection of desirable agronomic traits. From selected M_2 plants, seven M_3 lines and five M_4 lines of induced upland red rice were evaluated for yield and other traits. The mutant traits selected were successfully observed on many traits, such as number of filled grains/panicle, size of paddy, brown color of the rice. Grain yield of mutant lines was higher than that of the wild type. Phenotypic evaluation of the five M_4 lines, comparing to the wild type cultivar Dawk Kha 50, showed a higher number of filled grains/panicle, size of paddy and brown color of the rice in M_4 43-1-1-1 and M_4 43-2-1-3. The tenderness of cooked mutant rice was better than the wild type. It, hence, could be concluded that EMS improved agronomic traits of red rice. Moreover, the best mutant lines would be further used in developing commercial varieties or future breeding materials.

KEYWORDS: ethyl methanesulfonate, genetic variation, mutation breeding, *Oryza sativa* L., red rice

INTRODUCTION

Rice (*Oryza sativa* L.) is one of the most important food crops in the world, especially in Asia [1]. Upland rice is a type of rice associated with a land ecosystem. In Southeast Asia it is grown on the hills and intercropped with young rubber or palm oil plantations under rainfall. Improving upland rice yield is important to farmers in the tropics [2]. Currently, upland red rice is very important for producing functional foods. Upland red rice, such as Dawk Kha 50, is rich in antioxidants acting against aging and free radicals [3] and reducing risks of chronic diseases [4]. Farmers do not desire to grow red rice because its productivity is very low (2–3 t/ha). Thus, improvement of superior red rice cultivars is necessary, and mutation and hybridization can be used to improve high yielding and other desirable agronomic traits.

Mutation breeding is an effective method to induce genetic variability and new mutant lines with improved agronomic traits [5, 6]. Several traits; such as higher yield, early maturity, disease resistance, short stem, shattering resistance, and easier harvesting, were reported on mutant cultivars [6]. The induction of mutations involves physical and chemical mutagens to create variations at molecular level. Chemical mutagen is more efficient as it provides an inexpensive

and straightforward way for point mutation relating to base pair changes, which are traceable; whereas the physical one causes unintended deletions of part of the DNA. Among chemical mutagens, Ethyl methanesulfonate (EMS) is the most widely used in various crop plants, including wheat, rice, soybean, cotton, sesame, and maize [7], tomato [8], rice [9, 10], peanut [11], rapeseed [12], and carrot [13]. EMS can be applied to induce desirable functions in mutants as well as to recognize the functional roles of single amino acid residues. There are several types of base changes of mutants in M_2, M_3 or M_4, such as C/G to T/A, A/T to G/C, A/T to T/A, and A/T to C/G [14].

The aim of this study was to determine the important traits in M_2, M_3, and M_4 generations for selecting and improving yield components and overall yield in mutated population of upland red rice that had undergone EMS induced mutations.

MATERIALS AND METHODS

Experimental methods

The experimental approach in this study was illustrated in Fig. S1. The parental upland rice, Dawk Kha 50 variety, was induced for mutagenesis by EMS. It is a non-glutinous, photoperiod sensitive, and red brown rice type. Healthy seeds were supplied by

the Krabi Rice Research Center, Rice Research and Development Division, Thailand.

Two thousand and five hundred seeds were subjected to 1.23% EMS for 12 h based on root length of LD_{50} [15]. They were grown in the Plant Science field of Agricultural Innovation and Management Division, Faculty of Natural Resources, Prince of Songkla University (7°00′19.2″ N, 100°29′58.2″ E). One seed/hill was grown along with peat to ensure better germination. Seeds were sown in simple rows by maintaining distances between rows and plants at 5 cm and 10 cm, respectively. Thirty-two plants of Dawk Kha 50 cultivar were also grown for comparison between the treated and the untreated plants. The segregated mutant plants were tagged before harvesting. The germination percentage of the 1.23% EMS treated seeds was 70.84%, i.e. 1771 out of 2500; and only 43 plants survived and gave seeds. The mutant phenotypes of M_1 generation were reported in Sarhad Journal of Agriculture [15].

All the seeds from individual M_1 plants, the M_2, were harvested separately. Eight healthy seeds from each plant were sown in plant progeny row basis for marking M_2. M_3 seeds were collected from five high yielding M_2 mutant lines for subsequent M_3 generation. The seeds were sown and grown at sixty plants per mutant line. All experiments were completely randomized design with five replications. The observations were recorded from the M_3 mutant plants and ten Dawk Kha 50 plants. The highest seed yield potential produced to M_4 generation was used to evaluate the stability of yield traits.

The five best selected seeds of M_4 mutant lines were grown in the experimental field at the Agricultural Innovation and Management Division. The experimental design was a randomized complete block design with three replications. The treatments were five M_4 lines and three controls (Dawk Pa-yawm, Mai Tahk and Dawk Kha 50). In each experimental unit, the seeds were sown in four rows with 25×30 cm spacing between plant and row, and each row was four m long.

NPK (15-15-15) fertilizer was applied at the rate of 219 kg/ha at planting. Urea (46-0-0) was applied on day 30 after planting at the rate of 219 kg/ha. NPK fertilizer (0-0-60) was applied at boosting stage at the rate of 250 kg/ha in each generation. Agronomic management, such as weed control, was made manually; and insect control was done through the application of 40 ml cypermethrin 10% v/v EC in 20 l water solution.

Data collection

The traits: plant height (cm), days to maturity (day), number of tillers/plant (no.), number of panicles/plant (no.), panicle length (cm), number of filled grains/panicle (no.), weight of one thousand grains (g), and yield/plant (g) were recorded for the M_2–M_4
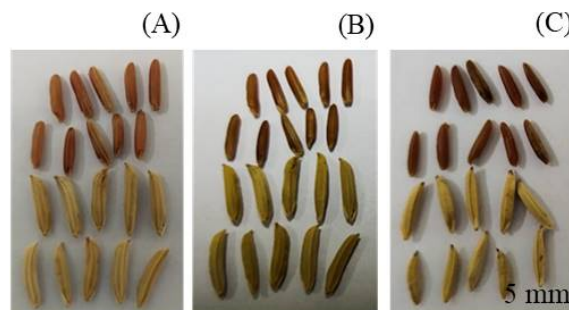
Fig. 1 Size and color of rice husk and dehusked rice grains of: (A), Dawk Kha 50; (B), M_4 43-1-1-1; and (C), M_4 43-2-1-3.

generations and the Dawk Kha 50 wild type. In M_4 generation, these traits were recorded on ten plants of each genotype in each replicate. The other recorded traits for the selected M_4 lines and the Dawk Kha 50 wild type were length and width of paddy (mm), amylose (%), gel consistency (mm), alkali test, and elongation ratio (lengths of cooked rice to uncooked rice). Color of husk and dehusked grain were determined by R.H.S. color chart of the Royal Horticultural Society.

Statistical analysis

For M_3 and M_4 generations, the data were subjected to analysis of variance (ANOVA). Multiple comparisons among M_3 or M_4 mutant lines and the Dawk Kha 50 wild type were achieved using the least significant difference (LSD) test at the 0.05 probability level of the R program [16]. The LSD values for comparisons among mutant lines and the wild type at 5% of significance were used to identify the best mutants.

RESULTS AND DISCUSSION

Microplastics abundance in shellfish

Only 27 M_2 families survived and gave healthy seeds. The comparison mean values of different quantitative traits of an average of Dawk Kha 50 and the selected M_2 mutants were shown in Table 1. The selected M_2 mutants showed ranges of 118–151 cm plant height, 72–100 days to maturity, 8–21 tillers/plant, 7–17 panicles/plant, 25–33 cm panicle length, 62.50–207.70 filled grains/panicle, 19–33 g weight of 1000 grains, and 9.10–54.80 g/plant yield. The common botanical morphology of each of M_2 plants (except the agronomic traits) did not change. For Dawk Kha 50, the means with standard deviations of plant height, days to maturity, number of tillers/plant, number of panicles/plant, panicle length, number of filled grains/panicle, weight of one thousand grains, and yield/plant were 134±15.89 cm, 104±0.00 days, 11±1.87 tillers/plant, 8±1.21 panicles/plant, 27±1.64 cm, 56.00±13.22 filled grains/

Table 1 Agronomic trait of selected phenotypic M_2 mutants.

Plant id	Plant height (cm)	Days to maturity	No. of tiller	No. of panicles	Panicle length (cm)	No. of filled grains/panicle	1000 grain weight (g)	Yield/ plant (g)
M_2 1-2	118	100	15	11	25	62.5	23	9.1
M_2 2-1	121	100	21	17	29	97.6	33	54.8
M_2 36-1	151	90	10	10	33	155.7	25	38.9
M_2 43-1	142	72	8	8	29	143.8	30	34.1
M_2 43-2	147	72	9	7	27	207.7	19	27.6
Dawk Kha 50[†]	134.0 ± 15.89	104.0 ± 0.00	11.0 ± 1.87	8.0 ± 1.21	27.0 ± 1.64	56.0 ± 13.22	34.0 ± 4.46	14.9 ± 4.45

[†] mean ± SD.

Table 2 Plant height, days to flowering, days to maturity, number of tillers/plant, and number of panicles/plant of M_3 mutants.[†]

Genotype	Plant height (cm)	Days to flowering	Days to maturity	No. of tillers/plant	No. of panicles/plant
M_3 1-2-1	148 ± 14	94 ± 0	107 ± 0	3.60 ± 0.55	4.40 ± 0.55
M_3 1-2-2	124 ± 2	94 ± 0	107 ± 0	2.80 ± 0.45	3.40 ± 0.89
M_3 2-1-1	119 ± 7	99 ± 2	114 ± 2	6.80 ± 2.68	6.20 ± 2.17
M_3 36-1-1	111 ± 11	99 ± 2	117 ± 4	5.80 ± 2.68	3.80 ± 2.05
M_3 43-1-1	122 ± 8	86 ± 0	107 ± 0	3.60 ± 0.55	4.20 ± 1.90
M_3 43-2-1	140 ± 4	83 ± 0	106 ± 0	3.80 ± 0.84	4.00 ± 0.71
M_3 43-2-2	127 ± 4	86 ± 0	106 ± 0	3.40 ± 0.55	3.00 ± 0.00
Dawk Kha 50	124 ± 3	103 ± 0	138 ± 0	4.20 ± 0.45	3.80 ± 0.45
CV (%)	6.06	1.05	1.29	33.69	29.75
LSD 5%	11.00	1.00	2.00	1.84	1.57

[†] mean ± SD.

panicle, 34±4.46 g, and 14.90±4.45 g/plant, respectively. The performance of Dawk Kha 50 mutants (except the M_2 1-2) was far better than the Dawk Kha 50 wild type; and, hence, provided a good basis for improving the yield of Dawk Kha 50. The M_2 1-2 plant had the lower panicle length, number of filled grains/ panicle, weight of one thousand grains and yield/plant than the Dawk Kha 50 (Table 1). These results showed the accuracy of breeding program because of the availability of high genetic variability in the M_2 segregating generation [17]. The efficiency transgressive segregate discovery could be raised if the environmental variation influencing the expression of the quantitative traits in the populations was small. Thus, it needs to grow superior progenies and compare the progeny means with the parent means grown in an analogous environment [18].

In the M_3 generation plants, the effect of EMS treatment on yield component, yield and other traits was studied. Based on good yield component and grain yield from M_2 segregating in each line, seven M_3 lines were selected. The performances of selected M_3 lines were analyzed. Table 2 and Table 3 show the performances of the mutants. The completed analysis of variances for plant height, days to flowering, days to maturity, number of tillers/plant, number of panicles/plant, panicle length, and yield/plant indicated significant differences among treatment means; while number of filled spikelets/panicle and the weight of

1000 grains did not exhibit any differences. The selected M_3 mutants showed ranges of 111–148 cm plant height, 83–99 days to flowering, 106–117 days to maturity, 2.80–6.80 tillers/plant, 3.00–6.20 panicles/plant, 28.70–34.58 cm panicle length, 134.00–231.00 filled grains/panicle, 22.28–31.40 g weight of 1000 grains, and 10.38–36.40 g/plant yield. The common botanical morphology of each of M_3 plants, except the agronomic traits, did not change. The plant height, days to flowering, days to maturity, number of tillers/plant, number of panicles/plant, panicle length, number of filled grains/panicle, weight of one thousand grains, and yield/plant means of Dawk Kha 50 were 124 cm, 103 days, 138 days, 4.20 tillers/plant, 3.80 panicles/plant, 35.60 cm, 202.00 filled grains/ panicle, 21.64 g, and 17.18 g/plant, respectively. In M_3 generation, the populations were still segregated; and some of the M_3 plants could transmit the mutations classified in the M_2 parents. EMS showed variability for yield component and grain yield in M_2 and M_3 rice lines [19].

In order to explore stabilization of variability and mean, M_4 plants were tested in a more significant appearance. Five M_4 lines were selected based on good yield component and grain yield from M_3 segregating in each line. The performances of selected M_4 lines were analyzed. The completed analysis of variances for plant height, days to flowering, days to maturity, number of tillers/plant, number of panicles/plant, number

Table 3 Panicle length, number of filled grains/panicle, grain weight/panicle, 1000 grain weight, and yield/plant of M_3 mutants.[†]

Genotype	Panicle length (cm)	No. of filled grains/panicle	1000 grain weight (g)	Yield/plant (g)
M_3 1-2-1	34.58 ± 3.13	195.20 ± 30.38	23.44 ± 3.95	17.32 ± 7.37
M_3 1-2-2	31.12 ± 2.72	157.20 ± 32.46	23.92 ± 2.60	10.38 ± 3.74
M_3 2-1-1	30.86 ± 3.38	212.20 ± 85.76	25.53 ± 5.23	36.40 ± 23.36
M_3 36-1-1	32.76 ± 3.10	205.80 ± 69.93	22.28 ± 2.28	19.00 ± 14.71
M_3 43-1-1	33.14 ± 1.60	174.80 ± 19.74	25.24 ± 2.26	15.74 ± 2.72
M_3 43-2-1	32.04 ± 2.16	231.00 ± 132.53	31.40 ± 12.35	29.12 ± 13.53
M_3 43-2-2	28.70 ± 1.15	134.00 ± 14.63	28.43 ± 2.85	12.70 ± 0.61
Dawk Kha 50	35.60 ± 2.19	202.00 ± 26.27	21.64 ± 4.02	17.18 ± 3.34
CV (%)	7.85	34.02	21.56	57.66
LSD 5%	3.27	ns	ns	14.66

[†] mean ± SD; ns = not significant.

Table 4 Plant height, days to flowering, days to maturity, number of tillers/plant, and number of panicles/plant of M_4 mutants.[†]

Genotype	Plant height (cm)	Days to flowering	Days to maturity	No. of tillers/plant	No. of panicles/plant
M_4 1-2-1-3	116 ± 2	103 ± 1	123 ± 1	4.67 ± 1.15	4.00 ± 1.00
M_4 2-1-1-1	121 ± 8	103 ± 1	122 ± 1	10.33 ± 2.51	10.33 ± 2.51
M_4 36-1-1-1	107 ± 12	103 ± 0	123 ± 0	3.67 ± 0.58	3.17 ± 10.76
M_4 43-1-1-1	96 ± 5	103 ± 0	123 ± 0	5.94 ± 1.53	5.65 ± 1.70
M_4 43-2-1-3	110 ± 3	103 ± 1	123 ± 1	6.70 ± 1.18	6.16 ± 1.28
Dawk Kha 50	102 ± 1	108 ± 1	125 ± 1	11.00 ± 1.00	10.00 ± 0.00
Dawk Pa-Yawm	104 ± 6	103 ± 1	122 ± 1	12.00 ± 1.00	11.00 ± 1.73
Mai Tahk	125 ± 3	110 ± 0	140 ± 0	3.53 ± 0.18	3.72 ± 0.35
CV (%)	5.36	0.39	0.45	18.68	19.52
LSD 5%	10.00	1.00	1.00	2.36	2.31

[†] mean ± SD.

of filled spikelets/panicle, and yield/plant indicated significant differences among the treatment means, while panicle length and the weight of 1000 grains did not show any significant differences (Table 4 and Table 5). The M_4 43-1-1-1 and M_4 43-2-1-3 lines showed a higher grain yield (23.65 and 23.26 g/plant, respectively) and, otherwise, better performance compared with the Dawk Kha 50. The common botanical morphology of each of M_4 plants, except the agronomic traits, did not change. Dawk Kha 50 had a grain yield of 22.37 g/plant, but these three genotypes were not significantly different. Plant height, number of panicles/plant and days to flowering should be used as selection criteria in the early segregating generation, but grain yield was to be used in a later generation. However, the results were not in agreement with those of Barmana and Borahb [20], which reported that M_2 and M_3 generations could be effective for selecting the high yielding mutants in an early generation, while the M_4 generation was proper for quality traits in bread wheat. As in this study, the grain yield of M_4 lines was not influenced significantly in desirable direction, which might be due to polygenic mutations with equally distributed positive and negative effects [21].

The two M_4 lines were selected based on high grain yield and other good agronomic traits and tested to identify size of paddy rice as well as eating and cooking qualities. The length and width of the paddy rice and the brown rice of the two selected M_4 lines and the Dawk Kha 50 exhibited significant variations (Table 6). The two selected M_4 lines and the Dawk Kha 50 had the paddy rice length of 10.69, 10.54 and 10.23 mm and the paddy rice width of 2.15, 2.17 and 2.04 mm, respectively; while the values for the brown rice were 7.87, 7.87 and 7.51 mm in length and 1.90, 1.21 and 2.06 mm in width, respectively. In Fig. 1, greyed-orange group 162C was for rice husk color of Dawk Kha 50 cultivar, greyed-yellow group 162B for M_4 43-1-1-1, and greyed-yellow group 162C for M_4 43-2-1-3. Color of dehusked rice grains of Dawk Kha 50 cultivar was greyed-orange group 172C; while colors of dehusked rice grains of M_4 43-1-1-1 and M_4 43-2-1-3 were greyed-orange groups 173C and 175C, respectively. The size of paddy rice and brown rice in M_4 lines was larger than the Dawk Kha 50. Color of rice husk and dehusked rice grains of some mutants were different from the parental cultivar. A similar result was obtained from Mohapatra et al [22], who reported

Table 5 Panicle length Number of filled grains/panicle, grain weight/panicle, 1000 grain weight, and yield/plant of M_4 mutants.[†]

Genotype	Panicle length (cm)	No. of filled grains/panicle	1000 grain weight (g)	Yield/plant (g)
M_4 1-2-1-3	30.31 ± 1.48	87.88 ± 20.89	20.65 ± 0.47	10.11 ± 2.95
M_4 2-1-1-1	30.13 ± 1.00	89.93 ± 22.23	21.25 ± 0.38	17.93 ± 0.09
M_4 36-1-1-1	28.44 ± 3.19	95.12 ± 19.14	22.00 ± 0.85	16.43 ± 1.97
M_4 43-1-1-1	28.00 ± 0.11	102.25 ± 3.96	20.80 ± 1.04	23.65 ± 0.30
M_4 43-2-1-3	28.38 ± 0.69	103.40 ± 32.71	20.51 ± 1.99	23.26 ± 0.57
Dawk Kha 50	29.75 ± 2.10	87.67 ± 10.86	19.72 ± 1.24	22.37 ± 2.64
Dawk Pa-Yawm	30.03 ± 1.14	41.09 ± 12.95	17.99 ± 2.98	22.23 ± 3.14
Mai Tahk	30.07 ± 0.63	51.60 ± 21.18	19.39 ± 1.80	19.04 ± 0.79
CV (%)	5.71	23.99	7.98	10.57
LSD 5%	ns	4.61	ns	3.58

[†] mean ± SD; ns = not significant.

Table 6 Length and width of paddy and brown rice, amylose, gel consistency, alkali test, and cooked rice elongation ratio of the selected M_4 mutants and Dawk Kha 50.[†]

Genotype	Paddy rice		Brown rice		Amylose (%)	Gel	Alkali test	Elongation
	Length (mm)	Width (mm)	Length (mm)	Width (mm)	(mm)	consistency		ratio
M_4 43-1-1-1	10.69 ± 0.04	2.15 ± 0.01	7.87 ± 0.03	1.90 ± 0.00	23.58 ± 0.28	83.64 ± 0.01	6.00 ± 0.00	1.22 ± 0.01
M_4 43-2-1-3	10.54 ± 0.06	2.17 ± 0.02	7.87 ± 0.07	2.06 ± 0.02	23.89 ± 0.17	84.11 ± 0.01	7.00 ± 0.00	1.21 ± 0.00
Dawk Kha 50	10.23 ± 0.16	2.04 ± 0.06	7.51 ± 0.20	1.78 ± 0.10	22.21 ± 0.44	84.50 ± 0.00	5.00 ± 0.00	1.22 ± 0.00
CV (%)	0.93	1.58	1.45	3.08	1.35	0.09	0.00	0.34
LSD 5%	0.20	0.07	0.24	0.18	1.00	0.24	ns	ns

[†] mean ± SD; ns = not significant.

that EMS induced mutants of grain color of upland rice cultivar Nagina22.

The amylose contents and gel consistencies of the two selected M_4 lines and Dawk Kha 50 were significantly different, but the alkali test and elongation ratio did exhibit significant variations (Table 6). The two selected M_4 lines and Dawk Kha 50 had 23.58, 23.89 and 22.21% of amylose, respectively. The gel consistencies of the two selected M_4 lines and Dawk Kha 50 were 83.64, 84.11 and 84.50 mm, respectively. In mutants and parental cultivar, the amylose contents and gel consistencies ranged within 22.21–23.89% and 83.64–84.50 mm, respectively, indicating that the cooking and eating qualities of these alternatives did not differ. With 20–25% amylose, rice is soft and not sticky [23]. The cooled gel length of 61–100 mm indicated that the cooked rice was tender, which is widely preferred by consumers [24].

The selected mutants should be carried over to later generations (M_6–M_7) using single seed descent method to produce homozygous plants or genetic stability [25]. The number of filled grains/panicle in M_2–M_4 lines was better than in Dawk Kha 50. Thus, this trait can be used as one of the selection criteria in an early generation for yield improvement. There must be the accuracy in selection for selective mutation breeding to be effective [21]. In certain situations, hybridization between various progenies'

selections led to a clearly higher yield, which could be a result of the impacts of heterosis for mutant genes [18]. Nakwilai et al [26] reported that pure-line selection in four generations could be used to improve the homogeneity, grain yield and cooking quality of japonica rice cv. DOA2. All selected mutants, M_2, M_3 and M_4 were not tested for the detail molecular genetic variations. In future research, phenotypic variation could be described using genetic analyses, such as genetic linkage maps, whole-genome sequencing, and RNA sequencing, giving a complete image of gene expression differences and single nucleotide polymorphisms contrasted to wild type, based on the source of rice genome. Furthermore, the high rice grain quality genes can be introgressed to rice cultivars having the low grain qualities using a marker-assisted pseudo-backcross method [27].

CONCLUSION

The results of the present study indicated that inducing mutations can be used to improve number of filled grains/panicle, size of paddy and brown color of the wild type. The preliminary yield evaluation showed that the selected mutant lines, namely M_4 43-1-1-1 and M_4 43-2-1-3, were high yielding, and cooked mutant rice had good tenderness. The desirable upland red rice lines could be chosen from each segregating mutants of advance generation. The selected mutant lines

could be used as germplasm and breeding materials in future upland red rice breeding programs.

Appendix A. Supplementary data

Supplementary data associated with this article can be found at http://dx.doi.org/10.2306/scienceasia1513-1874. 2023.040.

REFERENCES

1. FAO (2021) *The State of Food Security and Nutrition in the World*, Rome, FAO.

2. Saito K, Asai H, Zhao D, Laborte AG, Grenier C (2018) Progress in varietal improvement for increasing upland rice productivity in the tropics. *Plant Prod Sci* **21**, 145–158.

3. Jati IRAP, Darmoatmodjo LMYD, Suseno TIP, Ristiarini S, Wibowo C (2022) Effect of processing on bioactive compounds, antioxidant activity, physicochemical, and sensory properties of orange sweet potato, red rice, and their application for flake products. *Plants* **11**, 440.

4. Shahidi F, Yeo J (2018) Bioactivities of phenolics by focusing on suppression of chronic diseases: A review. *Int J Mol Sci* **19**, 1573.

5. Awan FS, Sadia B, Altaf J, Habib M, Hameed K, Hussain S (2021) Genetic variability through induced mutation. In: Maia RT, Campos MDA (eds) *Genetic Variation*, Intechopen.

6. Chaudhary J, Deshmukh R, Sonah H (2019) Mutagenesis approaches and their role in crop improvement. *Plants* **8**, 467.

7. Oladosu Y, Rafii MY, Abdullah N, Hussin G, Ramli A, Rahim HA, Miah G, Usman M (2016) Principle and application of plant mutagenesis in crop improvement: A review. *Biotechnol Biotechnol Equip* **30**, 1–16.

8. Sikder S, Biswas P, Hazra P, Akhtar S, Chattopadhyay A, Badigannavar1 AM, D'Souza1 SF (2013) Induction of mutation in tomato (*Solanum lycopersicum* L.) by gamma irradiation and EMS. *Indian J Genet* **73**, 392–399.

9. Takagi H, Tamiru M, Abe A, Yoshida K, Uemura A, Yaegashi H, Obara T, Oikawa K, et al (2015) MutMap accelerates breeding of a salt-tolerant rice cultivar. *Nat Biotechnol* **33**, 445–449.

10. Viana VE, Pegoraro C, Busanello C, Oliveira ACD (2019) mutagenesis in rice: the basis for breeding a new super plant. *Front Plant Sci* **10**, 1326.

11. Chen T, Huang L, Wang M, Huang Y, Zeng R, Wang X, Wang L, Wan S, et al (2020) Ethyl methyl sulfonate-induced mutagenesis and its eects on peanut agronomic, yield and quality traits. *Agronomy* **10**, 655.

12. Amosova AV, Zoshchuk1 SA, Volovik VT, Shirokova AV, Horuzhiy NE, Mozgova GV, Yurkevich OY, Artyukhova MA, et al (2019) Phenotypic, biochemical and genomic variability in generations of the rapeseed (*Brassica napus* L.) mutant lines obtained via chemical mutagenesis. *PLoS One* **14**, e0221699.

13. Wu Z, Liu Z, Chang S, Zhao Y (2020) An EMS mutant library for carrot and genetic analysis of some mutants. *Breed Sci* **70**, 540–546.

14. Tang S, Liu D, Lu S, Yu L, Li Y, Lin S, Li L, Du Z, et al (2020) Development and screening of EMS mutants with altered seed oil content or fatty acid composition in *Brassica napus*. *Plant J* **104**, 1410–1422.

15. Awais A, Nualsri C, Soonsuwon W (2019) Induced mutagenesis for creating variability in Thailand's upland rice (cv. Dawk Pa-yawm and Dawk Kha 50) using ethyl methane sulphonate (EMS). *Sarhad J Agric* **35**, 293–301.

16. Mendiburu FD (2020) *Agricolae: Statistical Procedures for Agricultural Research*. Available at: https://cran. r-project.org/web/packages/agricolae/index.html.

17. Pavadai P, Girija M, Dhanavel D (2010) Effect of gamma rays on some yield parameters and protein content of soybean in M_2, M_3 and M_4 generation. *J Exp Sci* **1**, 8–11.

18. Poehman JM (1979) *Breeding Field Crops*, AVI Publ. Com. Inc., Connecticut, USA.

19. Chakravarti SK, Kumar H, Lal JP, Singh S (2014) Induced genetic variability for yield and yield traits in aromatic rice (*Oryza sativa* L.). *Afr J Agric Res* **9**, 2345–2357.

20. Barmana D, Borahb SP (2012) Effect of selection response on F_3 and F_4 generation for yield and yield component characters in mutant rice strain (*Oryza sativa* L.). *APCBEE Procedia* **4**, 183–187.

21. Balkan A (2018) Genetic variability, heritability and genetic advance for yield and quality traits in $M_{2–4}$ generations of bread wheat (*Triticum aestivum* L.) genotypes. *Turkish J Field Crop* **23**, 173–179.

22. Mohapatra T, Robin S, Sarkala N, Sheshashayee M, Singh AK, Singh K, Singh NK, Mithra SVA, et al (2014) EMS induced mutants of upland rice cultivar Nagina22: Generation and characterization. *Proc Indian National Sci Acad* **80**, 163–172.

23. Khammari M, Khani MAM, Khammari DA, Nia MOR (2016) The eating and cooking qualities of rice: A review. *Int J Sci Res Sci Technol* **2**, 134–139.

24. Bhat FM, Riar CS (2016) Physicochemical, cooking, and textural characteristics of grains of different rice (*Oryza sativa* L.) cultivars of temperate region of India and their interrelationships. *J Texture Stud* **48**, 160–170.

25. Parry MAJ, Madgwick PJ, Bayon C, Tearall K, Hernandez-Lopez A, Baudo M, Rakszegi M, Hamada W, et al (2009) Mutation discovery for crop improvement. *J Exp Bot* **60**, 2817–2825.

26. Nakwilai P, Cheabu S, Narumon P, Saensuk C, Arikit S, Malumpong C (2020) Evaluation of japonica rice (*Oryza sativa* L.) varieties and their improvement in terms of stability, yield and cooking quality by pure-line selection in Thailand. *ScienceAsia* **46**, 157–168.

27. Cho KS, Kongsil P, Wangsawang T, Sreewongchai T (2020) Marker-assisted pseudo-backcross breeding for improvement of amylose content and aroma in Myanmar rice cultivar Sinthukha. *ScienceAsia* **46**, 539–547.
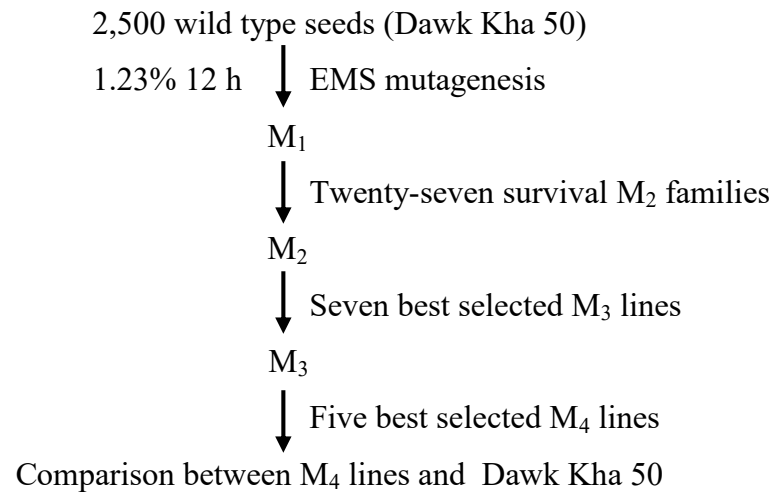
Appendix A. Supplementary data

<div align="center">

2,500 wild type seeds (Dawk Kha 50)

1.23% 12 h ↓ EMS mutagenesis

M_1

↓ Twenty-seven survival M_2 families

M_2

↓ Seven best selected M_3 lines

M_3

↓ Five best selected M_4 lines

Comparison between M_4 lines and Dawk Kha 50

</div>

Fig. S1 Illustration of EMS induced mutagenesis.