Original Article

Lethal Dose 50 and the first generation mutant of upland purple rice cultivar Med Fai 62

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Abstract

Inducing mutations is a breeding tool that creates genetic variability. In this study of inducing mutations in the Indica upland purple rice cultivar Med Fai 62 with mutagenic Ethyl Methane Sulfonate (EMS), a completely randomized experimental design was used with four treatments (0, 0.5, 1.0, and 1.5%) and five replications to find an appropriate EMS dose. Analysis of variance of treatments for germination rate showed highly significant differences ($P < 0.01$). Trend comparisons, and seeking 50% germination (Lethal Dose 50), showed that a 0.2% concentration of EMS was suitable for Med Fai 62 cultivar. Thus, mutations were induced in 5,000 seeds by infusion of 0.2% EMS. After planting for 15 days, 43.74% germination was found. The agricultural traits of the mutated plants were better in several cases than those of Med Fai 62 cultivar, for example in days to flowering, days to maturity, flag leaf length and width,

plant height, and 1,000 grain weight. These traits could be useful for the future upland rice breeding programs.

Keywords: Ethyl Methane Sulfonate, Lethal Dose, Mutant, Upland Rice

1. Introduction

Upland rice is an important crop in Asia for food security and used as a staple food. Furthermore, Upland rice is most suited for small plots of farmland. Most farmers produce upland rice for local consumption, while the rest of upland rice production is commercially distributed. A rice field can provide great savings in food costs as well as income for farmer households (Nokkoul *et al*., 2017).

Upland rice is popularly grown in rubber or oil palm plantations that are 1-3 years old. It is an alternative plant for food production in conditions with a limited water supply and cropping systems (Gupta and O'Toole, 1986). It has been reported photoperiod-sensitive upland rice varieties can be grown only in the rainy season (Watcharin *et al*., 2020). The local upland rice varieties are able to adapt to the environment, as their root system adapts well to a wide range of culture conditions. They give less broken seeds and are easy to de-hull (Nokkoul *et al.*, 2017). Therefore, native rice cultivars are important as genetic sources to a breeding program.

Mutagenic induction is a breeding technique that can quickly generate genetic variability for producing new genetic traits in plants (Poehlman, 1979), and Ethyl Methane Sulfonate (EMS) is an example of a mutagenic substance. Mutant varieties have been developed of rice and other crops, such as sesame, cucumber, and beans (Rao, 1977; Chen *et al*., 2018; Awais, Nualsri, & Soonsuwon, 2019; Shahwar, Khan, & Ansari, 2020). Med Fai 62, indigenous Indica rice cultivar, has been certified by the

Department of Agriculture in Thailand. It is a purple rice cultivar that can inhibit or prevent the spread of infectious bacteria as an antioxidant, and also inhibits the metastasis of cancer cells (Nakhon Si Thammarat Rice Research Center, 2019).

The objectives of this study were to find a near optimal concentration of EMS in seeds for inducing mutations in Med Fai 62 cultivar, and to study the agricultural traits in mutated seeds. These mutant traits will bring alternatives to the original traits for use in future upland rice crossing programs.

2. Materials and Methods

Plant Material and Ethyl Methane Sulfonate Treatments

The experiment was laid out at a laboratory of Agricultural Innovation and Management Division (Plant Science), Faculty of Natural Resources (FNR), Prince of Songkla University (PSU), Hat Yai district, Songkhla province, Thailand. The seeds of Med Fai 62 cultivar were supplied by Nakhon Si Thammarat Rice Research Center, Rice Department, Ministry of Agriculture and Cooperatives of Thailand. The seeds of Med Fai 62 cultivar were induced with EMS. The experimental design was a completely randomized design with 5 repetitions and 4 treatments (0 or control, 0.5, 1.0 and 1.5% EMS v/v).

For control 25 seeds of each replication were placed in a 125 ml glass flask, 25 ml of pure water (distilled water) was added, and the seeds were soaked in water at room temperature (25 °C) with the bottles on a shaker set at 150 rpm for 32 h.

For EMS-induced mutagenesis, 100 seeds of each replication were put in a 125 ml glass flask, 5 ml distilled water was added, and the seeds soaked in water at room temperature (25°C). For aeration, the flasks were placed on a shaker at 150 rpm for 20

h, then 0.5, 1.0 or 1.5% EMS was added, with distilled water topping up the volume to 25 ml, and soaking was on a shaker at 150 rpm and room temperature for 12 h. After this, the seeds were washed with 100 ml distilled water 5 times for 4 min and then washed with 200 ml distilled water 4 times, each time for 15 min, and then the seeds in each EMS treatment were washed with tap water for 4 h. The seeds of each treatment were transplanted into seedling trays. The trays had their bottom holes covered with peat moss and 1 seed was used per well. These trays were placed in the field at Agricultural Innovation and Management Division (Plant Science), FNR, PSU.

Lethal Dose Determination

At 15 days after germination each treatment was assessed for germination percentage. LD was analyzed based on the completely randomized design and a trend comparison, by studying the response to EMS regarding germination of Med Fai 62 cultivar, whether there was any response such as a straight line or a curve (Gomez & Gomez, 1984). Then the concentration of EMS was calculated for a 50% survival rate of Med Fai 62 rice (LD_{50}). The mean values of each treatment were compared using the Least Significant Difference (LSD) method at 95% and 99% confidence levels. Statistical analysis was conducted using the R program (Mendiburu & Simon, 2007).

M_1 Generation

The experiment was laid out at Agricultural Innovation and Management Division (Plant Science), FNR, PSU. Five thousand Med Fai 62 rice seeds were induced with EMS. The concentrations used were chosen based on the experiment for lethal dose determination. This gave a 50% survival rate to Med Fai 62 cultivar, when induced as described above. The induced seeds were planted in the experimental field. Each experimental row was 4 m long with 0.30 m spacing between the rows, and 0.10 m

between the plants in a row. The common Med Fai 62 rice seeds were grown in 4 rows for comparison of growth traits between wild-type and mutant plants.

Water was given 2 times a day (morning-evening) for 15 min each time with an automatic watering system. Insect control was done through the application of 40 ml chlorpyrifos 40% w/v EC per 20 L of water. Weeding was done using a hoe for 3 weeks and 4 months after planting. 15-15-15 fertilizer was applied at the rate of 156 kg/ha when the rice plant was 30 days old. Then urea (46-0-0), 219 kg/ha, was applied at 60 days old, and 0-0-60 fertilizer, 250 kg/ha was applied at booting stage.

M_1 Data Collection

The records of 10 normal plants and the mutants in M_1 generation (all plants yielding) were days to flowering (day), days to maturity (day), flag leaf width and length (cm), plant height (cm), number of tillers/plant, number of panicles/plant, panicle length (cm), number of grains/panicle (cm), grain weight/panicle (g), 1,000 grain weight (g) and yield/plant (g/plant). Color of husk and dehusked grain were determined by R.H.S. colour chart of the Royal Horticultural Society.

Statistical Analysis in M_1 Generation

Analysis of agricultural traits of the mutants in M_1 generation by analyzing mean, minimum and maximum values used comparisons to the original cultivar (Dowdy, Weardon, & Chilko, 2003)

3. Results and Discussion

Analysis of variance and LD_{50}

Percentage of germination showed highly significant variations as seen in Table 1. The results of trend comparison indicate that the first, second and third order terms in

polynomials were highly significant. Thus, the relationship between percentage of germination and the concentration of EMS exhibited a cubic response curve.

The highest 86.6% germination of the Med Fai 62 cultivar was found with the control treatment. At 0.5% EMS dose the percentage of germination was 16.2%. The percentage of germination of Med Fai 62 rice cultivar was the lowest at 0% when the concentration of EMS was 1.0 or 1.5% (Table 2). In contrast, Mohapatra *et al*. (2014) reported that 72-92% germination of Upland Rice Cultivar Nagina22 was found at 0.6-1.0% EMS doses. Increasing the concentration of EMS decreased the germination rate. This result is consistent with Awais, Nualsri, and Soonsuwon (2019) in a study of mutagenic induction of variability in Thai upland rice, as the root and shoot lengths of seedlings decreased with EMS concentration.

For 50% germination of the Med Fai 62 cultivar (i.e., LD_{50}) the concentration of EMS was estimated as 0.2% (Figure 1). This was considered a near optimal choice of EMS dose.

Agricultural traits of the mutants

For Med Fai 62 cultivar 5,000 seeds were soaked with 0.2% EMS, and at 15 days after planting the number of germinated seeds was only 2,187, giving 43.74% germination percentage. Med Fai 62 mutants had 68 seedlings surviving (3.11%) and only 36 seedlings gave yielding.

The agricultural traits of the Med Fai 62 mutants in M_1 generation are shown in Table 3. Several agricultural traits of the Med Fai 62 mutants in M_1 generation were better than those of the Med Fai 62 wild type.

The days to flowering of M_1 36 was the shortest 114 days, followed by M_1 2, 4, 14, 21, 34 and 35 that took 115-117 days. The days to flowering of the original Med Fai

62 cultivar was 118 days. Mohapatra *et al*. (2014) reported that some upland rice mutants showed early flowering at 5-11 days. The days to maturity of M_1 mutants 4, 14, 21, 30, 33 and 36 were the shortest 138 days, followed by M_1 2, 3, 5, 9,11, 12, 18, 20, 22, 23, 27, 32 and 34 that took 139-141 days. The days to maturity of the original Med Fai 62 cultivar was 143 days.

The flag leaf length of the M_1 18 was the largest at 51.00 cm, followed by M_1 3, 4, 9, 11, 12, and 14 that gave 48.60- 44.40 cm. The flag leaf width of the M_1 2, 4, 12, 14 and 18 was the widest at 1.80 cm. The flag leaf width of Med Fai 62 cultivar was 1.75 cm. The lowest plant height of M_1 33 mutants was 92.00 cm, followed by M_1 2- 5, 9, 11, 14, 18, 20-23, 30, 32 and 34-36 that gave 99.00-121.00 cm. The plant height of Med Fai 62 cultivar was 126.50 cm. Mohapatra *et al*. (2014) and Awais, Nualsri, and Soonsuwon (2019) reported that some upland rice mutants had reduced plant height compared to the wild type.

The panicle lengths of M_1 4 and 21 were the largest at 35.20 cm. The panicle length of Med Fai 62 cultivar was 26.00 cm. The 1,000 grains weight of M_1 32 was the largest 18.30 g, followed by M_1 3-5, 9, 11, 12, 14, 20, 27, 30, 33 and 36 that gave 17.76-14.34 g. The 1,000 grains weight of Med Fai 62 cultivar was 12.88 g. In contrast, Awais, Nualsri, and Soonsuwon (2019) found that the seed size of mutants (27-29 g) was not large when compared to parental upland white and red rice varieties (35-37 g) because of the different of genetic basis of seed sizes of purple, white and red rice.

Color of rice husk of mutants such as M_1 14 and 32 were greyed-yellow group 161A-162C and color of rice husk of Med Fai 62 cultivar was greyed-yellow group 162A (Figure 2). Color of dehusked rice grains of all mutants was black group 202A, and color of dehusked rice grains of Med Fai 62 cultivar was brown group 200A.

Similar to this study, Mohapatra *et al*. (2014) reported that grain colour of original upland rice cultivar was also changed.

4. Conclusions

A 0.2% concentration of mutagenic EMS for treating Med Fai 62 rice cultivar decreased the percentage of germination to 50%. Assessment of agricultural traits of the mutants in the first phase showed that the agricultural traits of the mutant plants had more outstanding traits than the original Med Fai 62 cultivar, in days to flowering, days to maturity, flag leaf length and width, plant height, and 1,000 grain weight. The selected mutant lines of Med Fai 62 in M_1 generation with good agricultural traits were M_1 4, 14, 32, 33 and 36. Such mutants will be useful for further upland rice breeding programs.

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Figure

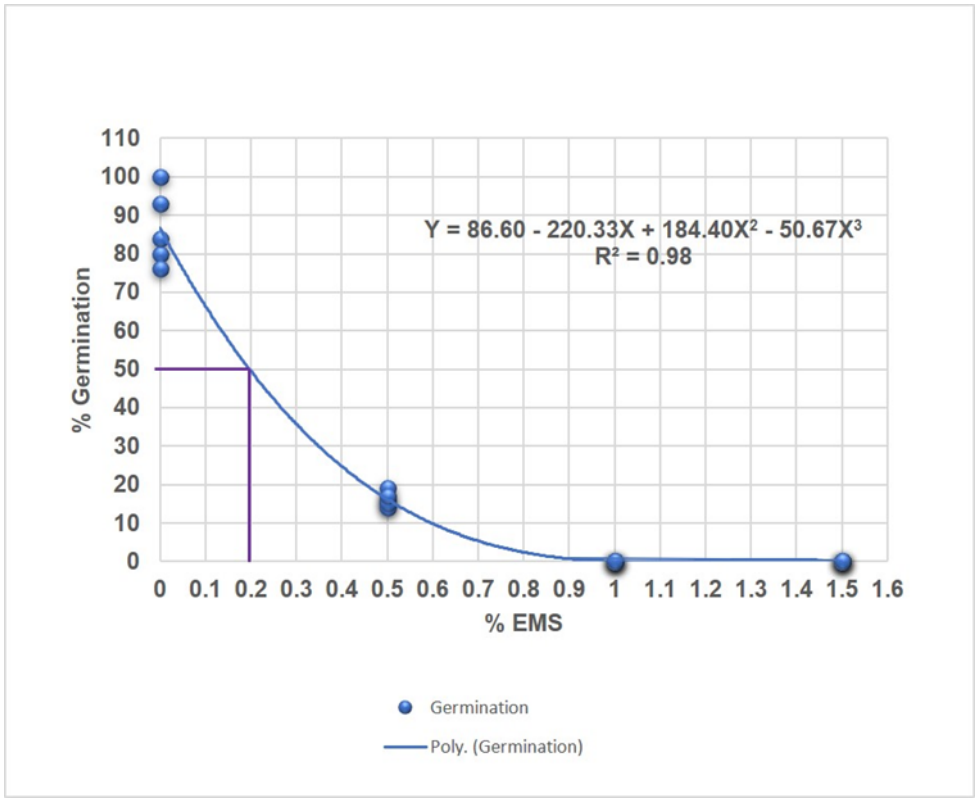

Figure 1 Effects of EMS concentration on germination rate of Med Fai 62 cultivar

Poly. = Polynomial fit third degree

Figure 2 Color of rice husk and dehusked rice grains of Med Fai 62 cultivar and mutants

Table 1 Analysis of variance for polynomial terms in model of germination rate of Med Fai 62 cultivar

Source	df	Mean Square
Treatment	3	8,533**
Linear	1	19,044**
Quadratic	1	6,195**
Cubic	1	361**
Error	16	25

*, ** significant at 5% and 1% level of probability, respectively.

Table 2 Comparing mean germination rates of Med Fai 62 cultivar by treatment group

Genotype	Concentration of EMS (%)	Germination of seeds (%)
Med Fai 62	0.00	86.60
	0.50	16.20
	1.00	0.00
	1.50	0.00
LSD 0.01		9.22
LSD 0.05		6.69
C.V. (%)		19.14

Table 3 Means of agricultural traits of Med Fai 62 cultivar and mutants in M_1 generation

Genotype/ id Plant	DF	DM	FLL (cm)	FLW (cm)	PH (cm)	NT/P	NPa/P	PL (cm)	NG/Pa	GW/Pa	1,000GW (g)	Y/P (g)
Med Fai 62±SD	119 ±1.50	144 ±1.50	37.53 ±2.11	1.75 ±0.06	126.50 ±6.03	6.75 ±1.71	5 ±1.63	26.00 ±3.39	239.80 ±73.12	3.17 ±1.30	12.88 ±1.58	15.79 ±7.28
M_1 2	117	139	36.20	**1.80**	113.00	3.00	2	24.10	15.00	0.10	6.67	0.20
M_1 3	118	141	**48.60**	1.70	116.00	3.00	3	23.10	53.00	0.76	14.34	2.28
M_1 4	116	**138**	44.40	**1.80**	101.00	4.00	3	**35.20**	42.00	0.69	16.43	2.07
M_1 5	118	140	30.20	1.60	99.00	4.00	4	23.40	47.00	0.71	15.11	2.84
M_1 9	120	141	39.00	1.60	99.10	4.00	3	24.10	53.00	0.89	16.79	2.67
M_1 11	120	140	47.60	1.60	121.00	4.00	3	24.10	47.00	0.72	15.32	2.16
M_1 12	119	139	**48.60**	**1.80**	126.00	4.00	4	23.10	43.00	0.72	16.74	2.88
M_1 14	116	**138**	**48.60**	**1.80**	109.00	4.00	4	23.40	49.00	0.87	17.76	3.48
M_1 18	120	140	51.00	1.50	111.00	2.00	2	23.10	15.00	0.06	4.00	0.12
M_1 20	120	141	30.50	1.60	121.00	3.00	3	23.10	48.00	0.85	17.71	2.55
M_1 21	115	**138**	32.30	1.50	118.00	4.00	3	**35.20**	39.00	0.44	11.28	1.32
M_1 22	120	140	32.40	1.60	111.00	5.00	4	24.10	28.00	0.31	11.07	1.24
M_1 23	120	141	29.00	1.60	108.00	5.00	4	23.10	39.00	0.41	10.51	1.64
M_1 27	119	141	33.40	1.60	126.00	3.00	2	23.40	48.00	0.79	16.46	1.58
M_1 30	118	**138**	28.10	1.60	112.00	3.00	3	25.60	57.00	1.00	17.54	3.00
M_1 32	120	141	27.60	1.60	121.00	3.00	2	23.10	53.00	0.97	**18.30**	1.94
M_1 33	119	**138**	32.50	1.50	**92.00**	3.00	3	23.40	42.00	0.72	17.14	2.16
M_1 34	116	139	32.30	1.60	121.00	2.00	2	20.70	47.00	0.49	10.43	0.98
M_1 35	115	**138**	33.40	1.60	95.00	2.00	2	19.00	23.00	0.17	7.39	0.34
M_1 36	**114**	**138**	30.50	1.60	101.30	4.00	3	24.10	41.00	0.69	16.83	2.07
Mean M_1 ±SD	118 ±1.97	139 ±1.24	36.81 ±7.89	1.63 ±0.10	111.1 ±10.10	3.45 ±0.86	3 ±0.74	24.42 ±3.81	41.42 ±11.86	0.62 ±0.28	13.90 ±4.15	1.88 ±0.93

DF = Days to flowering, DM = Days to maturity, FLL = Flag leaf length, FLW = Flag leaf width, PH = Plant height, NT/P = Number of tillers/plant, NPa/P = Number of panicles/plant, PaL = Panicle length, NG/Pa = Number of grains/panicle, GW/Pa = Grain weight/panicle, 1,000GW = 1,000 grains weight and Y/P = Yield/plant