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Ethyl Methanesulfonate (EMS)-Mediated Mutagenesis of Cucumber (*Cucumis sativus* L.)

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Abstract

Ethyl methanesulfonate (EMS) is a stable and effective chemical mutagen. In this study, cucumber (*Cucumis sativus* L. cv. "Shannong No. 5") seeds were treated by 1% EMS for 12 h, 24 h and 48 h to optimize EMS mutagenesis and determined median lethal dose of EMS (1% EMS and 24 h) for "Shannong No. 5". After treated by 1% EMS for 24 h, 541 M1 plants were grown in greenhouse for phenotype investigation. The fertility of M1 cucumbers was very low, and only 79 lines produced seeds after self crossing. 60 independent M2 families comprising 600 M2 plants were investigated for phenotypic alteration, and 11 individual mutant lines were isolated into six groups: *short-fruit* mutants, *long-fruit* mutants, *small-flower* mutants, *big-flower* mutants, *opposite-tendril* mutants and *clustered-leaf* mutants. The mutation frequency was 18.3%. Two selected representatives, *short-fruit* mutants and *clustered-leaf* mutants, showed 1:3 of segregation ratio in M2 populations. This ratio is consistent with classic Mendelian model, indicating that the two kinds of mutants may be controlled by a single recessive gene, respectively. *Long-fruit* phenotype was stably inherited and no segregation was observed in M3 generation, indicating that this mutant line may be homozygous.

Keywords

Cucumber, Ethyl Methanesulfonate, Mutagenesis

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1. Introduction

In plant breeding, a great challenge is the collection or development of a large amount of germplasm resources. To this end, several strategies, such as T-DNA or transposon insertional mutagenesis [1]-[3] chemically-induced mutagenesis [4] [5], have been used to develop different germplasms. However, the mutational spectrum of insertional mutagenesis with effect on gene function is mostly limited to gene knock-out disruptions, which often resulted in failure of functional dissection of mutants when mutations happen to lethal or highly pleiotropic genes [6]. Furthermore, the size of saturated populations is extremely large because each line carries only a rather small number of mutations [7].

Compared with insertional mutation, chemically-induced mutation has been shown some advantages such as high efficiency because each individual line can bear single point missense and nonsense substitutions in hundreds of genes [8]. Therefore, an allelic series of induced mutations with different effects on gene function can be easily isolated by screening a relatively small population of mutated plants [7]. Among the chemical mutagens, ethyl methanesulfonate (EMS) is considered as an effective one because it can from adducts with nucleotides efficiently, resulting in mispairing among these nucleotides with their complementary bases and thus introducing base changes after replication [5].

Cucumber is one of the most important horticultural crops and is now grown as either a fresh or processed vegetable in the world. Its fruits are rich sources of high quality protein and vitamins which provide an excellent supplement to the lower quality cereal or root and tuber protein consumed in much of the world [9]. Cucumber fruits vary markedly in size, shape and color, and thus always attract attentions from cucumber breeders. However, germplasm resources are still the big bottleneck for the present cucumber breeding. Here we determined the median lethal dose of EMS for cucumber cultivar "Shannong No. 5" and reported cucumber mutants of fruit, flower, tendril and plant size induced by EMS.

2. Materials and Methods

2.1. Plant Materials

Cucumber (*Cucumis sativas* L. cv. "Shannong No. 5") seeds were kindly provided by Dr. Chenxing Cao in Shandong Agricultural University, China, and were used in this research.

2.2. Determination of Median Lethal Dose

One hundred of cucumber seeds were soaked in 20 ml of distilled water at low speed shaker for 30 min, and then EMS (Sigma, USA) was added to the distilled water at the final concentration of 1% (v/v). These seeds were treated in 1% EMS solution for 12 h (EMS-1), 24 h (EMS-2) and 48 h (EMS-3) at low speed shaker, respectively. The treated seeds were incubated at 28˚C for 15 h after being washed with 1 M $Na_2S_2O_3$ solution, 100 mM $Na_2S_2O_3$ solution and distilled water, respectively. Seeds not treated by EMS were incubated as control under the same conditions as EMS-treated seeds. The lethal dose (LD) was calculated as follow:

$$LD\% = (1 - \text{Germination rates of treated seeds/Germination rates of Control}) \times 100$$

2.3. EMS-Mediated Mutagenesis

Mutant populations were constructed based on the work flow in **Figure 1**. In brief, wild type (WT) seeds (M0) were treated with 1% EMS solution for 24 h. The treated seeds were planted in greenhouse of Shandong Agricultural University, China, according to randomized block design. Each M1 plant was harvested separately and the seeds were sown in the next season in the greenhouse to grow M2 generation in a randomized block design followed by M3 generation. Mutants were detected by observing the plants through the whole growth stage in all generations.

2.4. Statistical Analysis

The results were subjected to one-way analyses of variance (ANOVA) and LSD test with SAS software (Statistica version 6.1, StatSoft, St. Tulsa, OK, USA) and presented as means ± standard error (SE) of twenty replicates. Different letters indicate a significant difference from control at 0.01 probability level.

3. Results and Discussion

Median lethal dose (LD50) is a critical parameter for chemically induced mutagenesis. This value is determined by both mutagen concentration and treatment time and varies in different species and even different cultivars [10]-[13]. Thus we tried to find LD50 of EMS for "Shannong No. 5" at first. With the increase of treatment time by 1% EMS, the number of survival seeds was decreased (**Table 1**). When the seeds were treated with 1% EMS for 24 h, LD value was 54%, which is very close to LD50. Therefore, the combination of 1% EMS and 24 h was used for subsequent mutagenesis of cucumber.

After induction by 1% EMS for 24 h, the treated seeds (M1) were planted in the greenhouse. Only 79 out of 541 M1 plants produced M2 seeds. Fertility rate of M1 plants was 14.6%. Low fertility is the common phenomenon observed in mutant plants by physical and chemical mutagenesis possibly due to severe damage of mutagens to plant genetic materials [5] [14]. The mutation frequency is one of the most dependable parameters for evaluating the genetic effects of mutagenic treatments [15] [16]. It was estimated by dividing the number of segregating M1 plant progeny with the determinate character by the total number of progeny. For the material grown in bulk, the mutant frequency was estimated by dividing the total number of mutants confirmed by the total number of M2 plants in the bulk population studied [5]. In this study, sixty lines (M2) from M1 cucumbers were raised for further investigation of phenotypes and calculation of mutation frequency. Eleven out of the sixty lines showed mutations including *short-fruit* mutants, *long-fruit* mutants, *small-flower* mutants, *big-flower* mutants, *opposite-tendril* mutants and *clustered-leaf* mutants compared with wild type plants (**Figure 2**). The mutation frequency was 18.3%. The main reason for this low mutation frequency is that most of the mutants bearing multi-mutational events may be lethal in the first generation [16]. The *short-fruit* mutants and *clustered-leaf* mutants showed 1:3 of segregation ratio in M2 populations (**Table 2** and **Table 3**). This ratio is consistent with classic Mendelian model, indicating that these mutant phenotypes may be controlled by a single recessive gene, respectively. Long-fruit phenotype was inherited stably and no segregation was observed in M3 generation (**Figure 3**). This evidence suggests that this mutant line may be homozygous at M3 generation.

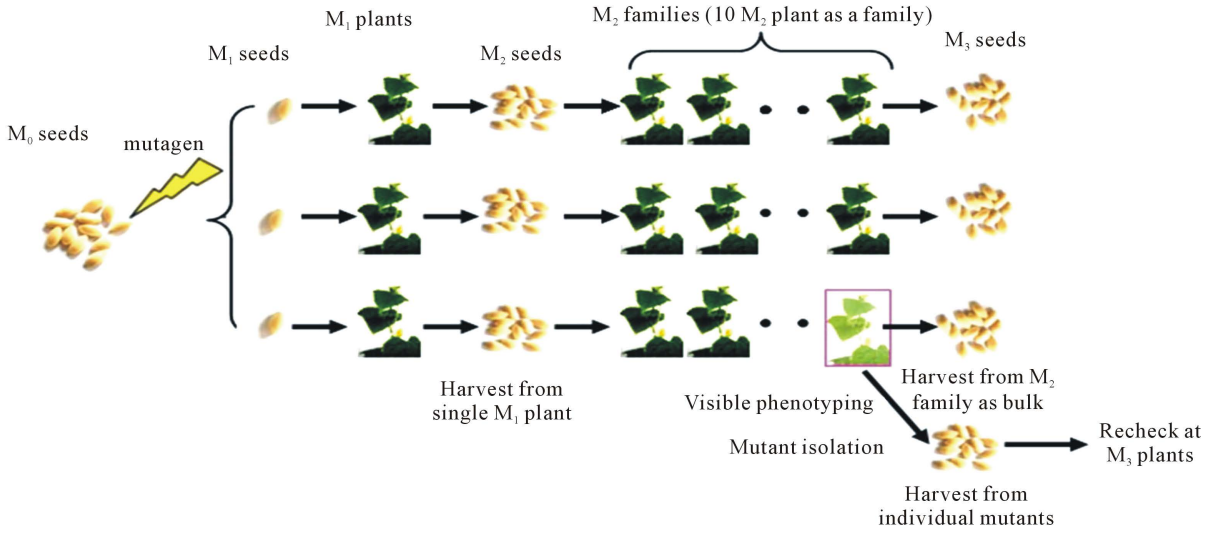

Figure 1. Flow chart for the construction of mutagenized populations.

Table 1. Effects of EMS treatments on the rate of survival in M1 plants and lethal dose.

Treatment	Seed number in M1	Survival number of M1 seeds	Germination rate of M1 seeds (%)	Lethal dose (LD%)
Control	100	100	100	0
EMS-1	100	100	98	2
EMS-2	100	46	46	54
EMS-3	100	2	2	98

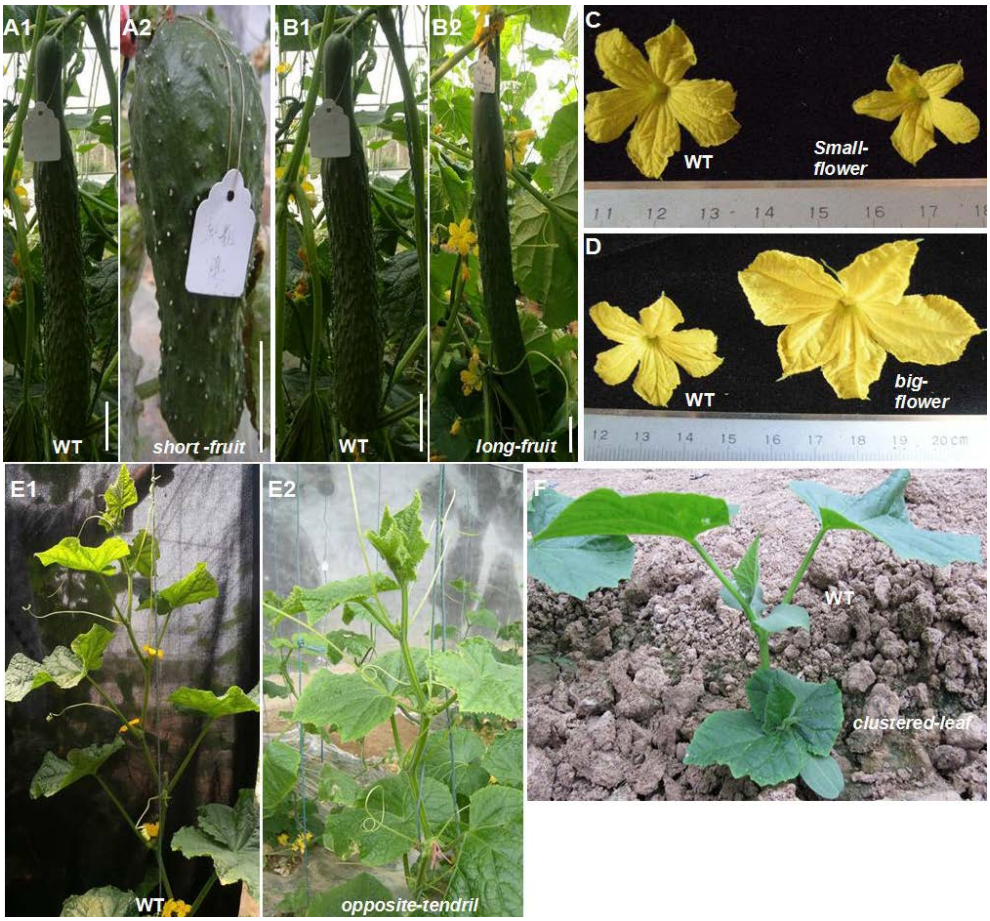

Figure 2. Mutant lines of cucumber by EMS observed at M2 generation. Wide type (WT) and *short-fruit* (A1 and A2); wild type (WT) and *long-fruit* (B1 and B2); wild type (WT) and *small-flower* (C); wild type (WT) and *big-flower* (D); wild type (WT) and *opposite-tendril* (E1 and E2); and wild type (WT) and *clustered-leaf* (F). Scale bar represents 3 cm.

Table 2. χ^2 of cucumber *short-fruit* mutant line in M2 population.

Item	f_o	f_e	$(f_o - f_e)^2/f_e$
Wilt type	7	8.25	0.19
Mutant	4	2.75	0.57
∑	11	11	$\chi^2 = 0.76$

Note: $\chi^2_{0.05}(1) = 3.84$, $\chi^2 < \chi^2_{0.05}(1)$. The segregation ratio of *short-fruit* mutant line is consistent with classic Mendelian model.

Table 3. χ^2 of cucumber *clustered-leaf* mutant line in M2 population.

Item	f_o	f_e	$(f_o - f_e)^2/f_e$
Wilt type	24	21	0.43
Mutant	4	7	1.29
∑	28	28	$\chi^2 = 1.72$

Note: $\chi^2_{0.05}(1) = 3.84$, $\chi^2 < \chi^2_{0.05}(1)$. The segregation ratio of *clustered-leaf* mutant line is consistent with classic Mendelian model.

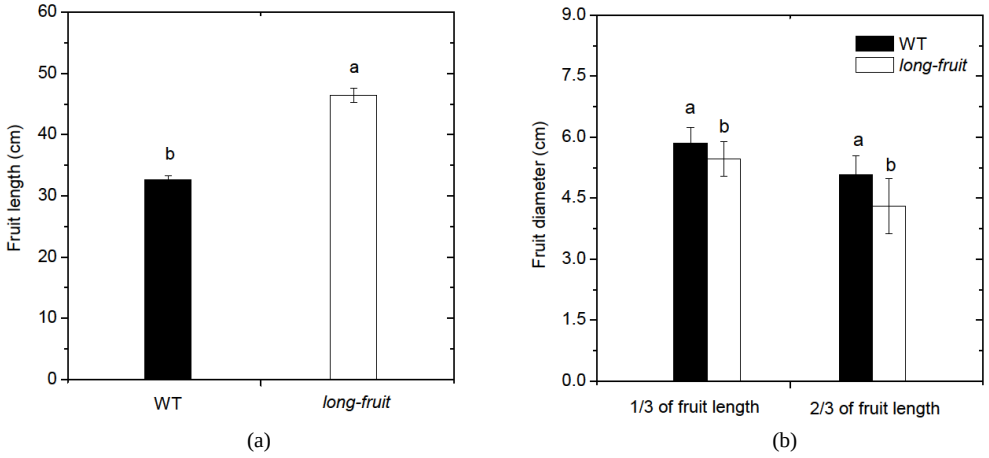

Figure 3. Fruit length (a) and diameter (b) of wild-type cucumbers and M3 *long-fruit* mutants. Fruit diameter was measured at 1/3 of fruit length and 2/3 of fruit length from fruit tip. Vertical bars represent the standard errors (n = 20). Different letters indicate significant differences between wild type plants (WT) and *long-fruit* mutants at 0.01 level.

All these mutant lines are now still under further investigation and we believe that these mutants can contribute greatly to our future cucumber breeding.

Acknowledgements

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