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# PHENOTYPIC VARIATION ANALYSIS OF ETHYL METHANE SULFONATE INDUCED MUTANT POPULATION OF PEPPER

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**Abstract**

In this study, ethyl methane sulfonate (EMS) was used to chemically mutagenize the seeds of Pepper Zunla-1 to increase the genetic variations of the peppers (Capsicum annuum). The lethal dose 50% ($LD_{50}$) for peppers was determined by analyzing the relative germination rates of pepper seeds in different concentrations of EMS at different mutagenesis durations, and the mutant phenotypes of 2,271 $M_1$ generation plants and 295 $M_2$ generation plants were investigated. In different developmental stages of the $M_1$ generation, mutations were observed in the leaf shape, floral organ, stem, leaf color, fertility, and fruit shape, and leaf-color and fertility chimeras were also identified. A total of 94 lines in the $M_2$ generation showed mutant phenotypes, with an overall mutation frequency of 31.86%. The types of mutations involved the leaves, stems, fruits, fertility, growth period, and floral organs, accounting for 20%, 34.55%, 21.82%, 7.27%, 12.12%, and 4.24%, respectively, of the overall mutation frequency. Moreover, the mutation types could be further divided into several subtypes. The diverse types of the $M_2$ generation mutants not only were valuable to the applications of genetic modification of peppers, but also had great implications for the discovery of new genes of peppers.

**Key words:** EMS; Pepper; Mutation; $M_1$ generation; $M_2$ generation; Phenotypic variation.

## Introduction

Pepper is an economic vegetable and spice crop widely grown in the tropical and subtropical regions, playing an important role in ensuring an equitable supply of vegetables and spices worldwide. After long-term artificial selection that pursues high yield, some of the modern pepper cultivars have deleted good genes and reduced genetic diversity. One of the ways to increase the genetic diversity of peppers is to induce mutations. By artificially inducing gene mutations, new genes can be generated, including both of those unfavorable and beneficial to practical production. Since the reference genome sequence of pepper can be used to identify the mutant genes of pepper, plants with unfavorable genes can be used as materials of forward or reverse genetics for the related gene mining and functional analysis (Emmanuel & Levy, 2002; Takagi *et al*., 2015), while those with favorable genes can be used to produce target variations using their dominant mutations, so as to modify the genetics of peppers (Bosland & Votava, 2012; Pathirana, 2011).

EMS is currently the most widely used chemical mutagen and is highly effective. By inducing alkylation of guanine to convert GC to AT, it generates a high proportion of point mutations that are randomly distributed throughout the genome (Talebi *et al*., 2012; Chen *et al*., 2013). In addition to its effectiveness, it can be detoxified by hydrolysis and thus is easier to handle compared with nitroso-based chemical mutagens (Pathirana, 2011). Point mutations induced by EMS can be analyzed in two ways: forward genetics, in which the obvious mutant phenotypes are located first and then the corresponding mutant genes are identified, and reverse genetics, in which the mutant genes are identified first and then the resulting phenotypic effects are explored (Peters *et al*., 2003).

At present, EMS mutagenesis is mainly used for food crops such as rice, corn, and soybean. In contrast, it is rarely used for plants from the Solanaceae family. Works on the EMS mutagenesis for pepper primarily focused on the optimization of mutagenic conditions (Alcantara *et al*., 1996; Arisha *et al*., 2014), investigation of the visible mutant phenotypes of mutagenized offspring (Jabeen & Mirza, 2002, 2004; Bosland, 2002; Arisha *et al*., 2015), and development of new cultivars (Daskalov, 1986). Based on official data published by the Joint FAO/IAEA program (http://mvd.iaea.org/), as of 2017, 3,275 mutants of 220 species have been released using mutagenic technology worldwide. Among them, 16 cultivars of peppers were bred by mutagenesis technology, with modified traits including growth period, yield, disease resistance, quality, plant type, and fruit shape.

Although several mutant groups of peppers had been established earlier, they were far from meeting the needs of diversified pepper breeding and functional genomics research. In the meantime, all of these mutant groups were derived from non-sequenced cultivars. To use the results of genome sequencing more directly and efficiently, the cultivar "Zunla-1" with published genome sequence was selected to optimize its mutagenic conditions. The mutant phenotypes of the $M_1$ and $M_2$ generations were investigated, and a new mutagenized population was formed, in an attempt to create new pepper materials that could meet the breeding objectives and be suitable for the functional genomics research.

## Materials and Methods

**Plants materials and EMS processing:** Pepper cultivar "Zunla-1" was used for all tests. Five soaking durations (8h, 10h, 12h, 14h, and 16h) and eight EMS concentrations (0, 0.6%, 0.7%, 0.8%, 0.9%, 1.0%, 1.1%, and 1.2%) were selected to optimize EMS concentration. Each treatment was repeated for three times, with 100 seeds repeated each time. The entire mutagenesis process was carried out in a shaker shaking at 110 rpm at 26℃. The treated seeds were rinsed three times with distilled water and immediately placed in a Petri dish of 90mm in diameter with two layers of wet filter paper for germination. The germination rate was calculated after 14 days of germination (Arisha *et al*., 2014, 2015).

**Formation of mutagenized group:** Six thousand pepper seeds were mutagenized by using 0.8% EMS for 12h to form the $M_1$ population. The mutagenized pepper seeds were separately planted in the field at a planting distance of 30cm×45cm after seedling. A total of 2,271 plants survived, and the seeds were separately harvested after maturity. 295 seeds were randomly selected to plant the $M_2$ generation by lines, with 12 plants per line (Espina *et al*., 2018).

**Phenotypic survey:** During the entire growth period of the $M_1$ and $M_2$ plants, the visible mutant phenotypes, including stems, leaves, fruits, floral organs, and fertility, were surveyed and recorded, and the growth periods were surveyed at the end. All observed mutant phenotypes were photographed and kept.

## Results and analysis

**Selection of lethal dose 50% ($LD_{50}$):** The mutagenesis duration extended from 8h to 16h, the germination rate gradually dropped; as the mutagen concentration increased from 0.6% to 1.2%, the germination rate also gradually dropped. The dose that resulted in germination of nearly 50% of the seeds through mutagenesis was taken as $LD_{50}$. $LD_{50}$ for "Zunla-1" was determined as treatment with 0.6% EMS for 16h, 0.7% EMS for 14h, 0.8% EMS for 12h, 1.0% EMS for 10h, and 1.2% EMS for 8h. LD50 with 0.8% EMS for 12h, selected as an optimization condition for further mutagenesis (Table 1).

**Analysis of field traits of the $M_1$ generation plants:** Observation of the field traits revealed that the $M_1$ generation plants showed mutant phenotypes in the seedling, flowering, and maturity stages (Fig. 1). Leaf-shape mutation was found in the seedling stage: Each one of the leaves seemed to be composed of two, with two leaf main veins and two leaf tips (Fig. 1-A). In the flowering stage, several types of mutant phenotypes were found, including flower clusters(Fig. 1-B), purple petal edges (Fig. 1-C), increased branches (Fig. 1-D), flat upper stems (Fig. 1-E), increased stem trichomes (Fig.1-F), extended main stem below the dichotomous branches (Fig. 1-G), uneven branching (Fig. 1-H), and leaf yellowing (Fig. 1-I). Other mutant phenotypes such as infertility (Fig. 1-J), deformed fruit shape (Fig. 1-K), and shortened plant height (Fig. 1-L) were found in the maturity stage.

In this study, some plants in the $M_1$ generation were found to be chimeras in two types. The first was the different phenotypes of the main and side branches, with the main being normal and the side showing mutant phenotypes. The second was the different phenotypes of the different subbranches of the main branch: With the site of the dichotomous branching as the border, the subbranch on one side showed normal phenotype, while the other showed mutant phenotypes. The types of chimeras included leaf color and fertility. There were four leaf-color chimeras (Fig. 2-A, B, C, D), with a mutation frequency of 0.18%, and five fertility chimeras (Fig. 2-G), with a mutation frequency of 0.22% (Table 2). The phenotypic mutation of the leaf-color chimeras was yellowing, which included four phenotypes (Fig. 2). The first type of yellowing was characterized by flattening of the leaves, with large areas of yellowed edges. There were still green areas in the middle, and the yellow-green border was obvious (Fig. 2-A). The second type of yellowing was bounded by the main vein of the leaf, with one half completely yellowed and the other still green (Fig. 2-B). The third type of yellowing was characterized by chlorosis of the whole leaf (Fig. 2-C). The fourth type of yellowing was characterized by the upward and downward curling of the blades, shrinkage, and marginal yellowing. The yellowing area was smaller than that of the first type, and the middle part was dark green, with an obvious yellow-green border (Fig. 2-D). The fertility chimeras were characterized by the differences defined by the dichotomous branches, with one branch normally fruiting while the other being infertile (Fig. 2-G).

**Analysis of field traits of the $M_2$ generation plants:** A total of 94 lines among the 295 $M_2$ generation lines showed mutant phenotypes, with an overall mutation frequency of 31.86%. The types of mutations could be divided into six categories involving the leaves, stems, fruits, fertility, growth period, and floral organs, which respectively accounted for 20%, 34.55%, 21.82%, 7.27%, 12.12%, and 4.24% of the overall mutation frequency (Table 3).

**Table 1. The germination rate of pepper seed under different concentration of EMS and mutagenesis duration.**

| EMS concentration | Mutagenesis duration | | | | |
|---|---|---|---|---|---|
| | 8h | 10h | 12h | 14h | 16h |
| 0.6% | (88.58 ± 8.70) | (88.52 ± 3.45) | (75.16 ± 0.95) | (65.33 ± 5.25) | (44.67 ± 2.49) |
| 0.7% | (87.91 ± 0.95) | (79.06 ± 4.38) | (67.11 ± 3.80) | (42.00 ± 4.32) | (26.67 ± 8.99) |
| 0.8% | (78.52 ± 5.93) | (71.63 ± 8.49) | (51.00 ± 5.02) | (26.67 ± 2.49) | (12.67 ± 3.40) |
| 0.9% | (75.16 ± 4.14) | (58.79 ± 5.97) | (40.94 ± 5.77) | (16.00 ± 2.83) | (8.67 ± 2.49) |
| 1.0% | (66.44 ± 4.93) | (50.68 ± 7.58) | (27.51 ± 5.02) | (10.00 ± 2.83) | (8.67 ± 3.40) |
| 1.1% | (62.41 ± 1.64) | (39.87 ± 6.89) | (12.75 ± 6.84) | (5.33 ± 1.89) | (3.33 ± 1.88) |
| 1.2% | (48.99 ± 4.14) | (30.41 ± 5.97) | (10.07 ± 2.85) | (1.33 ± 0.94) | (1.33 ± 0.94) |

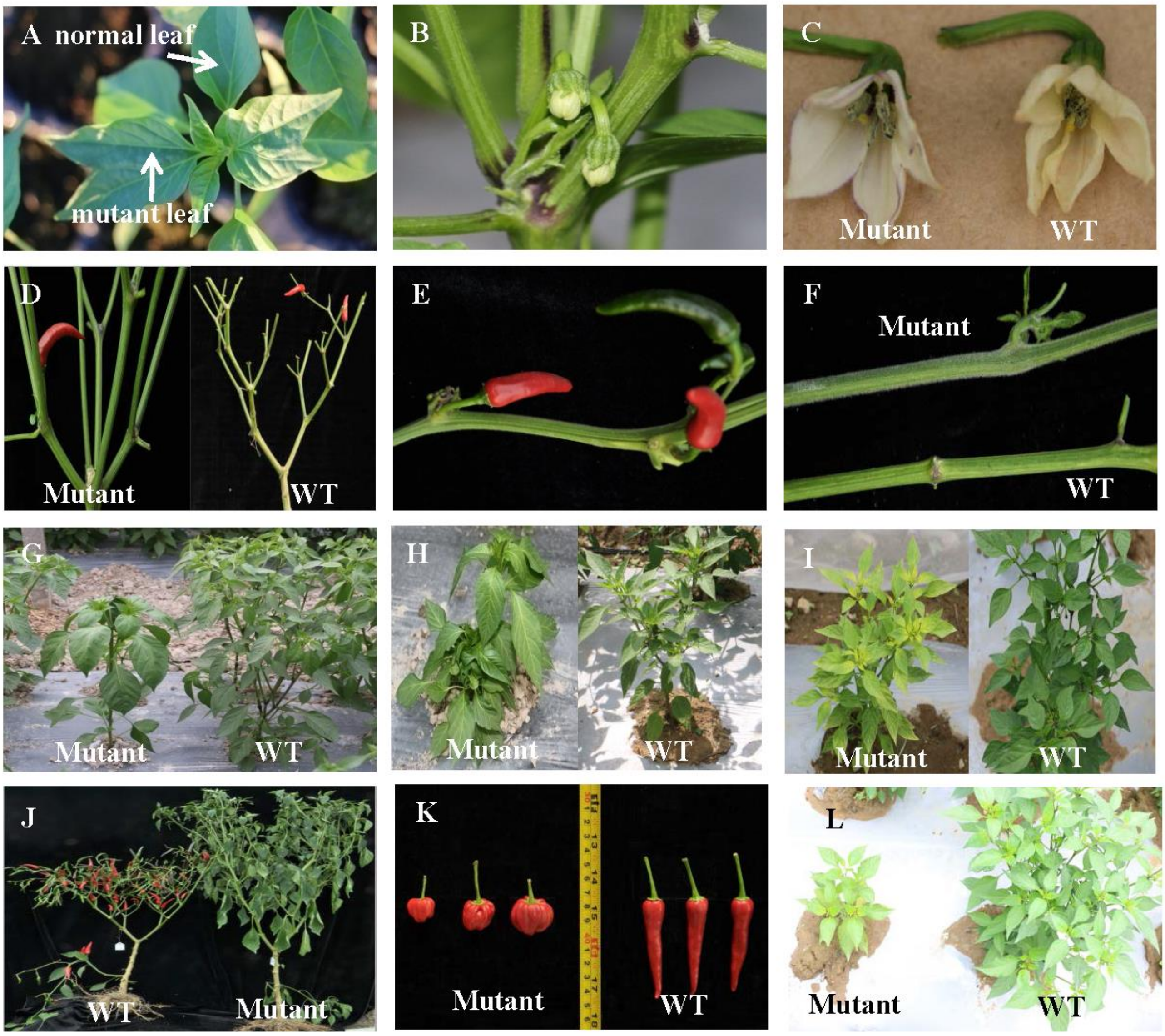


Fig. 1. The phenotypic variation of $M_1$ generation plants. (A)leaf-shape variation in the seedling stage , (B) flower clusters , (C) purple petal edges , (D) increased branches , (E) flat upper stems, (F) increased stem trichomes , (G) extended main stem below the dichotomous branches , (H) uneven branching , (I) yellow leaf , (J) infertility, (K) deformed fruit shape, (L) shortened plant height.

**Table 2. Chimeras induced by EMS in the $M_1$ generation.**

| Plant No. | Category | No. of mutant | Incidence (%) |
|---|---|---|---|
| 8 | Leaf color | 4 | 0.18 |
| 74 | Leaf color | | |
| 1012 | Leaf color | | |
| 2268 | Leaf color | | |
| 1417 | Fertility | 5 | 0.22 |
| 1428 | Fertility | | |
| 1449 | Fertility | | |
| 1699 | Fertility | | |
| 2177 | Fertility | | |

**Leaf mutations:** The types of leaf mutations involved leaf color (Fig. 3) and leaf shape (Fig. 4). Among them, the leaf-color mutations included whitened (Fig. 3-B) and yellowed (Fig. 3-C)plants with completely chlorotic leaves at the seedling stage, white and green leaves (photobleached blades) (Fig. 3-D), yellowed leaves (Fig. 3-F), dark green leaves (Fig. 3-G), and rough and dull leaves (Fig. 3-H). The leaf-shape mutations included narrowing (Fig. 4-B), widening (Fig. 4-C), curlingand shrinking (Fig. 4-D). Eight seedlings with cotyledons completely yellowed were found in No.2007 Line in the seedling stage. Compared with the wild type, the yellowing was very significant. The yellowed seedlings accounted for 28.57% of this line, with a separation ratio of 3:1, which was consistent with the classical Mendelian inheritance law (Table 4).

**Stem mutations:** The types of stems mutations involved plant height, main stem degradation, plant type, andtrichomes (Fig. 5). Among them, the plant height mutations included heightening and shortening (Fig. 5-A). The main stem degradation was characterized by obviously shortened internode length and significantly shortened height of plants (Fig. 5-B). The number of branches on the main stem was reduced but the number on the side branches was large The leaves were dense, so that the stems of multiple branches were clustered together. The plant type mutations included more compact growth (Fig. 5-C) and increased angle of the first branch. The trichome mutation was characterized by the dense trichomes growing on the stems (Fig. 5-D).


Fig. 2. Chimeras in the $M_1$ generation. (A) the first kind of leaf-color chimeras, (B) the second kind of leaf-color chimeras , (C) the third kind of leaf-color chimeras, (D) the fourth kind of leaf-color chimeras , (E) and (F) WT plant of leaf color , (G) the fertility chimera.

**Table 3. The types and numbers of phenotypic variation in the $M_2$ generation.**

| First category | Secondary category | Thirdly category | No of families | Incidence (%) |
|---|---|---|---|---|
| Leaf | Leaf color | Whitened plants with completely chlorotic leaves | 5 | 20 |
| | | Yellowed plants with completely chlorotic leaves | 4 | |
| | | Photobleachedblades | 1 | |
| | | Yellowed leaves | 9 | |
| | | Dark green leaves | 1 | |
| | | Rough and dull leaves | 3 | |
| | Leaf shape | Narrowing leaves | 4 | |
| | | Widening leaves | 2 | |
| | | Curling and shrinking leaves | 4 | |
| Stem | Plant height | Heightening plant | 1 | 34.55 |
| | | Shortening plant | 31 | |
| | Main stem degradation | | 12 | |
| | Plant type | More compact growth | 2 | |
| | | Increased angle of the first branch | 5 | |
| | Trichomes | | 6 | |
| Fruits | Fruit shape | Horn-shaped fruit | 1 | 21.82 |
| | | Finger-shaped fruit | 16 | |
| | | Deformed fruit | 1 | |
| | Fruit length | Lengthening fruit | 2 | |
| | | Shortening fruit | 5 | |
| | Fruit width | Thickening fruit | 6 | |
| | Fruit surface | | 4 | |
| | Fruit color | | 1 | |
| Fertility | Infertility | | 12 | 7.27 |
| Growth period | Extended growth period | | 20 | 12.12 |
| Floral organ | Purple petals | | 1 | 4.24 |
| | Deformed floral organs | | 6 | |

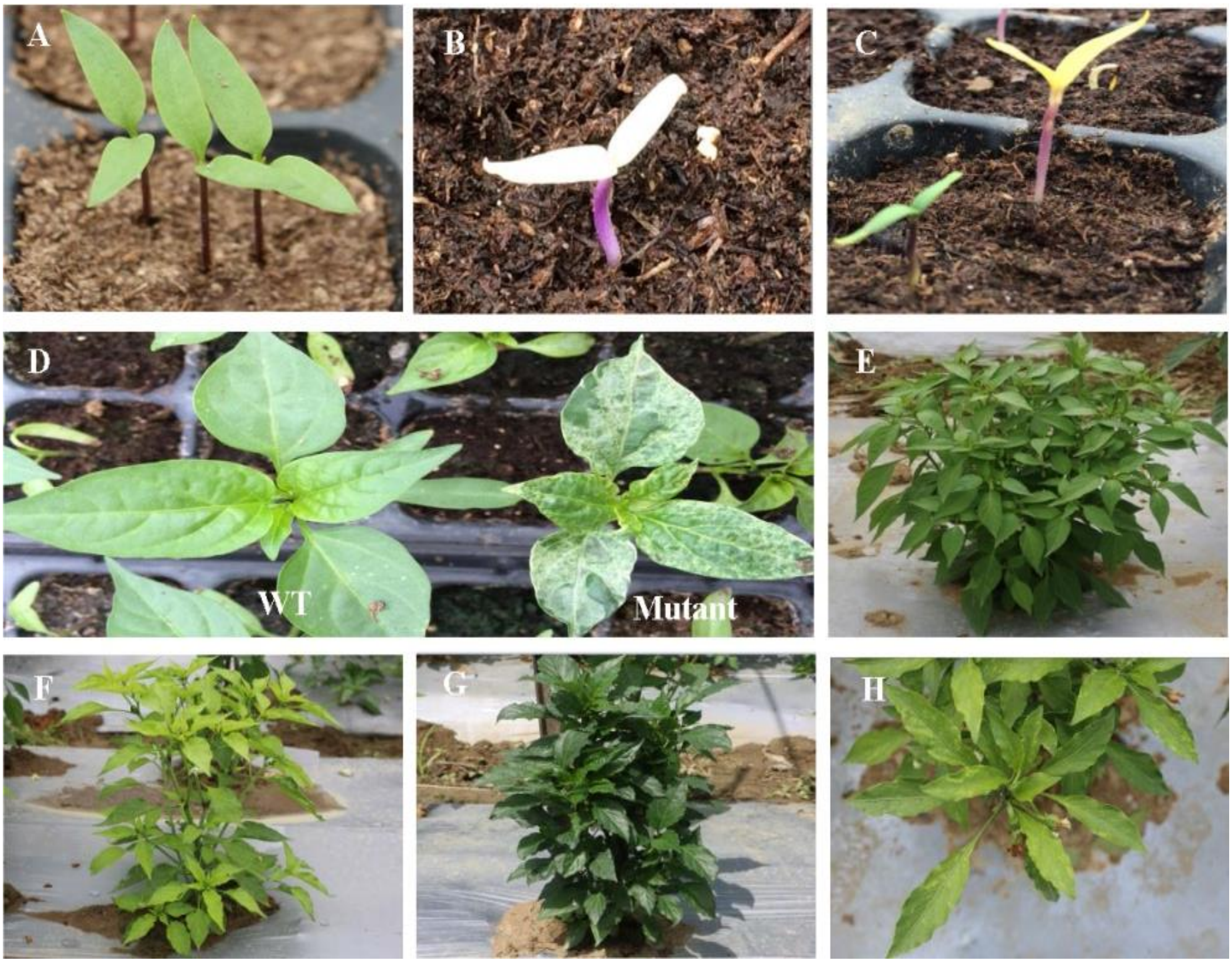


Fig. 3. The leaf-color mutations in the $M_2$ generation. (A) WT plant at the seedling stage, (B) whitened plants with completely chlorotic leaves at the seedling stage, (C) yellowed plants with completely chlorotic leaves at the seedling stage, (D)photo bleached blades , (E) WT plant for F to H , (F) yellowed leaves , (G) dark green leaves , (H) rough and dull leaves.


Fig. 4. The leaf- shape mutations in the $M_2$ generation. (A) WT plant of leaf- shape, (B) narrowing leaves, (C) widening leaves, (D) curling and shrinking leaves.

**Table 4.The leaf-color mutations at the seedling stage in the $M_2$ generation induced by EMS.**

| Family no. | Total no. of plants in the family | Albino seedings | | Yellow seedlings | |
|---|---|---|---|---|---|
| | | No. | % | No. | % |
| 718 | 41 | 9 | 21.95 | 0 | 0 |
| 753 | 30 | 8 | 26.67 | 0 | 0 |
| 953 | 15 | 3 | 20.00 | 0 | 0 |
| 1292 | 36 | 2 | 5.56 | 0 | 0 |
| 2120 | 22 | 3 | 13.64 | 0 | 0 |
| 54 | 24 | 0 | 0 | 6 | 25.00 |
| 394 | 46 | 0 | 0 | 12 | 26.09 |
| 926 | 30 | 0 | 0 | 5 | 16.67 |
| 2007 | 28 | 0 | 0 | 8 | 28.57 |

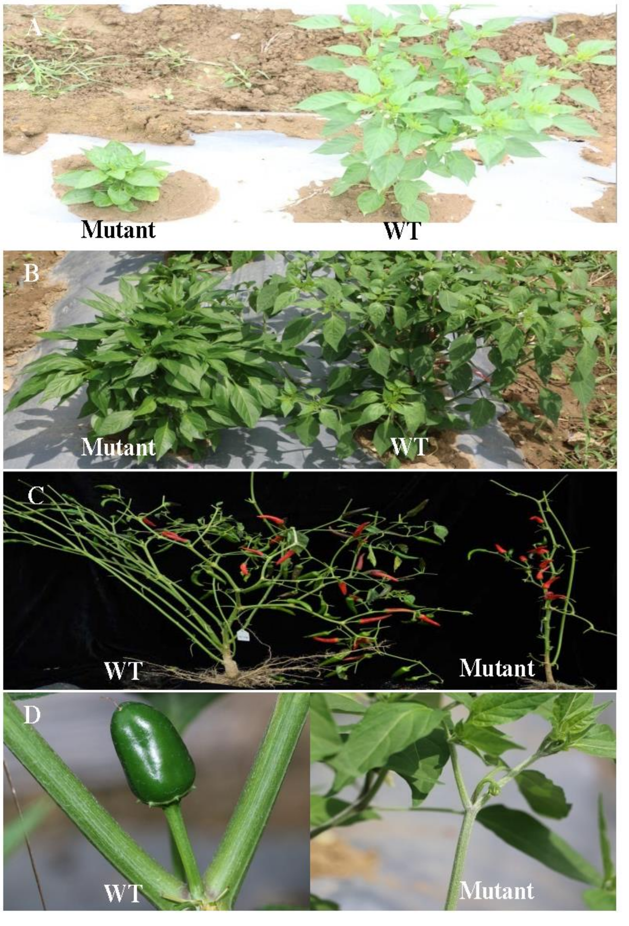


Fig. 5. The stem mutations in the $M_2$ generation. (A) shortening plant, (B) the main stem degradation, (C) more compact growth , (D) the obvious trichomeplant.

**Fruit mutations:** There were various types of fruits mutations, involving the fruit shape, fruit length, fruit width, fruit surface, and fruit color (Fig. 6). Among them, the fruit-shape mutations referred to the transformations of the fruits from cones to horn-shaped (Fig. 6-A), finger-shaped (Fig. 6-B), and deformed (Fig. 6-C). The fruit-length mutations included lengthening (Fig. 6-D) and shortening (Fig. 6-E). The fruit-width mutation was characterized by fruit thickening (Fig. 6-F). The fruit surface mutation referred to the brightening of the fruit surface (Fig. 6-G). The fruit-color mutation was expressed as the green ripe fruits turned purple.

**Fertility, growth period, and floral organ mutations:** The fertility mutations referred to the transition of plants from fertility into infertility, manifested as no fruit or parthenocarpy (Fig. 7-A). The growth period mutations referred to extended growth period and late ripening of the plants (Fig. 7-B). The floral organs mutations included purple petals (Fig. 7-C) and deformed floral organs (Fig. 7-D).

## Discussion

The mutagenesis dose was measured by the mutagen concentration and mutagenesis duration. A too large mutagenesis dose could cause excess physical damages to the plants, resulting in a low survival rate of plants. However, a too small mutagenesis dose could lead to a too low mutation frequency of the offspring. For this reason, the selection of a proper mutagenesis dose is a prerequisite for producing a rich mutant phenotype, obtaining high frequency target mutations, and efficiently constructing a mutant library (Wani, 2009). The $LD_{50}$ is usually used as the optimal mutagenesis dose. The $LD_{50}$ varies greatly from crop to crop. The optimal mutagen concentration of EMS is less than 1%V/V for rice, soybean, and tomato (Talebi *et al*., 2012; Espina *et al*., 2018; Sikder *et al*., 2013), but can be as high as 1.5% V/V for pepper (Alcantara *et al*., 1996). Compared with previous findings, it was found that mutagenesis with the same concentration of EMS could take a longer duration, which was four hours longer than that with the pepper $LD_{50}$ measured by Arisha and six hours longer than that measured by Zhou Shudong (Arisha *et al*., 2015). Such difference might be caused by the different cultivars or the different experimental settings used.

In general, the $M_1$ generation is extremely unlikely to exhibit a mutant phenotype. Only the dominant mutations can be identified, and these dominant mutants may be haploids or aneuploids that are parthenocarpic. As a result, they fail to flower or fruit, even subject to lethal mutations, and cannot offer genetic stability (Alcantara *et al*., 1996). In this study, compared with the wild type, the $M_1$ generation plants showed mutant phenotypes in the seedling, flowering, and maturity stages, with a variety of mutation types involving the stems, leaves, floral organs, and fruits. This was consistent with the findings of Jabeen. N *et al*., who found that he $M_1$ of the pepper showed diverse mutant phenotypes (Jabeen & Mirza, 2002). Nevertheless, most of these mutant phenotypes were only found in $M_1$ and could not be passed to the $M_2$ generation. This coincided with the findings of Alcantara *et al*., (1996) and Arisha *et al*., (2015). Alcantara *et al*., (1996) used EMS to mutagenize the pepper seeds and obtained various mutants in the $M_1$ generation, but most of these mutants could not be stably inherited. Arisha *et al*., (2015) used only one mutant with obvious mutant phenotype among 939 pepper $M_1$ plants.

In this study, the leaf-color and fertility chimeras were also found in the $M_1$ generation. Similarly, Hermelin et al. found chimeras in the $M_1$ generation of pepper mutagenized by radiation (Hermelin *et al*., 1983). Plant chimeras refer to the plant tissues or whole plants that are developed from two or more different genetic cells (Marcotrigiano, 1997). The reason for the generation of chimeras might be that the mature seeds were used as the experimental materials. Their embryo structure was complex, composed of multiple cells. As a result, mutations would take place only in the cells that actually absorbed mutagens, while the other cells would show no mutations. Mutant cells and normal cells jointly developed into chimeras with both mutant and normal traits. Studies using mutagenesis to generate chimeras in other crops have been reported. Previous efforts had been made to mutagenize rice with different mutagens, and chlorophyll chimeras were obtained in the $M_1$ generation. Compared with plants that showed normal phenotypes in the $M_1$ generation, such chlorophyll chimera plants had a higher mutation frequency of chlorophyll in the $M_2$ generation (Karunakaran & Kiss, 1971). The use of radiation mutagenesis to induce ornamental plants to produce leaf-color and flower-color chimeras has been studied (Kumari *et al*., 2014), and these chimeras could enhance the ornamental value of plants.

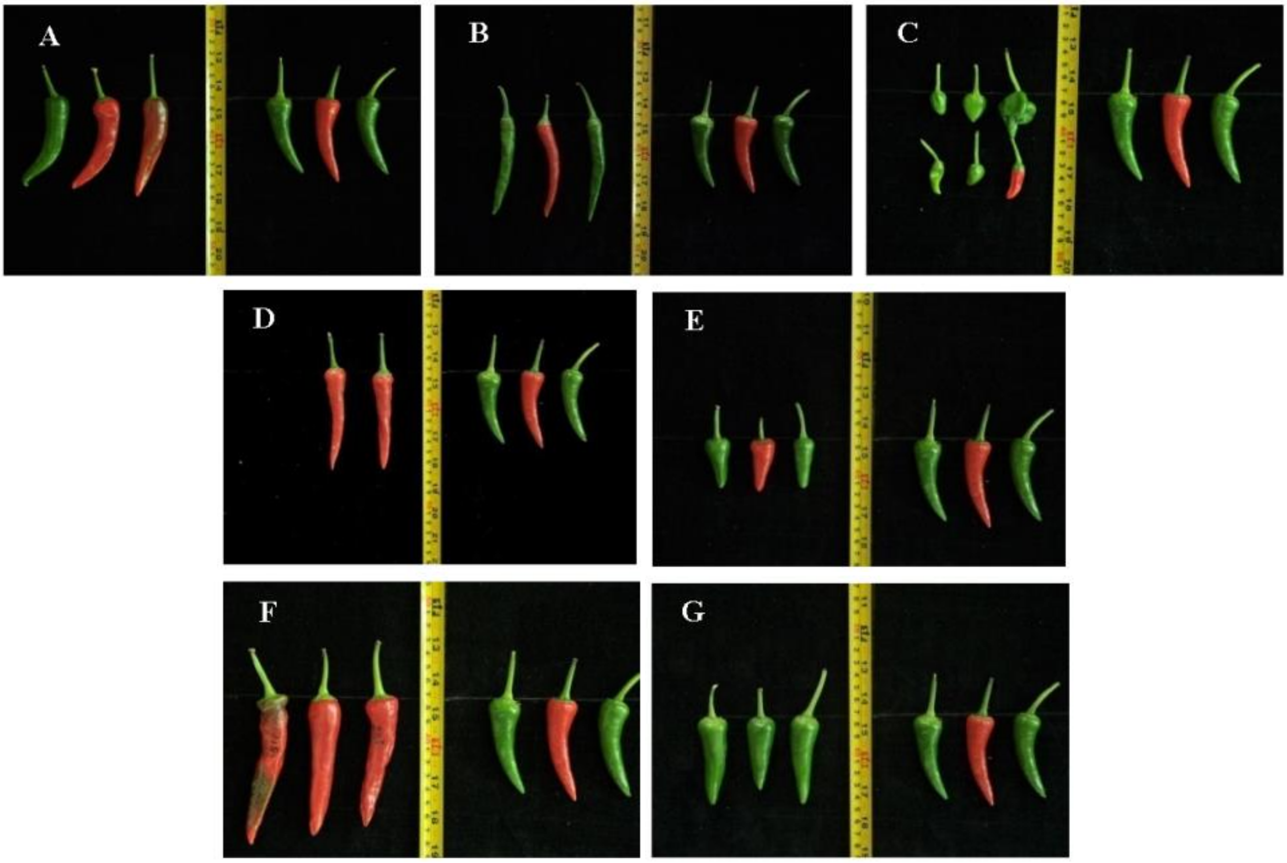


Fig. 6. The fruit mutations in the $M_2$ generation. (A) horn-shaped fruit, (B) finger-shaped fruit, (C) deformed fruit, (D) lengthening fruit, (E) shortening fruit, (F) thickening fruit, (G) the brightening fruit surface, the left fruits in A-G are mutants and the right ones are wild type.

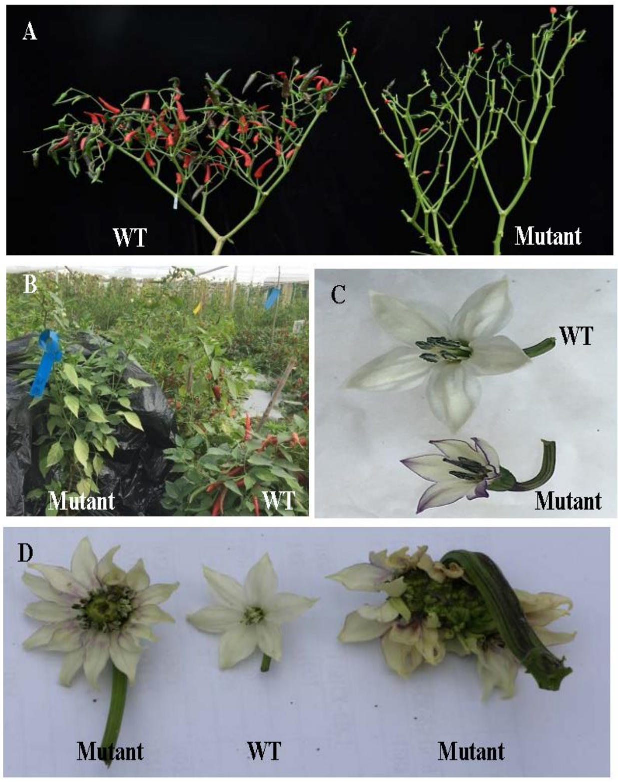


Fig. 7. The fertility, growth period, and floral organ mutations in the $M_2$ generation. (A)the fertility mutation, (B) the extended growth period mutation, (C) purple petal, (D)deformed floral organ.

The $M_2$ generation expressed the recessive mutations and exhibited dramatic trait separation, resulting in a large number of mutant phenotypes (Arisha *et al*., 2015). Therefore, the $M_2$ generation was the optimal generation for identifying the mutant phenotypes. Chlorophyll mutants such as yellowing, whitening, and photo bleaching of the leaves were observed in the $M_2$ generation of this group. The presence of chlorophyll mutants was the most reliable indicator for assessing whether mutagenesis produced genetic effects (Arisha *et al*., 2015). This demonstrated that EMS could indeed induce the pepper to produce genetic mutations. Consistent with our findings, previous studies showed that after EMS mutagenesis, the $M_2$ generation of pepper was found with mutants of whitened and yellowed leaves (Alcantara *et al*., 1996; Arisha *et al*., 2015). In contrast, compared with the yellowed mutants obtained by Arisha *et al*., the yellowed mutants obtained in this studyhad a more pronounced degree of yellowing. Moreover, among the lines in the $M_2$ generation, eight out of 28 seedlings were yellowed, with a separation ratio of 3:1, in line with the classical Mendelian inheritance law. However, the separation ratio was 10:1 in Arisha's study. In addition, compared with the wild type, the plant height of the yellowed mutants in this study was obviously lower, and the growth period was significantly longer. Leaf-color mutants are ideal materials for exploring the chlorophyll biosynthesis, chloroplast structure and development, photosynthesis mechanisms, gene functions, and nuclear-to-plasma interactions (Wu *et al*., 2007; Davis *et al*., 1999; Kohehi *et al*., 2001; Terry & Kendrick, 1999). In the meantime, in practical production, leaf-color mutations can be used a marker trait to identify the purity of the seeds and to breed new ornamental cultivars with high photosynthetic efficiency (Coschigano *et al*., 1998; Gan *et al*., 1995).

Moderate curling of the leaves helps to keep the blades upright, improve the light-receiving posture of the plants, and increase the utilization of light energy. Meanwhile, it helps to reduce the transpiration of the leaves and improve the drought tolerance of the plants. Therefore, curling-leaf mutants are important resources for cultivating the ideal plant type and drought-resistant breeding (Erik *et al*., 1999; Horton, 2000; Sinclair & Sheehy, 1999). During the survey of the leaf mutations, it was also found that there was a type of mutants with curly and shrunken leaves. The cotyledons and all true leaves of the mutants were curled and shrunk throughout the growth period, which did not change with moisture and temperature. Moreover, these mutants had several features including obviously lower plant height, more brittle stems, reduced side branches, fewer fruits, and thicker and shorter shape of fruits. Pepper mutants with curled leaves had been reported (Bosland, 2002), but the curling of mutant *flc* varied with moisture and temperature. In particular, the leaves were flattened at night or under suitable moisture, but curled during the daytime or under excessive moisture. A genetic analysis of the curling mutants had been preliminarily carried out and found that such mutant trait was controlled by a single recessive nuclear gene.

Dwarf mutants are important not only for studying the regulation mechanism of plant development, but also for breeding lodging-resistant cultivars. During the survey of the stem mutations, multiple dwarf mutants were found. They were characterized by obviously shortened internode length, usually accompanied by mutations of other traits, including darkened leaf color, longer and thicker leaves, dense leaves, changes in branching patterns, and extended growth period. Existing studies have shown that dwarf mutations in plants are associated with defects in gibberellin (GA) synthesis or loss of GA response pathways (Gao *et al*., 2010; Sun & Gubler, 2004), as well as the blocked signal transduction of brassinolide (BR) (Bishop & Yokota, 2001) and the signal transduction of ethylene (Yang *et al*., 2015; Eeker, 1995).

Fruit shape is the major indicator for qualifying and pricing the horticultural crops. Fruit-shape mutants have important application value for breeding new cultivars with ideal fruit shape. A variety of fruit-shape mutants were found among the fruits mutations in this study. Particularly, increased fruit length and width could lead to a larger overall size of the fruit, which had great application value in breeding practice. Although the scattered peppers with small fruits were difficult to pick, an enlarged fruit shape would make it much easier, substantially lowering the labor cost. However, since the fruit shape is tricky to measure and it is a quantitative trait that is greatly affected by the environment, little attention has been paid to the effects of mutagenesis on fruit shape. In the study using non-mutated pepper fruit shape, it was found that many QTLs controlling the pepper's measures, including the fruit size, length, width, and shape, were closely linked, clustered, and accumulated on the same chromosome (Ben Chaim *et al*., 2001; Ben Chaim *et al*., 2006; Rao *et al*., 2003; Zygier *et al*., 2005).

## Conclusion

In this study, EMS was used to induce the DNA mutations in peppers, so as to increase the genetic variations of pepper and form a new mutagenized group of peppers. By doing so, a variety of mutant phenotypes were obtained, involving the leaves, stems, fruits, fertility, growth period, and floral organs. Specifically, the chlorophyll, curled-leaf, dwarf, large-fruit, and dull-fruit-top mutants were suitable for pepper breeding. These mutants will be further studied to explore the genetic and molecular mechanisms responsible for these mutant phenotypes. The proposed mutagenized group will be used as a tool of forward genetics to mine new genes that cause mutant phenotypes in peppers. As a tool of reverse genetics, it will be used to identify the functions of about 35,000 predicted genes in peppers. Pepper is used in various sectors, such as food, seasonings, medicine, cosmetics, and pesticides. The mutagenized group can be used to select the valuable traits, which will in turn contribute to these sectors as an important resource. In addition, the proposed mutants will also be used to share and communicate with pepper researchers and breeders.

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