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Phenotypic variants among ethyl methanesulfonate M_2 mutant lines in *Capsicum annuum*

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Abstract

Induction of mutations using chemical mutagens has proved to be a useful tool in crop improvement and has advantages over transgenic approaches in view of legislative restrictions and intellectual property. Among the chemical mutagens, ethyl methanesulfonate (EMS) has been widely used to generate novel traits. In this study, we constructed an EMS mutant population consisting of 3945 M_2 mutant lines using a Korean landrace of *Capsicum annuum* 'Yuwol-cho'. In total, 1480 M_2 mutant lines were evaluated for novel traits. The mutant lines showed phenotypic variations such as plant growth (small size and dwarfism), development of leaves (variegation, colour and morphological changes), flowers (inflorescence, morphological and organ colour changes), and fruits (morphological and colour changes). Most of these mutant phenotypes were inherited recessively. Many of the mutant phenotypes were unique in pepper, while some were similar to those of known mutants in other plant species. These mutant lines will be useful for the study of gene function in *C. annuum*.

Keywords: ethyl methanesulfonate; mutant lines; novel traits; pepper

Introduction

Pepper (*Capsicum annuum* L.) is a commercially important vegetable because of its traits related to pungency, colour and high vitamin C content. Pepper is used as a food product, in the cosmetic industry, and for pharmaceutical purposes such as in anticancer and anti-inflammatory drugs, as well as in aerosol sprays (Bosland and Votava, 2012).

A range of natural genetic resources have been collected and managed for *Capsicum* (Bosland, 1992). Lists of genes affecting traits including plant architecture and leaf, flower and fruit phenotypes have been reported in a previous study (Wang and Bosland, 2006). By contrast, genetic studies using induced mutants are scarce due to the limited numbers of mutants in pepper.

Mutant populations have been developed in a range of species using mutagens such as ionizing radiations and chemical mutagens (Lippert *et al.*, 1964; Alcantara *et al.*, 1966). Among the chemical mutagens, ethyl methanesulfonate (EMS) is the most widely used mutagen because of its high mutation rate, low lethality and ease of handling (Tadmor *et al.*, 2007). EMS generates nucleotide substitutions and concomitant amino-acid changes, which can result in altered protein composition with phenotypic loss or gain effects (Parry *et al.*, 2009).

Mutagenesis has been successfully used in the breeding programmes of many vegetable crops. Notable examples

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are the improvement of starch content in potato (Muth *et al.*, 2008) and delayed ripening for long shelf life and reduced height and yellow fruit colour in tomato (Triques *et al.*, 2007; Okabe *et al.*, 2011). EMS-induced mutant populations have also been utilized for genetic studies in pepper. A single recessive mutant, *flaccid*, has been used for studies of turgor pressure and drought stress physiology in pepper (Bosland, 2002).

Previously, we had generated M_1 mutant lines from Yuwol-cho (*C. annuum* L.) with 1.5% EMS (Jeong *et al.*, 2011). The objective of the present study was to select EMS mutants from this population and characterize the various morphological changes in the M_2 mutant lines.

Materials and methods

Plant materials and mutagenesis methods

A Korean local landrace, *C. annuum* 'Yuwol-cho', was treated with EMS. Seeds were pre-soaked in distilled water at 24°C and shaken in an incubator for 18 h. The seeds were then drenched in 1.5% EMS (Sigma-Aldrich, Saint Louis, Missouri, USA) solution in 0.1 M phosphate buffer, pH 7.0, and subsequently incubated at 20°C for 12 h. EMS-treated seeds were washed with 0.5% (v/v) ethyl acetate (Sigma-Aldrich, Saint Louis, Missouri, USA) in 0.1 M phosphate buffer (pH 7.0) for 50 min (Jeong *et al.*, 2011). M_1 and M_2 seeds were sown in 72-plug trays in a glass greenhouse and then cultivated in small pots in a greenhouse (Seoul National University, Suwon, Korea).

Construction of mutant lines

Mutant lines derived from Yuwol-cho have been constructed since 2009. A total of 3945 M_2 mutant lines were used in this study (Table S2, available online). To evaluate the mutant lines, the phenotypic variations of 1480 M_2 mutant lines (about 37.5% of the population) were screened (Table 1). For each mutant line, ten individuals were grown to carry out phenotypic evaluation.

Phenotype screening in M_2 mutant lines

Phenotypes were evaluated in M_2 mutant lines. Phenotypic variations were characterized and categorized according to four classes and ten subclasses (Menda *et al.*, 2004; Minoia *et al.*, 2010). The various mutant phenotypes described were as follows: plant growth (small size and dwarfism), leaf development (variegation, colour and morphological changes), flower development (inflorescence, morphological and organ colour changes) and fruit development (morphological and colour changes).

Results and discussion

Classification of mutants

Among the 3945 M_2 mutant lines, 1480 were screened to evaluate traits that could be useful for crop improvement. Phenotypic alterations were categorized into four classes and further subcategorized into ten subclasses (Table 1). The mutation frequencies in each category varied. Mutants were given names according to their phenotypic characteristics (Wang and Bosland, 2006), with new names being designated if there were no previous examples of similar phenotypes (Table S1, available online). Most of the mutant phenotypes segregated at a ratio of 1:3, indicating that they were inherited recessively (Table 1). In addition, 123 of the 1480 M_2 lines exhibited pleiotropic phenotypes, in which more than one phenotypic trait was affected (Menda *et al.*, 2004). For example, downward-curling leaves and replicated petals with small round fruits were observed among the M_2 mutant lines.

Mutants exhibiting plant growth and leaf abnormalities

Among the 1480 M_2 mutant lines, abnormal phenotypic variations were observed in 353 (24%) mutant lines compared with the wild type (Fig. 1(a)–(f)). The highest number of morphological variations was observed for leaf morphology. In the plant growth category, 96 M_2 mutant lines were significantly shorter than the wild type, Yuwol-cho (Fig. 1(g)). Moreover, dwarf plants that exhibited retarded growth and generated no flowers were observed in 11 M_2 mutant lines (Fig. 1(h) and (i)), similar to mutants reported by Daskalov (1973; 1974).

Leaf colour changes and variegated patterning were observed in 100 M_2 mutant lines. Among these, 60 mutant lines had mottled white, pale-green–dark-green, generally yellowish leaves, and a half-ivory-coloured leaf (Fig. 1 (j)–(q)). Pale-green and yellow leaves were observed in 40 M_2 mutant lines (Fig. 1(r)–(t)). There were 120 M_2 mutant lines that exhibited abnormal leaf morphology. These included hair-like leaves, squid-leg leaves, Chinese cabbage-like leaves, scabrous leaves, downward- and upward-curling leaves, undulating leaves, wilting leaves, compact leaves, broad or narrow leaves, and elongated petiole leaves (Fig. 1(u)–(am)). Most of the lines exhibiting phenotypic variations in leaf morphology did not generate flowers and fruits, except for those with downward-curling leaves.

Mutants exhibiting abnormal flower or fruit development

Five distinct changes in flower characteristics were observed in the mutant lines. These included a flowerless

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Table 1. List of phenotypic categories and mutant characteristics

Classes	Subclasses		No. of mutant lines	Rate (%)	Segregation[a] (mutant:wild type)
Plant growth	Small size		96	27.2	3:7
	Dwarfism		11	3.1	3:7
Leaf	Variegation	White type	46	13.0	3:7
		Pale-green–green type	10	2.8	1:9
		Yellow type	3	0.8	2:8
		Ivory type	1	0.3	1:9
	Colour	Pale-green type	35	9.9	3:7
		Yellow type	5	1.4	2:8
	Abnormal morphology	Hair-like type	2	0.6	2:8
		Squid-leg type	16	4.5	1:9
		Chinese cabbage-like type	4	1.1	2:8
		Scabrous type	3	0.8	1:9
		Downward-curling type	2	0.6	4:6
		Upward-curling type	4	1.1	3:7
		Undulation type	1	0.3	3:7
		Flaccid type	1	0.3	1:9
		Bushed type	53	15.0	3:7
		Narrow leaf type	7	2.0	3:7
		Elongated petiole type	24	6.8	1:9
Flower	Inflorescence	Flowerless type	3	0.8	1:9
		Fasciculation type	2	0.6	3:7
	Morphology	Sunflower type	1	0.3	1:9
		Two flowers on one calyx type	1	0.3	1:9
		Replicated petal type	2	0.6	3:7
	Organ colour	Black stamen type	1	0.3	1:9
		White calyx and petiole type	1	0.3	1:9
Fruit	Morphology	Tailed type	1	0.3	2:8
		Round and small fruit type	8	2.3	3:7
		Two fruits on one calyx type	1	0.3	1:9
	Colour	Two colour fruits per one plant type	1	0.3	1:9
		Variegation type in immature fruits	1	0.3	3:7
		Yellow colour type	1	0.3	2:8
		Orange colour type	5	1.4	2:8
Total			353	100	

[a] The number of progeny exhibiting wild-type or mutant phenotype in the M_2 mutant lines is reported.

type and differences in the number of flowers produced on one branch (Fig. 1(an)–(ap)). These phenotypes were similar to those of mutants reported by Van der Beek and Ltifi (1990) and Elitzur *et al.* (2009). There were also three floral organ phenotypes such as sunflower shape, two flowers per one calyx and replicated petals (Fig. 1(aq)–(as)), and two altered stamen colour mutants (black stamen and white calyx and petiole) were observed (Fig. 1(at)–(au)). In addition, tailed fruits, small and round fruits, and two fruits from one calyx were observed in ten M_2 mutant lines (Fig. 1(av)–(ax)). Orange and yellow fruits, with variegation at the immature stage (variegated and pale-green fruits), were observed in eight M_2 mutant lines (Fig. 1(ay)–(bb)).

In conclusion, we evaluated EMS-induced phenotypes in 1480 M_2 mutant lines (approximately 37.5% of the total M_2 mutant lines). The various mutant phenotypes can be used as materials for breeding and genetic studies. For example, the ivory variegation in leaves and dwarf plants can be used for generating ornamental plants, as well as the squid-leg leaves, Chinese cabbage-like leaves and hair-like leaves are useful for genetic studies. Specifically, Paran *et al.* (2007) reported that hair-like leaves (*wiry* leaf mutation) often occur in tomato (2% of total), but are rarely found in pepper. Nevertheless, two wiry mutant lines were observed in the mutant population evaluated in the present study. These examples could be used for either forward genetics using EMS mutant populations and phenotypic data or reverse genetics through a high-throughput TILLING platform in pepper. Therefore, the phenotypic data obtained in this study will contribute to the identification of novel genes controlling phenotypes related to crop improvement and genetic studies in *C. annuum*.

Fig. 1. Examples of various mutant phenotypes. (a)–(f) Wild type: horizontal and vertical whole plant, normal leaf, normal flower, immature fruit and mature fruit, (g) small plant, (h)–(i) dwarf plant, (j)–(q) variegated leaf: white, pale green–green, mottled yellow, and ivory colour, (r)–(t) leaf colour: pale green and yellow, (u)–(am) abnormal morphology: wiry mutant, squid-leg leaf, cabbage-like leaf, scabrous leaf, downward- and upward-curling leaf, flaccid, compact and fused leaf, narrow leaf, and elongated petiole leaf, (an)–(ap) inflorescence: flowerless and formation of floral clusters similar to the fasciculate mutant type, (aq)–(as) flower morphology: many petals and floral organs, two flowers on one calyx and replicated petals, (at) and (au) flower organ colour: black stamen and white calyx and petiole, (av)–(ax) fruit morphology: tailed fruit with no seed and abnormal ovary, small and round fruit, and two fruits on one calyx, and (ay)–(bb) fruit colour: orange and yellow fruits on one plant, variegation type in immature stage and orange, yellow and orange fruits.

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Supplementary material

To view supplementary material for this article, please visit http://dx.doi.org/10.1017/S1479262114000434

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