Construction of a Pea EMS Mutant Library and Screening for Superior Mutants (Postprint)

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Abstract

To generate more diverse pea mutant materials and obtain superior mutated pea germplasm, this study utilized 'Qingjian No. 1' pea as the experimental material and ethyl methanesulfonate (EMS) as the mutagen. An EMS concentration of 1% and treatment duration of 8 h were employed as semi-lethal mutagenesis conditions. Under these conditions, mutation types in pea plants were analyzed, important phenotypic trait data of mutants were obtained, a pea phenotypic mutant library was established, and superior mutant materials were screened by integrating field phenotypic data. The results demonstrated: (1) Following EMS mutagenesis of 10,000 pea seeds at 1% concentration for 8 h, 4,682 seedlings survived in the M1 generation, and 342 pea mutants were identified in the M2 generation. (2) The study revealed relatively abundant mutation types in phenotypic traits of mutant peas, with the coefficient of variation for dry seed weight per plant being the largest, reaching 0.965. (3) Through comprehensive analysis of field phenotypic data, 10 superior pea mutant germplasms were selected. These findings enrich pea germplasm resources and can provide a reference basis for mining and research of pea-related functional genes as well as for breeding of improved varieties.

Full Text

Construction of EMS Mutant Library and Screening of Excellent Mutants in Pea

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Abstract

To create a broader range of pea mutation materials and acquire superior mutant pea germplasm, this study used 'Qingjian No. 1' pea as experimental material and ethyl methanesulfonate (EMS) as mutagen. An EMS concentration of 1% and treatment duration of 8 hours were selected as semi-lethal mutagenesis conditions. The study analyzed mutation types in pea plants under these conditions, obtained important phenotypic data for mutants, established a pea phenotypic mutant library, and screened for excellent mutant materials based on field phenotypic data. The results showed: (1) Treatment of 10,000 pea seeds with 1% EMS for 8 hours yielded 4,682 M1 plants and 342 pea mutants in the M2 generation. (2) Mutant peas exhibited diverse phenotypic mutation types, with the coefficient of variation for individual seed dry weight being the largest at 0.965. (3) Comprehensive analysis of field phenotypic data identified 10 superior pea mutant germplasms. These findings enrich pea germplasm resources and provide a reference for functional gene mining and breeding of superior pea varieties.

Keywords: pea, ethyl methanesulfonate (EMS), mutant library, germplasm resource, excellent mutant

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Pea (*Pisum* L.) is an annual cool-season legume crop in the family Leguminosae (Liu et al., 2020; Yi and Pang, 2022). It possesses characteristics such as cold tolerance, drought resistance, salinity-alkalinity tolerance, and strong adaptability, making it one of the world' s important cultivated crops (Heydisz et al., 2020; Lu et al., 2020). Pea has symbiotic nitrogen fixation capability that helps improve soil fertility and promote sustainable agricultural development (Mabrouk et al., 2018). Qinghai Province is located in the northeastern part of the Qinghai-Tibet Plateau with unique geographical location and natural environment (Deng et al., 2023). Through long-term natural and artificial selection, distinctive agricultural germplasm resources with strong cold tolerance, stress resistance, and adaptability have been formed, making it one of the regions with the richest agricultural germplasm resources on the Qinghai-Tibet Plateau. Pea also demonstrates strong adaptability under the unique geographical location and climatic conditions of high altitude and intense radiation in Qinghai. Therefore, in-depth research on pea growth patterns and stress resistance mechanisms, combined with biotechnology approaches to improve main traits such as seed size, color, and quality, and the establishment of pea germplasm resource phenotypic databases and mutant libraries, are of great significance for breeding new superior pea varieties with comprehensive resistance and for product

development.

A mutant library comprises individuals with various gene mutations (Zhu and Li, 2024). International research on pea molecular breeding has progressed rapidly, with multiple loci and genes related to important agronomic traits, disease and pest resistance, and stress tolerance having been mapped, including stem length, internode number, seed yield, protein content, and maturity (Tar' an et al., 2004). Constructing crop mutant libraries is an important foundation for studying functional genes and breeding new crop varieties. Methods for constructing plant mutant resource libraries are mainly divided into three categories: spontaneous mutation, insertion mutation, and physical/chemical mutagenesis (Jung et al., 2003). Compared with traditional breeding methods, mutagenesis breeding offers advantages such as high mutation frequency, broad variation range, and short breeding period (Tasaka, 2011; Liu, 2014). Physical and chemical mutagenesis is a widely applied chemical mutagenesis method. Ethyl methanesulfonate (EMS) is a widely used chemical mutagen with many advantages including low cost, simple operation, high mutagenic efficiency, few chromosomal aberrations, genetic stability, and ease of screening, making it an optimal approach for obtaining rich and diverse mutant materials and constructing plant mutant libraries (Zhang et al., 2010). However, EMS mutagenesis has uncertainty and randomness, with mutagenic efficiency varying significantly among different materials. Research by Bai (2018) showed that EMS treatment near the semi-lethal dose (LD50) resulted in higher mutation efficiency and more mutants, making it the optimal condition for EMS mutagenesis. EMS concentration and treatment duration are key factors affecting mutagenic efficiency (Qu et al., 2014). Excessively high EMS concentration leads to excessive lethality of mutagenized materials, while excessively low concentration yields poor mutagenic effects. Therefore, appropriate mutagenic conditions are crucial for obtaining ideal mutants with high mutation frequency, broad mutation spectrum, and abundant heritable variations (Xue, 2023). Kang et al. (2024) used EMS mutagenesis to create weak photosensitivity rice germplasm resources, preliminarily demonstrating the feasibility of EMS mutagenesis technology for improving rice photosensitivity and its importance for enriching rice genetic resources and accelerating breeding processes. Fan et al. (2021) constructed a spring rapeseed EMS mutant library to provide phenotypically diverse germplasm resources for rapeseed functional genomics research and molecular breeding. Liu (2022) used EMS mutagenesis of purple-grain wheat to create new wheat germplasm. Ma (2023) created and identified herbicide-resistant wheat mutants based on EMS mutagenesis, obtaining herbicide-resistant mutants that further enriched the genetic basis of herbicide resistance in wheat and promoted breeding of herbicide-resistant wheat varieties. Dong et al. (2006) used EMS mutagenesis to study stem segments of tetraploid potato cultivars, exploring the effectiveness of EMS mutagenesis in potato. These studies demonstrate that EMS mutagenesis is an important method for creating crop mutants. Currently, there are few reports on pea mutants, and using EMS mutagenesis of pea seeds is an important approach for obtaining rich pea mutant materials.

This study used 'Qingjian No. 1' pea as experimental material. Based on the research results of Hao et al. (2012) and preliminary experiments, an EMS concentration of 1% and treatment duration of 8 hours were selected for mutagenesis treatment. Under these conditions, 10,000 seeds of 'Qingjian No. 1' pea were mutagenized and planted (M1). Seeds were harvested from individual plants and planted the following year to record and document field plant mutant phenotypes and agronomic traits. Principal component analysis, correlation analysis, and membership function analysis were performed on various traits to screen for superior mutants. This study not only creates richer pea variation materials but also obtains superior mutant pea germplasm, with results providing a material foundation for functional gene mining and new variety breeding in pea.

Materials and Methods

1.1 Materials and Reagents

The experimental material 'Qingjian No. 1' pea was provided by the Northwest Institute of Plateau Biology, Chinese Academy of Sciences. Ethyl methanesulfonate (EMS) was used as mutagen, and 0.01 $mol \cdot L^{-1}$ phosphate buffer solution was used as mutagenic buffer.

1.2.1 Construction of Pea Mutant Library

This experiment used 10,000 'Qingjian No. 1' pea seeds as material, with 1% EMS treatment for 8 hours as the mutagenic condition. Seeds treated with 0.1 $mol \cdot L^{-1}$ phosphate buffer for 8 hours served as control, with three replicates. After mutagenic treatment, seeds were thoroughly washed and sown in the experimental field using single-seed dibbling, with row length of 2 m, row spacing of 0.25 m, and plant spacing of 0.1 m. Conventional field management was conducted, and plant variations were recorded and counted in detail throughout the experiment. Individual plants were harvested at maturity and sown in single rows in the same experimental field the following year to obtain M2 plants. Mutant and trait investigations were conducted on individual M2 plants.

1.2.2 Investigation of Agronomic Traits

Based on the research results of Gao et al. (2016), relevant main agronomic traits were investigated, including growth period, grains per pod, total pods per plant, and dry grain weight per plant for both M1 and M2 generations. Pea plants were harvested individually at maturity and data were recorded.

1.3 Data Analysis

Excel 2010, SPSS 20.0, and Origin software were used to analyze differences and variations in traits of mutant peas. The membership function fuzzy evaluation method was used for comprehensive evaluation. The membership function

formulas were as follows:

$$\text{Membership value} = \frac{X_{\text{obs}} - X_{\text{min}}}{X_{\text{max}} - X_{\text{min}}}$$

$$\text{Inverse membership value} = 1 - \frac{X_{\text{obs}} - X_{\text{min}}}{X_{\text{max}} - X_{\text{min}}}$$

Where X_{obs} is the observed value, X_{min} is the minimum value of each treatment index, and X_{max} is the maximum value of each treatment index. The membership value formula was used for traits positively correlated with main characteristics, while the inverse membership value formula was used for negatively correlated traits. Mutants were ranked according to the average membership function values.

Results

2.1 Statistical Analysis of Mutant Traits

EMS-mutagenized pea seeds (M1) (1% EMS concentration, 8 h treatment) were sown individually at the Balang experimental site in Xining, Qinghai Province (121°29 21.8 E, 31°24 18.97 N). A total of 4,682 seedlings were obtained, with a survival rate of 46.82%. Agronomic traits of M1 pea plants at various stages were recorded and counted in detail throughout the experiment, and seeds (M2 seeds) were harvested from individual plants at maturity.

The individually harvested M2 pea seeds were sown in single rows. Throughout the pea growth period, phenotypic and agronomic traits of individual plants were recorded in detail, revealing 342 mutant pea plants. The following sections document mutations in different developmental stages and plant parts of M2 mutant pea plants.

2.1.1 Leaf Color Among M2 mutant plants, 7 plants with leaf trait mutations were obtained, accounting for 2.05% of total mutants, mainly showing partial leaf yellowing mutations. Comparison of leaf phenotypes between wild-type and mutant plants revealed that mutant pea leaves exhibited yellowing, though leaf morphology showed no obvious difference from wild-type plants [Figure 1: see original paper].

2.1.2 Flowering Characteristics Among M2 mutant plants, 21 plants with flowering characteristic mutations were obtained, accounting for 6.14% of total mutants. Field observation and statistical analysis of flowering characteristics in wild-type and mutant plants showed that mutant pea plants had essentially consistent plant architecture and height with wild-type plants, with no significant differences. However, mutant plants flowered later than wild-type plants, with delayed full-bloom stage and fewer total flowers per plant [Figure 2: see original paper].

2.1.3 Seeds Seeds were harvested from wild-type and mutant plants that could flower and set fruit. Comparison of seed size between wild-type and mutants revealed that mutant grain diameter was significantly smaller than that of wild-type grains [Figure 3: see original paper].

2.2 Phenotypic Diversity Analysis of Mutant Peas

Comprehensive analysis of data from 342 mutant plants obtained in this study revealed large differences in dry grain weight per plant, total pods per plant, grains per pod, branches per plant, plant height, and total projected area of individual seeds per plant, smaller differences in early flowering stage, full-blossom stage, and fruiting stage, and no differences in seedling stage, branching stage, and mature stage.

Statistical analysis of important agronomic traits in 342 mutant pea germplasm resources was conducted to establish a phenotypic database for the mutant library . At the same sowing stage, pea seedling stage, branching stage, and mature stage were essentially consistent with small variation. However, six main field phenotypic traits showed large variation: dry grain weight per plant, total pods per plant, grains per pod, branches per plant, plant height, and total projected area of individual seeds per plant, with coefficients of variation ranging from 0.099 to 0.965. Dry grain weight per plant showed the maximum coefficient of variation (0.965), with a maximum value of 109.400 g and minimum of 1.860 g. Total pods per plant ranked second, with a maximum of 155 pods, minimum of 1 pod, and coefficient of variation of 0.761. Grains per pod and branches per plant also showed large coefficients of variation at 0.631 and 0.431, respectively, indicating significant variation. In summary, EMS mutagenesis generated rich genetic variation in phenotypic traits of pea mutants, providing a material foundation for breeding new pea varieties.

2.3 Correlation Analysis of Main Agronomic Traits of Mutant Peas

Correlation analysis was performed on main traits of 342 pea mutants from the mutant library. As shown in , early flowering stage showed extremely significant positive correlation with branches per plant, significant positive correlation with full-blossom stage and fruiting stage, but negative correlation with total pods per plant. Plant height showed extremely significant positive correlation with total pods per plant and branches per plant. Total pods per plant showed extremely significant positive correlation with branches per plant and dry grain weight per plant, and correlation with total projected area of individual seeds per plant. Branches per plant showed significant positive correlation with dry grain weight per plant, and dry grain weight per plant also showed significant positive correlation with total projected area of individual seeds per plant, but negative correlation with full-blossom stage. These results indicate that pea is mainly affected by early flowering stage, plant height, and dry grain weight per plant, suggesting that agricultural production could improve pea yield by regulating early flowering stage and breeding tall varieties.

2.4 Principal Component Analysis of Main Traits of Mutant Peas

Principal component analysis was conducted on nine main phenotypic traits of 342 pea mutants. As shown in , five principal components (eigenvalue > 1) were identified, with contribution rates of 52.511%, 16.248%, 12.253%, 10.997%, and 7.991%, respectively, and a cumulative contribution rate of 92.044%. These components comprehensively reflected most information on the nine main traits of 342 pea mutants and could be used for comprehensive evaluation of phenotypic traits in pea mutant germplasm resources. The first principal component had high factor contribution (52.511%) and mainly represented factors related to pea growth period, including early flowering stage, branching stage, full-blossom stage, and fruiting stage. The second principal component (16.248%) mainly represented total pods per plant (0.888) and plant height (0.676). The third principal component (12.253%) mainly represented dry grain weight per plant (0.928). The fourth principal component (10.997%) mainly represented seedling stage (0.979). The fifth principal component (7.991%) mainly represented mean total projected area of individual seeds per plant (0.979).

2.5 Comprehensive Membership Function Analysis of Main Phenotypic Traits of Mutant Peas

Membership function analysis was performed on five important indicators of 342 mutant peas: plant height, total pods per plant, branches per plant, dry grain weight per plant, and total projected area of individual seeds per plant. The average membership function value for each mutant material was calculated, and materials were comprehensively ranked based on these values. The top 10 materials were selected as superior mutants, all belonging to leaf yellowing mutation types accompanied by reduced flower number and decreased grain diameter . These 10 mutant peas showed superior comprehensive traits compared to other mutants, with plant height above 105 cm, total pods per plant exceeding 85, branches per plant above 5, dry grain weight per plant above 60 g, and total projected area of individual seeds per plant above 30 cm^2.

Discussion and Conclusion

Plant seeds are ideal mutagenic materials due to simple operation, ability for large-batch processing, and long-lasting biological effects (Liu et al., 2024). This study selected pea seeds as mutagenic material. Through extensive preliminary experiments, an EMS concentration of 1% and treatment duration of 8 hours were identified as optimal conditions where pea seeds reached semi-lethal mutagenesis, with seed germination rate of 51.16% and survival rate of 46.82%. Therefore, these conditions were selected as the optimal mutagenic conditions for pea mutant library construction.

3.1 Establishment of Pea Mutant Library

Mutagenic treatment induces random mutation sites that may cause phenotypic mutations in various tissues and organs at different developmental stages, with varying mutation frequencies (Fan et al., 2021). Due to the multidirectional nature of chemical mutagenesis, phenotypic variations may occur at various growth stages and in different tissues and organs (Liu et al., 2024). Currently, few reports exist on EMS-induced pea mutant library construction, though many related studies have been conducted in other plants. Fang et al. (2023) found that EMS mutagenesis produced various mutant types in tartary buckwheat, including leaf yellowing, plant dwarfing, compact plant architecture, and grain color changes. Other studies found that EMS mutagenesis of *Isodon lophanthoides* var. *graciliflorus* seeds produced variations in leaf color, leaf shape, and plant architecture (Liu et al., 2024). Li et al. (2024) used EMS mutagenesis on *Andrographis paniculata* seeds and identified 379 mutant lines with morphological trait variations including leaf shape, leaf color, and plant architecture, with significant differences in stress resistance and active component accumulation among mutants. EMS mutagenesis of seed-used pumpkin identified plant architecture mutants, seedless fruit mutants, seed number and size mutants, and pollen abortion mutants (Sun and Wang, 2024). Jung et al. (2003) reported that in rice and other crops, leaf color and plant architecture were two major mutant phenotypes in EMS-induced mutants, with leaf color mutations resulting from mutations in key genes controlling chlorophyll biosynthesis that cause abnormal chlorophyll metabolism and phenotypes such as yellowing, albinism, and bright green. Thus, EMS mutagenesis can cause variations in leaves, plant architecture, and seed morphology, creating diverse mutant types.

Similar to previous research methods, this study treated pea seeds with 1% EMS for 8 hours (semi-lethal condition for pea). After treatment, seeds were sown individually. At maturity, seeds were harvested from individual plants (M1). The harvested M1 seeds were sown in single rows the following year to obtain 342 pea mutant materials in the M2 generation. Observation and recording of agronomic traits of mutant materials revealed obvious variations in leaf color, plant architecture, and grain size. Dry grain weight per plant showed the largest coefficient of variation (0.965), followed by total pods per plant and grains per pod with coefficients of 0.761 and 0.632, respectively. In the M2 mutant library, mutant peas had fewer flowers and lower seed setting rates. Some mutant plants exhibited multiple mutation phenotypes, such as dwarf plants with weak growth, small pods, and few grains. This study constructed a pea mutant library by recording and statistical analysis of mutant traits, obtaining abundant leaf yellowing mutants and plant architecture mutants that provide a material foundation for further investigation of molecular mechanisms underlying pea yellowing and plant architecture mutations.

3.2 Screening of Superior Pea Mutant Materials

This study obtained 342 pea mutants related to leaf color, plant architecture, flowering period, and yield in the M2 generation and constructed a pea mutant library. Through statistical analysis of five important indicators—plant height, total pods per plant, branches per plant, dry grain weight per plant, and total projected area of individual seeds per plant—combined with correlation analysis, principal component analysis, and membership function analysis, 10 superior pea mutant materials were selected. These 10 mutant peas showed superior comprehensive traits compared to other mutants, with plant height above 105 cm, total pods per plant exceeding 85, branches per plant above 5, dry grain weight per plant above 60 g, and total projected area of individual seeds per plant above 30 cm^2. These superior mutant materials can be applied to both functional gene mining and research in pea, as well as to new variety breeding and product development.

This study used 'Qingjian No. 1' pea, widely cultivated on the Qinghai-Tibet Plateau, as experimental material. Through EMS mutagenesis treatment, a pea mutant library was established and superior pea mutant materials were screened, enriching the pea germplasm resource pool. The research results provide a material foundation and reference basis for functional gene development and new variety improvement in pea.

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Note: Figure translations are in progress. See original paper for figures.