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## Research Article

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# Dose-dependent effects of gamma radiation on seed vigor and genetic mutational spectrum in *Brassica juncea* var. *multiceps*

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## Abstract

**Background** Shuidong mustard (*Brassica juncea* var. *multiceps*), a geographically indicative crop, faces production threats from downy mildew. Radiation mutagenesis offers a potent tool for breeding resistant varieties. However, the precise dose-effects of gamma radiation on its seed vigor and genomic stability remain unclear, hindering breeding optimization.

**Results** We exposed Shuidong mustard seeds to a gradient of <sup>60</sup>Co-γ radiation (0–4000 Gy). Low-doses (200–500 Gy) exerted a hormetic effect, enhancing germination potential (85.3% at 200 Gy), root length (3.46 cm, +19.3%), and stress resistance. Genomic analysis revealed this range induced controllable genetic variations, predominantly C→T transitions (42.5%). In stark contrast, high doses (≥1000 Gy) severely inhibited seed vitality, reducing germination to 75.3% and fresh weight by 51.5% at 4000 Gy. Genomic stability was catastrophically compromised, evidenced by decreased Q30 (93.8%), aberrant GC content, and prevalent large-fragment Indels (deletions ≥10 bp accounting for 18.7%). High-dose mutagenesis was characterized by a shift towards G→T transversions (28.4% at 2000 Gy).

**Conclusion** Our study deciphers the dose-dependent phenotypic and genomic landscapes of γ-radiated Shuidong mustard. We establish 200–500 Gy as the optimal mutagenic window, providing a scientific foundation for precision breeding to improve yield and stress resistance. The identified mutational signatures offer valuable insights into the mechanisms of radiation-induced DNA damage in plants.

**Keywords** Shuidong mustard, Radiation mutagenesis, Seed vigor, Genetic effects

## Background

Shuidong mustard (*Brassica juncea* var. *multiceps*) is a high-quality agricultural product unique to the South of China. With its unique taste and excellent quality, it has become a national geographical indication protected product [1]. At present, Shuidong mustard has formed an industrialization

1 model of “base + cooperative + farmer”, with a planting area of over 40000 acres and an annual  
2 output of 80000 tons in the Dianbai area, becoming a pillar industry in the main vegetable producing  
3 areas.

4 However, despite the thriving development of the mustard industry in Shuidong, it still faces  
5 multiple challenges. Shuidong mustard is still mainly planted outdoors, and its production is easily  
6 affected by weather conditions [2], especially during periods of heavy rain and high air humidity in  
7 spring and summer. The pathogen of downy mildew is prone to infect the leaves and colonize,  
8 forming white mold like sporangia and sporangial stalks [3]. This disease can cause symptoms such  
9 as tissue swelling or deformity in plants, resulting in significant yield reduction. Therefore, in order  
10 to improve mustard's stress resistance, it is urgent to accelerate the breeding process of mustard and  
11 cultivate new strains that are resistant to high temperature, humidity, as well as diseases and pests.

12 Radiation-induced breeding can induce genetic variations in organisms using X-rays, gamma rays,  
13 and other methods, thereby facilitating the development of new varieties [4, 5]. Among them,  
14 gamma rays are characterized by the highest energy and shortest wavelength, marking them the  
15 most commonly used mutagens in breeding [6, 7]. It can not only changes in genetic material, but  
16 also causes chromosomal aberrations [8], thereby laying the material foundation for screening  
17 mutants with target traits. Due to their advantages of cleanliness, environmental friendliness, easy  
18 dose control, and wide mutation spectrum,  $^{60}\text{Co}$ - $\gamma$  rays have been widely applied in the field of  
19 mutagenesis breeding. They have been successfully used in breeding research on crops such as  
20 *Sesame* [9], *Sunflower* [10], *Alfalfa* [11], and *Syringa* [12].

21 Seed vitality is a core indicator of seed quality, containing germination rate, development speed,  
22 seedling uniformity, and seedling growth potential [13], among which germination rate is one of the  
23 most important parameters in seed vitality research. The 2,3,5-triphenyltetrazolium chloride (TTC)  
24 method is an internationally recognized and effective method for determining seed vitality. Its  
25 principle is based on the ability of dehydrogenases in viable seed embryo cells to reduce colorless  
26 TTC solution in an oxidized state to insoluble red triphenylformazan (TF) [14]. Therefore, seed  
27 vitality can be determined by observing the staining status of the embryo. This method has been  
28 widely used in the vitality detection of plants such as peach seeds and fir seeds.

29 It is crucial to study the genetic stability of seeds exposed to gamma radiation. Gamma radiation  
30 can cause different types and degrees of damage to seed DNA, and the seed's own DNA repair  
31 mechanism can cope with these damages. By using molecular marker techniques such as single  
32 nucleotide polymorphism (SNP) [15, 16], the genomic polymorphism of irradiated offspring can be  
33 analyzed, and gene marker annotation can be used to evaluate the genetic diversity changes and  
34 genetic stability of Shuidong mustard seeds induced by radiation.

35 However, a comprehensive study integrating phenotypic vigor assessments with high-throughput  
36 genomic sequencing to decipher the dose-dependent effects of gamma radiation on Shuidong  
37 mustard is still lacking. This study is the first to systematically bridge this gap. We employed a  
38 combination of germination tests, TTC staining, and whole-genome resequencing (SNP/Indel  
39 analysis) to: (1) quantify the physiological and biochemical responses of seeds to a gradient of  
40 radiation doses; (2) characterize the resulting mutational spectra and genome stability; and (3)  
41 identify the optimal radiation dose window for mutagenesis breeding in Shuidong mustard. Our  
42 work provides a mechanistic understanding and a practical framework for accelerating the breeding  
43 of this economically important crop.

## **Materials and methods**

### **Plant materials**

The seeds of Shuidong Mustard were provided by Dianbai Shuidong Mustard Development Co., Ltd. A certain number of plump and uniformly sized seeds were selected and packaged in separate sealed bags. These seeds were then subjected to  $^{60}\text{Co}$ - $\gamma$  radiation treatment at six different doses (0, 200, 500, 1000, 2000, 4000 Gy) at the Institute of Crop and Nuclear Technology Utilization, Zhejiang Academy of Agricultural Sciences, followed by refrigerated storage.

### **Experimental methods**

#### **TTC staining method**

2,3,5-triphenyltetrazolium chloride (TTC) solution is purchased from Beijing Biolab Technology Co., Ltd. Before TTC staining, five round, plump, uniformly sized, and undamaged seeds of Shuidong mustard should be selected from each radiation gradient, along with five seeds exhibiting heat-induced germination disorders to serve as controls. The seeds to be tested are soaked in a 75% ethanol solution for sterilization, then transferred to test tubes containing 1 mL of tap water and soaked at 30°C for 24 h. This allows the seeds to absorb water and soften, causing the seed coats to expand and fall off naturally, thereby exposing the embryos. If the seed coats do not crack spontaneously, they can be removed by puncturing or squeezing to extract intact embryos.

Place the processed seeds from each group into separate 5 mL centrifuge tubes and add 1 mL of TTC staining solution to ensure complete immersion of the seeds. Subsequently, incubate the centrifuge tubes in a 30°C water bath for 1 h to accelerate the reaction. Once the reaction is complete (i.e., when obvious coloration is observed), decant the TTC solution and rinse the seeds 2-3 times with distilled water. After removing the seeds and arranging them neatly, immediate comparative observation is necessary to prevent fading.

#### **Seed germination test**

To investigate the effect of gamma radiation on the agronomic traits of Shuidong mustard seeds, cut filter paper (9.5 cm  $\times$  9.5 cm) was spread flat in the center of square Petri dishes (10 cm  $\times$  10 cm  $\times$  1.5 cm), with 15 mL of tap water added to each dish to fully saturate the filter paper. For each specific radiation dose, 150 round, plump, undamaged, and uniformly sized Shuidong mustard seeds were selected. These seeds were disinfected with a 75% ethanol solution for 20 min and then rinsed 5 times with laboratory distilled water.

Three groups were used as parallel experimental units, with 50 Shuidong mustard seeds from each group inoculated onto moist filter paper. After covering the Petri dishes with lids, they were placed in an artificial climate chamber at 25°C with 80% humidity and 5000 Lx light exposure for 7 days. During this period, the seeds were observed three times daily (morning, midday, and evening), and any moldy or rotten seeds were removed. The number of germinated seeds was recorded, with germination defined as embryonic root emergence of at least 1.0 mm. On the 7th day of cultivation, 10 seedlings were randomly selected from each group to measure root length, stem length, and fresh weight.

#### **The resequencing method of Shuidong mustard seedlings**

##### **Sequencing process of Illumina**

The experimental procedure for Shuidong mustard seedling samples treated with different radiation doses is as follows: first, genomic DNA is extracted from the samples and subjected to quality

assessment; subsequently, ultrasonic fragmentation technology is used to randomly break the long strands of intact genomic DNA into fragments ranging from 200 bp to 500 bp; next, the fragmented DNA is subjected to pre-processing steps such as end repair, 3'-tail addition, and sequencing adapter ligation to complete the construction of the sequencing library. In the process of library construction, a pre-experiment stage for ligation products should be established up to verify adapter binding efficiency and ensure the quality of library construction.

After completing the above preprocessing steps, magnetic bead purification technology should be used to specifically screen for target fragments ranging from 200 bp to 500 bp, thereby removing free adapters and non-target fragments that are not effectively ligated. Following screening, the obtained library is subjected to PCR amplification, which not only increases the library concentration but also optimizes the library structure. After amplification, further tests on the library's concentration, fragment size distribution, and integrity are required to ensure it meets the sequencing requirements.

Finally, library DNA that meets the standards is immobilized on the surface of the sequencing flow cell and amplified in situ using PCR technology to form monoclonal DNA clusters. During the sequencing stage, the first-end sequence of the DNA fragment (i.e., Read 1) is read first, followed by the reverse-end sequence (i.e., Read 2) by adjusting the orientation of the clusters, thereby achieving paired-end sequencing. This paired-end sequencing strategy can effectively improve sequence reading accuracy and assembly quality, providing a reliable data foundation for subsequent bioinformatics analysis.

### **Bioinformatics analysis process of resequencing of Shuidong mustard seedlings**

Firstly, quality control and filtering are performed on the raw sequencing data (Reads), including removing adapter sequences and eliminating a large number of unknown bases (N) or low-quality Reads; Subsequently, data quality is validated by evaluating indicators such as fragment distribution, coverage, base quality, and nucleotide type distribution. Next, the filtered Reads are aligned to the reference genome, and alignment efficiency and sequencing depth are calculated to ensure uniform data coverage. On this basis, single nucleotide polymorphisms (SNP) and small-fragment insertions-deletions (INDEL) are detected. The biological significance of these variations is then analyzed through functional annotation, ultimately providing reliable genomic variation information for genetic research.

### **Data Processing**

#### **Descriptive statistics**

Use Microsoft Excel to process data on seedling height, root length, fresh weight, germination rate, and survival rate of Shuidong mustard. All data are analyzed for significance using IBM SPSS Statistics software and the results are labeled in the graph.

#### **Excel spreadsheet processing of seed vitality indicators**

Both germination vigor and germination rate are extremely important indicators of seed vitality and quality. Germination vigor emphasizes the ability of seeds to germinate within a relatively short period of time, mainly reflecting the vitality of seeds and the degree of neatness during germination; the germination rate emphasizes the long-term germination ability of the seed, which reflects the overall vitality and quality of the seed. The specific calculation formulas are as follows:

$$\text{Germination rate} = \frac{T_1}{T_{\text{tot}}} \times 100\%$$

$T_1$ : seed germination number mainly selected the number of germination recorded on the evening of 7th day;  $T_{\text{tot}}$ : total number of test seeds selected in the initial period

$$\text{Germination vigor} = \frac{T_2}{T_{\text{tot}}} \times 100\%$$

$T_2$ : seed germination number mainly selected the number of germination recorded on the evening of 3th day;  $T_{\text{tot}}$ : total number of test seeds selected in the initial period

The germination index is calculated by weighting the number of germinated seeds per day, and it is a commonly used indicator to evaluate the speed and uniformity of seed germination.

$$GI(\text{Germination index}) = \sum_{t=1}^n \frac{G_t}{D_t}$$

$G_t$ : the number of germinated seeds on day  $t$

$D_t$ : the corresponding number of germination days (days)

$n$ : the total number of days for the specified germination test

Seedling growth vigor belongs to the quantitative indicators of seedling morphology or biomass, where the biomass indicators mentioned include indicators such as root length, stem length, fresh weight, and so on. It focuses on reflecting the growth status and robustness of seedlings during the 7-day period after seed germination. In practical applications, it is often used to evaluate the quality of seed vitality and the potential of field seedlings.

$$\text{Seedling growth power index} = a \times \text{Root length} + b \times \text{Stem length} + c \times \text{Fresh weight}$$

When reviewing research literature on plant stress resistance, the weight coefficients " $a=0.4$ ,  $b=0.3$ ,  $c=0.3$ " were selected to study the stress resistance of Shuidong mustard.

The seed vitality index in this study refers to the comprehensive adaptability of seeds to adverse environments; therefore, root length is selected as the main object of research and analysis.

$$VI(\text{Vitality index}) = \text{Root length} \times \text{Germination rate}$$

By organizing and calculating raw data using Microsoft Excel to, seed germination kinetics parameters such as germination rate and germination vigor can be obtained.

## Seed TTC detection image quantization processing

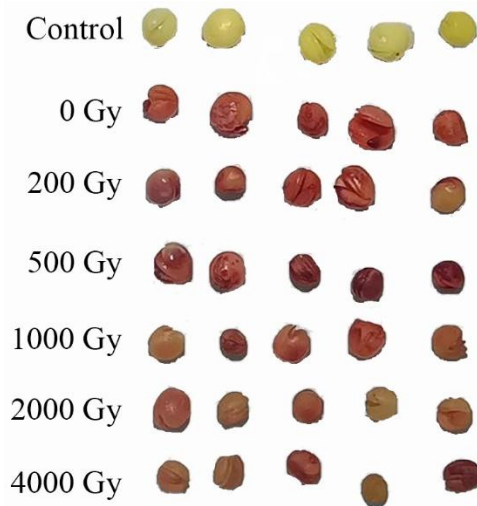
This study used Image Pro Plus 7.0 software to perform  $L^*a^*b^*$  color space analysis, with a focus on extracting  $a$ -value as a quantitative indicator of seed vitality. In the  $L^*a^*b^*$  color model,  $a$ -value represents the red-green axis parameter: the larger the positive value, the darker the red color, indicating stronger seed vitality and more vigorous metabolic activity; the smaller the positive value, the lighter the red color, indicating poorer seed vitality and weaker metabolic activity; negative values reflect a green or unstained signal, indicating that the seed has died. By comparing the average  $a$ -values of the control group (boiled seeds with no vitality) with different radiation dose groups, the dose effect of gamma rays on seed metabolic activity can be quantitatively analyzed, while avoiding errors caused by human factors.

1 **Results**

2 **Effects of different <sup>60</sup>Co-γ radiation doses on seed vitality of Shuidong mustard**

3 From Figure 1 and Table 1, it can be seen that different doses of <sup>60</sup>Co-γ radiation had varying degrees  
4 of impact on the a-value. The a-value of dead seeds was -3.57, which is significantly negative, thus  
5 excluding background interference. The a-value of the control group (0 Gy) was as high as 33.39,  
6 indicating that the non-irradiated seed embryos had the deepest staining and the strongest metabolic  
7 activity. As the radiation dose increased from 200 Gy to 4000 Gy, the a-value gradually decreased,  
8 suggesting that as the radiation dose increased, the seed vitality gradually decreased. Among these  
9 groups, the average a-value of the 200 Gy group was 26.29, a decrease of 21.3% compared to the  
10 control group, indicating that low-dose radiation slightly inhibited seed vitality. The 4000 Gy group  
11 had the lowest a-value of 17.92, a 46.3% decrease relative to the control group, indicating that high-  
12 dose radiation caused nearly half of the damage to seed vitality.

13 In addition, at low doses (≤ 1000 Gy), the decrease in a-value was relatively gradual, which might  
14 be due to the mild oxidative stress-induced by radiation activating cellular repair mechanism. At high  
15 doses (≥ 2000 Gy), the a-value decreased sharply, presumably due to its close association with  
16 DNA-double strand breaks, excessive free radical accumulation and mitochondrial dysfunction.  
17 Therefore, the results indicate that low-dose radiation (≤ 1000 Gy) has a significant promoting effect  
18 on the target process, whereas high-dose radiation (≥ 2000 Gy) may induce inhibitory effects.



19 **Fig. 1** The effect of different <sup>60</sup>Co-γ radiation doses on the staining degree of Shuidong  
20 mustard seeds  
21

22 **Table 1** Effects of different <sup>60</sup>Co-γ radiation doses on seed vitality (a-value) of Shuidong mustard

| Radiation<br>concentration | Blank<br>control<br>group | 0Gy   | 200Gy   | 500Gy   | 1000Gy  | 2000Gy  | 4000Gy  |
|----------------------------|---------------------------|-------|---------|---------|---------|---------|---------|
| a-value                    | -3.57                     | 33.39 | 26.29   | 24.62   | 23.97   | 18.64   | 17.92   |
| Change<br>rate/%           | /                         | /     | -21.26% | -26.27% | -28.21% | -44.17% | -46.33% |

# Effects of different <sup>60</sup>Co-γ radiation doses on seed germination of Shuidong mustard

According to Table 2 and Fig. 2, different <sup>60</sup>Co-γ radiation doses exerted varying effects on the germination of Shuidong mustard seeds. Overall, the germination vigor and germination rate of Shuidong mustard seeds exposed to gamma radiation remained relatively high. Among these groups, it was found that the germination vigor and germination rate of Shuidong mustard seeds exposed to 500 Gy radiation reached their peak, at 90.67% and 96.00% respectively, indicating that low-dose (≤ 500 Gy) gamma ray radiation doses have a certain promoting effect on the germination and growth of Shuidong mustard seeds. However, as the gamma radiation doses continued to increase, the germination vigor and germination rate of Shuidong mustard seeds decreased significantly, with the lowest dropping to 73.33%, indicating that high-dose (≥ 1000 Gy) gamma radiation doses inhibit the germination and growth of Shuidong mustard seeds.

The germination index of Shuidong mustard seeds exposed to 200 Gy and 500 Gy radiation was relatively high, with values of 75.59 and 74.29, respectively. This indicates that low-dose (≤ 500 Gy) gamma radiation doses can promote the rapid germination of Shuidong mustard seeds, enabling them to sprout and grow quickly in the early stages and shortening the plant's growth cycle. However, as the radiation dose continued to increase, the germination index of the seeds decreased significantly, reaching a minimum of 55.97. This phenomenon indicates that high doses (≥ 1000 Gy) of gamma ray radiation can inhibit the germination and growth rate of Shuidong mustard seeds, which is unfavorable for their rapid germination.

At the same time, the germination uniformity of Shuidong mustard seeds under radiation doses of 200 Gy and 500 Gy was relatively high, which is beneficial for maintaining good consistency in subsequent field growth of the plant. However, as the radiation dose continued to increase, the germination uniformity of Shuidong mustard seeds showed a significant decrease, indicating that high-dose gamma ray radiation caused considerable damage to the uniformity of the seed population.

According to a comprehensive analysis of the growth potential and vitality index of Shuidong mustard seedlings, seeds exposed to 200 Gy and 500 Gy radiation exhibited higher growth potential and vitality index. Among these, the growth potential and vitality index of seeds exposed to 200 Gy radiation reached as high as 3.304 and 1.698, respectively. This indicates that low-dose (≤ 500 Gy) gamma ray radiation is beneficial for the germination and growth of Shuidong mustard seeds, which will facilitate the breeding of stress resistance in Shuidong mustard in later research. As the radiation dose increased, however, the growth potential and vitality index of seeds decreased significantly, with the lowest values dropping to 0.478 and 0.473, respectively, suggesting that high-dose (≥ 1000 Gy) gamma ray radiation inhibits the germination of Shuidong mustard seeds and is not conducive to their stress resistance (Table 2).

Table 2 Effects of different <sup>60</sup>Co-γ radiation doses on seed germination of Shuidong mustard

| Radiation doses | Germination rate /%      | Germination vigor/%      | Germination index        | Vitality index          | Seedling growth vigor  |
|-----------------|--------------------------|--------------------------|--------------------------|-------------------------|------------------------|
| 0Gy             | 87.33±3.06 <sup>ab</sup> | 85.33±2.31 <sup>ab</sup> | 69.25±5.35 <sup>ab</sup> | 2.53±0.21 <sup>b</sup>  | 1.49±0.02 <sup>a</sup> |
| 200Gy           | 88.00±7.21 <sup>ab</sup> | 85.33±8.33 <sup>ab</sup> | 75.59±9.79 <sup>a</sup>  | 3.03±0.02 <sup>ab</sup> | 1.71±0.14 <sup>a</sup> |
| 500Gy           | 96.00±3.46 <sup>a</sup>  | 90.67±6.11 <sup>a</sup>  | 74.29±6.10 <sup>a</sup>  | 3.30±0.87 <sup>a</sup>  | 1.70±0.32 <sup>a</sup> |
| 1000Gy          | 87.33±3.06 <sup>ab</sup> | 85.33±3.06 <sup>ab</sup> | 68.36±1.63 <sup>ab</sup> | 2.49±0.22 <sup>b</sup>  | 1.47±0.06 <sup>a</sup> |
| 2000Gy          | 85.33±5.77 <sup>ab</sup> | 83.33±5.77 <sup>ab</sup> | 63.77±5.16 <sup>ab</sup> | 1.54±0.13 <sup>c</sup>  | 1.00±0.06 <sup>b</sup> |
| 4000Gy          | 75.33±14.05 <sup>b</sup> | 73.33±14.05 <sup>b</sup> | 55.97±10.50 <sup>b</sup> | 0.48±0.11 <sup>d</sup>  | 0.47±0.04 <sup>c</sup> |

Note: The different letters marked in each column indicate significant differences between groups in LSD analysis ( $P < 0.05$ )

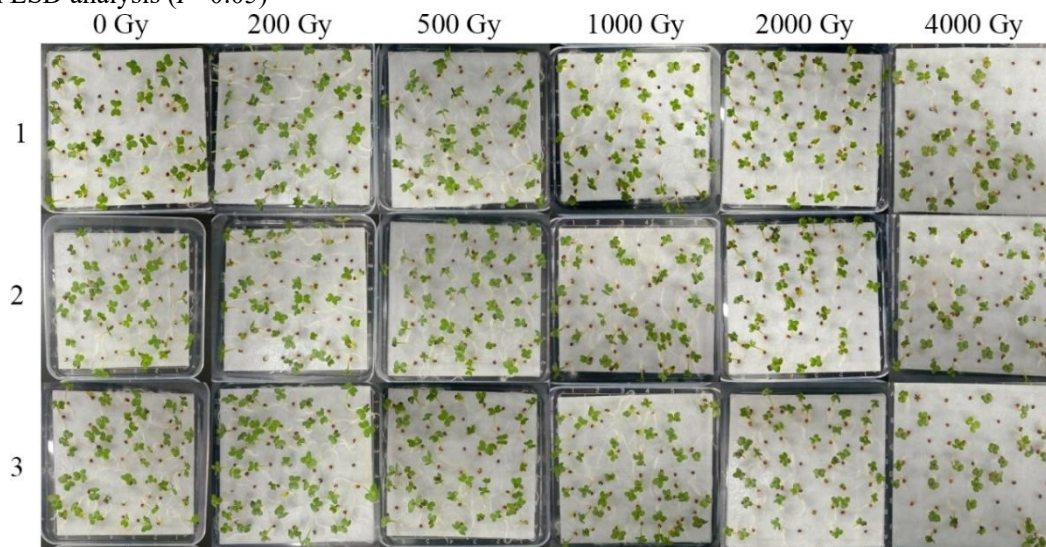


Fig.2 Effects of different  $^{60}\text{Co}$ - $\gamma$  Radiation Dose on Seed Germination of Shuidong Mustard

## Effects of different $^{60}\text{Co}$ - $\gamma$ radiation doses on the growth of Shuidong mustard seeds and seedlings

### The effect of different radiation doses on seedling root length

According to Table 3, after treatment with low-dose radiation (200 Gy, 500 Gy), the root length increased to  $3.46 \pm 0.30$  cm and  $3.43 \pm 0.88$  cm, respectively, which were significantly higher than that of the control group ( $2.90 \pm 0.14$  cm) ( $P < 0.05$ ). This indicates that low-dose radiation may promote root cell division or elongation by inducing plant stress response. However, when the radiation dose reached 1000 Gy, the root length decreased to  $2.85 \pm 0.15$  cm, which was similar to that of the control group. The root length of the 2000 Gy and 4000 Gy treatment groups decreased sharply to  $1.80 \pm 0.14$  cm and  $0.63 \pm 0.06$  cm, respectively, accounting for only 62.1% and 21.7% of the control group. This indicates that high-dose radiation has an inhibitory effect on roots growth, which may be related to the exacerbation of DNA damage and the inhibition of cell division.

### The effect of different radiation doses on seedling stem length

According to Table 3, the sensitivity of stem length to radiation was lower than that of root length. The stem length of the 200 Gy to 1000 Gy treatment groups remained between  $1.06 \pm 0.07$  cm and  $1.07 \pm 0.11$  cm, with no significant difference compared to the control group ( $1.06 \pm 0.13$  cm) ( $P > 0.05$ ). However, the stem length of the high-dose radiation treatment groups (2000 Gy and 4000 Gy) decreased to  $0.92 \pm 0.04$  cm and  $0.72 \pm 0.08$  cm, respectively, representing a decrease of 13.2% and 32.1% compared to the control group. This indicates that stem growth exhibits a strong tolerance to radiation and is only significantly inhibited at extremely high doses, which may be related to the lower metabolic rate or stronger repair capacity of stem cells.

### The effect of different radiation doses on the fresh weight of seedlings

According to Table 3, fresh weight, as a comprehensive growth indicator, exhibited a continuous downward trend with increasing radiation dose. The fresh weight of the 200 Gy treatment group was  $0.033 \pm 0.005$  g, representing a 16.1% decrease compared to the control group. The fresh weight of the 500 Gy and 1000 Gy treatment groups further decreased to  $0.029 \pm 0.005$  g and  $0.027 \pm 0.007$  g, respectively.

g, respectively. At doses of 2000 Gy and 4000 Gy, the fresh weight was only  $0.022 \pm 0.006$  g and  $0.019 \pm 0.005$  g, respectively, corresponding to a 43.6% and 51.5% decrease compared to the control group. This trend indicates that radiation inhibits photosynthesis, damages cell membrane structures, or reduces water absorption efficiency, thereby hindered biomass accumulation.

Table 3 Effects of different  $^{60}\text{Co}$ - $\gamma$  radiation doses on the growth of Shuidong mustard seedlings

| Radiation doses | Root length/cm    | Stem length/cm    | Fresh weight/g       |
|-----------------|-------------------|-------------------|----------------------|
| 0 Gy            | $2.90 \pm 0.14^a$ | $1.06 \pm 0.13^a$ | $0.0392 \pm 0.002^a$ |
| 200 Gy          | $3.46 \pm 0.30^a$ | $1.07 \pm 0.07^a$ | $0.0329 \pm 0.005^b$ |
| 500 Gy          | $3.43 \pm 0.88^a$ | $1.06 \pm 0.16^a$ | $0.0286 \pm 0.005^b$ |
| 1000 Gy         | $2.85 \pm 0.15^a$ | $1.07 \pm 0.11^a$ | $0.0267 \pm 0.007^b$ |
| 2000 Gy         | $1.80 \pm 0.14^b$ | $0.92 \pm 0.04^a$ | $0.0221 \pm 0.006^c$ |
| 4000 Gy         | $0.63 \pm 0.06^c$ | $0.72 \pm 0.08^b$ | $0.0190 \pm 0.005^c$ |

Note: Different letters indicate significant differences in LSD analysis ( $P < 0.05$ )

### Genetic Effects Analysis of Shuidong Mustard Seedlings

After obtaining genomic data of each group of seedlings using high-throughput sequencing technology, a systematic evaluation was conducted from three aspects: sequencing data volume, data quality, and base composition. This evaluation aimed to reveal the impact of radiation dose on DNA integrity and sequencing stability.

### Quality analysis of sequencing data

Clean\_Reads (effective read count) and Clean\_Base (effective base number) are core indicators for measuring sequencing data volume. Table 4 showed that the mean Clean\_Reads of the 0 Gy group (Group A) was 47,781,854, and the Clean\_Base was  $14.24 \times 10^9$ , indicating stable data output. As the radiation dose increased, the data volume exhibited non-monotonic fluctuations: the mean Clean\_Reads of the 200 Gy group (Group B) increased to 51,251,227, which might be related to the activation of DNA repair mechanisms by low-dose ( $\leq 500$  Gy) radiation. However, at doses above 1000 Gy (Group D), the data volume decreased significantly. For example, the mean Clean\_Reads of the 4000 Gy group (Group F) decreased to 47,137,503, a decrease of approximately 7.3% compared to the 0 Gy group. This indicates that high-dose radiation ( $\geq 1000$  Gy) may induce DNA double-strand breaks or crosslinks, reduce template integrity, and lead to a decrease in sequencing library construction efficiency.

Q20 (the ratio of base with  $\geq 99\%$  accuracy) and Q30 (the ratio of base with  $\geq 99.9\%$  accuracy) are key parameters for evaluating sequencing accuracy. Overall, the Q20 values of all radiation groups excelled 97.4%, and the Q30 values excelled 92.2%, indicating that the sequencing quality was generally controllable. However, as the dose increased, the quality showed a gradient decreasing trend: the Q20 values of the low-dose group ( $\leq 500$  Gy) remained stable at 98.2%–98.8%, and the Q30 values reached 94.9%–96.5%, indicating a lower degree of DNA damage and no significant increase in sequencing error rate. Both the Q20 and Q30 values of the high-dose group ( $\geq 1000$  Gy) showed a significant decline. For example, the mean Q20 value of the 4000 Gy group (Group F) was 97.7% (with the sample F2 as low as 97.62%), and the mean Q30 value was 93.8%, which were 0.5 and 1.6 percentage points lower than those of the 0 Gy group, respectively. This

phenomenon may be related to gamma ray-induced base oxidation (e.g., 8-oxoguanine formation) and the accumulation of single-strand breaks, which are prone to misreading during PCR amplification or sequencing, thereby leading to a decrease in quality score.

The GC content (%) reflects the characteristics of genomic base composition, and its abnormal fluctuations may indicate DNA structural damage or selective degradation. The GC content of the low-dose groups ( $\leq 500$  Gy) remained stable at 37.72%–39.53%, with no significant difference compared to the non-irradiated group (Group A with an average of 38.0%), indicating that the overall stability of the genome was not compromised. In contrast, the GC content in the high-dose groups ( $\geq 1000$  Gy) showed a polarized trend. For example, the GC content of sample E4 in the 2000 Gy group (Group E) reached as high as 40.9%, while the GC content of sample F4 in the 4000 Gy group (Group F) soared to 44.15%, far exceeding the normal range (38%–40%). It is speculated that high-dose radiation may lead to preferential breakage of AT-enriched regions (such as telomeres or repetitive sequences), resulting in a relative increase in GC content. Meanwhile, some samples (e.g., F5) exhibited a decrease in GC content to 38.61%, which may be related to the selective loss of GC-rich regions after DNA fragmentation.

Therefore, these results indicate that the effect of  $^{60}\text{Co}$ - $\gamma$  radiation on the genome of Shuidong mustard is dose-dependent: low-dose radiation (0 Gy–500 Gy) causes slight DNA damage, but the sequencing data quality remains stable, making it suitable for constructing a mutagenesis breeding library. In contrast, high-dose radiation ( $\geq 1000$  Gy) significantly reduces DNA integrity.

Table 4 Statistics of Sample Coverage Ratio

| Radiation doses | Sample_ID | Q20(%) | Q30(%) | GC(%) |
|-----------------|-----------|--------|--------|-------|
| 0Gy             | A1        | 98.20  | 94.96  | 38.00 |
|                 | A2        | 98.29  | 95.23  | 37.72 |
|                 | A3        | 98.24  | 95.04  | 38.09 |
|                 | A4        | 97.40  | 92.21  | 38.16 |
|                 | A5        | 98.08  | 94.65  | 39.53 |
| 200Gy           | B1        | 98.78  | 96.37  | 38.76 |
|                 | B2        | 98.76  | 96.30  | 38.81 |
|                 | B3        | 98.81  | 96.50  | 39.12 |
|                 | B4        | 98.72  | 96.12  | 38.69 |
|                 | B5        | 98.78  | 96.38  | 38.46 |
| 500Gy           | C1        | 98.83  | 96.53  | 38.60 |
|                 | C2        | 98.78  | 96.39  | 39.26 |
|                 | C3        | 98.75  | 96.26  | 39.11 |
|                 | C4        | 98.71  | 96.19  | 38.20 |
|                 | C5        | 98.76  | 96.29  | 38.54 |
| 1000Gy          | D1        | 98.71  | 96.14  | 38.92 |
|                 | D2        | 98.32  | 95.29  | 37.65 |
|                 | D3        | 98.68  | 96.06  | 39.42 |
|                 | D4        | 98.56  | 95.75  | 40.97 |
|                 | D5        | 98.21  | 94.93  | 41.65 |
| 2000Gy          | E1        | 97.87  | 94.09  | 38.32 |
|                 | E2        | 98.51  | 95.45  | 41.34 |

|        |    |       |       |       |
|--------|----|-------|-------|-------|
| 4000Gy | E3 | 98.79 | 96.41 | 39.74 |
|        | E4 | 98.90 | 96.78 | 40.90 |
|        | E5 | 98.92 | 96.85 | 39.94 |
|        | F1 | 98.40 | 95.54 | 42.24 |
|        | F2 | 97.97 | 93.94 | 41.56 |
|        | F3 | 97.82 | 93.90 | 40.63 |
|        | F4 | 97.62 | 93.26 | 44.15 |
|        | F5 | 97.81 | 93.81 | 38.61 |

Note: A-F represent radiation doses of 0, 200, 500, 1000, 2000, and 4000 Gy, respectively. Numbers 1-5 represent different individual plants

### SNP variation analysis

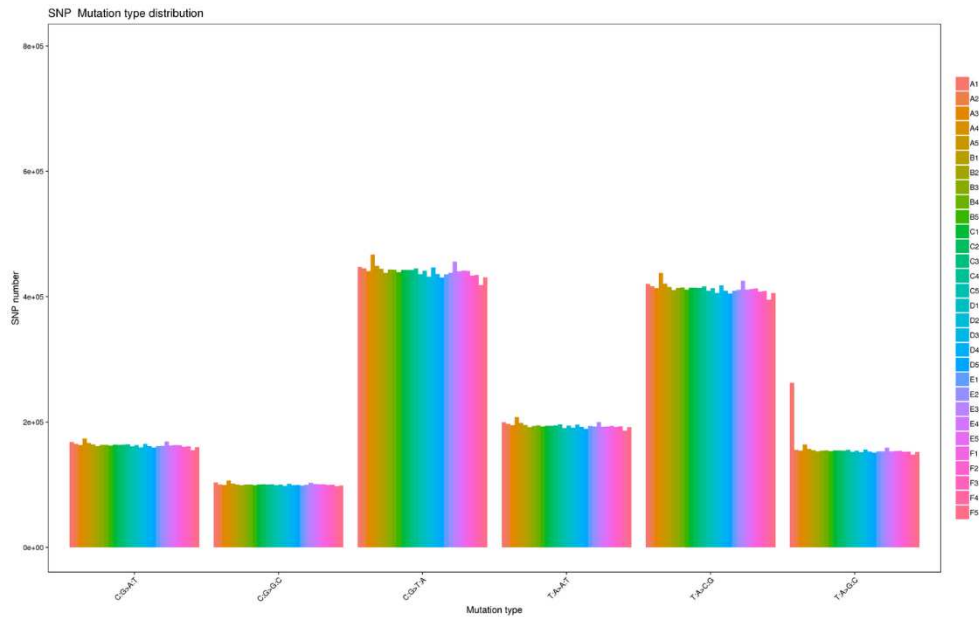
This study applied gradient dose radiation (0 Gy–4000 Gy) to Shuidong mustard seeds using  $^{60}\text{Co}$ - $\gamma$  rays, with 5 biological replicates per group (each replicate representing an individual plant). Combined with high-throughput sequencing technology, the distribution characteristics of single nucleotide polymorphism (SNP) mutation types and their association with radiation dose were systematically analyzed. As a core genetic marker for radiation mutagenesis, SNP type preference and frequency changes can reflect the effectiveness of DNA damage repair mechanisms and the biological effects of radiation.

As shown in Figure 3, in the non-irradiated group (0 Gy), SNP mutations were dominated by transitions, accounting for 72.3%. Among these, C→T and G→A transitions were the most common, which might be related to spontaneous deamination. Transversions accounted for 27.7%, with A→T and T→G being the main types. Synonymous mutations accounted for 64.2% of total SNPs, missense mutations accounted for 32.7%, and nonsense mutations for only 3.1%, indicating that natural mutations are mostly preserved through neutral selection. As the radiation dose increased, the total frequency of SNP mutations exhibited a "first increasing then decreasing" trend: SNP density in the low-dose groups ( $\leq 500$  Gy) increased significantly compared to the control group, with the average SNP frequency in the 200 Gy group increasing by 50%. At this stage, mutations were mainly characterized by C→T transitions and G→T transversions, which may be related to insufficient repair efficiency and base oxidation induced by gamma rays. The SNP frequencies of the 1000Gy and 2000G groups reached their peak, and the proportion of G→T transversions increased significantly. For example, the proportion of G→T transversions in the 2000Gy group reached 24.7%, indicating that high-dose radiation activates error-prone repair mechanisms by inducing double strand breaks. The sharp decrease in SNP frequency in the ultra-high dose group (4000 Gy) may be due to excessive DNA damage leading to repair system collapse or cell apoptosis.

The distribution of mutation types exhibits a significant dose-dependent pattern. Low-dose radiation (200–500 Gy) significantly increased the frequency of C→T transitions, especially in CpG island-enriched regions, which may be associated with increased cytosine deaminase activity and epigenetic regulation. The frequency of G→T transversions showed a strong positively correlation with radiation dose, accounting for 28.4% in the 2000 Gy group. Such mutations were mostly caused by 8-oxoguanine mismatches and were significantly correlated with decreased antioxidant enzyme activity. The proportion of missense mutations increased significantly at doses above 1000 Gy, with enrichment primarily in stress-related genes (e.g., the heat shock protein gene *BnHSP70*). These

1 mutations may enhance plant stress resistance by altering protein structure, but excessive mutations  
2 could lead to functional proteins inactivation. It is worth noting that mutation heterogeneity exists  
3 among samples within the same dose group. For example, sample B3 in the 200 Gy group exhibited  
4 an abnormally high C→T transition frequency (51.2%), which was related to its low expression of  
5 DNA glycosylases. The G→T transversions in sample E4 of the 2000 Gy group were enriched in  
6 the mitochondrial genome, indicating that mitochondrial DNA is more susceptible to radiation  
7 damage. In the 4000 Gy group, sample F2 showed a sharp decrease in the SNP frequency, with the  
8 proportion of synonymous mutations as high as 78.3%, which may be attributed to the  
9 downregulation of tolerance to extreme damage due to reduced global translation efficiency.

10 Overall,  $^{60}\text{Co}$ - $\gamma$  radiation regulates the distribution of SNP mutation types in Shuidong mustard  
11 through a dose-dependent mechanism. Low-dose preferentially induces C→T transitions, which  
12 may be utilized for targeted modification of the epigenome; high-doses radiation drives the  
13 evolution of stress resistance through G→T transversions and missense mutations.



14 Fig.3 Distribution of SNP detection in Shuidong mustard sequencing under different doses of  
15  $^{60}\text{Co}$ - $\gamma$  radiation  
16 Note: A-F represent radiation doses of 0, 200, 500, 1000, 2000, and 4000 Gy, respectively. Numbers 1-5  
17 represent different individual plants  
18

### 19 Indel variation analysis with different radiation doses

20 This study used  $^{60}\text{Co}$ - $\gamma$  rays to irradiate the Shuidong mustard seeds with gradient dose radiation (0  
21 Gy–4000 Gy), and combined with high-throughput sequencing technology, systematically analyzed  
22 the length distribution characteristics of insertion/deletion (Indel) and its correlation with radiation  
23 dose. As an important type of genomic structural variation, the length and distribution patterns of  
24 Indel can reflect the impact of radiation on DNA strand breakage and repair mechanisms.

25 As shown in Fig. 4, Indel lengths in the non-irradiated group (Group A) were mainly concentrated  
26 in small-fragment deletions of 1–3 bp, accounting for 68.4% of the total Indel events, with 1 bp  
27 deletions having the highest proportion (42.7%). This may be related to sliding mismatches during  
28 DNA replication. The proportion of insertion events was relatively low (21.3%), and their lengths  
29 were mostly concentrated in 1–2 bp, indicating that the Indel formation mechanism under natural

conditions tends to be a repair process relying on small microhomology fragments. With increasing radiation dose, significant changes were observed in the length distribution of Indel: the total number of Indel in the 200 Gy group (Group B) increased by 1.3-fold compared to the control group, and the frequency of 3–5 bp deletion increased to 34.5%, indicating that low-dose radiation activates the non-homologous end-joining (NHEJ) repair pathway by inducing single strand breaks or base damage. In the 500 Gy group (Group C), the proportion of long-segment deletions (>10 bp) exceeded 5% for the first time, and some samples (e.g., C3) even exhibited large-segment deletions of 30–50 bp, which may be associated with the accumulation of double strand breaks induced by gamma rays and the reduced efficiency of microhomology-mediated end-joining (MMEJ).

When the radiation dose increased to 1000 Gy (Group D) or above, the Indel length distribution exhibited a clear polarization feature. On the one hand, small-fragment (1–3 bp) deletions still dominated (with a mean of 55.2% in Group D), but their proportion decreased by approximately 13% compared to the low-dose group. On the other hand, the frequency of large-segment ( $\geq 10$  bp) deletions increased significantly. In sample E3 of the 2000 Gy group (Group E), 20–50 bp deletions accounted for 18.7%, and insertions of abnormal length 10–15 bp were detected for the first time in insertion events (3.9%). This trend was further exacerbated in the 4000 Gy group (Group F). For instance, the proportion of 50–100 bp deletions in sample F4 reached as high as 12.4%, accompanied by scattered long-fragment insertions ( $\geq 20$  bp) within the genome, indicating that ultra-high-dose radiation increases the randomness of DNA breakpoints, preventing the repair system from effectively recognizing microhomologous sequences and instead completing repair through a non-template-dependent end-joining mechanism. It is worth noting that the Indel distribution of some high-dose samples (e.g., E5 and F2) deviated from the intra-group mean. The proportion of 1-bp deletions in sample E5 rebounded to 65.3%, while the frequency of insertion events in sample F2 abnormally increased to 38.1%, which may be related to differences in their unique DNA repair enzyme activity or chromatin accessibility.

From the perspective of genomic regions, Indel events in the non-irradiated group were mainly enriched in non-coding regions (accounting for 82.6%), particularly in transposable elements and simple repeat sequence regions, which may be related to the structural instability of DNA in these regions. Under low-dose radiation (200 Gy–500 Gy), the proportion of Indels in coding regions (CDS) gradually increased. The Indel density in the CDS region of sample B2 in the 200 Gy group was 2.1-fold higher than that in the control group, and the proportion of 3-bp deletions in gene exons reached 27.8%, significantly increasing the risk of frameshift mutations. High-dose radiation ( $\geq 1000$  Gy) caused more extensive genomic damage: with Indel density in the CDS region of sample D4 increased by 4.3-fold compared to the control group, and over 50% of large deletions spanning multiple exons, which may directly lead to loss of gene function. In addition, the Indel frequency of mitochondrial genome was abnormally elevated in the 2000 Gy group (Group E); the proportion of mitochondrial DNA deletion events in sample E1 reached 15.3%, far exceeding that in the nuclear genome (2.8%), indicating that mitochondria are more susceptible to radiation damage due to the lack of histone protection.

In summary,  $^{60}\text{Co}$ - $\gamma$  radiation regulates the length distribution and genomic localization of Indels in *Shuidong* mustard through a dose-dependent mechanism. Low-dose radiation mainly induces small-fragments of Indels, which may enable trait improvement by finely regulating gene function; high-dose radiation tends to produce large structural variations, providing resources for screening mutants with extreme phenotypes.

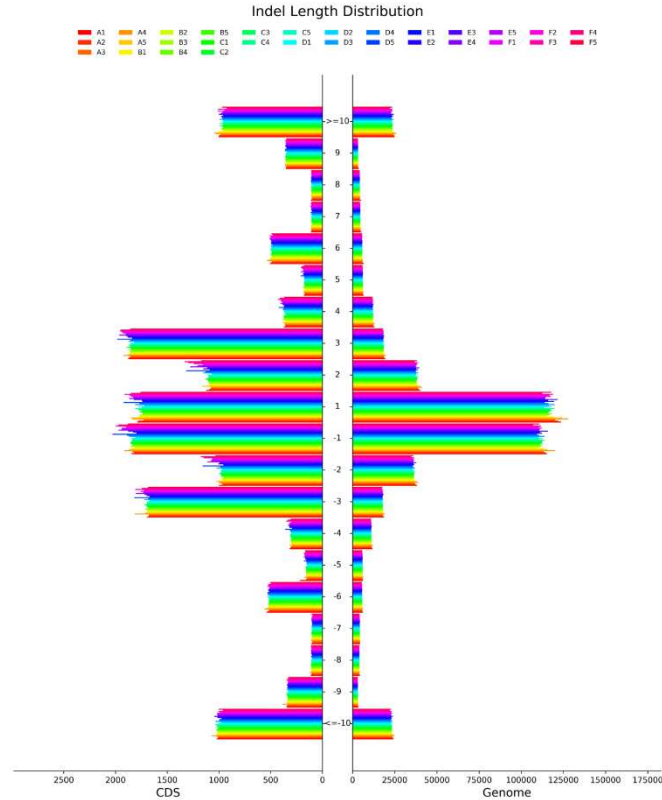


Fig. 4: statistical plots of Indel lengths in the CDS region and genome-wide range for samples irradiated with different doses of  $^{60}\text{Co}$ - $\gamma$  radiation

Note: A-F represent radiation doses of 0, 200, 500, 1000, 2000, and 4000 Gy, respectively. Numbers 1-5 represent different individual plants

## Discussion

This study systematically analyzed the genetic effects of  $^{60}\text{Co}$ - $\gamma$  radiation on Shuidong mustard, revealing the "hoemetic effect" of low-dose radiation and the mechanism of irreversible damage caused by high-dose radiation. Calabrese et al. [17] found that the dose-response relationship for stimuli such as radiation and heavy metals conforms to "hormesis", which is characterized by low-dose stimulation and high-dose inhibition; this is consistent with the results of this study. The increase in seed germination potential and root length observed at low-doses ( $\leq 500$  Gy) may be related to the activation of repair pathways induced by mild oxidative stress caused by radiation. For example, in the 200 Gy group, the root length of seedlings increased to 3.46 cm, and the TTC staining a-value decreased by only 21.3%, indicating that metabolic activity was not significantly impaired. Gudkov et al. [18] found that low-dose ionizing radiation can stimulate the upregulation of antioxidant enzyme (e.g., SOD, CAT) genes, effectively enhancing antioxidant enzyme activity and alleviating oxidative damage. However, high-doses ionizing radiation inhibit enzyme activity, leading to the accumulation of free radicals beyond the clearance capacity, which in turn inhibits cell growth, and in severe cases, induces cell apoptosis. This phenomenon is consistent with the findings of Zhang Jie et al. [19] in their study on radiation-treatment cotton: the activities of SOD and POD enzymes exhibit a trend of first increasing and then decreasing with increasing radiation dose, with low dose promoting and high-doses inhibiting activity. Meanwhile, consistent with the

1 results of Zhao Xiaodong et al. [20] in their study on irradiated sesame, low-dose  $^{60}\text{Co}$ - $\gamma$  radiation  
2 can increase the activities of SOD, CAT, and POD in sesame seedlings leaves. At high doses, enzyme  
3 activity decreases, indicating that a certain dose of  $^{60}\text{Co}$ - $\gamma$  radiation can enhance the salt resistance  
4 of sesame by increasing antioxidant enzyme activity. On the other hand, low-dose  $^{60}\text{Co}$ - $\gamma$  radiation  
5 can upregulate the expression of genes encoding antioxidant enzymes. However, compared with  
6 other crops, Shuidong mustard is more sensitive to high-dose radiation: the germination rate of the  
7 4000 Gy group drops sharply to 75.3%, the fresh weight decreases by 51.5%, and the Q30 value  
8 decreases significantly (1.6 percentage points lower than the control group). This indicates that the  
9 accumulation of DNA damage exceeds repair capacity.

10 Genomic analysis further revealed the molecular mechanisms underlying radiation effects. SNP  
11 detection showed that low-dose radiation-induced C $\rightarrow$ T transitions were enriched in CpG islands  
12 (47.3% in the 500 Gy group), which may be associated with increased cytosine deaminase activity  
13 and epigenetic reprogramming. Consistent with the findings of Laanen et al. [14], radiation was  
14 found to increase cytosine deaminase activity, accompanied by demethylation in the transposon  
15 region.

16 High dose radiation ( $\geq 1000$  Gy) induced G $\rightarrow$ T transversions (28.4% in the 2000 Gy group),  
17 mainly caused by 8-oxoguanine mismatches, and significantly correlated with decreased antioxidant  
18 enzyme activity. This is consistent with the conclusion of Shibutani et al. [21], who revealed that  
19 the molecular basis of gene mutations induced by 8-oxodG-mediated base mismatches under  
20 oxidative stress (e.g., ionizing radiation) is closely related to the exacerbation of oxidative damage  
21 resulting from radiation-induced decreases in antioxidant enzyme activity.

22 Indel analysis showed that low-dose radiation preferentially induced small-fragments deletion (1  
23 –3 bp; 34.5% in group B), which rely on MMEJ. In contrast, high-dose resulted in large-fragment  
24 deletions (50–100 bp; 12.4% of sample F4), indicating the failure of NHEJ repair.

## 25 **Conclusions**

26 This study definitively demonstrates a dose-dependent hormetic response of Shuidong mustard to  
27  $\gamma$ -radiation. The optimal dose window for mutagenesis breeding is precisely identified as 200–500  
28 Gy, which enhances physiological vigor and induces a spectrum of manageable genetic variations,  
29 predominantly C $\rightarrow$ T transitions. Doses exceeding 1000 Gy are detrimental, causing severe  
30 physiological suppression and genomic instability characterized by large-fragment deletions and G  
31  $\rightarrow$ T transversions. These findings provide a robust scientific basis for the precise application of  
32 radiation mutagenesis in Shuidong mustard breeding programs aimed at improving agronomic traits.

## 33 **Author's contributions**

34 Yuxing Huang: Methodology, Experimentation. Yongguo Zhao: Data visualization, curation and  
35 analysis. Xiaoyang Ou: Funding acquisition, Data visualization, curation and analysis,  
36 Experimentation, Writing-original draft. Guangyuan Lu: Project management, Methodology,  
37 Funding acquisition, Writing-review & editing. All authors have reviewed and agreed to the  
38 published version of the manuscript.

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(2020rc046).

## Data availability

The datasets supporting the conclusions of this article are included within the article and its additional files, and the raw sequence data are publicly accessible at <https://ngdc.cncb.ac.cn/gsa> (access number: CRA024718).

## Declarations

## Consent for publication

Not applicable.

## Conflict of interest

The authors declare no competing interests.

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