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Irradiation with carbon ion beams affects soybean nutritional quality in early generations

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With the increased demand for healthy life, seed nutritional quality of soybean has become as important as yield trait. This study aimed to develop soybean lines with beneficially altered seed compositions by using an early carbon ion beam mutant population generated by different irradiation doses (100, 120 and 140 Gy). Eleven quality traits, including protein, oil, sucrose, soluble sugar, Fe, Mn, Zn, Cu, daidzein, glycitin, and genistin concentrations were analyzed to evaluate the variation of seed nutritional quality of the M_2 and M_3 generations. Protein and oil concentrations changed by 38.5-42.9 % and 18.8-23.8 % in M_2 and M_3 generations, respectively, while soluble sugar and sucrose concentrations changed by 48.1-123.4 mg/g and 22.7-74.7 mg/g, with significant effects by 140 Gy across two generations. In two generations, the responses of Fe, Mn, Zn, and Cu to irradiation doses were different, but the inhibitory effect on Mn concentration was enhanced as the irradiation dose was raised. In general, irradiation raised isoflavone concentrations, but 140 Gy had an inhibitory effect on isoflavone concentrations in the M3 generation. Other indicators, with the exception of sucrose and soluble sugar, were still separated from the M2 generation to the M3 generation. Some nutritional quality traits of soybean can be screened in early generations using ion carbon beam irradiation. A lower irradiation dose is preferable when breeding targets are higher isoflavones and Mn concentrations. A higher irradiation dose can be tried if the breeding targets are higher protein, oil, sucrose, soluble sugar, Fe, Zn, and Cu.

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ABSTRACT

With the increased demand for healthy life, seed nutritional quality of soybean has become as important as yield trait. This study aimed to develop soybean lines with beneficially altered seed compositions by using an early carbon ion beam mutant population generated by different irradiation doses (100, 120 and 140 Gy). Eleven quality traits, including protein, oil, sucrose, soluble sugar, Fe, Mn, Zn, Cu, daidzein, glycitin, and genistin concentrations were analyzed to evaluate the variation of seed nutritional quality of the M_2 and M_3 generations. Protein and oil concentrations changed by 38.5-42.9 % and 18.8-23.8 % in M_2 and M_3 generations, respectively, while soluble sugar and sucrose concentrations changed by 48.1-123.4 mg/g and 22.7-74.7 mg/g, with significant effects by 140 Gy across two generations. In two generations, the responses of Fe, Mn, Zn, and Cu to irradiation doses were different, but the inhibitory effect on Mn concentration was enhanced as the irradiation dose was raised. In general, irradiation raised isoflavone concentrations, but 140 Gy had an inhibitory effect on isoflavone concentrations in the M3 generation. Other indicators, with the exception of sucrose and soluble sugar, were still separated from the M2 generation to the M3 generation. Some nutritional quality traits of soybean can be screened in early generations using ion carbon beam irradiation. A lower irradiation dose is preferable when breeding targets are higher isoflavones and Mn concentrations. A higher irradiation dose can be tried if the breeding targets are higher protein, oil, sucrose, soluble sugar, Fe, Zn, and Cu.

Keywords: irradiation, soybean, protein, oil, isoflavones, carbohydrates, trace elements

1. Introduction

Soybean is a unique legume crop because of its diverse nutritional value including protein, oil, isoflavones, trace element, and metabolizable energy (Schmutz et al., 2010; Liu et al., 2019). The seed protein concentration of soybean is about 40%, which is about 2-4 times higher than that of corn, rice and wheat. Soybean seed oil concentration is about 20%, contains 8 kinds of amino acids necessary for human, especially lazy acid and tryptophan which cannot be synthesized by human body (Patil et al., 2017). The percentage of carbohydrates in soybean seed is about 20-30%, but the functions are diverse owing to its complex composition (Cober et al., 2000). The ratio of sucrose, starch and dietary fiber in soybean seed influence its nutritional value greatly (Karr-Lilienthal et al., 2005). Soybean seeds are also rich in isoflavones and trace elements, which are essential for human diet (Wu et al., 2020).

Plant germplasms are the most important sources in crop breeding. Mutation breeding compared to cross breeding has an irreplaceable position in breaking the bottleneck of existing germplasm resources and thus creating more beneficial resources. Higher mutation rate and wider mutation spectrum of soybean seed nutritional quality have been reported by physical mutagenesis and chemical mutagenesis (Hajduch et al., 2000; Espina et al., 2018). Carbon ion beams (CIBs), a new mutation mutagenic resource with higher linear energy transfer (LET), higher mutation rates, and wider mutation spectra under lighter damage, has been increasingly applied to the mutation breeding in various plants including soybean (Arase et al. 2011). Therefore, studying the effects of carbon ion beam irradiation on soybean seed quality traits could have greater potential in accelerating the breeding of high-quality soybean varieties.

There was evidence that combined γ-radiation and EMS improved the concentration of soybean oil with higher levels of oleic and lower levels of linolenic acid (Patil et al., 2007). It has also been reported that irradiation of grain cereals and leguminous crops seeds leads to increased protein content and higher carbohydrate and vitamin levels (Jan et al., 2012). Mikuriya et al. (2017) demonstrated that the populations mutagenized by carbon ion beam irradiation exhibited lower isoflavone concentration in soybean seeds, which was closely related to the reduction of leaf chlorophyll concentration. The increased concentration of genistin, genistein, daidzin and glycitein, but decreased concentration of glycitein and daidzein in soybean seedlings were found by laser irradiation (Jin et al., 2011). The increased irradiation dose increased the concentration of Fe, Cu and Zn in soybean seedlings by mutation breeding (Alikamanoglu et al., 2011). Therefore, carbon ion beam irradiation could have greater potential in accelerating the breeding of high-quality soybean varieties. However, the general information of CIBs on soybean population is not available, especially on seed trace elements and isoflavones.

The present study investigated the seed protein, oil, isoflavones, carbohydrates and trace elements in M_2 and M_3 generation generated by carbon ion beam mutagen treatment with the aims in developing soybean lines with beneficially altered seed composition.

2. Materials & Methods

2.1. Plant material and experiment design

Populations of plants harboring mutants of soybean cultivar 'Dongsheng28' were previously obtained through carbon ion beam irradiation (CIBR) (Wang et al., 2021). The irradiation was directly targeted at the hilum of each seed with 960 Mev carbon ion beam, the dose for mutagenesis was 0 Gy (control), 100 Gy, 120 Gy and 140 Gy separately.

The treated seeds were planted in the field at the Agronomy Farm of the Northeast Institute of Geography and Agroecology, Chinese Academy of Science (45°73′N, 126°61′E). In the M_1 generation, M_0 seeds were planted according to the irradiation dose in 2018, the seeds of all survival plants were harvested separately. In the M_2 generation (2019), the seeds of individual plants harvested from the last generation were sown in a single line. In total, 47 lines in the treatment of 100 Gy group, 26 lines in the treatment of 120 Gy group and 18 lines in the treatment of 140 Gy group. For each single line, five seeds were harvested from every individual plant to form a block. Half of the block were for the determination of seed nutritional quality and the other half were used as seed for the next generation. In the M_3 generation (2020), 100 seeds were sown for every single line, the harvest method was the same as the M_2 generation.

2.2. Chemical analysis of plant samples

Crude protein

The crude protein concentration was determined using the method of combustion nitrogen analysis by Elementar-Vario (Elementar Analysensysteme GmbH E-III, Germany) (Li et al., 2012). A conversion factor of 6.25 was used to convert total nitrogen to crude protein concentration (Saldivar et al., 2011).

Crude oil

Total oil concentration in seeds was determined by the Soxhlet extraction method. Approximately 0.5 g of dried soybean seed sample with a piece of weighed filter paper was wrapped up, then put in a Soxhlet apparatus in a 60 °C water bath. Adding 200 mL ethyl ether to the Soxhlet apparatus for extracting oil. After a 48-h extraction, the defatted sample was placed in an oven at 45 °C about 12 h, the oil concentration was calculated according to Li et al. (2014).

Soluble total sugar and sucrose

The determination of soluble sugar and sucrose was based on the method of Tu *et al.* (2017). About 0.5 g sample was extracted by 4 mL 80 % ethanol, 80 °C water bath for 30 min. Then the mixture was centrifuged at 4500 r/min $^{-1}$ for 3 min, the supernatant was removed to a new 15 mL -centrifuge tube. Adding 3 mL 80 % ethanol to the precipitate and repeated above operation twice. Volumed up the supernatant to 10 mL finally. Added 4 mL anthrone (1000 mL 80% H_2SO_4 + 2.5 g anthrone) to 1 mL supernatant, bath in 90 °C water for 10 minutes, measured at 620 nm (Xinshiji T6, Beijing). The determination method of sucrose was basically the same as soluble sugar, but before adding anthrone, it was necessary to add 25 μL 12 mol/L of NaOH and water bath at

100 °C for 10 min in order to remove the monosaccharides.

Trace element

Trace element was determined with the modified method of Xue et al. (2006). About 0.5 g sample was weighed and placed in a crucible. After carbonization, it was placed in a muffle furnace at 510 °C for 2 h. After cooling, add 1 mL hydrochloric acid and 1 mL deionized water to dissolve the ash. Volumed up to 25 mL after filtering. The analysis of the trace element was performed by AAS (TAS-990, Beijing).

Isoflavones

Isoflavones determination was according to the method described by Hoeck et al. (2000) with high-performance liquid chromatography (HPLC). Weighing about 0.5 g sample into a 15 mL centrifuge tube, and 9 mL 80 % methanol was added. Then ultrasonically extracted at 60 °C for 30 min, centrifuged at 5000 rpm for 5 min, the supernatant was collected into a 25 mL volumetric flask. Adding 6 mL 90 % methanol to precipitation twice. Finally diluted the supernatant to 25 mL with 10 % methanol. The filtrate through 0.45 μm filter membrane was used for the determination of isoflavones. The conditions of HPLC were: RPC18 stainless steel chromatography column; the mobile phase was 0.1% acetic acid and 0.1% acetic acid acetonitrile; the flow rate was 1.0 mL/min; the column temperature was 40 °C and the wavelength was 260 nm.

2.3. Statistical Analysis

Excel 2016 and SPPS 25.0 were used for the analysis of statistical data. The frequency distribution histograms were created by SPPS 25.0. The figures of the effects of different irradiation doses on the quality traits were created with Graphpad Prism 8. The line was the average under the different irradiation dose. Pearson method was used to analyse the correlation of different nutrition quality traits.

3. Result

3.1. Effects of CIBR on soybean protein and oil concentration

As a result of mutagenic treatments, a wide range of variability for protein concentration and oil concentration was found in the M_2 and M_3 generation (**Fig 1**).

In the M_2 generation, the range of protein and oil concentration of all three CIBR doses was 38.5-42.9% and 18.8-23.8% respectively. The coefficient variation of protein ranged 2.0-2.2 % in M_2 generation and ranged 5.1-6.9 % in M_3 generations. In the M_3 generation, seed protein generally increased compared with control. To oil, compared with 100 Gy, higher irradiation doses (120 Gy and 140 Gy) exhibited positive effects on seed oil concentration.

In the M_3 generation, 140 Gy treatment exhibited positive effects on both protein and oil concentration, and their concentrations of most lines were higher than control in this group.

3.2. Effects of CIBR on seed soluble sugar and sucrose concentration

Compared to protein and oil concentration, the variation of the soluble sugar and sucrose concentration was more diverse. Besides the variations were relatively consistent in the M_2 and M_3 generations.

In terms of the concentration of soluble sugar, the range in the M_2 generation and M_3 generation was 78.0-123.4 mg/g and 48.1-98.8 mg/g respectively. Compared with the control treatment, mutagenic treatments generally increased the concentration of soluble sugar, especially at 140 Gy. The range of sucrose concentration was 39.6-74.7 mg/g and 22.7-71.8 mg/g in the M_2 generation and M_3 generation respectively. Overall, the distribution of sucrose was basically the same as soluble sugar, higher doses induced a more significant positive effect on sucrose concentration (**Fig. 2**).

3.3 Effects of CIBR on seed trace element concentrations

The effects of carbon ion beam irradiation on Fe, Mn, Zn and Cu concentrations are showed in **Fig 3**.

In the M_2 generation, Zn concentration ranged from 24.9-35.8 μg/g for all CIER doses. The maximum concentration of Zn under CIER treatment was 28.7 % higher than control (27.8±0.53 μg/g). In the M_3 generation, the Zn concentration ranged from 31.5 to 42.6 μg/g, and 89.0 % lines were concentrated at 36.0-42.0 μg/g. In the M_2 generation, irradiation treatments showed obvious positive effect on Zn concentration, especially at 100 Gy and 140 Gy doses with the highest coefficient variation of 7.25 % at 100 Gy. In M_3 generation however, higher irradiation dose (120 Gy and 140 Gy) increased Zn concentration.

Irradiation treatment generally decreased Fe concentration in the M_2 generation. The range of Fe concentration was 63.7-89.5 μg/g. Compared to control (80.66±1.35 μg/g), only 12.1% lines showed higher Fe concentration. In the group of 140 Gy, the Fe concentration of 55.6% lines was higher than control. In the M_3 generation, the range of Fe was 31.9-69.8 μg/g, and over 70.3% lines were concentrated at 40.0-55.0 μg/g. On average across the different irradiation dose groups, the Fe concentration in over 50% lines was higher than control, and the concentration over 90% lines at 140 Gy was higher than control.

To Mn element, the concentration range in the M_2 and M_3 generations was 17.1 - 23.3 μg/g and 13.8-32.5 μg/g, respectively. No significant differences were found among different irradiation doses in the M_2 generation. However, in the M_3 generation, the 100 Gy and 120 Gy treatment generally increased Mn concentration. In the group of highest irradiation dose (140 Gy), Mn concentration in most lines was lower than control.

The ranges of Cu concentrations in M_2 and M_3 generations were 9.1-15.0 μg/g and 5.6-15.6 μg/g, respectively. In the M_2 generation, irradiation treatment generally increased Cu concentration, while the effects were different in the M_3 generation with most significant effect at 120 Gy.

3.4. Effects of CIBR on seed isoflavones concentration

The concentration of different isoflavones all showed diverse variation in the M_2 generation and M_3 generation (**Fig 4**).

To daidzin, in the M_2 generation, the range was 265-851 μg/g. Compared to control (421±16μg/g), irradiation treatment generally increased the daidzin concentration. Higher CV was found in 100 Gy and 120 Gy treatment than 140 Gy, while treatment of 140 Gy had a more significant positive effect on daidzin concentration, and daidzin concentration in this group was higher than control. In the M_3 generation, the range was 215 -735 μg/g. Since the range in control was 468±18 μg/g, the positive effect was decreased. In the group of 120 Gy, about half lines showed higher daidzin concentrations, while the daidzin concentration of most lines in the group of 140 Gy was lower than control.

To glycitin, the range was similar in the two generations, which were 30.3-98.6 μg/g and 36.5-111.8 μg/g respectively. The distribution was different though there were no significant differences among different irradiation doses. The lines having glycitin concentration higher or lower than the average of control was all about 50%.

Genistin had higher CV compared with the other two isoflavones, reaching 514-1821 μg/g in M_2 generation and 104-1147 μg/g in M_3 generation. The maximum genistin concentration was 169 % and 53 % higher than control in M_2 and M_3 generation. The treatment of 100 Gy and 120 Gy consistently showed a discrete distribution in the two generations. However, 140 Gy exhibited a positive effect on genistin concentration in the M_2 generation but a negative effect in the M_3 generation.

3.5. Correlation analysis of different nutritional quality indexes in M_2 and M_3 generations

In order to compare the correlation among different nutritional quality indexes, the correlation of 11 quality indexes was analyzed in this study, as showed in **Table 1** and **Table 2**.

In the M_2 generation, protein concentration was negatively correlated with oil and soluble sugar, but positively correlated with Zn and Cu concentration. While oil concentration was negatively correlated with Zn and Cu concentration. Sucrose concentration was positively correlated with Fe and Cu concentration. Zn concentration was negatively correlated Fe and glycitin concentration, but positively correlated with Cu concentration. Daidzein, glycitin and genistin concentrations were significantly correlated.

In the M_3 generation, protein concentration was positively correlated with Zn concentration, but significantly negatively correlated with Fe concentration. Oil concentration was significantly positively correlated with the concentrations of sucrose and soluble sugar, but negatively correlated with Cu concentration. Sucrose and soluble sugar concentrations were positively correlated. Sucrose concentration was negatively correlated with Mn concentration, while Fe and Mn concentrations were both positively correlated with that of daidzin and genistin. Consistent with M_2 generation, daidzein, glycitin and genistin concentrations were significantly correlated.

Classifying the lines in M_3 generation by clustering 11 quality indicators (**Fig 5**), four categories: A, B, C, and D could be classified as shown in Table 3. Category A includes 16 lines with relatively higher concentration of Zn, Cu, Fe and sucrose; Category B includes 21 lines with relatively higher oil, sucrose and soluble sugar concentration; Category C includes 27 lines with relatively higher protein, Mn and isoflavone concentration; Category D contains 27 lines with higher concentration of soluble sugar, Fe, Mn, Cu and isoflavones.

4. Discussion

Seed protein and oil concentration are important quality indicators of soybean. Studies have shown that mutation breeding is an effective way to break the significant negative correlation between protein and oil, which can create germplasm resources with high protein and oil concentration (Chaudhary et al., 2015). The change range of the protein and oil concentration is about 5%, similar results in fast neutron irradiation methods are also revealed (Bolon et al., 2011). Though the negative correlation between protein and oil concentration were still found from the M_2 generation to the M_3 generation, higher irradiation doses induced the high-oil and high-protein mutants with the possibility of stable inheritance, thus the irradiation treatment may break the negative correlation between protein and oil. This was consistent with the results of Patil et al. (2007). Therefore, if the screening population is large enough, higher irradiation dose has the potential in creating lines with both high-oil and high-protein mutants in breeding program.

Hayashi and Aoki (1985) demonstrated that the irradiation treatment promoted the accumulation of sucrose in potatoes. EMS treatment can increase seed soluble sugar concentration up to two times compared to control in soybean (Espina et al., 2018). Consistent with these results, in present study, a wider range changes in sucrose and soluble sugar concentrations from the offspring population by carbon ion beam irradiation were also found. The distribution and variation were similar in the M_2 and M_3 generation, and irradiation treatments generally increased the concentration of soluble sugar and sucrose. Furthermore, the higher irradiation dose had more positive effect on their concentrations. In this regard, the trait of soluble sugar and sucrose may be more easily inherited stably in the early generations.

The concentration of trace elements is closely related to the varieties, planting conditions, soil and other environmental factors (Wang et al., 2000). Correlation analysis in present study showed that trace elements were closely associated with protein, oil, carbohydrate and isoflavone ingredients. Therefore, the position of trace elements in soybean seed nutrition quality traits is irreplaceable. The different distribution of trace elements in the M_2 generation and M_3 generation demonstrated that these traits were not stabilized in early generations. Although irradiation treatment had positive effect on the concentration of trace elements in this study, the responses of Fe, Mn, Zn, Cu to irradiation dose were different. For example, lower irradiation dose exhibited more significant positive effect on Mn than other trace elements, while 120 Gy was a better choice to increase the concentration of Cu element.

Much progress has been achieved in breeding high-isoflavone germplasm resources of soybean through the selection of existing resources and crossing combinations in recent years (Wu et al., 2020). The present study showed that higher radiation doses of carbon ion beam reduced the isoflavone concentration in the M_3 generation, which was different from other nutrition quality indicators. Therefore, in order to get more high-isoflavone mutants, the radiation dose should be appropriately reduced. There was evidence that isoflavone concentration is negatively correlated with protein concentration, but positively correlated with 100-seed weight, and small seed and brown coat seed showed higher isoflavone concentration (Seguin et al., 2004; Sakai et al., 2005; Lee et al., 2008). In this study, isoflavone concentration was closely related with trace element concentration, but the correlations were not consistent in the M_2 and M_3 generations, which indicates that the traits have not been stably inherited in these generations. Moreover, many studies demonstrated that isoflavone concentration is greatly affected by environmental factors (Sivesind et al., 2005). Therefore, the application of radiation mutation breeding to screen specific high-isoflavone germplasm still requires more systematic investigation.

It is difficult to screen mutants with all excellent nutrition quality indicators in breeding program, nevertheless it is feasible to focus some indicators. Our cluster analysis of the M_3 generation lines provides the reference for the screening of mutants in breeding. As the key generations of mutation breeding, the M_2 and M_3 generations can reflect the mutagenic effects of some traits, however, many nutrition quality traits are still separated in these generations. Therefore, further research is needed to determine in which generation these traits can stably be inherited.

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Legend of figures

Figure 1. The distribution of seed protein and oil concentration in M_2 and M_3 generations.

(**A** and **B**. Frequency distribution of protein concentration; **C** and **D**. Frequency distribution of oil concentration; **E** and **F**. Effect of different irradiation doses on protein concentration; **G** and **H**. Effect of different irradiation doses on oil concentration; M_2 generation: **A**, **C**, **E** and **G**; M_3 generation: **B**, **D**, **F** and **H**)

Figure 2. The distribution of seed soluble sugar and sucrose concentration in M_2 and M_3 generations.

(**A** and **B**. Frequency distribution of soluble sugar concentration; **C** and **D**. Frequency distribution of sucrose concentration; **E** and **F**. Effect of different irradiation doses on soluble sugar concentration; **G** and **H**. Effect of different irradiation doses on sucrose concentration; M_2 generation: **A**, **C**, **E** and **G**; M_3 generation: **B**, **D**, **F** and **H**)

Figure 3. The distribution of seed trace elements concentration in M_2 and M_3 generations

(**A** and **E**. Frequency distribution of Zn concentration; **B** and **F**. Frequency distribution of Fe concentration; **C** and **G**. Frequency distribution of Mn concentration; **D** and **H**. Frequency distribution of Cu concentration; **I** and **M**. Effect of different irradiation doses on Zn concentration; **J** and **N**. Effect of different irradiation doses on Fe concentration; **K** and **O**. Effect of different irradiation doses on Mn concentration; **L** and **P**. Effect of different irradiation doses on Cu concentration;M_2 generation: **A-D, I-L;**M_3 generation: **E-H, M-P)**

Figure 4. The distribution of seed isoflavones concentration in M_2 and M_3 generations

(**A** and **D**. Frequency distribution of daidzin concentration; **B** and **E**. Frequency distribution of Glycitin concentration; **C** and **F**. Frequency distribution of Genistin concentration; **G** and **J**. Effect of different irradiation doses on daidzin concentration; **J** and **N**. Effect of different irradiation doses on Glycitin concentration; **K** and **O**. Effect of different irradiation doses on Genistin concentration; M_2 generation: **A-C, G-H;** M_3 generation: **D-F, J-L)**

Figure 5. Cluster analysis of seed quality traits in M_3 generation lines

(The number in the figure is the line number, 1-47 is the treatment of 100 Gy, 48-73 is the treatment of 120 Gy and 74-91 is the treatment of 140 Gy)

Legend of tables

Table1. Spearman correlation analyses of seed quality traits in the soybean M_2 population

	Protein	Oil	SUG	Sucrose	Zn	Fe	Mn	Cu	Daidzin	Glycitin	Genistin
Protein	1.000										
Oil	-0.260*	1.000									
SUG	-0.208*	0.266*	1.000								
Sucrose	0.127	0.030	-0.144	1.000							
Zn	0.302**	-0.319**	-0.120	0.019	1.000						
Fe	-0.087	0.144	0.153	0.277**	-0.248*	1.000					
Mn	-0.030	0.017	-0.132	-0.076	-0.004	0.075	1.000				
Cu	0.230*	-0.398**	-0.188	0.243*	0.397**	-0.046	-0.201	1.000			
Daidzin	-0.026	0.090	-0.057	0.020	-0.133	-0.061	0.043	0.075	1.000		
Glycitin	-0.114	0.145	-0.061	0.036	-0.241*	-0.080	-0.048	0.057	0.881**	1.000	
Genistin	-0.046	0.181	0.072	0.137	-0.048	0.071	0.120	-0.050	0.715**	0.649**	1.000

Notes: ** and *represent significant difference at p = 0.01 and 0.05 level

Table2. Spearman correlation analyses of seed quality traits in the soybean M_3 population

	Protein	Oil	SUG	Sucrose	Zn	Fe	Mn	Cu	Daidzin	Glycitin	Genistin
Protein	1.000										
Oil	0.110	1.000									
SUG	0.172	0.357**	1.000								
Sucrose	-0.097	0.381**	0.572**	1.000							
Zn	0.212*	-0.020	-0.176	0.131	1.000						
Fe	-0.271**	-0.163	-0.074	0.066	0.113	1.000					
Mn	-0.082	-0.061	-0.019	-0.407**	-0.130	0.413**	1.000				
Cu	-0.060	-0.364**	-0.107	-0.071	0.131	-0.058	-0.204	1.000			
Daidzin	-0.101	0.002	0.135	-0.105	-0.281**	0.216*	0.383**	-0.132	1.000		
Glycitin	-0.153	0.035	0.088	-0.062	-0.200	0.117	0.268*	-0.164	0.756**	1.000	
Genistin	-0.084	0.007	0.107	-0.137	-0.339**	0.232*	0.448**	-0.119	0.961**	0.655**	1.000

Notes: ** and *represent significant difference at $p = 0.01$ and 0.05 level

Table 3. The average of quality traits based on the cluster analysis in M_3 generation

Category	Protein	Oil	SUG	Sucrose	Zn	Fe	Mn	Cu	Daidzin	Glycitin	Genistin
A	38.14	23.02	72.93	48.74	40.04	48.42	22.92	11.73	264.80	51.18	402.58
B	38.29	24.06	77.85	49.33	38.52	44.18	23.48	9.55	368.41	65.31	584.89
C	38.75	23.35	73.91	42.29	38.28	44.90	25.72	9.51	480.40	74.57	803.09
D	37.84	23.46	78.15	46.88	37.93	52.26	27.37	10.38	575.16	78.45	1003.17

Figure 1

Figure 1. The distribution of seed protein and oil concentration in M_2 and M_3 generations.

(A and B. Frequency distribution of protein concentration; C and D. Frequency distribution of oil concentration; E and F. Effect of different irradiation doses on protein concentration; G and H. Effect of different irradiation doses on oil concentration; M_2 generation: A, C, E and G; M_3 generation: B, D, F and H)

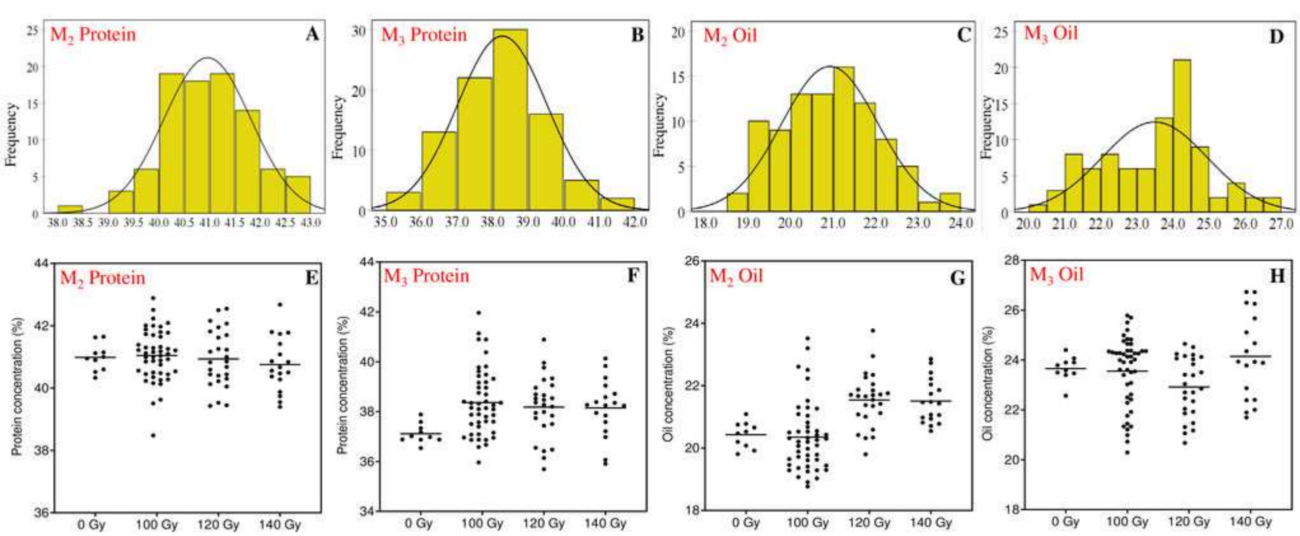

Figure 2

Figure 2. The distribution of seed soluble sugar and sucrose concentration in M_2 and M_3 generations.

(**A** and **B**. Frequency distribution of soluble sugar concentration; **C** and **D**. Frequency distribution of sucrose concentration; **E** and **F**. Effect of different irradiation doses on soluble sugar concentration; **G** and **H**. Effect of different irradiation doses on sucrose concentration; M_2 generation: **A**, **C**, **E** and **G**; M_3 generation: **B**, **D**, **F** and **H**)

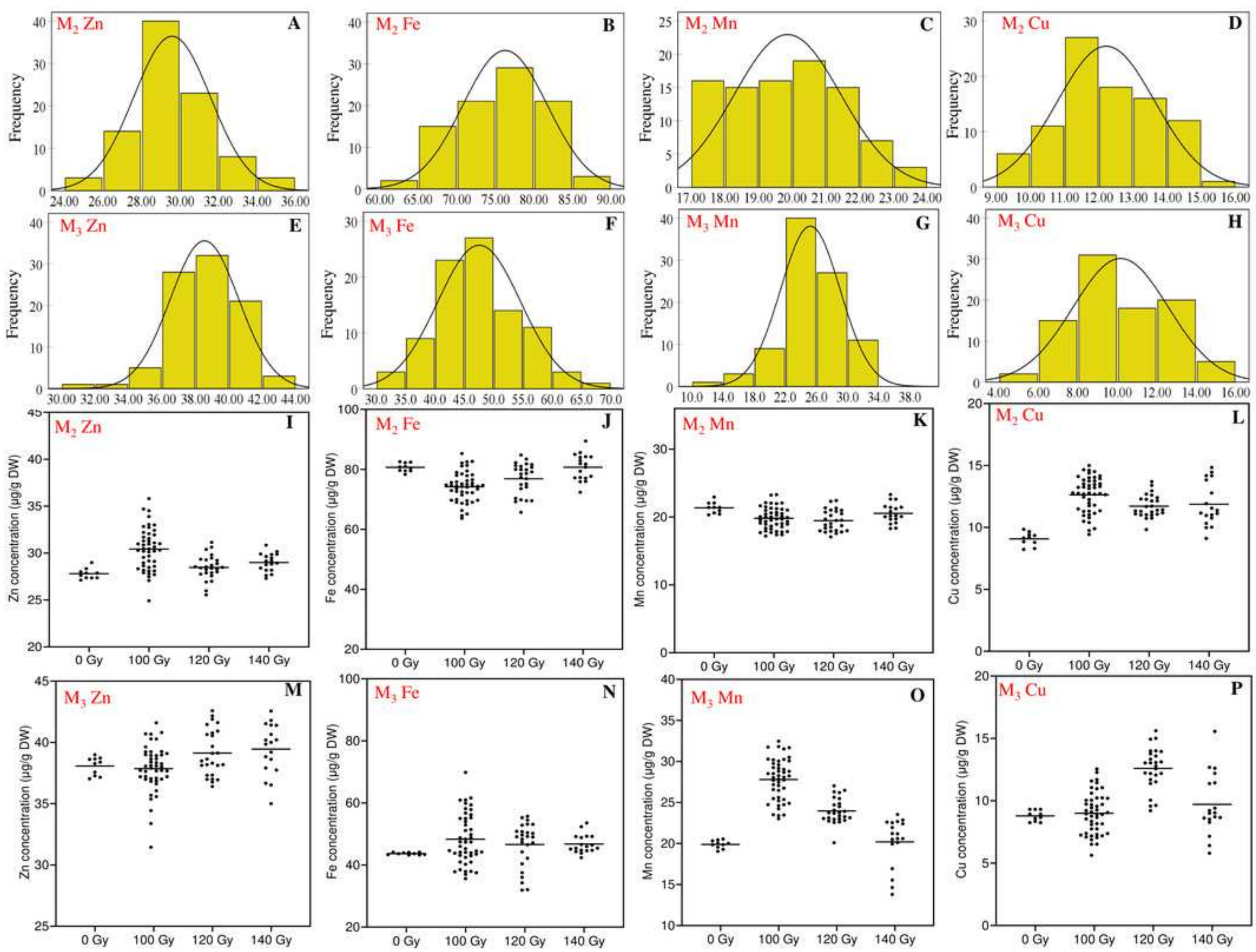

Figure 3

Figure 3. The distribution of seed trace elements concentration in M_2 and M_3 generations

(**A** and **E**. Frequency distribution of Zn concentration; **B** and **F**. Frequency distribution of Fe concentration; **C** and **G**. Frequency distribution of Mn concentration; **D** and **H**. Frequency distribution of Cu concentration; **I** and **M**. Effect of different irradiation doses on Zn concentration; **J** and **N**. Effect of different irradiation doses on Fe concentration; **K** and **O**. Effect of different irradiation doses on Mn concentration; **L** and **P**. Effect of different irradiation doses on Cu concentration;M_2 generation: **A-D, I-L;**M_3 generation: **E-H, M-P)**

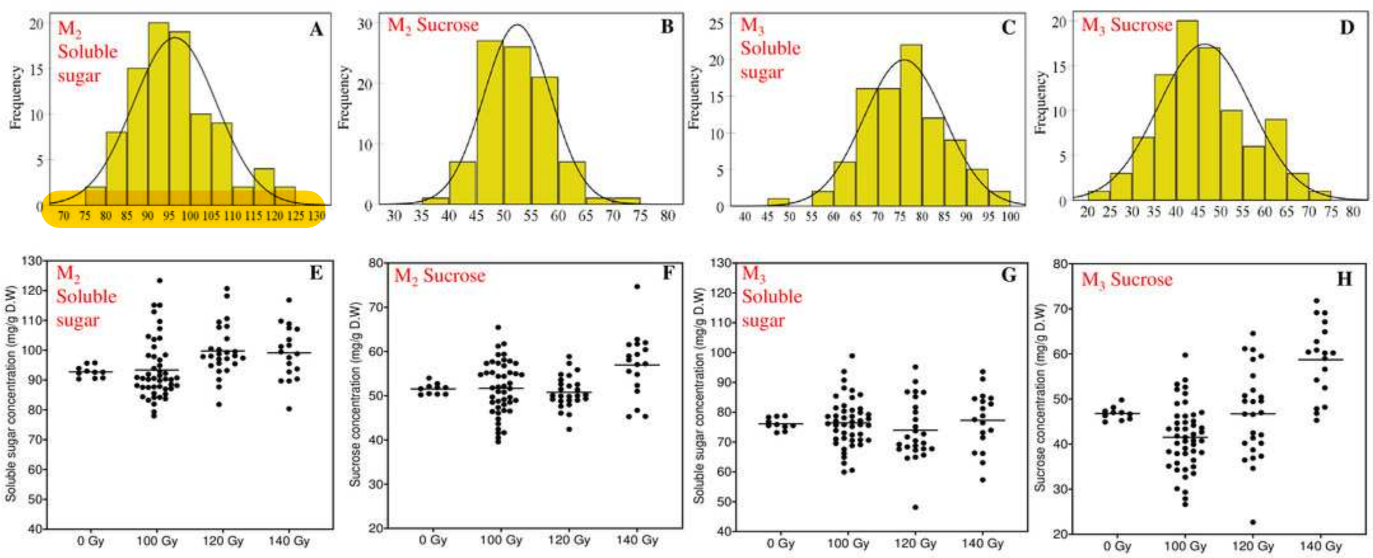

Figure 4

Figure 4. The distribution of seed isoflavones concentration in M_2 and M_3 generations

(**A** and **D**. Frequency distribution of daidzin concentration; **B** and **E**. Frequency distribution of Glycitin concentration; **C** and **F**. Frequency distribution of Genistin concentration; **G** and **J**. Effect of different irradiation doses on daidzin concentration; **J** and **N**. Effect of different irradiation doses on Glycitin concentration; **K** and **O**. Effect of different irradiation doses on Genistin concentration; M_2 generation: **A-C, G-H;** M_3 generation: **D-F, J-L)**

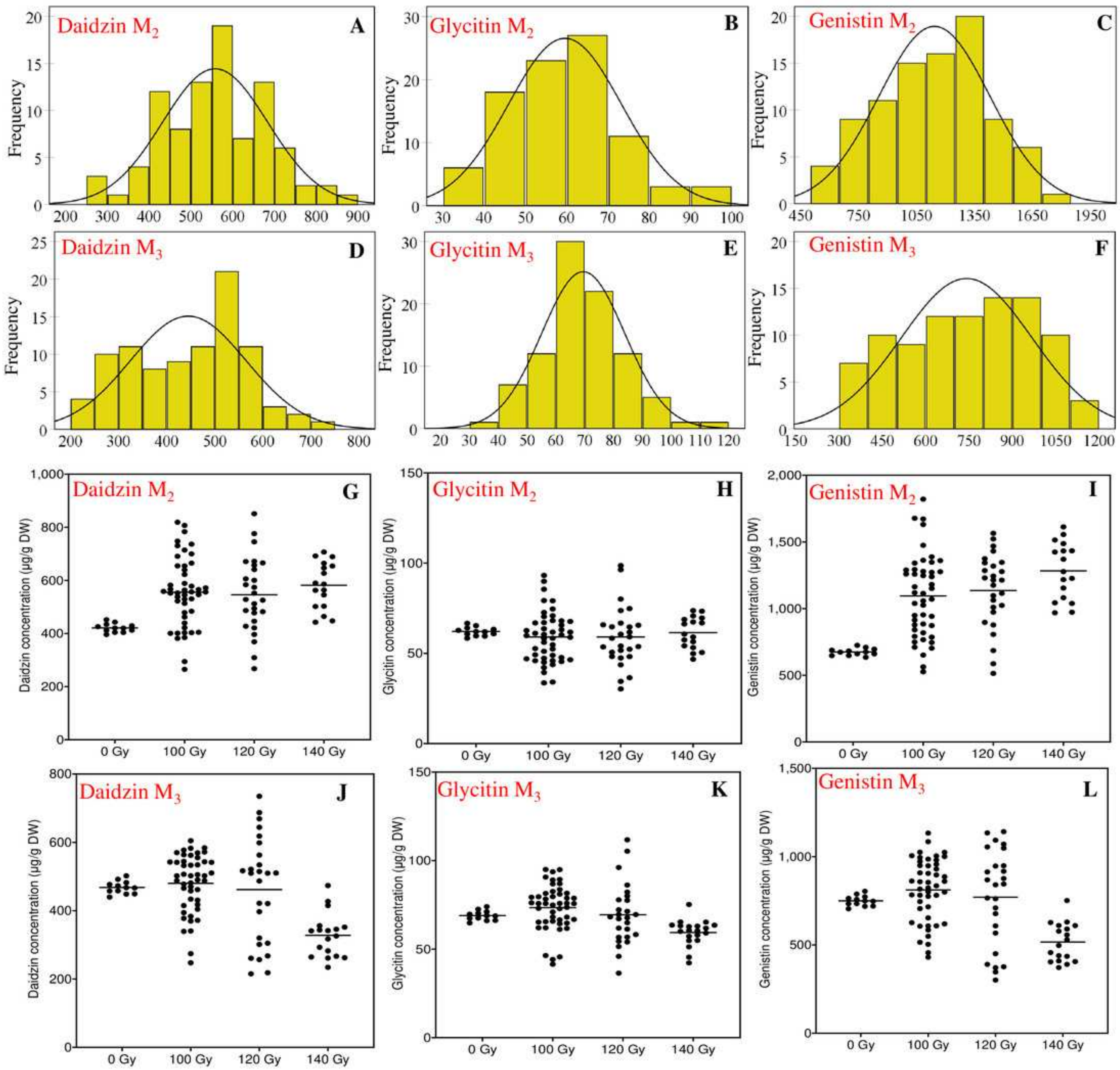
Daidzin M2
A
Frequency
Glycitin M2
B
Genistin M2
C
Daidzin M3
D
Glycitin M3
E
Genistin M3
F
Daidzin M2
G
Daidzin concentration (µg/g DW)
0 Gy
100 Gy
120 Gy
140 Gy
Glycitin M2
H
Glycitin concentration (µg/g DW)
Genistin M2
I
Genistin concentration (µg/g DW)
Daidzin M3
J
Glycitin M3
K
Genistin M3
L

Figure 5

Figure 5. Cluster analysis of seed quality traits in M_3 generation lines

(The number in the figure is the line number, 1-47 is the treatment of 100 Gy, 48-73 is the treatment of 120 Gy and 74-91 is the treatment of 140 Gy)