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Full Length Article

Studies on Application of Stay-Green Mutant in Radiation Mutagenesis Breeding of *Glycine max*

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Abstract

The stay-green mutant can change the genetic diversity of *Glycine max* Merr. mutants progeny and help to breed high-yielded and high-quality soybean varieties. In this study, the ray of ^{60}Co-γ radioactivity source was used to treat soybean stay-green mutant and methods as correlation analysis, principal component analysis, cluster analysis, PCR amplification, genetic diversity analysis and so on were applied to analyze the agronomic traits of soybean and the genetic variation of mutagenesis progeny. The results showed that the yield per plant was negatively correlated with the stay-green trait of soybean stay-green mutant after radiation, the agronomic traits of the mutant progeny had different degrees of genetic variation and the traits were interrelated and constrained. They were divided into five principal components and four groups. The mutant materials were classified into six categories by clustering based on the calculation of genetic distance. In conclusion, this study revealed the effects of radiation mutagenesis on soybean stay-green mutant, and provided a reference for the application of soybean stay-green mutants in radiation mutagenesis breeding programs. © 2020 Friends Science Publishers

Keywords: Agronomic traits; Genetic variation; *Glycine max*; Radiation mutagenesis; Stay-green mutant; SSR marker

Introduction

As a predominant source of protein and oil, soybean (*Glycine max* Merr.) is an indispensable crop for people's life, but the yield level and nutritional quality standard of soybean in China are often difficult to meet the rapidly increasing human population, resulting in an increase in the import of soybean purchases abroad (Wilson 2008; Ray *et al.* 2013). Therefore, it is extremely important to overcome the bottlenecks confronting low yield level and quality components of soybean in China.

Stay-green refers to that chlorophyll is not or insignificantly degraded during leaf senescence, especially in the later stage of plant growth and development, and it is an important character to improve grain number (Jagadish *et al.* 2015; Zhang *et al.* 2019). The leaves can remain green for a longer time, maintain an active leaf area for photosynthesis, improve photosynthetic activity and net photosynthetic efficiency and continue to fill their grains normally under stress (Liu *et al.* 2019). The stay-green mutants can be divided into two groups due to their different stay-green traits and mechanisms such as mutants and non-functional mutants (Thomas and Howarth 2000). Functional mutants can produce much more total dry matter than the plants without a stay-green trait because of their slow leaf senescence and long photosynthesis time. Cha *et al.* (2002) found that chlorophyll concentration in leaves of stay-green mutant decreased slowly than that of wild type during grain filling. Stay-green mutants have been reported in Arabidopsis (Armstead *et al.* 2007), *Lycopersicon esculentum* (Hu *et al.* 2011), *Zea mays* L. (Asakura *et al.* 2004). Furthermore, stay-green genes of some stay-green mutants have been analyzed. For example, the stay-green gene CL of the *Capsicum annuum* L. stay-green mutant was caused by mutation of homologous genes on chromosome 1 (Efrati *et al.* 2005). Ma and Gan have found that the yield of stay-green mutant varieties increased significantly in maize and *Nicotiana tabacum* research (Gan and Amasino 1995; Ma and Dwyer 1998). Studies have further shown that there are two recessive stay-green genes D1 and D2 controlling cotyledons and seed coats respectively in soybean (Fang *et al.* 2014; Nakano *et al.* 2014).

In addition, to the improved agronomic traits mentioned above, the stay-green mutants obtained by radiation mutagenesis have many advantages. First, compared with traditional natural variation and hybrid breeding, mutation breeding and molecular breeding have shorter breeding years and higher success rate, and it has been performed in a number of crop species such as *Oryza sativa* L. (Yao *et al.* 2018) and tomatoes

(Chaudhary *et al.* 2019). Second, radiation mutagenesis refers to a breeding method that uses some physical factors (x-ray, γ-ray, β-ray, etc.) to irradiate the plant and breed new varieties by altering plant genetic material. So it can not only create new germplasm resources and research materials, but also has no food safety issues similar to genetically modified (GM) crops (Wang and Hu 2002). Third, radiation mutagenesis has been widely used in breeding, since the late 1950s and radiation mutation breeding in China has played a great role in promoting mutation breeding and the mutant varieties have a leading advantage in quantity and planting area (Liu *et al.* 2009). People have used radiation mutation to breed new varieties of tobacco (Zhou *et al*. 2008), wheat (Xue *et al.* 2014) and rice (Sun *et al.* 2017). With the development of radioactive cobalt source ^{60}Co-γ rays are widely used and the breeding effects are remarkable (Zhao and Liu 2017). Fourth, radiation breeding is also environmental friendly. For instance, Japanese scientists have bred rice varieties with low cadmium accumulation by high energy heavy ion mutagenesis (Ishikawa *et al.* 2012). Scientists in United States, Canada and other countries have created a number of environmental friendly, low phytic acid mutants of maize, soybean and barley using mutation breeding approach (Sparvoli and Cominelli 2015).

Radiation mutation breeding has the advantages of high breeding efficiency, large variation range and mutagenesis progeny are safe (Wang and Hu 2002). Combining with radiation mutagenesis, stay-green mutants can be bred quickly to meet the requirements of new soybean varieties and the needs of actual agricultural production. In this study, soybean stay-green mutants were used as mutagenic materials and treated with ^{60}Co radiation to analyze the genetic variation of phenotypic traits and molecular markers of stay-green mutants induced by radiation. This study provided theoretical support for the application of stay-green mutants in radiation mutation, and combined with phenotypic data analysis. In addition, molecular experiments were done to selected new high-quality stay-green mutants, which can provide a practical basis for the innovation and development of soybean germplasm resources.

Materials and Methods

Plants material

Selected a soybean stay-green mutant and its progenies M_3 and M_4 (89 materials in total) were used to analyze the character variation of the progeny of stay-green mutant. The mutant with the characteristics of stay-green is produced under natural conditions. The leaves and seeds are green, plant type is compact, flower color is purple, semi-determinate, pubescence color is brown, and with few branches (generally 0~2), the growth period is generally 115~120 days (Table 1).

Table 1: Biological characteristics of stay-green soybean and mutagenesis progeny

Number of lines	Leaf shape	Flower color	Pubescence color	Maturity type
1 (ck)	Narrow	purple	brown	normal
2	Narrow	purple	brown	mid-late maturity
3	broad	purple	gray	normal
4	broad	white	gray	mid-late maturity
5	narrow	purple	brown	normal
6	narrow	purple	brown	normal
7	narrow	purple	brown	normal
8	narrow	purple	brown	normal
9	narrow	purple	gray	normal
10	broad	white	gray	normal
11	narrow	purple	brown	mid-late maturity
12	narrow	purple	brown	normal
13	narrow	purple	gray	early maturity
14	narrow	purple	brown	normal
15	narrow	purple	gray	normal
16	narrow	purple	brown	normal
17	narrow	purple	gray	normal
18	narrow	purple	brown	early maturity
19	narrow	purple	brown	mid-late maturity
20	narrow	purple	brown	normal
21	narrow	purple	brown	normal
22	narrow	purple	brown	normal
23	narrow	purple	gray	mid-early maturity
24	narrow	purple	gray	normal
25	narrow	purple	gray	normal
26	narrow	purple	gray	mid-late maturity
27	narrow	purple	brown	normal
28	broad	white	gray	normal
29	narrow	purple	gray	normal
30	narrow	purple	gray	normal
31	narrow	purple	brown	normal
32	narrow	purple	brown	normal
33	narrow	purple	brown	mid-late maturity
34	narrow	purple	brown	normal
35	narrow	purple	brown	normal
36	narrow	purple	brown	normal
37	narrow	white	gray	late maturity
38	narrow	purple	brown	mid-early maturity
39	narrow	purple	brown	normal
40	broad	white	gray	mid-late maturity
41	narrow	purple	brown	normal
42	broad	white	gray	normal
43	narrow	purple	brown	normal
44	broad	purple	gray	mid-late maturity
45	broad	white	brown	normal
46	narrow	purple	brown	early maturity
47	narrow	purple	brown	normal
48	narrow	purple	brown	mid-late maturity
49	narrow	purple	brown	normal
50	narrow	purple	brown	mid-early maturity
51	narrow	purple	brown	normal
52	narrow	purple	brown	late maturity
53	narrow	purple	brown	normal
54	narrow	purple	brown	normal
55	narrow	purple	brown	normal
56	narrow	purple	brown	mid-late maturity
57	narrow	purple	brown	normal
58	narrow	purple	brown	normal
59	narrow	purple	brown	normal
60	narrow	purple	brown	normal
61	broad	white	brown	normal
62	narrow	purple	brown	mid-late maturity
63	narrow	purple	brown	normal
64	narrow	purple	brown	normal
65	narrow	purple	brown	mid-early maturity

Table 1: Continue

66	narrow	purple	Brown	mid-late maturity
67	narrow	purple	Brown	Normal
68	narrow	purple	brown	Normal
69	narrow	purple	brown	mid-late maturity
70	broad	white	gray	Normal
71	narrow	purple	brown	Normal
72	narrow	purple	brown	Normal
73	narrow	purple	brown	Normal
74	narrow	purple	brown	Normal
75	narrow	purple	brown	late maturity
76	broad	purple	brown	mid-early maturity
77	narrow	purple	brown	normal
78	narrow	purple	brown	normal
79	narrow	purple	brown	normal
80	broad	purple	gray	normal
81	narrow	purple	brown	mid-late maturity
82	broad	white	gray	normal
83	broad	purple	gray	early maturity
84	narrow	purple	brown	normal
85	narrow	purple	brown	mid-late maturity
86	narrow	purple	brown	early maturity
87	narrow	purple	brown	normal
88	narrow	purple	brown	normal
89	narrow	purple	brown	mid-early maturity

Selection of mutations and their mutant progenies and the extraction of DNA

First, M_1 generation was obtained by air-dried mutant seeds of soybean with stay-green and irradiated with ^{60}Co 100R/min radiation mutation. Then the M_2 generation was obtained by planting, the excellent mutant plants and special mutant individuals were selected from M_2 generation for cultivation to get M_3 and M_4 generations of the selected plants. Using the method of randomized block design, M_3 generation was planted with a row spacing of 0.5 m, row length of 0.5 m and plant spacing of 0.25 m, repeated three times.

To analyze of SSR genetic diversity of stay-green mutant progeny, stay-green mutant progeny M_5 was planted in 2015, when the first alternate compound leaves emerged from the seedling Genomic DNA of stay-green mutants and its progeny M_5 were extracted based on the SDS method (Guan *et al.* 2003) and the oxidative reaction of (DNA) was prevented by adding β-mercaptoethanol in the experiment DNA was extracted twice and then dissolved and preserved in (TE) buffer. The extracted DNA was of high purity and moderate concentration, which was suitable for subsequent molecular experiments. In subsequent molecular experiments, the genetic diversity of mutant progeny was analyzed by SSR markers.

Character analysis

The SPAD values in leaves of mutant progeny at the seedling stage, blooming stage and seed filling stage was measured by portable chlorophyll meter SPAD-502 (Minolta Camera Co., Japan). After soybean matured, 10 individual plants were randomly selected to measure 17 agronomic traits. Measured plant height (cm) and pod height (cm) with ruler, and measured stem diameter (cm) with vernier caliper. Electronic balance was used to measure plant weight (g), 100-seed weight (g) and seed weight per plant (g). Then count main stem node number, branch number, main stem pod number, branch pod number, number of one seed per pod, number of two seed per pod, number of three seed per pod, number of four seed per pod, number of blighted pods, total pod number, insect herbivory number. After that, the protein content (%) and fat content (%) were measured by InfratecTM 1241 Grain Analyzer V5.00.

Statistical analysis

Mutagenic progeny M_4 were seeded in 2015, with the same planting season and method as before. SPSS19.0 software was used to collate the data of phenotypic traits, correlation analysis, principal component analysis (PCA) and cluster analysis were carried out.

The data of SSR were processed by Popgene Version 1.32 (Yeh *et al.* 1999) and the allele variance, genetic distance and Shannon index were obtained:

$$PIC = 1 - \sum_{j=1}^{n} p_{ij}^2$$

The p_{ij} in the formula denotes the probability of the occurrence of the *j* allele of marker *i*,

Shannon index (H′) = -∑pilnpi (Duan *et al.* 2003),

The pi in the formula is the probability of the occurrence of the I allele variation, and the ln is the natural logarithm. We used the STRUCTURE software 2.3.4 to analyze the genetic structure of stay-green mutants and their mutant progenies (Evanno *et al.* 2005). The optimum number of main groups was determined by the value of Ln P (D) obtained by the software. When the software runs, the K value was set to 1–15 and each K value was run 15 times. And used SAS 8.0 (SAS Institute Inc., Cary, NC, USA) to statistic the mean, standard deviation (SD) and coefficient of variation, etc. (Zondervan and Cardon 2004).

Results

Analysis of trait variation in the progenies of stay-green mutants induced by radiation mutagenesis

Trait difference analysis: The growth period of the contrast material was 118 days, while the change range of the growth period of mutagenic progenies was 96~142 days, which was mainly around 120 days, accounting for 67.0% of the mutant progenies. Early maturity accounted for 5.7%, middle-early maturity accounted for 8.0, 15.9 and 3.4% of them were middle-late and late maturity (Fig. 1).

The pods number per plant of contrast material was 53, and the pods number per plant of the mutant progenies was mainly happened between 56 and 65.

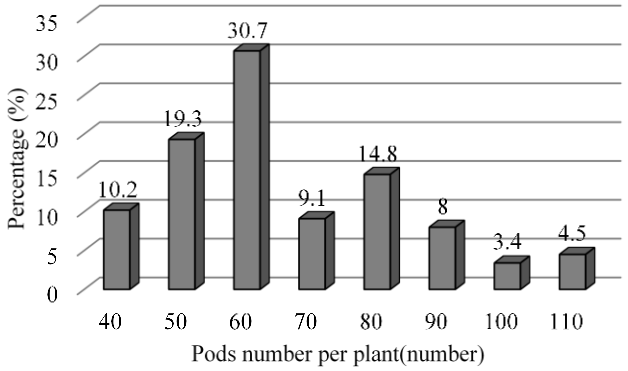

Fig. 1: Percentage of mutation progeny according to the different growth period

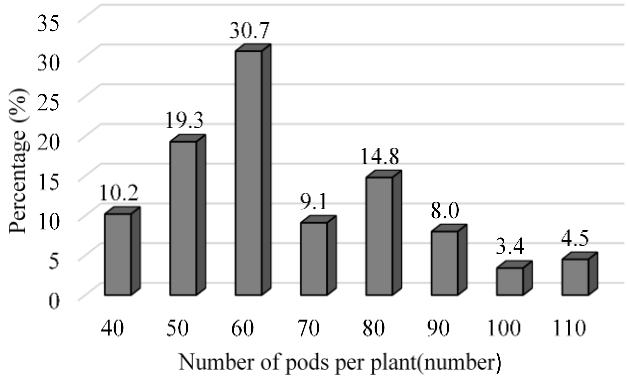

Fig. 2: Percentage of mutation progeny according to the different pods number per plant

Less than 45 of them accounted for 10.2% of the progeny population and more than 95 of them accounted for 7.9%, mutagenesis results in a higher variable rate of the pods number per plant (Fig. 2). One hundred-seed weight of contrast material was 22.08 g, the variation range of 100-seed weight of mutant progenies was 14.70~26.16 g, mainly distributed between 21.22~22.24 g, which accounted for 21.6% of the mutant progenies. Less than 16.22 g accounted for 2.2% of the mutant progenies and 5.7% of the progenies were above 12.11 g (Fig. 3). The seed weight per plant of the control material was 32.67 g, the seed weight per plant of mutant progenies was mainly between 25.55 and 35.55 g, accounting for 26.1% and there were 11.4% of the mutant progenies that seed weight per plant exceeded 65.55 g (Fig. 4).

In this study, the protein content of control material was 44.6% and the protein content of mutant progenies was mainly between 44.4 and 45.4%, which accounted for 68.2% of the mutant progeny population. The progenies whose protein content exceeded 45.4% accounted for 10.2% of the population, whilst the protein content of 5.7% mutant progenies was lower than 42.4% (Fig. 5). The fat content of the control material was 21.6%, the fat content of mutant progenies was mainly varied between 21.3% and 21.7%,

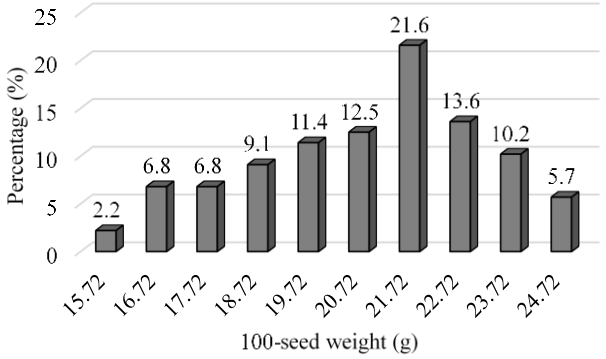

Fig. 3: Percentage of mutation progeny according to the 100-seed weight

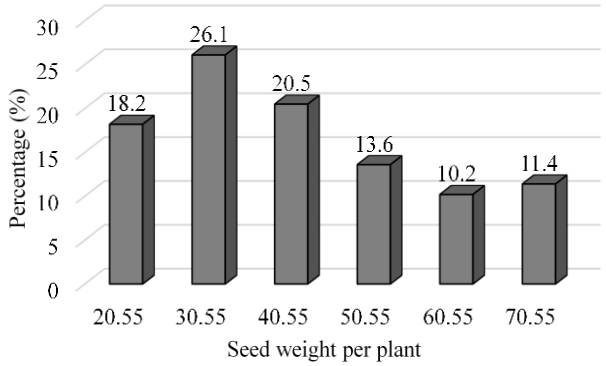

Fig. 4: Percentage of mutation progeny according to the different seed weight per plant

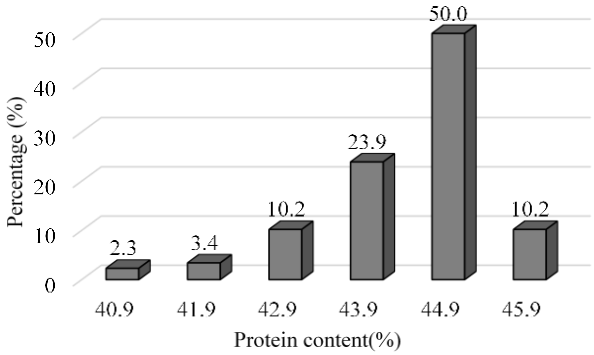

Fig. 5: Percentage of mutation progeny according to the different protein content

which accounted for 68.2% of the mutant progeny population. After mutagenesis, the progenies whose fat content exceeded 21.7% accounted for 20.4% of the population and less than 21.3% accounted for 11.4% (Fig. 6).

The SPAD values in the leaves of the control materials at the seedling stage, full bloom stage and filling stage were 38.2, 45.7 and 49.2, respectively. The SPAD values in the leaves of the mutant progenies at the seedling stage were mainly between 38.0 and 39.0 (Fig. 7). The SPAD values of the mutant progenies at flowering stage and seed filling stage remained in the range of 45.0–46.0 and 45.5–47.5, respectively.

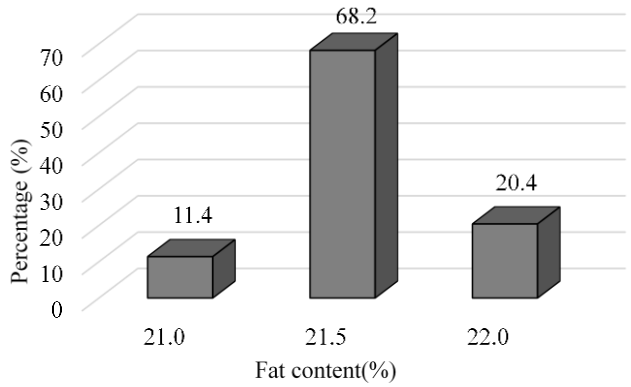

Fig. 6: Percentage of mutation progeny according to the different fat content

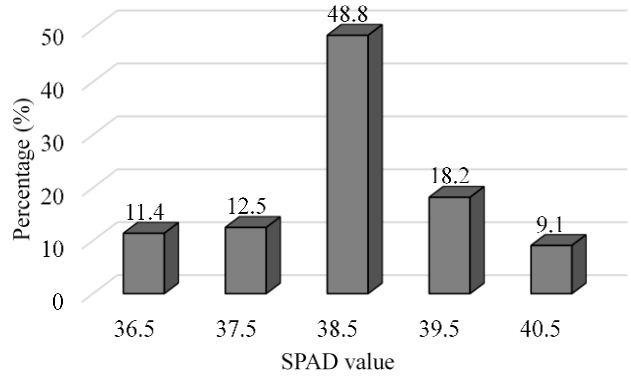

Fig. 7: Percentage of mutation progeny according to the different seeding SPAD value

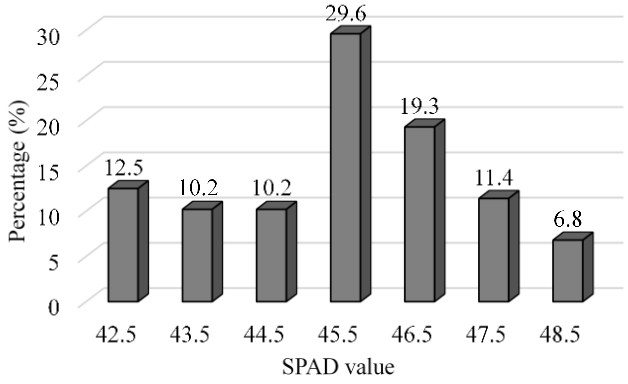

Fig. 8: Percentage of mutation progeny according to the different SPAD value on the full bloom stage

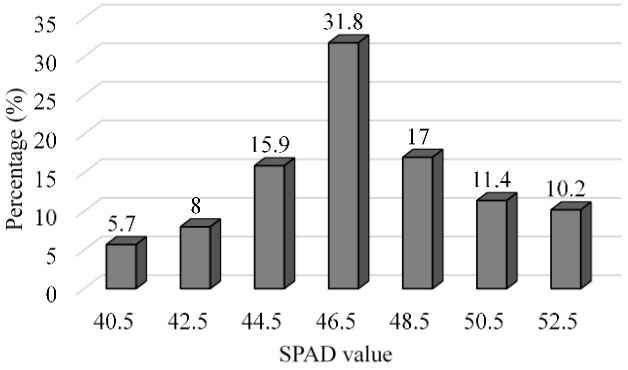

Fig. 9: Percentage of mutation progeny according to the different SPAD value in the seed filling period

The SPAD values of mutant progenies at seed filling stage were generally lower than that of soybean stay-green mutants and 21.6% of progenies had higher SPAD value than that of control materials. This indicated that mutation treatment caused changes in stay-green trait of some progeny populations, and mutation can easily weaken the stay-green property of soybean stay-green mutants (Fig. 8 and 9).

At the same time, it cannot be denied that mutation can also destroy some gene functions of the stay-green mutants, resulting in some dwarf plants, semi-sterile plants, and sterile plants. Because only one kind of soybean stay-green mutants was selected in this experiment, the results of this study cannot represent all the stay-green mutants of soybean and other varieties, but it also has very rich reference significance.

Analysis of genetic variation of phenotypic traits: Through statistical analysis of agronomic traits data of mutant progenies, the genetic variation degree of different materials on traits was compared by the coefficient of variation. It was noted that greater the coefficient of variation of a trait, greater was difference of this trait in the later generations. M_3 and M_4 are significantly higher than the control in plant weight (g), plant height (cm), protein content (%) and fat content (%) after mutagenesis, and these germplasms have great potential to become high-quality and high yield varieties (Table 2). In the analysis of coefficients of variation, in the M_3 generation, maximum coefficient of variation of number of four seed per pod was r=1.42 and the minimum coefficient of variation of protein content and fat content were r=0.02 and r=0.01, respectively. From these data, we noted that the phenotypic traits of M_3 and M_4 generations changed to some extent. The varying degree of number of four seed per pod, branch number and branch pod number and other traits were higher, but the difference of protein content (%) and fat content (%) of mutant progenies were not obvious, while the variations in the quality traits were lower. Moreover, the coefficient of variation of most traits in M_4 generation was slightly smaller than in M_3 generation, indicating that the traits of mutant progeny tended to be stable (Table 2).

Correlation analysis of the agronomic traits: Through correlation analysis of plant weight (g), plant height (cm) and other agronomic traits data of mutant progeny, the correlation between agronomic traits of progeny can be understood. Seven pairs of mutant progenies reached a significant level, 48 pairs reached an extremely significant level, accounting for 35.95% of the total (Table 3).

Table 2: The average of different agronomic traits and coefficient of variation in the mutants

Trait	CK		Max.		Min.		Mean		SD		*CV*	
	M_3	M_4	M_3	M_4	M_3	M_4	M_3	M_4	M_3	M_4	M_3	M_4
Plant weight (g)	50.84	58.96	118.47	122.87	18.74	25.13	55.21	62.47	21.87	21.61	0.40	0.35
Plant height (cm)	77.60	81.70	110.60	116.20	32.40	42.50	76.20	76.80	14.68	14.23	0.19	0.19
Pod height (cm)	11.80	14.10	37.20	21.80	2.30	2.30	10.40	9.30	7.48	3.78	0.72	0.41
Stem diameter (cm)	1.11	0.89	1.47	1.64	0.35	0.52	0.96	0.91	0.23	0.21	0.24	0.23
Main stem node number	17	21	20	24	11	10	19	19	3.44	3.09	0.18	0.16
Branch number	0	0	4	4	0	0	1	2	1.26	1.22	1.26	0.76
Main stem pod number	41	69	88	122	17	32	42	54	13.61	14.57	0.32	0.27
Branch pod number	0	0	72	90	0	0	12	25	14.98	22.49	1.25	0.90
No. of one seed per pod	2	5	36	34	1	2	9	9	6.93	5.76	0.77	0.66
No. of two seeds per pod	8	12	51	86	3	6	18	22	8.94	13.24	0.50	0.59
No. of three seeds per pod	17	33	38	103	2	3	19	34	12.04	18.63	0.63	0.54
No. of four seeds per pod	10	12	38	29	0	0	4	6	5.70	6.42	1.42	1.00
No. of blighted pods	4	7	14	19	0	1	4	7	3.21	3.27	0.80	0.45
Total pod number	41	69	142	168	19	38	55	79	22.42	28.06	0.41	0.35
Insect herbivory number	8	8	27	15	1	2	7	7	4.91	3.06	0.70	0.43
100-seed weight (g)	22.52	22.44	26.54	26.40	16.22	14.52	22.14	19.90	2.22	2.48	0.10	0.12
Seed weight per plant (g)	20.13	39.47	71.26	79.33	5.29	11.92	24.11	36.20	11.34	15.07	0.47	0.42
Protein content (%)	44.20	44.30	46.10	46.50	40.50	40.90	44.10	44.00	0.88	0.95	0.02	0.02
Fat content (%)	21.50	21.60	22.00	22.00	20.50	20.70	21.40	21.50	0.35	0.20	0.02	0.01

Table 3: The simple correlation coefficient for agronomic traits of mutation progeny

Characteristics	Plant weight	Plant height	Pod height	Stem diameter	Main stem node number	Branch number	Main stem pod number	Branch pod number	No. of one seeded pods	No. of two seeded pods	No. of three seeded pods	No. of four seeded pods	No. of blight affected pods	Total pod number per plant	Insect herbivory number	100-seed weight
Plant height	0.121															
Pod height	-0.038	0.376**														
Stem diameter	0.607**	-0.05	0.063													
Main stem node number	0.107	0.573**	0.096	0.196												
Branch number	0.511**	0.025	-0.111	0.218*	0.105											
Main stem pod number	0.583**	0.09	0.059	0.325**	0.1	-0.145										
Branch pod number	0.762**	-0.107	-0.204	0.416**	-0.004	0.788**	0.105									
No. of one seed per pod	0.269*	0.019	-0.129	0.299**	0.184	0.320**	0.125	0.305**								
No. of two seeds per pod	0.521**	0.053	-0.076	0.298**	-0.05	0.329**	0.349**	0.483**	0.249*							
No. of three seeds per pod	0.668**	-0.16	-0.111	0.299**	-0.027	0.396**	0.441**	0.625**	-0.03	-0.046						
No. of four seeds per pod	0.481**	0.135	0.088	0.278**	0.187	0.201	0.297**	0.419**	-0.08	0.115	0.277**					
No. of blighted pods	0.301	0.061	-0.114	0.318	0.121	0.243*	0.449**	0.471	0.267*	0.392	0.283	0.321				
Total pod number	0.914**	-0.039	-0.133	0.502**	0.049	0.557**	0.604**	0.856**	0.310**	0.568**	0.730**	0.490**	0.611**			
Insect herbivory number	0.214	0.016	-0.195	0.262*	0.172	0.283	0.182	0.334	0.28	0.078	0.321	0.103	0.343	0.363		
100-seed weight	0.14	0.105	0.024	0.308**	0.003	0.008	-0.055	-0.049	0.244*	0.11	-0.194	-0.229*	0.115	-0.068	-0.06	
Seed weight per plant	0.932**	0.022	-0.049	0.505**	0.03	0.524**	0.532**	0.804**	0.174	0.454**	0.736**	0.524**	0.452	0.921**	0.216	0.103

Note: * and ** indicate significant difference and extremely significant difference, respectively

Pearson's correlation revealed that 26 pairs were negative correlated, accounting for 17.0% of the total. Data showed that higher was the pod height, lower was the yield per plant. Therefore, the appropriate pod height should be selected when breeding varieties. In breeding programs, it is easier to breed new soybean varieties with high quality and yield by considering the mutual restriction and correlation of agronomic traits (Table 3).

PCA and cluster analysis: We used PCA to analyze the phenotypic traits of yield in order to select excellent varieties with the high efficiency as mentioned by Evanno *et al.* (2005). Five principal components were obtained from the dimensionality reduction of 17 agronomic traits by PCA. The first principal component was crop yield, including total pod number, plant weight, seed weight per plant, branch pod number; The second principal component was the plant type, including plant height, main stem node number and pod height, which was related to plant type; The third principal component was determinate nature of plants, including the number of one seed per pod, the 100-seed weight and the number of two seed per pod, which is called 'pod factor'; The fourth principal component was insect herbivory, which exhibited the most significant correlation with the insect herbivory number, including the insect herbivory number, branch number and the main stem node number. The fifth principal component includes the main stem node number, pod height and stem diameter, namely plant stem type (Table 4).

Cluster analysis was used to classify the stay-green mutants and their progenies into four groups. The first group had higher pod height, lower plant weight and yield per plant, the stay-green mutant of the control material was found in this group. The plant weight and yield per plant of the second group and its progeny maintained a medium level, while the number of two seed per pod was higher and the insect herbivory number were fewest. The third group had better plant type, more branches, higher plant weight and yield. The fourth group had the strongest stem, medium height, good plant growth, the highest plant weight and yield, which had high yield ability (Table 5–7).

Table 4: Component matrix table

Trait	Components				
	1	2	3	4	5
Plant weight	0.941	0.09	-0.053	-0.109	0.112
Plant height	0.026	0.821	-0.092	0.281	0.104
Pod height	-0.12	0.539	-0.306	-0.07	0.423
Stem diameter	0.602	0.249	0.189	-0.213	0.372
Main stem node number	0.128	0.694	-0.002	0.464	0.431
Branch number	0.608	-0.18	0.287	0.541	0.343
Main stem pod number	0.548	0.243	-0.348	-0.525	-0.351
Branch pod number	0.864	-0.269	0.119	0.271	0.238
No. of one seed per pod	0.348	0.154	0.655	0.069	-0.225
No. of two seeds per pod	0.541	0.076	0.301	-0.306	0.273
No. of three seeds per pod	0.691	-0.309	-0.387	0.101	-0.123
No. of four seeds per pod	0.514	0.154	-0.49	0.137	0.126
No. of blighted pods	0.649	0.134	0.09	-0.17	-0.243
Total pod number	0.977	-0.09	-0.085	-0.055	0.009
Insect herbivory number	0.44	-0.028	0.149	0.604	-0.59
100-seed weight	0.042	0.295	0.61	-0.365	0.172
Seed weight per plant	0.938	-0.034	-0.131	-0.078	0.125

Table 5: The average of phenotypic traits

Plant traits	Plant weight	Plant height	Pod height	Stem diameter	Main stem Node number	Branch number	Main stem pod number	Branch pod number
First kind	45.05	72.45	10.10	0.83	19	1	49	5
Second kind	69.29	75.10	8.90	0.96	19	2	57	33
Third kind	108.62	81.80	8.30	1.15	20	2	97	37
Fourth kind	105.32	83.30	9.40	1.02	21	3	62	69

Table 6: The average of phenotypic traits

Plant traits	No. of one seed per pod	No. of two seeds per pod	No. of three seeds per pod	No. of four seeds per pod	No. of blight affected pods	Total pod number	Insect herbivory number	100-seed weight	Seed weight per plant
First kind	5	14	25	4	5	54	8	19.66	24.62
Second kind	11	31	35	5	8	90	5	19.38	36.68
Third kind	13	24	70	15	12	134	9	19.10	62.63
Fourth kind	11	45	48	16	11	127	9	20.42	60.67

Analysis of genetic diversity of SSR in mutant progenies of stay-green mutants

Polymorphism analysis and cluster analysis of SSR markers in mutant progenies of stay-green mutants: In this study, 70 pairs of SSR primers were selected to amplify 89 materials of stay-green mutants and their mutant progenies and 34 pairs of primers with rich polymorphism were selected for genetic diversity analysis: a total of 96 allele variations were detected. The range of allele variations of each primer ranged from 2 to 5, with an average of 2.8 and the maximum allelic variation detected by primers Sat_385 and Sat_333 was 5, while the minimum number of allele variances was only 2. The variation range of polymorphic information quantity (PIC) was 0.049–0.693, and the average was 0.362. Among primers, Sat_385 has the largest polymorphic information. The range of the Shannon index was 0.1157–1.4128, with an average of 0.6998. Among them, the Shannon index of primer Sat_385 was the largest. According to the calculation of genetic distance, 89 materials were classified into 6 categories, including 1 material, 10 materials, 12 materials, 12 materials, 53 materials and 1 material, respectively. The genetic variation of No. 50 material of category 6 was relatively large, and it showed excellent performance in stay-green and phenotypic trait. It belonged to the third category in the clustering results of agronomic traits and had the potential to cultivate new varieties of high quality. And Fig. 10 showed the annular clustering of stay-green soybean and mutation progenies.

Analysis of genetic structure of mutant progeny: LnP (D) values derived from software processing results according to Evanno *et al.* (2005):

$$\triangle K = m(|L(K+1)-2L(K)+L(K-1)|)/s[L(K)]$$

The $\triangle K$ line chart is drawn (Fig. 11). Eighty eight mutant progeny materials were divided into 5 groups by population genetic structure analysis, including 22, 15, 13, 17 and 21 materials, which facilitated further analysis of the population genetic structure of mutant progenies with stay-green mutants (Fig. 12).

Discussion

It was found that radiation mutagenesis could change many traits of soybean such as the oil content of soybean can be increased after ^{60}Co mutagenesis (Guo *et al.* 2005). The stay-green mutant can maintain carbon assimilation over an extended period and maintain grain weight,

Table 7: Information of 18 SSR locus and diversity statistics

Number	Primers	Allele number	PIC	Shannon Index (H´)
1	Satt235	3	0.364	0.7081
2	Satt400	2	0.287	0.5232
3	Satt406	3	0.457	0.8455
4	Satt248	3	0.388	0.7156
5	Satt165	3	0.562	0.9825
6	Sat_385	5	0.693	1.4128
7	Satt321	2	0.103	0.2661
8	Sat_332	2	0.361	0.6714
9	Sat_201	3	0.513	0.9753
10	Satt450	3	0.265	0.4852
11	Sat_153	3	0.352	0.6254
12	Satt322	4	0.652	1.3465
13	Satt361	2	0.211	0.3562
14	Satt195	3	0.436	0.9768
15	Satt257	2	0.312	0.5763
16	Sat_272	2	0.107	0.1956
17	Sat_149	3	0.244	0.4015
18	Satt453	4	0.621	1.2237
19	Satt355	2	0.121	0.2681
20	Satt326	2	0.338	0.6028
21	Satt247	2	0.154	0.3627
22	Sat_091	3	0.445	0.8819
23	Satt624	3	0.563	1.0552
24	Satt514	4	0.517	0.9553
25	Sat_333	5	0.673	1.3624
26	Satt413	3	0.248	0.5126
27	Satt412	3	0.224	0.4528
28	Satt469	2	0.206	0.4124
29	Sat_200	2	0.146	0.3575
30	Satt243	3	0.522	0.9826
31	Sat_331	3	0.411	0.7784
32	Sat_108	2	0.378	0.6963
33	Satt156	3	0.347	0.6682
34	Satt723	2	0.049	0.1157
Average		2.8	0.362	0.6998
Total		96	12.3	23.7922

quality and nutrient efficiency (Jagadish *et al.* 2015; Rebetzke *et al.* 2016; Shi *et al.* 2016). In this study, ^{60}Co radiation mutagenesis was used to obtain stay-green mutants. Through analysis of 19 agronomic traits, we found that the yield per plant of mutant progeny was significantly increased, mainly due to the increase of effective total pod number, which was similar to Han's research (Han *et al.* 2008). The protein and fat content also changed. But in the aspect of stay-green trait, mutagenesis could decrease the stay-green trait of soybean stay-green mutant, which showed that SPAD values in leaves of most mutant materials was less than that of control materials without radiation treatment at seed filling stage.

Correlation analysis showed that there was a strong correlation between agronomic traits, which acted synergistically or antagonistically and affected plant growth. The results showed that stem diameter, plant weight, branch number, main stem pod number, branch pod number and total pod number were significantly positively correlated with yield per plant, and negatively correlated with pod height. Li (2018) showed that the breeding high-yield vegetable soybeans should consider all agronomic traits comprehensively, instead of pursuing plant height carelessly.

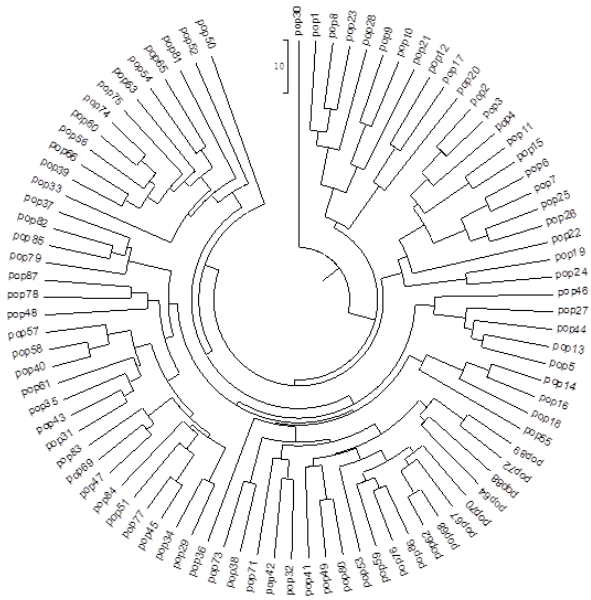

Fig. 10: Annular clustering figure of stay-green soybean and mutation progeny

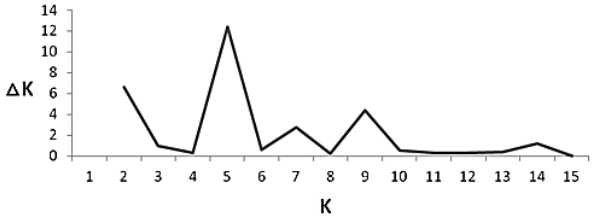

Fig. 11: △K determine the mutagenesis best group manager for several generations

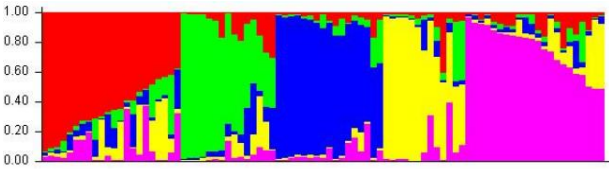

Fig. 12: Mutagenesis progeny population genetic structure

This study also indicates that when selecting a good progeny in mutant breeding, the material with low pod height should be given priority, which makes it easier to breed new varieties.

The PCA has been widely used in the character evaluation and the comprehensive evaluation of germplasm resources in many soybeans and vegetable soybeans (Wu and Chen 2007; Li 2018). This study used PCA transformed several variables into a few important variables. Similarly, 17 agronomic traits of 88 materials were dimensionally reduced to simplify the analysis method and reduce the data variables. Xin *et al.* (2019) have used this method to reduce the dimensionality of the quality evaluation indexes of wax gourd wine. After that, it would be easier to analyze the

yield components of mutant progeny. Then the factors affecting the growth of mutant progeny were divided into five main components such as yield factor, stem type factor, pod number factor, insect pest factor and plant type factor. These factors can be considered comprehensively in the selection of progeny materials to facilitate the selection of new varieties with high quality.

Finally, the data were processed by the systematic clustering method. Clustering analysis can not only reveal the genetic differences and relationships among populations, but also can revealed the genetic similarities among varieties within populations (Xue *et al.* 2019). By using cluster analysis and PCA, the genetic characters of main soybean varieties in different regions were analyzed, and the difference of genetic distance between different varieties was found out, which provided theoretical basis for soybean cross breeding and parent selection (Hu 2004; Kang *et al.* 2009; Zhao *et al.* 2017). In this experiment, the stay green mutants and their mutant progenies were divided into four categories, the first category belongs to low yield materials, the second category belongs to middle yield materials, the third category belongs to high yield materials and the fourth category belongs to extremely high yield materials. The hybrid combination of different categories of soybean is beneficial to the breeding of soybean varieties with good comprehensive characters. The stay-green mutants belong to the first category, and their yield is relatively low. After mutagenesis, the yield per plant of progeny increased significantly, and some extremely high yield materials appeared. This indicated that radiation mutagenesis could change the agronomic traits of the mutants with low yield and increase its ability to yield. After the clustering analysis, 89 materials were divided into 6 groups with different phenotypic traits. Among them, the genetic variation of No. 50 material in the sixth group was great, the stay-green trait was obvious, and the yield per plant was high. Therefore, it should be taken as the key research object in the next study.

Conclusion

The yield per plant of soybean stay-green mutant was negatively correlated with its stay-green trait after radiation mutagenesis and genetic variation of different traits occurred in varying degrees. Seventeen agronomic traits of mutant progenies can be divided into five main components: yield factor, pod factor, stem type factor, plant type factor and pest factor; and after systematic clustering, it can be divided into four groups: low yield, middle yield, high yield and extremely high yield. In this study, 96 allelic variations were detected by SSR genetic diversity analysis. The range of allelic variation of each primer ranged from 2 to 5, with an average of 2.8. Variation ranges of PIC were 0.049–0.693, with an average of 0.362. The Shannon's index of SSR markers ranged from 0.1157 to 1.4128, with an average of 0.6998. At last, according to the calculation of genetic distance, 89 materials were clustered into six categories.

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