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Quality trait variations in [^{60}Co]-irradiated wheat and high-molecular-weight glutenin subunit mutant identification

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ABSTRACT: With 300 Gy of [^{60}Co] γ-ray radiation of dry wheat seeds of Vortex 9722, the protein content, wet gluten content, sedimentation value, and hardness variation were analyzed in 341 lines in M4. Using over population mean ± 2X standard deviation as the screening standard, 8 lines with higher protein and wet gluten content and 4 lines with lower protein and wet gluten content were selected. In the M5 generation, the quality traits - silty parameters and high molecular weight glutenin subunits (HMW-GS) - were further analyzed in these 12 lines. The results showed that in the M5 generation, the quality traits in some variants were significantly different from those in the parents; the farinograms varied greatly. Eleven variants had significantly different HMW-GS bands compared to their parents. The parents had a HMW-GS composition of 5 + 14 + 15 + 12 + 9, and the variants had HMW-GS of 11 + 5 + 7 + 9 + 12 subunits or 1 + 5 + 7 + 8 + 12 subunits, indicating that the glutenin loci of these lines were mutated.

Key words: Wheat; [^{60}Co] γ-rays; Quality traits; Farinograms; HMW-GS

INTRODUCTION

Mutant varieties have effectively promoted agricultural production throughout the world and have produced significant economic benefits (Ahloowalia et al., 2004). Radiation mutation breeding techniques can create new materials and new germplasm; there is no safety problem (Xu, 1997). Ahloowalia and Maluszynski (2001) reported that at least 1800 kinds of cultivars have been created in more than 50 countries using radiation mutagenesis. In recent years, the number of mutant variety-bred plants, cultivated area with mutant varieties, and economic aspects in China have shown greater advantage over the rest of the world. We have bred 802 mutant varieties in 45 kinds of plants (Liu et al., 2009). Many studies have shown that radiation mutagenesis can improve the protein content of wheat, and the effect is apparent. Li et al. (1994) used [^{60}Co] γ-rays, lasers, and neutron radiation on wheat seeds. In the M5 generation of screening, the grain protein was mutated to have higher or lower content. The effect on protein content was clearly improved, where the maximum was 9.09% higher than in the control material. Sun et al. (1996) irradiated spring wheat 8131 with [^{60}Co] γ-rays to create a number of wheat germplasms with desirable qualities. The protein and lysine contents in some grain mutants were as high as 21.20 and 0.48%, respectively. However, so far, subunit mutation studies of radiation-induced silty traits and wheat glutenin have been rarely reported. In-depth quality improvement of wheat has become an important component of wheat breeding (He et al., 2006). A farinograph measures important flour properties to evaluate the quality of wheat and the quality of grain to help guide wheat flour and food production. These properties are also an important basis in the evaluation of high-quality wheat varieties, high-quality wheat, and gluten flour (Wei et al., 2010). High-molecular-weight glutenin subunits (HMW-GS) have important implications in the baking quality of wheat (Liu and Li, 2000; Payne et al., 1983). In this study, dry seeds of Vortex 9722 common wheat were subjected to [^{60}Co] γ radiation, and the mutants were screened in the M4 generation. Four quality traits - protein content, wet gluten content, sedimentation value, and hardness - were evaluated in M5 generation variants. Farinogram factors were determined using a Brabender farinograph; HMW-GS detection was performed with SDS-PAGE to explore the mutagenic effects of [^{60}Co] γ irradiation on wheat processing quality and to explore new ways in wheat quality improvement.

MATERIAL AND METHODS

Test materials and mutagenic treatment

The wheat varieties were Vortex 9722. The dry seeds received 300 Gy [^{60}Co] γ-ray acute radiation treatment for 10 min at the Institute of Atomic Energy of Anhui Academy of Agricultural Sciences in 2007.

Cultivation and identification of mutagenic descendants

The irradiated dry seeds were sown in October 2007. After maturation of M1, the surviving main spike of harvest seed plants was collected, and the seeds were mixed and then saved. M2 was planted with the mixed group, and a single plant was preserved. M3 planting

lines-341 lines selected according to field traits-formed the M4 groups. Quality traits of the M4 lines were measured. Mutants were determined so that protein and wet gluten content was more than the population mean ± 2X standard deviation. Screening produced twelve mutant lines, which, along with the parents, were planted. A randomized block design was adopted with two repetitions, and each material was planted in 4 rows; the rows were 2.0 m long and spaced 25 cm apart, and the plants were 5 cm apart.

Determination of quality traits

A DA 7200 infrared wave quality analyzer (Botong, Sweden) was used for the quality measurement of the wheat grain. Measurement indicators were protein content, wet gluten content, sedimentation value, and hardness. A 880110 laboratory flour mill and 810108 farinograph (Brabender, Germany) were used for the determination of silty indicators and silty map drawings. Five farinogram indicators - water absorption, dough development time, stability, softness, and evaluation - were obtained.

High-molecular-weight glutenin extraction and electrophoretic analysis

High-molecular-weight wheat gluten was extracted from M5 generation variants. HMW-GS was identified by SDS-PAGE electrophoresis; Zhongguo Chun (null, 7 + 8.2 + 12), Yumai 34 (1.7 + 8.5 + 10), and Wanmai 38 (1.7 + 8.2 + 12) were used as controls. The variants were interpreted, and the subunit naming was according to the Payne naming system (Nakamura, 2000).

One seed in each material was selected and crushed; it was placed in a 1.5-mL centrifuge tube. Each sample was extracted with 500 μL 50% (v/v) isopropanol, and the extraction was at 60°C for 30 min with intermittent mixing. The sample was then centrifuged at 10,000 rpm for 1 min. The supernatant was discarded, leaving the pellet; the procedures were repeated twice, and gliadin was removed. Next, 400 μL extraction buffer were added (62.5 mM Tris-HCl, pH 6.8, 10% glycerol, 2% SDS, 5% β-mercaptoethanol, and 0.002% bromophenol blue), and the sample extracted at 60°C for 2 h. After centrifugation at 10,000 rpm for 10 min, the supernatant was collected. Concentrated SDS-PAGE gel electrophoresis buffer was 0.5 M Tris-HCl, pH 6.8. Separation gel buffer was 3.0 mM Tris-HCl, pH 8.5. The electrophoresis buffers were 25 M Tris, 192 mM glycine, and 0.1% SDS, pH 8.3. A vertical plate electrophoresis tank was used; the separating gel contained 30% acrylamide and 0.8% bisacrylamide. Electrophoresis was run at 20-25 mA constant voltage for 8-10 h. After electrophoresis, the plastic sheet was placed at 12% trichloroacetic acid and fixed for 10 min; 0.1% Coomassie brilliant blue, 7% acetic acid, and 40% methanol were added for staining for more than 4 h. Destaining was performed with 7% acetic acid and 40% methanol solution. A gel imaging system (Gel Dec EQ, Bio-Rad, USA) was used for the photograph (Feng et al., 2000).

Data processing

The statistical analysis software Excel and DPS were used for data processing and charting.

RESULTS

Quality performance of variants in M4 generation

According to protein and wet gluten contents in various lines, over population mean ± 2X standard deviation was used for determining mutant lines. Eight lines with high protein and wet gluten contents were selected. Four lines had low protein and wet gluten contents; the mutant and parental quality traits are listed in Table 1.

Four quality traits were evaluated by analysis of variance in 12 M5 generation mutants and parental lines, and the F test results are shown in Table 2. As can be seen from Table 2, protein content, wet gluten content, and sedimentation value reached a significance level of 0.01 with the F test, but the difference in hardness was not significant.

Table 3 lists the multiple comparison results between the variants and the parent Vortex 9722. In the high-protein and wet gluten content mutants, protein content and wet gluten content of 226 lines showed a very significant difference compared to the parents ($P < 0.01$), and sedimentation showed a significant difference ($P < 0.05$). Wet gluten content of 166 lines showed a very significant difference compared to the parents ($P < 0.01$), whereas protein content and sedimentation showed a significant difference ($P < 0.05$). The wet gluten content of lines 34, 199, and 275 also showed significant or a very significant difference relative to the parents. In lines with low protein and wet gluten contents, the protein content and sedimentation value of lines 27, 205, and 253 showed a very significant difference compared to the parents ($P < 0.01$). The protein content of line 174 showed a significant difference ($P < 0.05$). The quality traits in other lines were not significantly different compared to their parents.

Table 1. Quantity characters of mutant lines in M4 and parent.

	Lines	Content of protein (%)	Content of wet gluten (%)	Sedimentation (mL)	Hardness
Higher protein and gluten content lines	34	15.8	38.8	56.6	42.1
	166	14.6	36.5	53.0	38.3
	188	15.9	39.3	56.7	38.5
	199	16.3	39.4	56.5	43.7
	219	14.9	34.9	45.9	43.7
	226	16.3	39.8	55.2	50.5
	256	14.6	37.3	54.5	43.6
	275	15.8	40.2	56.2	48.5
Lower protein and gluten content lines	27	12.2	31.1	45.2	39.3
	174	12.0	29.7	40.3	32.4
	205	12.3	30.6	40.6	38.0
	253	12.3	30.0	38.7	24.1
Parent		13.9	34.1	49.0	38.8

Table 2. Analysis of variance for quantity characters of mutant lines in M5 and parent.

Quantity characters	MSt	MSe	F value
Content of protein (%)	4.43	0.37	11.97**
Content of wet gluten (%)	14.20	1.42	10.00**
Sedimentation value (mL)	167.79	13.42	12.50**
Hardness	20.20	9.84	2.05

**Significant differences at 0.01 level.

Table 3. Difference of quality characters between mutant line and parent.

	Lines	Content of protein (%)	Content of wet gluten (%)	Sedimentation value (mL)	Hardness
Higher protein and gluten content lines	34	16.8	34.1**	64.6	43.2
	166	17.0*	33.9**	66.6*	42.9
	188	15.6	31.6	57.5	41.5
	199	16.7	32.8*	65.2	39.7
	219	14.7	29.7	52.9	39.8
	226	18.6**	37.6**	67.2*	41.0
	256	14.9	30.4	51.5	42.4
	275	16.2	33.2*	62.9	40.5
Lower protein and gluten content lines	27	13.7**	29.8	44.1**	40.8
	174	14.3*	29.9	55.0	37.2
	205	13.7**	27.7	42.4**	38.9
	253	13.5**	28.3	40.4**	36.2
Parent		15.7	30.1	58.2	36.8

*Significant difference at 0.05 level;**significant difference at 0.01 level.

Silty trait analysis in M5 mutants

Silty traits in M5 mutants are presented in Table 4. The results showed that water absorption, dough development time, stability, and degree of softening in mutants were different from those in parental lines. Mutants with greater water absorption difference were lines 199 and 275, which had high protein and wet gluten content. Lines 34, 188, 219, and 226 with high protein and wet gluten content and lines 174, 176, 205, and 253 with low protein and wet gluten content showed large differences in dough development time compared to the parent. Lines 219 and 226 with high protein and wet gluten content and lines 176, 205, and 253 with low protein and wet gluten content were quite different from their parents. The weakening of parents was high, in addition to low protein and wet gluten content of line 27; the remaining lines showed lower levels than those in the parents. The silty mass index in parents was lower, in addition to low protein and wet gluten content of line 27; the remaining lines had higher levels than those in the parents.

Table 4. Farinograph parameters of mutant lines in M5 and parent.

	Lines	Absorption (%)	Development time (min)	Stability time (min)	Degree of softening	Valorimeter value
Higher protein and gluten contents lines	34	64.6	5.2	5.5	65	86
	166	65.8	4.3	4.7	62	77
	188	64.2	4.9	5.9	67	79
	199	67.3	3.0	2.9	87	52
	219	61.4	4.9	6.8	58	93
	226	64.6	4.7	6.0	55	103
	256	64.3	4.2	5.3	77	69
	275	68.2	3.4	3.3	84	55
Lower protein and gluten contents lines	27	62.1	3.4	3.3	103	47
	174	61.1	4.5	4.3	79	68
	205	60.2	4.9	7.5	66	86
	253	63.8	4.5	8.0	80	81
Parent		64.9	3.2	2.6	98	50

HMW-GS analysis of M5 mutants

As seen in Figure 1, the parentals and mutants all had five bands each, and the

HMW-GS composition of Vortex 9722 was +9 + 15 + 14 + 12 5 subunits. The HMW-GS in the mutant could be roughly divided into two categories. The first category was 1 + 5 + 7 + 9 + 12 subunits, which had a two-subunit difference with the parents. The second category was 1 + 5 + 7 + 8 + 12 subunits, which had three different subunits compared to the parents. Lines 27, 34, 166, 188, 219, 226, 256, and 275 belonged to the first category of variation types; lines 174, 205 and 253 belonged to the second category of variation types. The above-mentioned results indicated that the screened mutants had significant differences in bands compared with the parental lines, suggesting that mutant lines had mutated in the glutenin loci.

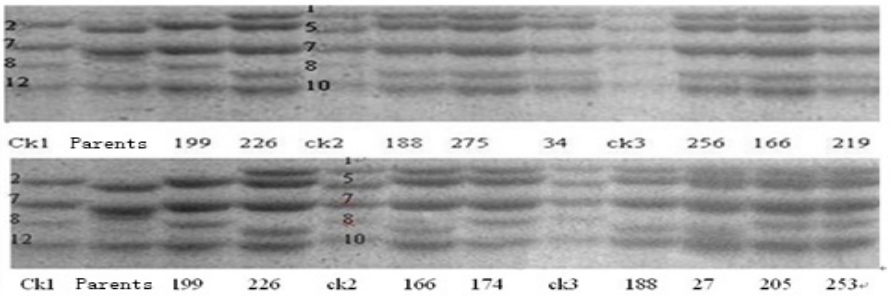

Figure 1. Electrophoresis pattern of HMW-GS: ck_1 = Chinese spring, ck_2 = Yumai 34; ck_3 = Wanmai 38, parent-Guo9722, other paths are mutant lines.

DISCUSSION

This study showed that with 300 Gy [^{60}Co] γ irradiation of dry wheat seeds, quality traits of mutants had larger separations. The four quality traits protein content, wet gluten content, sedimentation value, and hardness in M4-generation groups were normally distributed. According to statistical theory, the population mean ± 2X standard deviation [$P(|u| \geq 1.96\sigma) \leq 0.05$] was taken as the standard; protein and wet gluten contents were used to screen out the high and low variability of lines. Despite the generation differences, the grouping trend to line screening with the method was the same. M4 screened high and low protein, and wet gluten content lines were still in their respective categories in M5. It demonstrated that the use of this method in the M4 generation for quality traits screening of mutants was effective. In the direction of the protein content variations, Li et al. (1994) used [^{60}Co] γ irradiation of dry wheat seeds; the protein content in the M5 generation was detected. They found that the direction of development was divided into both high and low protein content variations; the effect of the protein content increase was significant, which was up to 9.09%. In this study, we selected eight lines of high-protein content and four lines of low-protein content in the M4 generation of Vortex 9722, which was consistent with previous findings.

Dough is the basic transition shape from flour to food in wheat. The good control of the process of dough properties produces bread, pasta, biscuits, and other foods with good quality. Accordingly, the dough rheological properties are the main indicators for determining the quality and processing of wheat flour. The farinograph is the instrument for measuring dough rheological properties. It was designed according to the resistance in kneading the dough. Re-

sistance is automatically recorded as a particular curve through the mobile imitation device, which is called a silty map; it is the basis for analyzing the quality of the dough. From the silty chart, we can be very intuitive to judge the gluten strength of the flour. Water absorption rate is the water quality required for mixing a given weight of flour in the production of dough; the water absorption rate directly affects the end product rate. Dough development time is the mixing time from 0:00 of dough (start of water addition) until it reaches a maximum consistency. It is positively correlated with the protein content. Dough stability explains the degree of mixing resistance of the dough; the quantity and quality of protein are the main factors affecting stability time. The long dough stability time is the description of strong gluten strength in wheat flour. Degree of softening is the destruction rate in the dough mixing process. The greater the indicator values, the weaker the gluten strength is, which is negatively correlated with the quantity and quality of the protein. Evaluation value is the composite score of the silty map according to the dough development time and dough softening degree. The greater the indicator values, the greater the gluten strength is. According to the silty map, wheat flour can be divided into weak force powder (the dough development time and stability time are short), medium force flour (the dough development time and stability time are longer), and strong flour (the dough development time and stability are the longest) (Lv et al., 2011). Silty analysis showed that parental Vortex 9722 belonged to the weaker force powder; dough development time (3.2 min) and stability time (2.6 min) were shorter. Degree of softening was higher (98), and the evaluation value was lower (50). We found that with [^{60}Co] γ-ray mutagenesis, the mutated lines can be obtained with silty index mutations. The dough development time in 34 lines was 5.2 min, which was higher than that in the parents. The dough stability time of 219 lines was 6.8 min, which was longer than 4.2 min in the parents. The evaluation value of 226 lines was 103, which was twice that in the parents.

Domestic and international studies have shown that HMW-GS have a great influence on the quality of wheat. Certain subunits (e.g., quality subunits 5 + 10) can significantly improve the baking quality of wheat (Sun et al., 2000; Li et al., 2000; Ma et al., 2004; Liu and Li, 2000). As can be seen in HMW-GS electrophoresis, there were significant differences in bands between the variants and the parental lines. There were two main HMW-GS combinations in variants. The protein content and its HMW-GS in different lines had no evident relationship, which is consistent with the results of Li et al. (2000) and Lu and Ma (2000). HMW-GS mainly affected gluten strength; they had less effect on protein content. Li et al. (2009) reported the space flight of wheat variety Baiyingdong No. 2; two HMW-GS mutants were discovered in 92 lines SP2. Our study demonstrated that [^{60}Co] γ irradiation may also cause HMW-GS variation.

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REFERENCES

Ahloowalia BS and Maluszynski M (2001). Induced mutations - A new paradigm in plant breeding. *Euphytica* 118: 167-173.
Ahloowalia BS, Maluszynski M and Nichterlein K (2004). Global impact of mutation derived varieties. *Euphytica* 135: 187-204.

Feng Y, Min DH and Sun DJ (2006). Composition of HMW-GS of wheat cultivars grown in Huanghuai wheat production area. *J. Tritical Crops* 26: 68-71.

He ZH, Yan YM and Zhang QS (2006). Establishment of quality evaluation system and utilization of molecular methods for the improvement of Chinese wheat quality. *Sci. Agric. Sinica* 39: 1091-1101.

Li BY, Wang YG, Liu FM, Sun H, et al. (2000). Relationships between high molecular weight glutenin subunits (HMW-GS) and quality traits in wheat (*Triticum aestivum* L.). *Acta Bot. Boreali-Occidental Sinica* 26: 322-326.

Li P, Sun MZ, Zhang F, Zhang FY, et al. (2009). Screening and identification of HMW-GS mutants in wheat. *Acta Agric. Nucleatae Sinica* 23: 935-938.

Li XZ, Lv SY and Pan B (1994). Radiation influence protein content of wheat. *He Nong Xue Tong Bao* 5: 53-55.

Liu GT and Li BY (2000). The inheritance and improvement of quality traits in common wheat. *J. Agric. Biotechnol.* 8: 307-314.

Liu LX, Guo HJ, Zhao LS, Li JH, et al. (2009). Current status and outlook perspectives of induced for plant improvement. *Acta Agric. Nucleatae Sinica* 23: 1001-1007.

Lu Y and Ma CX (2000). Variations of HMW glutenin subunits and their association with bread making quality in wheat. *J. Tritical Crops* 4: 32-36.

Lv XY, Wang F and Zhang HT (2011). Studies on flour rheological properties and steamed bread qualities of medium-gluten wheat. *Flour Milling* 6:17-22.

Ma X, Ren ZL, Zuo BJ and Zhang HQ (2004). Study on effect of high molecular weight glutenin subunits and protein components on bread-baking quality characteristics of wheat. *J. Sichuan Agric. Univ.* 22: 10-14.

Nakamura H (2000). Alleic variation at high molecular weight gluten in subunit loci Glu-A1, Glu-B1 and Glu-D1 in Japanese and Chinese hexaploid wheats. *Euphytica* 2: 187-193.

Payne PI, Holt LM and Lawrence GJ (1983). Detection of a novel high molecular weight subunit of glutenin in some Japanese hexaploid wheats. *J. Cereal Sci.* 1: 3-8.

Sun H, Li BY, Wang YG, Zhang SZ, et al. (2000). Correlation between gluten in subunits and bread-making quality characteristics of common wheat. *Sci. Agric. Sinica* 5: 18-24.

Sun JT, Si AX and Shang XW (1996). Developing high protein germplasm by x-Ray induction in spring wheat. *Xi Bei Nong Ye Xue Bao* 5: 27-30.

Wei YM, Zhang B, Guan EQ, Li WJ, et al. (2010). Comparisons of instruments in measuring dough rheological properties. *Sci. Agric. Sinica* 43: 4265-4270.

Xu GR (1997). Introduction to Nuclear Agriculture. 1st edn. Yuan Zi Neng Press, 10-15.