Induced effect of irradiated exogenous DNA on wheat*

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Abstract Irradiated exogenous DNA introduced into wheat can give rise to break of DNA-chain and damage of part of alkali radicals. Introducing exogenous DNA irradiated by γ rays could increase Do fructification rate and decrease seed size and plumpness. These tendencies became obvious with dose increase. In comparison with control DNA, introducing DNA irradiated could raise evidently mutagenic effect of pollen tube pathway technique.
Keywords Irradiation on exogenous DNA, Introduction, Wheat, Biological radiation effects

1 Introduction

Recent years, plant molecular biologists have done lots of work on the vectors, introduction paths and technique operation of plant gene engineering and obtained a great achievement. Generally, Ti and Ri plasmid were being used as vectors in dicotyledon, but in monocotyledon the direct transfer methods were being applied. Pollen tube pathway technique is a simple and rapid method of introducing DNA directly. It is unlimited to recipients and has been applied to cotton, paddy rice, wheat and soybean in China.

In order to increase transfer rate and mutagenic effect of direct transfer method, exogenous DNA irradiated was introduced into wheat and some results were obtained.

2 Materials and methods

2.1 Drawing and irradiating of exogenous DNA

With chloroform-iso-amylalcohol-RNAase method, the DNA of *Elymus Giganteus*, triticale (92k1, 92k230), wheat (91k4) and allooctoploid agrotuitcum (Zhong No.5) were drawn as donors. At 230, 260, 280 nm, the optical dencity (O.D.) values of ultraviolet light of DNA solution were determined, A260/230>1.90, A260/280>1.85, so the purity of DNA was achieved standard.

The DNA solutions of donors were irradiated respectively under 2, 20, 200, 400 Gy of X-rays and 400, 800 Gy of γ rays and the agrose gel electrophoreses of the DNA solutions were done using λDNA+Hind III as standard DNA. The break effects of donors DNA were observed and photographed. The O.D. values of irradiated DNA solution were determined at 230, 260, 280 nm.

2.2 Introducing of exogenous DNA

The styli of wheat 91B10, Xinkehan No.9, 92K375 and Longfu No.3 were excised 30—45 min after pollination, and then, the donor DNA solutions were smeared respectively on those sections. Otherwise, the 1×SSC buffer solution was smeared as control. To keep their humidity, the treated ears should be covered with plastic sacks for 1~2 h. The ripe ears were collected.

2.3 Counting of D_0 and D_1 variation

Fructification rate, seed size and plumpness of D_0 generation were counted. In the second year, extensive variation was observed such as stem strength, height, heading time, spike shape, awns and mature stage. The D_1 variation friquencies of all combinations were counted.

3 Results and analyses

3.1 Effect of radiation on exogenous DNA

Spectrum of the agrose gel electrophoresis shows that the lengths of λDNA+Hind III are, in turn, 23.7, 9.46, 6.57, 4.26, 2.26 and 1.98 kb; the lengths of DNA drawn from donors were situated between 25 kb and 4.26 kb; the lengths

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of Zhong No.5 DNA irradiated with 400 Gy of γ rays were situated between 23 kb and 1 bp, showing that irradiation broke a lot of DNA-chains into short pieces; after irradiation with 800 Gy of γ-rays, the DNA concentration of Zhong No.5 was obviously decreased, indicating that a lot of alkali radicals of DNA-chains were damaged; after irradiation with 400 and 800 Gy of γ-rays, the DNA lengths of 92k1 were not changed obviously.

As shown in Table 1, O.D. values of DNA solutions of Zhong No.5 and 92k1 were decreased after irradiation and these tendencies became obvious with dose increase, indicating that the bigger the dose was, the more obvious the break effect of DNA-chains were.

3.2 Effect of exogenous DNA on D_0 fructification rate

Table 2 shows that irradiating exogenous DNA with 2 and 20 Gy of X-rays could increase D_0 fructification rate obviously.

Table 1 O.D. values of irradiated DNA

λ/nm	Zhong No.5				92k1			
	Control	γ400Gy	γ800Gy	X400Gy	Control	γ400Gy	γ800Gy	X400Gy
230	0.203	0.130	0.126	0.125	0.280	0.264	0.259	0.233
260	0.396	0.321	0.309	0.274	0.574	0.538	0.514	0.490
280	0.210	0.149	0.123	0.115	0.311	0.274	0.258	0.148

Table 2 Effect of X-rays irradiated exogenous DNA on D_0 fructification rate

Dose/ Gy	92k1+ Xinkehan No.9	91k4+ 92k375	91k4+ Xinkehan No.9	Zhong No.5+ Longfu No.3	92k230+ Longfu No.3	Zhong No.5+ Xinkehan No.9	Average
Buffer	0.226	0.667	0.526	0.622	0.622	0.667	0.555
0	0.296	0.630	0.393	0.744	0.350	0.486	0.483
2	0.531	0.762	0.472	0.641	0.760	0.524	0.615
20	0.486	0.793	0.541	0.633	0.850	0.412	0.619
200	0.459	0.486	0.387	0.689	0.600	0.473	0.516

Table 3 Average sizes (long×wide×high, mm^3) of D_0 grains

Combinations	Donor	Recipient	Buffer	control	X2Gy	X20Gy	X200Gy
Zhong No.5+Longfu No.3	48.91	61.44	30.88	30.45	23.78	22.30	27.66
92k230+Longfu No.3	50.83	61.44	30.88	20.33	16.16	18.16	15.85

Table 4 Distribution of plump, less plump and shrivelled grains of D_0 generation

Combinations	Buffer			control			X2Gy			X20Gy			X200Gy		
Zhong No.5+Longfu No.3	2	14	0	3	19	3	3	13	2	0	22	2	0	23	0
92k230+Longfu No.3	2	14	0	—	—	—	0	10	3	0	10	0	0	5	3
Zhong No.5+Xinkehan No.9	4	26	0	0	10	7	0	16	6	0	16	5	0	9	14

3.3 The effects of irradiated exogenous DNA on D_0 seed size and plumpness

After introducing exogenous DNA irradiated with X-rays into recipients, average size of D_0 seed was smaller than those of donors and recipients (see Table 3).

The distribution of plump, less plump and shrivelled grains of D_0 was investigated (see Table 4) and a certain law can be found. The buffer solution was introduced and D_0 grains of that were all plump or less plump. Shrivelled grains appeared in introduction of no-irradiated DNA. With increase in irradiation dose, plump grains were decreased, but shrivelled grains were increased. Do grains of 3 combinations with 20 Gy and 200 Gy were all less plump or shrivelled.

3.4 The effect of irradiated exogenous DNA on D_1 variation

Table 5 shows that D_1 variation frequencies are remarkably raised with introducing exogenous DNA irradiated. On D_1 plant of combination *E. Giganteus* + 91B10, the new characters emerged such as erect stem, narrow and sharp leaves, which were similar to those of *E. Giganteus*. Meanwhile, on some plants of

the combination, the pagoda type of ear became the square type. In combination 92k1 + Xinkehan No.9, the character of less plumpness emerged in D_1 grains. It is between donor of shrivelled triticale and recipient of plump wheat. In D_1 of combination Zhong No.5 + Xinkehan No.9, different introduction treatments gave rise to different heading time. After introducing buffer solution and no-irradiated DNA, D_1 heading time was the same as that of recipient. After introducing DNA irradiated, the heading time of D_1 was later for 2 or 3 d than that of recipient, approaching that of donor. Otherwise, the variations appeared in the traits such as the awn type, ear type, leaf color and waxiness. Especially, with 200 Gy, the characters emerged in D_1 plants such as the thick vein, dense leaf color, bristle and creeping stem, which the donor had originally. In D_1 plants of other combinations, characters of more plants tended to recipients; but some plants tended to donors; some plants had the new characters which the parents had not ever had.

Table 5 Effect of irradiated exogenous DNA on variation frequency of D_1 plants

Combinations	Buffer control	X2Gy	X20Gy	X200Gy	
E.Giganteus+91B10	0	0	0.833	—	—
92k1+Longfu No.3	0	—	0	0.500	0.500
91k4+92k375	0.300	0	0	0.600	0
92k1+Xinkehan No.9	0	1.000	0.800	0.667	1.000
Average	0.075	0.330	0.408	0.589	0.500

4 Discussion

As shown in the experimental results, through irradiation with ionization rays, the molecular weight of exogenous DNA became small and O.D. value of ultroviolet light decreased. The results showed that irradiated exogenous DNA gave rise to break of DNA-chains and damage of alkali radicals.

Radioactive biology[1] has pointed out that the ionization rays act on DNA solution through two ways: direct action and indirect one. The former indicates that ionization rays deposit energy directly on DNA molicules and make them broken or damaged. The latter indicates that ionization rays act on H_2O molecules and engender hydroxyl free radicals ·OH and hydration electron e_{aq}^-, then these products attack DNA molecules. The attacked DNA molecules will be broken or damaged and engendered some alkali free radicals and ribose free radicals. Because the broken DNA molecules are short, the introduced DNA fragment was easy to be conformed to recipient's chromosomes and the chromosomes with donor DNA were easy to be mated. This might be the cause that exogenous DNA irradiated can raise D_0 fructification rate.

The free radicals of biological molecules have strong character of oxidation or reduction. In the cell, those can break peptide bonds and –S–S– bonds of proteins, and oxydize SH radicals, and make carbohydrate dehydrogenated and then open the rings, and make unsaturated bonds of lipid oxidized and broken. Therefore, the plasmalemmas and nuclear memebranes of cell endomembrane system were easy to be damaged by biological free radicals, and that was favourable for small DNA passages to get into egg cells. This was one of the reasons that exogenous DNA irradiated could raise D_0 fructification rate. Irradiation gave rise to some damage of donor DNA; broken DNA was easy to conform to recipient's chromosomes; these were the reasons that D_1 variation frequency rised. The study on character variation of subsequent generations is in progress.

Reference

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