Study on Variation Effects Caused by Ion Beam-mediated Transformation Whose Transformation Receptors are Wheat's Segregation Population Seeds

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Abstract: To provide theory evidence for ion beam transformation used in traditional breeding easily, the variation effects were studied that wheat's segregating population seeds were used as transformation receptor via ion beam implantation. Wheat's F2 segregating population seeds of two hybridization combination were used as transformation receptor via ion beam implantation, and the genome DNA of Hongmang wheat and Hexaploid Triticale were used as transformation donor.The result showed that the germination rate of different transformation combination was different. The coefficient variability of spike length of wheat main axic increased significantly. The average of plant height had decreasing trend. Both average and coefficient variability of grain's quality characters had decreasing trend. All the above results show that after doing ion beam-mediated transformation to segregation population seed, the coefficient variability of some characters could increase significantly, in another word, ion beam-mediated transformation could widen the variation spectrum of some characters.
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Key words: Transformation mediated by ion beam implantation; Wheat; Segregating population

1. Introduction

Transformation technique has combined with conventional breeding technique and applied in wheat genetic improvement. It could improve the actuality that wheat's genetic basis is strait, genomic resources are lack. Now, the main wheat genetic transformation methods are particle bombardment (Takumi et al., 1994, Chugh and Khurana, 2003, TANG et al., 2006), pollen-tube pathway (LIU et al., 2005, YIN et al., 2004), agrobacterium-mediated transformation (Supartana et al., 2006, Bi et al., 2006, Haliloglu and Baenziger, 2003), et al. Since Yu reported the successful GUS and CAT gene transfer into suspension cells and mature rice embryos following the 20–30 keV argon ion bombardment (Yu et al, 1993), a number of reports have emerged on ion bombardment which induced foreign molecule transfering into plant tissues and bacteriacells (Yang et al, 1994, Li et al, 2001, Wu et al, 2000, Wu et al, 2000) . It showed special characters in wheat's transformation. The chitinasegene (RCH8) in plasmid vector pCAMBIA1308 had been reported to be delivered into three wheat cultivars (Yangmai158, Wan9210, Wanmai32) by a Low energy Ar^+ beam-mediated method, and molecular blotting assays confirm stable integration of alien DNA fragments into wheat genome (Wu et al, 2000). The plant transformation frequencies which depend on the plant species and ion fluence ranged from 0.5 to 3.8%. Jiao et al. (2006) reported wheat was transformed with soybean DNA through ion beam mediation and its high-protein offspring plants were obtained through field selections and protein determinations in the four consecutive generations. The application of ion beam-mediated transformation on wheat, especially transferring foreign genomic DNA into wheat, indicated that the merging of foreign genomic DNA could lead receptor's genomic DNA sequences' change, and could enrich wheat's genetic resources.

In present, ion beam-mediated transformation process is that put stable varieties and strains as transformation receptor first, and then obtain transformation material via choice, and then modify and use these transformation materials. While wheat traditional breeding process is choicing single plants from F2 segregation population. To combine the dominance of ion beam-mediated transformation and traditonal breeding and improve traditional breeding via ion beam-mediated transformation, the variation effects caused by ion beam-mediated transformation which put wheat's segregation population seed as transformation receptor were studied.

2. Material and Methods

2.1 Material

2.1.1 Receptor wheat material

Two wheat crossing combinations were named combination 1(Yunong 118×Shannong 520853) and

combination 2 (Yi 12×Lankaoaizao 8).

2.1.2 Donor material

When Hongmang wheat and hexaploid triticale grew to 3-5 leaf, extracted leaf genomic DNA using SDS alkaline lysis method. The OD260/OD280 =1.89-1.95. The extracting leaf genomic DNA was used as transformation donor DNA.

2.2 Method

2.2.1 Ion beam-mediated transformation method

Seeds of F2 of each combination were divided into two groups. Each group had 500 seeds. One of the two groups was named "control group" which was not implanted by ion beam. The other was named "treatment group" which was implanted by $3\times10^{17}N^{+}/cm^{2}$. Implantation instrument was TITAN pulse implantation instrument. Frequency of pulse was 25 Hz. Width of pulse was 400μs. Energy of ions was 30keV. Type of ions was N^{+}. Flux of ion beam was 2mA. Wheat seeds were put into the holes of the sample plat one by one with embryo upwards and were implanted by ions. After the treatment group of combination 1 was implanted, these seeds were dipped into 300μg/ml genomic DNA solution of Hongmang wheat. The temperature of solution maintained at 25℃. After 12h, refreshed the DNA solution and dipped another 12h. Then the seeds of control group and treatment group were planted in field at the same time. Distant between plants was 10cm. After the treatment group of combination 2 was implanted, these seeds were dipped into 300μg/ml genomic DNA solution of hexaploid triticale wheat. The dip method was same as the above. After dip, the seeds of control and treatment group were planted in field at the same time. The condition of planting was same as the above.

2.2.2 Agronomic traits' statistication

Statistication of germination rate: 100 seeds were placed on wet filter papers in the condition of 25℃. 7 days late, germination rate was statisticated. Germination rate=the number of germinating seeds/100. The rate of survival plant was statisticated after seed-setting. The rate of survival plant=the number of plants/the number of plant seeds.The control and treatment groups were observed and noted in the whole growth period. All the single plants were pulled out after maturity and several indices, for example plant height, spike length of main axic and effective panicle number, were measured.

2.2.3 Measurement of grain quality characters

Bulk density, protein and wet gluten were measured using FOSS NIRS5000 analyzer. One day ahead of spectrum scanning, seeds were placed in the same room with the NIR analyzer in order to make seeds had same enviroment condition with analyzer. The scanning internal of wavelength was 1100-2498nm, and scanning step was 2nm. The reflect intensity(R) was collected. Each material of seeds was scanned 30 times by analyzer automatically and analyzer obtained the mean spectrum automatically. Each material was scanned 3 times repeatedly. Difference of average and coefficient variability between control and treatment group was analyzed using *t*-test and *u*-test in statistical analysis. The difference of coefficient variability could express the condition of trait segregation.

3. Result

3.1 Statistication of germination rate and survival plant rate of each transformation combination

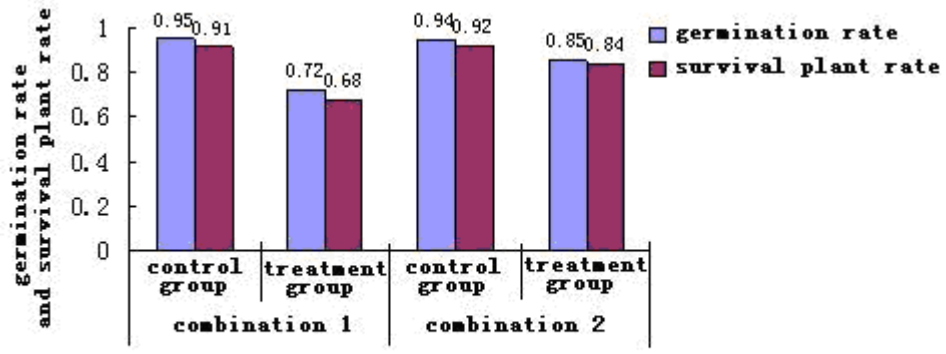

Figure 1. Germination rate and survival plant rate of different transformation combination

Transformation combination included 4 groups: control group of combination 1, treatment group of combination 1, control group of combination 2, treatment group of combination 2. Figure1 showed that survival plant rate of each group decreased little compared with germination rate. It indicated that ion beam-mediated transformation had little influence to plant growth after the seeds' germination. The germination rate of treatment group of combinatin 1 decreased by 24.2% compared with that of the control group. The germination rate of treatment group of combination 2 decreased by 9.6% compared with that of the control group. It indicated that ion beam-mediated transformation had more influence to germination rate of combinatin 1 compared with combination 2.

3.2 Agronomic traits' statistication

Table 1 showed the statistication result of plant height. To combinatin 1, the number of plants which plant height were between 70cm and 80cm in treatment group decreased from 60.0% to 18.1% compared with that of the control group, the number of plants which plant height were between 80cm to 90cm in treatment group decreased from 6.7% to 0 compared with that of the control group, the mean of plant height of treatment group decreased from 71.1cm to 63.4cm compared with that of the control group, achieved to significance level (α=0.01). Range of treatment group of combination 2 decreased from 60 cm to 47 cm compared with that of its control group, coefficient variability decreased from 21% to 16%, achieved to significance level (α=0.05).

Table 2 showed the statistication result of spike length of main axic. The mean of treatment group of combination 1 decreased to significance level (α=0.01) compared with that of the control group. The mean of treatment group of combination 2 decreased to significance level (α=0.05) compared with that of the control group, too. The coefficient variability of both combination 1 and 2 increased to significance level (α=0.05), and the result showed ion beam-mediated transformation could widen the variation spectrum of spike length of main axic.

Table 3 showed the statistication result of 1000-seed weight. The mean of treatment group of both combination 1 and 2 had no significant change compared with that of the control group. The coefficient variability of treatment group of combination 1 had no significant change compared with that of the control group, while the coefficient variability of treatment group of combination 2 decreased to significant level (α=0.05).

The maximum of effective panicle number of combination 1 and 2 appeared in treatment group. The maximum of effective panicle number of combination 1 was 26, and the maximum of effective panicle number of combination 2 was 32, while the maximum of effective panicle number of two control groups were 23.

Table 1. Statistication result of plant height

		combination 1		combination 2	
		control group	treatment group	control group	treatment group
Plant height distribu-tion range （cm）	<50(take **% of total plant number）	0.0	0.0	6.7	9.4
	50-60（as above）	5.0	23.6	29.8	18.8
	60-70（as above）	28.3	58.3	32.7	28.1
	70-80（as above）	60.0	18.1	25.0	23.4
	80-90（as above）	6.7	0.0	5.8	18.8
	>90（as above）	0.0	0.0	0.0	1.6
maximum（cm）		86	78	90	87
minimum（cm）		53	52	30	40
Range（cm）		33	26	60	47
Mean （cm±SD）		71.1±6.7	63.4±6.3**	66.8±11.0	64.2±10.3
Coefficient variability（%）		9.7	9.9	21.0	16.0*

(*significance level α=0.05,** significance level α=0.01)

Table 2. Statistication result of spike length of main axic

		combination 1		combination 2	
		control group	treatment group	control group	treatment group
Spike Length of Main Axic distribution range （cm）	5-7（take **% of total plant number）	0.0	7.0	0.0	9.6
	7-9（as above）	18.3	57.7	46.9	69.2
	9-12（as above）	81.7	35.2	53.1	21.2
maximum（cm）		11	10	11	12
minimum（cm）		7	5	7	5
Range（cm）		4	5	4	7
Mean（cm±SD）		9.3±1.0	8.0±1.1**	8.7±1.1	8.3±1.4*
Coefficient variability（%）		10.6	14.0*	12.4	16.5*

(*significance level α=0.05,** significance level α=0.01)

Table 3. Statistication result of 1000-seed weight

		combination 1		combination 2	
		control group	treatment group	control group	treatment group
1000-seed distribution range （g）	<30（take **% of total plant number）	0.0	0.0	9.4	4.8
	30-40（as above）	3.3	0.0	26.6	26.0
	40-50（as above）	36.7	45.8	32.8	50.0
	50-60（as above）	56.7	52.8	29.7	18.3
	>60（as above）	3.3	1.4	1.6	1.0
maximum（g）		63	60	60	61
minimum（g）		35	40	20	24
Range（g）		28	20	40	37
Mean（g±SD）		50.3±5.1	49.7±4.6	42.8±7.8	43.5±7.7
Coefficient variability（%）		10.3	9.3	22.7	17.7*

(*significance level α=0.05,** significance level α=0.01)

3.3 Statistication of grain quality characters

Table 4 showed the statistication result of protein content. The mean and coefficient variability of treatment group of combination 1 had no significant change compared with that of its control group. The mean and coefficient variability of treatment group of combination 2 decreased to significant level (α=0.01) compared with that of the control group.

Table 5 showed the statistication result of bulk density. The mean of treatment group of two combination had no signification change compared with that of the control group. The coefficient variability of treatment group of combination 1 decreased to significant level(α=0.05), and that of treatment group of combination 2 decreased to significant level (α=0.01).

Table 6 showed the statistication result of wet gluten. The mean and coefficient variability of treatment group of combination 1 had no significant change compared with that of the control group. The mean and coefficient variability of treatment group of combination 2 decreased to significant level (α=0.01) compared with that of its control group.

Table 4. Statistication result of protein content

		combination 1		combination 2	
		control group	treatment group	control group	treatment group
Protein content distribution range （ dry basis %）	11-13（take **% of total plant number）	5.0	0.0	6.25	14.4
	13-15（as above）	51.7	47.2	53.1	67.3
	15-17（as above）	41.7	50.0	32.8	18.3
	17-19（as above）	1.7	2.8	6.3	0
	〉20（as above）	0.0	0	1.6	0
maximum（dry basis %）		17.7	17.3	23.6	16.6
minimum（dry basis %）		11.9	13.3	12.0	11.8
Range（dry basis %）		5.8	4.0	11.6	4.8
Mean（dry basis %±SD）		14.9±1.0	15.0±1.1	14.9±1.5	14.1±0.8**
Coefficient variability（%）		7.1	7.1	12	7.3**

(*significance level α=0.05,** significance level α=0.01)

Table 5. Statistacation result of bulk density

		combination 1		combination 2	
		control group	treatment group	control group	treatment group
bulk density distribution range（g/l）	<780（take **% of total plant number）	3.3	1.4	6.3	4.2
	780-800（as above）	6.7	4.2	18.8	23.6
	800-820（as above）	25.0	23.6	35.9	50.0
	820-840（as above）	50.0	58.3	26.6	59.7
	>840（as above）	15.0	12.5	12.5	6.9
maximum（g/l）		855.8	847.9	849.5	848.1
minimum（g/l）		761.2	764.3	719.5	774.1
Range（g/l）		94.6	83.6	130	74
Mean（g/l±SD）		823.5±18.3	824.6±14.6	813±23.1	816±16.2
Coefficient variability（%）		2.3	1.8*	2.8	2.0**

(*significance level α=0.05,** significance level α=0.01)

Table 6. Statistication result of wet gluten

		combination 1		combination 2	
		control group	treatment group	control group	treatment group
wet gluten distributon range（%）	<25（take **% of total plant number）	3.3	0.0	6.25	6.7
	25-30（as above）	35.0	37.5	51.6	72.1
	30-35（as above）	58.3	54.2	37.5	20.2
	>35（as above）	3.3	8.3	4.7	1.0
maximum（%）		37.2	37.1	38.5	35.3

minimum（%）	22.3	25.1	23.1	22.5
Range（%）	14.9	12	15.4	12.8
Mean（%±SD）	30.4±2.8	30.8±2.9	29.9±3.3	28.3±2.3**
Coefficient variability（%）	9.2	9.4	14.6	8.8**

(*significance level α=0.05,** significance level α=0.01)

4. Discussion

Ion beam-mediated transformation have different influence to the germination rate of different receptor, for example the germination rate of treatment group of combination 1 decreased more compared with combination 2 (Figure 1). So before doing ion beam-mediated transformation, suitable implanting dose should be decided through previous dose experiment.

All the above results show that after doing ion beam-mediated transformation to segregation population seed, the coefficient variability of some characters could increase significantly, in another word, ion beam-mediated transformation could widen the variation spectrum of some characters. So in breeding process, both the treatment and control group of segregation population shoule be planted and choice good plants from the population, it is benefit to increase breeding efficiency.

Transformation receptor were mature seeds in this research, which is convenient to be obtained. And according to experiment object, the transformation receptor could be immature embryo, root, root tip, ovary, anther, shoot, shoot tip, stem and leaf, or callus of them.

The result showed that mean of plant height had the decreasing trend. It reached an agreement on Yang et al. (2002) and Wang et al. (1998). And at the same time, the mean of spike length of main axic had the decreasing trend, too. It had relationship with damage of ion beam implantation.

Segregation population as transformation receptor could be F2 segregation population, or other generation segregation population, for example F3, F4 and F5 generation, and could be segregation population including mixed single plants obtained from F2 and its offsprings, it could widen genotype of transformation receptor. In addition, donor gene segments accelerates gene's recombination, widens traits segregation, raises select chance and breeding efficiency.

In wheat breeding process, the choice of agronomic traits should be paid attention, and the choice of grain quality characters should be paid attention, too, for example, protein, Bulk density, wet gluten, flour yield , et al. The statistication result of grain quality characters showed, in groups which mean and coefficient variability had significant change compared with that of its control groups, the mean and coefficient variability were decreasing. Is this decreasing a universality case leading by damage of ion beam implantation or a coincidence related to the choice of transformation donor? Supposing it is the former, if every offspring is traced, do grain quality characters appear different case or not? Supposing it is the latter, if we choice different transformation donor according to breeding aim, could grain quality characters be other result? These are questions which need to be studied more.

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