将外源DNA注入幼荚实现大豆遗传转化*

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摘 要

目前,基因转移的方法已发展到很多作物上,但大多都不是很有效的,并且对于育种家来说又太复杂。本项实验的目的是研究能否通过将外源DNA注入幼荚的方法来转化大豆。实验中,利用一个玉米自交系和一个栽培大豆品种作为供体,另一个栽培大豆品种作为受体。两个组合的突变率分别为10.00%和5.32%。后代在很多农艺性状方面产生了可遗传的变异,例如:熟期、种子蛋白质含量、株高、结荚习性、茎粗、倒伏性、每株分枝数、每株荚数、每株粒数和百粒重。外源DNA可以通过维管系统或胞间连丝系统进入分裂时期的细胞,或通过核膜通道进入细胞核,然后聚集到基因并在受体上得到表达。该项技术简单、有效、育种家容易掌握。利用该技术可创造新的种质,实现不同作物种间、属间、种间基因交换。

GENETIC TRANSFORMATION OF SOYBEANS BY INJECTING EXOGENOUS DNA INTO YOUNG PODS

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ABSTRACTS

Recently many gene transfer methods have been developed for many crops, but most are not very effective and sophisticated for breeders. In this experiment, our objective is to study whether exogenous DNA can transform soybeans or not by injecting exogenous DNA into young

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soybean pods. One maize inbred line and one soybean cultivar were used as DNA donor parents, one soybean cultivar was used as recipient parent in the study. Mutation rates are 10.00% and 5.32% in two combinations respectively. There exist heritable variations in many agronomic characters, such as maturity, seed protein content, plant height, stem termination, stem diameter, lodging, number of branches per plant, number of pods per plant, number of seeds per plant and 100-seed weight. Exogenous DNA may be transported through vascular system or plasmodesa system to the cells in division stage, and enter the nuclei through openings on nucleus membrane, then integrated to the genome and expressed in the recipient. This technique is simple, effective and easy for breeders. It can be used to create new germplasms and realize gene exchangement of different species, genera, and families.

INTRODUCTION

Recently many gene transfer methods have been developed for many crops. There are transgeinc plants now in tobacco, tomato, maize, wheat, soybean etc. But most methods need tissue culture techniques or regeneration of mature plants from protoplasts, and tissue culture techniques. Regeneration of mature plants from protoplasts are not so secessful in a lot of crops until now. They are also complex and expensive for conventional breeders. Some scientists tried to treat seeds with exogenous DNA or inject exogenous DNA into generating tissues or ovaries after self-pollination in order to transform plants in rid of tissue culture or regeneration of mature plants from protoplasts. Huang et al. (1986), in experiment with cotton, injected exogenous DNA into young bolls about one or two days after selfpollination, several superior lines with higher resistance to deseases and high yield were found in the progenies. Duan and Chen(1985) injected donor parent DNA to recipient parent' s ovary 2-4 days after self-pollination in rice, a new type of rice, which is very dwarf, was created. Zhang et al. (1991) injected donor DNA to the intercalary meristem of young wheat plants, heritable variations were found for many characters. Zhu et al. (1988) soaked geminating broad bean seeds with exogenous DNA, a lot of variations were observed. In this experiment, we injected maize total DNA and soybean total DNA into young pods of soybeans, the objective is to test (1) whether we can transform one soybean cultivar by injecting another soybean cultivar' s total DNA into its young pods; (2) whether we can transform soybeans by injecting another family' s total DNA into its young pods.

MATERIALS AND METHODS

Donor parent total DNA was extracted according to Chen(1979). DNA fragment was suspended in 0.1 × SSC buffer. Donor parents are soybean cultivar "Dongnong 36"(maturity group 000, protein content 45.36%, indeterminate) and maize inbered line "Luozao 4". Recipient parent is soybean cultivar "Dongnong 8179"(maturity group I. protein content 42.32%, in-

determinate). Two combinations were made: Dongnong 8179 + Luozao 4 (DNA-1) and Dongnong 8179 + Dongnong 36 (DNA-2). On 27 July 1990, two donor parents' DNA was injected into young pods (0.5～1.5cm/ong) of the recipient parent respectively in the fields on our campus. We also injected 0.1 × SSC buffer into the young pods of Dongnong 8179 as CK. Seeds from these pods are called D_0 generation, the next generation is called D_1 generation, and so on. 200 D_0 seeds were harvested in combination DNA-1, 395 in DNA-2, and 107 in CK. We planted all D_0 seeds on Xiangfang Farm in Harbin in 1991, most plants were similar with the recipient parent Dongnong 8179, but some were obviously different from Dongnong 8179. No variations were found in CK, so untreated Dongnong 8179 was used as CK in 1992. We harvested all these mutants and planted them as plant rows on Xiangfang Farm in 1992. Maturity and stem termination were investigated in the fields. 10 plants were harvested for each plant row. A total of 41 plant rows (including 410 plants) were studied for several agronomic characters.

RESULTS AND DISCUSSIONS

In 1991, all of the D_1 plants were similar with the recipient parent before folwering. Later we found a few plants in DNA-1 and DNA-2 which were apparently taller than others in both combinations and the recipient. In the autumn, 20 earlier, taller and semideterminate plants were selected from combination DNA-1, 21 earlier, taller and semideterminate plants were selected from combination DNA-2. After threshing, we found that all 41 mutants had smaller seeds than the recipient Dongnong 8179 although they had large pods. There are 200 D_0 seeds in DNA-1 and 395 D_0 seeds in DNA-2, so the mutation rates are 10.00% and 5.32% respectively in DNA-1 and DNA-2. Because there were no variations in all D_1 plants from D_0 seeds of the CK (treatment with 0.1 × SSC buffer), we know the variations in DNA-1 and DNA-2 were induced by exogenous DNA.

All 41 plants were planted as plant rows in the fields in 1992, no difference was observed among these plant rows and between them and the recipient before flowering. Later, all of them were taller than the recipient, and lodged before maturation. The recipient was resistant to lodging. All 41 plant rows matured about 10 days earlier than the recipient. They are semideterminate, Dongnong 8179 is indeterminate. All the characters above are heritable from D_1 to D_2. After harvesting, we investigated several agronomic characters (Table 1, Table 2 and Table 3), only small variations existed among the plant rows, but they showed great difference with Dongnong 8179. On average, D_2 plant rows had fewer branches, thinner main stem, higheer height, more pods and seeds per plant, smaller seed size and lower seed weight per plant in comparison with Dongnong 8179. Their seed protein contents were significantly higher. Protein content of Dongnong 8179 was 42.32%, but those of DNA-1 and DNA-2 on average were 44.46% and 45.09% respectively. The most interesting thing is that plant rows from both combinations look like similar regardless of the donor parents. There were little variations within a plant

row for all characters studied.

Table 1 Performance of D_2 Lines of the combination DNA-1

Line	No. of branch /plant	Stem diam. (mm)	Plant height (cm)	No. of pods /plant	No. of seeds /plant	Seed wt(g) /plant	100-seed wt(g)
1	0.3	0.61	89.70	39.0	83.6	10.81	13.18
2	1.5	0.61	88.77	38.0	82.4	11.67	14.43
3	1.8	0.64	92.39	40.4	88.3	11.63	13.26
4	1.3	0.64	81.00	31.3	61.8	11.83	20.57
5	1.1	0.62	97.89	33.2	70.5	9.63	14.07
6	1.9	0.68	86.88	55.2	116.7	15.72	13.60
7	2.0	0.68	97.33	58.8	120.7	16.40	13.92
8	1.7	0.66	89.30	55.6	114.6	15.59	13.70
9	2.1	0.64	89.40	46.2	98.2	13.19	13.52
10	1.5	0.70	84.07	49.9	107.0	14.92	14.13
11	1.8	0.63	86.77	42.0	85.1	11.17	13.22
12	1.6	0.57	89.46	31.6	66.6	9.18	13.30
13	1.6	0.63	89.64	38.8	81.3	10.95	13.42
14	1.6	0.71	101.18	52.0	107.4	16.56	15.67
15	1.5	0.69	104.10	44.2	95.2	13.40	14.35
16	2.1	0.68	74.59	45.9	101.0	17.01	17.09
17	1.6	0.71	87.82	54.4	113.8	15.31	13.44
18	0.9	0.64	85.70	40.9	89.4	11.59	13.20
19	1.1	0.65	87.80	43.1	88.0	12.10	13.99
20	1.4	0.63	93.03	29.5	61.4	8.68	14.30
Mean	1.5	0.65	89.84	43.5	91.7	12.87	14.32
CK	2.4	0.73	79.80	33.4	68.1	14.08	20.88

These results indicate that exogenous DNA can induce variations of many characters in soybeans, and induced variations are heritable. Maize and soybean DNA was used in different combinations, but no difference was found in the progenies. Therefore, exogenous DNA maybe only altered the expression of the genes of the recipient in this experiment, no exotic genes were integrated into and expressed in the recipient Dongnong 8179 as in the donor parents. Because the induced variations are heritable, we think some exogenous DNA has integrated in to the genome of the recipient parent. As for how exogenous DNA emter the nuclei of the recipient, we postulate that exogenous DNA is transported through vascular system and plasmodesma system to the cells in division stage. As we know, there were openings on karyotheca, exogenous DNA may enter nuclei through those openines. The cell cannot destroy these exogenous DNA because there are

too much exogenous DNA there. Another possibility for induced variations in that mutable genes may function in this experiment.

Table 2 Performance of D_2 Lines of the combination DNA-2

Line	No. of branch /plant	Stem diam. (mm)	Plant height (cm)	No. of pods /plant	No. of seeds /plant	Seed wt(g) /plant	100-seed wt(g)
1	2.0	0.72	92.04	56.6	124.5	17.68	14.53
2	1.6	0.65	92.20	43.9	97.6	12.70	14.32
3	1.5	0.75	101.58	55.7	122.5	16.68	13.77
4	1.3	0.69	94.30	48.8	103.2	13.63	13.36
5	0.7	0.62	87.78	36.1	76.2	10.04	13.32
6	0.7	0.68	99.67	40.6	83.0	11.51	14.16
7	1.5	0.67	94.94	46.2	97.0	14.00	14.72
8	1.9	0.71	101.95	50.7	104.5	14.82	14.57
9	0.9	0.67	97.70	48.6	97.3	14.55	15.29
10	1.1	0.69	95.04	47.3	87.9	15.29	15.49
11	2.1	0.75	99.24	58.6	120.1	18.02	15.35
12	1.2	0.68	98.67	48.2	103.6	14.47	14.15
13	1.2	0.69	96.17	51.5	108.7	15.13	14.07
14	1.3	0.64	95.45	40.6	84.5	12.24	14.73
15	1.3	0.66	91.08	43.2	90.0	12.83	14.75
16	1.4	0.62	86.59	36.2	77.1	10.31	13.63
17	1.8	0.63	89.80	40.5	84.9	11.58	13.79
18	1.4	0.64	98.67	40.6	82.4	11.62	14.49
19	1.8	0.71	92.02	48.1	103.9	14.32	14.14
20	1.7	0.70	104.34	49.3	101.0	14.81	15.19
21	1.0	0.72	102.23	45.2	90.7	12.28	13.57
Mean	1.4	0.68	95.78	46.5	97.2	13.74	14.35
CK	2.4	0.73	79.80	33.4	68.1	14.08	20.88

This technique is simple, effective, economical and easy to operate for breeders. It may not be very effective for developing cultivars, but it can be used to create new germplasms and help exchangement of genes between different species, genera and families. We can improve the technique by using specific genes, not the total DNA.

CONCLUSION

The results show both total maize DNA and total soybean DNA induced variations of some

agronomic chacters in soybeans by injecting them to young soybean pods. This technique is simple, effective and easy for bredders. It can be used to create new germplasms and realize gene exchangement of different species, genera, and families.

Table 3 Seed protein contents of transformed plant rows in soybeans

Line	CK	DNA-1	DNA-2
1	42.48*	45.17	45.47
2	42.27	44.81	45.38
3	42.15	44.90	44.71
4	42.54	43.06	46.60
5	42.18	44.46	43.92
6		45.60	46.27
7		43.23	45.00
8		43.55	46.18
9		44.29	45.22
10		45.30	44.45
11		43.95	43.99
12		44.73	45.90
13		44.63	43.92
14		44.29	44.42
15		42.92	44.90
16		43.82	44.91
17		44.87	46.33
18		45.32	45.29
19		44.97	44.91
20		45.41	45.79
21			43.20
Mans	42.32	44.46*	45.08*
Std Dev	0.18	0.81	0.91

*On dry matter basis.

* Significant in comparison with CK at 0.05 level in T-test.

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