

Potential variability of trans-lysozyme gene rice under ecological conditions of Yunnan Province, China

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Abstract Using trans-lysozyme gene rice line D2-1-2 and its restorer Zhonghua No.9 as test materials, we studied their agronomic characters and grain qualities under two ecological conditions of Yunnan Province. The results show that there were no significant differences in the agronomic characters between D2-1-2 and Zhonghua No.9, but the seed setting rate and plant height of D2-1-2 were significantly lower, while the protein, amino acid and mineral element (Ca, Fe and Zn in particular) contents in its crude grain were higher than those of Zhonghua No.9. It is suggested that there could be some potential genetic variances in the transgenic plant and the possibly induced environmental risk should be evaluated in multi-plots for several years.

Keywords trans-lysozyme gene rice, agronomic character, quality character, security

1 Introduction

Plant genetic engineering breeding program is conceived to introduce a target gene and only target characters are expressed stably in the receptor plant. Since only a few loci in the receptor are expected to change, the offspring of the transgenic plant should soon be stable, and thus a new cultivar could come into being. However, current studies revealed that morphological changes, physiological characteristic changes and biochemical characteristic changes occurred in the transgenic plants besides the expected character (Liu et al, 2006). Such changes may bring unexpected effects on yield, metabolism of the receptor and safety to the environment. Therefore, analyzing multiple variables is the basis for the safe evaluation of transgenic plants (Ding et al, 2001).

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Mineral elements are an important component of genes and enzymes and also play a key role in physiological and biochemical reactions (Yu and Tang, 1998; Marschner, 1995; Wang, 2000). Changes of mineral elements of grain reflect the changes in physiological and biochemical properties. Analysis of these important components in the transgenic rice and non-transgenic parent rice in different growing stages provides the basis for the ecology and safety evaluation with regard to the metabolism of the transgenic rice (Wang et al, 2005; Jia et al, 2005). Since the foreign gene was successfully introduced into rice with the gene-gun method by Christou et al (1991), many transgenic rice lines and new germ plasm resources have been obtained throughout the world. However, some unlooked-for variabilities were brought at the same time (Tang et al, 2001). Lysozyme is a widely existing enzyme family with chitinase activity. It can decompose the β -1, 3-glucoside bond in the cell wall of bacteria or fungi and thus help resist pathogenic infection (Xu et al, 2003).

In order to estimate the potential variance of transgenic rice, seven agronomic characters besides the resistance to rice blast, the contents of protein, 17 kinds of amino acids and 11 kinds of mineral elements in brown rice were compared between transgenic rice and the receptor planted under the Yunnan ecological condition in this study.

2 Materials and methods

2.1 Materials

The pure transgenic line D2-1-2 and the receptor cultivar Zhonghua No.9 were used. D2-1-2 was a T7 generation line selected from the plant of receptor cultivar Zhonghua No.9 with introduced lysozyme gene from T4 phage by the Institute of Genetics and Development Biology of the Chinese Academy of Sciences under the ecological conditions of Beijing.

2.2 Methods

2.2.1 Planting

The transgenic line D2-1-2 and receptor Zhonghua No.9 were planted under two different ecological conditions in Yunnan Province, China during May to October 2003 and 2004. In the rice field of the Yiliang County (altitude 1500 m), the two materials were raised in a 66.67 m² plot, respectively. The plants were grown under normal culture practices. In the yard of the Institute of Biotechnology of the Yunnan Agricultural Academy of Sciences, Kunming City (altitude 1900 m), the two materials were planted in 100 pods, respectively, with no influence of the rice plant. The duration of day in Kunming City and Yiliang County was almost the same because of similar latitudes, but temperature was not because of different altitudes.

2.2.2 Investigation of agronomic characters

Days from sowing to heading, resistance to rice blast, plant height, tillers, panicle length, filled grains per panicle, seed setting percentage and 1000-grain weight were investigated.

2.2.3 Analysis of protein, amino acids and mineral element content

The analysis was performed in the Analysis and Testing Center in the Yunnan Academy of Agricultural Sciences.

Three random grain samples of 0.1 kg were extracted from each material from Yiliang and the chaff were wiped off by hands. Brown rice was obtained. Then, crushed samples of the brown rice were analyzed in two replicates.

Total protein contents were analyzed with semi-micro Kjeldahl method (Chinese National Standard GB 5511–85).

Analysis of amino acid content: The flour from the brown rice was hydrolyzed for 24 h in HCl at 110°C ± 1°C. The acids of filtered hydrolysate through the filter paper were dislodged in 60°C water bath. The amino acids of the diluted hydrolysate were analyzed on an amino acids analysis meter (Hatachi 835–50).

Analysis of mineral elements: 0.5 g of each unpolished rice samples was precisely weighed and put into a beaker. Five mL of nitric acid and 1 mL of perchloric acid were added. The beaker was heated with electricity to nitrify and decompose rice in it until the solution became clear. The clear solution was continuously heated and evaporated to dry. The residue was dissolved with 5 mL of 1:1 hydrochloric acid and the solution was transferred into a graduated bottle of 50 mL for test. The sample was reduced to ashes with high temperature or decomposed with warm acid decomposition. The residue was dissolved in an acid solution in order to transform the elements to be tested into inorganic ion. Then this solution was properly

diluted and analyzed on an ICP-AES meter.

Adding percentage over the receptor

$$= \left[\frac{\text{(the value of transgenic line} - \text{the value of the receptor)}}{\text{the value of the receptor}} \right] \times 100\%$$

3 Results

3.1 Agronomic character variability of the transgenic rice line

The resistant capability to rice blast (target character) of the line D2-1-2 was enhanced than that of the receptor (Table 1). However, there was no significant difference ($P > 0.05$) between line D2-1-2 and the receptor with regard to agronomic characters (such as days from sowing to heading, tillers, panicle length, grains per panicle and 1000-grain weight), while the plant height and the seed setting percentage of line D2-1-2 were significantly lower than those of the receptor ($P < 0.05$).

The seed setting percentage of Zhonghua No.9 in Kunming (high altitude) was lower than that in Yiliang (middle altitude), indicating that Zhonghua No.9 was sensitive to temperature. The seed setting percentage of the transgenic line D2-1-2 with lysozyme gene was lower than that of the receptor in Kunming and Yiliang. Furthermore, more differences were found between the seed setting percentage of the transgenic line in Kunming (nearly sterile) and that in Yiliang. Apparently, the transgenic rice became more sensitive to temperature.

3.2 Comparison analysis of mineral elements in brown rice between the transgenic line and the receptor

The difference of mineral elements in plants reflects the difference in the metabolism of the elements which were actively absorbed by the seeds. Therefore, mineral elements of brown rice were compared between the transgenic rice and the receptor (Table 2).

As shown in Table 2, 11 mineral elements of Zhonghua No. 9 and D2-1-2 were all identically ranked based on the value of elements contents in the following order: P, K, Mg, S, Ca, Fe, Zn, Mn, Cu, B and Mo. Such a consistency suggests that foreign genes did not change the elements' order. Except for elements K and B in D2-1-2, the contents of elements were all higher, especially Ca, Fe, Zn and Cu, which increased fourfold, 91.51%, 60.22% and 42.27%, than that in Zhonghua No. 9 ($P < 0.05$). As a result, the total content of elements in D2-1-2 increased 18.04% than that in Zhonghua No. 9, indicating that metabolism relating to these elements changed profoundly.

Table 1 Agricultural characters of transgenic line D2-1-2 and the restorer line Zhonghua No. 9

characters	year	Yiliang		Kunming	
		D2-1-2	Zhonghua No.9	D2-1-2	Zhonghua No.9
days from sowing to heading	2003	115	110	120	117
	2004	118	112	122	119
plant height*	2003	92.45 ± 2.30	104.22 ± 3.20	85.32 ± 2.43	100.10 ± 3.67
	2004	94.22 ± 3.11	105.31 ± 2.98	83.22 ± 2.65	98.22 ± 4.22
tillers	2003	6.17 ± 1.60	6.50 ± 1.82	5.60 ± 1.52	5.90 ± 1.90
	2004	5.98 ± 1.44	6.2 ± 1.56	5.42 ± 1.43	5.40 ± 1.77
panicle length	2003	18.49 ± 1.27	17.46 ± 2.08	16.33 ± 1.31	15.23 ± 2.34
	2004	17.31 ± 1.11	15.41 ± 2.0	15.00 ± 1.10	13.22 ± 1.65
grains per panicle	2003	131.1 ± 30.21	125.1 ± 28.61	128.2 ± 24.33	127.3 ± 25.43
	2004	125.1 ± 20.36	124.2 ± 24.33	125.4 ± 28.12	124 ± 28.33
seed setting percentage*	2003	43.72 ± 12.83	79.56 ± 11.86	5.91 ± 4.63	65.44 ± 10.65
	2004	30.2 ± 15.30	76.2 ± 14.3	4.12 ± 3.22	66.78 ± 10.68
1000-grain weight	2003	21.03	23.23	20.11	21.30
	2004	20.34	24.20	21.31	21.58
disease index for rice leaf blast	2003	1	2	–	–
	2004	1	2	–	–
disease index for rice panicle neck blast	2003	1	2	–	–
	2004	1	1	–	–

*: $P < 0.05$

3.3 Comparison analysis of the content of amino acids in brown rice between the transgenic line and the receptor

Comparison analysis of the protein contents and amino acids in brown rice between the transgenic line and the receptor was conducted to determine if the foreign gene affected the content of nutritional substance.

As shown in Table 3, the protein content in D2-1-2 was increased by 29.31% than that in Zhonghua No.9 and the contents of amino acids in D2-1-2 was increased than that in Zhonghua No.9 from 13.28% to 29.84%. According to the value of amino acids contents, the ranking order of amino acids in Zhonghua No.9 was Glu, Asp, Leu, Arg, Val, Ala, Phe, Ser (Gly), Pro, Ile Tyr, Lys, Thr, His, Met and Cys, while the ranking order of amino acids in D2-1-2 was almost the same as Zhonghua No.9 except for Pro and Gly. This result

indicated that the metabolic capability of protein and amino acids was enhanced in the transgenic line.

4 Discussion

Foreign genes introduced in receptor plants are often derived from non-relative species or compounded artificially. It is difficult to predict accurately the phenotype and side effect of these genes in a new genetic background and the effects of these genes on human beings and the environment have not been clarified because of interaction and multi-effects of genes. The transgenic line with lysozyme gene from Bacteriophage T4 had been predicted to only acquire resistance to fungi and bacteria, but variability of agronomic characters, quality characters and mineral element contents was also found in high generation, showing that heritable variability occurred. These variances were probably caused by three factors (Jahne et al, 1995; Labra et al, 2001; Lynch et al, 1995). The first was the introduced foreign gene. Different insertion loci and copy number of foreign gene in receptor genome could result in foreign gene silence. Multi-effect of foreign genes and interaction of foreign gene and receptor gene could also induce changes in characters. The second was the gold powder used in the gene-gun method, which may destroy the receptor gene and induce mutation. The third was that variance occurred in the tissue culture process. However, further studies are needed to elucidate the exact mechanism.

Our results also show that the expression of foreign gene was a complicated process and the introduction of foreign gene probably brings a series of variances producing both beneficial and harmful effects (Piao et al,

Table 2 Comparison of 11 kinds of mineral elements contents between D2-1-2 and Zhonghua No. 9

element	Zhonghua No. 9	D2-1-2	addition percentage/%
S	953 ± 17	1195 ± 24	25.39
P	3503 ± 16	3949 ± 37	12.73
K	2633 ± 61	2570 ± 19	-2.20
Ca*	127 ± 12	670 ± 8	427.56
Mg	1232 ± 14	1544 ± 10	25.32
Fe*	49.5 ± 3.9	94.8 ± 2.4	91.51
Zn*	17.6 ± 6.1	28.2 ± 3.2	60.22
Cu*	2.92 ± 0.11	4.16 ± 0.08	42.47
Mn	14.2 ± 0.28	17.3 ± 0.14	21.83
Mo	1.53 ± 0.01	1.65 ± 0.03	7.84
B	1.93 ± 0.01	1.67 ± 0.01	-13.47
Total	8535.68	10015.78	18.04

*: $P < 0.05$

Table 3 Comparison of protein, starch, amino acids contents between D2-1-2 and Zhonghua No. 9 (mg/100 mg)

amino acids	Zhonghua No.9	D2-1-2	addition percentage/%
protein/%	9.28 ± 0.32	12.0 ± 0.18	29.31
Asp	0.771 ± 0.029	0.935 ± 0.014	23.61
Thr	0.284 ± 0.003	0.337 ± 0.007	18.66
Ser	0.384 ± 0.012	0.460 ± 0.022	19.79
Glu	1.572 ± 0.052	1.908 ± 0.029	21.37
Gly	0.384 ± 0.001	0.435 ± 0.004	13.28
Ala	0.494 ± 0.001	0.608 ± 0.002	23.08
Val	0.559 ± 0.007	0.686 ± 0.011	22.72
Met	0.158 ± 0.006	0.184 ± 0.008	16.46
Ile	0.330 ± 0.003	0.421 ± 0.002	27.58
Leu	0.722 ± 0.006	0.913 ± 0.009	26.45
Tyr	0.322 ± 0.003	0.405 ± 0.002	25.78
Phe	0.439 ± 0.002	0.570 ± 0.005	29.84
Lys	0.290 ± 0.001	0.337 ± 0.001	16.21
His	0.170 ± 0.001	0.199 ± 0.002	21.18
Arg	0.673 ± 0.007	0.777 ± 0.009	17.06
Pro	0.368 ± 0.004	0.436 ± 0.001	15.45
Cys	0	0	0
NH ₃	0.203 ± 0.005	0.246 ± 0.007	18.48
total	8.124	9.857	21.33

2005; Xiong, 2004). For example, the decline of the seed setting percentage is disadvantageous to yield, while the increase in the content of mineral elements (Ca, Fe and Zn), protein and amino acids is beneficial to quality characters. The change of rice color from white to grey was perhaps related to the change of the content of mineral elements. Mineral elements play an important role in the process of plant metabolism (Jiang et al, 2004).

Since the expression of characters is the result of interaction of genes and the environment, some characters will not be expressed unless a fitting environment is provided. There was no difference between the transgenic line D2-1-2 and its receptor in their original place with regard to the seed setting percentage, but a significant variance was found between the two materials under the Yunnan ecological conditions. The results indicated that the introduction of the foreign gene brought some potential variances which may not be expressed unless under certain conditions. This is in conformity with the views of Yang (2003). Therefore, safety of the transgenic plant should be evaluated in multiple ecosystems. Moreover, it has been regarded that there could be some potential genetic variances in a transgenic plant and the possibly induced environmental risk should be evaluated in multi-plots for several years.

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