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Research Article

Inheritance and expression of transgene in SOD2-Transgenic petunia descendants and their morphological traits

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Abstract This study was conducted to determine the inheritance and expression of transgene in descendants (T_1 to T_2 generation) of SOD2-transgenic petunia by PCR and RT-PCR analysis. The trangene was segregated as Mendelian inheritance pattern (3:1 or 1:0) in most of T_1 and T_2 generation lines. Transgenic homozygous lines were obtained in T_2 generation. It was identified that the transgene expressed stably in examined all plants of 6 T_2 lines. The representative morphological traits (plant height, flower diameter, and flower color) of T_2 plants were compared with those of non-transgenic plants.

Keywords PCR, RT-PCR, Mendelian, Segregation, Pattern

Introduction

Current global climate change urges us to develop cultivars resistant to environmental stress (or abiotic stress) in many crops. However, it is not easy to develop new cultivars resistant to the abiotic stress through conventional breeding technique. So, many researchers have studied to develop plants resistant to abiotic stresses through transfer of superoxide dismutase (SOD) or ascorbate peroxidase (APX) or NDP kinase (NDPK) genes in many crops (Fang *et al*. 2002; Moon *et al*. 2003; Tang *et al*. 2004a; Tang *et al*. 2004b; Kim *et al*. 2005; Tang *et al*. 2007). Bhatnagar-Mathur *et al*. (2008) reported a review paper on transgenic approaches for abiotic stress tolerance in plants. One of characteristics, which should be improved in petunia, is resistance to abiotic stresses such as rainfall, humidity, and air pollution. In our previous study, we got T_1 seeds and proved their resistance to abiotic stress (Lee *et al*. 2009) after we tried the transfer of MnSOD (SOD2) gene into purebred lines of petunia. Meanwhile, the success of plant genetic transformation needs to be followed by the stable inheritance and expression of a transgene in descendants (Zhang *et al*. 2005). Hence, this study examined whether the transgene transmits to T_1 or not, and we let T_1 plants, which were identified as transmitter of the transgene, self-pollinated to get T_2 generation. It was also investigated whether the transgene transmitted to and expressed in T_2 plants or otherwise. Furthermore, it was investigated whether their morphological traits changed in comparison with non-transgenic plants or not.

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Materials and Methods

Plant materials

T_1 four lines (A2-36-1-1-2, A2-36-2-1-1, A2-19-3-1, and A2-19-4-1) and T_2 six lines (A2-36-1-1-2-5, A2-36-2-1-1-35, A2-19-3-1-8, A2-19-3-1-37, A2-19-4-1-15, and A2-19-4-1-43) were used as plant materials. T_1 generation was obtained by selfing SOD-transgenic primary plants, in which introduction of only one copy of transgene had been identified by Southern analysis in our previous study (Lee *et al*. 2009). T_2 generation was obtained by selfing of each T_1 line.

Analysis of inheritance and expression of transgene in progenies

Inheritance of transgene in T_1 and T_2 generations was examined depending on PCR analysis, and its expression in T_2 generation was investigated by RT-PCR analysis. Both DNA and RNA used for PCR and RT-PCR analysis were

extracted from leaves of the transgenic plants grown for three months after having been sown in the greenhouse. PCR and RT-PCR analysis were conducted as described by Lee *et al*. (2009).

Examination of morphological traits in progenies

Data for two morphological characters (plant height and flower diameter) in transgenic (T_2) lines was recorded on the basis of the survey standard in agricultural experiment and research (Rural Development Administration, 1995) and in application and evaluation of petunia new varieties (National Seed Management Office, 1997), when transformants bloomed.

Results and discussion

With successful development of transgenic plants, many researchers have been reported on inheritance pattern of transgene in descendants by PCR analysis (Chareonpornwattana *et al*. 1999; Zhang *et al*. 2005; Sriskandarajah *et al*. 2007). In this study, segregation pattern of transgene was also analyzed in the process of obtaining progenies (T_2 to T_3), which will exhibit and express transgene, from SOD2-transgenic (T_1) petunias as obtained in our previous study. Results are shown in Table 1~2. One hundred eighteen out of 174 plants of T_1 4 lines (33 out of 50 A2-19-3-1, 37 out of 49 A2-19-4-1, 25 out of 34 A2-36-1-1-2, and 23 out of 41 A2-36-2-1-1) exhibited the transgene. By chi-square analysis, segregation pattern of transgene in 3 out of T_1 4 lines except of A2-36-2-1-1 was not significant at the 5% level, but it coincided with the expected ratio of Mendelian inheritance pattern (3:1) (Table 1). This finding was in contrast with Deroles and Gardner (1988) who reported that approximately half of the transgenic T_1 petunia plants showed normal Medelian inheritance in resistance to kanamycin. Meanwhile, all T_2 lines obtained by selfing each T1 line exhibited segregation ratio (3:1 or 1:0) suitable for the expected ratio of Mendelian inheritance. There was no plant which did not have transgene in T_2 4 lines (A2-19-3-1-8, A2-19-3-1-37, A2-19-4-1-43, and A2-36-2-1-1-35). In other words, in this study to develop SOD2 transgenic plant, transgenic homozygous lines were obtained in T_2 generation. On the other hand, by chi-square analysis on the segregation pattern of the transgene in T_2 two lines (A2-19-4-1-15 and A2-36-1-1-2-5) where the transgene segregated, A2-19-4-1-15 (where 58 out of 88 plants exhibited the transgene) was not segregated significantly at the 5% level, whereas A2-36-1-1-2-5 (where 66 out of 83 plants had the transgene) was segregated significantly (Table 2).

To succeed plant genetic transformation, it is necessary to produce descendants where transgene must be not only transmitted but also expressed stably (Zhang *et al*., 2005). Six plants per T_2 line were selected to examine whether the transgene were stably expressed or not. Of course, 12 plants from 2 lines (A2-19-4-1-15 and A2-36-1-1-2-5) where trans-

Table 1 Segregation ratio of transgene in SOD2-transgenic (T_1) petunias

Genotype	No. of analyzed plants	No. of plants with SOD2 gene	Expected ratio	X^2*
A2-19-3-1	50	33	3:1	2.160
A2-19-4-1	49	37	3:1	0.006
A2-36-1-1-2	34	25	3:1	0.039
A2-36-2-1-1	41	23	3:1	7.813

* $X^2_{0.05} = 3.841$(*d.f.*=1)

Table 2 Segregation ratio of transgene in SOD2-transgenic (T_2) petunias

Genotype	No. of analyzed plants	No. of plants with SOD2 gene	Expected ratio	X^2*
A2-19-3-1-8	84	84	1:0	0.000
A2-19-3-1-37	56	56	1:0	0.000
A2-19-4-1-15	88	58	3:1	3.878
A2-19-4-1-43	51	51	1:0	0.000
A2-36-1-1-2-5	83	66	3:1	0.903
A2-36-2-1-1-35	73	73	1:0	0.000

* $X^2_{0.05}$=3.841(*d.f.*=1)

Table 3 Mean for 2 quantitative traits in SOD2-transgenic (T_2) petunia plants

Genotypes	No. of plants examined	Plant height (cm)	Flower diameter (cm)
A2-36-1-1-2-5	54	28.5±3.3[z]	5.3±0.1
A2-36-2-1-1-35	57	32.3±3.7	5.5±0.1
A2-36 (Control)	10	21.3±2.2	5.9±0.2
A2-19-3-1-8	58	35.6±7.2	7.3±0.1
A2-19-3-1-37	51	31.7±6.4	7.3±0.2
A2-19-4-1-15	50	35.0±6.5	6.8±0.1
A2-19-4-1-43	52	30.4±6.3	7.2±0.2
A2-19 (Control)	10	35.0±4.7	7.2±0.2

[z] Mean±Standard deviation

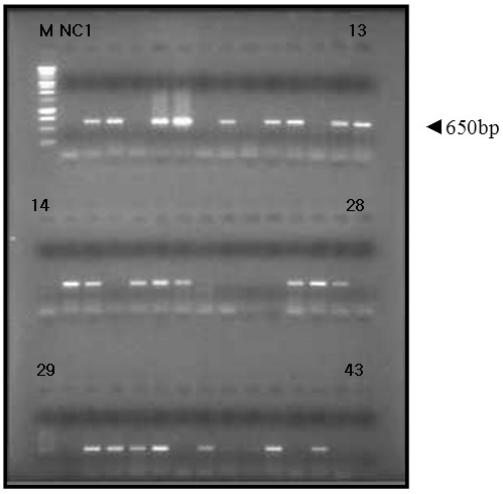

Fig. 1 PCR analysis of SOD2 transgenic T_1 (A2-19-3-1) 43 plants M, molecular marker; NC, negative control

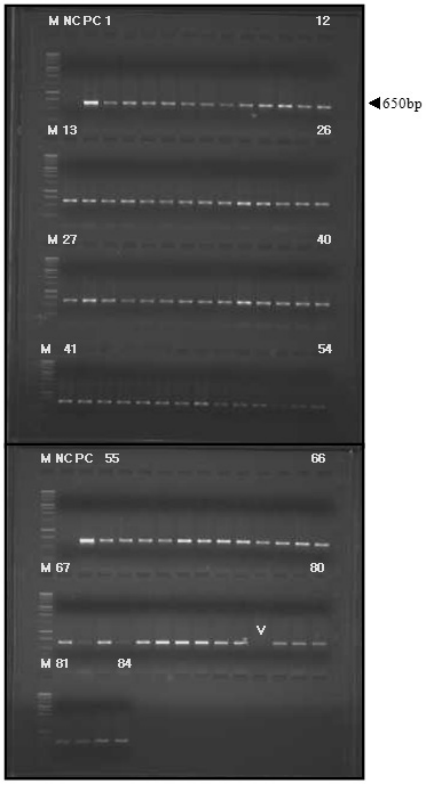

Fig. 2 PCR analysis of SOD2 transgenic T_2 (A2-19-3-1-8) 84 plants. M, molecular marker; NC, negative control; PC, positive control

gene was segregated by PCR analysis were selected as what was identified as transmitters of the transgene. By RT-PCR analysis, we confirmed the expression of the transgene at only 29 out of 36 plants of six lines (Fig. 3A). However, at a retrial experiment of RT-PCR analysis where amount of RNA sample was used twice, we confirmed the expression of the transgene in the rest of seven plants where the expression of the transgene was insufficient in the first experiment (Fig. 3B). After all, the expression rate of the transgene in T_2 lines examined in this study was 100% .

Many researchers hoped that only target characters in transformed plant will be changed by the transfer of the gene. However, there are many reports on the change of morphological trait that is not desirable in transgenic plant (Han *et al*. 2007; Han *et al*. 2008). So, we investigated the representative morphological traits (plant height, flower color, and flower diameter) of T_2 lines compared to non- transgenic plants. Flower color of transgenic lines was not different with that of non-transgenic plant except for A2-36- 1-1-2-5 (Fig. 4). Transgenic lines obtained from A2-36 showed a tendency to lengthen in plant height compared to non- transgenic plants. The plant height of the latter was 21.3±2.2 cm, whereas that of A2-36-1-1-2-5 and A2-36-2-1-1-35 was 28.5±3.3 cm and 32.3±3.7 cm, respectively. On the other hand, their flower size was a little smaller than that of the latter. The flower diameter of the latter was 5.9±0.2, whereas their flower diameter was 5.3±0.1 and 5.5±0.1, respectively. A2-36-2-1-1-35 was taller in plant height and larger in flower size than A2-36-1-1-2-5. Meanwhile, transgenic lines obtained from A2-19 did not show a tendency in plant height

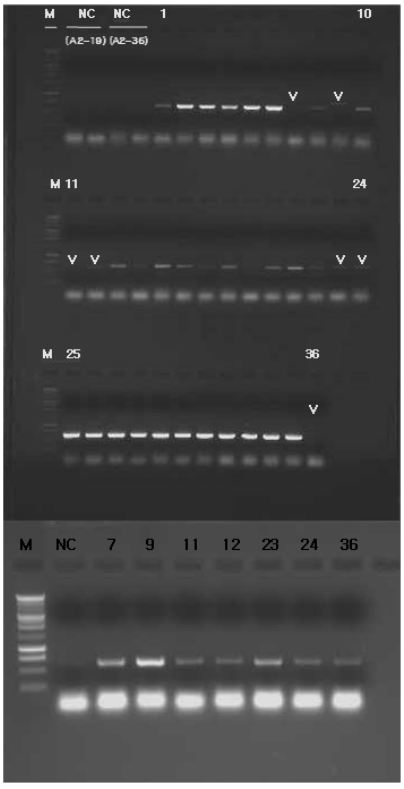

Fig. 3 Expression of transgene in SOD2 transgenic petunia T_2 36 plants by RT-PCR analysis. M, molecular marker; NC, negative control; 1-6, A2-36-1-1-2-5; 7-12, A2-19-3-1-37; 13-18, A2-36-2-1-1-35; 19-24, A2-19-3-1-8; 25-30, A2-19-4-1-43; 31-36, A2-19-4-1-15

Fig. 4 Comparison of flower color between SOD-transgenic lines from A2-36, A2-36-1-1-2-5 (A) and (B) A2-36-2-1-1-35 (C) and non-transgenic plant (B)

or in flower size compared to non-transgenic plants. A2-19-3-1-8 was taller in plant height as well as larger in flower size than non-transformant. A2-19-3-1-37 and A2-19-4-1-43 were shorter than non-transgenic plants in plant height, whereas they were the same or a little larger than non-transformant in flower size. A2-19-4-1-15 was almost similar to non-trangenic plant in plant height, but it was smaller than control in flower size (Table 3).

In conclusion, in this study, we found that transgene of SOD2-transgenic petunias inherited into their progeny and it expressed well in them based on PCR and RT-PCR analysis. Until now, there were few papers on the comparison of morphological characters between transgenic and non-transgenic plant except Lee *et al*. (2004) who reported on the distinct modification of morphological characters between transformed and non-transgenic plants of *Taraxacum platycarpum*. Therefore, the transgenic petunia progeny (seeds) as well as results obtained in this study will be valuable data and genetic materials for breeding petunia new cultivar resistant to abiotic stress.

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