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RESEARCH ARTICLE

Analysis on virus resistance and fruit quality for T4 generation of transgenic papaya

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Abstract Molecular biological characterization, fruit characters, and nutrients were analyzed for T4 generation of transgenic papaya. All transgenic papaya plants with the mutated replicase (RP) gene from papaya ringspot virus (PRSV) showed high resistance or immunity against PRSV in the field. The RP transgene can be steadily inherited to, and expressed at RNA level, the progenies. The growth characteristics of transgenic papaya were much better than non-transgenic papaya in the field. The non-transgenic papaya seedlings began to show typical symptoms caused by PRSV after being inoculated with PRSV. They died quickly and never grew to produce fruit. The adult trees developed yellow leaves and produced smaller fruits and were doomed to a slow death after some time, while most of transgenic papaya plants (about 91.8%) did not show any symptoms caused by PRSV, and produced more, bigger, and high quality fruits. Compared with non-transgenic plants, the fresh fruit length of T4 generation of transgenic papaya increased 2.6%–5%, and the diameter decreased 0.6%–1.5%. The flesh thickness of fresh fruit increased 12%–15%, which made it fitter for eating. Although the fresh fruit quality changed, there was no significant difference between transgenic and non-transgenic papaya. The quality characteristics of dry fruit including the contents of water, lipid, N, protein, reduced sugar, vitamin A, vitamin C, and carotene in the T4 generation of transgenic papaya were all the same as their non-transgenic parents. This means that transgenic plants and non-transgenic plants are substantially equivalent, and the transgene has no effect on dry fruit quality. In this study, we found that vitamin A and vitamin C in red-fleshed papaya were 1.4–1.8 and 1.78–2.07 times more than the yellow-fleshed ones, respectively, while N and protein were only 84.2%–92.1% and 82.1%–98.9% of the yellow-fleshed ones.

Keywords transgenic papaya, molecular detection, fruit quality

1 Introduction

Transgenic plants or genetically modified (GM) plants have developed fast since their emergence in the 1980s, and large quantities of GM plants have come into the world and thousands of field trials have been conducted at different locations. In 2005, GM crops were grown on 90 million ha in more than 21 countries, which was an increase of 50 times in area since their first commercialization of GM plants in 1996[1)] (James, 2005). With the development and continued release and commercialization of GM plants, they brought huge advantages for human beings and society, while they could possibly cause negative effects to human beings' health and environmental safety (Kerlan et al., 1992; Scheffler et al., 1993; Lewellyn and Fitt, 1996). Its potential effects on environment and food safety have become a hot topic especially after 1998 (Khan et al., 1973; Losey et al., 1999; Zhou and Li, 2000; Lu et al., 2002; Liu et al., 2003). Now, the biosafety of GM plants has caused a growing public concern around the world and have become economic, trade, and political problems from being just simple scientific problems even if there is limited evidence on the influence of GM plants and their products on the environment and food (Jia, 1997; Zhang and Guo, 2000). In order to solve the controversy on the biosafety of GM plants and foods, the principle of substantial equivalence was first described in 1993 and implemented

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in 1998 by the Organization for Economic Cooperation and Development (OECD) (Kearns and Mayers, 1999; Garard, 2003). It was introduced to value the biosafety of GM foods by means of comparison with existing foods or food components with a known history of safe use. The comparison with natural non-GM counterparts was seen as a means to provide a guiding principle and useful tool for regulatory scientists engaged in biosafety assessments (Kearns and Mayers, 1999; Garard, 2003).

Papaya is a perennial fruit crop, widely grown throughout the tropics and subtropics. It is an important fruit and food in many countries, and is one of the most nutritious fruit crops and the industrial raw material in many fields. The enzyme, papain, obtained from immature fruit is used in the pharmaceutical and food industry. Papaya ringspot virus (PRSV) induces one of the most destructive diseases in papaya. This disease has become a major threat to papaya cultivation throughout world. All papaya seedlings show typical symptoms of PRSV when planted on a large scale (Purcifull et al., 1984). They die quickly and never grow to produce fruit. The adult trees develop yellow leaves and produced smaller fruits and are doomed to a slow death after October. In order to overcome PRSV, we transform the mutant replicase (RP) from PRSV into the papaya genome and obtain 14 transgenic lines, four of them are highly resistant to PRSV and three of them are immune to PRSV (Ye et al., 1996; Chen et al., 2001). Field release and PRSV inoculation experiments show that all transgenic lines can flower and bear fruit as non-transgenic papaya and two stably hereditary and highly resistant transgenic lines are screened out from the 14 transgenic lines and authorized by the Ministry of Agriculture, China, for field release in 2002 (the authorized number is 200-002) (Ye et al., 2002). In order to industrialize the transgenic papaya earlier, this biosafety study of the T4 generation of transgenic papaya was conducted by comparing their growth, horticultural traits, hereditary stability and fruit nutritional component with those of non-transgenic papaya. The results obtained could provide useful information for the assessment of the biosafety of GM plants and the establishment and administration of industrial law.

2 Materials and methods

2.1 Cultivation of transgenic papaya

Seeds of the T3 generation of two screened transgenic papaya lines, the Zhongkang 1, yellow-fleshed papaya and the Zhongkang 2, red-fleshed papaya, were collected in 2001. The non-transgenic papaya lines were Meizhonghong (red-fleshed papaya) and Guanghong (yellow-fleshed papaya). Seeds were germinated in plastic trays containing a commercial soil mix (peat moss- and perilte-based fortified with fertilizers) in January 2002. In March 2002, the papaya seedlings were about 20 cm in height (10-leaf stage) and all of transgenic papaya seedlings were inoculated with PRSV and the disease status was observed after 15 days. Then the ill transgenic papaya seedlings were washed out, while the healthy transgenic papaya seedlings were replanted at Guangzhou Agricultural Scientific Research Institute. Each type of transgenic papaya seedlings had two mu and the density of plants were 200 plants per mu. Twelve transgenic papaya seedlings were planted outside the greenhouse in Sun Yat-sen University campus so that the symptoms of PRSV could be observed the following year after the plants cultivated in the field had been cut down. In order to prevent the spreading and diffusion of transgenic papaya pollen, the non-transgenic papaya plants were cultivated around the transgenic papaya and used as control. In May, the transgenic papaya plants were inoculated with PRSV again and ill plants were removed. All transgenic papaya plants were cut down and stacked and maturated to fertilizer after the release experiments were finished in January 2003.

2.2 Molecular analysis of transgenic papaya DNA and RNA

Total genomic DNA was extracted from papaya leaves (three from Zhongkang 1 plants and three from Zhongkang 2) using the CTAB method (Aldrich and Cullis, 1993). The primer systems that target the 5′ and 3′ region of RP were designed with the Oligo 4.0 program (Bioasia Biotechenology Co. Ltd., China) based on the sequences reported by Ye et al. (1996).

The oilgonucleotide RP primers, yielding a fragment size of 1602 base pairs, were

5′ – CGAGGATCCATGGATAAGTTACACGGCAATCT - 3′
5′ – CACGGTACCTTACTTAGACTGGTGAAACACAT - 3′

PCR mixes contained 0.1 μmol of both forward and reverse primers, 3.75 mM $MgCl_2$, 0.2 mM deoxynucleoside triphosphates, 1 × buffer, and 2.5 U *Taq* polymerase (MBI). Amplification involved a 7-min denaturation step at 94°C and 35 cycles consisting of 1 min denaturation at 94°C, 1 min primer annealing at 56°C, and 1.5 min primer extension at 72°C followed by a final 10-min extension step at 72°C. PCR products were analyzed on 0.8% (w/v) agarose gels with tris borate EDTA buffer (TBE). In order to reduce the false positive, the positive PCR products were purified with a Qiaquick Gel Purification Kit (Qiagen, Hilden, Germany) and used as template for nested PCR. The non-transgenic papaya plants were cultivated around the transgenic papaya and used as control. The nucleotide sequence used for nested PCR amplification of a 246 -bp DNA fragments were

5′ – CGAGGATCCATGGATAAGTTACACGGCAATCT - 3′
5′ – AACATCGTGGTCAACTTCACC- 3′

The PCR mixes and amplification were the same as PCR except the 0.5 min primer annealing at 50°C. DNA was applied to a nylon Hybond-N membrane (Osmonics, USA) and hybridized with α-^{32}P-dCTP-labelled probes after being

digested by *Bam H*I and *Kpn*I. The probe was obtained by the extension of random oligonucleotides with 3′ downstream primer. The hybridization was conducted as described by Sambrook and Russell (2001).

Total RNA was digested by DNase I to remove the presumable DNA from samples after being extracted from transgenic papaya leaves with Guanidinium Isothiocyanate and used as template for RT-PCR with RP primer systems (Sambrook and Russell, 2001). The PCR mixes and amplification were the same as PCR and the results were analyzed on 0.8% (w/v) agarose gels with TBE.

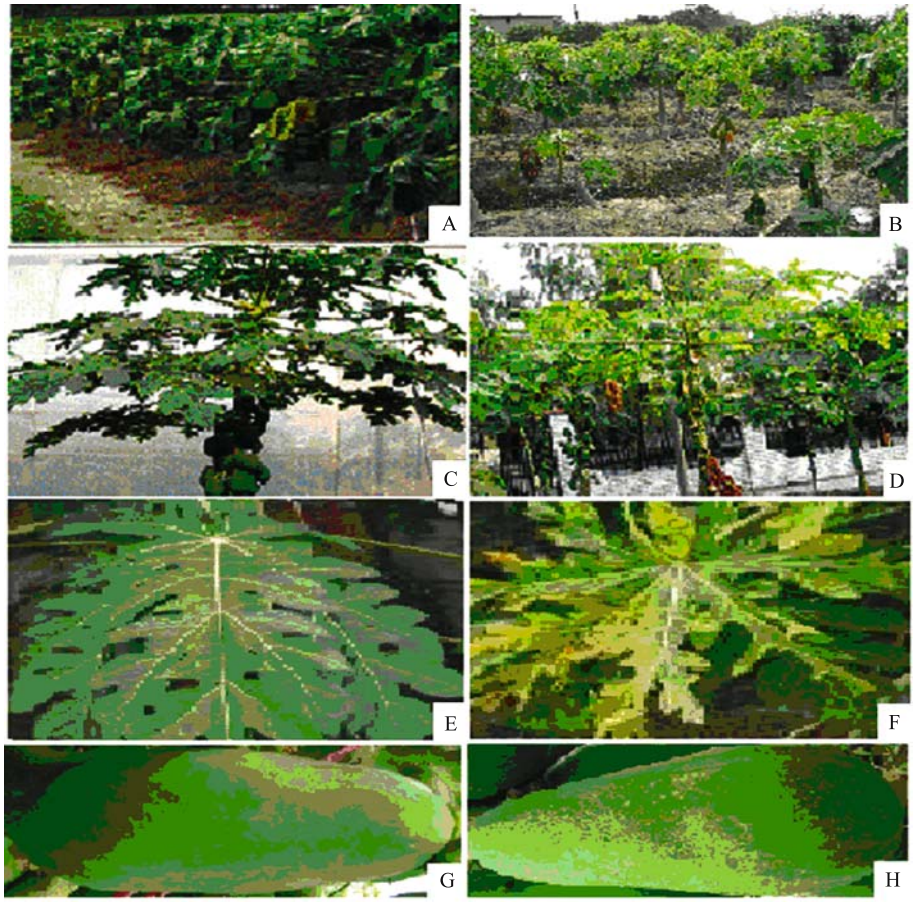
2.3 Analysis on horticultural traits and the content of nutritional component of transgenic papaya fruits

Ten ripe papaya fruits were selected randomly from each field planted with yellow-fleshed transgenic papaya, red-fleshed transgenic papaya, yellow-fleshed non-transgenic papaya or red-fleshed non-transgenic papaya. The horticultural traits including fruit length, fresh weight per fruit, fruit shape, fruit color and luster, and flesh thickness were measured. The fruits were dried at 65°C after taking out the seeds and powdered by a muller (TUV GS). Water (GB 5009.3-85), nitrogen (GB 5009.5-85), protein (GB 5009.5-85), lipid (GB 5009.6-85), reduced sugar (GB 5009.7-85), carotene (GB 12389.90), vitamin A (GB 12388-90), and vitamin C (GB 12392-90) were determined according to the national standard methods. All data are reported on the basis of oven-dried fruit and compared statistically by ANOVA and the least significant difference (LSD) test at the 5% level with SPSS (V 12.0).

3 Results

3.1 Growth and virus resistance of transgenic papaya

The growth characteristics of transgenic papaya were much better than non-transgenic papaya in the field (Fig. 1). The non-transgenic papaya seedlings began to show typical symptoms caused by PRSV two to three weeks after inoculation with PRSV. Initially the symptoms appeared as yellow spots on the leaves, which later coalesced to produce a mosaic pattern. In severe conditions there was a complete distortion of leaf lamina producing a shoestring effect, which gives rise to a bushy canopy. The plants begin to taper at the top and finally collapse and never grow to produce fruit. When planted on a large scale, all non-transgenic papaya plants showed typical symptoms of PRSV after October. The adult trees displayed a



A, C, E, G: Transgenic papaya; B, D, F, H: Wild papaya

Fig. 1 Growth and virus resistance of transgenic papaya in field

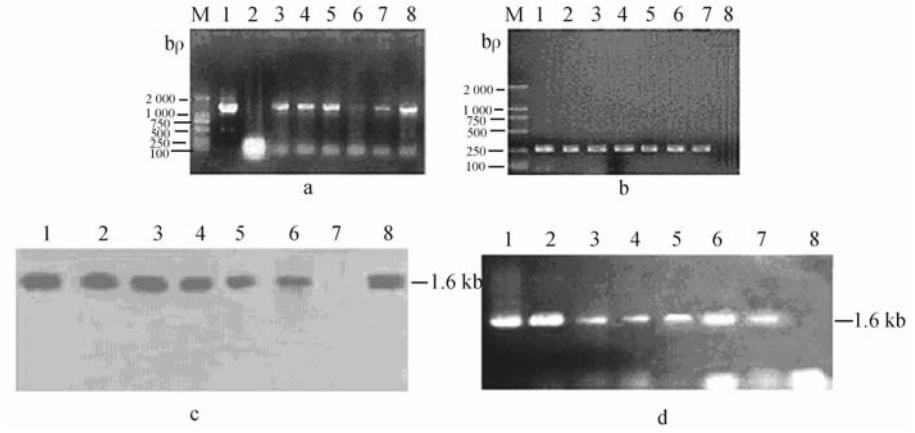
yellow mosaic structure with leaf distortion and produced smaller or malformed fruits with similar mottle symptoms and doomed to a slow death. They were insipid in taste due to reduction of sugar content and reduced papain content in the fruits, which had adversely affected small-scale industries engaged in papain processing for pharmaceutical use. Most of the transgenic papaya plants (about 91.8%) did not show any symptoms of PRSV, and produced more, bigger, and high quality fruits. Although 75 of the total 812 plants of transgenic papaya (about 9.2%) showed slight symptoms of PRSV, they still had more fruits and fewer mottle symptoms on fruits than non-transgenic papaya (Fig. 1). Because of PRSV, non-transgenic papaya must be cut down and replanted every year. However, we found that the transgenic plant could grow very health in the second year. Even in the next two or three years, the transgenic papaya plants still grew very well and showed no symptoms of PRSV.

3.2 Hereditary stability of transgenic papaya

The DNA extracted from papaya leaves was subjected to PCR amplification and electrophoresed through agarose. Samples from transgenic papaya leaves were identified by the presence of an amplified product of 1602 bp, representing the RP sequence in plasmid pRPTW, while the non-transgenic papaya had no PCR products that were detected (Fig. 2a). The presence of the 1602-bp RP fragment was confirmed by nested PCR and 255-bp DNA fragments equaling the theoretical value was observed from all the positive PCR products (Fig. 2b). Genomic DNA from those transgenic papaya plants had strong hybridization signals with the same size, which showed the foreign gene; the RP gene was a single-copy in transgenic papaya genome (Fig. 2c). A 1602-bp cDNA fragment could be detected from all RNA samples extracted from transgenic papaya plants, which was the same size as the foreign RP gene, while no cDNA fragments could be observed from the RNA samples extracted from the non-transgenic papaya (Fig. 2d).

3.3 Horticultural traits of transgenic papaya fruit

In 2002, the fruit fresh weight of Zhongkang 1 was from 505 g to 1240 g and the average fresh weight was 701.6 g per fruit, while the fruit fresh weight of Zhongkang 2 was from 245 g to 435 g and the average fresh weight was 360.6 g per fruit, which had no significant difference from their parents, the non-transgenic papaya, Guanghong and Meizhonghong (Tables 1–3). Compared with non-transgenic papaya, the fruit length of transgenic papaya (Zhongkang 1 and Zhongkang 2) were longer than their parents, while the reverse was true for fruit diameter of transgenic papaya, but they were not significantly different from the non-transgenic papaya (Tables 1–3). Flesh thickness of Zhongkang 1 and Zhongkang 2 were 15% and 12% thicker than their parents, which made the transgenic papaya have more papain content in the fruits and higher edible value than the non-transgenic papaya. Compared with non-transgenic papaya fruits, it was observed that transgenic papaya had more fruits and less mottle symptoms on the fruit, which showed that the transgenic papaya had higher resistance to PRSV (Fig. 1).



a: PCR for RP gene. Template DNA from 1: pRPTW; 2: Wild type papaya; 3–8: Transgenic papaya; b: PCR for nested PCR. Template DNA from 1–6: Purified from 3–8 line in a; 7: pRPTW; 8: Wild type papaya; c: Southern hybridization of papaya genomic DNA. 1–6: DNA from transgenic papaya; 7: DNA from wild type papaya. 8: pRPTW; d: RT-PCR for RP gene. Template RNA from 1: pRPTW; 2–6: Transgenic papaya; 8: Wild type papaya;

Fig. 2 Molecular analysis for transgenic papaya

Table 1 Fruit characters of T4 generation of transgenic papaya ($n = 10$, SE)

Line	Fruit length /cm	Fruit diameter /cm	Fresh weight per fruit /g	Flesh thickness /cm
Zhongkang 1	19.9 ± 3.3	8.64 ± 1.18	701.6 ± 181.9	2.34 ± 0.46
Guanghong	19.4 ± 5.4	8.77 ± 1.29	705.6 ± 188.2	2.04 ± 0.47
Zhongkang 2	16.7 ± 3.36	6.87 ± 1.06	360.6 ± 61.8	1.85 ± 0.25
Meizhonghong	15.9 ± 3.37	6.91 ± 1.27	358.6 ± 65.5	1.65 ± 0.46

Table 2 Nutrients of T4 generation of transgenic papaya ($n = 10$, SE)

Line	Water /%	Nitrogen /%	Protein /%	Lipid /%	Reduced sugar /%	Vitamin A /(mg·100 g^{-1})	Vitamin C /(mg·100 g^{-1})	Carotene /(μg·g^{-1})
Guanghong	91.9±1.6	1.9±0.3	11.2±1.5	1.0±0.2	7.0±1.7	0.41±0.1	82.9±24.8	31.8±9.7
Zhongkang 1	91.1±1.2	1.7±0.4	9.5±2.1	0.87±0.2	8.6±1.1	0.47±0.1	73.7±13.4	40.9±7.0
Meizhonghong	89.9±1.6	1.6±0.4	9.4±2.4	1.1±0.2	6.4±1.2	0.66±0.2	147.4±40.5	46.2±8.6
Zhongkang 2	90.6±1.4	1.6±0.3	9.2±1.8	1.2±0.2	7.7±1.9	0.74±0.2	152.9±35.3	38.7±10.9

Table 3 Differences between transgenic papaya and non-transgenic papaya

Line	Transgenic 1 and Guanghong			Transgenic 2 and Meizhonghong		
Fruit characters	*F*	*t*	*P*	*F*	*t*	*P*
Fruit length	0.8151	0.1615	0.8251	0.0650	0.4224	0.6980
Fruit diameter	0.0508	0.1766	0.8485	0.0698	0.0384	0.9683
Fresh weight per fruit	0.0005	0.0374	0.9612	0.0000	0.0541	0.9806
Flesh thickness	0.1770	1.1175	0.2060	2.2850	1.0490	0.2885
Water	0.4881	1.1054	0.2319	0.1700	0.8401	0.3946
Nitogen	0.2478	1.6898	0.1406	2.8214	0.2275	0.8013
Protein	0.3327	1.8171	0.1125	1.9095	0.1555	0.8679
Lipid	0.7292	1.5855	0.1574	1.1719	0.2603	0.7909
Reduced sugar	6.4964	0.8617	0.4355	0.3950	1.0820	0.5698
Vitamin A	0.1067	1.0827	0.3456	0.2166	0.8217	0.3213
Vitamin C	1.8255	0.5661	0.6730	0.8035	0.2026	0.8280
Carotene	1.7545	1.2502	0.2668	0.0011	1.3738	0.8619

3.4 The contents of nutritional components of transgenic papaya fruits

Although the contents of measured nutritional components in transgenic papaya fruits changed after the foreign genes were introduced into the papaya genome, though to different extent and direction with different type of transgenic papaya, there was still no significant difference in the contents of measured nutritional components in fruits between transgenic papaya and non-transgenic papaya (Tables 2 and 3). The contents of water, lipid, nitrogen, protein, and vitamin C of fruit of Zhongkang 1 were lower than non-transgenic papaya, while the reverse was true for the content of reduced sugar, carotene, and vitamin A. Compared with non-transgenic papaya, the contents of lipid, protein, and vitamin C in transgenic fruits were reduced by 13%, 15% and 11.1%, respectively, while the contents of carotene and vitamin A increased 28.6% and 22.9%, respectively. For Zhongkang 2, the contents of reduced sugar, vitamin C and vitamin A in fruits had 20%, 12%, and 3.7% more than their parents respectively, while the contents of carotene in fruits was 16.2% lower than non-transgenic papaya, and the contents of the other measured nutritional components of transgenic papaya fruits almost had no change.

3.5 The contents of nutritional components of red-fleshed papaya fruits and yellow-fleshed papaya fruits

The contents of fruits nutritional components were significantly different between red-fleshed papaya (transgenic and non-transgenic) and yellow-fleshed papaya (transgenic and non-transgenic), especially for the contents of vitamin A, vitamin C, and carotene. The contents of nitrogen, protein, and reduced sugar of red-fleshed papaya fruits were lower than that of yellow-fleshed papaya fruits, while the reverse was true for the vitamin and carotene. Compared with the red-fleshed papaya, the contents of nitrogen and protein of yellow-fleshed papaya fruits were 6.3%–18.8% and 1.1%–21.7% higher than those of red-fleshed papaya fruits, while the contents of vitamin A and vitamin C of yellow-fleshed papaya fruits were 1.4–1.8 and 1.78–2.07 times higher than those of red-fleshed papaya fruits.

4 Discussion

Papaya ringspot virus causes one of the most prominent diseases in papaya (*Carica papaya* L.) and occurs wherever it is grown (Purcifull et al., 1984). PRSV, a member of the genus potyvirus, is non-persistently transmitted by aphids to papaya and members of the Chenopodiaceae and Cucurbitaceae families (Purcifull et al., 1984). Once PRSV diseases occur in a papaya orchard, and they spread very rapidly within the orchard by winged aphids. Roguing infected plants away from the orchard is effective to reduce the rate of infection. Considerable efforts have been made to overcome PRSV, but the difficulty encountered in controlling a non-persistent aphid-borne virus like PRSV is due to the fact that many of the available pesticides do not kill incoming vectors before they transmit the virus to the crop. In the late 1980s, a papaya transformation system was developed whereby young embryos from papaya seedlings of the commercial Hawaiian solo cultivar ‘Sunset’ were transformed with the coat protein gene of a PRSV isolated from Hawaii and a promising transgenic papaya line (55-1) that showed resistance to PRSV from Hawaii was identified in 1991 (Lius et al., 1997). The transgenic papaya created by American scientists had been commercialized by the Food and Agriculture Organization (of the United Nations) in 1997. However, the results of field release showed that the transgenic papaya plants only postponed the outbreak of PRSV to two months and the plants had to be cut down and replanted every year (Lines et al., 2002). Some transgenic papaya lines developed in our laboratory in 1996 and the T0 generation plants of PRSV-resistant papaya were transplanted outside the greenhouse in Sun Yat-sen University campus in October 1997. Although the T0 generation plants of transgenic papaya inoculated with PRSV many times by manual and natural friction in the six-year duration,

two of the transgenic plants proved to be RP transgenic papaya that did not show any symptom of PRSV until now. Transgenic plants, which had higher resistance and could be kept longer, have not been reported in any other papers. The results of the field release and middle test showed all screened transgenic papaya plants had high resistance or immunity against PRSV and were proved to be RP transgenic papaya lines by molecular analysis. Furthermore, the foreign genes could be steadily inherited to, and expressed at RNA level, the progenies.

The results of American field release and our experiments showed that the RP transgenic papaya plants had so much higher resistance than CP transgenic papaya plants. The CP transgenic papaya lines were washed out while the RP transgenic papaya lines were kept down during screening, which has been reported by other scientists (Carr et al., 1994; Smith et al., 1995; Brederode et al., 1995; Guo et al., 2001). The growth of transgenic papaya plants showed that the RP transgenic papaya lines had overcome the harm of PRSV and made the perennial fruit crop not only resume growth for many years but also bear more fruits with higher quality. Because papaya is an allogamy plant, the segregation of characteristics could be observed among the progenies of transgenic papaya. However, after screening at the seedling stage, only a few transgenic papaya plants (about 9.2%) showed slight symptoms of PRSV when cultivated in the field, which had little effect on the output and quality of papaya fruits, which may mean achieving the commercial need.

Compared with non-transgenic plants, the fresh fruit length of T4 generation of transgenic papaya fruits increased 2.6%–5%, while the diameter decreased 0.6%–1.5%, which made the shape of fruits become more elliptic. The flesh thickness of fresh fruit increased 12%–15%, which made it fitter for eating. In order to reduce the errors, the fruits in the same sex plants were sampled and used to analyze the horticultural traits because the sex expression of papaya plants had enormous effects on the horticultural traits of the fresh fruits. Although the horticultural traits of the fresh fruits had some changes, there was still no significant difference between transgenic papaya and non-transgenic papaya. Thus, we were also unsure whether the changes of the horticultural traits of the fresh fruits were caused by insertion of foreign genes. Although the quality characteristics of dry fruits including the contents of water, lipid, N, protein, reduced sugar, vitamin A, vitamin C, and carotene of T4 generation of transgenic papaya were different from their parents, the non-transgenic papaya, there was still no significant difference that could be observed between transgenic papaya and their parents, the non-transgenic papaya, which means that transgenic plants and non-transgenic plants were substantially equivalent, and the transgene had no effect on the contents of nutritional components and fruit quality of transgenic papaya fruits. Although some studies showed the contents of the fruits' nutritional components, such as vitamin C, this might change when the samples are dried. However, in our studies, dryness had few effects on the contents of all measured nutritional components because the samples were treated with the same times and methods. Furthermore, the contents of nutritional components of transgenic papaya fruits were the same as the papaya reported by others (Popenoe, 1974).

In this study, we also found that the contents of fruit nutritional components were significantly different between red-fleshed papaya and yellow-fleshed papaya. The contents of vitamin A and vitamin C of red-fleshed papaya fruits were about 1 times more than those of the yellow-fleshed papaya fruits, while the contents of nitrogen, protein, and reduced sugar of red-fleshed papaya fruits were only 84.2%–92.1%, 82.1%–98.9%, and 91.4%–91.7% more than those of the yellow-fleshed papaya fruits, respectively; that is why the the red-flesh papaya is usually the favorite and priced higher than the yellow-fleshed papaya. The red-fleshed papaya is often used as fruit, while the yellow-fleshed papaya is mainly used for industrial production because of its terrible smell. Our results showed that the terrible smell might be nitrogenous compounds or protein, which had been confirmed by Fitch et al. (1992), Paull et al.(1999) and Yasar and Donald (2003).

Although the biosafety of GM plants has been a hot topic and has caused a growing public concern around the world since their first emergence in the 1980s, it is still not known whether GM plants and their products have effects on the environment and food (Fitch et al., 1992; Jia, 1997). However, transgenic papaya has not caused any biosafety problems since CP transgenic papaya was commercialized in America in 1998. Compared with the transgenic papaya (CP) invented by American scientists, the RP transgenic papaya invented in our laboratory had no GUS gene, which might have less possible horizontal gene transfer and few biosafety problems than the CP resistant papaya. Furthermore, our results showed there was no significant difference between RP transgenic papaya and non-transgenic papaya. Results indicated that the RP transgenic papaya and non-transgenic plants were substantially equivalent. Considering that the mutated RP gene was cloned from plant virus and the selection gene was widely used kanamycin resistance gene, the RP transgenic papaya would be safe for eating and cultivation.

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