

Effects of ^{60}Co γ -ray radiation on kiwifruit grafted buds

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Abstract

The aim of this study was to determine the feasibility of radiation treatment of the grafted buds in kiwifruit improvement. After treated with 50 Gy ^{60}Co γ -ray, buds from different fruit spurs of *Actinidia* were top grafted onto the heavily pruned mature vines with 50-100 grafted buds per stock plants and the grafted plants were under intensive cultural practices for high survival rate of the grafting. The experimental results showed that treatment with ^{60}Co γ -ray increased SSR diversity and phenotypic variations of the kiwifruit canes produced from the grafted buds. Selection of the favourable variants from a large population (canes from different plants) in a limited area of kiwifruit orchard was possible and some of favourable variants from the grafted populations were selected and rapidly propagated for further utilization in kiwifruit improvement.

Key words: Kiwifruit, radiation, top grafting, ^{60}Co γ -ray, breeding

Introduction

Domestication from wild kiwifruit plants, variant selection from cultivated kiwifruit plants, and screening from kiwifruit hybrids are main sources for kiwifruit (*Actinidia*) improvement (Huang, 2000). Domestication of wild kiwifruit plants is one of the great successes of horticulture in the 20th century (Huang and Ferguson, 2007). However, it will become less important in the future because the poor characters of wild kiwifruit plants are difficult to improve and new wild kiwifruit resources are limited. Kiwifruit plants are naturally cross-pollinating plants. There are extensive variations in hybrid generations for screening in kiwifruit improvement (Beatson *et al.*, 2007; Cho *et al.*, 2007; Liu *et al.*, 2006). Nevertheless, hybrid breeding of kiwifruit face difficulties in identifying hybrid plants, discerning juvenile male and juvenile female plants, and screening for flowers and fruits because of the long juvenile phase of the seedling plants. To search for a more efficient, rapid and practical method for kiwifruit improvement, we characterized simple sequence repeat (SSR) marker diversity and phenotypic variation of grafted populations from treated kiwifruit spurs with ^{60}Co γ -ray radiation. Feasibility of applying radiation treatment plus multiple top grafting on kiwifruit improvement is discussed in this study.

Materials and methods

Plant materials: One year old fruiting spurs from mature vines of *Actinidia chinensis* 'Wuzhi No.3' (China) and *A. deliciosa* 'Heping No. 1' (China), grown in the germplasm collection of the Fruit Research Institute of Heping County (Guangdong, China), were harvested and put in ice boxes on October 28, 2006, then transferred to the laboratory in Guangzhou (China) and stored at 0-4 °C for later use.

Radiation treatment: The fruiting spurs were cut into lengths of 30-40 cm and irradiated with 50 Gy (8 Gy/min) of ^{60}Co γ -ray on November 26, 2006 based on our previous experiment results (Hu *et al.*, 2008). The treated fruiting spurs were put in plastic

bags and stored at 0-4 °C for grafting in December.

Grafting: Grafting was carried out in December, 2006. Buds from un-radiated fruiting spurs and radiated fruiting spurs were top grafted onto heavily pruned mature stock plants (more than ten years old) with 50-100 buds per plant. There were 30-40 grafted plants in each treatment. The grafted plants were under intensive cultural practices including heavy dressing of ground fertilizer and manuring, sufficient water supply, removal of new shoots from the stock plants, timely shortening of shoots from the grafts when they were 40 cm long or more, and spraying water against high temperature and sunburn in the summer. Growth and the characteristics of the grafts were observed and recorded.

SSR assay: Genomic DNA was extracted from the young kiwifruit leaves according to the modified SDS extraction method (Ke *et al.*, 2006; Liu *et al.*, 2008). A pair of primers 5'-GTGCTCCTCCGTCATGTAT-3' and 3'-CGTCCTCTCTCGCCATTTA-5' was selected to amplify each DNA sample in PCR. PCR reactions were set up with 10 ng template DNA, 50 pmol of each primer, 0.8 μL of Taq polymerase (2 U/ μL), and 600 μmol of dNTPs in a 30 μL reaction system. The amplification cycle was: 94 °C (45 s) \rightarrow 54 °C (40 s) \rightarrow 72 °C (55 s) for 38 times and 72 °C 10 min for last extension. The PCR products were then separated on denaturant polyacrylamide gels by electrophoresis and the genetic diversity analysis was based on SSR polymorphism. Quantitatively, genetic diversity was estimated by the polymorphism index coefficient (PIC) of SSR loci (amplification bands) as following formula:

$$PIC = 1 - \sum_{j=1}^n f_{ij}^2 \quad (f_{ij} = \text{frequency of } j \text{ DNA band of } i \text{ primer pair})$$

Variant survey: Variants of leaf shape, blossom (start to flower 7 d earlier or 7 d later) and fruit shape were estimated through field observation. Some of variants with exploitation value and utilization prospect for future breeding programs were selected and propagated by means of micro-grafting. Differences between the treatment and the control were analyzed statistically.

Results

Canes from the radiated fruiting spurs, grafted in the winter of 2006 grew more slowly than the control in 2007 and grew normally in 2008 and in the later years. More than 90% of the grafted buds could sprout next spring. About 30% of the canes sprouted from the grafted buds flowered in 2007 and the later years. Most of the rest could flower both in 2008 and the later years. There was more SSR diversity in the populations produced from the grafted buds treated by ^{60}Co γ -ray and some phenotypic variants were also observed.

SSR diversity: SSR diversity could be found among different kiwifruit plants of *A. chinensis* 'Wuzhi No. 3' (Fig. 1) with the *PIC* value 0.453, which suggested that natural variation occurred in a long period of cultivation. Irradiated treatment with 50 Gy of ^{60}Co γ -ray increased SSR diversity of *A. chinensis* 'Wuzhi No. 3' as the canes emerging from the radiated buds showed more SSR diversity among the different fruiting spurs (Fig. 2), with *PIC* value 0.907. The radiated fruiting spurs of *Actinidia deliciosa* 'Heping No. 1' expressed similar tendency.

Phenotypic variations: Variants in leaf shape, blossom (early flowering or late flowering) and fruit shape were investigated through field observation (Table 1). In both varieties, the variant rates in the radiated treatment are significantly higher than the controls. After irradiated with ^{60}Co γ -ray, the variants of fruit shape were relatively more than leaf or blossom, however, there were not statistically significant differences among these three agricultural characters both in *A. chinensis* 'Wuzhi No. 3' and *A. deliciosa* 'Heping No. 1'. There was no significant difference in variation rate between above two varieties.

In 2008 fall, flowering and fruiting of the canes from the grafted buds irradiated with ^{60}Co γ -ray were screened at the orchards for utilization in breeding. A large fruit variant (fruit weight 138.25 ± 8.76 g, 22.32 g more than the control) and a long-fruit (axis ratio of length to width 1.91 ± 0.22 , 0.60 more than the control) and high sugar variant with total sugar content $21.23 \pm 2.28\%$ ($14.45 \pm 2.04\%$ in the control) of *A. chinensis* 'Wuzhi No. 3' were selected (Fig. 3). An early-flowering variant of *A. deliciosa* 'Heping No. 1' has also been selected (Fig. 4), with the blooming 10 d earlier than the control. By means of micro-grafting, each of these variants was propagated into a population over 100 plants for further observation and selection.

Discussion

Feasibility of radiation and multiple top grafting on kiwifruit improvement: Top grafting is one of techniques used for rejuvenation and conversion of cultivars in fruit orchards (Bao, 1996; Hu, *et al.*, 2003; Huang, 2006; Liu and Wang, 2006; Huang and Li, 2007). Since mature kiwifruit vines have large canopy, a very large area of kiwifruit orchard will be required for variant selection because of low natural mutation frequency, which greatly increases the cost in the kiwifruit breeding. Moreover, utilization of kiwifruit hybrids is a long term process

Table 1. Variant survey of two kiwifruit variety

Variety	Treatment	Grafting Number (survival)	Leaf variant	Flowering period variant	Fruit shape variant	Variant rate (%)
<i>A. chinensis</i>	50 Gy	3061	4	3	5	0.39*
'Wuzhi No.3'	Control	3038	0	0	1	0.03
<i>A. deliciosa</i>	50 Gy	3123	4	2	5	0.35*
'Heping No.1'	Control	2947	0	0	1	0.03

* Difference at $P=0.01$ levels by contrast to the control

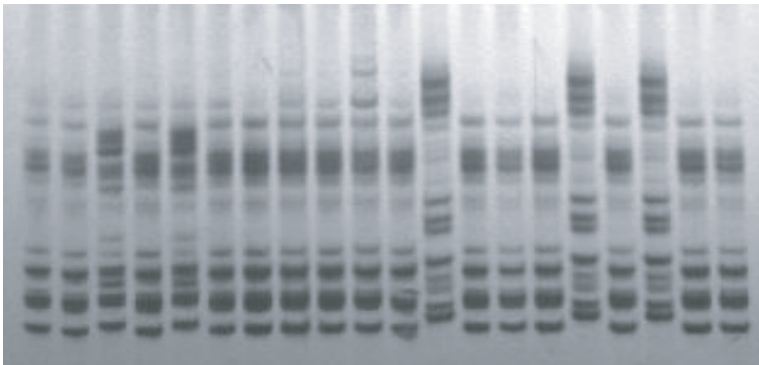


Fig. 1. SSR electrophoretogram of *A. chinensis* 'Wuzhi No. 3' from different spurs.

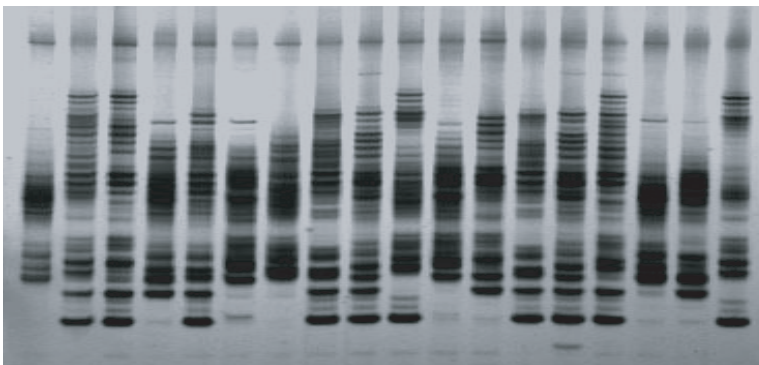


Fig. 2. SSR electrophoretogram from different spurs of *A. chinensis* 'Wuzhi No. 3' irradiated with 50 Gy ^{60}Co γ -ray.



Fig. 3. Fruit shape variants of *A. chinensis* 'Wuzhi No. 3' irradiated with 50 Gy of ^{60}Co γ -ray.



Fig. 4. Blossom variant of female *A. deliciosa* canes from grafted buds irradiated with 50 Gy of ^{60}Co γ -ray. A. control; B. early flowering variant

because the seedling kiwifruit plants may undergo a long juvenile phase before they flower and bear fruits.

Bud variants resulted from natural mutagenesis; induced mutation and sexual crossing are essential sources of new fruit cultivars. Practical utilization of new variants must be reviewed through agronomical characters and fruit quality. In phenotype, radiation treatment in this study might have little mutation effect because both varieties are polyploid and most of the mutations were recessive. Since very few of the mutations are favourable, a large population with numerous individuals is required for screening. Nevertheless, a large area of kiwifruit orchard for selecting favourable variants is very difficult to obtain because only 600~900 plants can be accommodated in a hectare of kiwifruit orchard. By means of multiple top grafting, theoretically, there are approximately 30000 to 45000 different canes per hectare for selection. In this study, two favourable fruit variants and an early blossom variant have been selected for further studies.

Genetic diversity in cultivated kiwifruit plants: Genetic diversity is the basis of plant breeding. Most of kiwifruit cultivars came from domestication of wild kiwifruit plants and variant selection from cultivated kiwifruit plants. As natural mutations might occur and accumulate in a long period of cultivation and asexual propagation, the cultivated kiwifruit will display genetic diversity. And radiation treatment can increase genetic diversity as this study showed. However, a lot of genetic diversity at molecular level such as SSR diversity is difficult to observe morphologically. Anyway, SSR diversity increase can be used to estimate the effectiveness of radiation treatment. When cane variants from the treated buds flower and bear fruits, selections based on some dominant characters or characters with high heritability such as fruit-shape, fruit quality, leaf shape and blooming may be effective.

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