**Journal of the American Pomological Society 70(3): 165-168 2016**

# DNA Markers Based on *PSY* Genes Can Differentiate Yellow- and White-Fleshed Loquats

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**Additional index words:** *Eriobotrya japonica,* fruit flesh color, marker-assisted selection

**Abstract**

White-flesh is one of the most appreciated traits in loquat. The development of new white-fleshed cultivars is costly and time consuming, involving the generation and selection of thousands of progeny each year. Marker-assisted selection (MAS) is an excellent technique to accelerate conventional breeding approaches. In this study, a specific DNA marker based on the sequence of phytoene synthase (*PSY*) gene was identified as an ideal DNA marker for the identification of fruit flesh color. We screened 31 cultivars derived from different countries and 24 $F_1$ hybrids developed from 4 cross combinations, and analyzed the relationships between flesh color and the DNA marker. The results showed that the DNA marker not only can distinguish yellow- and white-fleshed cultivars accurately, but can be used for accurate and early selection of yellow and white flesh progeny derived from controlled hybridizations.

Loquat (*Eriobotrya japonica* Lindl.), a member of the Rosaceae, accumulates carotenoids as the main pigments in mature fruit. The fruit can be divided into two groups, yellow- and white-fleshed, according to the color of the flesh (Lin, 2008; Deng et al., 2009). White-fleshed cultivars have become more popular because of their delicious flesh and higher retail selling price. However, breeding loquat is still limited at the seedling selection stage because of the long juvenile period (Wang et al., 2012). Marker-assisted selection (MAS) can shorten the breeding process and enhance breeding efficiency, and is widely utilized in many crops (Lahogue et al., 1998; Dalbo et al., 2001; Chu et al., 2011; Kong et al., 2013; Xu, 2013). With the development of loquat specific markers, DNA markers are widely used in loquat for various purposes, such as assessing genetic diversity(Vilanova et al., 2001; Chen et al., 2007; Wang et al., 2010; Wu et al., 2015), genetic linkage mapping (Gisbert et al., 2009) and germplasm identification (Watanabe et al., 2008; Fan et al., 2012). However, few DNA marker studies have focused on fruit color. Studies by Sheng et al. (2006) and Xie et al. (2012) only studied several white-fleshed cultivars as experimental materials. Phytoene synthase (*PSY*) gene is a very important structural gene of the carotenoid biosynthesis pathway, many studies showed that an increase in the expression of *PSY* leading to a massive carotenoids accumulation (Giuliano et al., 1993; Lois et al., 2000; Kato et al., 2004). Fu et al. (2014) revealed that the white-fleshed loquats contain only the mutant sequence *EjPSY2A*$^d$ and the yellow-fleshed loquats may contain both normal sequence *EjPSY2A* and mutant sequence *EjPSY2A*$^d$ or only normal sequence *EjPSY2A*. However, this hypothesis requires additional support because only 14 Chinese loquat cultivars (7 yellow-fleshed and 7 white-fleshed), were used as experimental materials. In this study, we hypothesized that sequence diversity of *PSY* gene may provide a means of developing a molecular marker for early selection of

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yellow- and white-fleshed loquat. A total of 31 cultivars and 24 $F_1$ hybrid progeny from 4 cross combinations were used as experimental materials to test our hypothesis.

## Materials and Methods

*Plant materials.* Thirty-one cultivars, 21 yellow-fleshed and 10 white-fleshed, were collected from the Loquat Germplasm Resources Garden, College of Horticulture, South China Agricultural University (SCAU). The details of these cultivars are listed in Table 1.

In 2010, 4 cross combinations (Zaozhong No.6×Javierin, Zaozhong No. 6×Peluches, Zaozhong No.6×Ullera, and Zaozhong No. 6×Marc) were hybridized and 24 $F_1$ hybrid plants were selected and kept for desirable traits. Among them, there were 11 yellow-fleshed and 13 white-fleshed selections.

*DNA extraction and PCR amplification.* DNA was extracted from young leaves according to the manual of a DNA extraction kit (Beijing Kangwei Biotech Inc., Beijing, China). A biophotometer was used to assess DNA concentration and purity.

A pair of gene specific primers EjPSY-2AUP1 5′-TATGAACCATTGATTAGT-CTAGC-3′ and EjPSY2ADP1 5′-GTTATT-GTCACCGTAGTCGC-3′ were used for amplification (Fu et al., 2014). PCR amplification was performed in a total volume of 25µl, including 0.5µl of each primer (10µM), 1µl template DNA, 10.5µl sterilized $ddH_2O$, and 12.5µl of 2× Mix buffer (Shanghai Shenggong Inc., Shanghai, China). The PCR profile was initiated with a preliminary step of 5 min at 95°C, followed by35 cycles at 95°C for 30 s, 55°C for 30 s, and 72°C for 1 min, and was terminated with a final extension for 7 min at 72°C. All amplification products were separated on 1.2% (W/V) agarose gels, and stained with ethidium bromide. Photos of the gels were taken and saved with BioradGelDoc XR system (Bio-Rad, USA).

## Results

*Marker genotyping of loquat cultivars.* The results clearly showed that PCR amplification of the *PSY* gene yielded two fragments: a long fragment, 1013bp and a short fragment, 319 bp (Fig.1). All white-fleshed cultivars investigated possessed only the short fragment, indicating these cultivars contained only the mutant sequence *EjPSY-2A*$^d$. However, all 21 yellow-fleshed cultivars showed the long (1013bp) fragment. Of these 21 yellow-flesh cultivars, 13 also showed the

**Table 1.** Name of loquat cultivars, flesh color and country of origin used in the study.

| Code | Cultivar | Flesh color | Origin | Code | Cultivar | Flesh color | Origin |
|---|---|---|---|---|---|---|---|
| 1 | Dawuxing | yellow | China | 16 | Ullera | yellow | Spain |
| 2 | Guangrongben | yellow | China | 17 | Javierin | yellow | Spain |
| 3 | Huabao No.3 | yellow | China | 18 | Peluches | yellow | Spain |
| 4 | Luoyangqing | yellow | China | 19 | Dazhong | yellow | China |
| 5 | Wanzhong | yellow | China | 20 | Baozhuqingzhong | yellow | China |
| 6 | Meihuaxia | yellow | China | 21 | Jiefangzhong | yellow | China |
| 7 | Zhongzhong No.6 | yellow | China | 22 | Baiyu | white | China |
| 8 | Xiangzhong | yellow | China | 23 | Baili | white | China |
| 9 | Moeiowase | yellow | Japan | 24 | Wugongbai | white | China |
| 10 | Golden Nugget | yellow | USA | 25 | Ruantiaobaisha | white | China |
| 11 | Mogi | yellow | Japan | 26 | Shiromogi | white | Japan |
| 12 | Dahongpao | yellow | China | 27 | Ninghaibai | white | China |
| 13 | Chuannao | yellow | China | 28 | Biqizhong | white | China |
| 14 | Nagasakiwase | yellow | Japan | 29 | Bingtangzhong | white | China |
| 15 | Marc | yellow | Spain | 30 | Tianzhong | white | China |
| 31 | Guifei | white | China | | | | |

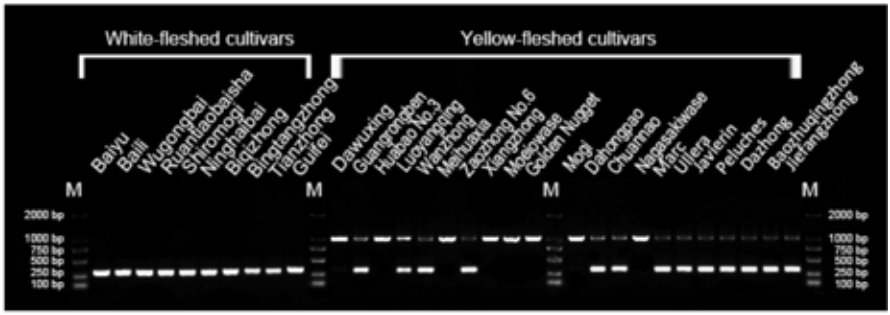


**Fig. 1.** PCR amplifications of 31 loquat cultivars using *PSY* gene specific marker. M represents DNA Ladder.

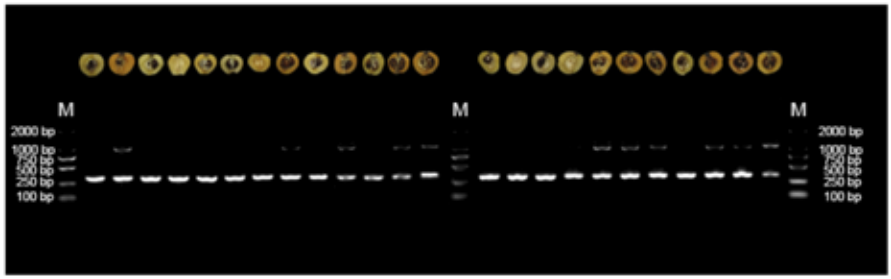


**Fig. 2.** PCR amplifications of 24 loquat plants derived from $F_1$ using *PSY* gene specific marker. M represents DNA Ladder.

319bp fragment as well, indicating these cultivars were heterozygous for this gene. The differences in amplification products clearly differentiated the 31 cultivars into yellow- and white-fleshed phenotypic groups.

*Marker genotyping of $F_1$ hybrid plants.* In this study, 24 $F_1$ hybrid plants derived from 4 different cross combinations, which had flowered and bore fruit, were used for marker genotyping. The results revealed that hybrid plants with white-fleshed fruit yielded only one short fragment, while hybrid plants with yellow-fleshed fruit produced two fragments (Fig.2). The PCR amplification results of $F_1$ hybrid selections were consistent with the known cultivars. This evidence supports our hypothesis that the polymorphisms revealed in the *PSY* gene amplification products using the specific primers described can be used as a molecular marker for early selection of yellow- and white-fleshed loquats.

## Discussion

There are also yellow-fleshed and white-fleshed varieties in peach, the yellow color of leaf vein can serve as a user-friendly marker for selection of yellow-fleshed varieties (Ma et al., 2014). However, there is no agronomic/phenotypic character that can differentiate between yellow-and white-fleshed cultivars at the seedling stage in loquat, so the presence of molecular markers will be helpful for accelerating the breeding of loquat. MAS is a very effective technique for fruit trees because it assists in accurate and rapid selection at the seedling stage for various important agronomic traits. The availability of DNA markers is of great significance in a species like loquat with a long period of juvenility. At present, an increasing number of growers prefer to plant white-fleshed cultivars for its high market price, and it is of paramount interest to develop a suitable method to select white-fleshed progeny from controlled cross-

es at the seedling stage. Our study demonstrated that the molecular DNA marker based on the structure of *PSY* gene can successfully distinguish yellow- and white-fleshed cultivars, and $F_1$ hybrid progeny with 100 % accuracy.

**Acknowledgments**

This research was partially supported by Guangzhou science and technology project (201504010028). Professor Jules Janick helped with the language editing of this manuscript.

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