Preliminary Study on Screening Loquat Hybrid Seedlings for Flesh Color Using *PSY* Gene Molecular Markers

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Abstract [**Objectives**] To distinguish loquat flesh color, thereby guiding early-age selection in loquat hybrid progeny. [**Methods**] Molecular markers based on the phytoene synthase gene (PSY) of loquat were applied to a hybrid progeny population derived from a cross between 'Zaozhong 6' and a local wild white-fleshed loquat resource 'DB1'. [**Results**] Among the 49 hybrid progeny, 24 were identified as white-fleshed loquat resources and 25 as yellow-fleshed loquat resources. [**Conclusions**] The molecular marker of the *PSY* gene can effectively distinguish loquat flesh color and is of significant importance for guiding early-age selection in loquat hybrid breeding.

Key words *PSY* gene, Molecular marker, Loquat, Flesh color

0 Introduction

Loquat originated in China with a cultivation history of over 2 000 years. It was first introduced to Japan from China, where it was known as "Tang Loquat," and later spread to many subtropical regions and countries[1]. Since 2003, Sichuan Province has become the largest loquat cultivation area in China, surpassing Fujian Province, traditionally the leading region for loquat cultivation[2]. Currently, the main cultivated varieties in Sichuan are 'Dawuxing' and 'Longquan No. 1'. Among newly developed loquat plantings, 80% are of the 'Dawuxing' variety (*Eriobotrya japonica* (Thunb.) Lindl.), while the cultivation area of 'Zaozhong 6' is also rapidly increasing. Based on the flesh color, loquats can be divided into two types: yellow (or red) flesh and white flesh. Yellow-fleshed fruits have orange flesh, firmer texture, thicker peel, better storage and transport tolerance, and stronger stress resistance. White-fleshed fruits have creamy white or pale yellow flesh, tender texture, abundant juice, and sweet flavor. Due to their superior flavor and taste, white fleshed loquats are more favored by consumers, while the promotion of yellow-fleshed loquats has been significantly constrained.

Previous research and practice have demonstrated that phytoene synthase (PSY) is a key regulatory enzyme in the carotenoid metabolic pathway, and this gene is often selected as the primary target gene in plant molecular marker studies[3]. In loquat, researchers have successfully cloned the *PSY* gene. The *PSY* gene produces two fragments: a long fragment of 1013 bp and a short fragment of 319 bp. The studied pure white-fleshed varieties possess only the short fragment, whereas yellow-fleshed varieties possess both the short and the long fragment. This difference in amplification products can clearly distinguish yellow-fleshed from white-fleshed loquats[4]. Therefore, in this experiment, the *PSY* gene was used as an auxiliary molecular marker for early selection of white-fleshed and yellow-fleshed varieties in loquat germplasm resources. The flesh color of reciprocal cross progenies between 'Zaozhong 6' (represented as "ZZ" in this study) and DB1 (a local wild white-fleshed loquat resource, temporarily designated) was investigated, aiming to obtain white-fleshed loquat resources and lay a solid foundation for the future breeding of white-fleshed loquat varieties.

1 Experimental materials

The test materials in this study consisted of leaves collected from the F1 seedlings obtained through reciprocal crosses between 'Zaozhong 6' (ZZ) and DB1.

2 Experimental materials

2.1 Extraction and quality detection of genomic DNA from loquat leaves

2.1.1 Genomic DNA extraction from loquat leaves. DNA of loquat leaves was extracted using the Chengdu Foregene Co., Ltd. Plant Genomic DNA Extraction Kit. The obtained DNA exhibited high purity and good integrity.

2.1.2 Quality test of template DNA. The concentration and purity of the loquat leaf DNA were measured using a NanoDrop 2000 ultra-microvolume spectrophotometer (Thermo Fisher Scientific, USA).

2.2 PCR amplification

2.2.1 SSR primer synthesis. The selected primers were EjPSY2-AUP1 5'-TATGAACCATTGATTAGTCTAGC-3' and EjPSY2ADP1 5'-GTTATTGTCACCGTAGTCGC-3', which were synthesized by Tsingke Biotechnology Co., Ltd.

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2.2.2 PCR reaction conditions. The PCR reaction conditions are shown in Fig. 1.

Predenaturation	95 ℃	5 min	
Denaturation	95 ℃	30 sec	35 cycles
Annealing	55 ℃	30 sec	
Extension	72 ℃	30 sec	
Polymerization	72 ℃	10 min	
Ramp	12 ℃	Incubation	

Fig. 1 PCR reaction conditions

2.2.3 PCR reaction components. The 2 × Taq PCR Master Mix was purchased from Sangon Biotech (Shanghai). PCR amplification was performed using a BIO-RAD C1000 Touch Thermal Cycler.

Table 1 PCR reaction components

Component	Concentration	Aliquot//μL
Template DNA	50 – 100 ng	
10 μM Primer I	0.2 pmol/μL	0.5 μL
10 μM Prime II	0.2 pmol/μL	0.5 μL
2 × Taq PCR Master Mix	1 ×	12.5 μL
ddH_2O		
Total		25.0 μL

2.3 Detection of PCR products The PCR products were detected by 1.2% agarose gel electrophoresis, and images were captured using a Fusion Fx vilber lourmat biomolecular imaging system.

3 Experimental results

As shown in Table 1, using 'Zaozhong 6' as the female parent and 'DB1' as the male parent, hybrid progeny seedlings were obtained. A total of 19 samples were submitted for testing. DNA was successfully extracted from all samples, and detection bands were obtained, resulting in a success rate of 100%. Among the detection results, samples showing only a short *PSY* gene band (319 bp) were classified as white-fleshed loquats, totaling 6 samples. Samples exhibiting both the short *PSY* gene band and the long *PSY* gene band (1 013 bp) were classified as yellow-fleshed loquats, totaling 13 samples. Among the hybrid loquat seedling resources, the ratio of white-fleshed to yellow-fleshed loquats was 6 ∶ 13.

Analysis of Table 2 shows that using 'DB1' as the female parent and 'Zaozhong 6' as the male parent, hybrid progeny seedlings were obtained. A total of 30 samples were submitted for testing. DNA was extracted from all samples, and detection bands were successfully obtained for all 30 samples, resulting in a success rate of 100%. Among the detection results, samples showing only a short *PSY* gene band (319 bp) were classified as white-fleshed loquats, totaling 18 samples. Samples exhibiting both the short *PSY* gene band and the long *PSY* gene band (1 013 bp) were classified as yellow-fleshed loquats, totaling 12 samples. Among the hybrid loquat seedling resources, the ratio of white-fleshed to yellow-fleshed loquats was 3 ∶ 2.

Table 2 Detection of molecular markers based on the *PSY* gene in loquat (ZZ × DB1)

No.	Variety (No.) name	DNA concentration ng/μL	PCR amplified bands 319 bp	PCR amplified bands 1 013 bp	Flesh color
1	ZZ × DB1-1	6.1	YES	YES	Yellow
2	ZZ × DB1-2	105.2	YES		White
3	ZZ × DB1-3	54.5	YES	YES	Yellow
4	ZZ × DB1-4	99.8	YES		White
5	ZZ × DB1-5	147.2	YES	YES	Yellow
6	ZZ × DB1-6	18.1	YES	YES	Yellow
7	ZZ × DB1-7	9.7	YES	YES	Yellow
8	ZZ × DB1-8	5.2	YES		White
9	ZZ × DB1-9	18.7	YES	YES	Yellow
10	ZZ × DB1-10	3.2	YES	YES	Yellow
11	ZZ × DB1-11	5.9	YES		Yellow
12	ZZ × DB1-12	4.4	YES	YES	Yellow
13	ZZ × DB1-13	2.7	YES	YES	Yellow
14	ZZ × DB1-14	13.1	YES		White
15	ZZ × DB1-15	3.8	YES	YES	Yellow
16	ZZ × DB1-16	28.6	YES	YES	Yellow
17	ZZ × DB1-17	6.5	YES		White
18	ZZ × DB1-18	5.2	YES	YES	Yellow
19	ZZ × DB1-21	85.1	YES		White

Table 3 Detection of molecular markers based on the *PSY* gene in loquat (DB1 × ZZ)

No.	Variety (No.) name	DNA concentration ng/μL	PCR amplified bands 319 bp	PCR amplified bands 1 013 bp	Flesh color
1	DB1 × ZZ-1	7.2	YES		White
2	DB1 × ZZ-2	53.7	YES		White
3	DB1 × ZZ-3	24.0	YES	YES	Yellow
4	DB1 × ZZ-4	26.5	YES		White
5	DB1 × ZZ-6	70.2	YES		White
6	DB1 × ZZ-7	12.0	YES	YES	Yellow
7	DB1 × ZZ-8	32.3	YES		White
8	DB1 × ZZ-9	152.5	YES		White
9	DB1 × ZZ-10	57.5	YES		White
10	DB1 × ZZ-11	67.0	YES		White
11	DB1 × ZZ-12	47.3	YES	YES	Yellow
12	DB1 × ZZ-13	111.1	YES	YES	Yellow
13	DB1 × ZZ-14	43.4	YES		White
14	DB1 × ZZ-15	109.8	YES		White
15	DB1 × ZZ-16	6.2	YES	YES	Yellow
16	DB1 × ZZ-17	24.7	YES		White
17	DB1 × ZZ-18	8.8	YES	YES	Yellow
18	DB1 × ZZ-19	14.2	YES		White
19	DB1 × ZZ-20	12.2	YES		White
20	DB1 × ZZ-21	72.4	YES		White
21	DB1 × ZZ-22	12.8	YES	YES	Yellow
22	DB1 × ZZ-23	18.2	YES	YES	Yellow
23	DB1 × ZZ-26	11.6	YES	YES	Yellow
24	DB1 × ZZ-27	13.1	YES		White
25	DB1 × ZZ-28	5.8	YES		White
26	DB1 × ZZ-29	41.5	YES	YES	Yellow
27	DB1 × ZZ-30	50.1	YES		White
28	DB1 × ZZ-31	27.9	YES	YES	Yellow
29	DB1 × ZZ-32	74.2	YES	YES	Yellow
30	DB1 × ZZ-33	70.2	YES		White

4 Discussion

4.1 White-fleshed loquat resources Flesh color is an agronomic trait of significant focus in agricultural production and serves as an important basis for horticultural crops to attract consumers[5]. In this study, using seedlings derived from the reciprocal crosses of Zaozhong 6 and DB1 as experimental materials and employing molecular markers based on the *PSY* gene as a detection method, we screened for loquat resources with white flesh. This work lays a solid foundation for the future breeding of white-fleshed loquat varieties. In this study, a total of 49 samples were tested, including 19 seedlings from the ZZ × DB1 cross and 30 seedlings from the DB1 × ZZ reciprocal cross. Among these, 24 were identified as loquat resources with white flesh, while 25 were identified as resources with yellow flesh, representing a proportion of 48% for the white-fleshed loquat resources. Molecular markers based on the *PSY* gene are of great significance in guiding the breeding of new loquat varieties with desired flesh color[6]. Therefore, emphasis will be placed on observing these 24 white-fleshed loquat resources to select those with excellent traits, strong stress resistance, and high yield. This will be followed by the promotion and extension of new loquat varieties.

4.2 Yellow-fleshed and white-fleshed loquat hybrid The flesh color of loquat is regulated by the *PSY* gene. By crossing Zaozhong 6 (which has yellow flesh) with DB1 (which has white flesh), the resulting offspring include both white-fleshed and yellow-fleshed loquats. From this, it can be inferred that the *PSY* gene in Zaozhong 6 loquat must carry one long band and one short band. In the direct cross, the ratio of white-fleshed to yellow-fleshed loquat resources was 6 ∶ 13, while in the reciprocal cross, the ratio was 3 ∶ 2. No clear inheritance pattern was observed from these results.

4.3 Limitations of this study The sample size obtained in this experiment was relatively small, allowing only for the screening of white-fleshed loquat resources. It remains difficult to identify patterns regarding whether the offspring from crosses between yellow-fleshed and white-fleshed loquats produce white flesh, as well as the underlying genetic probabilities. In the future, the experimental sample size must be expanded to better explain the inheritance patterns of flesh color in hybrid progeny of loquats with different flesh colors.

References

[1] FU Y, YANG C, WANG YQ, *et al.* Botany character investigation and fruit quality comparison between cultivars of *Eriobotrya japonica* Lindl. from Japan[J]. South China Fruits, 2011, 40(1): 13 – 15. (in Chinese).

[2] JIANG JM, CHEN XP, DENG CJ, *et al.* Analysis and development strategy of the advantages and disadvantages of loquat industry in China [J]. Chinese Horticulture Abstracts, 2019(4): 46 – 48, 68. (in Chinese)

[3] FU X, CHAO F, WANG C, *et al.* Involvement of multiple phytoene synthase genes in tissue- and cultivar-specific accumulation of carotenoids in loquat[J]. Journal of Experimental Botany, 2014, 65(16): 4679 – 4689.

[4] ZOU SC, ZHAO CB, WANG M, *et al.* Study on the inheritance of fruit color in loquat based on *PSY* gene marker[J]. Journal of Fruit Science, 2020, 37(2): 175 – 182. (in Chinese)

[5] WANG XT, XU XW, CHEN XH. Progress on fruit flesh color in horticultural crops[J]. Molecular Plant Breeding, 2022, 20(3): 1014 – 1025. (in Chinese)

[6] SONG HY, SUN SX, CHEN D, *et al.* Development and application of molecular marker for the key gene PSY2A of flesh color regulation in loquat[J]. Journal of Sichuan Agricultural University, 2022, 40(2): 172 – 178, 205. (in Chinese).

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[3] DONG HJ, LI XY. Rural reform and development in China in the past hundred years: Policy evolution, structural dilemma and innovation path [J]. Journal of Central South University (Social Sciences), 2025, 31(6): 158 – 172. (in Chinese).

[4] XU YY, MENG Y. New quality productive force empowering rural industrial revitalization: Practical basis, key tasks and systematic framework [J]. Seeker, 2025(6): 173 – 182. (in Chinese).

[5] YIN J, WANG YL. New quality agricultural productive forces and new production relations: An analysis from the perspective of political economics[J]. Journal of Sichuan University (Philosophy and Social Science Edition), 2025(6): 23 – 34, 225 – 226. (in Chinese).

[6] GAO M, HUANG Z. Strategies for accelerating the development of new quality productive forces in agriculture[J]. Economic Review Journal, 2024(11): 46 – 54. (in Chinese).