Postprint: Identification of a Superior Early-Ripening Peach Bud Sport Germplasm 'Wuyuehong' from Taiwan

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Date: 2018-12-04T00:00:00+00:00

Abstract

Peach is an important fruit tree species in China with extensive distribution, ranking fourth in national fruit production; however, cultivars suitable for southern cultivation remain scarce. Through germplasm resource investigation, a novel bud sport germplasm was identified on a large branch of Taiwan early-ripening peach in the arboretum of Guangxi University, designated as 'Wuyuehong'. Comprehensive observations and analyses were subsequently conducted on its principal biological characteristics, botanical traits, and fruit economic attributes, while SRAP molecular markers were utilized to detect genetic divergence from the maternal Taiwan early-ripening peach. The results demonstrated that the 'Wuyuehong' bud sport germplasm exhibits a maturity period approximately 20 days earlier than the maternal cultivar, ripening in early to mid-May, accompanied by increased fruit size, reduced stone size, enhanced edible flesh percentage, uniform coloration, and significantly elevated soluble solids content, while preserving the desirable traits of low chilling requirement, superior quality, and excellent flavor characteristic of Taiwan early-ripening peach, thereby constituting an extra-early ripening premium germplasm resource. Agarose electrophoresis detection revealed that the SRAP amplification primer combination me9/em5 produced a specific band of approximately 1200 bp in 'Wuyuehong' DNA samples, confirming genetic variation at the DNA level from the maternal Taiwan early-ripening peach and establishing its status as a novel extra-early ripening germplasm resource. The discovery of 'Wuyuehong' bud sport germplasm will provide valuable breeding material for developing extra-early ripening peach cultivars adapted to southern cultivation, contribute to optimizing the cultivation structure of varieties with different maturity periods, and consequently promote the enhancement of economic returns in southern peach cultivation industries.

Full Text

Preamble

Identification of a New Bud Sport Germplasm 'May Red' from Taiwan Precocious Peach

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Abstract

Peach is an important fruit crop in China, widely distributed and ranking fourth in national fruit production. However, few varieties are suitable for cultivation in southern regions. Through germplasm surveys, a novel bud sport was discovered on a large branch of a Taiwan precocious peach tree in the specimen garden of Guangxi University. This mutant was designated as 'May Red' and subsequently characterized through systematic observation of its primary biological characteristics, botanical traits, and fruit economic properties. Genetic divergence from the maternal Taiwan precocious peach was assessed using SRAP molecular markers. The results demonstrated that 'May Red' matures approximately 20 days earlier than Taiwan precocious peach, reaching harvest maturity in early to mid-May. The mutant produces larger fruits with smaller stones, resulting in improved edible flesh percentage and uniform coloration. Notably, the soluble solids content increased significantly while retaining the maternal cultivar' s advantages of low chilling requirement, excellent quality, and superior flavor, establishing it as an exceptionally early-maturing and high-quality germplasm resource. Agarose electrophoresis of SRAP amplification products using primer combination me9/em5 revealed a specific band of approximately 1200 bp in 'May Red' DNA samples, confirming genetic variation at the DNA level and validating its status as a novel, exceptionally early-maturing germplasm resource. The discovery of 'May Red' will provide valuable breeding material for developing very early-maturing peach varieties adapted to southern cultivation conditions, helping optimize cultivar structures across maturity periods and ultimately enhancing the economic viability of peach production in southern China.

Keywords: peach, bud sport, early maturity, germplasm resources, breeding

Introduction

Peach (*Amygdalus persica* L.), belonging to the family Rosaceae and genus *Prunus*, is one of China' s most ancient domesticated fruit crops with a long cultivation history and extensive distribution, ranking fourth in national fruit production. Currently, over 5,000 peach cultivars exist worldwide, with approximately 1,000 in China [**?**]. The primary commercial production regions in China

are concentrated in North and East China, including Beijing, Shandong, Henan, Hebei, Shaanxi, Gansu, Sichuan, and Liaoning provinces. Southern regions such as Taiwan, Guangxi, Guangdong, Zhejiang, and Jiangsu have limited cultivation areas. As China' s peach industry rapidly expands, southern production zones are becoming increasingly critical for optimizing cultivar structures, particularly for early- and mid-season varieties. However, several challenges persist: First, southern peach production holds a disadvantaged position in the national market, generally characterized by small fruit size, inferior quality, and soft flesh that limits distribution to local markets, with an extremely small proportion of very early-maturing varieties. Second, postharvest preservation of peaches is difficult, and the concentrated maturity period during June-July frequently leads to low market prices, severely constraining economic benefits for southern peach growers. A primary factor underlying these issues is the scarcity of high-quality, very early-maturing peach cultivars suitable for southern production conditions.

Southern Chinese peach production regions are better suited for early- and mid-season cultivars due to chilling requirement limitations. Leveraging the region' s natural environmental advantages to develop earlier-maturing varieties could extend the fresh fruit supply season, capture early market opportunities, effectively regulate production periods, and improve profitability. During previous germplasm surveys, our research team identified a bud sport mutant on a Taiwan precocious peach tree, which we designated as 'May Red' . Taiwan precocious peach itself possesses numerous desirable traits including broad adaptability, rapid growth, high yield, and early maturity, with visually appealing fruits of excellent quality. At the firm-ripe stage, the flesh is fine-textured, crisp, and sweet without acidity; at full ripeness, the flesh becomes soft and juicy with rich aroma, making it an excellent fresh-eating peach cultivar suitable for southern cultivation [**?**]. 'May Red' retains these superior characteristics while advancing maturity by approximately 20 days, reaching harvest in early to mid-May, thus representing a very early-maturing, high-quality germplasm resource. This discovery provides important breeding material for developing very early-maturing peach varieties and will facilitate optimization of cultivar structures, advancement of market availability, and extension of fresh peach supply periods, ultimately promoting improved economic returns for the peach industry.

Materials and Methods

1.1 Plant Materials

Experimental materials consisted of Taiwan precocious peach and its bud sport mutant 'May Red' . Samples were collected from the fruit tree specimen garden at the Agricultural College of Guangxi University (three-year-old grafted trees). Mature leaves were harvested from healthy plants, cleaned, flash-frozen in liquid nitrogen, and stored at -20°C. Two biological replicates were collected from different individual plants for both 'May Red' and Taiwan precocious

peach. EX Taq DNA polymerase and dNTPs were purchased from TIANGEN, while DL2000 DNA Marker and plant DNA extraction kits were obtained from Shanghai Sangon Biotech. Related primers were also synthesized by Shanghai Sangon Biotech.

1.2 Sample Collection and Processing

Fruit samples were collected at commercial maturity using completely randomized sampling. Five representative plants with similar growth vigor were selected for both Taiwan precocious peach and 'May Red' , with collection performed on May 14, 2018. From each tree, four healthy, uniformly sized, consistently colored, pest- and damage-free fruits were harvested from the upper canopy in each of four cardinal directions (east, south, west, north), totaling 16 fruits per tree. Fruits were sealed in plastic bags and transported to the laboratory for processing.

1.3 Experimental Methods

1.3.1 Observation of Biological and Botanical Characteristics In 2014, an early-maturing, superior bud sport was discovered on a lateral branch of a Taiwan precocious peach tree in the specimen garden of Guangxi University (Xixiangtang District, Nanning). This mutant was preliminarily designated as 'May Red' . In 2015, scions were collected from the mutant branch and grafted onto two-year-old Taiwan precocious peach rootstocks for further observation, initiating breeding research on this superior bud sport germplasm. The grafted 'May Red' plants began normal flowering and fruiting in the second year. From 2016 to 2018, biological characteristics, botanical traits, fruit economic properties, and genetic stability were observed and evaluated for three consecutive years according to the *Specification and Data Standard for Description of Peach Germplasm Resources* [**?**], while molecular SRAP marker analysis was conducted concurrently.

1.3.2 Fruit Quality Assessment From the collected fruit samples, ten fruits were randomly selected for economic trait evaluation, including fruit transverse and longitudinal diameters, stone dimensions, soluble solids content, and individual fruit weight. Mean values were calculated and subjected to significance testing. Measurements were performed as follows: fruit dimensions were measured directly using vernier calipers; soluble solids content was determined using a PAL-1 digital refractometer (Japan); individual fruit weight was measured using an electronic balance and averaged; edible flesh percentage was calculated based on mean values of whole fruit weight and flesh weight.

1.3.3 Genomic DNA Extraction Pre-collected mature leaf samples of 'May Red' and Taiwan precocious peach were retrieved from storage. Total DNA was extracted following the peach leaf DNA extraction method described by Zhang et al. [**?**], with final DNA concentration adjusted to 50 ng/μL and stored at -20°C.

Two biological replicate DNA samples were extracted from different individual plants for both 'May Red' and Taiwan precocious peach.

1.3.4 SRAP Analysis PCR amplification was performed using 24 published SRAP primer combinations [**?**, **?**]: me1/em1, me2/em2, me3/em3, me4/em4, me5/em5, me1/em5, me3/em6, me3/em11, me4/em10, me4/em11, me6/em5, me6/em6, me7/em4, me7/em6, me7/em10, me7/em11, me9/em5, me9/em6, me9/em8, me9/em11, me10/em2, me10/em5, me10/em7, and me10/em9. Primer sequences are listed in Table 2 . PCR amplification was conducted on a MasterCycler Gradient thermal cycler (Eppendorf) using EX Taq DNA polymerase (TaKaRa) in a 20 µL reaction volume following the manufacturer' s instructions. The amplification program consisted of: initial denaturation at 94°C for 5 min; 5 cycles of 94°C for 1 min, 35°C for 1 min, and 72°C for 1 min; followed by 35 cycles of 94°C for 1 min, 50°C for 1 min, and 72°C for 1 min; with a final extension at 72°C for 10 min. Amplification products were separated and detected using 1.5% non-denaturing polyacrylamide gel electrophoresis.

Results

2.1 Biological Characteristics

Both Taiwan precocious peach and 'May Red' exhibited identical flowering periods in Nanning, Guangxi, with anthesis occurring around February 10 and fruit set approximately on March 16. However, significant differences were observed in fruit color development stages. 'May Red' entered the color-turning period around mid-April, whereas Taiwan precocious peach began color development in late April. The maturity date for 'May Red' was approximately May 7, compared to May 28 for Taiwan precocious peach, representing a 20-day difference. Since both cultivars share the same flowering time but differ in fruit maturation, the primary cause of early maturity in 'May Red' appears to be accelerated fruit development.

2.2 Botanical Traits

The 'May Red' bud sport exhibited vigorous growth with an open tree architecture. The trunk surface was gray-brown, rough, and fissured. One-year-old shoots displayed reddish-brown coloration on sun-exposed surfaces and green on shaded sides, with numerous small lenticels. Leaves were elliptic-lanceolate, measuring approximately 14 cm × 4 cm (length × width), with cuneate bases and intersecting lateral vein termini. Leaf glands were reniform, numbering 2–3 per leaf, with acuminate apices and bluntly serrated margins. The adaxial leaf surface was green and glabrous, while the abaxial surface was light green. Flowers were solitary, opening before leaf emergence, with single-petaled rose-type blooms. Petals were oblong-elliptic to broadly obovate and pink. Pedicels were extremely short, with bell-shaped calyx tubes covered by short 绒毛. Sepals were

ovate with rounded apices and short 绒毛 on the outer surface. Stamens were deep pink with orange-yellow anthers and abundant pollen. The style exceeded stamens in length, and the ovary was 绒毛-covered.

2.3 Primary Fruit Economic Traits

Comparative analysis of major economic and biological traits between 'May Red' and Taiwan precocious peach fruits is presented in Table 1 and Table 2 , respectively. No differences were observed between the two cultivars in fruit shape, suture depth, fruit symmetry, pubescence density, stalk cavity dimensions, pericarp background color, overcolor intensity, coloration degree, coloration pattern, peelability, flesh color, red pigment content, fruit cracking rate, stone adhesion, fresh stone color, juice content, stone splitting rate, flavor, fiber content, or aroma. However, three major distinctions were evident: First, 'May Red' matured significantly earlier. In Nanning, Guangxi, Taiwan precocious peach typically matures in late May, while 'May Red' reaches maturity in early May, representing a 20-day difference. Second, 'May Red' produced larger fruits (Figure 1 [Figure 1: see original paper]) with mean individual fruit weight, longitudinal, transverse, and side diameters of 55.78 g, 52.00 mm, 46.98 mm, and 47.90 mm, respectively, compared to 43.43 g, 50.08 mm, 43.96 mm, and 44.64 mm for Taiwan precocious peach. Concurrently, 'May Red' developed smaller stones (Figure 2 [Figure 2: see original paper]) with mean fresh stone weight, length, width, and thickness of 2.96 g, 27.24 mm, 15.69 mm, and 13.58 mm, versus 3.33 g, 30.08 mm, 17.98 mm, and 13.80 mm for the maternal cultivar. Consequently, 'May Red' achieved a higher edible flesh percentage of 94.69% compared to 92.33% for Taiwan precocious peach. Third, fruit quality was superior in 'May Red' , with soluble solids content reaching 16.83% versus only 12.4% in Taiwan precocious peach. These results demonstrate that 'May Red' maintains the excellent quality and flavor of Taiwan precocious peach while exhibiting larger fruit size, smaller stones, higher edible flesh percentage, and increased soluble solids content with enhanced aroma, confirming its status as a very early-maturing, high-quality germplasm resource.

2.4 SRAP Molecular Marker Identification

Screening of 24 SRAP primer pairs identified me9/em5 as capable of distinguishing between Taiwan precocious peach and its bud sport 'May Red' . As shown in Figure 3 [Figure 3: see original paper], both biological replicates of 'May Red' displayed a specific band at approximately 1200 bp, while neither replicate of Taiwan precocious peach showed this band. These results confirm that 'May Red' has undergone mutation at the DNA level, establishing it as a novel, very early-maturing, superior germplasm resource.

Conclusion and Discussion

China is a major peach-producing country where the crop represents an important economic resource for farmers. However, the current production structure is imbalanced, with mid-season cultivars dominating while very early- and very late-maturing varieties are scarce. The majority of cultivars mature during June-July, and because peaches are difficult to store postharvest, this concentration leads to market oversupply, low prices, and significant economic losses for growers despite high yields. Therefore, developing high-quality, extremely early- or late-maturing cultivars to extend the fresh fruit supply season represents a critical solution to this production concentration problem.

Bud sport mutation is an important pathway for cultivar development in horticultural crops, referring to spontaneous genetic alterations in meristematic cells that modify heritable traits, including stress resistance, botanical characteristics, and economic properties such as fruit shape, size, aroma, color, nutritional composition, sweetness, edible flesh percentage, and seed dimensions. Our initial field investigation identified the 'May Red' bud sport mutant on Taiwan precocious peach. Subsequent observations of biological characteristics, botanical traits, and primary fruit economic properties revealed that 'May Red' retained the desirable features of Taiwan precocious peach—including high yield, rapid growth, attractive fruit appearance, and sweet flesh—while exhibiting beneficial mutations in key economic traits: earlier maturity, larger fruit size, smaller stones, increased soluble solids content, and enhanced aroma. To genetically confirm these mutations, we conducted SRAP molecular marker analysis, which revealed genetic divergence between 'May Red' and its maternal cultivar. These findings collectively establish 'May Red'as a novel, very early-maturing, superior germplasm resource.

Phenological observations revealed identical flowering and fruit-set periods between 'May Red' and Taiwan precocious peach, with maturity differences arising from distinct color development stages. Fruit coloration in peach represents anthocyanin biosynthesis and accumulation, resulting in vibrant red skin at maturity. Current research indicates that anthocyanin biosynthesis occurs via the flavonoid metabolic pathway, with key structural genes including anthocyanidin synthase (ANS), UDP-glucose:flavonoid 3-O-glucosyltransferase (UFGT), flavanone 3-hydroxylase (F3H), chalcone isomerase (CHI), and chalcone synthase (CHS) [**?**, **?**]. Recent studies demonstrate that three major transcription factor families—MYB, basic helix-loop-helix (bHLH), and WD40 proteins—interact to form MBW (MYB-bHLH-WD40) complexes that regulate expression of these structural genes and control anthocyanin biosynthesis [**?**, **?**]. The earlier color-turning period in 'May Red' may result from mutations in anthocyanin synthesis genes or regulatory transcription factors such as MYB. Another significant mutation is the combination of larger fruit size with increased soluble solids content. Enhanced fruit size may stem from accelerated cell division and cell expansion rates during fruit development compared to Taiwan precocious peach. Soluble solids primarily comprise soluble sugars including monosaccharides, dis-

accharides, and polysaccharides. The simultaneous increase in fruit size and soluble solids in 'May Red' may be attributed to mutations in sugar metabolism pathway genes that accelerate soluble sugar synthesis or impede metabolism and transport. Future research will employ high-throughput sequencing-based bioinformatics and molecular biology techniques to investigate the molecular mechanisms underlying these mutations in key fruit economic traits.

'May Red' exhibits low chilling requirement, very early maturity, significantly larger fruit size, and maturity approximately 20 days earlier than Taiwan precocious peach, with notably superior fruit quality. While chilling requirements limit major peach production regions in southern China, the region' s geographical and climatic advantages can be leveraged to develop low-chilling, very early-maturing cultivars as an important strategy for optimizing production structures. Currently, few excellent low-chilling cultivars are available for southern cultivation. The discovery of 'May Red' provides valuable breeding material for developing very early-maturing peaches adapted to southern Chinese conditions.

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Note: Figure translations are in progress. See original paper for figures.