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# Transmission of early ripening trait related loci in grapevines from backbone cultivar Pearl of Csaba to its descendants

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ABSTRACT

In order to dissect the inheritance of the grapevine early ripening trait, Pearl of Csaba, 32 derived varieties of Pearl of Csaba and 5 other parents for the breeding of the derived varieties were analyzed based on the SSR (Simple Sequence Repeats) markers. Fifty SSR markers evenly distributed on 19 chromosomes of grapevine were initially employed and only 36 markers could transmit to all the descendants of four generations. Then, the false positive loci were excluded by tracing the high steadily transmitted loci in one consecutive line including three generation varieties (11 markers left), comparing to the other 5 medium and late ripening parents used in the formation of the descendants (8 markers left), considering the stability of the inheritance frequency from the generations to generations (3 markers left) and three SSR markers, VVMD5, VVMD32 and VMC4F8 were identified as the candidate. These three markers could be stably inherited in four generations and their inheritance frequency, genetic contribution rate and allele frequency were all above 40%, 70% and 0.4, respectively. The genomic loci corresponding to these markers and their adjacent chromosomal regions may be associated with the inheritance of early ripening trait in the derived varieties of Pearl of Csaba.

## 1. Introduction

Grapevine (*Vitis vinifera* L.) has important economic value that could be consumed as fresh berry, wine, juice, jam, raisins and seed oil (Zo et al., 2016). For the table grapevine market, the ripening date of grapevine berry was one of the important factors affecting the economic benefit of grapevine growers. The breeding of the abundant excellent early, medium and late ripening varieties could extend the supplying period of fresh grapevine berry and better satisfy the demands of the market. However, the early ripening varieties which have comprehensive excellent performance are relatively few at present in China. In order to accelerate the breeding efficiency of the early ripening grapevine varieties, it is necessary to explore the inheritance mechanism of early ripening trait in grapevine.

The backbone varieties are with eminent traits which have stable and strong inheritance ability in the derived varieties and play a key role in the formation of derived cultivar groups with the same trait in the long-term breeding progress (Yuan et al., 2010). In Chinese table grapevine breeding history, there were many grapevine varieties employed as the parents, such as Muscat Hamburg, Kyoho, Pearl of Csaba, Thomson Seedless, and so on (Liu et al., 2002). Muscat Hamburg, Kyoho and Pearl of Csaba were the top three varieties which were directly used or their derived varieties were further used as the parents to breed the new varieties in China. As one of the most prominent early ripening grapevine variety in the world, Pearl of Csaba has been successfully used to breed a number of valuable early ripening grapevine varieties by the domestic breeders (Liu et al., 2002). And the complete pedigree of Pearl of Csaba comprised 32 varieties of four generations derived from it was summarized (Fan et al., 2010). Most of the derived varieties from Pearl of Csaba were early ripening varieties according to the standard of Liu et al. (2004); i.e., grapevine varieties with BDP (berry development period, the period from flowering to physiological ripening) $\leq$80 days, 80–100 days and $>$100 days were classified as early ripening, medium ripening and late ripening types, respectively. Therefore, the early ripening varieties group derived from Pearl of Csaba was the valuable germplasm resource to trace and identify the inheritance loci of early ripening trait in grapevine.

Molecular markers have been widely used in plant research. At present, the transmission of the important traits from the backbone parents to the derived varieties have been extensively studied in crops, for example, in wheat (Wu et al., 2016), soybean (Samanfar et al., 2016), grapevine (Vouillamoz et al., 2007), and sweet cherry (Rosyara et al., 2013). Most of the researches focus on the traits of high yield, good quality and disease resistant. These studies revealed the inheritance of these traits to a certain extent, and preliminarily identified the approximate loci that control them. However, there were few

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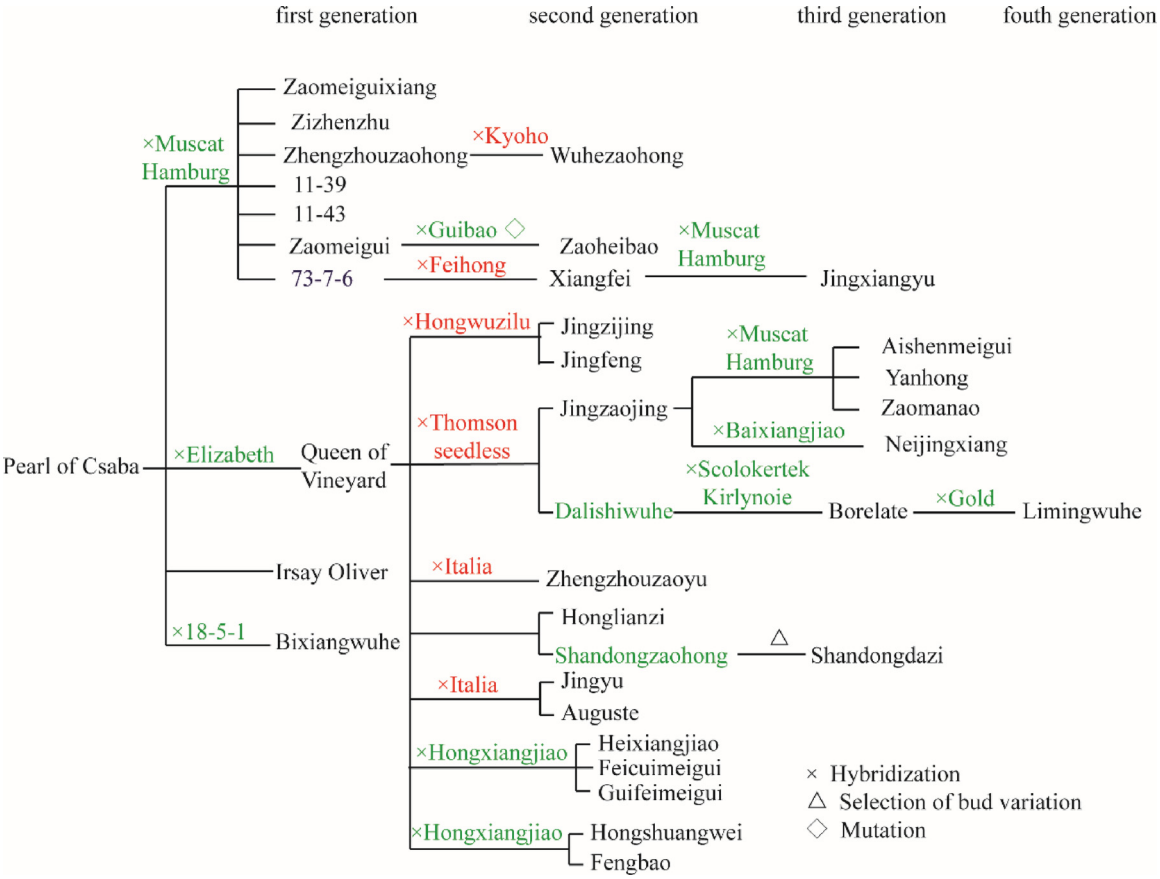


**Fig. 1.** The pedigree map of Pearl of Csaba and the materials used in this study (revised based on Fan et al. (2010)). The varieties with names in black color were employed as the derived varieties of Pearl of Csaba, while in green color were not available in this study. The varieties with names in red color were the other five parents analyzed in this study. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article).

studies on the backbone parents of grapevine, especially for the early ripening trait.

In this study, Pearl of Csaba, 32 derived varieties of Pearl of Csaba and 5 other parents (Fig. 1) for the breeding of the derived varieties were analyzed based on SSR molecular markers. It was aimed to explore the inheritance of early ripening trait of Pearl of Csaba in its derived varieties at the DNA level.

## 2. Materials and methods

### 2.1. Plant material and extraction of DNA

The young leaves of 38 grapevine varieties were collected in the middle of May 2017 and immediately stored in the liquid nitrogen after the sampling and packaging. All materials were collected from the National Grape Germplasm Reporsitory, Zhengzhou Fruit Research Institute of the Chinese Academy of Agricultural Sciences. The pedigree map of Pearl of Csaba was shown in Fig. 1. The ripening data of these 38 grapevine varieties were presented in Supplementary Table S1.

Total genomic DNA was isolated from the fresh leaves following the modified CTAB method (Borges et al., 2009). The quality and concentration of the DNA were checked in Nanodrop (America) and the DNA was diluted to 50 ng/μL for SSR analysis. Both the stock and diluted DNAs were stored at -20℃.

### 2.2. SSR analysis

Pearl of Csaba, 32 derived varieties and 5 other parents were screened with 50 SSR markers including VMC, VVS, VVMD, VRZAG series (Fanizza et al., 2005; Mejia et al., 2007) and markers retrieved from NCBI database (https://www.ncbi.nlm.nih.gov/genome/gdv/browser/?context=genome&acc=GCF_000003745.3). All primers were synthesized by Shanghai bioengineering technology company and the primer sequences were shown in Supplementary Table S2.

The SSR amplifications were performed in 25 μl reaction system including DNA 30 ng, *Taq* enzyme Buffer 1×, $MgCl_2$ 2 mmol· $L^{-1}$, dNTP 0.2 mmol· $L^{-1}$, each primer 0.4 μmol $L^{-1}$, Ex *Taq* 1 U. Polymerase chain reaction (PCR) were conducted as the following: the initial denaturing step was 95 °C for 3 min, subsequent denaturing was at 95 °C for 30 s, annealing was 55–67 °C for 1 min, extension was 72 °C for 30 s, and repeated for a total of 29 cycles with a final extension at 72 °C for 10 min. PCR products were resolved by capillary electrophoresis with 3730XL instrument (Shanghai bioengineering technology company).

### 2.3. Statistical analysis

Chromatograms were analyzed using the software GeneMapper 4.0. The genetic similarity coefficient (Liu and Muse, 2005) was calculated by Powermarker v3.25 software, and the clustering tree was constructed by UPGMA (unweighted pair grouping method with arithmetic mean) (Drummond and Rodrigo, 2000) with Powermarker.

## 3. Results

### 3.1. SSR amplification profile

Fifty primers distributed evenly on 19 chromosomes of the whole grapevine genome were initially selected for SSR analysis in Pearl of Csaba and its derived varieties. Two markers, VVIV37 and VMC5H2,