



## Androecious Genotype ‘Male 8’ Carries the CPCNA Gene Locus Controlling Natural Deastringency of Chinese PCNA Persimmons

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### Abstract

The androecious genotype of *Diospyros* spp. ‘Male 8’, which is distributed in Dabie Mountain area of the central China, was probably derived from the hybrids or natural variation of Chinese pollination constant and non-astringent (CPCNA) persimmon. Its application potential as the breeding parent of pollination constant and non-astringent (PCNA) is undefined. In this study, we identified the astringency type of a hybrid individual (H8-2) derived from the cross between ‘Huashi 1’ (pollination variant and astringent, PVA) and ‘Male 8’. Through comparison with the known genotypes of CPCNA, Japanese PCNA (JPCNA) and non-PCNA, the results of soluble/insoluble tannin content and tannin cell size measure showed that H8-2 was a PCNA genotype with the characteristic of natural deastringency of CPCNA. According to the currently known genetic features of PCNA persimmon, a new individual of PCNA may be generated in F<sub>1</sub> population when two JPCNA genotypes are crossed or when the CPCNA genotype is used as a parent. Thus, the study verifies that androecious genotype ‘Male 8’ carries the dominant gene locus that controls the non-astringent trait of CPCNA, indicating its potential to be used as pollen donor for the genetic improvement of PCNA persimmon.

**Keywords:** Chinese androecious persimmon; PCNA; soluble tannin content; tannin cell size; genetic improvement

### 1. Introduction

Based on the presence/absence of astringency in the fruit at maturity and the genetic characteristics of astringency, persimmon (*Diospyros kaki* Thunb.) cultivars are classified into two types: pollination constant and non-astringent (PCNA) and non-PCNA. According to the differences in the genetic characteristics of the PCNA trait, the PCNA type comprises Chinese PCNA (CPCNA) and Japanese PCNA (JPCNA) (Akagi et al., 2011). Based on the presence/absence of astringency and release of volatile compounds in seeds, the non-PCNA type can be further classified into pollination variant non-astringent (PVNA), pollination-variant astringent (PVA), and pollination constant astringent (PCA). In Japan, the genetic improvement of persimmon

has been focused on the breeding of PCNA type since the 1930s, and a series of new PCNA varieties have been released, including ‘Taishuu’ (Yamane et al., 2001) and the first nonaploid seedless variety in the world ‘Akiou’ (2n = 9 × = 135) (Chijiwa et al., 2008). However, the genetic diversity among JPCNA cultivars is low (Kanzaki et al., 2000a, 2000b), which causes obvious inbreeding depression on fruit quality, yield and vigor in the hybrids (Yamada et al., 1994; Yonemori et al., 2000). In addition, the non-astringent trait of JPCNA is recessive compared with that of non-PCNA genotypes. Generally, a new individual of PCNA may be generated in F<sub>1</sub> population in the hybridization among JPCNA types, while the F<sub>1</sub> offspring from the cross between JPCNA and non-PCNA are all new non-PCNA genotypes (Ikeda et al., 1985; Yamada and Sato, 2002).

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A previous study has shown that CPCNA and JPCNA originated and evolved independently of each other, and CPCNA has more abundant genetic diversity (Du et al., 2009). The natural deastringency trait of CPCNA is controlled by a single dominant gene locus, which is significantly different from the case of JPCNA. As a result, 50% of the progenies may be PCNA genotypes in F<sub>1</sub> population from the cross of CPCNA with JPCNA or non-PCNA (Ikegami et al., 2004, 2006). Thus, CPCNA could be an ideal parent for the genetic improvement of PCNA persimmon. A series of androecious genotypes were found in the Dabie Mountain region in central China (Luo et al., 2005). The cluster analysis with various molecular markers showed that these androecious materials have close genetic relationships with CPCNA (Guo and Luo, 2006; Du et al., 2009). Zhang and Luo (2006) and Xu et al. (2008) have demonstrated that androecious genotypes are characterized by the stable trait of bearing only staminate flowers, abundant amount of pollen, high germinability *in vitro* and pollination affinity with the main cultivars. Moreover, the RO2 marker (Ikegami et al., 2011), which is linked to the allele associated with the non-astringent trait of CPCNA, was detected in 6 androecious genotypes (Pei et al., 2013), suggesting that several Chinese androecious genotypes, such as ‘Male 8’, might carry the *CPCNA* locus that controls the natural deastringency of CPCNA. However, there have been no reports about the determination of astringency in the hybrid population derived from the cross between non-PCNA and ‘Male 8’. Hence, this study was aimed to determine the astringency traits of this hybrid population, and to explore the potential of ‘Male 8’ to be used as a breeding parent for PCNA genotypes. The results of the present study may expand the scope of the breeding parents for PCNA persimmon, which is of great significance for the genetic improvement of persimmon cultivar.

## 2. Materials and methods

### 2.1. Materials

The 6 known genotypes of controls and F<sub>1</sub> offspring of 9 combinations were used in this study, which were grown in the Persimmon Repository of Huazhong Agricultural University,

Wuhan, China (Table 1). Except that the H8-2 persimmon fruits were sampled at 20 and 25 weeks after flowering (WAF), other materials were collected at 20 WAF. In addition, three biological replicates with 5 fruits for each replicate were performed for all samples except for H1-72. All the samples were frozen with liquid nitrogen and stored at -80 °C until analysis.

### 2.2. Measurement of tannin content

Soluble and insoluble tannin contents were measured with the printing method (Eaks, 1967) and Folin-Ciocalteu (Oshida et al., 1996).

### 2.3. Determination of tannin cell size

Tannin cells were separated according to Ikegami et al. (2006), and then at least 100 cells with 3 replicates were observed and photographed using OLYMPUS BX61 fluorescence microscope. SPSS software (version 12.0) was used for significance analysis.

## 3. Results

### 3.1. Brown spot distribution of fruit transection

As shown in Fig. 1, the brown spot area was positively correlated with the number of seeds in the fruits of F<sub>1</sub> progenies H1-68, H1-71 and H1-73, and the transection of fruit was covered by brown spots when the seed number exceeded 3, which is similar to the case of PVNA persimmon ‘90-1-10’. However, different from PVNA fruit, the fruit of H1-72 formed only a small amount of brown spots around the seeds, which was similarly observed in PVA persimmon ‘Huashi 1’ (Fig. 1). Brown spots were not observed in the fruit of other hybrids, suggesting that these individuals may be PCA or PCNA persimmon (Fig. 1).

### 3.2. Soluble tannin content and tannin cell size of fruit

The results showed that the soluble tannin contents of H1-68, H1-71 and H1-73 were below 0.20% (threshold value to distinguish PCNA and non-PCNA persimmon), indicating that

**Table 1** The innovated F<sub>1</sub> materials and known astringent genotypes used for identification of astringency loss characteristics

Origin	Genotype	Individuals	Astringent type	Sexuality	
Japan	Youhou		JPCNA	♀	
China	Luotian-tianshi		CPCNA	♀	
	Eshi 1		CPCNA	♀	
	90-1-10		PVNA	♀	
	Huashi 1		PVA	♀♂	
	Gongcheng-shuishu		PCA	♀	
	H1 (Huashi 1 × Luotian-tianshi)		H1-13	Unknown	♀♂
			H1-68	Unknown	♀
			H1-71	Unknown	♀
			H1-72	Unknown	♀
			H1-73	Unknown	♀
	H2 (Huashi 1 × Male 3)		H2-6	Unknown	♀♂
		H2-9	Unknown	♀	
		H3-5	Unknown	♀♂	
H3 (Eshi 1 × Luotian-tianshi)		H3-5	Unknown	♀♂	
H8 (Huashi 1 × Male 8)		H8-2	Unknown	♀♂	

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