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Genetic diversity among wild androecious germplasms of *Diospyros kaki* in China based on SSR markers

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ABSTRACT

A lack of male flowers strongly restricts controlled pollination in persimmons. To address this problem, we collected and conserved male persimmon resources from various provinces in China in 5 successive years, from 2012 to 2017. Approximately 120 wild androecious persimmons and 10 monoecious persimmons were collected in Hubei, Jiangxi, Jiangsu, Hunan, Shaanxi, and Henan Provinces as well as Guangxi Zhuang Autonomous Region in China. To determine the genetic diversity and relationships among these androecious persimmons, as well as the relationship among Chinese persimmons, Japanese persimmons and closely related species, 47 wild androecious, 2 wild andromonoecious, 10 monoecious, and 1 female persimmons from China, 12 Japanese persimmons and 15 closely related species, were analyzed based on simple sequence repeat (SSR) markers. A total of 179 alleles and 456 genotypes were obtained from 15 loci, with a mean of 11.93 alleles and 30.40 genotypes per locus. The 87 persimmon resources investigated in this study were divided into XIII clusters using the unweighted pair group method with arithmetic mean average (UPGMA) and the androecious persimmons were distinguished completely from their closely related species. The androecious persimmons originating from the same provinces displayed a close genetic relationship and most Japanese resources could be classified into a same cluster exclusively. A C-PCNA type called 'Baohuatianshi' was genetically closely related to three Chinese androecious persimmons ("Macheng Yeshi-1″, "Zhanfanghou-male" and "Qinghua-male") and two monoecious pollination-constant astringent (PCA) type ('Xiangyang Niuxinshi' and "Laojianshan-5″). These results provide useful information for the effective conservation and utilization of androecious persimmons.

1. Introduction

Persimmon (*Diospyros kaki* Thunb.) is an important temperate fruit tree species in China and has been widely cultivated for more than 2000 years (Luo and Wang, 2008). Kajiura (1946) classified persimmons into four types based on the level of astringency loss in fruits: pollination-constant non-astringent (PCNA), pollination-constant astringent (PCA), pollination-variant non-astringent (PVNA), and pollination variant astringent (PVA). According to the genetic traits controlled by different loci, Akagi et al. (2011) divided PCNA into J-PCNA (Japanese PCNA) and C-PCNA (Chinese PCNA). The genetic trait of the J-PCNA type is controlled by a recessive locus named *AST* (Astringency), while the C-PCNA type is controlled by a dominant locus (*CPCNA*) (Yonemori et al., 2000; Ikegami et al., 2006). Although a total of approximately 1000 cultivars of *D. kaki* have been identified, several chanllenges remain, such as the lower rate of cultivation of improved cultivars and the limited popularity of the fruits among consumers due to their astringency; thus, the abundant persimmon trees growing in China are an under-utilized resource (Sun et al., 2017). To address these issues, it is important to strengthen the breeding of superior varieties for specific use (e.g., fresh fruit, medicine, and health products).

Complex flowering characteristics with bisexual (hermaphrodite) and unisexual (male and female) flowers exist in persimmon (Zhang et al., 2016a, b). Pistillate (female) flowers, locating in the axils of leaves, are usually solitary with a central flower per inflorescence and two lateral aborted flowers. Male (staminate) flowers are presented in cymose clusters of three to five flowers with fertile stamens but rudimentary, arrested carpels, and smaller than female flowers (pistillate),

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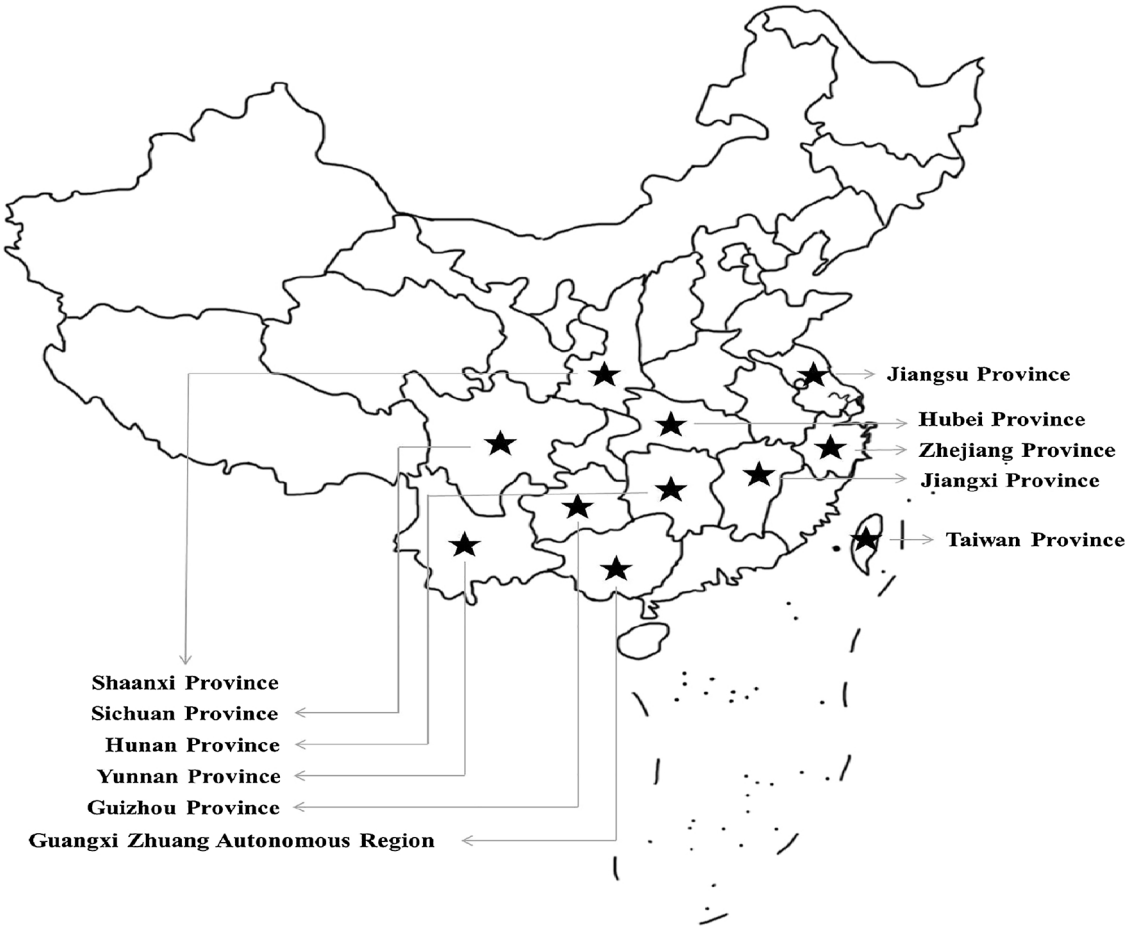

Fig. 1. Map showing origins for the resources specific in China investigated in this study. Stars represent 11 origins located in China.

which are generally solitary (Spongberg, 1979; George et al., 1997; Akagi et al., 2014). Occasionally, the staminate flower is a hermaphrodite bearing much smaller fruits than those originating from normal female flowers (George et al., 1997; Tetsumura et al., 2009). Yonemori et al. (1993) divided persimmon into three types at the individual level sex expression: i) bear only female flowers (gynoecious-type), ii) both pistillate and staminate flowers on the same plant (monoecious-type), and iii) hermaphroditic, pistillate, and staminate flowers on the same plant (polygamomonoecious-type). In addition, a type bearing only male flowers (androecious-type) was found in Dabie Mountain area from China (Xu et al., 2013; Zhang et al., 2009). According to the phenotype of sex among various persimmon cultivars, most varieties are gynoecious while just a few are monoecious, and the androecious are extremely rare. Meanwhile, as a key means of germplasm innovation, controlled pollination can combine favorable traits and the offspring may inherit these traits. Thus, the advancement of cross-breeding is significantly limited by the scarcity of male persimmon resources. Interestingly, a certain number of androecious persimmon germplasms, as well as a few andromonoecious (persimmon bearing both hermaphrodite and staminate flowers) randomly occur in the various wild regions in China by our research team in 5 successive years, from 2012 to 2017. Morever, most persimmon cultivars present strong parthenocarpic ability, whereas, seeds and seedlings with new genotypes will not be obtained without pollens provided by male parents. Thus, the male persimmon trees (including androecy, monoecy, andromonoecy and polygamomonoecious trees which can bear male flowers and produce active pollens) are crucial for the controlled pollination of persimmons. Therefore, systematic collection and arrangement of androecious persimmon resources, as well as elucidating the genetic diversity of these resources, could offer theory basis for the selection of male parent and hybrid combination of persimmon cross breeding.

Simple sequence repeat (SSR) is regarded as a co-dominant marker, which is distributed throughout the genome abundantly and has high levels of polymorphism as well as medium reproducibility (Du et al., 2009a, b; Ismail et al., 2016; Yang et al., 2016). In recent years, genetic analyses of persimmon have primarily examined by the use of series of molecular markers, particularly via the SSR method (Naval et al., 2010; Liang et al., 2015), other genetic diversity studies made in persimmon using other markers are ITS (Yonemori et al., 2008a) and AFLPs (Yonemori et al., 2008b). However, most studies focused on PCNA and various female cultivars (Liang et al., 2015). Thus, to the best of our knowledge, there have been no systematic studies on the genetic relationships among androecious persimmons, either interspecific or intraspecific, collected in China or overseas, which has limited the cultivation and use of androecious persimmons, as well as controlled pollination activities.

In this study, we investigated and collected wild persimmons from various provinces in China, and selected 87 individuals including four types of *D. kaki* (60 Chinese germplasm resources originating from various areas consisting of 47 androecious, 2 andromonoecious, 10 PCA monoecious and 1 C-PCNA, and 12 Japanese samples) and related species. Subsequently, 15 highly polymorphic microsatellite markers were used to evaluate genetic diversity among the androecious persimmon resources and to determine the genetic relationships among Chinese persimmons, Japanese persimmons, and closely related species. The results of this study will promote the use of androecious persimmons and provide useful information for male parent selection for the controlled pollination of persimmons.