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# Germplasm conservation, molecular identity and morphological characterization of persimmon (*Diospyros kaki* Thunb.) in the NFGP of China

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ABSTRACT

Germplasm conservation and evaluation has been regarded as one of the most successful methods for preserving and utilizing genetic resources in commercial crops. However, for commercial fruit crop in East Asia, persimmon (*Diospyros kaki*), the understanding of its genetic diversity remains largely unclear and is of great interest both for conservation and breeding purposes. Here, we phenotyped and genotyped 495 persimmon accessions from 4 countries of origin in the National Field Genebank for Persimmon (NFGP) using both morphological and simple sequence repeat (SSR) markers. In the 495 persimmon accessions, a relatively complete morphological profile was built by twenty-five morphological markers, including tree traits, fruit appearance and inner qualities, and postharvest indexes, suggesting highly variable relationships among the indicated germplasms. The molecular profile of twelve SSR loci were used to compare genetic diversity parameters, to characterize genetic differentiation, and to examine factors responsible for the maintenance of genetic diversity and population structure in persimmon. By combining SSR and morphological markers, we successfully identified 10 synonymous groups containing 24 persimmon germplasms in the NFGP. Moreover, we also selected 52 varieties to reveal their genetic and morphological relationships, which showed potential for breeding applications due to their unique molecular and morphological characters. The overall results suggest that the diverse genetic and morphological variations in persimmon have prevented the occurrence of a genetic bottleneck. Our genetic and morphological profiles can be used to accelerate persimmon breeding by identifying potential persimmon parents exhibiting traits of agronomic interest.

## 1. Introduction

Persimmon (*Diospyros kaki* Thunb.), as a typical subtropical and deciduous fruit crop, belongs to the Ebenaceae family, and is believed to originate in southern China with dissemination to Korea and Japan for centuries, and was later cultivated in other countries, such as Brazil, Spain, Turkey, Italy, Israel and New Zealand (Luo and Wang, 2008; Tang et al., 2018; Woolf and Ben-Arie, 2011; Yamada et al., 2012). This fruit crop is rich in vitamins, trace elements, antioxidants and other nutrients that are vital for human health and has been used in various medicinal and chemical industries and for commercial fruit consumption (Giordani et al., 2011; Luo and Wang, 2008; Woolf and Ben-Arie, 2011). Recently, the world's total persimmon production and acreage has increased and expanded rapidly, suggesting that persimmon is becoming an important fruit crop worldwide.

Germplasm conservation has been regarded as one of the most successful methods for preserving the genetic diversity and agronomic traits of endangered plants and commercial crops in a resource house (gene bank/library) (Bhatia, 2015). For fruit crops, these resources are often conserved in an *ex situ* resource house (gene bank/library), which also assists in the *in vitro* testing of germplasms before the release of commercial varieties. The National Field Genebank for Persimmon (NFGP) in China was first built in 1987 in Meixian County, Yangling, China, and was moved to the experimental farms of Northwest A&F University in 2003 (Wang et al., 1997; Yang et al., 2013). Currently, the NFGP contains more than 1000 persimmon resources from different regions in China as well as a relatively high number of varieties from other countries, such as Japan, Korea, the United States, Italy and Israel. Moreover, some related *Diospyros* species, such as *D. lotus*, *D. glaucifolia*, *D. rhombifolia*, *D. cathayensis*, *D. oleifera*, and *D. virginiana*

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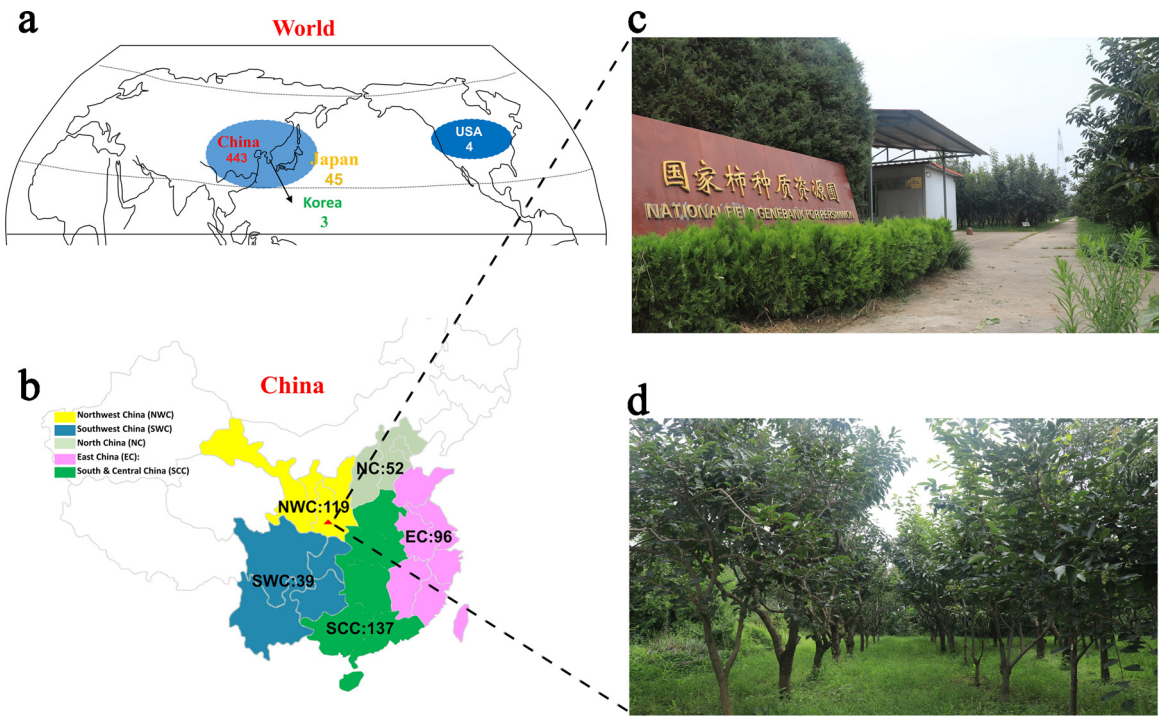


**Fig. 1.** Distributions, locations and descriptions of 495 persimmon cultivars. (a) The 495 samples were collected from China (443), Japan (45), America (4) and Korea (3). (b) The 443 samples were from different regions in China (excluding the South China Sea). 52 samples were collected from North China (NC, light green), 96 from East China (EC, pink), 137 from South & Central China (SCC, dark green), 119 from Northwest China (NWC, yellow) and 39 from Southwest China (SWC, gray). No *D. kaki* were distributed in the blank area. (c, d) The representative photos of the National Field Genebank for Persimmon (NFGP), located in Yangling, Shaanxi, China. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

are also included in the NFGP to be used as rootstocks in most cases. In China, the NFGP is important for germplasm conservation and provides vital support for the investigation, collection, conservation, evaluation, and utilization of current persimmon resources.

The breeding goals for persimmon emphasize improved fruit appearance quality, such as fruit weight, fruit shape, skin color and fruit cracking; enhanced fruit inner quality, such as fruit texture, soluble solids content (SSC), fruit flavor and fruit astringent quality; prolonged fruit shelf and storage life; ameliorated fruit ripening time; increased productivity; selected parthenocarpy and female-flower-only sexuality pattern; and the expanded usage of fruit (consumption as fresh or dried fruit or use in ornamental and industrial applications). In terms of consumption as a type of fruit, persimmon is usually consumed as a fresh fruit, but there can also be relatively high production of dried fruit depending on whether astringency can be naturally reduced on the tree (Luo and Wang, 2008; Woolf and Ben-Arie, 2011). Thus, selection for pollination-constant non-astringent (PCNA) cultivars has always been regarded as attractive and crucial for persimmon breeding because the fruits of such cultivars are edible when still firm and exhibit the natural loss of astringency after maturation on the tree and regardless of artificial de-astringency (Luo and Wang, 2008; Sato and Yamada, 2016; Yesiloglu et al., 2018). However, only a small number of cultivars from Japan and China are of the PCNA type. In addition to the PCNA type, three other types (based on the astringency of the fruit at maturity): pollination-variant astringent (PVA), pollination-variant non-astringent (PVNA), and pollination-constant astringent (PCA) cultivars are classified as non-PCNA cultivars. Thus, the fruit of non-astringent types is attractive and serves as an important breeding aim for persimmon selection.

Molecular and morphological markers play an essential role in the evaluation of persimmon germplasm resources. Several researchers have characterized persimmon cultivars through various molecular markers, including random-amplified polymorphic DNA (RAPD) (Badenes et al., 2003; Luo et al., 1995), sequence-related amplified polymorphism (SRAP) (Guo and Luo, 2006), sequence-specific amplification polymorphism (SSAP) (Du et al., 2009), amplified fragment length polymorphism (AFLP) (Parfitt et al., 2015; Yonemori et al., 2008b), inter-retrotransposon-amplified polymorphism (IRAP) and start codon-targeted (SCoT) (Guan et al., 2020), and simple sequence repeat (SSRs) (Guan et al., 2019b; Liang et al., 2015; Naval et al., 2010; Wang et al., 2018), in relation to the diversity of genetic performance among/within persimmons. In addition, marker-assisted selection (MAS) has been developed in selection of astringent and sexual traits in persimmon as an efficient tool for breeding efficiency (Akagi et al., 2014; Ikegami et al., 2004; Pei et al., 2013; Zhang et al., 2016b, 2016c). In consideration of morphological markers, few reports have mentioned evaluating the characteristics of persimmon germplasms. Remarkably, two reports have provided insights into the morphological attributes of fruit shape and other parameters, which are related to the agronomic traits of breeding potential in persimmon (Maeda et al., 2018; Martínez-Calvo et al., 2013). However, the genetic characterization, especially for morphological markers, for the evaluation of persimmon germplasm in the NFGP is still lacking and of great potential for breeding application in persimmon. Here, we collected 495 varieties of persimmon from different regions in China (443 samples) and related countries (52 varieties), which were *in vitro* conserved in the NFGP. We investigated the genetic characterization with the combination of morphological and SSR markers in these 495 persimmon germplasms. In the present study, we aim to achieve four main goals: (a) to investigate the morphological characteristics and performance of persimmon germplasms in the NFGP; (b) to update the SSR profiles for the evaluation of genetic diversity and relationships in the expanding number of persimmon samples; (c) to identify some misnamed or synonymous germplasm with the combination of morphological and SSR profiles in the NFGP; and (d) to select some potential varieties for the future application of breeding parents for persimmon.

## 2. Materials and methods

### 2.1. Plant material

We studied 495 accessions of cultivated persimmon (*D. kaki* Thunb.) from 4 countries: China (443), Japan (45), Korea (3), and the United States of America (4) (Table S1 and Fig. 1a). Among the 443 germplasms in China, 52 samples were obtained from North China (NC), 96 from East China (EC), 137 from South & Central China (SCC), 119 from Northwest China (NWC) and 39 from Southwest China (SWC) (Table S1 and Fig. 1b). These persimmon germplasm resources have been continuously collected from different regions since 1960 and are conserved in the NFGP located in Yangling City, Shaanxi Province, China (34°17'52.55?N, 108°04'05.58?E; Fig. 1c, d). The morphological traits have been observed for at least two years after grafting on *D. lotus* or *D. kaki* rootstocks grown in the NFGP.

### 2.2. Morphological traits

The morphological traits of the accessions were characterized according to *Descriptor and data standard for persimmon* (Yang and Wang, 2006). Briefly, we firstly investigated tree traits (growth potential and tree sexuality) following the description in this book. Then, we visually observed the fruit traits, which included fruit shape, skin color, grooves on fruit side, fruit indent, cross-concave on fruit apex, fruit transect, fruit apex shape, and sepal-extension direction. Moreover, we measured the average fruit weight, greatest fruit weight, vitamin C content, fruit rust speckle, speckle in the flesh, soluble solids content, soluble tannin content and astringent type. Finally, we investigated postharvest traits, including the texture of soft fruit, fruit juice content, fruit flavor, texture of firm fruit, days over which the fruit remain crisp, and usage of fruit. Samplings were performed by randomly collecting 12 leaves, 12 flowers and 12 fruits from each plant, with three replicates. We independently carried out the evaluation of appearance or tree traits with three continuous observations over at least 2 years. For some fruit inner qualities, we evaluated at least 3 replicates in each year during three continuous years.

### 2.3. DNA extraction and SSR marker

Young leaves were collected from the experimental farm and stored in a -80?°C freezer after liquid nitrogen freezing. Total DNA was extracted according to the cetyltriethylammnonium bromide (CTAB) method with minor modifications, following a previous report (Guan et al., 2020, 2019b). The stable SSR primers were the same as those in our previous report (Guan et al., 2019b) and modified with 3 fluorescent markers at the 5'-end, including FAM (6-carboxy-fluorescein), HEX (hexachloro-fluorescein) and TAMRA (carboxy tetramethyl-rhodamine). The PCR reaction was performed as described in our previous report (Guan et al., 2019b). Capillary electrophoresis (CE) was conducted on an ABI 3130 genetic analyzer (Applied Biosystems, Carlsbad, CA). The amplified loci of SSR markers were analyzed using GeneMapper software (ver 4.0; Applied Biosystems).

### 2.4. Genetic data analysis

The SSR marker data were analyzed as follows (Guan et al., 2019b; Liang et al., 2015; Naval et al., 2010). The NJ (neighbor-joining) tree or UPGMA (unweighted pair group method with arithmetic mean) tree was performed on the DARwin software (http://darwin.cirad.fr/) or NTSYS-PC software (Rolf, 2000). The population genetic structure in different regions of China was examined with the STRUCTURE 2.31 (Pritchard et al., 2000) software. The STRUCTURE HARVESTER software (Earl and von Holdt, 2012) was used to determine the value of the estimated ln probability of the data, ln P(K), and to obtain the best-fit K value for the data. The significant differences between groups and samples were tested by analysis of molecular variance in GenAlEx 6.5 (Peakall and Smouse, 2012). The proportion of genetic diversity components within and among locations was determined by analysis of molecular variance (AMOVA). As we have previously evaluated genetic diversity and relationship of 228 persimmons in the NFGP using the same SSR methods (Guan et al., 2019b), our present study produced the genetic data of SSR markers to (a) update the investigation of genetic relationships among the expanding 495 cultivars; (b) complement the use of morphological markers; (c) verify cases of synonymous germplasms.

### 2.5. Morphological data analysis

Data for all 25 morphological traits were standardized for use in the calculation of the Euclidean distance and genetic similarity among the 495 accessions and were then analyzed with Q-mode clustering and principal component analysis (PCA) with SPSS 17.0 software (SPSS Inc., USA). A dendrogram of the morphological characters was constructed using SAS software version 9.4 (SAS Institute Inc., Cary, NC). In the standardized analysis, morphological traits described in words were coded using the number of encoding levels (Martínez-Calvo et al., 2013; Yang and Wang, 2006), by which dualistic traits were labeled as “0” or “1”, ordered multimodal traits were labeled as “1”, “2”, “3”, and so on (Table S2). The average values of the numeric traits were directly used in the next calculation.

## 3. Results

### 3.1. Persimmon germplasm collection

In the present study, 495 germplasms collected from China (443), Japan (45), America (4) and Korea (3) were used for evaluation of morphological and molecular markers (Table S1 and Fig. 1). The persimmon germplasms in the NFGP exhibited abundant diversity for diverse commercial applications. For example, some male-flower-only resources (such as ‘Male No. 8’) tended to be pollen donors for selecting potential PCNA cultivars (Zhang et al., 2016a). The fruits of most cultivars are regarded as fruits for commercial consumption, such as ‘Fuping Jianshi’ and ‘Mopanshi’, which are used as dry or fresh fruit after artificial deastringency, while some PCNA cultivars, such as ‘Eshi No. 1’ and ‘Taishu’, are used as fresh fruit. In this study, we investigated the diverse utilization and morphological phenotypes of 495 germplasms. Following the use of SSR molecular markers, we characterized the genetic diversity and relationships among these persimmon cultivars. We also verified some synonymous accessions and selected potential varieties for breeding applications based on their phenotypes and genotypes.

### 3.2. Relationship analysis using morphological markers

We first investigated a total of 25 morphological traits of 495 persimmon accessions. The 25 morphological attributes included 2 tree traits (growth potential and tree gender), 10 fruit appearance traits (fruit shape, grooves on fruit side, fruit indent, cross-concave fruit apex, fruit apex shape, fruit transect, fruit skin color, average fruit weight, largest fruit weight, sepal-extension direction), 11 indexes of fruit inner quality (fruit rust speckle, speckle in the flesh, texture of soft fruit, texture of firm fruit, fruit juice content, fruit flavor, soluble solids content, content of vitamin C, content of soluble tannins, and astringent type), one index for fruit storage (days over which fruit maintain crispness) and usage of fruit. Based on a previous description standard for persimmon germplasms (Yang and Wang, 2006), the morphological profile of each sample was the combination of its level of expression for each of the 25 traits that were evaluated (Table S1). Some morphological traits that are crucial for appearance and inner quality were summarized according to their categorizations (Table 1 and Fig. 2). For

**Table 1**
Classification of various morphological characteristics among the 495 samples.

| | | | | | |
|---|---|---|---|---|---|
| **Grooves on fruit side** | **None** | **No obvious** | **Shallow** | **Deep** | |
| **Numbers** | 263 | 38 | 59 | 44 | |
| **Crosss-concave on fruit apex** | **None** | **No obvious** | **Shallow** | **Deep** | |
| **Numbers** | 84 | 146 | 152 | 23 | |
| **Fruit indent** | **Absent** | **Present** | | | |
| **Numbers** | 280 | 122 | | | |
| **Fruit apex shape** | **Sunken** | **Flat** | **Round** | **Embossing** | |
| **Numbers** | 41 | 154 | 111 | 98 | |
| **sepal-extension direction** | **Sag** | **Flat** | **Incline** | **Upright** | |
| **Numbers** | 32 | 138 | 156 | 12 | |
| **Fruit transect** | **Square-round** | **Round** | **Square** | **Multi-edge** | |
| **Numbers** | 150 | 167 | 66 | 21 | |
| **Fruit rust speckle** | **None** | **Strip shape** | **Band shape** | **Patch shape** | |
| **Numbers** | 264 | 76 | 36 | 26 | |
| **Fruit shape** | **Cordate** | **Flat-square** | **Flat-rotundity** | **Low-flat** | **Others** |
| **Numbers** | 126 | 67 | 59 | 53 | 102 |
| **Fruit skin color** | **Orange-red** | **Orange-yellow** | **Dark-orange** | **Deep-orange** | **Others** |
| **Numbers** | 161 | 158 | 57 | 20 | 10 |
| **Astringent type** | **PCA** | **PCNA** | **PVNA** | **PVA** | |
| **Numbers** | 454 | 28 | 9 | 4 | |
| **Usage of fruit** | **Firm for fresh** | **Soft for fresh** | **Processing** | **Other** | |
| **Numbers** | 79 | 215 | 49 | 17 | |
| **Texture of soft fruit** | **Slight glutinous** | **Glutinous** | **Liquid** | **Slight soft-sponge** | **Soft-sponge** |
| **Numbers** | 41 | 187 | 111 | 8 | 38 |
| **Fruit juice content** | **Much more** | **More** | **Intermediate** | **Very tiny** | **Tiny** |
| **Numbers** | 34 | 152 | 79 | 11 | 110 |
| **Flavor of fruit** | **Insipid** | **Slight insipid** | **Slight sweet** | **Sweet** | **Strong sweet** |
| **Numbers** | 8 | 28 | 83 | 206 | 61 |
| **Texture of firm fruit** | **Soft gentle** | **Slight tender** | **Tender** | **Slight firm dense** | **Firm and dense** |
| **Numbers** | 66 | 15 | 97 | 9 | 67 |
| **Average fruit weight** | **Very big** | **Big** | **Middle** | **Small** | **Very small** |
| **Numbers** | 30 | 104 | 199 | 64 | 5 |
| **largest fruit weight** | **Very big** | **Big** | **Middle** | **Small** | **Very small** |
| **Numbers** | 107 | 103 | 153 | 32 | 2 |
| **Soluble solid content** | **Very high** | **High** | **Middle** | **Low** | **Very low** |
| **Numbers** | 14 | 51 | 152 | 124 | 17 |
| **Content of Vitamin C** | **Very high** | **High** | **Middle** | **Low** | **Very low** |
| **Numbers** | 23 | 71 | 172 | 43 | 10 |
| **Content of solible tannim** | **Very high** | **High** | **Middle** | **Low** | **Very low** |
| **Numbers** | 1 | 57 | 200 | 41 | 22 |
| **Days of hold fruit crisp** | **No resistence to storage** | **Storage resistence** | **High storage resistence** | | |
| **Numbers** | 88 | 214 | 23 | | |
| **Growth potential** | **Strong** | **Intermediate** | **Weak** | | |
| **Numbers** | 174 | 124 | 33 | | |
| **Flower sex** | **Only female** | **Only male** | **Monoecious** | **Male, female hermaphrodite flowers** | |
| **Numbers** | 373 | 7 | 20 | 16 | |
| **Speckle in the flesh** | **None** | **Few** | **Middle** | **More** | |
| **Numbers** | 272 | 85 | 19 | 19 | |
| **Chromoso me ploidy** | **6X=90** | **9X=135** | **2X=30** | | |
| **Numbers** | 397 | 1 | 1 | | |

example, fruit shape could be classified into 8 types, *i.e.*, low-flat, flat-square, flat-rotund, globular, elliptical, cordate, long-heart and Chinese-millstone-like (Fig. 2a), while 8 types of fruit peel colors were identified in these persimmons, namely, green-yellow, orange-yellow, dark orange, deep orange, orange-red, bright red, dark red and black (only one, 'Heishi') (Fig. 2b). We then screened some unique germplasms based their morphological traits, such as 'Heishi', displaying a black fruit peel color, 'Mopanshi' and 'Mancheng Lianhuashi', with a Chinese-millstone-like fruit shape, 'Yuncheng Tailihong', with an orange-red color and good taste, 'Fuping Jianshi', with a relatively heavy

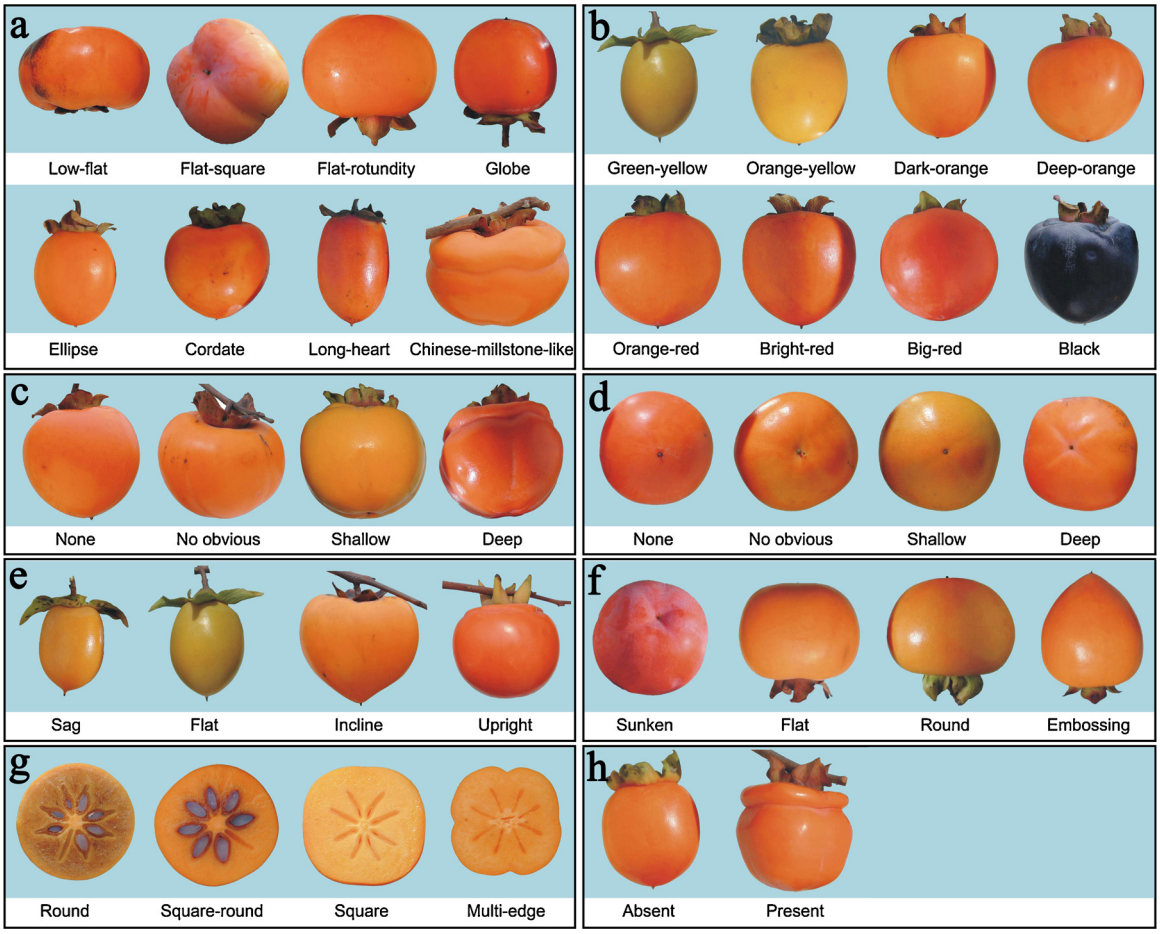


**Fig. 2.** The classification standard for various morphological characteristics. (a) Fruit shape, (b) Fruit skin color, (c) Grooves on fruit side, (d) Cross-concave on fruit apex, (e) Sepal extending direction, (f) Fruit apex shape, (g) Fruit transect, (h) Fruit indent. Few photos were rearranged from our previous report (Guan et al. 2019b).

fruit weight (mainly used as dry fruit), and 'Dabaoshi', with the largest fruit weight (395.33?g on average and 469.8?g maximum weight). For the fruit astringent trait, most cultivars are of the PCA type (423), some are PCNA (31), and few are PVA (6) and PVNA (8). In terms of flower sexuality, most are of the female-flower-only type, while 7 germplasms could bear male flowers only ('Male No. 8'), 20 germplasms were monoecious (bearing both male and female flowers), and 16 germplasms exhibited male, female and hermaphrodite flowers on one tree.

To further investigate the relationships of 495 accessions based on the 25 morphological traits, we assigned the morphological profiles of the samples into digitized values for visualization analysis. The assignment of the traits was based on the description standards (Table S2), as previously reported (Martínez-Calvo et al., 2013; Yang and Wang, 2006). UPGMA cluster analysis was performed for multivariate analysis (Fig. 3). The consensus dendrogram was obtained after bootstrapping with 1000 repeats and 35% replacement. The coefficient of cophenetic correlation was 0.81 according to the Mantel analysis (p? = ?0.02, with 100 permutations), which indicates consistency between the generated dendrogram and the matrix of distances. The dendrogram obtained with 495 samples based on 25 quantitative and qualitative morphologic characteristics separated the persimmon genotypes into four groups (P1, P2, P3 and P4) at a similarity index value of 0.27 (Fig. 3). Cluster P1 comprised 195 different morphological accessions plus most of the samples from the USA and Korea. Cluster P2 consisted of only one sample, 'Huixian Xiaoshi'. The P3 group had the largest number of *Diospyros* genotypes (214) and exhibited PCNA characteristics. Group P4 contained the other 85 different morphological accessions. Most male persimmons were clustered into the P4 cluster, representing important germplasms for breeding new varieties. The most important trait for the classification of persimmon is the type of fruit astringency (PVNA, PCA, PVA or PCNA) because postharvest treatment for persimmon consumption depends on this qualitative variable. The dendrogram showed that the accessions of the Chinese PCNA (C-PCNA) and Japanese PCNA (J-PCNA) types were mostly clustered into the P1 and P3 groups, respectively. Together, these data revealed that the 495 persimmon germplasms showed highly varying relationships among all samples on the basis of the morphological markers.

### 3.3. Relationship and structure analysis using SSR markers

Next, we updated to investigate the genetic diversity and relationship of 495 persimmon samples genotyped with 12 SSR markers as previously reported (Guan et al., 2019b). Our previous report have evaluated the genetic diversity and relationship of 228 persimmon accessions based on SSR marker (Guan et al., 2019b), which were included in the 495 persimmon accessions in the NFGP. The genotyping analysis of the SSR data and the subsequent evaluation of the genetic relationships and structure among these accessions allowed the assessment of the accuracy of the 495 persimmon germplasms. We first constructed an unrooted neighbor-joining (NJ) tree using DARwin software (Fig. 4). In this tree, 495 samples were divided into four clusters (C1, C2, C3 and C4), which are indicated in orange, green, purple, and blue, respectively. The C1 group was mainly composed of NWC (89), SCC (68), and Japan (9) samples; the C2 cluster mainly consisted of Japan (19), EC (21) and SCC (39) samples; the C3 group was mainly composed of EC (50) and SCC (42) samples; the C4 cluster mainly consisted of SWC and SCC samples. Next, we assessed the

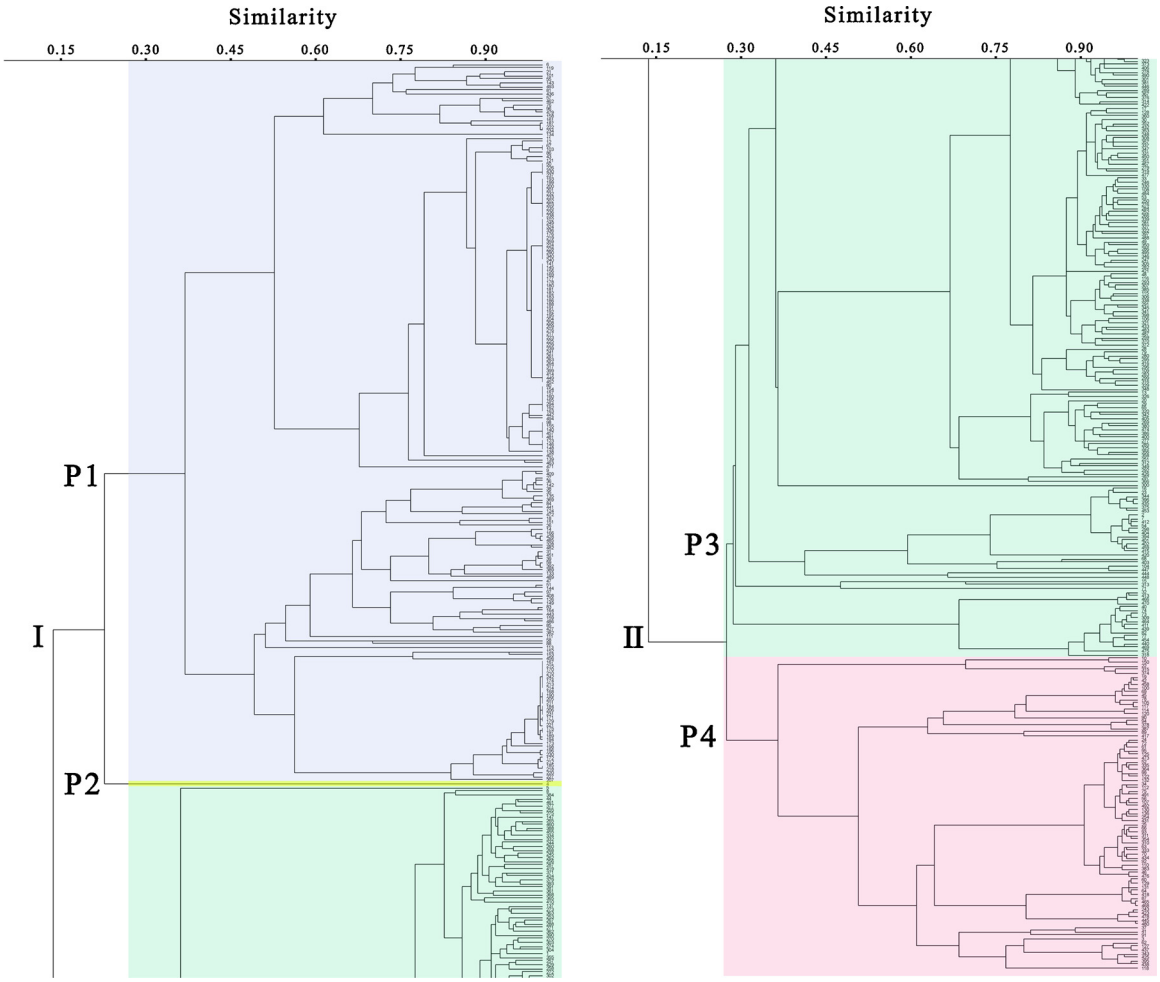


**Fig. 3.** Cluster dendrogram using the morphological characteristics of 495 samples. Based on the dendrogram, the 495 samples were divided into two major groups (group I and II) and further grouped into four subgroups (group I contains subgroups P1 and P2; subgroups P3 and P4 are included in group II).

genetic structure of the 495 samples using STRUCTURE software. The K was tested from 2 to 9 with ten replicates (Fig. S1), and K? = ?4 provided the most congruent arrangement of cultivars (delta K value was highest) (Fig. 5a). Based on the structure analysis, four groups were identified: Pop1, Pop2, Pop3 and Pop4 (Fig. 5b). The Pop1 group comprised 101 samples, and the samples from the EC and SCC regions accounted for 78% of all the samples. Pop2 contained 88 samples, which were mostly from Japan and the SCC region. Pop3 included the largest number of samples (208), most of which came from the EC and SCC regions. The remaining 98 samples were included in the Pop4 cluster, which were mainly from the SWC region. The samples from the USA and Korea were clustered into the Pop3 and Pop2 groups, respectively. We then explored the genetic distance (GD) and genetic identity (GI) to evaluate the diversity associations among the five regions. In our study, the GI among the five regions ranged from 0.000656 to 0.001376, while the GD was between 0.998242 and 0.999345 (Table S3). In addition, the AMOVA of the data from the 443 samples from the respective five regions revealed that a large proportion of the genetic variation (98%) existed among individuals within the regions, and only 2% existed among the regions (Table S4). The genetic differentiation among the regions was significant (p? = ?0.001, Fst? = ?0.024). Consistently, our updated SSR profile of 495 persimmons show similar results to those in a previous report (Guan et al., 2019b), suggesting that the persimmons originating from the five regions frequently underwent genetic exchange.

### 3.4. Identification of synonymous germplasm in the NFGP

Further, we performed the authentication of the 443 cultivars from different regions in China by comparing their morphological traits and profiles of SSR markers. Based on the SSR data, we first compared the genetic similarity coefficient and genetic diversity index values among these 443 samples of Chinese origin and then combined them with the morphological trait values to identify synonymous germplasms. A total of 24 samples were identified that belonged to 10 synonymous groups (Table 2). Among these 10 synonymous groups, 9 were newly identified (S1–S9), while one (S10) was consistent with one identified in our previous report (Guan et al., 2019b). The groups S1, S2, S3, S4, S5, S7 and S8 contained 2 samples, while S6 and S9 contained 3 samples. The last group, S10, included 4 samples, of which three of them ('Qianxian Huoshi', 'Huxian Dashi', and 'Binxian Jiandingshi') were previously reported to be synonyms (Guan et al., 2019b), and one of them, 'Meixianqizhen Xiaoniuxinshi', was newly identified to be synonymous. The genetic similarity coefficient of the synonymous samples ranged from 0.86 to 0.99. Most of the samples from the synonymous group were from the same region and had similar morphological phenotypes and a high genetic similarity coefficient. For example, the similarity coefficient for S1 was 0.91, which exhibits the common traits that the growth potential is intermediate and that the fruits show a flat-rotund shape, a fruit indent and no obvious cross-concave fruit apex. The texture of firm fruit is firm and dense, and the usage of the fruit is for processing or consumption of fresh fruit (Table 2).

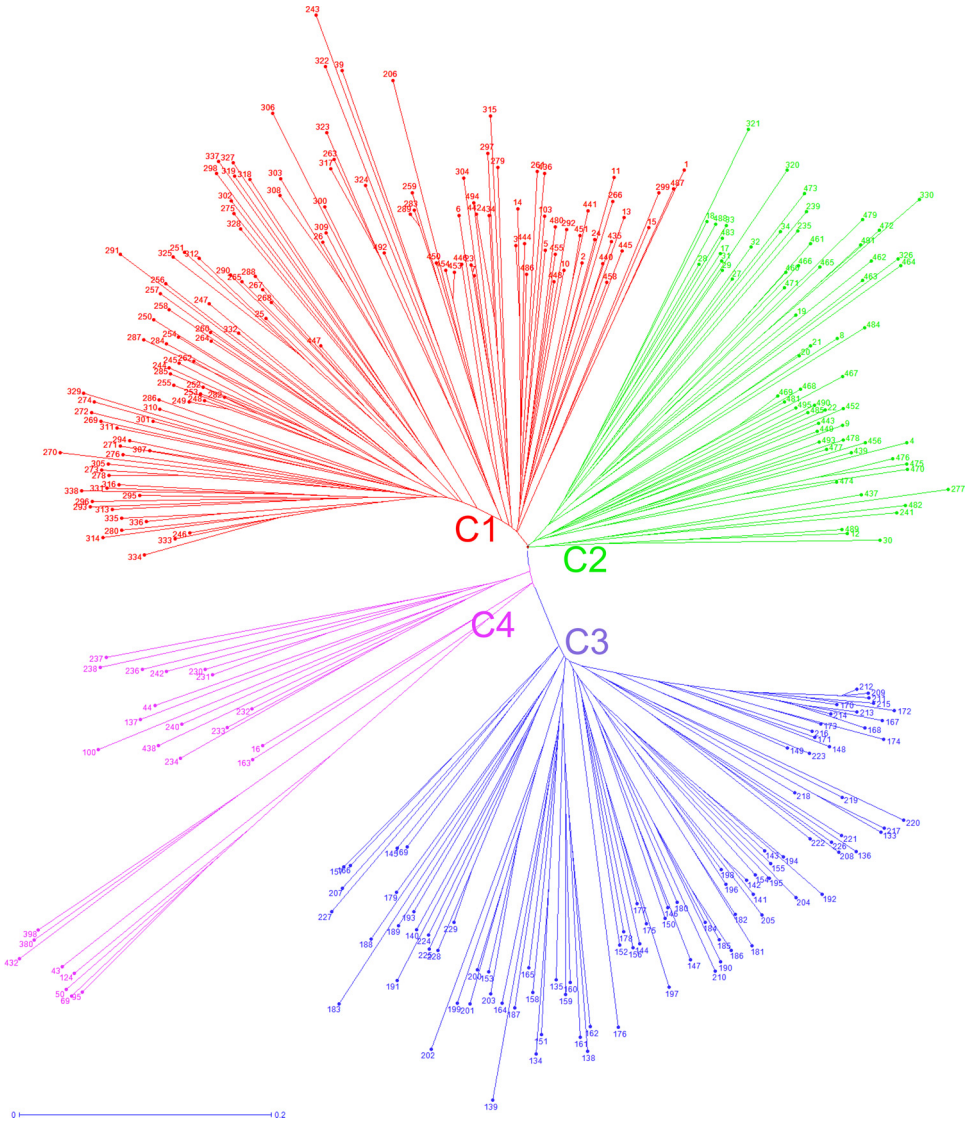


**Fig. 4.** Unrooted neighbor-joining tree of 495 *D. kaki* samples using SSR markers. A dendrogram was obtained from the UPGMA (unweighted pair group method based on arithmetic mean) using DARwin software. A total of 495 samples were divided into four clusters (C1, C2, C3 and C4), which are indicated in orange, green, purple, and blue, respectively. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

### 3.5. Selection of germplasms for potential breeding

To potentially promote the breeding program for persimmon, with the combination of morphological characteristics and SSR data, we identified 52 representative varieties (Table 3) that have excellent characteristics as breeding parents and can accelerate the breeding of new varieties. First, cluster analysis based on SSR data divided the 52 representative germplasms into three groups (G1, G2, and G3) (Fig. 6a). The G1 cluster comprised 27 samples, which were mainly from the NWC, NC, and Japan. The G2 group mainly consisted of 10 genotypes, most of which came from the SWC region. The remaining samples were clustered into the G3 group, which mainly came from the EC and SCC regions. We also performed PCA based on the SSR markers, which indicated the presence of relationships between the SWC region and the two regions of EC and SCC, while the samples from the NWC, NC and EC and SCC regions also had a close relationship (Fig. S2). Next, the cluster analysis based on morphological traits resulted in the same three groups (Fig. 6b). Together, we selected 52 germplasms that may have breeding potential.

## 4. Discussion

The evaluation of germplasm resources for cash crops and endangered plants represents a continuously promising new territory for breeding and conservation research. In regard to persimmon (*D. kaki*), one of the most important cultivated species of the *Diospyros* genus and believed to have originated in China (Luo and Wang, 2008; Tang et al., 2018; Yonemori et al., 2008a, 1998), there are still some barriers related to its genetic heritage due to its high levels of polyploidy and heterozygosity. Previous studies using several molecular markers, the chloroplast genome and comparative transcriptomic sequencing methods have revealed that *D. kaki* displays rich genetic variation within species and among related species (Guan et al., 2019a, 2019b; Li et al., 2018). Currently, in the NFGP of China, we are conserving more than 1000 persimmon germplasms of *D. kaki* and those of some variant and closely related species. These persimmon resources in the NFGP will contribute to illustrating the origination of *D. kaki* and make use of genetic relationships, breeding applications and cultivar selection for persimmon in China. In the present study, we combined morphological characteristics and molecular markers for evaluation of persimmon germplasms in the NFGP. Several molecular markers, especially SSR

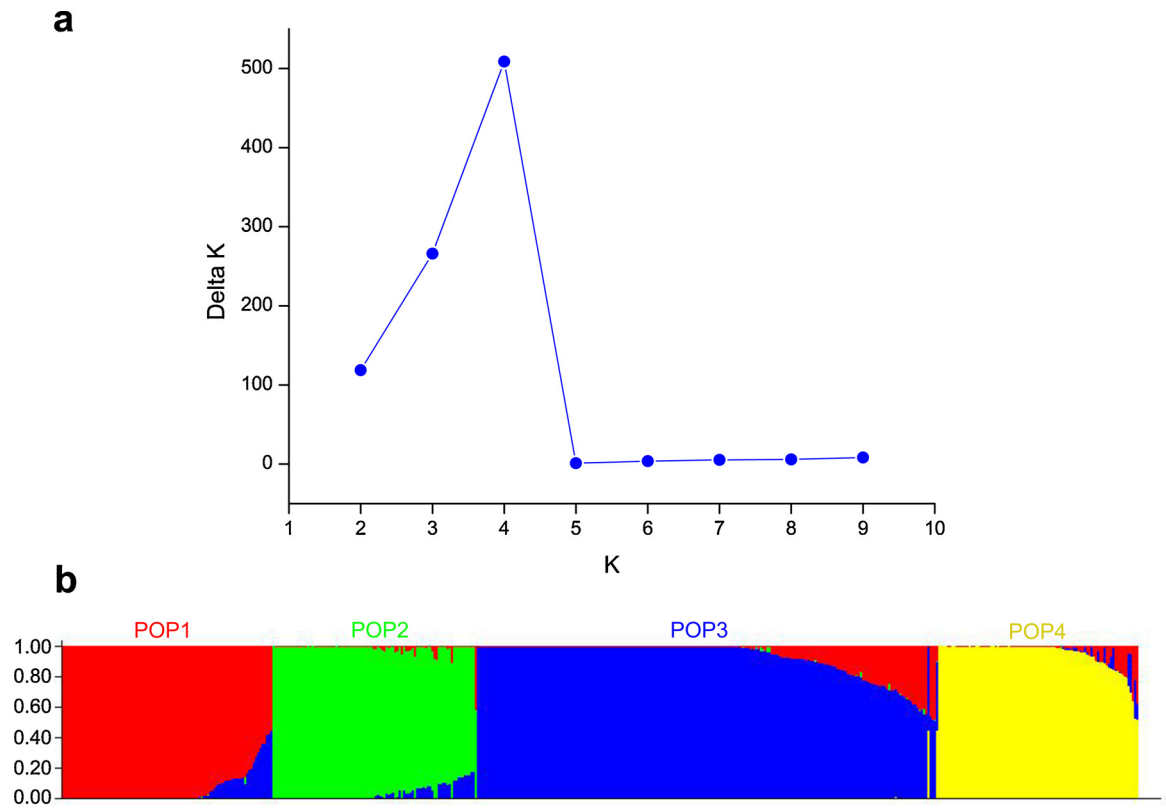


**Fig. 5.** Genetic structure of 495 samples using SSR markers. (a) The calculation of delta K *via* the Structure Harvester software. K? = ?4 values provided the most congruent arrangement of cultivars. The horizontal and vertical coordinates indicate the K value and delta K value, respectively. (b) The analysis of genetic structure showed that the 495 samples could be divided into four main clusters (POP1, POP2, POP3 and POP4) when K? = ?4.

**Table 2**
Identification of synonyms based on SSR markers and morphological traits.

| Group | Code | Accession name | Region | Chromosome | Similarity coefficient | Morphological traits |
|---|---|---|---|---|---|---|
| S1 | 245 | Xiangfen Bayuehong | NC | 6X? = ?90 | 0.91 | The growth potential is intermediate; fruit shows the flat-rotund shape, fruit indent and no obvious cross-concave fruit apex. Texture of firm fruit is firm and dense; usage of fruit is for processing or the consumption of fresh fruit |
| | 262 | Wenxi Pingdingshi | NC | 6X? = ?90 | | |
| S2 | 253 | Baishi | NC | 6X? = ?90 | 0.95 | The growth potential is weak or intermediate; fruit shows cordate shape and shallow cross-concave fruit apex. Texture of firm fruit is firm and dense; usage of soft or firm fruit is for fresh consumption |
| | 282 | Meipishi | NWC | 6X? = ?90 | | |
| S3 | 322 | Jinpingshi | EC | 6X? = ?90 | 0.92 | The growth potential is intermediate; fruit shows elliptical shape, orange-yellow skin, no indent and no obvious cross-concave fruit apex. Texture of firm fruit is tender; usage of soft fruit is for fresh consumption |
| | 363 | Cangshan Niuxinshi | EC | 6X? = ?90 | | |
| S4 | 331 | Ganmaokui | NWC | 6X? = ?90 | 0.96 | The growth potential is strong; fruit shows long-heart shape, dark-orange skin, no indent and no obvious cross-concave fruit apex. Texture of firm fruit is tender; usage of fruit is for the processing of soft fruit or fresh consumption |
| | 338 | Zhouqu Niuxinshi | NWC | 6X? = ?90 | | |
| S5 | 335 | Dangshan Niuxinshi | EC | 6X? = ?90 | 0.86 | The growth potential is intermediate; fruit shows the flat-rotund shape, dark-orange skin, round transect, an indent and no obvious cross-concave fruit apex. Usage of soft fruit is for fresh consumption |
| | 333 | Dangshan Ehuangshi | EC | 6X? = ?90 | | |
| S6 | 362 | Heze Bayuehuang | EC | 6X? = ?90 | 362/360? = ?0.91; | The growth potential is intermediate; fruit shows flat-rotund shape, deep-orange fruit skin, fruit indent and no obvious cross-concave fruit apex. Texture of firm fruit is tender, usage of fruit is for processing |
| | 360 | Daercao | EC | 6X? = ?90 | 362/361? = ?0.89 | |
| | 361 | Xiaoercao | EC | 6X? = ?90 | | |
| S7 | 373 | Yuanguanhong | SCC | 6X? = ?90 | 0.89 | The growth potential is weak; fruit shows cordate shape, red fruit skin, no fruit indent and no obvious cross-concave fruit apex. Texture of firm fruit is firm and dense, usage of soft fruit is for fresh consumption |
| | 440 | Yanguohong | SCC | 6X? = ?90 | | |
| S8 | 379 | Huaxian Dahongshi | SCC | 6X? = ?90 | 0.91 | The growth potential is intermediate; fruit shows flat-rotund shape, deep-orange fruit skin, fruit indent and no obvious cross-concave fruit apex. Texture of firm fruit is tender, usage of fruit is for processing |
| | 472 | Guangzhou Dahongshi | SCC | 6X? = ?90 | | |
| S9 | 470 | Huangbian Xiaojixin | SCC | 6X? = ?90 | 470/475? = ?0.95; | The growth potential is wreak or intermediate; fruit shows elliptical or cordate shape, and cross-concave fruit apex. Texture of soft fruit is liquid, usage of soft fruit is for fresh consumption |
| | 475 | Guangzhou Jixinshi | SCC | 6X? = ?90 | 470/476? = ?0.94 | |
| | 476 | Guangzhou Guihuashi | SCC | 6X? = ?90 | | |
| S10 | 450 | Qianxian Huoshi | NWC | 6X? = ?90 | 450/454? = ?0.96; | The growth potential is strong; fruit shows cordate shape, and shallow cross-concave fruit apex. Texture of firm fruit is firm and dense, texture of soft fruit is glutinous, usage of soft or firm fruit is for fresh consumption |
| | 454 | Huxian Dashi | NWC | 6X? = ?90 | 450/453? = ?0.94; | |
| | 453 | Binxian Jiandingshi | NWC | 6X? = ?90 | 450/206? = ?0.89 | |
| | 206 | Meixianqizhen Xiaoniuxinshi | NWC | 6X? = ?90 | | |

**Table 3**
Selection of unique germplasms for breeding potential in persimmon.

| Numbers | Accession name | Fruit shape | Fruit skin color | Grooves on fruit side | Fruit apex shape | Fruit transect | Speckle in the flesh | Fruit juice content | Flavor of fruit | Texture of firm fruit | Astringent type | Usage of fruit |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | Zhengyang Dongshi | Cordate | Orange-yellow | None | Round | Round | None | More | Slight sweet | Soft gentle | PCA | Processing, soft for fresh |
| 3 | Dengfeng Jiaodingshi | Cordate | Orange-yellow | None | Embossing | Square | Few | More | Sweet | | PCA | Soft for fresh |
| 30 | Zhoushan Changshi | Ellispe | Orange-yellow | None | Round | Round | None | Intermediate | Sweet | Firm and dense | PCA | Soft for fresh |
| 39 | Jousaiwasefuyuu | Low-flat | Orange-red | None | Round | Round | None | Intermediate | Sweet | Tender | PCNA | Firm for fresh |
| 48 | Youhou | Low-flat | Orange-red | None | Flat | Round | None | Intermediate | Sweet | Tender | PCNA | Firm for fresh |
| 79 | Mancheng Lianhuashi | Chinese-millstone-like | Dark-orange | None | Round | Square-round | None | Much more | Sweet | Slight tender | PCA | Soft for fresh |
| 94 | Caoxian Zaosiban | Ellispe | Dark-orange | None | Embossing | Square-round | Few | More | Sweet | Slight firm dense | PCA | |
| 107 | Haian Xiaofangshi | Flat-rotundity | Orange-yellow | Deep | Sunken | Multi-edge | None | Tiny | Slight sweet | | PCA | Soft for fresh |
| 115 | Taishu | Low-flat | Dark-orange | None | Flat | Square-round | None | Tiny | Sweet | Tender | PCNA | Firm for fresh |
| 116 | Tianbaogai | Low-flat | Dark-orange | None | Flat | Round | None | Much more | Sweet | Soft gentle | PCA | Firm for fresh |
| 125 | Wuhetuoshi | Chinese-millstone-like | Dark-orange | Deep | Flat | Square | Few | More | Slight sweet | | PCA | Soft for fresh |
| 126 | Sandaijinshi | Flat-square | Orange-yellow | Deep | Flat | Square-round | None | Intermediate | Strong sweet | | PCA | Processing, soft for fresh |
| 135 | Xiaoguotianshi | Globe | Dark-orange | None | Round | Round | Few | Tiny | Sweet | Tender | PCA | Other |
| 167 | Shibingshi | Low-flat | Orange-red | Shallow | Flat | Square | None | Intermediate | Sweet | | PCA | |
| 246 | Mexinshi | Flat-square | Orange-yellow | None | Flat | Round | None | More | Slight sweet | Firm and dense | PCA | Soft for fresh |
| 247 | Daojiushi | Cordate | Orange-yellow | None | Embossing | Square | Few | More | Slight sweet | Firm and dense | PCA | Soft for fresh |
| 295 | Dahuoguantoushi | Flat-square | Orange-yellow | Shallow | Embossing | Square | None | More | Sweet | Firm and dense | PCA | Soft for fresh |
| 296 | Chutoushi | Long-heart | Orange-yellow | Shallow | Embossing | Round | None | More | Sweet | Tender | PCA | Soft, firm for fresh |
| 299 | Xiangyang Niuxinshi | Cordate | Deep-orange | None | Round | Round | Few | Tiny | Slight sweet | Tender | PCA | Soft for fresh |
| 300 | Qiyuezao | Flat-square | Orange-yellow | None | Embossing | Square | Few | Intermediate | Sweet | Soft gentle | PCA | Soft for fresh |
| 302 | Zhaijiahong | Flat-rotundity | Dark-orange | Shallow | Sunken | Square | None | Very tiny | Slight sweet | Tender | PCA | Soft for fresh |
| 305 | Zhaotianhong | Flat-rotundity | Deep-orange | Shallow | Flat | Square | None | Tiny | Slight sweet | Firm and dense | PCA | Soft, firm for fresh |
| 308 | Laopige | Flat-rotundity | Orange-red | Deep | Sunken | Round | None | Tiny | Slight insipid | Tender | PCA | Soft for fresh |
| 313 | Luoyang Fangtianshengshi | Flat-rotundity | Orange-yellow | Shallow | Flat | Square-round | None | Tiny | Sweet | Tender | PCA | Soft for fresh |
| 314 | Gongcheng Yueshenshi | Low-flat | Deep-orange | Shallow | Sunken | Round | None | More | Sweet | Firm and dense | PCA | Processing |
| 321 | Fuyuu | Low-flat | Bright-red | No obvious | Round | Round | Few | More | Sweet | Tender | PCNA | Firm for fresh |
| 322 | Jinpingshi | Ellispe | Orange-yellow | None | Round | Round | None | Much more | Sweet | Tender | PCA | Processing |
| 330 | Zhaoan Yuanxiaoshi | Flat-rotundity | Green-yellow | None | Flat | Square-round | None | Tiny | Sweet | Slight firm dense | PCA | Processing |
| 331 | Ganmaokui | Cordate | Orange-red | None | Embossing | Square-round | Few | Tiny | Sweet | Tender | PCA | Processing, soft for fresh |
| 332 | Heixinshi | Flat-rotundity | Orange-red | No obvious | Flat | Round | More | Tiny | Sweet | Tender | PCA | Soft for fresh |
| 334 | Denglongshi | Long-heart | Bright-red | Deep | Flat | Square | None | More | Sweet | Tender | PCA | Soft for fresh |
| 341 | Huixian Jianjianshi | Long-heart | Orange-red | None | Round | Round | None | Tiny | Sweet | Tender | PCA | Processing, soft for fresh |

(*continued on next page*)

**Table 3** (*continued*)

| Numbers | Accession name | Fruit shape | Fruit skin color | Grooves on fruit side | Fruit apex shape | Fruit transect | Speckle in the flesh | Fruit juice content | Flavor of fruit | Texture of firm fruit | Astringent type | Usage of fruit |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 345 | Tongguan Lianhuashi | Cordate | Orange-yellow | Deep | Flat | Round | None | Tiny | Slight sweet | Tender | PCA | Soft for fresh |
| 351 | Matian Fangshi | Cordate | Orange-red | Shallow | Round | Square-round | Few | More | Slight insipid | Tender | PCA | Firm for fresh |
| 353 | Heishi | Chinese-millstone-like | Dark | None | Flat | Square-round | None | Tiny | Strong sweet | Slight tender | PCA | Soft for fresh |
| 355 | Xiaoyi Niuxinshi | Cordate | Orange-red | No obvious | Embossing | Square-round | More | Tiny | Slight sweet | Soft gentle | PCA | Processing |
| 362 | Heze Bayuehuang | Flat-rotundity | Orange-yellow | None | Flat | Round | Few | Tiny | Slight insipid | Slight tender | PCA | Soft for fresh |
| 367 | Xingtai Taishi | Cordate | Orange-red | Deep | Flat | Square-round | None | Tiny | Strong sweet | Tender | PCA | Soft for fresh |
| 372 | Zenjimaru | Globe | Orange-red | None | Round | Round | More | More | Sweet | Firm and dense | PVNA | Firm for fresh |
| 379 | Huaxian Dahongshi | Ellispe | Orange-red | None | Embossing | Round | Few | Tiny | Sweet | Tender | PCA | Soft for fresh |
| 381 | Chaoyang Yuanxiaoshi | Flat-rotundity | Green-yellow | None | Flat | Round | None | Tiny | Slight insipid | Slight firm dense | PCA | Processing |
| 384 | Rongxian Jingshi | Ellispe | Orange-red | None | Round | Round | None | Tiny | Slight insipid | Firm and dense | PCA | Soft for fresh |
| 386 | Luotian Tianshi | Low-flat | Dark-orange | None | Round | Round | Few | Tiny | Sweet | Firm and dense | PCNA | Firm for fresh |
| 393 | Biantashi | Low-flat | Orange-yellow | None | Flat | Round | None | Much more | Sweet | Tender | PCA | Soft for fresh |
| 396 | Xunyang Banyetian | Cordate | Orange-red | None | Embossing | Square-round | None | Tiny | Sweet | Firm and dense | PCA | Soft for fresh |
| 416 | Hangzhou Gaofangshi | Flat-square | Orange-red | Shallow | Flat | Square-round | Few | Much more | Strong sweet | Soft gentle | PCA | Soft, firm for fresh |
| 419 | Shagu 1 | Chinese-millstone-like | Orange-red | None | Flat | Square | None | More | Strong sweet | Soft gentle | PCA | Soft for fresh |
| 422 | Hiratanenashi | Low-flat | Orange-red | None | Flat | Square | None | Much more | Slight sweet | Tender | PVA | Soft, firm for fresh |
| 424 | Wenxian Jianjianshi | Ellispe | Orange-yellow | No obvious | Embossing | Round | None | Very tiny | Insipid | Firm and dense | PCA | Processing |
| 450 | Qianxian Huoshi | Cordate | Orange-red | Shallow | Embossing | Square | None | Intermediate | Strong sweet | Firm and dense | PCA | Soft, firm for fresh |
| 469 | Baoshan Shuishi | Cordate | Orange-yellow | None | Round | Square-round | None | More | Sweet | Soft gentle | PCA | Soft for fresh |
| 495 | Zhengyang Baheshi | Cordate | Deep-orange | None | Embossing | Round | None | Tiny | Sweet | Soft gentle | PCA | Soft, firm for fresh |

Note: represent no data.

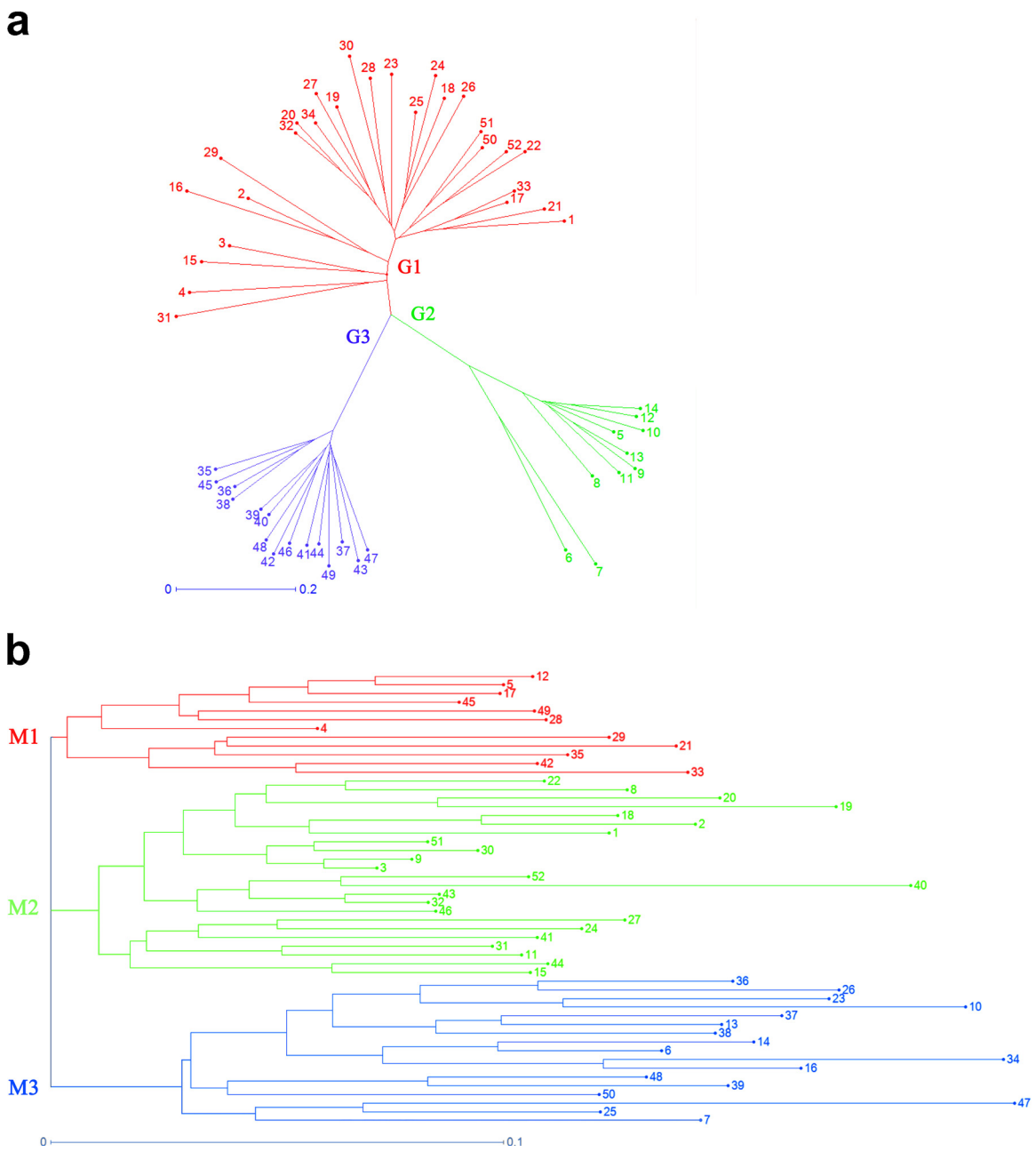


**Fig. 6.** Dendrograms of 52 representative germplasms with breeding potential. (a) Cluster analysis of SSR markers. (b) Cluster analysis of morphological characteristics.

markers, have been reported in persimmon (Guan et al., 2019b; Liang et al., 2015; Naval et al., 2010; Wang et al., 2018). We chose to investigate 495 germplasms of *D. kaki* conserved in the NFGP that tend to be of continuous agronomic interest to broaden our understanding of genetic and morphological relationships in persimmon, as an important cash crop. Very recently, our other study reported the genetic relationships among 228 persimmon germplasms in the NFGP (Guan et al., 2019b), which were verified by SSR data. Our tasks are not only to identify and conserve persimmon germplasms but also to characterize their variability and attributes. For this reason, we combined the morphological markers and SSR markers for identification purposes to provide optimized markers to further investigate the relationships and variability among the germplasms in the NFGP.

In this study, we genotyped and phenotyped both domestic and abroad persimmon germplasms and evaluated their relationships using efficient reference SSR markers and morphological traits. The analysis of GD and GI were also carried out to evaluate the diversity associations among different clusters (Table S5). Moreover, the AMOVA of the data from the 495 samples revealed that a large proportion of the genetic variation (96%) existed among individuals within four clusters, and only 4% existed among clusters (Fig. 4; Table S6). The genetic profiles of 495 persimmon germplasms were constructed with two lines of experiments involving 25 morphological traits that showed important agronomic value and 12 efficient SSR loci. Together with the two types of markers for evaluation in relatively larger germplasms, our study presents a profile of the relationships among persimmon cultivars. Moreover, our SSR and morphological marker profile data have allowed us to reveal several unique features of the relationships in a relatively large population of persimmons: (1) we report 10 synonymous groups including 24 germplasms, and (2) 52 varieties with potential for breeding applications were selected (main selection criteria for 52 representative varieties: specific or excellent fruit characters; covering 25

trait types; higher genetic diversity representing 495 copies of persimmon resources to a greater extent). In fact, through decades of hard work, we have only conducted a more detailed evaluation for more than 500 persimmons from 1000 resources preserved in NFGP of China. The evaluation of germplasm resources will be a continuously interesting topic in further investigations regarding persimmon.

Thanks to the long-term historical cultivation and widespread distribution of persimmon, several previous reports have showed abundant genetic diversity and morphological trait variation in persimmon germplasms in China (Du et al., 2009; Guan et al., 2019b; Guo et al., 2006; Liang et al., 2015; Luo et al., 1995; Wang et al., 2018), leading to synonyms. For management studies, the identification of synonyms is an essential tool for persimmon germplasm management. In this study, combination of SSR and morphological markers analysis suggested that it had highly variable relationships among the 495 germplasms. In addition, 10 synonymous groups including 24 germplasms were identified, showing similar results to those in a previous report (Guan et al., 2019b; Liang et al., 2015). Moreover, we also selected representative 52 varieties to reveal their genetic relationships and morphological profiles, which showed their potential for breeding applications due to their unique character. Our results indicate that SSR markers can also serve as an efficient tool for the identification of genotypes with desired morphological and physiological traits. The overall results suggest that the diverse genetic and morphological variations in persimmon have prevented the occurrence of a genetic bottleneck. The genetic and morphological profiles generated in this study can be used to accelerate persimmon breeding by identifying potential persimmon parents with traits of agronomic interest.

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## CRediT authorship contribution statement

**Changfei Guan:** Conceptualization, Investigation, Methodology, Writing - original draft. **Yangfan Zhang:** Methodology, Writing - original draft, Resources, Validation. **Pingxian Zhang:** Methodology, Writing - original draft, Resources, Validation. **Sadaruddin Chachar:** Writing - review & editing. **Renzi Wang:** Software, Supervision. **Xiaoyun Du:** Project administration, Conceptualization, Validation, Writing - review & editing. **Yong Yang:** Project administration, Conceptualization, Validation, Writing - review & editing.

## Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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