Genetic relationship and species identification of *Dioscorea polystachya* Turcz. in Yams determined by ISSR, ISAP, SRAP and SCAR markers

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**Abstract** *Dioscorea polystachya* Turcz. is the authentic herb Yam in Chinese traditional medicine. In the medicinal marketplace, some related *Dioscorea* species are mislabeled as “Yams”, such as *D. alata* L., *D. exalata* C.T. Ting & M.C. Chang, *D. persimilis* Prain et Burkill, *D. fordii* Prain et Burkill and *D. japonica* Thunb. To determine the genetic relationships among these species and develop a rapid and accurate method for *D. polystachya* identification, Inter Simple Sequence Repeat (ISSR), Intron Sequence Amplified Polymorphism (ISAP), Sequence-Related Amplified Polymorphism (SRAP) and Sequence Characterized Amplified Region (SCAR) markers were used in this study. By combining ISSR, ISAP and SRAP markers, a high degree of variation among the *Dioscorea* species was detected. Moreover, a relatively high gene flow (*N_m* = 0.3293) among the species was observed. This may result from gene pool exchanges of wild materials. The phylogenetic tree and genetic similarity analysis showed that *D. japonica* had the closest genetic relationship with *D. polystachya*. Considering the lowest inter-species variation observed in *D. polystachya* and the highest genetic divergence within *D. japonica*, we suggest a taxonomy of a one-species complex, *D. polystachya*–*D. japonica*, for breeding and conservation purposes. The phylogenetic data in this study indicated that *D. alata* and *D. persimilis* are independent species, not cultigens. Furthermore, a SCAR marker was developed that could be used to discriminate the authentic herb *D. polystachya* from other *Dioscorea* species. Overall, these results are useful as guidelines for breeding programs and conservation actions.

**Keywords** *Dioscorea polystachya* · Authentic herb · Yams · Relative species · Genetic relationship · Molecular markers

**Introduction**

Yam (*Dioscorea* spp.) is not only an important tuber crop, but also an important pharmaceutical plant used in traditional Chinese medicine (Karnick 1969; Hodeba et al. 2008; Peng et al. 2017; Hang and Sun 2020). As a traditional herb, the pharmaceutical uses
of yam involve treating diabetes, strengthening stomach functions and alleviating anorexia (Hang 1995; Hang and Sun 2020). In addition, it is used as a health food (Shin et al. 2006). In the Chinese Pharmacopoeia, the authentic herb “Yam” exclusively refers to *Dioscorea polystachya* Turcz. in Dioscoreaceae (Pharmacopoeia of China 2020). In China, owing to its high market demand, other species in the genus *Dioscorea*, including *D. alata* L., *D. exalata* C. T. Ting & M. C. Chang, *D. persimili* Prain et Burkill, *D. fordii* Prain et Burkill and *D. japonica* Thunb. are commonly treated as “Yams” to be used in medicines (Lay et al. 2001; Hang et al. 2006; Li Mimi et al. 2012; Hang and Sun 2020). Because the nutrient and bioactive compositions quantitatively and qualitatively vary among many *Dioscorea* species (Wu et al. 2016), the confusion and misuse of non-authentic yams has had a great impact on the medicinal market, medicinal efficacy and herb growers. Furthermore, there are still controversies regarding the taxonomy of some edible *Dioscorea*. For example, a study reported that *D. persimili* is a cultivar of *D. polystachya* on the basis of an 18S sequence analysis (Liu et al. 2000). Additionally, the status of *D. alata* as a true species or a putative cultigen remains unresolved (Lebot et al. 1998; Wilkin et al. 2005; Mengesha et al. 2013a).

To date, species identification in *Dioscorea* has predominantly depended on morphological and micromorphological descriptors. For example, leaf morphology, stem twining direction, tuber anatomy and leaf epidermal cell micromorphology have been used for the identification of *Dioscorea* species (Hang et al. 2006; Li Mimi et al. 2012; Adeniran and Sonibare 2016; Hang and Sun 2020). However, morphological identification requires conditionality, including a suitable plant developmental stage or sophisticated assessment tools.

Developed molecular tools, such as Random Amplified Polymorphic DNA, Amplified Fragment Length Polymorphism, Inter Simple Sequence Repeat (ISSR), Microsatellites, Simple Sequence Repeat (SSR) and Restriction Fragment Length Polymorphism markers, have become important means of identifying *Dioscorea* species (Lay et al. 2001; Tostain et al. 2006; Mengesha et al. 2013b; Mukherjee and Bhat 2013). Using a combined SSR and ISSR analysis, many edible yam species in West have been identified, including cultivated species of *D. prahensilis*, *D. abyssinica* and wild species of *D. sagittifolia* in Nigeria (Nascimento et al. 2013). However, these molecular techniques may be ambiguous, pose time constraints and are costly because PCR amplification requires multiple reactions (Wu et al. 2009; Sun et al. 2012). To overcome these drawbacks, traditional molecular markers may be converted into stable and reliable markers, such as Sequence Characterized Amplified Region (SCAR) markers (Devaiah et al. 2011; Al-Qurainy et al. 2018). This approach has been demonstrated to work in species identification, including those of *Satureja montana* L., *Cucumis sativus* L. and *Casuarina equisetifolia* L (Ghosh et al. 2010; Marieschi et al. 2011; Saengprajak 2012). Furthermore, we have developed a SCAR marker to distinguish the landrace *D. polysstachya* ‘Tiegun’ from the other *D. polystachya* varieties (Peng et al. 2017).

In this study, ISSR, Intron Sequence Amplified Polymorphism (ISAP) and Sequence-Related Amplified Polymorphism (SRAP) markers were used to evaluate the genetic relationships among yam species. Moreover, a species diagnostic SCAR marker was developed to identify the authentic herb “Yam” *D. polystachya*.

### Materials and methods

#### Plant materials

Here, we collected not only cultivated *Dioscorea* materials, but also many wild *Dioscorea* materials in China (Table 1). Healthy young leaves from *Dioscorea* species were collected for genomic DNA extraction. The wild *D. zingiberensis* C. H. Wright was selected as the outgroup. An additional six to 10 individuals per species were assayed to validate the reliability of the SCAR marker using a double-blind test. When the validation was finished, the identities of the experimental materials were disclosed (Table 2). All the samples in this study were identified by Professor Yueyu Hang (Jiangsu Institute of Botany, Chinese Academy of Sciences).
| Plant materials used to determine the genetic relationships among *Dioscorea* species | Species | Code | Habit/cultivated name | Locality |
|---|---|---|---|---|
| **D. polystachya** | SY1 | Wild | Jiangsu jurong |
| | SYHZ | Cultivate/ ‘Huazi’ | Jiangsu Xuzhou |
| | SYWJSB | Cultivate/ ‘Wujiahuangbao’ | Jiangsu Xuzhou |
| | SYMI | Cultivate/ ‘Mishanyao’ | Jiangsu Xuzhou |
| | SYXCM | Cultivate/ ‘Xichangmao’ | Henan Wenxian |
| | SUHY | Cultivate/ ‘Huaying’ | Sichuan Guangan |
| | SYCSY | Cultivate/ ‘Cao’ | Shandong Heze |
| | SYMA | Cultivate/ ‘Ma’ | Henan Wenxian |
| | SYAS | Cultivate/ ‘Anshun’ | Guizhou Guiyang |
| | SYXBJZ | Cultivate/ ‘Xiaobaizui’ | Henan Wenxian |
| | SYCNGT | Cultivate/ ‘Cuniutui’ | Henan Wenxian |
| | SYJJH | Cultivate/ ‘Jiujinghuang’ | Henan Wenxian |
| | SYBY | Cultivate/ ‘Baiyu’ | Shandong Heze |
| | SYTG | Cultivate/ ‘Tiegun’ | Shanxi Taiyuan |
| | SYTG-2 | Cultivate/ ‘Tiegun’ | Jiangsu Xuzhou |
| | SYTG-1 | Cultivate/ ‘Tiegun’ | Henan wenxian |
| | SYTG-5 | Cultivate/ ‘Tiegun’ | Shandong Jingning |
| | SYTG-6 | Cultivate/ ‘Tiegun’ | Hebei Cangzhou |
| | SYTG-7 | Cultivate/ ‘Tiegun’ | Hunan Liuyang |
| | SYTG-3 | Cultivate/ ‘Tiegun’ | Sichuan Nanchong |
| | SYTG-4 | Cultivate/ ‘Tiegun’ | Guangdong Dongguan |
| **D. persimilis** | HB-1 | Cultivate | Fujian mingi |
| | HB-2 | Wild | Guangdong shaoguan |
| | HB-3 | Wild | Yunnan lijiang |
| | HB-4 | Wild | Fujian wuyishan |
| | HB-5 | Wild | Jiangxi lushan |
| | HB-6 | Wild | Guangxi nanning |
| **D. alata** | SS-1 | Cultivate | Guangdong shaoguan |
| | SS-2 | Cultivate | Hainan lingshui |
| | SS-3 | Cultivate | Hunan hengshan |
| | SS-4 | Cultivate | Yunnan lijiang |
| | SS-5 | Unknown | Jiangxi lushan |
| | SS-6 | Unknown | Yunnan jinghong |
| **D. exalata** | WC-1 | Cultivate | Guangxi guilin |
| | WC-2 | Cultivate | Yunnan lijiang |
| | WC-3 | Wild | Guangxi,guilin |
| | WC-4 | Wild | Yunnan jinghong |
| | WC-5 | Wild | Yunnan,linchang |
| | WC-6 | Wild | Guangxi,longzhou |
| **D. fordii** | SHANS-1 | Cultivate | Fujian,shanming |
| | SHANS-2 | Wild | Guangxi guilin |
| | SHANS-3 | Wild | Yunnan jinghong |
| | SHANS-4 | Wild | Hainan,limushan |
| | SHANS-5 | Wild | Guangdong,dinghushan |
| | SHANS-6 | Wild | Yunnan, kunming |
DNA extraction

Genomic DNA extraction was performed as described previously (Peng et al. 2017). The extracted DNA was subjected to agarose gel electrophoresis to confirm that it was intact and then stored at −20 °C for later use.

Table 1 (continued)

| Species   | Code | Habit/cultivated name | Locality           |
|-----------|------|-----------------------|--------------------|
| D. japonica |      |                       |                    |
| RB-1      | Wild |                       | Zhejiang, linan    |
| RB-2      | Wild |                       | Jiangxi, lushan    |
| RB-3      | Wild |                       | Hunan, yongshun    |
| RB-4      | Wild |                       | Fujian wuyishan    |
| RB-5      | Wild |                       | Guangdong lianshan |
| RB-6      | Wild |                       | Anhui guniiuijiang |

“Unknown” indicates that the material was collected from the wild and that there was no cultivation in this area.

Table 2 Plant materials for the validation of the developed SCAR marker

| Species   | Individuals | Habit | Location          |
|-----------|-------------|-------|-------------------|
| D. polystanchya | 2           | Cultivate | Henan Wenxian     |
|            | 2           | Cultivate | Jiangsu Xuzhou    |
|            | 2           | Cultivate | Shanxi Taiyuan    |
|            | 2           | Cultivate | Henan Wenxian     |
|            | 2           | Wild      | Jiangsu Nanjing   |
| D. persimilis | 10          | Cultivate | Jiangsu Yixing    |
| D. alata   | 10          | Cultivate | Jiangsu Yixing    |
| D. fordii  | 10          | Cultivate | Jiangsu Yixing    |
| D. exalata | 1           | Cultivate | Guangxi guilin    |
|            | 1           | Cultivate | Yunnan lijiang    |
|            | 1           | Wild      | Guangxi guilin    |
|            | 1           | Wild      | Yunnan jinghong   |
|            | 1           | Wild      | Yunnan, lijiang   |
|            | 1           | Wild      | Guangxi, longzhou |
| D. japonica| 1           | Wild      | Zhejiang, linan   |
|            | 1           | Wild      | Jiangxi, lushan   |
|            | 1           | Wild      | Hunan, yongshun   |
|            | 1           | Wild      | Fujian wuyishan   |
|            | 1           | Wild      | Guangdong lianshan|
|            | 1           | Wild      | Anhui guniiuijiang|

ISSR, ISAP and SRAP reaction system

Preliminary assays were conducted to determine the optimum primers for the Dioscorea genetic relationship analysis. Finally, 10 highly polymorphic ISSR primers were chosen for the PCR reactions (Supplemental Table S1). PCR amplifications were performed in a 20-µL reaction volume containing 1 µL DNA (20 ng·µL⁻¹), 2 µL 10× Buffer (Mg²⁺), 1.6 µL dNTP (2.5 mM), 0.8 µL (10 mM) primer, 0.2 µL Taq DNA polymerase (5 U·µL⁻¹) and ddH₂O up to 20 µL. PCR amplifications were carried out in a T1 Thermocycler (Biometra, Germany) using the following program: 1 min at 94 °C; 5 cycles of 94 °C for 45 s; 36 °C for 3 s and 72 °C for 60 s; 35 cycles of 94 °C for 45 s, 50 °C for 30 s and 72 °C for 75 s, followed by a final extension at 72 °C for 5 min. Then, 11 ISAP primer pairs were chosen for the PCR reactions on the basis of high polymorphism rates and good reproducibility (Supplemental Table S2). The ISAP analysis was performed in accordance with those described previously (Peng et al. 2017). Furthermore, 21 highly polymorphic primer pairs were selected for the SRAP analysis (Supplemental Table S3). PCR was carried out in a 20-µL reaction mixture containing 25 ng DNA as template, 0.7 mM primer, 0.2 mM Mg²⁺, 0.3 mM dNTPs and 1 U Taq DNA polymerase. The PCR reaction program was as follows: 94 °C for 1 min; 5 cycles of 94 °C for 45 s; 36 °C for 3 s and 72 °C for 60 s; 35 cycles of 94 °C for 45 s, 50 °C for 30 s and 72 °C for 75 s, followed by a final extension at 72 °C for 5 min. All the primers used in this study were synthesized by Invitrogen Trading (Shanghai) Co., Ltd.
Genetic relationship analysis

All the clearly detectable polymorphic and monomorphic bands were scored as present (1) or absent (0) in each sample during the analysis. POPGENE software was used to analyze the genetic relationships of species, as well as to calculate the indices of number of alleles \( (N_a) \), the number of effective alleles \( (N_e) \), Nei’s gene diversity index \( (H) \), the Shannon Index \( (I) \), gene differentiation coefficient \( (G_{st}) \) and gene flow \( (N_m) \) (Yeh et al. 1999). The observed total heterozygosity \( (H_t) \) and the average heterozygosity within the species \( (H_s) \) were calculated using GenAlEx v. 6.5 (Peakall and Smouse 2012). A Principal coordinate analysis (PCoA), genetic similarity coefficients and genetic distances were obtained using the NTSYS 2.10e software (Rohlf 2000). The intra-specific similarity coefficient \( (F_{is}) \) and inter-specific variation coefficient \( (F_{st}) \) were calculated using the Artificial Bee Colony (ABC) algorithm (Foll et al. 2008).

SCAR identification system

To screen the species-specific bands conveniently, all the individual DNAs of each species (Table 1) were mixed into one gene pool. The presence of a specific fragment in \( D. \) polystachya that is absent in all the other species was designated as an identification marker. A specific fragment of \( D. \) polystachya was obtained using an Agarose Gel Extraction Kit (TaKaRa, Dalian, China) and sequenced by Majorbio Co. (Shanghai, China). On the basis of the DNA sequence analysis results, a pair of specific primers were designed for the SCAR reaction using Primer Premier 5.0 (Lalitha 2000). PCR was carried out in a 20-μL reaction mixture containing 1 μL (10–25 ng) DNA as template, 13.3 μL double-distilled water, 2 μL Mg\(^{2+}\), 0.3 μL (10 mmol/L) dNTPs, 2 μL PCR buffer, 0.6 μL (10 pmol/L) each primer and 0.2 μL (1 U) Taq DNA polymerase. The PCR reaction program was as follows: 94 °C for 1 min; 30 cycles of 94 °C for 50 s, 60 °C for 45 s and 72 °C for 60 s, followed by an extension at 72 °C for 5 min. The PCR products were electrophoresed on a 1 % agarose gels, stained with ethidium bromide, observed and photographed with a gel imaging system. A 2,000-bp DNA ladder was used as a molecular weight marker.

Results

Genetic relationship between \( D. \) polystachya and its related species

Using the combination of ISSR, ISAP and SRAP markers, 523 bands were obtained. The number of polymorphic bands for these markers was 507, representing 96.94%. The \( N_a \) of Dioscorea species was 1.9963, whereas the \( N_e \) was 1.7724. The \( H \) value was 0.4950, and the \( I \) value was 0.6762. An analysis of the coefficient of genetic differentiation revealed that the \( H_t \) of the six species was 0.4821, and the \( H_s \) was 0.1769. The \( G_{st} \) among species was 0.6029, whereas the \( N_m \) among species was 0.3293.

The genetic similarity coefficient among Dioscorea species were 0.7126–0.9258, whereas the genetic distances were 0.0770–0.2825 (Table 3). The closest and farthest genetic relationships associated with \( D. \) polystachya were those of \( D. \) japonica (genetic similarity coefficient = 0.8430) and \( D. \) fordii (genetic similarity coefficient = 0.7126), respectively. In addition, among the six species, \( D. \) exalata and \( D. \) persimilis were the closest, with a genetic distance and a genetic similarity coefficient of 0.0770 and 0.9258, respectively.

The \( F_{is} \) ranged from 0.2096 to 0.4339, with the \( F_{is} \) of \( D. \) persimilis being the highest, and the \( F_{is} \) of \( D. \) japonica being the lowest. The \( F_{st} \) varied from 0.0497 to 0.1862. Compared with other species, \( D. \) fordii

| Species          |  \( D. \) polystachya |  \( D. \) alata |  \( D. \) japonica |  \( D. \) persimilis |  \( D. \) exalata |  \( D. \) fordii |
|------------------|----------------------|----------------|-------------------|-------------------|-----------------|---------------|
| \( D. \) polystachya | ****                | 0.8282         | 0.8430            | 0.8085            | 0.7954          | 0.7126        |
| \( D. \) alata    | 0.1885               | ****           | 0.8255            | 0.7539            | 0.7753          | 0.8059        |
| \( D. \) japonica | 0.1708               | 0.1918         | ****              | 0.8738            | 0.8679          | 0.8567        |
| \( D. \) persimilis | 0.2126              | 0.2825         | 0.1349            | ****              | 0.9258          | 0.9111        |
| \( D. \) exalata  | 0.2289               | 0.2545         | 0.1416            | 0.0770            | ****            | 0.8770        |
| \( D. \) fordii   | 0.3388               | 0.2158         | 0.1546            | 0.0930            | 0.1312          | ****          |
had the greatest variability, and *D. polystachya* had the lowest. The *P*-values of all results were less than 0.05, indicating high confidence levels (Table 4).

The phylogenetic tree revealed that individuals of each species clustered together. All the samples of *D. polystachya* were clustered in one branch, and they gathered with all the *D. japonica* individuals into a large branch. *Dioscorea alata*, *D. persimilis*, *D. exalata* and *D. fordii* clustered together into another large branch, which was located at the base of the phylogenetic tree (Fig. 1).

Interestingly, the PCoA using Euclidean similarity indices separated all the samples, except *D. exalata* and *D. persimilis* (Fig. 2).

### Specific identification of *D. polystachya* based on a SRAP-SCAR analysis

In this study, a specific band of *D. polystachya* was found by SRAP amplification (Fig. 3).

The sequencing results revealed that specific band in *D. polystachya* was 1228 bp (Fig. 4). The sequence was compared against the GenBank database, and no homologous sequences were found. A pair of SCAR primers (F: 5′-TTGCCCTCAAATGATTGC CC-3′/R: 5′-AGGTGATGGATATGGCCCCAAAT-3′) were designed in accordance with the primer design principle. Then, 46 *Dioscorea* individuals were prepared for a double-blind test. The specific band was observed in 10 individuals (Fig. 5). When the experimental materials were disclosed, all 10 of these individuals belonged to *D. polystachya*. In addition, the specific band was not amplified in individuals of the

| Species         | *F*-data | Mean  | *P*-value |
|-----------------|----------|-------|-----------|
| *D. polystachya* | *F*<sub>is</sub> | 0.2611 | 0.000744  |
| *D. persimilis*  | *F*<sub>is</sub> | 0.4339 | 0.002997  |
| *D. japonica*    | *F*<sub>is</sub> | 0.2096 | 0.000246  |
| *D. exalata*     | *F*<sub>is</sub> | 0.3054 | 0.000255  |
| *D. fordii*      | *F*<sub>is</sub> | 0.3248 | 0.000679  |
| *D. alata*       | *F*<sub>is</sub> | 0.4173 | 0.000587  |
| *D. polystachya* | *F*<sub>st</sub> | 0.0497 | 0.000889  |
| *D. fordii*      | *F*<sub>st</sub> | 0.1862 | 0.004133  |
| *D. japonica*    | *F*<sub>st</sub> | 0.0756 | 0.000392  |
| *D. exalata*     | *F*<sub>st</sub> | 0.0698 | 0.000487  |
| *D. persimilis*  | *F*<sub>st</sub> | 0.1088 | 0.000355  |
| *D. alata*       | *F*<sub>st</sub> | 0.1255 | 0.000873  |

Fig. 1 Phylogenetic tree of *D. polystachya* and its five relatives. Each terminal node is described by a corresponding code provided in Table 1.
Discussion

For nearly two decades, combinations of multiple molecular markers have been used to analyze genetic relationships. For example, Random Amplified Polymorphic DNA, ISSR, Inter-retrotransposon Amplified Polymorphism and Retrotransposon Microsatellite Amplified Polymorphism markers were used to reveal the genetic diversity and relationships among Citrus species (Biswas et al. 2010). Genetic relationships and modes of Robinsonia species were illustrated using Amplified Fragment Length Polymorphism and SSR markers (Takayama et al. 2015). Combining SSR and ISSR markers better reflected the genetic relationship of D. trifida than single markers (Nascimento et al. 2013). At the molecular level, the percentage of polymorphic bands, $H$, $N_e$, I, $H_t$, and $H_s$ are essential for evaluating the genetic diversity of germplasm (Mignouna et al. 2005; Narzary et al. 2009). In this study, ISSR, ISAP and SRAP molecular markers were used to analyze the genetic relationship between D. polystachya and its related species, and high levels of genetic diversity among Dioscorea species were observed with the above parameters. Our results also
indicated that the combined three markers detected a higher degree of variation among Dioscorea species compared with single markers, as reported previously (Shiwachi et al. 2000; Malapa et al. 2005; Zhou et al. 2008).

There was a relatively high $N_m$ (0.3293) among the six species used in this study, which is much higher than in previous studies ($N_m=0.1081$) that used four species, D. opposita, D. alata, D. persimilis and D. fordii, from 21 cultivar accessions (Wu et al., 2014). In addition, the $G_d$ of 0.6029 among species was lower than the previously reported $G_d$ value of 0.8222 (Wu et al. 2014). This might be because the experimental materials used in this study included more species and many wild individuals. In previous studies, the Dioscorea species were almost all cultivated crops. In fact, the main reproductive mode of cultivated Dioscorea crops is asexual reproduction, which limits the gene pool exchange between species (Mengesha et al. 2013a, 2013b; Takayama et al. 2015). This might be why there were $N_m$ values in some Dioscorea genetic diversity studies. Furthermore, we conducted a genetic structural analysis, which is typically assessed using $F$-statistics (Foll et al. 2008). The lowest $F_{st}$ was observed in D. japonica, which indicates that this species had the highest degrees of intraspecific differentiation and variation. The $F_{is}$ value of D. persimilis was the highest, indicating that the gene purity in the species was high, and the species may rarely undergo sexual reproduction. In contrast, the lowest $F_{st}$ was observed in D. polyschachya. This indicated that there was less reproductive isolation between D. polyschachya and the other Dioscorea species, which means it is a good parental Dioscorea material. The highest $F_{st}$ value was obtained in D. fordii, which indicated the highest differentiation between D. fordii and other five species.

The genetic relationships of some edible species of the genus Dioscorea have always been controversial. For example, on the basis of an18S sequence analysis, it was speculated that D. persimilis might be a cultivar of D. polystachya and thus should not be classified as an independent species (Liu et al. 2000). However, our results showed that all the individuals of D. persimilis had a distant relationship with D. polystachya. In addition, the phylogenetic tree showed that all the individuals of the two species were located on different branches. The evidence indicated that D. persimilis is an independent species. Another taxonomic issue in Dioscorea is whether D. alata is a true species or a putative cultigen. Some studies have indicated that D. alata might be a cultigen of D. persimilis (D. hamiltonii synon.) owing to the narrow genetic base (Hahn...
In fact, *D. alata* originates in North and East of the Bay of Bengal, and it is naturalized in China (Hang and Sun 2020). Our results showed that all the individuals of *D. alata* clustered together in the phylogenetic tree. This indicated that *D. alata* is a true species and not a putative cultigen. This phenomenon was also found in a previous study using an isozyme analysis (Lebot et al. 1998). Here, another interesting phenomenon was the close genetic relationship between *D. exalata* and *D. persimilis*. Furthermore, the PCoA analysis could not separate the individuals of the two species. Therefore, further work is required to establish the genetic relationship between the two species.

In this study, the phylogenetic tree and genetic similarity results showed that the genetic relationship between *D. japonica* and *D. polystachya* was the closest. Since the Song Dynasty, *D. japonica* has been called ‘Wild Yam’ (Hang and Sun 2020). The morphological characteristics of *D. japonica* are very similar to those of *D. polystachya*, and there is no reproductive isolation between them (Araki et al. 1983; Mizuki et al. 2010). Moreover, in pharmacological experiments, unlike other *Dioscorea* species, *D. japonica* and *D. polystachya* have the same efficacy level (Hang 1995). As mentioned previously, the highest degrees of intraspecific differentiation and variation were observed in *D. japonica*, indicating that it might be a suitable potential *Dioscorea*
breeding material. For subsequent breeding programs or conservation actions, we suggest that these species be referred to as the D. polystachya–D. japonica complex in accordance with the classification of Guinea yam (D. cayenensis–D. rotundata complex) (Martin and Rhodes 1978; Mengesha et al. 2013a, 2013b; Loko et al. 2015;)

Identification with SCAR makers

At present, the species identification of yam having medicinal properties is still obscure. Therefore, there is an urgent need for the development of a fast and robust identification method to distinguish D. polystachya from its related species. In this study, a species-specific band was obtained using SRAP and developed into a SCAR marker. To examine its accuracy and sensitivity, the species-specific SCAR marker was used in a double-blind test. All the individuals of D. polystachya amplified the species-specific band that was absent in the other species, confirming the specificity of the SCAR marker for species-specific identification. In short, a converted-SCAR technique proved to be a helpful tool in identifying Dioscorea species. The results of this study indicated that PCR products can be used to form SCAR markers, which may then be used in DNA barcoding for low-cost species identification.

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Declarations

Conflicts of Interest The authors declare that there is no conflict of interests regarding the publication of this paper.

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