



A comparative assessment of diversity of greater yam (*Dioscorea alata*) in China



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ABSTRACT

Dioscorea alata is a species of yam that is widely cultivated throughout the world, including China. Nevertheless, there is very limited knowledge on this type of yam diversity in China. In the present study, we assessed the genetic diversity of Chinese greater yam by analyzing their morphology, genotype, chromosome and ploidy. 142 cultivated greater yams were collected from eight geographical regions of China. 16 morphological characters were used for phenotypic variation assessment. Genotypic diversity was analyzed using 186 EST-SSR markers, while the chromosomes counting and ploidy evaluation were detected by flow cytometry. A broad morphological diversity was found, i.e. significant differences among accessions were found at each quantitative trait. The genetic similarity coefficients ranged from 0.42 to 0.91 with an average of 0.70. UPGMA and PCA indicated that the population was separated into two major clusters. The variation within pops accounted for 99.0%. 10 chromosome counts genotypes showed mainly $2n = 40$, Da176 was a mixoploid with 20 and 40 chromosomes. Four levels of ploidy were detected by flow cytometry technology, and 87.3% of accessions had 40 chromosomes. Our findings demonstrated a broad variation of cultivated greater yam at phenotypic, genotypic and ploidy levels in two groups of observed cultivars.

1. Introduction

Yam (*Dioscorea spp.*) is a multi-species, polyploid, and vegetatively propagated tuber crop. It is economically important as staple food crop in countries of Western and Central Africa, Pacific and Caribbean Islands, and in some Asian and Latin American countries (Mignouna et al., 2003; Sasaki et al., 2015). In these countries, yam is the third most important tropical root crop after cassava (*Manihot esculenta* Crantz.) and sweet potato (*Ipomoea batatas* L. Lam.) (Srivastava et al., 2012). According to the FAOSTAT (<http://www.fao.org/faostat/en/#data/QC>), we calculated the total production of yams from 1961 to 2016, Africa is the largest producer with production of 15 hundred million tonnes, followed by America and Oceania. *Dioscorea alata*, also known as greater yam or the water yam, has the largest world distribution and is one of the oldest cultivated yam species worldwide (Lebot, 2009; Mignouna et al., 2002b). The exact time when it was introduced to China still remains unknown. In China, *D. alata* is famous for its high production that may achieve 58 kg per plant. Moreover, because of its wide adaptability, greater yam can grow in both tropical and

subtropical regions, including temperate climate that is often found in different regions of China like Shandong and Jiangsu regions. In addition, *D. alata* is food crop with nutritional values, besides that its contribution for revenue generation and socio-cultural events are also significant.

Greater yam has an advantage for sustainable cultivation due to its comparatively good agronomic characteristics (Zekraoui et al., 2015). Currently, the development of new crop breeding methods is constantly improving food yield and quality of yam (Zekraoui et al., 2015). Yam breeding have been created by CIRAD (Centre International de Recherche Agronomique pour le développement), IITA (International Institute of Tropical Agriculture), CTCRI (Central Tuber Crops Research Institute, Trivandrum, India) and INRA (Institut National de la Recherche Agronomique), they planed to create new varieties that combine high and stable yields, anthracnose resistance and tuber characteristics adapted to commercial production (Scarcelli, 2016). In China, diseases such as anthracnose (*Colletotrichum spp.*) and root knot nematode are the major cause of decrease in greater yam quality and production. They also result in the disappearance of many cultivars;

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requiring new breeding methods. Currently, yam breeding approaches are facing problems such as long growth cycle (8 months or more), dioecy, poor to non-flowering plants, polyploidy, vegetative propagation, heterozygous genetic background, and poor knowledge of crop genes (Mignouna et al., 2003). Hence, a large collection of germplasms and diversity assessment can be very important in supporting breeding of *D. alata* (Narina et al., 2011; Ngo Ngwe et al., 2015; Velasco-Ramírez et al., 2014). The improvement of cultivars and the breeding of new crop varieties have always relied on the availability of biological diversity (Baranger et al., 2004). To date, genetic diversity analysis have been used to characterize greater yam germplasm, including the diversity of morphological traits, isozyme, ploidy and DNA. Nevertheless, there was almost no published data on genetic diversity of Chinese greater yam. Very little is known about the genetic background of *D. alata*, there is little information available on cultivar genealogies and *D. alata* has suffered from research neglect in general. In the present study, we aimed to investigate morphological characterization; to evaluate genotypic polymorphism by EST-SSR markers; to count the chromosome numbers of 10 samples, then selected one sample as nucleic acid content control to verify the various ploidy levels by flow cytometry. A better understanding of the available genetic diversity and the breeding potential of specific accessions is important for the choice of parents used in breeding programs (Mignouna et al., 2003). The results of genetic diversity of the population in this study, will help us to make collection and conservation strategies of greater yam; and implementation of future breeding programs.

2. Materials and methods

2.1. Plant materials

142 cultivars of greater yam were conserved in Yam Germplasm Resources of Hainan University, collected from eight provinces in south of China. (see Table 1), including the southernmost region of Baoting (Hainan province, geographic coordinates (gc): 18°38'N, 109°41'E), the northernmost region of Yam (Sichuan province, gc: 30°01' N, 103°02'E), the westernmost region of Ruili (Yunnan province, gc: 24°0'N, 97°51'E) and the easternmost region of Anxi (Fujian province, gc: 25°03'N, 118°10'E).

2.2. Morphological characterization

Morphological characteristics were observed in the field, and the data recording were as previously described (IPGRI/IITA, 1997), with some modifications. In this study, we evaluated variation in 16 traits related to leaf characters, petiole, stem, tubers and flowering. These observations were based on phenotypic average of 6 measurements for the quantitative descriptors for leaves, in each individual plant. We assessed a total of 11 quantitative and 5 qualitative traits, which were transformed into different classes, attributing codes to each class (Table 2).

2.3. Genotype identification

DNA samples were isolated from plant leaves. Modified CTAB (Cetyltrimethyl Ammonium Bro-mide) method was used for DNA extraction (Wu et al., 2015). Extracted DNA was analyzed on 1.0% (w/v) agarose gels, and the DNA concentration was measured using UV visible spectrophotometer (Thermo NanoDrop 2000, USA). Based on concentration estimations, all samples were diluted to 20 ng/μL and stored at -20 °C.

A total of 186 EST-SSR polymorphic primers (Narina et al., 2011) were chosen for this study (Table S1) (see Supplemental Data with this article). Amplification reactions were performed using a master mix solution of 20 μL containing 10 μL of 2 × Taq PCR mix (Aidlab, China), 1 μL of each primer (10 mM), and 1 μL of DNA template (20 ng. μL⁻¹).

Table 1
List and origin of the 142 accessions of *Dioscorea alata* in this investigation.

Geographical origin	Numbers	Accession No.
Hainan Province	40	Da83, Da84, Da86, Da87, Da88, Da90, Da93, Da94, Da99, Da100, Da101, Da102, Da103, Da105, Da107, Da108, Da112, Da114, Da115, Da117, Da118, Da119, Da126, Da128, Da134, Da139, Da140, Da141, Da142, Da143, Da150, Da151, Da152, Da154, Da159, Da164, Da166, Da173, Da174, Da176
Guangxi Province	28	Da29, Da30, Da31, Da32, Da37, Da38, Da39, Da40, Da42, Da44, Da46, Da47, Da56, Da77, Da130, Da137, Da168, Da189, Da199, Da206, Da213, Da223, Da229, Da239, Da246, Da250, Da259
Guangdong Province	3	Da4, Da24, Da201
Guizhou Province	13	Da193, Da203, Da204, Da205, Da207, Da210, Da216, Da234, Da244, Da248, Da253, Da258, Da262
Sichuan Province	14	Da202, Da219, Da220, Da224, Da225, Da226, Da233, Da237, Da238, Da249, Da252, Da265, Da267, Da274
Yunnan Province	14	Da62, Da68, Da70, Da73, Da74, Da80, Da169, Da190, Da191, Da228, Da235, Da260, Da273, Da279
Chongqing Province	10	Da196, Da200, Da215, Da217, Da227, Da232, Da236, Da242, Da247, Da256
Fujian Province	20	Da2, Da192, Da195, Da208, Da211, Da212, Da214, Da218, Da222, Da241, Da251, Da255, Da257, Da263, Da264, Da268, Da269, Da271, Da276, Da277

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