Volume-4, Issue-4, April-2015 • ISSN No 2277 - 8160

Research Paper Biology

Use of Its Dna Barcoding to Identify Ornamental Camellia

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ABSTRACT

Cha-hua is the world's famous flower, refers to which with the high ornamental values in Camellia, and has a long cultivation history. Due to Camellia cultivars a wide application and increasing number of new varieties, homonym and heteronym are very common phenomena, made the difficulties on identification, germplasm exchange and naming new species. In this study, we used DNA barcording methods, analyses the ITS sequences identification on Camellia. By the methods of calculating the distances in intraspecific and interspecific divergences evaluating DNA barcoding gap and constructing NJ and UPMGA phylogenetic trees. The sequence ITS performed best. In conclusion ITS sequences can be considered as a novel DNA barcode in Camellia genus other four sequences can be as combination barcode for identification.

KEYWORDS : - DNA barcoding; Camellia; ITS

INTRODUCTION

DNA barcoding is the use of the short DNA sequences for species identification, because of advances in sequencing and computational technologies; this method have been help us understanding of evolutionary and genetic relationships(Hajibabaei, Singer, Hebert, & Hickey, 2007; Lahaye et al., 2008). Since its inception as an approach for large-scale species identification(Blaxter, 2003), the mtDNA COI fragment as a core gene employed by DNA barcoding initiatives and has the potential to facilitate both the identification of known species and the discovery of new ones.Because it has simple operation, strong repeatability, the advantages of stable and reliable results, has become the international biological identification and a hotspot in the research of the diversity, attracted much attention in the world. In 2005, DNA barcode of this technique was in reference to the study of botany, because the plant mitochondrial genome evolution rate slower, therefore, COI gene is not applicable to the plant, barcode fragment selection, mainly on the chloroplast genome involved segments of the candidate area are mainly distributed in chloroplast genetic encoding. In 2009 by the international union of the barcode working group evaluate candidate barcoded DNA fragment of plant, recommended by rbcL+matK as the DNA sequences in plant(Hollingsworth et al., 2009).In addition the chloroplast gene spacer psbA-trnHand nuclear genesITShave also been more attention(Kress & Erickson, 2007).

In China, cha-hua refers to the ornamental trees of *Camellia*. There are 125species in this genus, China is regarded as the original place and the distribution center of *Camellia* with 104 species, also distributed in east and southeast Asia. Nowadays, *Camellia* being an important landscape ornamental flower and commercial flower cultivated in the world including three species: *Camellia* japonica L., *Camellia* reticulata Lindl., *Camellia* sasanqua Thunb. It is be famous of its beautiful appearance with leaves dark green and shiny and bright colorful flower-shaped. The most common in market are *C. reticulata* 'shizitou' *C. japonica* 'Daniel Webster' *C. sasanqua* 'Baifurong'et.. Especially the three species of above, due to cultivars a wide application and increasing number of new varieties, homonym and heteronym are very common phenomena, made the difficulties on identification, traditional taxonomy, germplasm exchange and naming new species. Therefore, in this study, we using modern biotechnology means to provided an effective evidence for identification and differentiation of these cultivars.

Materials and Methods

We got 81 ITS sequencesdata from NCBI GenBank (Table 1). In addition, using Ternstroemia_gymnantheraas out-group make cluster analysis.

Table 1 Sequences number of samples

Species	Number of samples	GenBank sequence
C. japonica	27	AY697418; AY697417; FJ432119; EF649690-AY701854; AY701855; AY701856AY701857-EU579723; HM061402; HM061404; HM061405 HM061406; HM061407; HM061408; HM061409; HM061410; HM061411; HM061412; HM061413-HM061414; HM061415; HM061416-HM061417; HM061418; HM061419-HM061420
C.reticulata	45	HM061401; HM061380; HM061386; JX666613; JX457340; JX457339; JX457338; JX500523' JX500527; JX500525; JX500520; JX500521; JX500522; JX500517; JX500513; JX500510; JX500508; JX500506; JX500505; JX500502' JX500501; JX500499; JX500496; JX500490; JX500488; JX500482; JX500481; JX500480; JX500479; JX500478; JX500477; JX500476; JX500475; JX500474; JX500473; JX500471; JX500469; JX500468; JX500466; JX500464; JX500463; JX500462; JX500461; JX500460; JX500458
C. sasanqua	9	EU579769; EF544762; EF544761; EF544760; EF544759; EF544758; EF544757; EF544756; EF544755

Sequences analysis

By using Bioedit software, in sequence alignment respectively, delete the extra sequence. Mega 6.0 software was used to analysis DNA sequence. Intraspecific compared to interspecific distances (K2P) were calculated. Also, the computer program Mega was used to provide information on the total variance, intraspecies variation, maximum intraspecific variation,interspecific variation,and minimum interspecific variation.Barcoding gapwas performed usingTaxonDNA, estimated the distribution of interspecies and intraspecies variation.Its effectiveness is verified by using molecular phylogenetic tree, and constructed by ML and NJ, makeTernstroemia_gymnantheraas out-groupmakes cluster analysis.

Results and discussion

Variation features

A total of 523 loci after sequence alignment, among them: conserved 187, account for 35.8%; variable 336, account for64.2%; parsim-into 253, account for 48.3%; Singleton 83, account for15.9%; A, T, C,G content were19.7%, 17.1%, 31.7%, 31.5%, respectively.The table shows that (Table 2), the ideal DNA barcode sequence should haveobvious interspecific variations, and intraspecific variation is small enough.The ITS sequence meets the criteria for an ideal DNA barcoding.

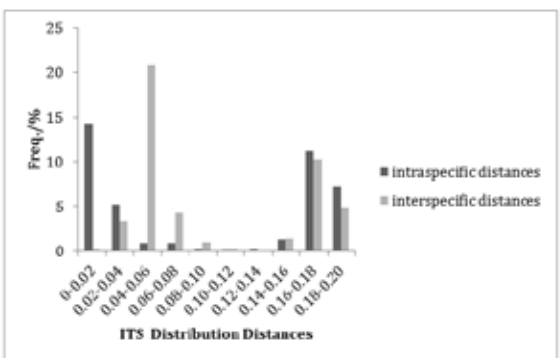
Table 2 The ITS sequences interspecies and intraspecies variation on *Camellia*

Mean	Total variance	Intraspecies variation	Maximun intraspecific variation	Interspecific variation	**Minimum** Interspecific variation
ITS	**0.1027± 0.0079**	**0.0550± 0.0036**	**0.1389± 0.0102**	**0.0892± 0.0098**	**0.0374± 0.0081**

Estimation of Barcoding Gap

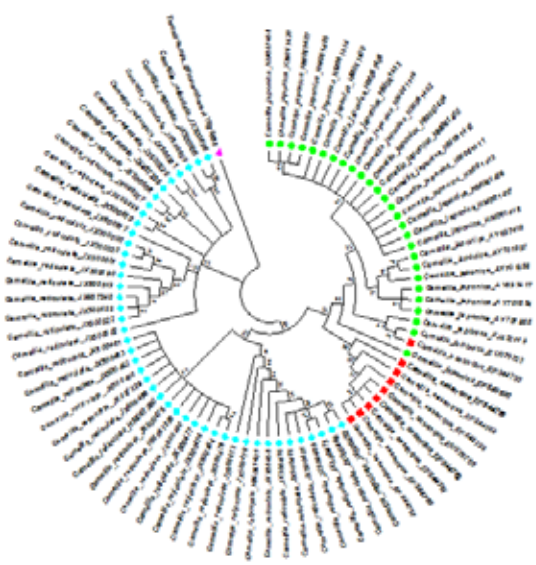
In an ideal situation, genetic variation between species should be exceeds variation within species of a DNA barcode,form an obvious gap area.the intraspecific variation will beon the one side of smaller numerical of the bar charts, and variation within species are focused on big numerical side. The ITS sequences obviously had barcoding gap (Figure 1).

Figure 1Genetic distance distribution map of ITS

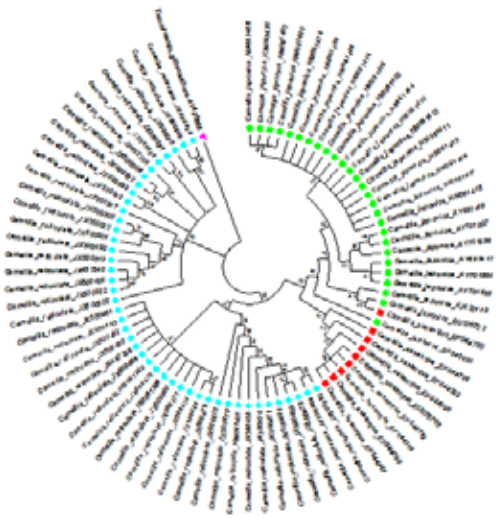


Identification of validity

Molecular phylogenetic tree constructed by UPGMA and NJ method - can be identification of cultivars species(Figure 2).



A. Molecular phylogenetic tree constructed by NJ



B. Molecular phylogenetic tree constructed by ML

Figure 2: Molecular phylogenetic tree constructed by ML and NJ

Conclusion

In This study, we analyzed the ITS sequences of DNA barcoding method used in Camellia.The results show that the ITS sequence not only can be showed significant interspecific differences, also have obvious barcoding gap,have less over lap on interspecies and intraspecies variation, can be identification of Camellia cultivars.The NJ and UPGMA phylogenetic trees showed that it is clearly classified all samples.In conclusion,

Recommends that ITS sequencesas the core DNA barcoding sequence identifying for Camellia ornamental plants, also in combination with other fragments as a combination of bar code.

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