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Research Article

Genetic diversity of *Kalopanax pictus* populations in Korea based on the nrDNA ITS sequence

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Abstract *Kalopanax pictus* is a long-lived deciduous perennial tree in the family Araliaceae mainly distributed in the East Asia. In Korea, this species is of ecological and medical importance. Because typical populations of this species are small and distributed in patches, *K. pictus* has been considered as a narrow habitat species. To understand the genetic diversity and population structure of this species, the sequence variation of the nuclear ribosomal DNA (nrDNA) internal transcribed spacer (ITS) region was analyzed among 18 different *K. pictus* populations in the present investigation. The nrDNA ITS sequences of Korean populations investigated in this study showed identical of 616 bp in length, and no any nucleotide variation was found in the entire nrDNA ITS region sequence. This result suggested that the *K. pictus* populations in Korea might belong to the same isolate, and no mutation was found in the nrDNA ITS region. Compared with other known ITS sequence sources from *K. pictus* populations, only four variable nucleotide sites were found within the entire ITS region. Very narrow genetic diversity appearing in the population level of *K. pictus* makes us hypothesize that their relatively isolated habitats. The long-lived traits might be one main reason. However, another probability was that the nrDNA ITS region might be noneffective in classifying populations of *K. pictus*. Thus, to further understand the phylogenetic relationship of *K. pictus* populations, more samplings should be performed based on more DNA sequences.

Keywords *Kalopanax pictus*, genetic diversity, nrDNA ITS, population structure

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Introduction

Kalopanax pictus (*K. septemlobus*), a deciduous tree in the genus *Kalopanax* (Araliaceae), is distributed mainly in the temperate regions of East Asia (Lee 1997). The stem bark of *K. pictus* has early been used in traditional medicine in neuralgia, rheumatic arthritis, lumbago, furuncle, carbuncle, wound, diarrhea and scabies (Namba 1994). In Europe and North America, this species has been cultivated as an ornamental tree because of the "tropical" appearance of its large palmate leaves. In Korea, *K. pictus* has been used as a vegetative or functional food (Park et al. 2005) and a medicinal muscle relaxant (Jung et al. 2003). Recently, a variety of biological activities have been found from the bark and stem of *K. pictus*, including antidiabetic, cytotoxic, antifungal, and anti-inflammatory properties (Park et al. 2001; Li et al. 2002; Hu et al. 2010).

Because *K. pictus* is usually found on subsites of several Korean mountains with elevations of 300 to 400 m, it is considered as a species with narrow habitat (Jung et al. 2003). Their typical populations are sometimes small and distributed in patches (Jung et al. 2004; Huh 2006). Despite this species is distributed in a wide geographic range, their populations are ecologically restricted, resulting in relatively isolated, local populations. As known, populations growing in different environmental conditions with species may produce some variations in morphological characteristics, genetic structure, environment adaptation and so on. Thus, the understanding of their phylogenetic relationships according to the genetic diversity becomes required.

For a better characterization and comparison of *K. pictus* germplasm diversity, morphological differences have been analyzed among Korean populations (Jung et al. 2004). A 16.2% of differentiation rate was found among nine Korean populations based on 23 morphological characteristics (Jung et al. 2004). Among these morphological characteristics, the number of lobe of palmatified leaf, minor groove of palmatified leaf, and length from basal sinus to central lobe apex were most useful in the infraspecies variation analysis of *K. pictus* in Korea (Jung et al. 2004). To gain detailed information on the levels and distribution of genetic variation and population structure, allozyme diversity was estimated between cultivated populations and wild populations (Jung et al. 2003). Based on variation analysis of 10 enzymes, the polymorphic rate of loci reached 55.6% and the genetic diversity at the species and population levels were 0.200 and 0.149, respectively. The genetic variation and structure of Korean populations have also been investigated using the random amplified polymorphic DNA (RAPD) method (Huh et al. 2005) and inter simple sequence repeat (ISSR) markers (Huh 2006). Using 12 RAPD primers, 49 loci were found among six *K. pictus* populations in Korea, of which 29 loci showed polymorphic, ranging the polymorphic rate of 59.2% (Huh et al. 2005). To analyze the phylogenetic relationships of Korean *K. pictus* populations, 11 ISSR primers were used and the polymorphic rate of the reproducible ISSR bands was 64.1% (Huh 2006). All these results suggested that geographical distribution and reproductive isolation between wild plants and cultivars plays important roles in shaping the population structure of this species. To further understand the genetic diversity and phylogenetic relationship of *K. pictus*, a clearer identification of this species is required.

As the demands of species discrimination increase, traditional organoleptic and chemical methods do not satisfy humans increasing needs. An accurate, universal, stable, and specific marker is undoubtedly beneficial. Compared with traditional classification, molecular identification sharing more advantages has been widely applied in phylogenetic studies (Hinrikson et al. 2005). The most frequently sequenced region for plant phylogenetic studies is the nuclear ribosomal DNA (nrDNA) internal transcribed spacer (ITS, Coleman 2003). Because of its high species discrimination, technical ease of amplification, and high primer universality, the nrDNA ITS region has been used in various organisms at the genus and species levels (Sun et al. 2010a, 2010b, 2011a, 2011b; Sun and Hong 2011). In this study, the genetic diversity and structure of 18 different *K. pictus* populations in Korea was detected based on the nrDNA ITS region. The objective of this study is to find out how various the ITS nucleotide sequences are among different *K. pictus* populations, and whether the geographic factors mainly affect the genetic diversity of this species. This work would help clear understanding of genetic diversity and population structure of *K. pictus* in Korea, as well as variety protection of this species.

Materials and Methods

Plant materials

Fresh leaf tissues were collected from 18 natural populations of *K. pictus* from different geographical origins in Korea (Table 1). Voucher specimens identified by plant classification expert, Wan-Geun Park, Department of Forest Resources, Kangwon National University, Chuncheon, Korea.

DNA extraction and PCR amplification

DNA was extracted from fresh leaf tissues of *K. pictus* populations using the modified cetyltrimethylammonium

Table 1 Geographical origin distribution and NCBI GenBank accession number of the 18 *K. pictus* populations in Korea

Population	Geographical origin	NCBI GenBank accession number
HCSN1	Hwacheon-gun Sanae-myeon	JQ048740
HCSN2	Hwacheon-gun Sanae-myeon	JQ048741
HCSN3	Hwacheon-gun Sanae-myeon	JQ048742
HCSN4	Hwacheon-gun Sanae-myeon	JQ048743
YGYG1	Yanggu-gun Yanggu-eup	JQ048744
YGYG2	Yanggu-gun Yanggu-eup	JQ048745
IJBU1	Inje-gun Buk-myeon	JQ048746
IJBU2	Inje-gun Buk-myeon	JQ048747
GPHA1	Gapyeong-gun Ha-myeon	JQ048748
GPHA2	Gapyeong-gun Ha-myeon	JQ048749
UJSE1	Uljin-gun Seo-myeon	JQ048750
UJSE2	Uljin-gun Seo-myeon	JQ048751
HSGC	Hoengseong-gun Gapcheon-myeon	JQ048752
HSUC1	Hoengseong-gun Ucheon-myeon	JQ048753
HSGG	Hoengseong-gun Gonggeun-myeon	JQ048754
HSUC2	Hoengseong-gun Ucheon-myeon	JQ048755
HNSS	Haenam-gun Samsan-myeon	JQ048756
IJIJ	Inje-gun Inje-eup	JQ048757

bromide (CTAB) method described by Doyle and Doyle (1987). Common ITS primer sets ITS5, 5'-GAA AGT AAA AGT CGT AAC AAG G-3' and ITS4, 5'- TCC TCC GCT TAT TGA TAT GC-3' were used to amplify the nrDNA ITS region including ITS1, 5.8S rRNA, ITS2 regions (White et al. 1990). PCR amplification was conducted using this set of primers with the following program: 35 cycles of denaturation at 95℃ for 1 min, annealing at 53℃ for 1 min, and a final extension step at 72℃ for 1.5 min. All PCR products were purified before DNA sequence analysis using a QIAquick PCR Purification Kit (QIAGEN, Korea) according to the manufacturer's instructions. Purified PCR products were then sequenced at MACROGEN Advancing through Genomics (Korea, http://dna.macrogen.com/kor/).

Sequence editing and alignment

For editing and assembly of the complementary strands, the software program DNAMAN version 6.0 (Lynnon Biosoft Corporation, USA, www.lynon.com) was used. Analogue of our sequences and nucleotide sequence comparisons were detected with Basic Local Alignment Search Tool (BLAST) network services against databases (http://www.ncbi.nlm.nih.gov/). The multiple sequence alignment of ITS region (ITS1, 5.8S rRNA gene, and ITS2) of all the 18 *K. pictus* populations was performed to detect single nucleotide polymorphisms, using DNAMAN version 6.0 software constructing firstly and then man-made checking.

Results and discussion

The nrDNA ITS region comprises the ITS1 intergenic spacer, 5.8S rRNA, and the ITS2 intergenic spacer, with entire ITS region less than 700 bp (Sudheer Pamidimarri et al. 2009). Due to its ease of amplification and high informative variations, the ITS region has been used in numerous systematic studies at genus and species levels of a wide array of plant taxa (Sun et al. 2010a, 2010b, 2011a, 2011b; Sun and Hong 2011). In this study, the entire ITS regions were successfully amplified by PCR using common primer sets, ITS4/ITS5, which is located in the 3' 18S rRNA and 5' 28S rRNA gene, respectively (White et al. 1990). The PCR products consisted of partial 18S rRNA gene, complete ITS1 region, 5.8S rRNA and ITS2 region, and partial 28S rRNA gene sequence (GenBank accession number, JQ048740 ~ JQ048757), with the entire ITS region of 616 bp identical in all the 18 *K. pictus* populations (Table 2, Fig. 1).

Environment is a good and strong factor to produce a phenotype having potentials to adapt to the environment by genetic variation. Morphological variation of *K. pictus* has been suggested to be distinct among Korean populations collected from different geographical habitats (Jung et al. 2004). In addition, *K. pictus* performs both asexual and sexual reproduction, and these both abilities could not only enhance genetic variation but maintain this enhanced variation (Bayer 1990; Jung et al. 2003). Thus, genetic diversity was predicted to be obtained among Korean populations based on the nrDNA ITS region sequence. However, through molecular analysis based on the nrDNA ITS region, very high identity of nucleotide sequence was found among Korean populations investigated in this study (data not shown). It was suggested that all the 18 *K. pictus* populations might belong to one same isolate. In addition, there are thousands of copies of the ITS region existing in Angiosperm genomes, and the nuclear ribosomal RNA gene complex is a tandem repeat unit of one to several thousand copies (Baldwin et al. 1995). This complex having several domains that evolve at varying rates (Jorgenson

Table 2 Integrity, sequence length (bp) and G+C content (%) of the nrDNA ITS region (ITS1, 5.8S rRNA, and ITS2) from different voucher specimens of *K. pictus*

NCBI GenBank accession number	ITS1 region			5.8S rRNA gene			ITS2 region		
	Integrity	Length (bp)	G+C content (%)	Integrity	Length (bp)	G+C content (%)	Integrity	Length (bp)	G+C content (%)
JQ048740*	+	223	62.33	+	160	54.38	+	233	63.95
AJ786228	+	222	63.96	+	162	51.85	+	226	63.27
AY304818	-	210	62.27	+	162	54.94	-	211	63.03
EF152175	-	223	62.78	+	160	54.38	+	234	64.10
AY256899	+	223	61.88	+	160	54.38	+	233	63.52
GU054645	-	214	62.62	+	163	54.60	-	242	63.64

*The detailed information of sequence JQ048740 presents JQ048740 ~ JQ048757

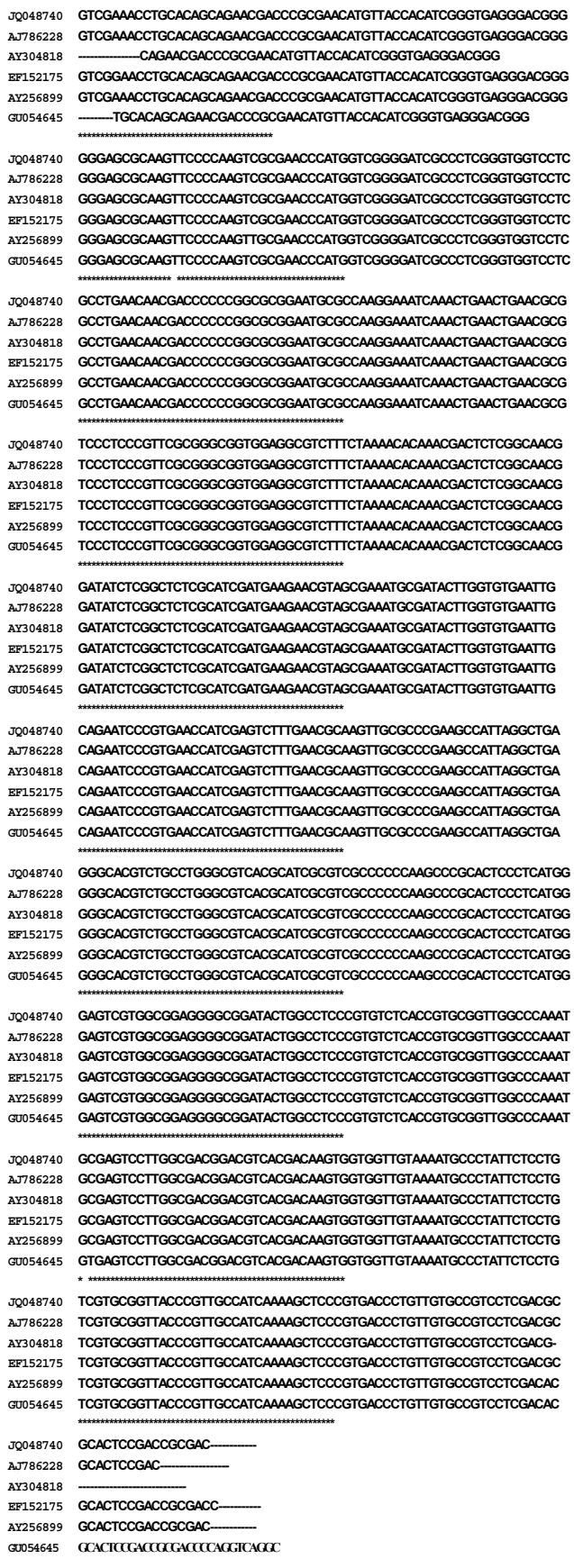

Fig. 1 Sequence alignment of different voucher specimens of *K. pictus*

and Cluster 1988), should possess various informative variations and different poylogenetic utilities, such as hybridization, concerted evolution. In this study, the absolutely identity of sequence in the nrDNA ITS region also indicated that there might be no hybridization, mutation or concerted evolution existing in Korean populations of this species. However, we did not eliminate the probability of low intraspecies variation level and inefficiency of the nrDNA ITS region. All available data suggested that the genetic diversity among *K. pictus* populations could not be evaluated based on the nrDNA ITS region sequence.

To further understand the genetic diversity and evolution relationship of *K. pictus* populations, we searched all the known ITS sequences from different *K. pictus* populations in the GenBank database of NCBI (http://www.ncbi.nlm.nih.gov/, Table 2). Except our sequences from 18 different populations, there were only five ITS sequence sources obtained from the GenBank database of NCBI, including two complete ITS sequences (AJ786228 and AY256899) and three partial ITS sequences (AY304818, EF152175 and GU054645, Table 2). Due to no clear criterion of region discrimination for ITS1 and ITS2 region, there were some shifts of several nucleotide sites found among these ITS sequences. This situation of obscure region definition is available in many sequence sources from the GenBank database of NCBI, such as *Hedyotis corymbosa* and *H. diffusa* previously analyzed in our laboratory work (data now published). Although this situation has nearly no affect on genetic diversity and phylogenetic analysis, it could result in length variation of the ITS region sequence. Our sequences of the nrDNA ITS region from 18 *K. pictus* populations showed the identical region discrimination to that of AY256899 provided by Wen et al. (2003). Due to sharing the same definition of the ITS1, 5.8S rRNA, and ITS2 region, there was no length variation between our sequences and AY256899 (Table 2). The ITS1 region of AJ786228 ended one nucleotide site earlier than our sequence, while that of GU054645 ended one nucleotide site later. Although the region definition between the ITS1 and 5.8S rRNA was the same in EF152175 as that of our sequence, the beginning location of the ITS1 region of EF152175 had exceeded that of our sequence, suggesting that the ITS1 region of EF152175 was longer than that of our sequence. The region definition for ITS2 was more disordered than the ITS1 region: compared to the region discrimination of AY256899 and our sequence, AJ786228 showed one nucleotide site later in the beginning of ITS2 region, AY304818 showed five, and GU054645 showed three; in the ending of ITS2 region, AJ786228 showed six

nucleotide sites earlier than that of AY256899 and our sequence, EF152175 showed one nucleotide site later than AY256899 and our sequence (Table 2, Fig. 1). In addition, GU054645 having exceeded 12 nucleotide sites was suggested to be partial ITS2 region, indicating that the ITS2 region of GU054645 was much longer than that of AY256899 and our sequence.

Despite the region discrimination for ITS1, 5.8S rRNA, and ITS2 was different among all the *K. pictus* ITS sequences, there was nearly no influence on genetic diversity and phylogenetic analysis. Compared with the entire ITS region sequences among all the *K. pictus* populations, there were only four variable nucleotide sites located in 5 bp, 80 bp, 480 bp, and 597 bp, respectively (Fig. 1). Our sequences were identical to AJ786228 and AY304818 in the ITS region, while EF152175, AY256899, and GU054645 were found to have their specific variable sites. This result suggested that this species showed very narrow genetic variation in the nrDNA ITS region. The main reason of narrow genetic variation was that the geographical habitats of this species are relatively isolated so that opportunites for genetic diversity were low.

In conclusion, very narrow genetic diversity was found in the nrDNA ITS region among Korean populations of *K. pictus*. Using the ITS region to analyze the nucleotide sequence variation could not achieve the understanding of *K. pictus* population structure. The main reason might be their isolated habitats of *K. pictus* populations in Korea. In order to further understand the genetic diversity among *K. pictus* populations, more samplings should be performed and a variety of DNA sequences should be used to investigate in further investigations.

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