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GENETIC CHARACTERIZATION OF SPECIES IN GENUS *POPULUS* BASED ON *TRNK* GENE

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Abstract

Many species in the genus *Populus* are ecologically and economically important forest tree species. In the present study, genetic characterization of selected species from all the six sections of *Populus* was evaluated using chloroplast *trnK* gene. Two nucleotide diversity parameters, π and θ_W reached 0.00341 and 0.00603 respectively, which meant relatively low level of nucleotide diversity of these species in *trnK* gene. Results of neutral tests showed no significance, indicating the neutral evolution of *trnK* gene. Phylogenetic analysis showed monophyly of this genus. All the species of genus *Populus* were separated into two clades in the phylogenetic tree. The phylogenetic pattern of some species was discussed in detail, such as *P. mexicana* and *P. tremuloides*. The present investigation illustrates the presence of genetic variability among species of *Populus* and signifies *trnK* gene as a potential marker in phylogenetic analysis.

Key words: *Populus*; *trnK*; Nucleotide diveristy; Phylogenetic construction

Introduction

The genus *Populus* comprising of many economically and ecologically significant forest tree species, distributed throughout the Northern Hemisphere, from subtropical to boreal forests (Stettler *et al.*, 1996; DiFazio *et al.*, 2011). Species in this genus are also well known for the rapid growth rate, tolerance to biotic and abiotic stress, profuse vegetative propagation and multiple usage of wood (Cronk, 2005). Since the completion of the genome sequence of *P. trichocarpa* (Tuskan *et al.*, 2006), species in genus *Populus* have become excellent research models in many fields of plant biology and plant genetics. Based on 76 morphological characteristics, *Populs* is divided into six sections consisting of 29 species, which are named *Turanga*, *Abaso*, *Leuce*, *Aigeiros*, *Tacamahaca* and *Leucoides* (Eckenwalder, 1996). This taxonomic classification of genus *Populus* has generally been accepted by many poplar taxonomists and researchers (Hamzeh & Dayanandan, 2004; Cervera *et al.*, 2005). Taxonomists in China have identified as many as 62 species, including six hybrid taxa and a number of *varietas* and *forma* (Wu, 1999).

Nucleotide diversity of *Populus* has been investigated using various methods, such as SSR and AFLP markers (Li *et al.*, 2007; Han *et al.*, 2017; Zong *et al.*, 2018). However, little has been conducted using sequencing data. Furthermore, phylogenetic analyses of *Populus* based on sequencing data as well as morphological characteristics supported the monophyly of this genus (Eckenwalder, 1996; Hamzeh & Dayanandan, 2004; Hamzeh *et al.*, 2006; Wang *et al.*, 2014; Liu *et al.*, 2016; Huang *et al.*, 2017). However, a reconstructed phylogeny of *Populus* using 151 AFLP markers from 28 species showed that *P. mexicana*, distributed exclusively in Mexico, North America, showed the highest differentiation from other species in the genus *Populus* and clustered as a single clade (Cervera *et al.*, 2005). This result supported the polyphyly of genus *Populus* to some extent. Thus, the underlying aim of the present study was to estimate the taxonomic status (monophyly or polyphyly) of the genus *Populus* and nucleotide diversity as well as evolutionary relationships among species within this genus based on chlorophast *trnK* gene. Previous literature also provides the utilization of other chloroplast regions for phylogenetic reconstruction of different plant families (Shinwari *et al.*, 2014; Zahra *et al.*, 2016; Shinwari *et al.*, 2018; Khan *et al.*, 2019).

Materials and Methods

Samples collection: 26 *Populus* species covering all the six sections and seven out-group species (*Idesia polycarpa*, *Poliothysis sinensis* and five *Salix* species) were collected (Table 1). Genomic DNA was extracted from silica gel-dried leaves for all sampled individuals using CTAB method (Doyle, 1987). Furthermore, sequencing data of the following species, *P. fremontii*, *P. maximowiczii* and *P. mexicana* as well as those of the outgroup species were directly collected from GenBank.

PCR amplification and sequencing: PCR was performed in a volume of 30 µL comprising of 1 µL genomic DNA (10 ng/µL), 1.2 µL of each primer (25 pM), 1.5 µL of each dNTP (2 mM), 1.5 µL $MgCl_2$ (25 mM), 0.3 µL ex Taq DNA polymerase (0.15 U) (TaKaRa, Shiga, Japan), 2.5µL of Taq buffer (10×) and 16.3 µL nano pure water (Wang *et al.*, 2014). The sequences of primers was as follows: F: 5'-TCAGTGCTGGTTATCCAATTACAG-3', R: 5'-ATTATCTGTCAGAGGGACTAATAC-3' (Liu *et al.*, 2016). Amplification was carried out in a temperature gradient 96 U thermocycler (Eppendorf, Germany) as follows: 10 min at 94°C followed by 35 cycles of 90 s at 94°C, 40 s at 55°C, 90 s at 72°C, and a final extension at 72°C for 5 min. The PCR products of each individual were examined by electrophoresis on 1.0% agarose gel and purified using a DNA Purifica-tion kit (Amersham Pharmacia Biotech, Piscataway, NJ, USA). Afterwards, the purified DNA products were sequenced in an ABI 3730 DNA analyzer (Applied Biosystems). For sequencing, a pair of new primers was developed, trnK-3F: 5'-TTTCTTAAGACTGTTCAAATTCCA-3', trnK-3R: 5'-ATTGGATTTGCTGTGATA-3'.

Table 1. The detailed information for the samples used in this study.

Genus	Section	Species	Geographic coordinates	GenBank accession no.
Populus	*Leuce*	*P. alba*	N47°43′ E86°52′	KF940864
		P. hopeiensis	N34°44′ E106°07′	KF940871
		P. tremula	N60°27′ E25°02′	KF940884
		P. tomentosa	N39°58′ E116°21′	KF940883
		P. adenopoda	N30°22′ E110°26′	KF940862
		P. davidiana	N30°17′ E110°28′	KF940867
		P. tremuloides	N48°24′ W123°23′	KF940885
		P. grandidentata	N48°24′ W123°22′	KF940870
	Leucoides	*P. lasiocarpa*	N30°23′ E110°26′	KF940873
	Aigeiros	*P. deltoides*	N35°32′ W86°35′	KF940868
		P. afghanica	N39°29′ E75°59′	KF940863
		P. nigra	N47°22′ E87°48′	KF940875
		P. fremontii	N41°38′ W111°55′	KJ664926
	Turanga	*P. pruinosa*	N38°32′ E70°05′	KF940877
		P. euphratica	N47°42′ E86°48′	KF940869
	Tacamahaca	*P. cathayana*	N45°58′ E126°35′	KF940866
		P. balsamifera	N51°11′ W115°35′	KF940864
		P. koreana	N47°02′ E129°02′	KF940872
		P. maximowiczii	NCBI	EF135587
		P. simonii	N38°11′ E100°16′	KF940879
		P. suaveolens	N53°07′ E123°05′	KF940880
		P. ussuriensis	N47°02′ E129°02′	KF940887
		P. trichocarpa	N48°26′ W123°22′	KF940886
		P. przewalskii	N36°55′ E101°43′	KF940878
		P. pamirica	N40°03′ E75°52′	KF940876
		P. talassica	N48°4′ E86°26′	KF940882
	Abaso	*P. mexicana*	N32°12′ W110°58′	KX454943
Salix		*S. amygdaloides*		EU790673
		S. chaenomeloides		EU790678
		S. exigua		DQ875034
		S. floridana		EU790674
		S. interior		DQ875027
Idesia		*I. polycarpa*		FJ670040
Poliothyrsis		*P. sinensis*		EF135586

Data analysis: Assembled contigs of each sequence for each individual were aligned in CLUSTAL X (Thompson *et al.*, 1997) and refined manually with Bioedit (Hall, 1999). The number of haplotypes (H) and segregating sites (S), haplotype diversity (H_d), as well as nucleotide variation parameters, π (Nei, 1987) and Watterson's θ_w (Watterson, 1975) were analyzed using DNASP 6 (Librado & Rozas, 2009). Fu and Li's D^* and F^* (Fu & Li, 1993) and Tajima's D (Tajima, 1989) were calculated using DNASP 6 (Librado & Rozas 2009) to test whether the data conformed to neutral evolution.

Phylogenetic analysis: The phylogenetic tree concerning all the sampled individuals were estimated in MEGA X (molecular evolutionary genetics analysis) (Kumar *et al.*, 2018) with neighbour-joining method. The support value of each node was evaluated using 100 bootstrap analysis.

Results and Discussion

The aligned sequence of *trnK* gene in all the species in the present study was 1198 bp in length and the number of segregation sites and haplotype were 27 and 20, respectively. Two nucleotide diversity parameters, π and θ_W reached 0.00341 and 0.00603, which meant relatively low level of nucleotide diversity of these species in *trnK* gene. Results of neutral test showed no significance, indicating the neutral evolution of *trnK* gene (Table 2).

Phylogenetic tree constructed using neighbour-joining method in MEGA X with *trnK* gene showed the monophyly of genus *Populus*, which was consistent with the results from other phylogenetic analysis based on sequencing data and morphological traits (Eckenwalder, 1996; Hamzeh & Dayanandan, 2004; Wang *et al*., 2014) (Fig. 1). Therefore, the result of Cervera *et al*., (2005) may relate to the method employed and/or the unique fragments generated from *P. mexicana*.

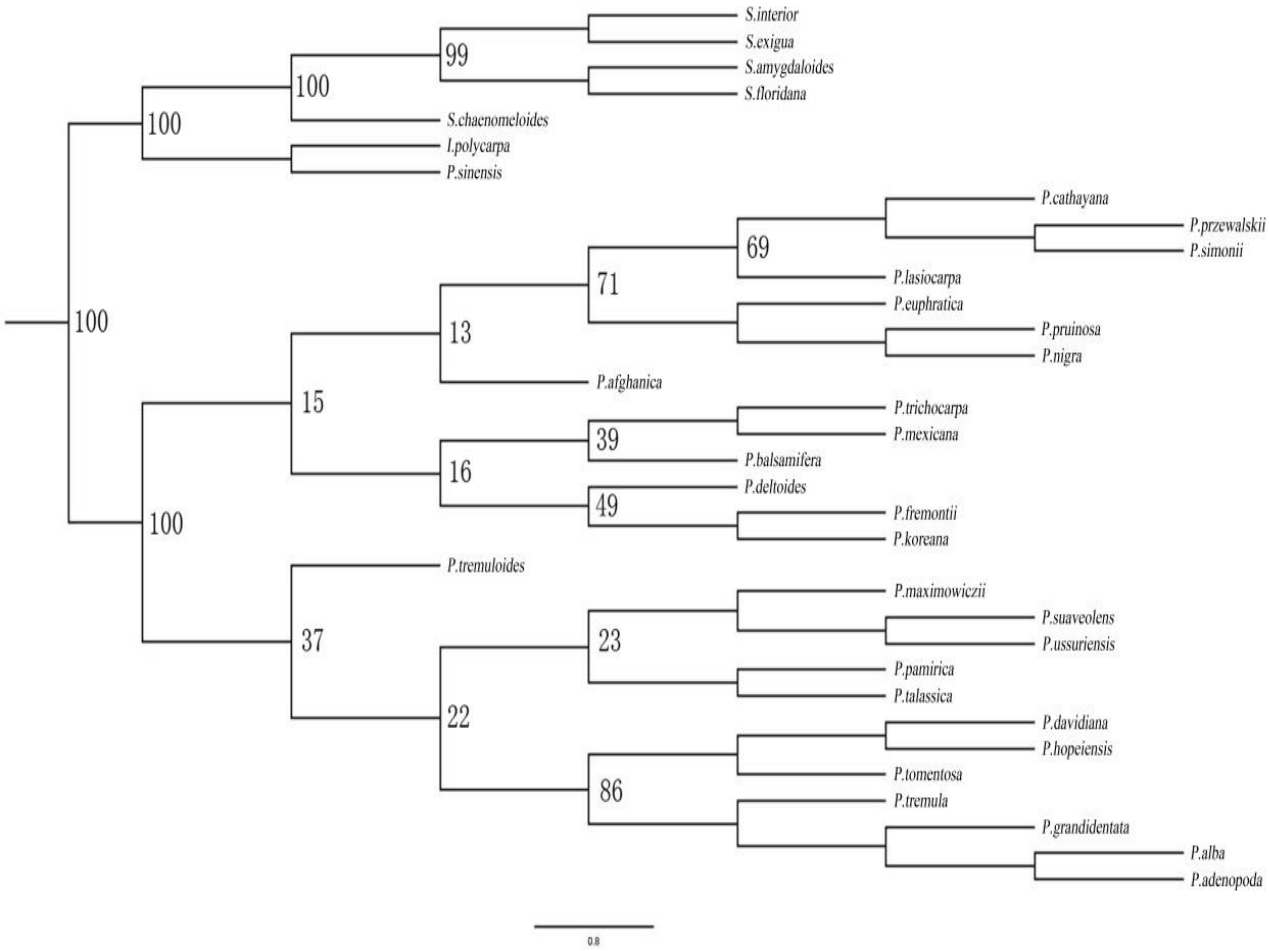

Fig. 1. Phylogenetic tree of *Populus* constructed using *trnK* gene.

Table 2. Characterization of *trnK* gene in selected *Populus* species.

	Length L (bp)	No. of segregation sites S	No. of haplotype H	Nucleotide diversity π	Nucleotide diversity θw	Tajima's D	Fu and Li's D*	Fu and Li's F*
trnK	1198	27	20	0.00341	0.00603	-1.742	-2.220	-2.431

All the *Populus* species were divided into two clades. Clade I was further divided into two clusters and included species from section *Abaso*, *Turanga*, *Leucoides*, *Tacamahaca* and *Aigeiros*. However, the bootstrap values of these nodes that represented the division were relatively low, indicating the low resolution of a single locus in resolving interspecific relationships (Wang *et al*., 2014; Liu *et al*., 2016). In clade I, it was found that species from *Aigeiros* and *Tacamahaca* showed close affinity to section *Leucoides*, *Turanga* and *Abaso*, such as the close relationship of *P. deltoides* and *P. fremontii*, *P. korean*, which suggested the relatively comprehensive origin of these two sections. This polyphyly phylogenetic pattern was consistent with other researches suggesting that the origin of these two sections involved species from other sections (Eckenwalder, 1996; Wang *et al*., 2014). Clade II comprised of species from section *Leuce* and *Tacamahaca*. Reproductive isolation existed between extant species from section *Leuce* and species from other sections, which corresponded well with the phylogenetic pattern that all the species of section *Leuce* clustering in a single clade and illustrated the terminal evolutionary position of this section in *Populus* (Zsuffa, 1975; Eckenwalder, 1996).

The origin of some species can be inferred based on the phylogenetic tree constructed in the present study. *P. mexicana*, the most ancient *Populus* species proposed based on morphological characteristics and fossil records (Manchester *et al*., 1986; Eckenwalder, 1996), did not illustrate a basal position to other species of *Populus* but clustered with species from section *Tacamahaca* although the bootstrap value was not high. Chloroplast capture was taken into consideration in explaining the special phylogenetic position of *P. mexicana* and thus the exact original location of genus *Populus* or the exact most primitive species or section in this genus need more exploration (Liu *et al*., 2016). *P. nigra* is a species with wide distribution in Northern Hemisphere. The putative maternal parent giving rise to *P. nigra* was *P. alba*, and some phylogenetic analysis have provided support for this hypothesis (Smith & Sytsma, 1990). However, in the present study *P. nigra* clustered with section *Turanga* in clade I, hence the origin of *P. nigra* would need further investigation. *P. tremuloides*, a species in section *Leuce* and showed basal position to other species in clade II. This phylogenetic pattern was similar to that in Wang *et al*., (2014), which might be resulted from the long branch attraction or the special nucleotide composition of this species.

Conclusion

The present investigation illustrates the presence of genetic variability among species of *Populus* and signifies *trnK* gene as a marker in phylogenetic analysis.

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