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A MOLECULAR PHYLOGENY OF *CERCIS* L. (FABACEAE) USING THE CHLOROPLAST *trn*L-F DNA SEQUENCES

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Abstract

This study included 13 *Cercis* taxa from different parts of the world and an outgroup, *Bauhinia faberi*. Although analysis of the *trn*L-F cpDNA sequences did not resolve the branches of the phylogenetic tree well, it did shed light to some extent on the phylogeny of *Cercis* taxa. Pairwise distances using Jukes-Cantor model among *Cercis* taxa for the *trn*L-F data was very low among many *Cercis* taxa (0.00%) as expected because the sequenced area is a coding region of the tRNAs. The highest distance was 0.064% between *C. racemosa* and *C. chingii* and *C. yunnanensis* and *C. chingii*. Our analysis indicated that *C. chingii* is very different from the other genus members and *C. yunnanensis*, *C. racemosa*, *C. glabra*, *C. gigantea* and *C. chuniana* formed well a clade.

Introduction

Cercis L., has a disjunct distribution in the Northern Hemisphere (Coskun&Parks, Submitted). This genus shows the characteristics of the subfamily Caesalpinioideae and comprises about 10 species of shrubs or small trees. A proper current review of the literature on this genus was given by Coskun (2003) and Coskun & Parks (Submitted).

This paper will discuss the phylogenetic relationships of *Cercis* by presenting evidence from *trn*L-F region of chloroplast DNA, (cpDNA), sequences and question the phylogenetic relationships among the ingroup taxa and between the ingroup and outgroup taxa.

Materials and Methods

Plant material and outgroup selection. Taxon sampling followed the methodology as described in Coskun & Parks (Submitted). We were able to obtain 13 *Cercis* taxa from all the geographic regions of the world in which this genus is present (Table 1). The taxa included in this analysis are *C. canadensis* var. *canadensis*, *C. canadensis* var. *texensis*, *C. canadensis* var. *mexicana*, *C. occidentalis*, *C. chingii*, *C. chuniana*, *C. gigantea*, *C. glabra*, *C. racemosa*, *C. siliquastrum*, *C. californica*, subspecies *californica* (not listed in the literature) and *C. yunnanensis*, now recognized as the synonym of *C. glabra*. Most of the taxa for this analysis is same with the ITS nrDNA data analysis of Coskun & Coskun (Submitted) except addition of the taxon *C. gigantea* and deletion of *C. chinensis* in this analysis.

Cercis plant materials collected and used in this study were also vouchered as herbarium specimens and were deposited in the Herbarium of the University of North Carolina (NCU). Outgroup selection for the study group of plants also followed Coskun & Parks (Submitted). Thus one *Bauhinia* species, *B. faberi*, was sampled as an outgroup that is closely related to *Cercis* in this analysis as well.

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Primer name	5' to 3' Primer sequence	Primer designed by	Based on (the source publication)
*trn*L-F			
Forward			
trnLe ----------	GGTTCAAGTCCCTCTATCCC	Taberlet *et al*., 1991	Taberlet *et al*., 1991
Reserse			
trnFf ----------	ATTTGAACTGGTGACACGAG	Taberlet *et al*., 1991	Taberlet *et al*., 1991

Fig. 1. ITS primers used in this study with their designers.

Genomic DNA isolation, amplification, and data analysis: Total genomic DNA isolation followed the same protocol outlined in Coskun & Parks (2009). Amplifications of *trn*L-F region of the chloroplast DNA were conducted by using the PCR temperature files suggested by Taberlet *et al*., (1991).

*trn*L-F molecular marker analyzed in this study belongs to the chloroplast genome (cpDNA). Polymerase Chain Reaction (PCR) amplifications of *trn*L-F cpDNA were performed using the primers designed by Taberlet *et al*., (1991) for all taxa included in this work (see Fig. 1).

PCR products were purified using 'Qiaquick PCR purification Kit' (Qiagen) and followed the instructions directed by the company. Both strands of DNAs were sequenced for all taxa and the sequences were generated from two or three different individuals for each taxon.

Cycle sequencing reactions, purification of sequenced products, manual checking of data and data analysis were performed as outlined in Coskun & Parks (2009). Generated DNA sequences were submitted to the Genbank and accession numbers obtained from Genbank were given in Table 1.

Results and Discussion

A complete, aligned DNA data matrix for trnL-F region of *Cercis* taxa can be seen in Appendix 1. Pairwise distances using Jukes-Cantor model among *Cercis* taxa for the *trn*L-F data was very low among many *Cercis* taxa (0.00%) as expected because the sequenced area is a coding region of the tRNAs. The highest distance was 0.064% between *C. racemosa* and *C. chingii* and *C. yunnanensis* and *C. chingii* (Table 2). Fig. 2 shows the single most parsimonious *trn*L-F tree following a Branch-and-Bound search. The *trn*L-F data analysis did not resolve well relationships among the *Cercis* taxa (Figs. 2 and 3). However, it indicated that *C. chingii* is very different from rest of the genus members and *C. yunnanensis*, *C. racemosa*, *C. glabra*, *C. gigantea* and *C. chuniana* formed a clade differing from all the other ingroup taxa with a moderately high bootstrap support (Figs. 2 and 3).

Simultaneous with our study, Davis *et al*., (2002) worked on the phylogeny and biogeography of the genus *Cercis* and they found similar but not the same results obtained by our study. Different results were possibly due to the following provisions: they used ITS marker with shorter sequences than the sequences generated in this study since they used different forward primer for sequencing reactions. They also employed less number of taxa than this work such as the use of 11 taxa in their analysis whereas this study used 14 number of taxa. Thus the results of this analysis and the analysis by Coskun & Parks (2009) are different.

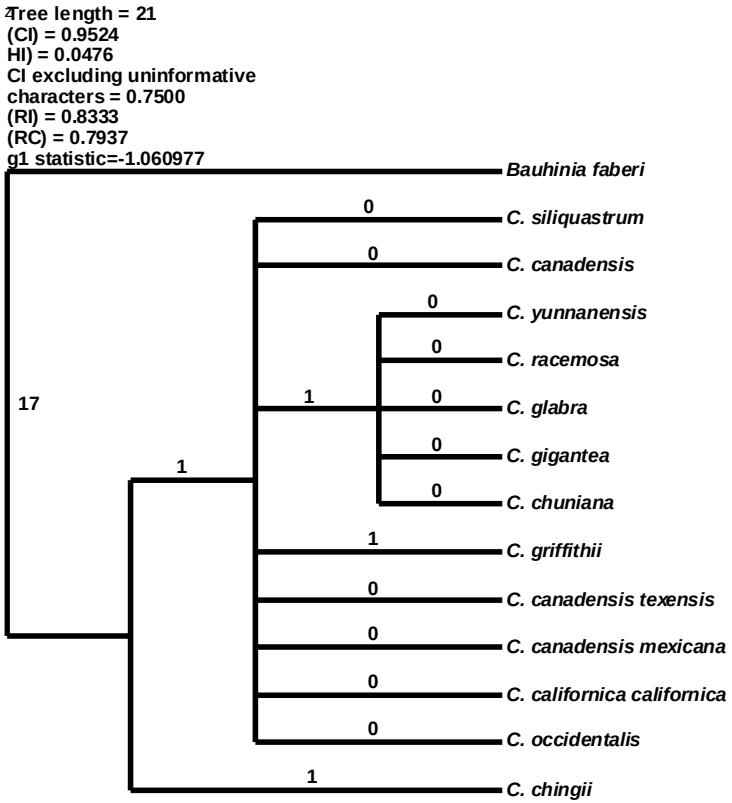

Fig. 2. Single MP *trn*L-F tree of *Cercis* taxa following a Brach-and-Bound search.

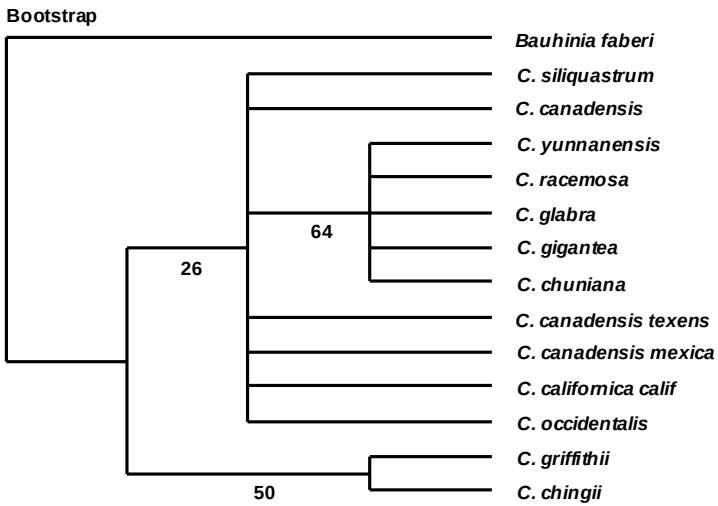

Fig. 3. Bootstrap support for single MP *trn* L-F tree of *Cercis* following a Branch-and-Bound search (bootstrap values below branches).

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