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Genetic diversity of *Dimocarpus longan* in China revealed by AFLP markers and partial rbcL gene sequences

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Abstract

Amplified fragment length polymorphism (AFLP) and partial rbcL gene sequencing were used to investigate genetic diversity among various longan (*Dimocarpus longan* Lour) accessions as well as a presumed closely related species *Dimocarpus confinis* How et Ho and litchi (*Litchi chinensis* Sonn). No significantly shared AFLP fragment was found between the three species, indicating that *D. confinis* and litchi are very far in genetic distance from any longan accession studied. Partial rbcL sequences of 501 bp from the first coding site in these species were obtained, which revealed several substitutes. One such DNA base pair substitute resulted in an amino acid difference between longan and litchi. Furthermore, another 4 bp resulted in a two amino acid difference between longan and *D. confinis*, which was consistent with AFLP results and indicated that *D. confinis* should be excluded from the longan genus, *Dimocarpus*. Within the longan species, no DNA substitute was found. Using nine primer combinations, a total of 66 AFLP markers were obtained from 41 longan accessions. One non-Chinese longan accession 'Miaoqiao' was distinctly different from all other longan cultivars collected in China, indicating that more genetic resources of longan might be collected also from longan production regions outside of China. AFLP markers might be developed

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to identify longan cultivars as well as expedite progeny screening in breeding programs of this perennial fruit tree.

Keywords: *Dimocarpus longan*; Litchi; Confinis (*Dimocarpus confinis*); Amplified fragment length polymorphism (AFLP); Ribulose-1,5-bisphosphatecarboxylase/oxygenase Large fragment (rbcL); Genetic diversity

1. Introduction

Longan and litchi are established tropical fruit crops across Asia, especially in China. While closely related to the subtropical fruit litchi, in the family *Sapindaceae*, the longan is placed in a separate genus, and is currently designated *Dimocarpus longan* Lour (Morton, 1987). Within the *Dimocarpus* genus, there is a presumed closely related wild species *Dimocarpus confinis* How et Ho. It is high yielding and the fruit are pleasant tasting, however, the fruit may be toxic to the human intestine (Law et al., 1985). Both longan and litchi have a number of good quality traits. Interspecific breeding programs have been initiated with the aim of combining these traits of the two species. McConchie (1994) investigated the breeding barriers between commercial litchi and longan cultivars by conducting reciprocal pollinations and obtained two intergeneric hybrids using litchi as the female parent. Unfortunately, interspecific crosses between longan and *D. confinis* have failed to generate any viable progeny up to now. Whether longan and *D. confinis* are cross compatible is not clear. Therefore, a closer examination of the interspecific genetic diversity among longan, litchi and *D. confinis* is needed.

Longan has been cultivated in China for more than 2000 years and over this period 300 cultivars have been selected (Li and Zhuang, 1983). However, a number of production issues remain, including the need to improve fruit quality and agronomic characteristics, the development of which is a key industry focus. To facilitate this process, germplasm of longan from both the field and breeding programs is being actively collected. This is being undertaken in conjunction with the development of efficient methods for identification and classification of these accessions. The genetic diversity in longan studied thus far is mostly based on morphological traits, such as leaf size, maturity time and seed shape have been studied (Ke et al., 1988; Liu and Lin, 1988). However, these morphological traits are largely dependent on environmental conditions, and a few protein markers detected did not allow separation of individual longan accessions. As a result much confusion currently exists regarding the precise indentification of specific cultivars. A comprehensive analysis of the extent and distribution of genetic variation in longan is therefore needed. While our previous studies employing RAPD method to analyze longan genetic diversity garnered some useful data (Lin et al., 1998), we found that RAPD was not powerful enough to solve some longan germline mysteries. Thus, AFLP (Vos et al., 1995) which is one of the most powerful methods developed for characterizing the genetic diversity within species was used.

The chloroplast encoded gene ribulose-1,5-bisphosphatecarboxylase/oxygenase Large fragment (rbcL) was used for the study of plant phylogeny. The full enzyme rbc/o is responsible for fixation of carbon dioxide in the Calvin cycle, and it sits at the nexus of the earth's carbon cycle. It was advantageous to use the rbcL gene for this study for two