Identification of *Acacia auriculiformis* and *A. mangium* using RAPD markers [*1]

(Preliminary study)

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Ⅰ．Introduction

Acacia auriculiformis A. Cunn. *ex*. Benth. and *A. mangium* Willd. are closely related *Acacia* species which belong to section *Juliflorae* (Benth.) Maiden & Betche in the subgenus *Phyllodineae* Benth. The two species are naturally distributed throughout Australia, Papua New Guinea, and Indonesia, and utilized for forest plantation and ornamental. Since *A. auriculiformis* and *A. mangium* overlap in their distribution and their relation is very close, they can naturally cross-hybridize. Interspecific hybridization of these species has been reported by Sedgley *et al*. (*6*). The hybrid possesses morphological characteristics intermediate between the two parents, and shows a remarkably hybrid vigor in growth. Another favorable characteristics such as better growth, straight stem, and pest and disease resistance are recognized in the hybrid *(3)*.

Some breeding programs of these species and the hybrid are in advance. Provenance and progeny trials of each species has been done in many countries and seed orchards for supplying have been established. Since the hybrid of these species shows better appearance than its parents, hybrid seed orchard establishment and controlled pollination activity are included in these species breeding programs.

Generally, *A. auriculiformis* and *A. mangium* can be distinguished by morphological characters such as leaf size and stem bark. *Acacia auriculiformis*, which has high genetic diversity (*6*), also shows a lot of morphological variation. Therefore, discrimination among *A. auriculiformis*, *A. mangium* and their hybrid become more difficult, and a discrimination method by using genetic information is needed. Up to now, there are no reports concerning this subject. Studies on species discrimination have been reported for *Lolium*, pine and *Populus* (*1, 2, 5*). The aim of this study is to identify RAPD markers which can discriminate *A. auriculiformis* and *A. mangium*.

Ⅱ．Materials and methods

Eight samples for each *A. auriculiformis* and *A. mangium* were used for primer screening. For investigating species-specific markers, forty-eight for each species were used. These forty-eight samples represented the natural distribution of the species. For *A. auriculiformis*, seeds were obtained from CSIRO, Australia. The seeds were germinated, and the seedlings were used for the experiment. For *A. mangium,* leafs were collected from provenance/progeny trial forests, which were established by Forest Tree Improvement Research and Development Institute, Indonesia cooperating with JICA, Japan.

Total genomic DNA was extracted from leaf and seedling using a modified CTAB protocol as described by Shiraishi and Watanabe (*7*). The DNA was purified using GENECLEAN III (BIO101) to make a template for the further analyses. RAPD analysis was performed according to Widyatmoko *et al*. (submitted for publication in *Silvae Genetica*).

Ⅲ．Results and discussion

A total of twenty-four 10-mer primers were screened in order to select primers which produced reliable species-specific markers. Of eight primers selected,

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seven primers were Operon Technologies primers and the remaining primer was designed in our laboratory.

RAPD profile of OPN-01 primer is shown in Fig. 1. Each species was representative of 12 individuals. The left 12 samples are *A. mangium*, and the right 12 samples are *A. auriculiformis*. This profile revealed that *A. auriculiformis* has a larger genetic diversity than *A. mangium* has. The same result was also reported by Khasa *et al.* (*4*). One monomorphic and two polymorphic fragments were recognized in each species. Species-specific fragments were one fragment for *A. mangium* and two for *A. auriculiformis*.

A species-specific marker must possess two properties: present in all individuals from one species; and absent in all individuals from the other species (*1*). To confirm the species-specifics of the fragments mentioned above, number of samples in each species was increased up to 48. As the result, some fragments which had been shown only in one species during primer screening were also detected in another species. The primary primer screening of OPN-01 using twelve samples in each species showed that fragment with length 410 bps was detected only in *A. mangium*. In the secondary screening using 48 samples, the 410-bp fragment was detected also in one sample of *A. auriculiformis*. Therefore, this fragment was not a true species-specific marker. Only three species-specific markers were still remained after the secondary screening. Of the three markers, one marker was *A. mangium* specific marker with length 640 bps; and the other two markers were *A. auriculiformis* with length 380 and 500 bps.

In this preliminary study, both *A. auriculiformis* and *A. mangium* species-specific markers were found using RAPD analysis with OPN-01 primer. In the primary screening, some putative species-specific fragments were also revealed in remaining seven primers. However, these markers should be certified to be true species-specific markers in the same manner as the three markers recognized in OPN-01 primer. Thus more species-specific markers can be obtained.

This study showed that RAPD analysis is a good tool for identifying species, since it showed easily multiple informative marker. Unfortunately, this analysis has some disadvantages concerning reproducibility and reliability of the data. Numerous studies have been reported that reproducibility of RAPD was altered by different parameters, such as the ratio of template DNA to primer, concentration of polymerase, and Mg^{2+} concentration. More reproducible and reliable polymerase chain reaction (PCR)-based technique is a sequence-characterized-amplified-region (SCAR). Developing of this marker based on these RAPD fragments is essential in future. The 5' and 3' ends of the DNA fragments should be sequenced and a pair of longer primers (more than 20 mers) homologous to the two ends be synthesized. Thus, species-specific markers of *A. auriculiformis* and *A. mangium* recognized in this study will be utilized as SCAR markers for identifying the two species and their hybrid with high reproducibility and reliability.

References

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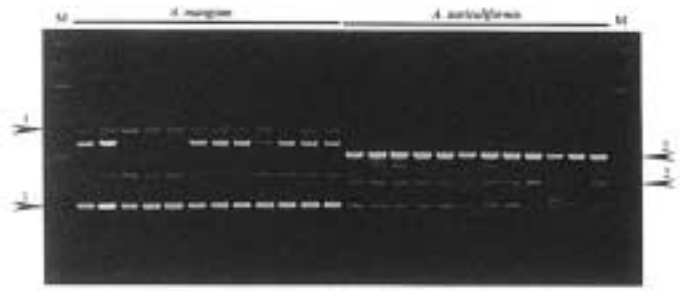

Fig.1 RAPD profile of primer OPN -01
➤1, *A. mangium* specific marker; 2, *A. auriculiformis* specific marker; 3, monomorphic marker for both species M, 100 bp ladder