Original paper

Species-specific RAPD markers for identification of *Acacia mangium*, *A. auriculiformis* and their hybrid [*1]

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Widyatmoko, A. Y. P. B. C. and Shiraishi, S. : **Species-specific RAPD markers for identification of *Acacia mangium*, *A. auriculiformis* and their hybrid Kyushu J. For. Res. 56：66-68, 2003** Identification of *Acacia mangium, A. auriculiformis* and their hybrid is one of the important activities in *Acacia* breeding program. In this study, Random Amplified Polymorphic DNAs (RAPDs) was used to investigate species-specific markers of those species. Three steps of screening were carried out for selecting the species-specific markers. From twenty-four RAPD primers in the first screening, a total of 44 putative species-specific markers of *A. mangium* and *A. auriculiformis* were selected. Since the number of samples of each species was increased from 8 to 48 (second screening) and only fragment with length between 250 and 700 bp was selected for SCAR development (third screening), a total of 7 and 8 species-specific markers were recognized for *A. mangium* and *A. auriculiformis*, respectively. These markers are useful for identifying both species and their hybrid (F_1) in any stage. In future, these markers will be used to regenerate SCAR with higher sensitivity and reproducibility.
Key words: species-specific markers, RAPD, identification, *Acacia*, hybrid

Ⅰ. Introduction

Acacia auriculiformis A. Cunn. *ex* Benth. and *A. mangium* Willd. are important fast growing species for pulp and paper production. The hybrid of these species become important since it possesses morphological characteristic between the two parents, such as better growth, straight stem, high wood density, and pest and disease resistance (*3*).

Recently, breeding program of the two species and their hybrid is going advantageously, such as seed orchard and hybrid seed orchard establishment. Although both *A. auriculiformis* and *A. mangium* can be distinguished morphologically, identification using DNA method will prove the most conclusive. *A. auriculiformis* was reported as ancestor of *A. mangium* and the two species cross-hybridize in nature and in controlled pollination (*9*). Due to intraspecific variation and the widespread potential for gene exchange between the two species, the detection, evaluation and interpretation of interspecific hybridization has been difficult.

RAPDs have been successfully applied as markers to identify species and their hybrid in *Lolium* (*1*), *Populus* (*7*), *Spruce* (*4*), *Larch* (*8*), *Quercus* (*6*) *and Picea* (*5*). Widyatmoko and Shiraishi (*10*) reported the possibility of identifying *A. mangium* and *A. auriculiformis* using RAPD marker. The aim of this study is to identify species-specific RAPD markers in *A. mangium* and *A. auriculiformis*. These markers will be used to generate sequence characteristic amplified regions (SCARs) for discriminating *A. mangium, A. auriculiformis* and their interspecific hybrid.

Ⅱ. Materials and methods

The materials and methods used in this study were same as reported by Widyatmoko and Shiraishi (*10*).

Three-step selections were carried out in order to select species-specific markers of *A. mangium* and *A. auriculiformis*. As mentioned by Delozier *et al.* (*1*), a species-specific marker must possess two properties: present in all individuals from one species; and absent in all individuals from the other species. The first step was screening the RAPD primers produced reliable species-specific markers in 8 samples of each species. The second step was screening the selected primers from step I by using 48 samples of each species. In order to generate SCARs markers that need cloning and sequencing process for recognizing a new primer, only markers with 250 bp to 700 bp of length were selected.

Ⅲ. Results and discussion

[*1] アントニウス・ウィダヤトモコ・白石 進：*A. mangium*, *A. auriculiformis* 及びそれらの雑種を識別するための種特異的な RAPD マーカー
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In the first screening, a total of twenty-four 10-mer primers were screened. The 24 primers were selected by Widyatmoko *et al*. (not published) for analyzing genetic diversity of 4 *Acacia* species of *Juliflorae*. Twenty-one primers revealed putative species-specific marker were selected. Number of putative species-specific marker for *A. mangium* and *A. auriculiformis* was 23 and 21 respectively (data not shown).

In order to recognize the species-specific marker, number of sample of each species was increased to 48 samples (the second screening). Total 7 primers were selected from 21 primers. All the primers were Operon Technologies primers (Table 1). As reported by Widyatmoko and Shiraishi (*10*), OPN-01 was one of the selected primers. This result showed that 8 samples of each species were not enough to select species-specific marker. The number of population for the second screening was 15 for *A. mangium* and 9 for *A. auriculiformis*. Distribution of the population was represented by the natural distribution of each species. Thus, using 48 samples was quite enough for selecting the markers as species-specific markers. Ten *A. mangium* species-specific markers and eight *A. auriculiformis* species-specific markers were selected. The decreasing number of species-specific markers from the first screening to the second screening could be distinguished into 3 chases: markers which are present in all individuals of one species but also in few individuals of the other species; markers which are present almost in one species but not in all individuals and which were also revealed in few individuals of the other species; markers which were absent in all individuals belonging to one species but not present in all individual of the other species.

RAPD analysis is a good tool for identifying species, since it is rapid, cheap and showed easily multiple informative markers. However, this method 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. A minor change in reaction conditions can significantly alter the number and intensity of the amplification products. The type of thermocycler also affected the number of band pattern (*2*). Many research reported that competition was a major source of errors in RAPD analysis.

In future, these markers will be used to generate sequence characterized amplified region (SCAR) with higher sensitivity and reproducibility. A good SCAR for identification should be present in all individuals belonging to one of the two species and absent in all individuals belonging to the other species. Species-specific bands are extracted, cloned and sequenced in order to design new primers of 20－30 mer that are specific to the DNA sequence (SCAR primers). Normally, the sequencer can read clearly until about 800 bp (include about 150 bp of vector's fragment). However, if the marker is too small, a clear and sharp band is not appeared. For the reason, in this study we only selected the marker with the length between 250 bp up to 700 bp. Finally, in the last screening (the third screening), a total of 15 species-specific markers were revealed. The primers and the number of species-specific markers of each primer for both *Acacia* species, including length of each marker, are shown in Table 1.

Table 1 Species-specific markers of *Acacia mangium* and *A. auriculiformis* that have been revealed in this study

No.	Operon primers	Species-specific markers	
		A. mangium	*A. auriculiformis*
1	OPA-08	1 *(390 bp **)	
2	OPC-06	1 (280 bp)	2 (250 bp, 380 bp)
3	OPC-18	1 (620 bp)	1 (550 bp)
4	OPH-15	1 (700 bp)	
5	OPN-01	1 (640 bp)	2 (380 bp, 500 bp)
6	OPW-06		2 (470 bp, 500 bp)
7	OPX-07	2 (470 bp, 490 bp)	1 (450 bp)
	Total	7	8

* : number of species-specific marker
** : length of the marker

Table 1.

The RAPD technique was shown to be convenient for detecting species-specific markers of *A. mangium* and *A. auriculiformis*. The total number of species-specific markers determined in this study is sufficient to identify both the species and their hybrid. Generating the selected markers into SCARs markers is a method to increase the reproducibility and reliability of the markers. The markers are very useful not only for species identification, but can also be used for quick identification of both species at any stage. Khasa and Dancik (*4*) have selected RAPD species-specific markers for white-Engelmann spruce which possible to estimate the hybrid fraction and indicates the true introgression between the two species. Selected species-specific markers can be used for identifying the hybrid of both *Acacia* species in early stages because morphologically it is quite difficult. Therefore, this information is important for counting the number of hybrid and future breeding program.

Even though the number of species-specific markers selected in this study is enough to determine both *Acacia* species and their F_1 hybrid, it is quite difficult to use for determining the F_2 hybrid or introgression of one species to the other species. Thus, additional species-specific markers are necessary for this kind of research.

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