In situ Hybridization to Metaphase Chromosomes in Six Species of *Phaseolus* and *Vigna* Using Ribosomal DNA as the Probe

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In situ hybridization with a biotin-labeled rice ribosomal DNA (rDNA) probe to the somatic metaphase chromosomes of six species of *Phaseolus* and *Vigna* (*P. angularis*, *P. calcaratus*, *P. coccineus*, *P. vulgaris*, *V. sesquipedalis* and *V. sinensis*) was done to determine the sites of rDNA. Hybridization signals were present in the terminal and subterminal chromosome regions of each of the six species. The number of rDNA sites was two in *P. angularis* and *P. calcaratus*, four in *P. coccineus* and *P. vulgaris*, and six in *V. sesquipedalis* and *V. sinensis*.

Key words: In situ hybridization — *Phaseolus* — Ribosomal DNA — Somatic metaphase chromosome — *Vigna*

In situ hybridization has been widely used to detect specific DNA sequences on the chromosomes of such plant species as, *Phaseolus coccineus* (Avanzi, et al. 1972), *Scilla siberica* and *Vicia faba* (Timmis et al. 1975) using radio-labeled probes, and on wheat (Rayburn and Gill 1985, Friebe et al. 1991, Tsujimoto 1993), *Brassica* (Iwabuchi et al. 1991), *pea* (Simpson et al. 1988), and *Allium* (Ricroch et al. 1992) using a biotin- or fluorescence-labeled probe. Only one study, on fluorescent in situ hybridization to soybean metaphase chromosomes (Griffor et al. 1991), has been reported for chromosomes less than 2 μm long.

Beans of economic importance such as adzuki bean (*Phaseolus angularis*), rice bean (*P. calcaratus*), and cow pea (*Vigna sinensis*) that belong to *Phaseolus* and *Vigna* of the Fabaceae have small, morphologically similar somatic metaphase chromosomes (Zheng et al. 1991). The average lengths of the 22 somatic metaphase chromosomes of *P. angularis*, *P. calcaratus*, *P. coccineus*, *P. vulgaris*, *V. sesquipedalis*, and *V. sinensis* range from 1.4 μm in *P. calcaratus* to 2.7 μm in *V. sesquipedalis* (Zheng et al. 1991). We analyzed these bean chromosomes by C-banding (Zheng et al. 1991) and by fluorescent banding (Zheng et al. 1993). Our results show that each species has distinctive C- and fluorescent banding patterns.

We here report a non-radioactive, in situ hybridization method with which to locate specific DNA sequences on these small bean chromosomes as a means of distinguishing between them.

Materials and Methods

Plant materials and chromosome preparations

The six species studied are listed in Table 1. Somatic metaphase chromosomes were prepared according to Zheng et al. (1991). Briefly, 4-day-old root tips were treated with 2 mM 8-hydroxyquinoline for 4 hr at 20°C then fixed in acetic-ethanol (1:3) at 5°C for 24 hr, after which they were treated at 37°C for 30 min with 4% Cellulase ONOZUKA RS (Yakult Honsha Co., Ltd.) and 2% Pectolyase Y-23 (Seishin Seiyaku Pharmaceutical Co., Ltd.). After being rinsed with deionized water and treated with a drop of acetic–ethanol solution, the chromosomes were stained with CMA, DAPI, 4′-6-diamidino-2-phenylindole.

<table>
<thead>
<tr>
<th>Species</th>
<th>Source</th>
</tr>
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<tbody>
<tr>
<td><em>P. calcaratus</em></td>
<td>Tokushima Prefecture</td>
</tr>
<tr>
<td><em>P. coccineus</em></td>
<td>Chiba</td>
</tr>
<tr>
<td><em>P. vulgaris</em></td>
<td>Commercial</td>
</tr>
<tr>
<td><em>V. sesquipedalis</em></td>
<td>Commercial</td>
</tr>
<tr>
<td><em>V. sinensis</em></td>
<td>India</td>
</tr>
</tbody>
</table>

Abbreviations : CMA, chromomycin A3 ; DAPI, 4′-6-diamidino-2-phenylindole.
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were stained with 4% Giemsa solution (Merk). The chromosome preparation was photographed and destained for periods of 5 min in 90, 70, 50, 30, and 15% ethanol then treated at 37°C for 1 hr with 100 μg/ml RNase A (Sigma) in 2XSSC (1XSSC is 150 mM sodium chloride and 15 mM sodium citrate, pH 7.0). After rinses in three

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**Fig. 1.** *In situ* hybridization to somatic metaphase chromosomes of *V. sesquipedalis* with biotin-labeled rice rDNA as the probe. The somatic metaphase plate first was stained with Giemsa solution (a) then hybridized with the biotin-labeled rice rDNA (b). The arrows and arrowhead respectively indicate *in situ* hybridization signals in the terminal and subterminal regions. Bar = 2 μm.