Theor Appl Genet (1993) 87:38–43

THEORETICAL AND APPLIED GENETICS

Fluorescent banding pattern analysis of eight taxa of *Phaseolus* and *Vigna* in relation to their phylogenetic relationships

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Received: 30 October 1992 / Accepted: 1 March 1993

Abstract. Phylogenetic relationships among eight taxa of seven species of *Phaseolus* and *Vigna* (*Phaseolus angularis, P. aureus, P. calcaratus, P. coccineus, P. vulgaris, Vigna sesquipedalis* and *V. sinensis*; 2n = 22 each) were studied by the fluorescent chromosome banding technique. Preparations of somatic metaphase chromosomes of each taxon were sequentially stained with Giemsa, GC-specific fluorochrome chromomycin A_3 (CMA) and AT-specific fluorochrome 4′-6-diamidino-2-phenylindole (DAPI). On the basis of the fluorescent banding patterns of the 22 chromosomes of each taxon, *P. angularis, P. coccineus* (from China and Korea) and *P. vulgaris* were grouped into one group ("*Phaseolus* group"), *P. aureus* and two *Vigna* species were grouped into another ("*Vigna* group") and *P. calcaratus* was grouped in an independent group.

Key words: Fluorescent chromosome banding patterns – *Phaseolus* – *Vigna* – Phylogenetic relationships

Introduction

The *Phaseolus* L. and *Vigna* Savi genera include many economically important species, such as *P. angularis* (adzuki bean), *P. vulgaris* (common bean) and *V. sinensis* (cowpea). Taxonomically, these two genera are considered to be closely related, and some species of *Phaseolus*, such as *P. angularis* and *P. calcaratus*, have been classified into the *Vigna* genus (Verdcourt 1970). Cytological studies on these species have mostly been limited to karyotype analysis (Bhattacharya 1978; Joseph and Bouwkamp 1978; Lackey 1980; Lavania and Lavania 1982; Mok and Mok 1976; Sarbhoy 1977; Schweizer and Ambros 1979; Sen and Bhowal 1960; Sen et al. 1989; Sinha and Roy 1979); no systematic studies using banding techniques have as yet been reported.

We previously reported cytological studies on six species of *Phaseolus* and *Vigna*, i.e. *P. angularis, P. calcaratus, P. coccineus, P. vulgaris, V. sesquipedalis* and *V. sinensis* by use of karyotype analysis and C-banding pattern analysis (Zheng et al. 1991). Our cytological results indicated that *P. angularis* and *P. calcaratus* had more similarities with *Vigna* species than with the other *Phaseolus* species. In this article we present our analysis of the fluorescent banding patterns of eight taxa of seven *Phaseolus* and *Vigna* species.

Materials and methods

Plant materials

The sources of the species used in this study are listed in Table 1. The names of these species are in accordance with those in the List of Genetic Resources of Plants (National Institute of Agrobiological Resources of Japan 1987) and Takashima et al. (1964).

Chromosome preparations

Somatic metaphase chromosomes were prepared according to the method of Zheng et al. (1991). Briefly, root tips were treated with 2 m*M* 8-hydroxyquinoline for 4 h at 20 °C, fixed in acetic-ethanol (1:3) at 5 °C for 24 h and then treated with 4% Cellulase "ONOZUKA" RS (Yakult Honsha Co) and 2% Pectolyase Y-23 (Seishin Seiyaku Pharmaceutical Co) at 37 °C for 30 min.

Sequential staining of chromosomes

The chromosome preparations were first stained with 4% Giemsa solution for 15 min at 20 °C and observed microscopically. They were subsequently stained with fluorescent dyes according to the method of Hizume et al. (1989) with a slight modification.

Communicated by K. Tsunewaki
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Table 1. Sources of the seven species (all 2n = 22) of *Phaseolus* and *Vigna* studied

Species	Source
Phaseolus angularis (Willd.) Wight cv 'Tanba-dainagon'	Commercial (Kyoto Pref.)
P. aureus Roxb.	NIAR 250007[a] (Iran)
P. calcaratus Roxb.	NIAR 070001[a] (Tokushima Pref.)
P. coccineus L.	Commercial (from China)
P. coccineus L.	Commercial (from Korea)
P. vulgaris L. cv 'Kuro-Kinugasa'	Commercial
Vigna sesquipedalis Wight	Commercial
V. sinensis Endl. ex Hassk.	NIAR 200001[a] (India)

[a] Strain code of the National Institute of Agrobiological Resources (NIAR) of Japan

All of the treatments described below were carried out at 20 °C in the dark unless otherwise specified.

The preparations were, after being destained with ethanol, incubated with McIlvaine buffer (pH 7.0) for 15 min followed by counterstaining with 0.1 mg/ml distamycin A (Sigma) for another 15 min. They were then washed with McIlvaine buffer supplemented with 5 m*M* $MgCl_2$, stained with 0.1 mg/ml chromomycin A_3 (Sigma) for 15 min and mounted with glycerol. After being stored at 4 °C for 24 h the preparations were observed and photographed under a Nikon epi-fluorescence microscope with BV filter cassette. They were then destained with acetic-ethanol (1:3) for 30 min, air-dried and incubated with McIlvaine buffer for 15 min followed by staining with 0.25 mg/ml actinomycin D (Sigma) for 15 min. After being rinsed with the buffer, they were stained with 0.2 mg/ml DAPI (Sigma) for 15 min and mounted with glycerol. The preparations were then observed and photographed under the fluorescence microscope with UV filter cassette.

Results

Figure 1 shows metaphase chromosomes of eight taxa of seven species stained sequentially with Giemsa, DA/CMA and AMD/DAPI. All of these species had the same chromosome number (2n = 22, see Table 1). The morphology of the Giemsa-stained chromosomes did not appreciably differ among these species (Fig. 1 a, d, g, j, m, p, s and v), but the fluorescent chromosome banding patterns were more or less species specific after staining with both DA/CMA (Fig. 1 b, e, h, k, n, q, t and w) and AMD/DAPI (Fig. 1 c, f, i, l, o, r, u and x). It should be noted that the morphology of the Giemsa-stained chromosomes did not change upon fluorescent staining and that the sequence of staining with CMA and DAPI did not affect fluorescent chromosome banding patterns (data not shown).

Each of the chromosomes shown in Fig. 1 was separated into three parts, i.e. one proximal (P) and two terminal parts [short arm terminal region (TS) and long arm terminal region (TL)], and these parts were observed to be stained (positive) or unstained (negative) with CMA or DAPI (see Fig. 1). Idiograms of the 22 chromosomes of the eight taxa were constructed by assigning one of the following four staining types to each part of the chromosomes (Table 2): CMA-positive and DAPI-negative type (p/n or G-type), CMA-positive and DAPI-positive type (p/p or P-type), CMA-negative and DAPI-positive type (n/p or B-type) and CMA-negative and DAPI-negative type (n/n or R-type). The capital letters for each type stand for the color of each staining type shown in Table 2.

In the proximal part of the chromosome, the G-type was the major (in *P. angularis*, *P. coccineus* (Korea) and *P. vulgaris*) or sole (in *P. calcaratus* and *P. coccineus* (China)) type present. The major staining type in this region was the P-type in *V. sesquipedalis* and *V. sinensis*, while it was the P- and B-types in *P. aureus*.

In the terminal parts of the chromosome, the major type was the B-type in *P. aureus*, *P. coccineus* (China), *P. coccineus* (Korea) and *P. vulgaris*, and it was the P-type in *P. calcaratus*. *P. angularis*, *V. sesquipedalis* and *V. sinensis* had all of the four fluorescent staining types, in varying proportions in these parts (see Fig. 1 and Table 2).

Accordingly, the characteristics of the overall staining types in the proximal and terminal parts of the chromosomes were similar in three taxa of *Phaseolus*, *P. coccineus* (China), *P. coccineus* (Korea) and *P. vulgaris*; this was also the case for two *Vigna* species, *V. sesquipedalis* and *V. sinensis*. However, the overall characteristics of the chromosomes of *P. angularis*, *P. aureus* and *P. calcaratus* were more or less complex, and thus in order to obtain a precise grouping of these species a detailed analysis of the fluorescent banding patterns of individual chromosomes seems to be required.

If we ignore the asymmetry of the chromosomes, if there is any, there are theoretically 40 types of fluorescent banding patterns in the three combinations of four different fluorescent staining types. For example, the fluorescent banding pattern of chromosome no. 1 of *P. angularis* (see Fig. 1 and Table 2) can be designated as "BGB".

Of the 40 types of fluorescent banding patterns theoretically possible, 20 of them were observed in the eight taxa studied here (Table 2). The diversity of fluorescent banding patterns differed among individual taxa: 9 types in *P. angularis* and *V. sinensis*; 6 types in *P. aureus* and *V. sesquipedalis*; 5 types in *P. vulgaris*; 4 types in *P. coccineus* (China) and *P. coccineus* (Korea); and 3 types in *P. calcaratus*.

Vigna sesquipedalis and *V. sinensis* shared very similar fluorescent banding patterns (*Vigna* type), although they had minor differences. *V. sesquipedalis* had 6 out of 40 theoretically possible types: 6 BPB, 6 BPG, 2 BPP, 5

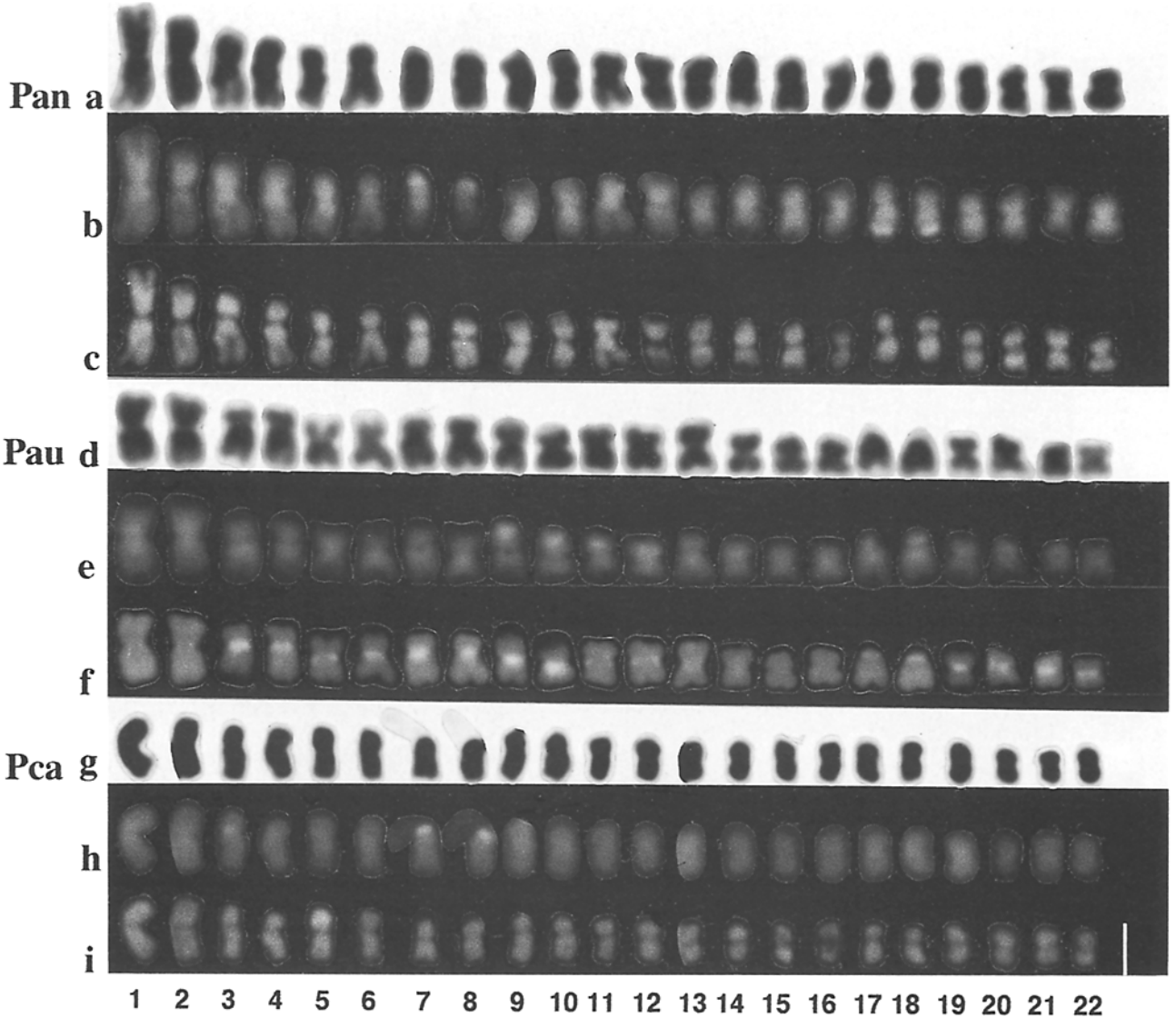
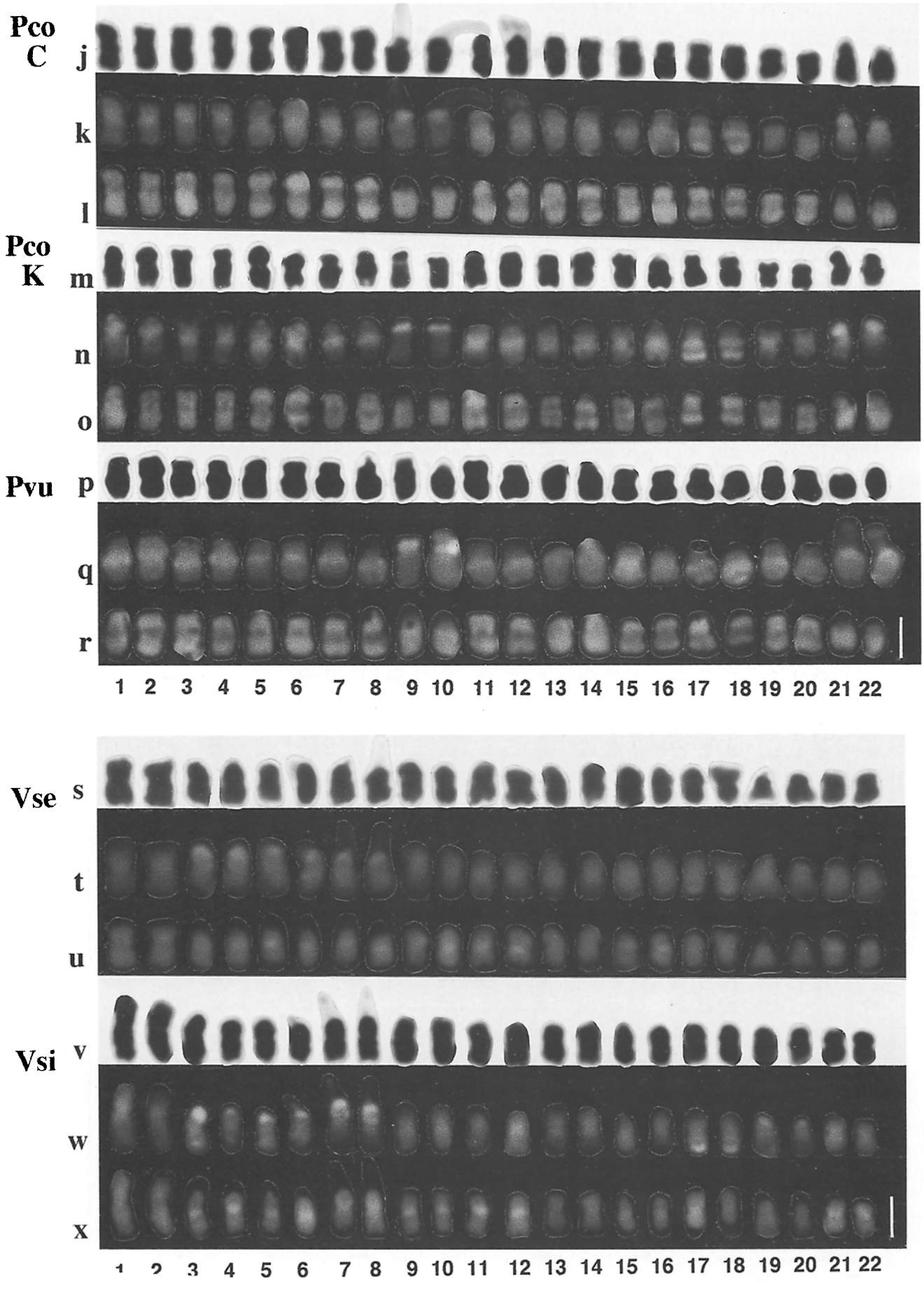


Fig. 1. **A** Mitotic metaphase chromosomes of *P. angularis* (*Pan*), *P. aureus* (*Pau*) and *P. calcaratus* (*Pca*) stained sequentially by Giemsa (*a, d, g*), DA/CMA (*b, e, h*) and AMD/DAPI (*c, f, i*). Chromosomes are numbered in the decreasing order of their lengths. See text for details. *Bar:* 2 μm. **B** Mitotic metaphase chromosomes of *P. coccineus* (China) (*Pco C*), *P. coccineus* (Korea) (*Pco K*) and *P. vulgaris* (*Pvu*) stained sequentially by Giemsa (*j, m, p*), DA/CMA (*k, n, q*) and AMD/DAPI (*l, o, r*). Chromosomes are numbered in the decreasing order of their lengths. See text for details. *Bar:* 2 μm. **C** Mitotic metaphase chromosomes of *V. sesquipedalis* (*Vse*) and *V. sinensis* (*Vsi*) by Giemsa (*s* and *v*), DA/CMA (*t* and *w*) and AMD/DAPI (*u* and *x*). Chromosomes are numbered in the decreasing order of their lengths. See text for details. *Bar*: 2 μm

PPG, 2 RGR and 1 PPP. *V. sinensis* had 9 types of which 5 were the same as in *V. sesquipedalis*: 3 BPB, 6 BPG, 5 BPP, 2 PPG, 2 RPG and 1 each of BPR, PPP, PPR and BGR.

P. coccineus (China), *P. coccineus* (Korea) and *P. vulgaris* also shared similar fluorescent banding patterns (*Phaseolus* type). *P. coccineus* (China) had only 4 out of 40 theoretically possible types: 11 BGB, 7 BGG, 3 BGP and 1 PGP. *P. coccineus* (Korea) also had 4 types of which 3 were the same as in *P. coccineus* (China): 11 BGB, 6 BGG, 3 BGP and 2 BPG. *P. vulgaris* had 5 types of fluorescent banding patterns of which the major ones were the same as those found in *P. coccineus* (China) and *P. coccineus* (Korea): 13 BGB, 6 BGG, and 1 each of BPP, PGP and PPP.

P. angularis, *P. aureus* and *P. calcaratus* had more or less species-specific fluorescent banding patterns. *P. angularis* had the most "diverse" fluorescent banding patterns in which 9 out of 40 theoretically possible types were observed (as in *V. sinensis*, see above). But *P. angularis* and the two *Vigna* species did not share any similarities in fluorescent banding patterns except for the


Pco C
j
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Pco K
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o
Pvu
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1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22
Vse
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1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22

Table 2. Idiogramic representation of fluorescent chromosome banding patterns of eight taxa of *Phaseolus* and *Vigna*

		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22
Pan	TS	n/p	n/p	n/p	n/p	n/p	n/p	p/n	p/n	n/p	n/p	p/p	n/p	n/p	n/p	n/p	n/n	n/p	n/p	p/p	n/p	n/p	p/p
	P	p/n	n/n	p/n	p/n	p/n	p/n	p/n	p/n	p/n	p/n	p/p	n/n	p/n	p/n	p/n	p/n	p/n	p/n	p/n	p/n	p/n	p/n
	TL	n/p	n/p	n/p	n/p	n/p	n/p	n/p	n/p	p/p	n/n	n/p	n/n	n/n	n/n	n/p	n/n	p/n	p/n	p/p	n/p	n/p	p/p
Pau	TS	n/p	n/p	n/p	n/p	n/p	n/p	n/p	n/p	p/n	p/n	p/p	p/p	n/p	n/p	n/p	n/p	p/p	p/p	n/p	n/p	n/p	n/p
	P	p/p	p/p	n/p	n/p	p/p	p/p	n/p	p/p	n/p	n/p	p/p	p/p	p/p	p/p	p/p	p/p	p/n	n/n	n/p	n/p	n/p	n/p
	TL	n/p	n/p	n/p	n/p	n/p	n/p	n/p	n/p	n/p	n/p	n/p	n/p	n/p	n/p	n/p	n/p	n/p	n/p	n/p	n/p	n/p	n/p
Pca	TS	p/p	p/p	p/p	p/p	p/p	p/p	p/n	p/n	p/p	p/p	p/p	p/p	p/p	p/p	p/p	p/p	p/p	p/p	p/p	p/p	p/p	p/p
	P	p/n	p/n	p/n	p/n	p/n	p/n	p/n	p/n	p/n	p/n	p/n	p/n	p/n	p/n	p/n	p/n	p/n	p/n	p/n	p/n	p/n	p/n
	TL	p/p	p/p	p/p	p/p	n/p	p/p	p/p	p/p	p/p	p/p	p/p	p/p	p/p	p/p	p/p	p/p	p/p	p/p	p/p	p/p	p/p	p/p
Pco C	TS	p/n	n/p	p/p	n/p	n/p	p/p	n/p	n/p	p/n	p/n	n/p	n/p	n/p	n/p	n/p	n/p	n/p	n/p	n/p	p/p	p/n	p/n
	P	p/n	p/n	p/n	p/n	p/n	p/n	p/n	p/n	p/n	p/n	p/n	p/n	p/n	p/n	p/n	p/n	p/n	p/n	p/n	p/n	p/n	p/n
	TL	n/p	n/p	n/p	n/p	n/p	n/p	n/p	n/p	n/p	n/p	p/p	n/p	n/p	n/p	n/p	n/p	p/n	p/n	n/p	p/p	n/p	n/p
Pco K	TS	p/n	p/n	n/p	n/p	p/p	p/p	n/p	n/p	p/n	p/n	n/p	n/p	n/p	n/p	n/p	n/p	n/p	n/p	n/p	n/p	p/n	p/n
	P	p/p	p/n	p/n	p/n	p/n	p/n	p/n	p/n	p/n	p/n	p/n	p/n	p/n	p/n	p/n	p/n	p/n	p/n	p/n	p/n	p/p	p/n
	TL	n/p	n/p	n/p	n/p	n/p	n/p	n/p	n/p	n/p	n/p	n/p	n/p	n/p	n/p	n/p	p/p	p/n	p/n	n/p	n/p	n/p	n/p
Pvu	TS	n/p	n/p	n/p	n/p	n/p	n/p	n/p	n/p	p/n	p/n	n/p	n/p	p/p	p/p	p/p	n/p	n/p	n/p	n/p	n/p	p/n	p/n
	P	p/n	p/n	p/n	p/n	p/n	p/n	p/n	p/n	p/n	p/n	p/n	p/n	p/p	p/p	p/n	p/n	p/n	p/n	p/n	p/n	p/n	p/n
	TL	n/p	n/p	n/p	n/p	n/p	n/p	n/p	n/p	n/p	n/p	n/p	n/p	n/p	p/p	p/p	n/p	p/n	p/n	n/p	n/p	n/p	n/p
Vse	TS	n/n	n/n	p/n	p/n	p/n	p/n	p/n	p/n	n/p	p/p	n/p	n/p	n/p	p/p	p/n	p/p	p/p	p/p	p/n	p/n	n/p	n/p
	P	p/n	p/n	p/p	p/p	p/p	p/p	p/p	p/p	p/p	p/p	p/p	p/p	p/p	p/p	p/p	p/p	p/p	p/p	p/p	p/p	p/p	p/p
	TL	n/n	n/n	n/p	n/p	n/p	p/p	n/p	p/p	n/p	n/p	n/p	n/p	n/p	n/p	p/p	p/p	p/n	p/n	n/p	n/p	n/p	n/p
Vsi	TS	n/p	n/n	p/n	p/n	p/n	p/n	p/n	p/n	n/p	n/p	n/p	p/p	n/p	p/p	n/p	n/p	n/p	n/p	p/n	p/n	p/p	p/p
	P	p/p	p/n	p/p	p/p	p/p	p/p	p/p	p/p	p/p	p/p	p/p	p/p	p/p	p/p	p/p	p/p	p/p	p/p	p/p	p/p	p/p	p/p
	TL	n/p	n/p	p/p	n/p	p/p	n/p	n/n	n/p	p/p	p/p	n/p	p/p	n/n	n/n	p/p	n/p	p/n	p/n	n/p	n/n	n/p	n/p

P, TS and TL correspond to proximal, and short and long arm terminal region, respectively, of a chromosome. p (or n)/p (or n) represents a staining type that is positive (or negative) with CMA and positive (or negative) with DAPI. For the abbreviations of the names of each taxa, see legend of Fig. 1. See text (Results) for explanation of colors

sharing of 1 type (BPP type). Instead, the chromosomes of *P. angularis* had more or less "*Phaseolus* type" characteristics with 8 BGB, 4 BGG and 1 BGP types, which were also the major types in *P. coccineus* and *P. vulgaris* (see Table 2). The remainder of the fluorescent banding patterns of *P. angularis* was 3 BGR, 2 PGP and 1 each of the BPP, BRB, BRR and RGR type. Most of these types were specific to *P. angularis*.

P. aureus had 6 types of fluorescent banding patterns, 2 of which were of the "*Vigna* type"; i.e. it had 9 BPB and 2 BPP types, which were also the major types in *Vigna* species. Thus, the chromosomes of *P. aureus* are considered to be similar to those of *Vigna* species. The remainder of the chromosomes were 7 species-specific (BBB) types, 2 BBG, and 1 each of BGP and BRP.

P. calcaratus had characteristically the smallest number (3) of fluorescent banding patterns, and 19 of its chromosomes were of the PGP type. The remainder, of its chromosomes consisted of 1 BGP and 2 PGG types. In contrast to *P. angularis* and *P. aureus*, *P. calcaratus* had no similarities in fluorescent banding patterns to either the *Vigna* species or the other *Phaseolus* species studied here.

Discussion

Based on the results of our fluorescent banding pattern analysis, the eight taxa can be grouped as follows. Two cultivars of *P. coccineus* and *P. vulgaris* can be separately grouped from two *Vigna* species (*Phaseolus* and *Vigna* groups, respectively).

Of the 22 chromosomes of *P. angularis* 13 are of the *Phaseolus* type in terms of fluorescent banding patterns, while only 1 is of the *Vigna* type (see Fig. 1 and Table 2 and discussion). Thus, this species can be placed in the *Phaseolus* group. On the other hand, 11 of the chromosomes of *P. aureus* are of the *Vigna* type, while only 1 is of the *Phaseolus* type. Thus, this species can be placed in the *Vigna* group. In contrast to *P. angularis* and *P. aureus*, *P. calcaratus* has no similarities in its fluorescent banding pattern with *Vigna* species or other *Phaseolus* species. Thus, this species can be placed in a group that is independent from either the *Phaseolus* or *Vigna* group.

We previously reported (Zheng et al. 1991) that the C-banding patterns of *P. angularis* and *P. calcaratus* are rather more similar to those of *V. sesquipedalis* and *V. sinensis* than those of *P. coccineus* and *P. vulgaris*. Also, *P. aureus* had a C-banding pattern similar to that of the *Vigna* species (unpublished results). These results, except for *P. angularis* and *P. calcaratus*, are in agreement with the present fluorescent banding analyses. We are currently studying restriction fragment length polymorphisms, of eight taxa to elucidate their phylogenetic relationships at a DNA level. The results of these analyses will be published elsewhere.

Acknowledgements. The authors are grateful to the National Institute of Agrobiological Resources (NIAR) and Hiroshima Botanical Garden of Japan for supplying the plant seeds used in this study. This research was supported in part by the Grants-in-Aid from the Ministry of Education, Science and Culture of Japan.

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