Karyomorphology of Several Species of *Phaseolus* and *Vigna*¹

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Of the nearly 500 species in the tribe Phaseoleae (Hutchinson 1964) karyotype has been completely described in only 5 species (Cionini and Avanzi 1972, De and Krishnan 1966a, 1966b, Frahm—Leliveld 1965, Marechal 1969). This is primarily due to the small chromosome size and the low frequency of cells in mitotic divisions under normal growing conditions.

Moh (1962) described the chromosome morphology of *Phaseolus vulgaris*, using a low temperature pre-treatment in a saturated solution of paradichlorobenzene (PDB) for 4-6 hours. Immersion in PDB at 4°C for 6 hours caused a build-up of early or pro-metaphase cells (Moh and Alan 1964). When brought back to room temperature for 30 minutes, the normal division processes resumed almost immediately.

Marechal (1969) provided total complement length, longest and shortest chromosome length and an average chromosome length within the complement for each of 8 species of *Phaseolus*. In other studies (Krishnan and De 1965, and Shrivastava *et al.* 1973) the individual chromosomes ranged from 0.44 to 3.33 microns and the total length ranged from 11.44 microns to 23.00 microns. The range of the longest chromosome divided by the shortest in a complement was 1.6 to 4.0.

The genus *Vigna* belongs to the tribe Phaseoleae. The diploid chromosome number of the genus as reported by Darlington and Wylie (1955) is 22. There are also some species with 20 chromosomes and some reported with 24 (Sen and Bhowal 1960).

In conjunction with the problem of classification, Sen and Bhowal (1959) studied 11 species of *Vigna* for taxonomic purposes. All the chromosomes of the 11 species are similar in that the number of long, medium, and short chromosomes was about equal. They stated that the gross morphology of the 11 species was similar.

Marechal (1969) reported on 21 plant introduction lines including 14 species of *Vigna*. The total length of the complements ranged from 13.03 microns to 20.90 microns. The ratio of longest chromosome divided by the shortest within each plant ranged from 1.75 to 2.36.

Materials and methods

Seeds of *P. bracteatus* Nees et Mart. (P 1 158831), *P. lathyroides* L. (P 1 316464),

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P. multiflorus Willd. (P 1 358087), *V. angularis* Willd. (P 1 322573), *V. caracalla* L. (P 1 145800), *V. radiata* L. (P 1 291366), *V. trilobata* Chiov. (P 1 286306), and *V. umbellata* Thumb (P 1 286300) were obtained and germinated in moist filter paper in petri dishes in darkness (Shrivastava *et al.* 1973). When the radicles were 1 cm long, they were pre-treated in a saturated solution of PDB for 2.5 hours. *P. multiflorus* and *V. angularis* were placed in the solution at 10°C as higher temperature did not condense and spread the chromosomes sufficiently. The other 6 species were treated at 16–17°C. The root tips were then immediately washed in dH₂O and transferred to a fixative described by Kita *et al.* (1959) for 15 minutes. The time between pre-treatment and fixation did not exceed 15 minutes (Moh 1962). The root tips were transferred to 70% ethyl alcohol (EA) and held at –6°C until they were observed (Darlington and LaCour 1962).

P. bracteatus was hydrolyzed in a 1:1 solution of 95% EA: conc. HCl for 4.5 minutes, transferred to a 70% solution of EA for 5 minutes, then placed in basic fuchsin (BF) for 10 minutes, and squashed in 2% acetopropiono-carmin in the usual manner. *V. caracalla* was hydrolyzed in 1N HCl at 60°C for 2 minutes, *V. umbellata* for 5 minutes at 55°C and the other 5 species for 5 minutes at 60°C. The individual treatments were found to be optimum for the individual species. The root tips of these 7 species were then given a wash in 70% EA for greater than 2 minutes. They were then transferred to BF for 10–15 minutes and squashed in aceto-propiono-carmin in the usual manner. Slides that were observed to have desirable cells were made permanent by the use of venetian turpentine according to the technique of Wilson (1945).

Slides that were found containing cells with 22 chromosomes visible were photographed. Photographs were also taken of a micrometer at the same power. The chromosome lengths were determined with a divider and measured on a 0.1 mm scale and were converted to microns by comparison of scale with the photo of the micrometer at the same enlargement as the photo of the chromosomes. The mean of the measurements of 2 cells from the same plant were taken when possible as in *P. lathyroides*, *P. multiflorus*, *V. angularis*, *V. caracalla*, *V. radiata*, and *V. umbellata*. For *P. bracteatus* and *V. trilobata* the measurements were taken from 1 cell.

Arm length was measured and other relationships were calculated such as arm ratio, relative length (individual chromosome length divided by total length of the complement times 100), ratio of longest chromosome to shortest in a complement. Centromere class was defined by the arm ratio as suggested by Shrivastava *et al.* (1973); median (M)=1.00–0.76, sub-median (SM)=0.75–0.51, and sub-terminal (ST)=0.50–0.26. When secondary constrictions were observed, they were designated by a slash (/) and the distance in microns from the centromere in the appropriate tables. Idiograms were then constructed for the chromosomes of each species.

Results and discussion

Data obtained are presented in Table 1 and idiograms drawn from the data in Fig. 3.

P. bracteatus was found to have the longest chromosome complement (21.63

Table 1. Chromosome arm length and relative length
of the chromosomes in the species:

(a) *V. angularis*; (b) *V. umbellata*; (c) *P. multiflorus*; (d) *V. trilobata*;
(e) *P. lathyroides*; (f) *V. radiata*; (g) *P. bracteatus*; and (h) *V. caracalla*.

Length in microns		Relative length ¹	Length in microns		Relative length ¹
short arm	long arm		short arm	long arm	
(a)			(b)		
1.03	1.03	12.0	0.95	1.51	13.1
0.83	1.19	11.8	0.64	1.27	10.2
0.87	0.95	10.6	0.87	1.03	10.2
0.79	0.95	10.2	0.79	1.11	10.2
0.48	1.19	9.7	0.87	0.87	9.3
0.64	0.79	8.3	0.79	0.95	9.3
0.48	0.95	8.3	0.79	0.79	8.5
0.56	0.79	7.9	0.64	0.87	8.1
0.64	0.64	7.4	0.64	0.79	7.6
0.48	0.71	6.9	0.48	0.95	7.6
0.44	0.75	6.9	0.56	0.56	5.9
(c)			(d)		
0.56	1.03	11.8	0.59	0.59	12.0
0.71	0.80	11.2	0.47	0.59	10.8
0.56	0.87	10.7	0.41	0.65	10.8
0.64	0.64	9.5	0.47	0.53	10.2
0.48	0.80	9.5	0.47	0.47	9.6
0.56	0.64	8.9	0.41	0.47	9.0
0.56	0.64	8.9	0.41	0.41	8.4
0.40	0.64	7.7	0.35	0.47	8.4
0.40	0.64	7.7	0.35	0.41	7.8
0.48	0.48	7.1	0.24	0.47	7.2
0.32	0.64	7.1	0.29	0.29	5.9
(e)			(f)		
0.64	0.79	12.8	0.48	0.91	11.7
0.64	0.79/0.38 ²	12.8	0.64	0.71	11.3
0.40	0.71	10.0	0.56	0.64	10.0
0.40	0.64	9.3	0.32	0.79	9.3
0.48	0.48	8.6	0.48	0.60	9.0
0.48	0.48	8.6	0.48	0.56	8.7
0.32	0.64	8.6	0.48	0.56	8.7
0.40	0.48	7.9	0.48	0.56	8.7
0.32	0.56	7.9	0.40	0.64	8.7
0.32	0.48	7.1	0.32	0.56	7.3
0.32	0.40	6.4	0.32	0.48	6.7
(g)			(h)		
1.15	1.31	11.4	0.64	0.79	12.6
0.87	1.51/0.87 ²	10.6	0.48	0.79	11.2
0.83	1.35	10.1	0.56	0.64	10.5
1.07	1.07	9.9	0.56	0.56	9.8
1.03	1.03	9.5	0.48	0.64	9.8
0.87	1.19	9.5	0.40	0.64	9.1
0.91	1.11	9.4	0.48	0.48	8.4
0.79	1.11/0.48 ²	8.8	0.32	0.64	8.4
0.64	0.95	7.3	0.40	0.48	7.7
0.56	0.91	6.8	0.32	0.48	7.0
0.71	0.71	6.6	0.32	0.32	5.6

¹ Relative length = chromosomes length $\times$ 100% / total complement length.

² a/b refers to total chromosome length — distance from centromere to secondary construction.

Figs. 1-2. Photograph of somatic pro-metaphase chromosomes of root tip cells. 1, *Phaseolus multiflorus* Willd. 2, *Vigna radiata* L.

Fig. 3. Idiograms of the several species drawn from the data in Table 1.

microns) and *V. trilobata* was the shortest (9.82 microns). Secondary constrictions were only observed in *P. bracteatus* and *P. lathyroides*. In "*P. bracteatus*, constrictions were located 0.87 microns from the centromere on the long arm of chromosome 2 and 0.48 microns from the centromere on the long arm of chromosome 8. In *P. lathyroides*, a constriction was observed 0.38 microns from the centromere on the long arm of chromosome 2."

All species had the least number of chromosomes in the sub-terminal centromere class, and, with the exception of *V. trilobata*, the remaining chromosomes were fairly evenly divided between median and sub-median centromere positions. *V. trilobata* was observed to have 9 chromosomes with median centromere position, but this may have been due to excessive condensation of the chromosomes.

Generally, *Phaseolus* and *Vigna* showed similar morphology. The range of total length of chromosomes in *Phaseolus* was from 11.11 to 21.63 microns and in *Vigna* from 9.82 to 18.73 microns. The range of length of individual chromosomes was from 0.71 to 2.46 microns for *Phaseolus* and 0.64 to 2.46 microns in *Vigna*. The range of chromosome ratio (longest divided by shortest in each complement) was also similar for *Phaseolus*, 1.67 to 2.00, and *Vigna* 1.72 to 2.25. This work is in agreement with respect to ranges of lengths with previous work on these genera. The centromere position did not remain constant for each chromosome in most species.

"Chromosome 3 had a median centromere position in all *Vigna* species except *V. trilobata* a sub-median centromere position in all *Phaseolus* species studied, but no other cytological evidence was found to support the genus change of 5 former *Phaseolus* species to *Vigna* as suggested by Gunn (1973)."

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