Cytogenetical Studies in Genus *Phaseolus* Linn. I and II. Somatic and meiotic studies in fifteen species of *Phaseolus* (Part 1)¹

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Phaseolus, Linn. is economically the most important genus of the family Leguminosae. According to Bessey (1915) and Hutchinson (1948, 1959 and 1964) the genus *Phaseolus* belongs to the family *Papilionaceae* or *Fabaceae*, the most evolved and largest family with about 482 genera and 12000 species, of the order Leguminales.

Bailey (1951) and Hutchinson (1964) recorded from 150 to 200 species of *Phaseolus* Linn. from warm regions of both southern and northern hemispheres. The first high grade taxonomic work on the Indian Flora was taken up by Hooker (1879). He described about 15 Indian species and classified them into four sections- 1) *Phaseolus proper* 2) *Macroptilium* 3) *Strophostyles* and 4) *Dysolobium*.

Verdcourt (1970) has concluded, on the basis of morphological and some biochemical evidences, that *Euphaseolus* Piper (1926) should be considered as true representatives of the genus *Phaseolus* Verdc. The yellow flowered species of *Phaseolus* Linn. under the section *Ceratotropis* Piper should be transferred to the genus *Vigna* subgenus *Ceratotropis* Verdc. and according to this revision *Phaseolus aureus* Roxb., *P. mungo*, Linn., *P. aconitifolius*, Jacq., *P. calcaratus*, Roxb; *P. trilobus*, Ait and *P. ricciardianus*, Tenore have been designated as *Vigna radiatus* (L.) Wilczek., *V. mungo* (L.) Hepper, *V. aconitifolius* (Jacq.) Marechal, *V. calcaratus* (Roxb.) Kurz. (= *V. umbellata* (Thunb.) Ohwi and Ohashi), *V. trilobata* (L.) Verdc. and *V. umbellata* (Thunb.) Ohwi and Ohashi, respectively. The purple flowered species of *Phaseolus* under the section *Macroptilium* Benth. have been placed under the new genus *Macroptilium* by Urban; and Verdcourt (1970) has supported it. This revision designates *Phaseolus bracteatus* Nees and Mart. and *P. lathyroides* as *Macroptilium longepedunculatum* (Benth.) Urb. and *M. lathyroides* (L.) Urb. var. *semierectum* (L.) Urb.

Various species and varieties of the genus *Phaseolus*, Linn. are classed as important 'Pulses and Beans', considered to be the most nutritious with high percentage of vegetable proteins next to soybean. Some of the common and important *Phaseolus* species viz. *Phaseolus mungo*, Linn, *P. aconitifolius*, Jacq., *P. lunatus*, Linn., *P. multiflorus*, Willd. and *P. vulgaris*, L. are cultivated as field crop or garden beans throughout India for its edible and nutritious seed which are consumed as 'Pulses'

¹ "Discussion and summary," "acknowledgements" and "Literature cited" are described in Part 2.

or 'Beans'. Plants of the important species of *Phaseolus* yield valuable kind of green fodder and green manure. Young pods are also eaten as vegetable.

The genus *Phaseolus*, Linn. has not received much attention from the cytological and cytogenetical point of view. Several scattered reports—Karpechenko (1952), Kawakami (1930), Tschechow and Kartaschowa (1932), Senn (1938), Turner (1956), Frahm-Leliveld (1953, 1957), Shibata (1962), Pritchard and Gould (1964), Dana (1964) and Singh and Roy (1970) of the determination of chromosome numbers only are available in the literature on the cytology of the genus *Phaseolus*, Linn. but consolidated studies dealing with diverse representatives of this genus are completely lacking. In order to have a generalized idea about the chromosomal behaviour of these important crop plants an attempt has been made to undertake a well knit cytogenetical studies in the genus *Phaseolus*, L.

The present paper deals with detailed mitotic and meiotic studies in fifteen species along with varieties and strains of the genus *Phaseolus*, Linn.

Materials and methods

The seeds of different species and varieties have been obtained from the following sources:

1. *Phaseolus atropurpureus*
(Moc. and Sesse) ex. DC. Fodder Botanist,
Haringhata, West Bengal
P. atropurpureus
E.C. 27615 I.A.R.I., New Delhi
2. *P. aureus*, Roxb.
T1, BR1 and BR2 Bihar Agriculture Institute,
Sabour (Bihar)
China Moong (Maharashtra),
yellow Moong and B1 (Bengal). Central Arid Zone
Jalgaon 781, Moong 305 Agriculture Research Station,
(Punjab), D-45-6 (Gujrat), Jodhpur
RS. 5, RS. 4 (Raj.), I.C.
7856, I.C. 4826, I.C. 682
and I.C. 616
PLM 1066 — I.A.R.I., New Delhi
3. *P. acutifolius*, Gray
EC. 22121 I.A.R.I., New Delhi
4. *P. aconitifolius*, Jacq.
PLMO. 130 I.A.R.I., New Delhi
C.A.Z.A.R.S., Jodhpur
5. *P. bracteatus*, Linn.
EC. 28858 I.A.R.I., New Delhi
6. *P. Calcaratus*, Roxb.
EC. 18565 C.A.Z.A.R.S., Jodhpur
I.A.R.I., New Delhi
7. *P. helvolus*, Linn.
EC. 28853 I.A.R.I., New Delhi

8. <i>P. lunatus</i> , Linn.
EC. 27870	I.A.R.I., New Delhi
9. <i>P. multiflorus</i> , Willd.
Crusader	Carters tested seed Ltd.,
Rayne's Park, London S.W. 20
10. <i>P. mungo</i> , Linn.
PLU. 155
ST. 8, BR. 10, BR-61,
and BR 68	I.A.R.I., New Delhi
Bihar Agriculture Institute
Sabour (Bihar)
11. <i>P. ricciardianus</i> , Tenore	Fodder Botanist, Haringhata
West Bengal
12. <i>P. triolobus</i> , Ait
EC. 37804 and I.W. 2214	I.A.R.I., New Delhi
13. <i>P. semierectus</i> , Linn.
EC. 38196	I.A.R.I., New Delhi
14. <i>P. vulgaris</i> , Linn.
var. Climbing (Peabean)	Carter's tested seeds Ltd. London
<i>P. vulgaris</i> , Linn.
var. Dwarf (The Prince)	" " "
<i>P. vulgaris</i> , Linn.
vars. Climbing and Dwarf	Sutton and Sons Ltd., Seed Store,
Calcutta-16,
13, D, Russell Street
15. <i>Tetraploid Phaseolus</i>
Species—Dana	Fodder Botanist,
Haringhata, West Bengal

For somatic chromosomal studies root tips were pretreated in aqueous saturated solution of paradichlorobenzene for 2–4 hours at 10–14°C (Sharma and Mookerjee 1955). Pretreatment was followed by fixation in acetic alcohol (1 : 3) for 2 to 6 hours. Root tips were then transferred to a mixture of 2% aceto-orcein and N.HCl in the ratio of 8 : 2 at 60°C for 30 to 50 seconds. Deeply stained portion of a root tip was squashed in a drop of 1% aceto-orcein (Naithani and Sarbhoy 1973).

For meiotic studies flower buds were fixed in Carnoy's fixative (3 ethyle alcohol : 1 acetic acid and 1 chloroform) with some crystals of iron acetate for 6 to 12 hours. Squash preparations were made following Bellings 1% aceto-carmin technique.

Temporary squash preparations were made permanent by passing the slide in tertiary butyl alcohol series and finally mounting in Euparal or as detailed before (Sarbhoy 1971, 1977 a, b, c).

Pollen viability was determined on the basis of stainability in aceto-carmin well stained pollen grains with regular shape were considered viable and empty ones without any stain were taken as sterile.

Photomicrographs were taken with the aid of Leitz 'Panphot' microscope fitted with photographic attachment at a magnification of 1800 ×, using oil immersion objective (100.01 ×) and 10 × eye piece.

Camera lucida drawings were made at table level with the aid of Leitz camera

lucida giving a magnification of about $1600\times$.

Observations

Phaseolus atropurpureus (Moc. and Sesse) ex DC.

Somatic chromosomes: The normal diploid chromosome number, in a exotic, drought-resistant Mexican-Australian hybrid species, has been found to be twenty two (Fig. 1). The size of the chromosome vary from 1.0μ to 1.80μ in length. On the basis of the size, the chromosomes could be classified into three categories.

1. Two pairs of long (1.80μ) chromosomes with nearly median primary constrictions.
 2. Six pairs of medium size chromosomes (1.30μ – 1.65μ) with submedian primary constrictions.
 3. Three pairs of short (1.0μ) chromosomes with median primary constructions.
- Total chromatin length : 30.90μ .

Figs. 1–15. Somatic metaphase stage of different species of *Phaseolus*. 1, *Phaseolus atropurpureus* $2n=22$. 2, *P. aureus* $2n=22$. 3, *P. acutifolius* $2n=22$. 4, *P. aconitifolius* $2n=22$. 5, *P. bracteatus* $2n=22$. 6, *P. calcaratus* $2n=22$. 7, *P. helvolus* $2n=22$. 8, *P. lunatus* $2n=22$. 9, *P. multiflorus* $2n=22$. 10, *P. mungo* $2n=22$. 11, *P. ricciardianus* $2n=22$. 12, *P. semierectus* $2n=22$. 13, *P. trilobus* $2n=22$. 14, *P. vulgaris* $2n=22$. 15, tetraploid *Phaseolus* species $2n=44$.

Meiosis: Studies of early prophase stages could not be undertaken due to delicate and slender nature of chromosomes. At diakinesis and metaphase I eleven bivalents have been observed (Figs. 16 and 17). Both ring and rod shaped bivalents have been observed. However, rod bivalents are greater in number. At anaphase I bivalents disjoin regularly and eleven chromosomes reached at each pole (Fig. 18). At telophase I two daughter nuclei have been included within the same pollen mother cell (Fig. 19).

Second prophase was very short and the chromosomes immediately entered into

metaphase II (Fig. 20). Eleven chromosomes were observed to reach each pole at anaphase II (Fig. 21). Four nuclei were observed within a PMC at telophase II (Fig. 22).

Pollen sterility was 3%.

Phaseolus aureus, Roxb. 'Green Gram or Moong'.

Somatic chromosomes: On critical observation of a number of metaphase plates the normal diploid chromosome number was found to be $2n=22$ (Fig. 2). No significant morphological difference in the chromosomes of different varieties could be observed. The chromosomes could be classified as follows:

1. Two pairs of long chromosomes ($2.8\mu-2.6\mu$).
2. Four pairs of medium size chromosomes ($2.0\mu-1.6\mu$).
3. Five pairs of short chromosomes ($1.4\mu-1.0\mu$).

Total chromatin length : 37.40μ .

Meiosis: At diakinesis eleven bivalents were observed. One bivalent is seen attached to the nucleolus (Fig. 23). All the eleven bivalents were observed to orient themselves at the equator at metaphase I (Fig. 24). One or two bivalents showed precocious anaphasic separation into two or four univalents. They were oriented on either side of the equator opposite their probable homologues or they occupied various positions inside the pollen mother cell.

Table 1 gives various types of configurations observed in 50 PMC's at metaphase I.

Table 1.

Types of configuration	Number of PMC's observed
11 _{II}	39
10 _{II} +2 _I	9
9 _{II} +4 _I	2
Total	50

Table 2.

Types of anomalies	Number of PMC's observed
Double chromatin bridge without fragments	4
Double chromatin bridge with fragment	1
Single chromatin bridge without fragment	5
Single chromatin bridge with fragment	2
Normal separation	88
Total	100

It is clear that about 22% of the PMC's showed univalents. The occurrence of two univalents was as high as 18% while 4 univalents were of comparatively rare occurrence (4%).

It seems that univalents in *Phaseolus aureus* arise either due to lack of synapsis at early prophase stages or precocious separation, or both these factors combined together were responsible for their formation. The univalents, resulting due to

precocious separation at metaphase I moved normally to the opposite poles.

Anaphase I was normal in most of the cases (Fig. 25) with eleven chromosomes at each pole. In about 12% cases double or single chromatin bridges with or without fragments were observed at anaphase I (Fig. 26). Table 2 gives the various types of anomalies observed at anaphase I.

At interphase two daughter nuclei were observed within common PMC (Fig. 27).

At metaphase II eleven chromosomes were observed to orient themselves at the equator (Fig. 2). Anaphase II was regular and showed normal separation. A lagging chromosome was observed in one of the poles (Fig. 29). All subsequent stages were normal.

Pollen sterility was 12%.

Phaseolus acutifolius, Gray

Somatic chromosome: The normal somatic chromosome number, in 'Tepary bean' a cultivated species, was found to be $2n=22$ (Fig. 3). The length of the chromosomes ranges between 1.0μ to 2.50μ . The chromosomes could be classified as follows:

1. Three pairs of long chromosomes ($2.2\mu-2.5\mu$).

Figs. 16–59. Meiosis in different species of *Phaseolus*. 16–22. *Phaseolus atropurpureus*. 16, diakinesis showing 11 bivalents. 17, metaphase I with 11_{II} . 18, anaphase I showing (11:11) normal separation of chromosomes. 19, interphase or telophase I—two nucleate stage. 20, metaphase II showing 11 chromosomes oriented on each equator. 21, anaphase II showing normal separation of chromosome with 11 chromosomes at each pole. 22, telophase II with four microspore nuclei. 23–29. *Phaseolus aureus*. 23, diakinesis showing 11_{II} . 24, metaphase I in polar view showing 11_{II} . One bivalent showed precocious separation into 2 univalents. 25, anaphase I showing 11 : 11 separation of chromosomes and a bivalent forming a bridge. 26, anaphase I showing double chromatin bridge. 27, telophase I with two nuclei. 28, metaphase II showing 11 chromosomes oriented on each equator. 29, anaphase II showing a lagging chromosome in one of the poles. 30–34. *Phaseolus acutifolius*. 30, diakinesis with 11_{II} . One bivalent is seen attached with the nucleolus. 31, metaphase I showing 11 bivalents oriented at the equator in equatorial view. 32, anaphase I showing 11 chromosomes at each pole. 33, metaphase II—in equatorial view. 34, anaphase II showing normal separation with 11 chromosomes at each pole. 35–4. *Phaseolus aconitifolius*. 35, diakinesis showing 11 bivalents. 36, metaphase I in polar view showing 11. 37, anaphase I with normal 11 : 11 separation of chromosomes. 38, metaphase II showing 11 chromosomes oriented at each equator. 39, anaphase II showing the failure of spindle mechanism in one of the two metaphase plates. 40, telophase II with four microspore nuclei. 41–47. *Phaseolus bracteatus*. 41, early prophase showing two nucleoli small and big together. 42, early prophase showing two nucleoli small and big separated apart. 43, diakinesis showing two nucleoli and 11 bivalents. Bivalents are seen attached to the nucleolus. 44, metaphase I with 8_{II} and 6_I in equatorial view. 45, anaphase I with normal 11 : 11 separation of chromosomes. 46, metaphase II with 11 chromosomes at each equator. 47, anaphase II with 11 chromosomes at each pole. 48–52. *Phaseolus calcaratus*. 48, diakinesis with 11_{II} . 49, metaphase I with 11_{II} in polar view. 50, anaphase I showing eleven chromosomes at each pole. 51, metaphase II showing 11 chromosomes arranged on each equator. 52, anaphase II showing normal separation. 53–57. *Phaseolus helvolus*. 53, diakinesis showing 11 bivalents. 54, metaphase I in polar view showing 11_{II} . 55, anaphase I with normal 11 : 11 separation. 56, metaphase II showing 11 chromosomes. oriented at each equator. 57, anaphase II showing normal separation with eleven chromosomes at each pole. 58–59. *Phaseolus lunatus*. 58, diakinesis showing 11 bivalents. 59, metaphase I with 11_{II} and one bivalent showing precocious separation into univalents.

2. Five pairs of medium size chromosomes ($1.5\mu-1.8\mu$).
3. Three pairs of short chromosomes (1.00μ).

Total chromatin length : 36.20μ .

Meiosis: Eleven bivalents were observed at diakinesis (Fig. 30). Invariably one bivalent was seen attached to the nucleolus. Both ring and rod shaped bivalents were encountered. However, rod bivalents, were greater in number.

At metaphase I all the eleven bivalents were observed to orient themselves at the equator (Fig. 31). Bivalents disjoined regularly and eleven chromosomes were seen at each pole at anaphase I (Fig. 32).

All the subsequent second stages, metaphase II and anaphase II were regular and normal (Figs 33 and 34).

Pollen sterility was 3%.

Phaseolus aconitifolius, Jacq.

Somatic chromosomes: Commonly known as 'Moth' or 'Maut' or 'Mat Bean'. The normal diploid chromosome number in this wild and cultivated species was found to be $2n=22$ (Fig. 4). The size of chromosomes ranges between 1.00μ to 1.85μ . The chromosomes could be classified as follows:

1. Two pairs of long chromosomes ($1.85\mu-1.70\mu$).
2. Four pairs of medium size chromosomes ($1.5\mu-1.3\mu$).
3. Five pairs of short chromosomes ($1.2\mu-1.0\mu$).

Total chromatin length : 29.14μ .

Meiosis: At diakinesis and metaphase I eleven bivalents were observed (Figs. 35 and 36). Maximum terminalization was achieved by bivalents at diakinesis. Both ring and rod shaped bivalents were observed. However, rod bivalents were greater in number. Bivalents disjoined normally and eleven chromosomes were seen at each pole at anaphase I (Fig. 37).

Metaphase II (Fig. 38), anaphase II (Fig. 39), telophase II (Fig. 40) were found to be regular and normal.

Pollen sterility was 3%.

Phaseolus bracteatus, Spreng.

Somatic chromosomes: The normal diploid number of chromosomes, in this plant believed to be native to Brazil, was found to be 22 (Fig. 5). The chromosomes size ranges between 1.0μ to 1.80μ . The chromosomes could be classified as follows:

1. Three pairs of long chromosomes ($1.80\mu-1.70\mu$).
2. Four pairs of medium size chromosomes ($1.60\mu-1.40\mu$).
3. Four pairs of short chromosomes ($1.2\mu-1.0\mu$).

Total chromatin length= 30.80μ .

Meiosis: Studies of early prophase stages could not be undertaken due to poor staining and delicate nature of the chromosome threads. However, two nucleoli of unequal size were generally observed at early prophase stage (Figs. 41 and 42).

At diakinesis generally eleven bivalents were encountered. In Fig. 43 two nucleoli, small and large, were observed and each was associated with at least one bivalent.

Eleven bivalents were generally found to orient themselves on the equator at metaphase I (Fig. 44). One or two bivalents showed precocious separation into 2 or 4 univalents respectively. They were oriented on either side of the equator opposite their homologues (Fig. 44).

Table 3 gives the chromosomal configuration at metaphase I in 50 PMC's.

Table 3.

Types of configuration	Number of PMC's observed
$10_{II}+2_I$	10
$9_{II}+4_I$	3
$8_{II}+6_I$	2
11_{II}	35
Total	50

It is clear from the above table that about 30% of the PMCs showed univalents. The occurrence of two univalents was as high as 20%, while 4 and 6 univalents were of comparatively rare occurrence.

The univalents were generally included in the anaphasic group of chromosomes. Anaphase I was normal with eleven chromosomes at each pole (Fig. 45).

At metaphase II eleven chromosomes became oriented at each equator (Fig. 46). Anaphase II was regular and showed normal 11 : 11 separation (Fig. 47).

Telophase II was normal.

Phaseolus calcaratus, Roxb. 'Rice Bean'

The normal diploid chromosome number was found to be 22 (Fig. 6). The size of chromosomes ranges between 1.00μ to 1.80μ . The chromosome could be classified as follows:

1. Two pairs of long chromosomes (1.80μ).
2. Six pairs of medium size chromosomes (1.20μ – 1.65μ).
3. Three pairs of short chromosomes (1.00μ).

Total chromatin length : 30.64μ .

Meiosis: Eleven bivalents were generally observed at diakinesis. One bivalent was seen attached to the nucleolus (Fig. 48). All the eleven bivalents were observed to orient themselves at the equator of metaphase I (Fig. 49). Anaphasic disjunction of bivalents was normal and eleven chromosomes were observed at each pole (Fig. 50). Telophase I was normal.

Second prophase was very short and chromosomes immediately entered into metaphase. Eleven chromosome became oriented at each equator at metaphase II (Fig. 51). Anaphase II (Fig. 52) and telophase II were observed to be normal.

Pollen sterility was 4%.

Phaseolus helvolus, Linn. 'Amberique Bean'

Somatic chromosomes: Twenty two was observed to be the normal diploid chromosome number (Fig. 7). The size of the chromosomes ranges inbetween 1.0μ to 1.85μ in length. The chromosomes could be classified as follows:

1. Two pairs of long chromosomes (1.85μ – 1.7μ).

2. Five pairs of medium size chromosomes (1.60μ – 1.3μ).
3. Four pairs of short chromosomes (1.2μ – 1.00μ).

Total chromatin length : 30.30μ .

Meiosis: During the course of meiotic studies not a single abnormality was observed. At diakinesis eleven bivalents were observed (Fig. 53). Invariably one bivalent was seen attached to the nucleolus. Eleven bivalents were observed to orient themselves at the equator of metaphase I (Fig. 54). The distribution of the chromosomes at anaphase I was normal (Fig. 55) with eleven chromosomes at each pole.

Eleven chromosomes were seen arranged at each equator at metaphase II (Fig. 56). Anaphasic disjunction of chromosomes was normal and eleven chromosomes were seen at each pole of anaphase II (Fig. 57).

Pollen sterility was 4%.