

**CYTOMORPHOLOGICAL STUDIES IN SOME  
SPECIES OF GENUS *VIGNA* SAVI**

By  
**H. E. SHASHIDHAR**

Department of Agricultural Botany  
**UNIVERSITY OF AGRICULTURAL SCIENCES**  
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**CYTOMORPHOLOGICAL STUDIES IN SOME  
SPECIES OF GENUS *VIGNA* SAVI**

By  
**H. E. SHASHIDHAR**

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in partial fulfilment of the requirements  
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Bangalore

August, 1980

*Dedicated to*

**MRS. AND MR. RAMANAGOWDA G. PATIL**

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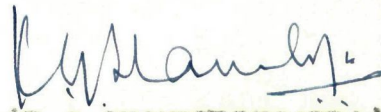
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UNIVERSITY OF AGRICULTURAL SCIENCES

Bangalore

CERTIFICATE

This is to certify that the thesis entitled "CYTOMORPHOLOGICAL STUDIES IN SOME SPECIES OF GENUS Vigna SAVI" submitted by Mr. H.E. SHASHIDHAR for the degree of MASTER OF SCIENCE (AGRICULTURE) in AGRICULTURAL BOTANY (PLANT BREEDING AND GENETICS) of the University of Agricultural Sciences, Bangalore, is a record of research work carried out by him during the period of his study in this University under my guidance and supervision, and the thesis has not previously formed the basis for the award of any degree, diploma, associateship, fellowship or other similar titles.



(K.G. SHAMBULINGAPPA)  
Geneticist (Sunflower)  
G.K.V.K. Bangalore

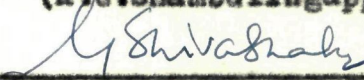
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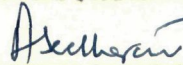
(K.G. Shambulingappa)

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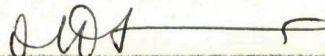
(G. Shivashankar)

2.



(A. Seetharan)

3.



(M. Mahadevappa)

4.



(H.T. Gurusiddappa)

5.



(M. Udaykumar)

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Bangalore

August 12 , 1980

*Shashidhar:HE*

(H.E.SHASHIDHAR)

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# **INTRODUCTION**

## I. INTRODUCTION

The importance of pulses in Indian agriculture is immense as they are the main source of protein component in the diets of cereal eating people. Pulses are generally grown as dryland crops and have, for centuries, been selected mostly for their survival under conditions of stress rather than productivity. Cowpea, (Vigna unguiculata (L.) Walp.) an important pulse crop, is grown in almost all parts of India excepting the high hills. It is a multipurpose crop grown for its green pod for use as vegetable, for grain as pulse and for fodder. Blackgram (Vigna munge (L.) Hepper, syn. Phaseolus munge L.) and Greengram (Vigna radiata (L.) Wilczek, syn. Phaseolus radiatus L.) also play an important role in protein nutrition mainly because of their easy cookability and digestibility.

This fascinating group of plants has defined precise classification since centuries. The classification of plants into families, genera and species, in general, is largely based on similarity of morphological characteristics. Emphasis was laid on floral characteristics like number of stamens, type of ovary, placentation and other floral features. Many decades were to go-by for this spectrum to be enlarged and other morphological characters made use of. Taxonomic classification on an extensive

scale was undertaken by Linnaeus (1753); Bentham and Hooker (1865); Hutchinson (1959), which represent classical work in this field. This information has ever since been the guidepost for all further taxonomic and biological investigations.

With the advancement of time and technology, it was realised that classification which was done by placing weightage on the morphological characteristics, was less reliable and often misleading and that, for a sound classification, a comprehensive and detailed study has to be conducted. The present concept of taxonomical study is multi-directional and includes two or more of the following aspects viz., morphological, cytological, cytogenetical, physiological, anatomical, biochemical and palynological aspects. By considering two or more disciplines together and extrapolating the results of different disciplines it should be possible to obtain reliable and sound grouping of the taxa so studied. Consideration of any one of the above mentioned disciplines for classification of the taxa may not be sound.

Significance of taxonomy to the plant breeder is profound. Breeders resort to hybridization involving plants of different varieties, species and genera. For this a sound taxonomic information is very useful as it denotes nearness or otherwise between the individuals involved. This will also be helpful for collaborative work at the International

level among scientists, for exchange of material and scientific knowledge.

Taxonomists in general have a disliking towards the study of cultivated crops, which is largely due to the great diversity and variation pattern found in domesticated species, but quite uncharacteristic of the wild ones (Heiser, 1969). Furthermore, the scattered distribution of the cultigens over the globe makes sampling a difficult task. The complex nature of the cultivated plants often present difficult nomenclature problems. As a result, very few groups of cultivated plants with their wild relatives have been the subject of detailed taxonomic study (Li, 1974). The observation that the taxonomy of many of the world's most important genera is so imperfect as to be of little practical use (Anderson, 1951), can be aptly applied to the genera Vigna and Phaseolus of Fabaceae.

Karyomorphological investigation particularly at meiosis, yields a wealth of information, utility of which was first demonstrated by McClintock (1929) working with Zea mays. Following the success of this classical analysis of pachytene chromosomes, the potential value of such studies have been realised in tackling numerous cytogenetic problems (McClintock, 1931; Rhoades and McClintock, 1935; Morgan, 1956; Rebbelen, 1960). An inclusive herbaria of the type envisaged by Anderson (1951) for maize would be very useful for the pulses, since little headway can be made in the

taxonomic treatment in this group by studies based on chromosome numbers only. In these, besides morphological characteristics, cytological features especially the data on morphology of pachytene chromosomes would be very useful. Therefore, an evaluation of karyomorphological differences amongst the various species with a view to findout features, if any, that can be correlated with morphological features would be very interesting and useful. This would necessitate the precise identification of each chromosome of the haploid set, a type of study which has been used in the study of maize and its relatives with great benefit.

The study on chromosome morphology especially at pachytene, has been neglected in this genus. Perhaps, the major reason for such negligence was the difficulty in obtaining suitable preparations of the early stages of microsporogenesis. However, the effort needed to obtain suitable preparations of the earlier stages can prove quite worthwhile, for the chromosomes at this stage are much less condensed than they are at mitotic metaphase, and hence, it could show a greater number of morphological landmarks which can help in identification of individual chromosome pairs. Furthermore, when the chromosomes are closely paired the occurrence of even small structural differences between them will be clearly broughtout at this stage.

The five species included in the study are cowpea - V. unguiculata, Blackgram - V. mungo (P. mungo), Greengram V. radiatus (P. aureus L.) and two wild species of genus Vigna viz., V. cylindrica and V. marina.

Briefly, the aims of the present investigation may be summarised as follows:

- 1) To study the karyomorphology of the five species both at somatic stage in root tip cells, and at pachytene in pollen mother cells with a view to find out principle diagnostic features which may be helpful in identification of individual chromosomes and also to study the karyotypic diversities in these species.
- 2) To study the chiasma frequency in the five species at diakinesis.
- 3) To trace the phylogenic relationship among the five species.
- 4) To provide cytological proof or otherwise for the transfer of Blackgram and Greengram to genus Vigna based on the suggestion of Wilesek (1954) and Hepper (1956).

## **REVIEW OF LITERATURE**

## II. REVIEW OF LITERATURE

The taxa belonging to the family Leguminosae and subfamily Papilionaceae have been extensively studied for taxonomic classification. The study has given rise to a lot of controversial views. For example, grouping some species belonging to genus Vigna under Phaseolus and vice versa, can be cited.

Linnaeus (1753) in *Species Plantarum* groups eleven species under Phaseolus including Greengram and Blackgram. The key that Linnaeus (1754) proposed in *Genera Plantarum* for this group is as follows:

Phaseolus: Carina spiraliter contra solem revolula.

Vigna : Carina cum contortis genitalibus spiralis  
essentials est nota.

Savi, (1822; 1824) distinguishes three genera in Papilionaceae viz., Phaseolus, Vigna and Dolichos. As per the key of characteristics given by Savi, Phaseolus - carina spiraliter, Convoluta, vel falcata, vel uncinati and Vigna - calycis labium superium integrum.

The distinction between Phaseolus L. and Vigna Savi is one of the major problems in the classification of the tribe Phaseoleae. In the past, differentiation between the

two genera was based on the degree to which the beak of the keel is incurved.

Bentham and Hooker, (1865) list 47 genera under Papilionaceae based on the key of characteristics given by Linnaeus. Phaseolus L. and Vigna Savi have also been grouped under Papilionaceae.

Hooker, (1897) states that Vigna has twisting herbs or shrubs with exactly the same habit of Phaseolus from which it differs by the style and keel being much less curved and lengthened out.

Hooker and Jackson, (1895) summarising the taxonomic classification from the period of Linnaeus, 1773 to 1885, retains Blackgram and Greengram under Phaseolus.

Bailey, (1949) identifies Black-gram and Greengram under Phaseolus and distinguishes Vigna sesquipedalis, V. sinensis and V. cylindrica as distinct species under genus Vigna. The interspecific differences between them being primarily the length, thickness and nature of pods (erect or drooping).

Wilezek, (1954) keys out the two genera as follows:

Phaseolus: Stipules not prolonged below the point of insertion; keel in a spiral of 1-5 complete turns; stigma

elongated, internal or sometimes very short and terminal or sub-terminal; with or without an apical appendage.

Vigna: Stipules prolonged below the point of insertion; keel erect-incurved, rarely making almost one complete turn; style ending beyond the stigma in a more or less distinct beak.

On this basis Wilczek transfers, for instance, P. radiatus to Vigna and retains P. schimperii Taub., in Phaseolus.

Maekawa (1955) separates the species P. radiatus from genus Phaseolus on the grounds that, the first foliage of in this species is sessile as in Vigna luteola (type species of genus Vigna) and not petiolated as characteristic of Phaseolus vulgaris (type species of genus Phaseolus). With this Maekawa has supported Wilczek's proposed change of nomenclature and has added a new point of distinction.

Hepper, (1956) transfers Blackgram viz., Phaseolus mungo L. into genus Vigna Savi, based on the key proposed by Wilczek (1954) and adds few more characters to distinguish between the two genera Phaseolus and Vigna.

Vigna: Stipules cordate or appendaged below the base; keel straight or spirally twisted; fruit septate.

Phaseolus: Stipules truncate at the base; keel spirally twisted; fruits not septate.

Tourneur (1958), in his studies on seeds and seedlings of some Phaseolus and Vigna species and considers that in Vigna the first pair of leaves, after the cotyledons is sessile, while in Phaseolus it is petiolate. He thus substantiates the transfer of P.radiatus and P.mungo by Wilczek and Hepper.

Taylor, (1966) studied pollen grains of Asiatic and American forms of Phaseolus and concludes that the pollen of P.radiatus and P.mungo is different from the type species, Phaseolus vulgaris by the reticulation pattern of the exine.

Casimir and Marchand, (1966) studied several species from Phaseolus, Dolichos and Vigna with respect to presence or otherwise of Pigeolic acid and reported that it was present in the type species, Phaseolus vulgaris L. but absent in V.luteola (Jacq) Benth, V.unguiculata (L.) Walp. and P.aureus. P.mungo L. was not included in the study. Further chemical study by Thurman and Derbyshire (1967), of globulin pattern separation in several species of the two genera Vigna and Phaseolus also lent support to the change of nomenclature proposed by Wilczek and Hepper.

Fawcett and Randle, (1970) compared the reticulation pattern on the pollen grains and suggested another character of difference between genus Vigna and the genus Phaseolus. They reported that Vigna is characterised by wide open reticulation pollen unlike that of Phaseolus L., which possesses narrow and smooth sculpture on the pollen grain.

Biochemical evidence in support of the transfer of Blackgram and Greengram to Vigna is given by Verdcourt (1970) by studies on protein spotting pattern. He found that P.mungo and P.radiatus showed spotting pattern similar to V.luteola and not like P.vulgaris.

Despite numerous attempts to re-define the distinction between Phaseolus and Vigna, no clear solution to the problem has been reached till now. Several efforts have been made to reduce the confusion by subdividing either Phaseolus or Vigna, or both. However, all such attempts were based on the study of relatively few of the total number of species and no sound decision could be reached until all the species of the sub-tribe have been examined.

According to Verdcourt, (1970; 1971) the type species of Phaseolus (P.vulgaris L.) and that of Vigna Savi (V.luteola (Jacq.) Benth.) are centrally placed examples of natural groupings, and since 'typical' Phaseolus and 'typical' Vigna are

clearly distinct, the merging of the two genera into one is no solution. Alternatively, the subdivision of the combined pair into a series of smaller genera would be premature and would involve creating a large number of new genera.

The best solution may be to separate-off difficult marginal groups as genera or subgenera. Consequently Verdcourt (1970) proposed to restrict Phaseolus to those species which closely resemble P. vulgaris, P. lunatus and P. coccineus in having no prolonged stipules; keel and thickened part of style twisted through more than 360°; keel without a 'Pocket', and having pollen grains with a fine (not coarsely reticulated) sculpture. Other groups previously retained in Phaseolus may be separated or moved to Vigna which is divided into a number of sub-genera pending further study.

The first report of the five species as presented in Index Kewensis is as follows:

- V. cylindrica (L.) Skeels., U.S. Dept. Agril. Bur. Pl. Ind. Bull., 32: 282, (1913).  
V. marina Merril., Interpr. Pump. Herb. Amboin., 285 (1916).  
V. unguiculata Walp., Rep. Bot., Syst. 1:779(1842).  
P. radiata L., Sp. Pl., 725, Hooker and Jackson P. mungo.  
P. aureus Roxb., Nert. Beag., 55, Fl. Ind., 111. 297.  
V. radiata (L.) Wilczek, Fl. Congo Belge and Ruande Urundi. VI. 386 (1954).  
V. mungo (L.) Hepper., Fl. Trop. W. Africa ed. 2, 1: 508(1958).

Karpachenko, (1925) has reported 11 as the haploid number of the genus Phaseolus and Vigna. This has later been confirmed by studies of Kawakami (1930); Tschekow and Kartaschowa (1932); Senn (1938); Turner (1956); Frahm Leli-veld (1953; 1957); Shibata (1962); Pritchard and Gould (1964); Dana (1964) and Singh and Roy (1970) have established 11 and 22 as the haploid and diploid number respectively.

The importance of pachytene analysis in cytotaxonomical study was recognised after the classic work of McClintock (1929) in maize.

Krishnan and De, (1965) studied pachytene chromosomes in Phaseolus aureus and reported range of chromosome length as  $73.3 \mu - 28.1 \mu$  and range in mitotic chromatin length as being  $3.3 \mu$  to  $1.4 \mu$ . Total chromatin length was reported as being  $45.5 \mu$  and  $13.5 \mu$  at pachytene and somatic metaphase respectively.

De and Krishnan, (1967a) conducted cytological studies in Phaseolus munge and reported diploid chromosome number as 22. Seventh and ninth chromosome were reported to be the nucleolar chromosomes at pachytene while second and third chromosomes were the nucleolar chromosomes at metaphase. The chromosomes are said to belong to the differentiated group.

Mukherjee, (1968) conducted analysis of chromosomes at pachytene and at somatic metaphase and reported range in

chromatin length as being  $44.30 \mu$  -  $19.4 \mu$  at pachytene and  $4.5 \mu$  -  $1.9 \mu$  at somatic metaphase.

Metaphase karyotypic study has also been conducted by several workers like, Sen and Bhowal, (1962); Faris, (1969); Marechal, (1970), Sarbhoy, (1977; 1978). The mitotic chromosome measurements have been reported as under.

Species	Diploid No.	Total length of chromatin	Range of chromosome length	Reference
<u>V. luteola</u> (Jacq.) Benth	22	40.62 $\mu$	1.33-2.53 $\mu$	Marechal, 1970
<u>P. vulgaris</u> L.,	22	42.26 $\mu$	1.5 -3.00 $\mu$	Sarbhoy, 1977
<u>P. mungo</u> Roxb.,	22	33.20 $\mu$	1.0 -2.2 $\mu$	Sarbhoy, 1977
<u>P. aureus</u> Roxb.,	22	37.4 $\mu$	1.0 -2.8 $\mu$	Sarbhoy, 1977

Crosses between P. mungo and P. radiata were first effected by Sen and Gosh (1960) and Boling et al. (1961). Cytological study of the cross was conducted by Dana (1966) and De and Krishnan (1967b). Based on these studies it has been concluded that the cytological difference between the two species is one deletion, one duplication and one translocation.

## **MATERIAL AND METHODS**

### III. MATERIAL AND METHODS

Seed material of the five species was procured from the All India Co-ordinated Project on Pulses, the University of Agricultural Sciences, Bangalore. The list of species and the corresponding variety selected for the study is given below:

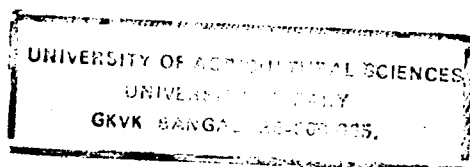
Sl.No.	Name of species	Variety
1	<u>V. unguiculata</u> L. Walp ssp <u>unguiculata</u>	C-152
2	<u>V. mungo</u> (L.) Hepper ( <u>P. mungo</u> L.)	T-9
3	<u>V. radiatus</u> (L.) Wilczek ( <u>P. radiatus</u> L.)	PS-16
4	<u>V. cylindrica</u> (L.) Skeels	-
5	<u>V. marina</u> Merrill	-

The seeds were sown in the Botanical garden in the kharif of 1976. The seeds were also sown at the same time in well prepared pots in the breeding cage. Necessary plant protection measures were taken so as to maintain the plants vigorous and healthy. Plants grown in the pots, inside the cage were used for detailed plant morphological studies. Five plants were considered for observation in each species.

Cytological technique: The material presents certain difficulties in getting well spread and stained preparations. The most common problem was the excessive staining nature of the cytoplasm. It was, therefore, quite necessary to find-

out suitable methods of fixation and staining. The fixatives and stains used vary according to the preference of the workers and material (see review by Sharma, 1956).

It is known that karyotypic analysis is aided by pre-treating the materials with certain chemicals prior to fixation. Numerous chemicals have been employed for this purpose and each has its own limitations. Pre-treatment before fixation has become a routine practice to prevent the clumping together of chromosomes. The use of chemicals like colchicine, 8-hydroxy-quinoline, para-di-chlorobenzene,  $\alpha$ -bromonaphthalene (see <sup>O</sup>hara, 1948; Tjio and Levan, 1950; and Kita et al., 1959) or cold treatment are now generally recommended. Success has often been shown to be due less to the particular fixative or stain used than to the choosing of good vigorous material at the correct stage with strongly growing root tips. Such pre-treatment helps to clarify the chromosome morphology by accentuating both primary and secondary constrictions and by bringing about spreading of the chromosomes. The way in which the pre-treatments influence the structure and behaviour of chromosomes is not clearly understood. It has been suggested that they may cause (1) changes in viscosity of the cytoplasm, (2) constriction of the chromosomes, (3) inhibition or breakdown of the spindle mechanism. It is not possible to single out any particular



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pre-treatment to be universally applicable as, different workers have stressed the superiority of one pre-treatment over the other. The success of any method depends to a large extent on the type of material being examined. The most satisfactory method successfully used in the present study is briefly described below:

For the study of mitotic chromosomes healthy root tips were taken from seeds germinated on wet filter paper in petri-dishes. The germination took place in 3-4 days. Maximum number of dividing cells were observed in the material fixed between 9.00 AM to 10.00 AM. When the root tips were about  $1\frac{1}{2}$  to 2 cms long, they were cut-off from the seeds and were kept for pre-treatment in a saturated solution of  $\alpha$ -Bromonaphthelene for two hours and forty five minutes at  $17^{\circ}$ - $18^{\circ}$ C in a Refrigerator. They were then removed and washed thoroughly in running water and fixed in Acetic-alcohol for 24 hours and stored afterwards in 70% alcohol. Three to four root tips were taken in a watchglass containing nine drops of 0.5% Acetocarmine stain and one drop of 1 N HCl. The watchglass with the contents was heated over a flame. Care was taken to avoid boiling of the stain. Later the root tips were washed with fresh stain to remove all the HCl and the root tips were left in the fresh stain for 2-3 hours for staining. Well stained portion of the root tips was taken and squashed in a drop of acetocarmine. To get good spread

of metaphase plates the coverslip was covered by a filter paper folded three times and pressed uniformly with a rubber backed pencil. Excess of stain was removed by a piece of filter paper. The coverslip was then sealed with wax. The temporary slides were studied and well spread metaphase plates were selected for detailed analysis. Measurements were done by employing camera lucida drawings and stage micrometer. Photomicrographs of the representative metaphase plate were taken from the fresh slides.

The standard acetocarmine method (Belling, 1926) has not been found useful here as cytoplasm absorbs a lot of stain and becomes dark in color. The staining reaction is said to depend on four factors each of which can be varied independently (1) constitution of prefixative, (2) strength of the stain, (3) duration of fixation, storage, and (4) amount of iron introduced. Numerous schedules based on altering these factors and aiming at greater standardization of the procedure have been suggested by different workers to suit the requirements of different crops (Marks, 1952; Hyde and Gardella, 1953; Swaminathan, 1954; Magoon, 1958). All the observations on microsporegenesis were made from temporary preparations.

Depiction of pachytene chromosomes: Various methods of depicting the pachytene chromosomes have been used depending mostly on the type of pachytene chromosomes. In several

species the chromosomes have been photographed and straight line drawings made (Barton, 1950; Gottschalk, 1954 in tomato; Krishnan and De, 1965 in P. aureus; Shambulingappa, 1962 in sorghum). Lima-de-Faria (1952) in secale and Agapanthus and Sarvella et al. (1958) in barley, on the other hand, have followed chromomeric pattern and their intensities. Since in genus Vigna and Phaseolus chromosomes were somewhat similar to tomato and sorghum, the former method developed by Swaminathan et al. (1954) and successfully used in the study of chromosome analysis in sorghum by Shambulingappa (1962), has been used. In this method, buds of the appropriate size are fixed in a mixture of three parts of absolute alcohol and one part of propionic acid saturated with ferric acetate. The appropriate time to collect and fix the material was found to be between 8.00 AM to 9.00 PM. Fixation at low temperature for 24 hours gave good results. The material can be kept in the fixative itself for a few months if necessary. The anthers are smeared in one or two drops of 1% propiano carmine. Judicious warming over the flame before and after tapping gave proper differentiation and spreading. A plated needle was used, to make the smear, otherwise, the staining becomes very dark owing to the addition of more iron by way of the needle. Propiano-carmine was prepared as described by Magoon et al. (1958).

Measurement of chromosomes: Measurement of chromosomes both at mitotic metaphase and at pachytene in pollen mother

cells were accomplished through camera lucida drawings. In order to do this, chromosomes of 6-8 good metaphase plates in root tips and not less than five cells at pachytene for each of the five species were done with the help of the following optical instruments. Meopta ocular 10 x, apochromatic objective 100 x and using a prism type camera lucida. Line drawings along with the morphological features especially for each pachytene chromosome were made at the table level. Each chromosome of the accurately analysable cell was moved to the centre of the microscopic field and drawn carefully thus minimising the amount of error introduced by the limitations of drawing projected image of chromosomes. Individual chromosome from the drawing was then measured with great care using a non-elastic twine thread.

The measurements of mitotic metaphase chromosomes was taken from the line drawing passing through the middle of each arm of the chromosome and extending from one end to the other. In case of pachytene chromosomes, measurements were made from each arm separately leaving the centromeric region (Shambulingappa, 1962).

Relative length: For the sake of convenience in comparing the chromosome sizes both at pachytene and mitotic metaphase relative length was used. Usually, there are two methods for finding out the relative length. (1) Percentage relative length in relation to the total chromatin length, and (2) the

ratio in percentage of the length of the individual chromosomes to that of the longest in the complement (Huziwara, 1956). In the present study the former method has been followed.

The chromosomes measured as described above, were drawn and average values for each chromosome was used in the preparation of the final idiogram for each of the species. In the idiogram of both mitotic as well as pachytene chromosomes the gap of the centromere is kept constant. The chromosomes in both cases are arranged in decreasing order of their length, the longest one occupying the first position and the shortest chromosome being the eleventh with all centromeres occupying the same position (for scale see idiogram).

Chiasma frequency: The cells at diakinesis stage were considered for the analysis of chiasma frequency. Thirty cells at diakinesis, in each of the five species, were scored for bivalents with - (a) three chiasma, (b) two chiasma, (c) one chiasma. Average chiasma per cell and per bivalent was calculated from the data. Recombination index, an useful method to study the extent of homology was also calculated as described by Stebbins (1946).

Photomicrographs: Photomicrographs were taken with a Carl/Zeiss 35 mm camera using a 80 ASA original-Wolfen black and white film, under an optical combination of Eye piece 10 x, apochromatic objective 100 x.

The index of asymmetry or symmetry of the karyotype was calculated using the method adopted by Stebbins (1958) and according to the principles of Levitzky (1931) who defines asymmetry as a condition where number of submedian chromosomes are more than the number of median chromosomes.

Morphological observations were made in plants grown in pots. Critical morphological differences have been depicted in the form of a polygraph (Anderson, 1957; Mehra, 1961). For plotting polygraph twelve morphological characteristics were selected and two to three index values were used. The characters chosen and the corresponding range of variation and respective index values are given in Table 1.

To construct a polygraph a key is made in the shape of a wheel with radiating spokes (Fig.31). The centre of the wheel is represented by a small circle. Each spoke represents one character and the distance along the spoke representing the variation for that character. Only twelve morphological characters were selected to represent twelve spokes. All the characters chosen have been observed objectively. In plotting a polygraph a graph sheet was used to aid precise plotting. After the spokes were marked for each species, the points were joined to form a polygonal figure.

Table 1: List of characters for plotting polygraph  
of the five species

No. of spokes	Character	Variation	Index value for polygraph
1	Habit	Erect	1
		Bushy	2
		Indeterminate	3
2	Duration	70-90 days	1
		90-110 days	2
		110 perennial	3
3	Germination	Epigeal	1
		Hypogeal	2
4	First leaf	Sessile	1
		Petiolate	2
5	Flower color	Yellowish green	1
		Purple	2
6	Flower size	Small	1
		Medium	2
		Large	3
7	Style and Keel	Straight rarely making one turn	1
		Curved making one or more turns	2
8	Apical appendage	Present	1
		Absent	2
9	Hairyness	Hairy	1
		Smooth	2
10	Stipules	Appendaged	1
		Not appendaged	2
11	Shattering nature	Present	1
		Absent	2
12	Length of pod	< 4"	1
		4" - 6"	2
		> 6"	3

## **EXPERIMENTAL RESULTS**

#### IV. EXPERIMENTAL RESULTS

The salient features of external morphology, the detailed study of somatic and pachytene chromosomes and course of microsporogenesis are studied critically in five species of Vigna, and the results are presented species-wise. With regard to external plant morphology, emphasis has been laid on a few key characteristics (Vegetative and reproductive) of diagnostic value in describing these species. As regards chromosome morphology, prepachytene stages are not easily amenable for critical analysis. However the observations made in several nuclei in each of these species showed that pairing initiates at the proximal end and progresses towards the distal end.

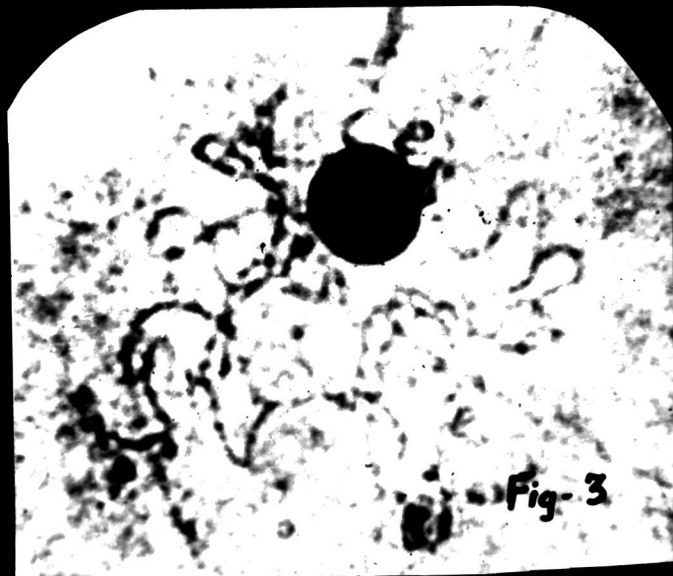
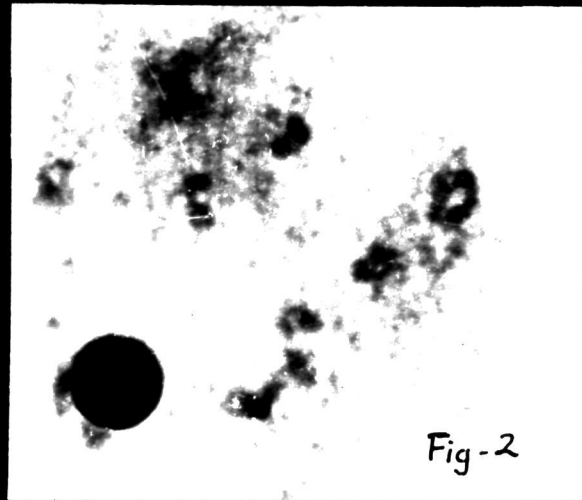
The features considered in the identification of the individual pachytene bivalents are - (1) total length, (2) position of centromere, (3) relative length, and (4) arm ratio.

Based on values obtained by accurate measurements of the chromosomes in several nuclei, where all the chromosomes could be traced from end to end, the eleven pachytene bivalents of each species have been arranged in the descending order of their length, chromosome 1 is the longest while chromosome 11 being the shortest in the complement.

**Fig.1: Plant of Vigna unguiculata (L.) Walp**

**Fig.2: Plate of meiosis in V.unguiculata - Diakinesis stage**

**Fig.3: Plate of meiosis in V.unguiculata - Pachytene stage**



The later stages of microsporogenesis were also studied in each of these species laying emphasis on the chiasma frequency at diakinesis. The data of the same is presented in Table 7.

The various aspects of the study are grouped under three headings viz., plant morphological observations, pachytene chromosomal analysis, studies in microsporogenesis and measurements of somatic chromosomes.

Vigna unguiculata (L.) Walp. ( $2n=22$ )

a) Morphological observations: The species is characterised by annual, erect-bushy, herbs. Germination of seeds being epigeal, the first pair of leaves are sessile in nature. The plants grow to a height of about 45 cms and bear long drooping pods on long peduncles. The leaves are pinnately compound with three angular unequal lobes. At the base of the pedicel a pair of stipules are present which are extended below the point of insertion to the main stem. The basal two leaflets possess a pair of stipellae while the terminal leaflet possesses a pair of stipellae (Table 4).

Inflorescence is borne on long peduncle. The flowers are large, symmetrical and papilionaceous. The standard petal has a purple coloration on the hind region. Keel petals are fused together and enclose ten diadelphous stamens. The style is incurved making an angle of about  $90^\circ$ . The style is

Table 2: Chromosome measurements at pachytene in *Vigna unguiculata* (L.) Walp.

Chromosome No.	Long arm ( $\mu$ )	Short arm ( $\mu$ )	Total length ( $\mu$ )	Relative length	Arm ratio	Type of chromosome
I	53.60	16.00	69.60	16.02	1:3.35	AS
II*	43.20	10.40	53.60	12.34	1:4.15	AS
III*	36.80	12.80	49.60	11.42	1:2.87	AS
IV	26.40	21.60	48.00	11.05	1:1.22	S
V	26.40	16.80	43.20	9.95	1:1.57	S
VI	24.80	8.80	33.60	7.74	1:2.82	AS
VII	20.80	12.00	32.80	7.55	1:1.73	S
VIII	16.80	14.40	31.20	7.18	1:1.16	S
IX	17.80	10.20	28.00	6.45	1:1.75	S
X	12.60	10.60	23.20	5.34	1:1.19	S
XI	13.60	8.00	21.60	4.97	1:1.70	S

\* Molecular chromosome pairs

AS Asymmetrical

S Symmetrical

extended beyond the stigma to form an apical appendage as characteristic of the genus Vigna. The species has a bracteole at the base of the calyx and this is larger in size than that present in V.mungo and V.radiatus(Figs.1,6).

b) Pachytene chromosomes: The absolute length of all the chromosomes measured at pachytene has been found to be 434.40  $\mu$ . The longest chromosome measures 69.60  $\mu$  while the smallest is 21.60  $\mu$ . Two pairs of chromosomes are associated with the nucleolus and measure 53.6  $\mu$  and 49.60  $\mu$  in length. Details of chromosome measurements are presented in Table 2 and the idiogram in Fig. 28.

Chromosome I: Measured 69.60  $\mu$  long. The long arm being 53.60  $\mu$  and the short arm 16.00  $\mu$ . It has an arm ratio of 1:3.35 and relative length of 16.02.

Chromosome II: This is the nucleolar chromosome with submedian centromere. It measures 53.60  $\mu$ . The secondary constriction being present on the short arm subterminally. The long arm measures 43.20  $\mu$  and the short arm 10.40  $\mu$ . Relative length and arm ratio are 12.34 and 1:4.15 respectively.

Chromosome III: Being submedian this chromosome pair is attached to the nucleolus. The secondary constriction is subterminal and located on the short arm. Total length of

**Fig.No.4: Camera lucida drawing of chromosomes at pachytene stage in Y.unguiculata**

**Fig.No.5: Camera lucida drawing of somatic chromosomes in Y.unguiculata**

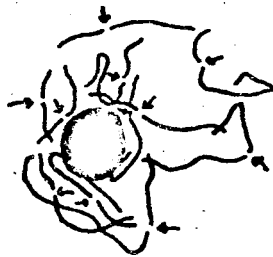


Fig-4

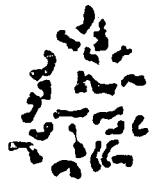


Fig-5

the chromosome is 49.60  $\mu$ , long arm and short arm are 36.80  $\mu$  and 12.80  $\mu$  long. Relative length and arm ratio are 11.42 and 1:2.87 respectively.

Chromosome IV: This median chromosome measured 48.00  $\mu$ . The length of the long arm and short arm are 26.40  $\mu$  and 21.60  $\mu$ . Relative length and arm ratio are 11.05 and 1:1.22.

Chromosome V: The submedian chromosome measures 43.20  $\mu$  and has a long arm measuring 26.40  $\mu$  and short arm of 16.80  $\mu$ . Arm ratio and relative length are 1:1.57 and 9.95 respectively.

Chromosome VI: Total length of this chromosome is 33.60  $\mu$  the long arm and short arm measures 24.80  $\mu$  and 8.80  $\mu$  respectively. Relative length is 7.74. The chromosome is submedian with an arm ratio of 1:2.82.

Chromosome VII: Being submedian the chromosome has an arm ratio of 1:1.73. Total length is 32.80  $\mu$ , while the long arm and short arm measure 20.80  $\mu$  and 12.00  $\mu$  respectively.

Chromosome VIII: It measures 31.20  $\mu$ . The chromosome is median with arm ratio of 1:1.16 and relative length is 7.18.

Chromosome IX: This submedian chromosome measures 28.00  $\mu$  in length with the long arm measuring 17.80  $\mu$  while short arm measures 10.20  $\mu$ . The relative length and arm ratio has been found to be 6.45 and 1:1.75 respectively.

Chromosome X: The chromosome has a total length of 23.20  $\mu$  and is median in centromeric position. The long arm measures 12.60  $\mu$  and short arm 10.60  $\mu$ . The relative length and arm ratio are 5.34 and 1:1.19.

Chromosome XI: Being the smallest chromosome in the complement the two arms measure 13.60  $\mu$  and 8.00  $\mu$  and the total length is 21.60  $\mu$ . Relative length and arm ratio being 4.97 and 1:1.70 respectively. The chromosome has been grouped as sub-median (Figs. 3, 4).

Meiosis: Regular bivalent formation both at diakinesis and metaphase-I and followed by normal disjunction at anaphase-I has been noticed. Pollen fertility was normal with 85-90% normal pollen.

Detailed studies on chiasma frequency in 30 cells at diakinesis has been carried out. Out of the 330 bivalents examined 70 bivalents have been found with one chiasma each; 243 bivalents with two chiasmata each; and 17 bivalents with three chiasmata. Total chiasmata is 607, average number of chiasmata per cell is 20.23 and number of chiasmata per bivalent is 1.84 (Table 7) (Fig. 2).

Mitotic chromosome: Absolute length of somatic chromosomes ranged from 2.60-1.20  $\mu$ , and the overall length of the chromatin being 38.80  $\mu$  (Table 3) (Fig. 29).

Table 3: Chromosome measurements at somatic metaphase of *Y. nanaiculata* (L.) Walp

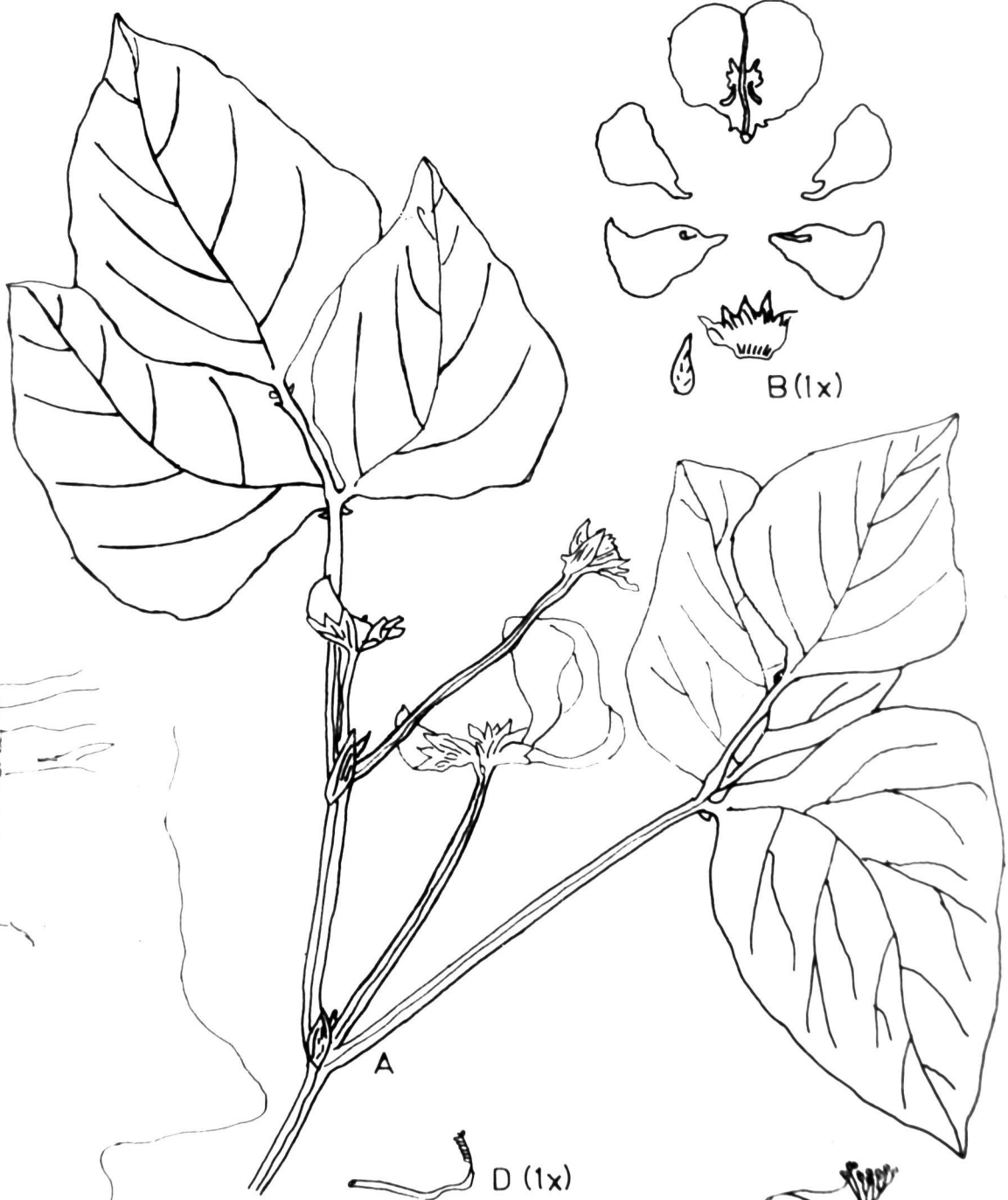
Group	No. of chromosome pairs	Arm length		Total length ( $\mu$ )	Relative length	Arm ratio	Type of chromosome
		Long ( $\mu$ )	Short ( $\mu$ )				
A	1	1.80	0.80	2.6	13.40	1:2.25	AS
B	2*	1.70	0.60	2.3	11.86	1:2.83	AS
C	4	1.00	0.70	1.7	8.76	1:1.43	S
D	3	0.70	0.70	1.4	7.22	1:1.10	S
E	1	0.70	0.50	1.2	6.19	1:1.40	S

\* Nucleolar chromosome pairs

AS Asymmetrical

S Symmetrical

**Fig.6: Figure of morphological features of Y.unguiculata  
plant**



B (1x)

A

D (1x)

C (1x)

E (32x)

V. unguiculata

Group A: Consists of one pair of submedian chromosomes measuring  $2.60 \mu$  in length. The long arm is  $1.8 \mu$  and short arm  $0.80 \mu$ . Relative length and arm ratio are 13.40 and 1:2.25 respectively.

Group B: Chromosomes with submedian centromeric position, possesses a secondary constriction on the short arm. It measures  $2.30 \mu$  in total, with the long arm measuring  $1.70 \mu$  and the short arm  $0.60 \mu$ . The relative length and arm ratio are 11.86 and 1:2.83 respectively. This group has two pairs of chromosomes.

Group C: This group has four pairs of median chromosomes. Length of the chromosome being  $1.70 \mu$ . The long arm is  $1.0 \mu$  and short arm  $0.7 \mu$ . Relative length is 8.76.

Group D: Has three pairs of median chromosomes measuring  $1.4 \mu$  while both arms measured  $0.70 \mu$ . Relative length and arm ratio are 7.22 and 1:1.0 respectively.

Group E: Comprises of one pair of submedian chromosomes measuring  $1.20 \mu$ . In this chromosome, the long arm is  $0.7 \mu$  and short arm  $0.50 \mu$ . Relative length and arm ratio are 6.19 and 1:1.40 respectively (Fig.5).

Vigna munge (L.) Hepper ( $2n=22$ )

a) Morphological observations: Being a domesticated species the plants of this species are annual and determinate

herbs. The plants grow to a height about 30 cms, the stem is partially pigmented and hard. Germination of seeds being epigeal the first pair of leaves are sessile and linear. Leaves are pinnately compound with three lobes. At the base of the petiole a pair of stipules are present. These are smaller than those of V. unguiculata and are extended below the point of insertion to the stem. Each of the basal leaflets have one stipellae while the terminal leaflet has a pair of stipellae.

Inflorescence is a raceme and is borne on a short peduncle. The flowers are relatively small and papilionaceous. The standard petal is greenish yellow in colour. The keel is fused and encloses a diadelphous stamen and a style which rarely makes one complete turn. The style ends in a stigma which has an apical appendage (Fig. 7, Plate II)

b) Pachytene chromosomes: Absolute length of all the chromosomes at pachytene is  $357.60 \mu$  and the range is between  $44.80 \mu$  and  $20.00 \mu$ . Detailed chromosomal measurements are given in Table 5 and pachytene idiogram in Fig. 28.

The regions flanking the centromere (proximal region) takes deep stain and the other regions stain lightly (Figs. 8, 9).

Chromosome I: It is a median type of chromosome with the long arm measuring  $24.00 \mu$  and the short arm  $20.8 \mu$ .

Fig. 7: Plant of Y. ~~YUNGA~~ (L.) Hepper

Fig. 8: Plate of meiosis in Y. ~~YUNGA~~ - Pachytene stage

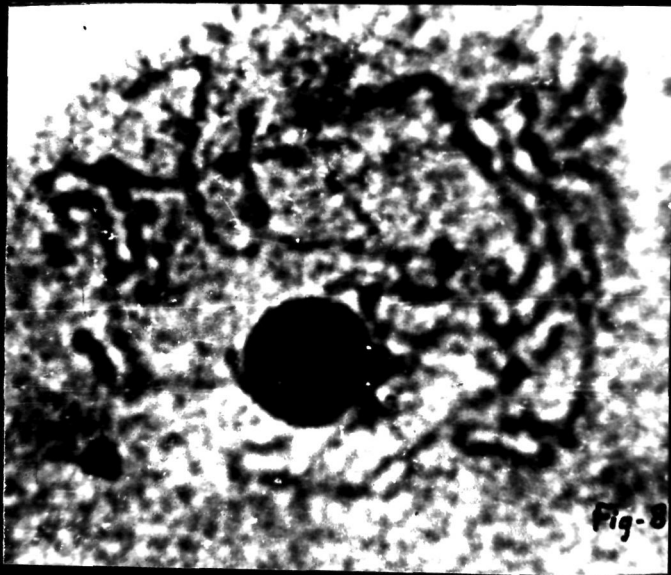


Table 4: Critical morphological differences amongst the five species of genus *Yucca*

Sl.No.	Characteristic	<i>Y. eruginosa</i>	<i>Y. elaeagnifolia</i>	<i>Y. radiata</i>	<i>Y. cylindrica</i>	<i>Y. mariae</i>
1	Habit	Erect - bushy	Erect	Erect	Bushy	Indeterminate and trailing
2	Duration	Annual	Annual	Annual	Annual	Perennial
3	Germination	Epigeal	Epigeal	Epigeal	Hypogeal	Hypogeal
4	First pair of leaves after cotyledon	Sessile	Sessile	Sessile	Petiole	Petiole
5	Flower colour	Purple	Yellow	Yellow-green	Yellow	Yellow
6	Flower size	Large	Small	Small	Medium	Large
7	Style and Keel	Incurved	Rarely making one turn	Rarely making one turn	Incurved	Incurved
8	Style-apical appendage	Present	Present	Present	Present	Present
9	Hairiness	Smooth	Hairy	Hairy	Smooth	Smooth
10	Stipules	Prolonged below point of insertion	Prolonged below point of insertion	Prolonged below point of insertion	Prolonged below point of insertion	Not prolonged below point of insertion
11	Bracteoles	Present	Present	Present	Absent	Present
12	Length of pod	30 cms	4-5 cms	5-6 cms	3-4 cms	5-6 cms
13	Pods drooping or erect	Drooping	Erect	Erect	Erect	Erect
14	Shattering or nonshattering	Nonshattering	Shattering	Shattering	Shattering	Shattering

Table 5: Chromosome measurements at pachytene in *Y. ENHAKA* (L.) Hepper

Chromosome No.	Long arm ( $\mu$ )	Short arm ( $\mu$ )	Total length ( $\mu$ )	Relative length	Arm ratio	Type of chromosome
I	24.00	20.80	44.80	12.53	1:1.15	S
II	33.60	9.60	43.20	12.08	1:3.50	AS
III	24.00	15.20	39.20	10.96	1:1.58	S
IV	24.80	13.60	38.40	10.74	1:1.82	S
V	17.60	17.60	35.20	9.84	1:1.00	S
VI	19.20	15.20	34.40	9.62	1:1.26	S
VII	20.80	12.80	33.60	9.40	1:1.62	S
VIII*	17.60	8.00	25.60	7.16	1:2.20	AS
IX*	14.40	7.20	21.60	6.04	1:2.00	AS
X	17.60	4.00	21.60	6.04	1:4.40	AS
XI	10.40	9.60	20.00	5.59	1:1.08	S

\* Aucleolar chromosome pairs

AS Asymmetrical

S Symmetrical

The over all length of the chromosome is  $44.80 \mu$ . The relative length is 12.53 and arm ratio 1:1.15.

Chromosome II: Being a submedian chromosome it measures  $43.20 \mu$ , long arm measuring  $33.60 \mu$  and short arm  $9.60 \mu$ . The arm ratio is 1:3.50 and relative length 12.08.

Chromosome III: Total length of the chromosome is  $39.20 \mu$ . The chromosome is submedian with long arm measuring  $24.00 \mu$  and short arm  $15.20 \mu$ . Relative length and arm ratio are 10.96 and 1:1.58 respectively.

Chromosome IV: This chromosome measures  $38.4 \mu$  with long arm measuring  $24.80 \mu$  and short arm  $13.60 \mu$ . The relative length and arm ratio are 10.74 and 1:1.82 respectively. The chromosome is submedian.

Chromosome V: The total length of the chromosome is  $35.20 \mu$ . The position of the centromere is median with both the arms measuring  $17.6 \mu$ . The relative length of this chromosome is 9.84.

Chromosome VI: The total length of the chromosome is  $34.40 \mu$  with the long arm measuring  $19.20 \mu$  and short arm  $15.20 \mu$ . This is found to be a median chromosome with an arm ratio of 1:1.26 and relative length is 9.62.

Chromosome VII: This chromosome measuring  $33.6 \mu$  long is submedian with long arm measuring  $20.80 \mu$  and short arms

**Fig. 9: Camera lucida drawing of chromosomes at pachytene stage in V.mungo**

**Fig.10: Camera lucida drawing of somatic chromosomes in V.mungo**

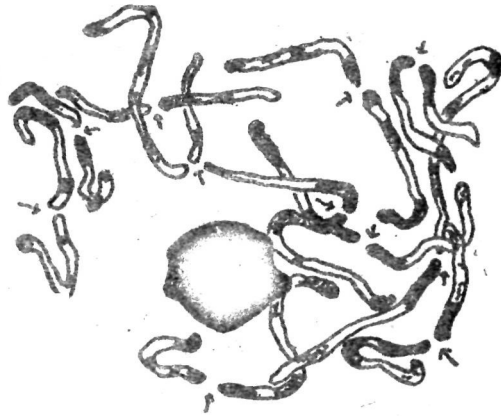


Fig-9.



Fig-10.

12.80  $\mu$ . Relative length and arm ratio are 9.40 and 1:1.62 respectively.

Chromosome VIII: This is a submedian nucleolar organising chromosome measuring 25.60  $\mu$  in length the long arm being 17.60  $\mu$  and short arm 8.00  $\mu$ . The nucleolus is attached to the short arm. Relative length is 7.16 and arm ratio 1:2.20.

Chromosome IX: Measuring 21.6  $\mu$  long, this pair also has a secondary constriction on the short arm which measures 14.40  $\mu$  long. Short arm of this submedian chromosome measured 7.20  $\mu$ , relative length 6.04  $\mu$  with arm ratio 1:2.0.

Chromosome X: Measuring 21.60  $\mu$  this is the most asymmetrical chromosome with an arm ratio of 1:4.40. Relative length is 6.04. Long arm and short arm measures 17.60  $\mu$  and 4.00  $\mu$  respectively.

Chromosome XI: Is the shortest chromosome in the complement and has submedian centromere. The chromosome measures 20.00  $\mu$ , with an arm ratio of 1:1.08 and relative length 5.59.

Meiosis: Later stages of meiosis such as diakinesis, Metaphase-I, Anaphase-I and second meiotic division are normal with pollen fertility upto 85-90%.

Chiasma analysis in 30 cells at diakinesis has been carried out. Of the 330 bivalents studied, 139 bivalents

Table 6: Chromosome measurements at somatic metaphase of *Vigna mungo* (L.) Hepper

Group	No. of chromosome pairs	Arm length		Total length ( $\mu$ )	Relative length	Arm ratio	Type of chromosome
		Long ( $\mu$ )	Short ( $\mu$ )				
A	1	1.3	0.9	2.2	11.82	1:1.44	S
B	3	1.6	0.5	2.1	11.29	1:3.20	AS
C	2*	1.0	0.7	1.7	9.14	1:1.43	S
D	4	0.7	0.7	1.4	7.53	1:1.00	S
E	1	0.7	0.4	1.1	5.91	1:1.75	S

\* Nucleolar chromosome pairs

AS Asymmetrical

S Symmetrical

showed one chiasma each; 177 bivalents possessed two chiasma to each; and 14 bivalents have three chiasmata each. Total number of chiasmata is 535 and average chiasmata per cell is 17.84, while average number of chiasmata per bivalent is 1.62 (Table 7)(Fig. 23).

c) Mitotic chromosomes: Somatic chromosomes ranged in length from 2.20  $\mu$  to 1.10  $\mu$ . The overall length of chromatin is 37.20  $\mu$ . The details with respect to length, centromeric position, relative length and arm ratio are presented in Table 6. Based on length the chromosome pairs they could be grouped into five groups (Fig. 29)

Group A: Possesses one pair of submedian chromosomes measuring 2.2  $\mu$ . Long arm of this chromosome is 1.3  $\mu$  and short arm 0.9  $\mu$ . Relative length and arm ratio were 11.82 and 1:1.44 respectively.

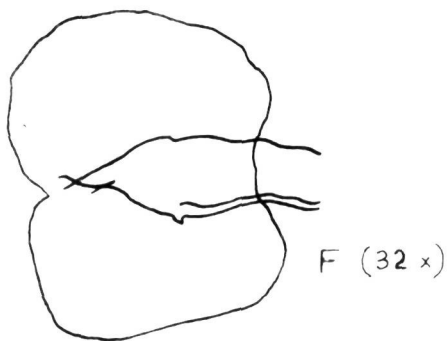
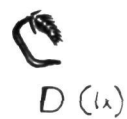
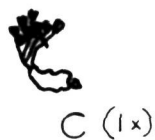
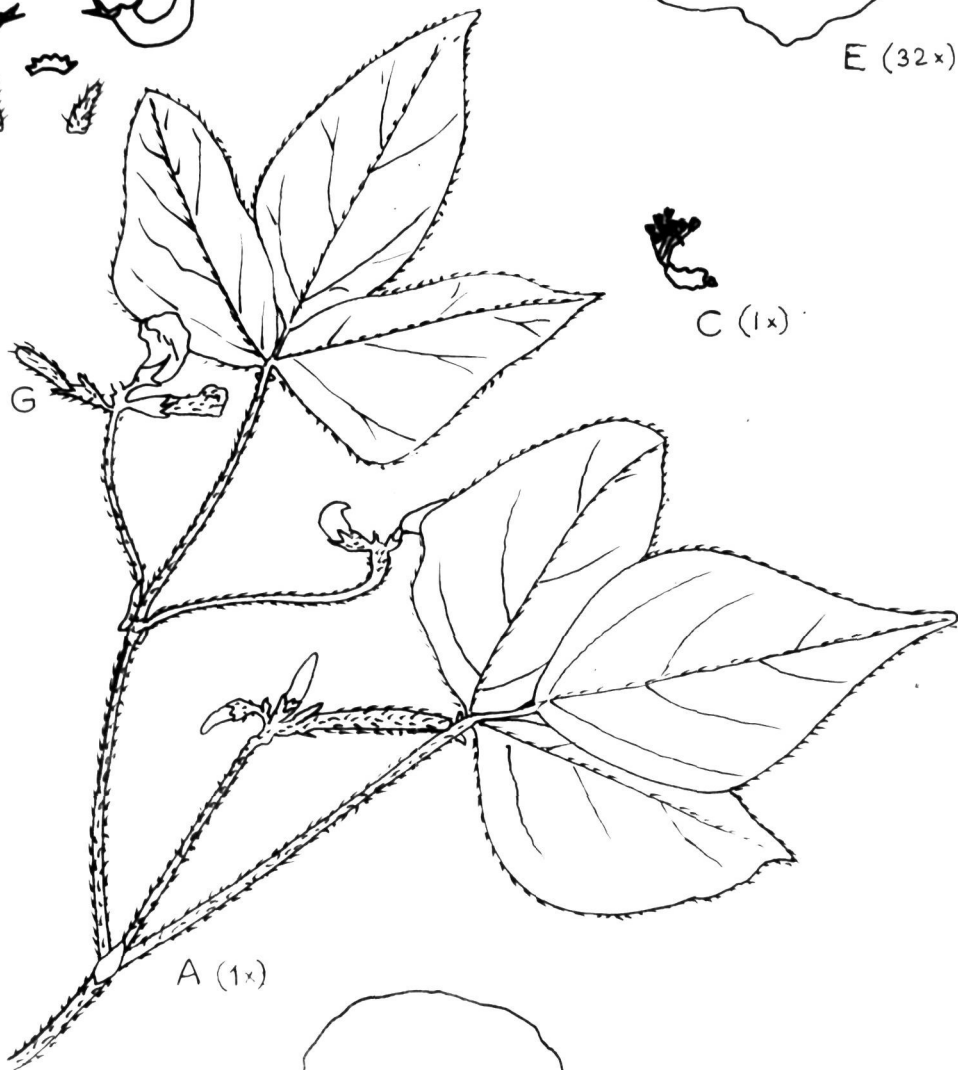
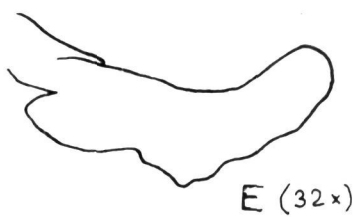
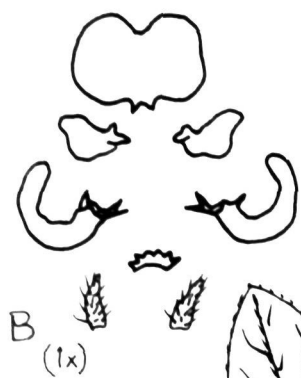
Group B: Comprises three pairs of chromosomes measuring 2.10  $\mu$ , long arm and short arm being 1.6  $\mu$  and 0.5  $\mu$  respectively. Nucleolus is attached to the short arm. The relative length and arm ratio of this submedian chromosome are 11.29 and 1:3.20 respectively.

Group C: Has two pairs of satellite chromosomes with submedian centromere and nucleolus is attached to the short arm. It measures 1.7  $\mu$  long with a long arm of 1.0  $\mu$  and short arm of 0.7  $\mu$ . Relative length and arm ratio being 9.14 and 1:1.43 respectively.

Table 7: Data on chlamydia studies at Makinosis in five species of *Y. m.*

No.	Species	No. of Bivalents analysed	Bivalents with			Total χ <sup>2</sup>	Average Chlamydia	
			1 χ	2 χ <sup>2</sup>	3 χ <sup>2</sup>		per cell	bivalent
1	<i>Y. maculata</i> (L.) Walp.	330	76	243	17	607	20.25	1.84
2	<i>Y. mungo</i> (L.) Hepper	330	139	177	14	535	17.84	1.62
3	<i>Y. radiata</i> (L.) Wilkes.	330	130	194	6	536	17.87	1.62
4	<i>Y. pallidus</i> (L.) Skene.	330	98	219	13	575	19.17	1.74
5	<i>Y. merrilli</i> (Conn.) Merrill.	330	90	231	9	579	19.30	1.76

**Fig.11: Figure of morphological features of I. gungu plant**



V. mungo

Group L<sub>1</sub> Includes four pairs of median chromosomes measuring 1.40  $\mu$ . Relative length is 7.53.

Group L<sub>2</sub> Smallest chromosome pair being submedian measures 1.10  $\mu$  in length with long and short arms measuring 0.70  $\mu$  and 0.40  $\mu$  respectively. Arm ratio and relative length are 1:1.75 and 5.91 respectively (Fig. 10)

Vigna radiata (L.) Wilczek (2n=22)

a) Morphological observations: An annual, erect, determinate herb. The species differs from V. mungo in few aspects.

Germination being epigeal the first pair of leaves are sessile, the hypocotyl is purple and the epicotyl green in colour.

Inflorescence is borne on a short peduncle bearing small flowers. The back of the standard petal is greenish yellow. The keel petal enclosed a stigma which is curved but not by one complete turn. The tip of the style ended in a stigma which has an apical appendage as in V. mungo. The leaves are trifoliate and have a linear stipule at the base of the petiole, extended below the point of attachment to the stem. Pods were 5-6 cms long and erect in nature (Table 4) (Figs. 12, 17).

Pachytene chromosomes: Chromosome length at pachytene ranged from 47.98  $\mu$  to 23.99  $\mu$ . The overall length of the pachytene chromosome complement was found to be 401.07  $\mu$ .

Table 8: Chromosome measurements at pachytene in *V. radiatus* (L.) Willd.

Chromosome No.	Long arm ( $\mu$ )	Short arm ( $\mu$ )	Total length ( $\mu$ )	Relative length	Arm ratio	Type of chromosome
I	28.91	19.07	47.98	11.96	1:1.52	S
II	33.83	12.32	46.15	11.51	1:2.74	AS
III	28.29	14.15	42.44	10.58	1:2.00	AS
IV	22.76	15.38	38.14	9.50	1:1.48	S
V	19.09	17.82	36.91	9.20	1:1.07	S
VI	18.45	17.84	36.29	9.04	1:1.03	S
VII*	22.45	12.61	35.06	8.74	1:1.77	S
VIII	19.68	14.15	33.83	8.43	1:1.39	S
IX	15.38	15.38	30.76	7.67	1:1.00	S
X	15.99	13.53	29.52	7.36	1:1.18	S
XI	15.38	8.61	23.99	5.98	1:1.79	S

\* Subleolar chromosome pair

AS Asymmetrical

S Symmetrical

The relative length ranged from 11.96 to 5.98 (Table 8, Figs 13, 28).

Chromosome I: It is a submedian chromosome measuring 47.98  $\mu$ . The long arm measured 28.91  $\mu$  while the short arm 19.07  $\mu$ . Relative length and arm ratio are 11.96 and 1:1.52 respectively.

Chromosome II: The chromosome measured 46.15  $\mu$ , the long arm being 33.83  $\mu$  while the short arm 12.74  $\mu$ . The relative length and arm ratio are 11.51 and 1:2.62 respectively.

Chromosome III: This has an overall length of 42.44  $\mu$  with submedian centromeric position. The chromosome has a long arm of 28.29  $\mu$  and a short arm of 14.15  $\mu$ . Relative length is 10.58 and arm ratio 1:2.

Chromosome IV: Measuring 38.14  $\mu$ , this submedian chromosome has a long arm measuring 22.76  $\mu$  and short arm measuring 15.38  $\mu$ . The relative length is 9.50 and arm ratio 1:1.48.

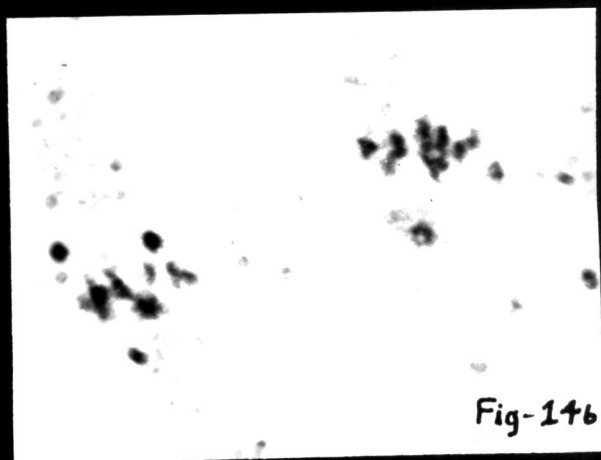
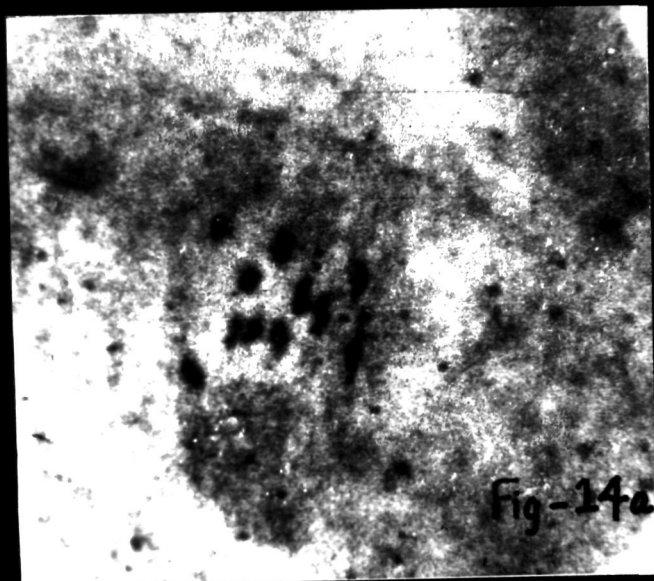
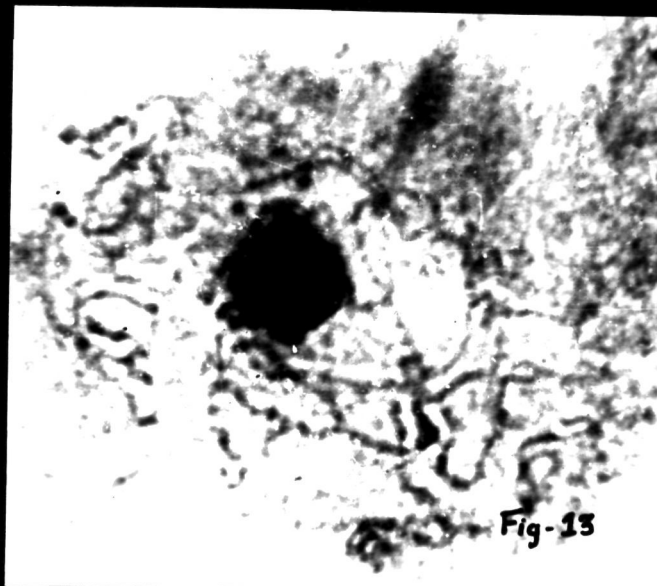
Chromosome V: This chromosome is 36.91  $\mu$  in length with median centromeric position has a long arm measuring 19.09  $\mu$  and short arm 17.82  $\mu$  long. Arm ratio and relative length are found to be 1:1.07 and 9.20 respectively.

Chromosome VI: This chromosome measured 36.29  $\mu$  and possesses median centromere. The long arm of this chromosome measures 18.45  $\mu$  and short arm 17.84  $\mu$ . The arm ratio and relative length are 1:1.05 and 9.04 respectively.

**Fig.12: Plant of Y.radiatus (L.) Wilson**

**Fig.13: Plate of meiosis in Y.radiatus - Pachytene stage**

**Fig.14: Plate of meiosis in Y.radiatus - Metaphase stage**



Chromosome VII: This has an overall length of 35.06  $\mu$ . The centromere is submedian in its position. The secondary constriction is present subterminally on the short arm. The arm ratio and relative length are 1:1.77 and 8.74 respectively. Long arm and short arm measured 22.45  $\mu$  and 12.61  $\mu$  respectively.

Chromosome VIII: This chromosome measures 33.83  $\mu$  in length the long arm and short arm measure 19.68  $\mu$  and 14.15  $\mu$ . The relative length and arm ratio are 8.43 and 1:1.39. The chromosome is subterminal.

Chromosome IX: The centromere in this chromosome is median and measures 30.76  $\mu$ , each arm measuring 15.38  $\mu$ . Relative length is 7.67.

Chromosome X: The long arm of this chromosome measures 15.99  $\mu$  and short arm 13.53  $\mu$ . Overall length of this median chromosome is 29.52  $\mu$ . Relative length and arm ratio are 7.36 and 1:1.18 respectively.

Chromosome XI: This submedian chromosome measures 23.99  $\mu$  in length. The long arm measures 15.38  $\mu$  and short arm 8.61  $\mu$ . Arm ratio and relative length are 1:1.79 and 5.98 respectively.

Meiosis: Later stages of meiosis such as diakinesis, Metaphase-I, Anaphase-I and second meiotic division are normal (Fig. 14).

Chiasma analysis in 30 cells at diakinesis has been carried out. Of the 330 bivalents, 130 bivalents have one

Table 9: Chromosome measurements at somatic metaphase of *V. radiatus* (L.) villosus

Group	No. of chromosome pairs	Arm length		Total length in $\mu$	Relative length	Arm ratio	Type of chromosome
		Long ( $\mu$ )	Short ( $\mu$ )				
A	1	2.1	1.2	3.3	15.79	1:1.75	S
B	1	1.8	0.6	2.4	11.48	1:3.00	AS
C	3	1.2	0.8	2.0	9.57	1:1.50	S
D	1	1.0	0.8	1.8	8.61	1:1.25	S
E	2	1.0	0.6	1.6	7.66	1:1.66	S
F	3	0.7	0.7	1.4	6.70	1:1.00	S

• Nucleolar chromosome pair

AS Asymmetrical

S Symmetrical

chiasma each; 194 bivalents possessed two chiasmata each; and 6 bivalents showed three chiasmata each. Total number of chiasmata in this species (for 30 cells) is 536. Average number of chiasmata per cell and per bivalent is 17.87 and 1.62 respectively (Table 7)(Fig. 32)

c) Mitotic chromosomes: The somatic metaphase chromosomes ranged in length from 3.30  $\mu$  to 1.40  $\mu$ . The second longest chromosome pair is the nucleolus organising chromosome. Based on the presence or absence of secondary constriction, length and position of centromere the complement could be divided into six groups (Table 9)(Fig. 29).

Group A: Has one pair of submedian chromosome with the two arms measuring 2.10  $\mu$  and 1.20  $\mu$ . The chromosome measures 3.30  $\mu$  in length.

Group B<sub>1</sub> One pair of satellited chromosomes with submedian primary constriction. Secondary constriction is present on the short arm. It measures 2.40  $\mu$  in length. Relative length is 11.48 and arm ratio being 1:3.00.

Group C: Has three pairs of submedian chromosomes measuring 2.00  $\mu$  in length. Relative length and arm ratio are 9.57 and 1:1.50 respectively.

Group D: One pair of median chromosomes measuring 1.80  $\mu$  in length. Arm ratio and relative length are 1:1.25 and 8.61 respectively.

**Fig.15: Camera lucida drawing of chromosomes at  
pachytene stage in V.radiatus**

**Fig.16: Camera lucida drawing of somatic chromosomes  
in V.radiatus**

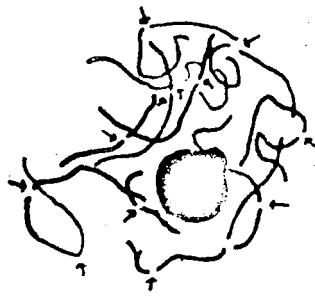


Fig - 15



Fig - 16

**Fig.17: Figure of morphological features of V.radiatus  
plant**



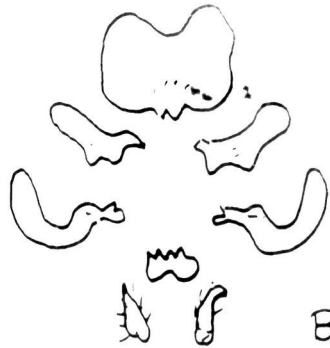
C (1x)



D



A



B

V. radiatus

Group E: Total length is 1.60  $\mu$ , and is subterminal with the long arm measuring 1.00  $\mu$  and short arm measuring 0.6  $\mu$ . This group consists of two pairs of chromosomes.

Group F: Consists of three pairs of median chromosomes, and each pair measures 1.4  $\mu$ . Relative length is 6.70. (Fig. 16).

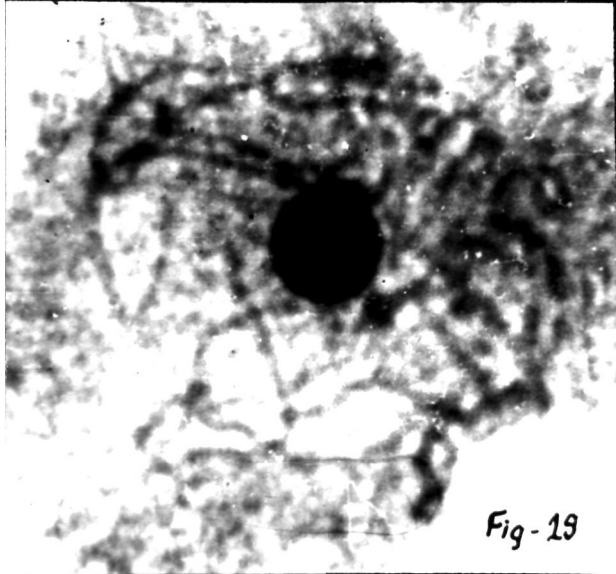
Vigna cylindrica Skeels (2n=22)

a) Morphological observations: A twining shrub this species is photosensitive in nature. The plants grow to a height of 60 cms. Leaves are angular and smaller compared to that of V. unguiculata. Flowers are borne on long peduncles. Flowers are not as big as those of V. unguiculata. Germination is hypogeal and the first pair of leaves are petiolate. Leaves are smooth and trifoliate. Base of petiole has a stipule which is not extended below the point of insertion to the stem. Pods are 5-6 cms long and erect in nature. Pods on drying dehisce and the seeds are dispersed. This being a wild character is conspicuous by its non-existence in species V. unguiculata, V. mungo and V. radiatus (Table 4) (Figs. 18, 22).

b) Pachytene chromosomes: The longest chromosome in this measures 80.00  $\mu$  while the shortest chromosome is 24.0  $\mu$ . Overall length of the chromosome complement at pachytene in this species was found to be 607.6  $\mu$ . Detailed measurements

**Fig.18: Plant of V.gylindrica (L.) Skeels**

**Fig.19: Plate of meiosis in V.gylindrica - Pachytene stage**



*Fig-19*

Fig.20: Camera lucida drawing of chromosomes at pachytene stage in Y.cylindrica

Fig.21: Camera lucida drawing of somatic chromosomes in Y.cylindrica



Fig - 20



Fig 21

of chromosomes are given in Table 10. Idiogram is presented in Fig. 28.

Chromosome I: It is 80  $\mu$  long. The chromosome has a median centromere. Relative length is 13.17.

Chromosome II: It is also a median chromosome measuring 70.40  $\mu$  long. The chromosome has a long arm measuring 36.4  $\mu$ , and short arm measuring 34.00  $\mu$ . Relative length is 11.59 and arm ratio 1:1.07.

Chromosome III: This chromosome has a submedian centromere with a total length of 65.60  $\mu$ . The long arm measures 45.00  $\mu$  and short arm 20.6  $\mu$  in length. Relative length and arm ratio are 10.80 and 1:2.18 respectively.

Chromosome IV: 63.20  $\mu$  long the chromosome has a submedian primary constriction. The long arm measures 40.00  $\mu$  and the short arm is 23.20  $\mu$ . Relative length and arm ratio are 10.41 and 1:1.72 respectively.

Chromosome V: The chromosome is 61.60  $\mu$  long with the centromere median in position. Both the arms measure 30.80  $\mu$ . The secondary constriction is located subterminally on the short arm. Relative length of this chromosome is 10.14.

Chromosome VI: This satellited chromosome measures 61.60  $\mu$ . The long arm is 44.20  $\mu$  and short arm 17.40  $\mu$ .

Table 10: Chromosome measurements at pachytene in *Vigna cylindrica* (L.) Steudl

Chromosome No.	Long arm (μ)	Short arm (μ)	Total length (μ)	Relative length	Arm Ratio	Type of chromosome
I	40.00	40.00	80.00	13.17	1:1.00	S
II	36.40	34.00	70.40	11.59	1:1.07	S
III	45.00	20.60	65.60	10.80	1:2.18	AS
IV	40.00	23.20	63.20	10.41	1:1.72	S
V*	30.80	30.80	61.60	10.14	1:1.00	S
VI*	44.20	17.40	61.60	10.14	1:2.54	AS
VII	30.60	23.00	53.60	8.82	1:1.33	S
VIII	28.30	19.30	47.60	7.83	1:1.47	S
IX	20.00	20.00	40.00	6.58	1:1.00	S
X	20.00	20.00	40.00	6.58	1:1.00	S
XI	20.00	4.00	24.00	3.95	1:5.00	AS

\* Bivalent chromosome pairs

AS Asymmetrical

S Symmetrical

Secondary constriction is located subterminally on the short arm. Relative length is 10.14 and arm ratio 1:2.54.

Chromosome VII: This chromosome is 55.60  $\mu$  in length. The primary constriction is nearly median with long arm measuring 30.60  $\mu$  and short arm 25.00  $\mu$ . Relative length and arm ratio of this chromosome are 8.82 and 1:1.35 respectively.

Chromosome VIII: This is submedian type of chromosome measuring 47.60  $\mu$ . The long arm is 28.3  $\mu$  and short arm 19.3  $\mu$  in length. Relative length and arm ratio being 7.83 and 1:1.47 respectively.

Chromosome IX and X: The two chromosome pairs measure 40.0  $\mu$  each and possessed a median primary constriction. Relative length is 6.58.

Chromosome XI: This is the shortest chromosome in the complement measuring 24.00  $\mu$ . This is the most asymmetrical type of chromosome with long arm and short arm measuring 20.0  $\mu$  and 4.0  $\mu$  respectively. Relative length is 3.95 and arm ratio 1:5.00 (Fig. 20).

Meiosis: All the subsequent stages of meiosis are found to be normal with normal pairing, disjunction of chromosomes. Pollen fertility is high.

Chiasma analysis in 330 bivalents from 30 cells at Diakinesis gave the following results. 98 bivalents with one

Table 11: Chromosome measurements at somatic metaphase of *V. gyllingiana* Skeels

Group	No. of chromosome pairs	Arm length		Total length ( $\mu$ )	Relative length	Arm ratio	Type of chromosome
		long ( $\mu$ )	short ( $\mu$ )				
A	1	1.60	1.40	3.00	13.33	1:1.10	S
B	2*	1.80	0.60	2.40	10.67	1:3.00	AS
C	3	1.20	0.80	2.00	8.89	1:1.50	S
D	4	0.90	0.90	1.80	8.00	1:1.00	S
E	1	0.90	0.60	1.50	6.67	1:1.50	S

\* nucleolar chromosome pairs

AS Asymmetrical

S Symmetrical

chiasma; 219 bivalents with two chiasmata each; 13 bivalents with 3 chiasmata each. Total number of chiasmata is 575. Average number of chiasmata per bivalent and per cell is found out to be 19.17 and 1.74 respectively (Table 7) (Fig. 23).

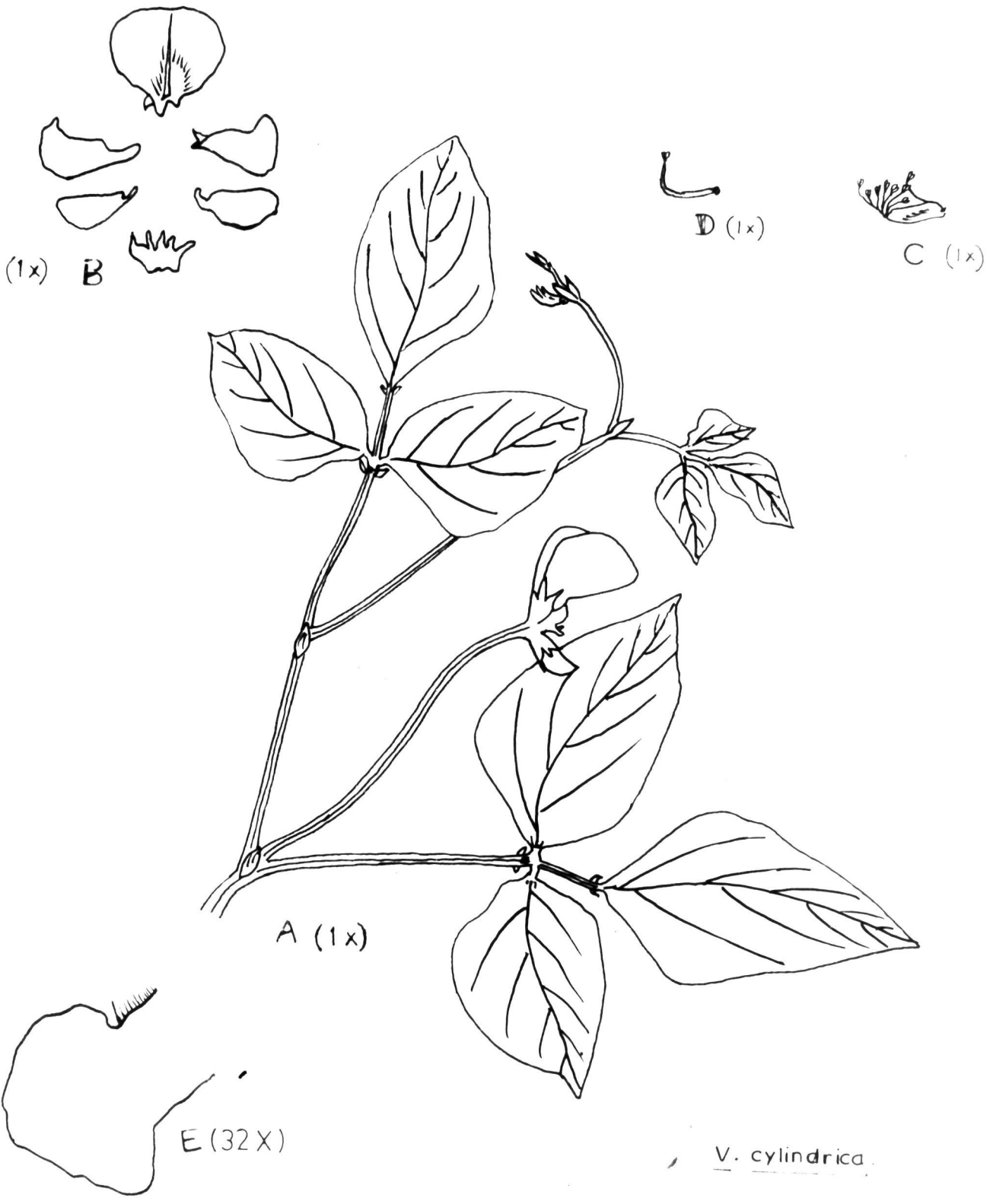
c) Mitotic aspects: The overall length of somatic metaphase chromosome complement is  $45.0 \mu$ . The longest chromosome is  $3.00 \mu$  while shortest  $1.5 \mu$ . Based on position of centromere, relative length and arm ratio the chromosomes have been grouped into five groups. Details of chromosome measurements is given in Table 11, Figs. 21.29.

Group A: Has one pair of submedian chromosomes measuring  $3.00 \mu$  in length. The long arm is  $1.60 \mu$  while the short arm is  $1.40 \mu$ . Relative length is 13.33 and arm ratio 1:1.11.

Group B: Comprises of two pairs of satellited chromosomes with submedian primary constriction with nucleolus being attached to short arm. Total length of chromosome is  $2.4 \mu$  long arm measuring  $1.8 \mu$  and the short arm measuring  $0.6 \mu$ . The relative length and arm ratio are 10.67 and 1:3.00 respectively.

Group C: Consists of three pairs of chromosomes, the long arm and short arm are  $1.2 \mu$  and  $0.8 \mu$  respectively. Total length is  $2.00 \mu$ . Arm ratio and relative length are 1:1.5 and 8.89 respectively.

**Fig.22: Figure of morphological features of V.cylindrica**



(1x) B

D (1x)

C (1x)

A (1x)

E (32x)

*V. cylindrica*

Group D: This group has four pairs of chromosomes measuring  $1.80 \mu$  with median primary constriction. The chromosome has equal arms measuring  $0.9 \mu$ . Relative length of the chromosome is 8.0.

Group E: This group has one pair of submedian chromosomes which measures  $1.50 \mu$  long. The long arm measures  $0.90 \mu$  while the short arm is  $0.60 \mu$ . Relative length and arm ratio are 6.67 and 1.5 respectively.

Vigna marina (Burn.) Merrill. ( $2n=22$ )

a) Morphological observations: The species is an undomesticated wild species. Germination is hypogeal and the plant grows into a trailing plant. Being photosensitive, the plant keeps growing all through the year and is perennial in nature. Leaves are thick and coated with a waxy layer. This species is resistant to powdery mildew and leaf septoria of cowpea. First pair of leaves is petiolate. The stem is woody. Base of the pedicel has a pair of stipules (Table 4). (Figs. 23, 27).

Inflorescence is a raceme and is borne on a long peduncle. Flowers are neither as large as in Cowpea nor as small as in Black-gram or Greengram. The stipule, at the base of the leaf does not extend below the point of insertion to the main stem. The standard petal is yellow, keel is fused and encloses diadelphous stamens and style which similar to that of cultivated Cowpea, by possessing an apical appendage

Table 12: Chromosome measurements at pachytene in *Vigna marina* (Burm.) Merrill

Chromosome No.	Long arm ( $\mu$ )	Short arm ( $\mu$ )	Total length ( $\mu$ )	Relative length	Arm ratio	Type of chromosome
I*	40.00	19.20	59.20	11.94	1:2.08	AS
II*	32.00	24.80	56.80	11.45	1:1.30	S
III	30.40	25.60	56.00	11.29	1:1.19	S
IV	31.20	18.40	49.60	10.00	1:1.70	S
V	30.40	17.60	48.00	9.68	1:1.75	S
VI	31.20	12.80	44.00	8.87	1:2.44	AS
VII	30.40	12.60	43.20	8.71	1:2.37	AS
VIII	24.00	16.00	40.00	8.07	1:1.50	S
IX	20.00	20.00	40.00	8.07	1:1.00	S
X	16.00	15.20	31.20	6.29	1:1.05	S
XI	20.00	8.00	28.00	5.64	1:2.50	AS

\* Bivalent chromosome pair

AS Asymmetrical

S Symmetrical

b) Pachytene chromosomes: Study of chromosomes at pachytene showed that overall chromosome length is 496.00  $\mu$  and the range being 59.20  $\mu$  to 28.00  $\mu$ . Details of chromosome measurements and idiogram are presented in Table 12, Figs. 24, 25, 28.

Chromosome I: Measures 59.2  $\mu$  in length. The long arm is 40.00  $\mu$  the short arm measures 19.20  $\mu$ . The secondary constriction on the short arm. Relative length and arm ratio are 11.94 and 1:2.08. This chromosome has a submedian centromere.

Chromosome II: This median chromosome measures 56.80  $\mu$  in length. The long arm measuring 32.00  $\mu$ , and short arm 24.8  $\mu$ . Relative length being 11.45, arm ratio is 1:1.30. This chromosome is the nucleolar chromosome with nucleolus attached to the short arm.

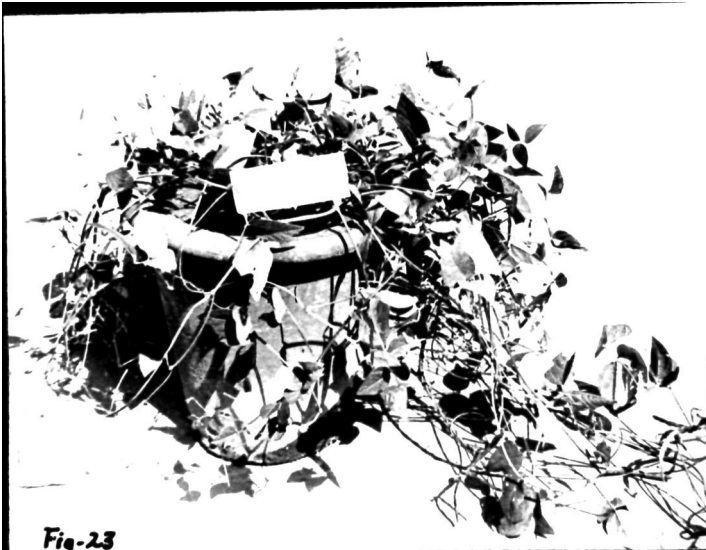
Chromosome III: Measuring 56.00  $\mu$  long, the chromosome has a median centromere. Long arm and short arm measure 30.40  $\mu$  and 25.60  $\mu$  respectively. Relative length and arm ratio are 11.29 and 1:1.19 respectively.

Chromosome IV: This submedian chromosome measures 49.60  $\mu$ . Long arm is 31.2  $\mu$  and the short arm is 18.40  $\mu$ . Relative length and arm ratio are 10.00 and 1:1.70 respectively.

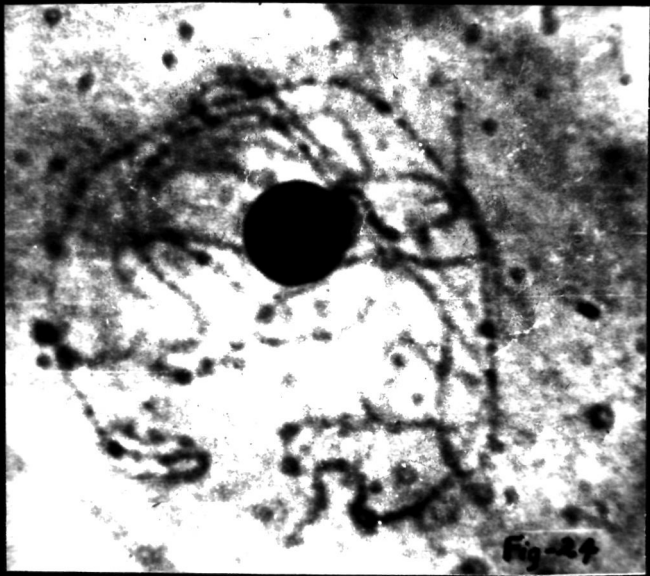
Chromosome V: This chromosome measured 48.00  $\mu$  long. The long arm and short arm of this chromosome are 30.40  $\mu$  and

Fig. 23: Plant of Y.marina (Burn.) Merrill.

Fig.24: Plate of meiosis in Y.marina - Pachytene stage



*Fig-23*



*Fig-24*

**Fig. 25: Camera lucida drawing of chromosomes at  
pachytene stage in Y.marina**

**Fig.26: Camera lucida drawing of somatic chromosomes  
in Y.marina**



Fig - 25



Fig - 26

17.60  $\mu$  long. Arm ratio and relative length have been found to be 1:1.73 and 9.68 respectively.

Chromosome VI: 44  $\mu$  long this chromosome has a submedian centromere with a long arm, measuring 31.20  $\mu$  and short arm measuring 12.80  $\mu$ . Relative length and arm ratio are 8.87 and 2.44 respectively.

Chromosome VII: Centromere being submedian the long arm measures 30.40  $\mu$  and short arm 12.80  $\mu$ . Total length is 43.20  $\mu$ . Relative length was 8.71 and arm ratio was 1:2.37.

Chromosome VIII: This chromosome also has submedian primary constriction. Total length is 40.00  $\mu$ . The long arm measures 24.00  $\mu$  and the short arm 16.00  $\mu$ . Relative length and arm ratio are 8.07 and 1:1.50 respectively.

Chromosome IX: It is 40.00  $\mu$  long with median centromere. The two arms of the chromosome are equal in length. Relative length is 8.07.

Chromosome X: This chromosome also possesses a median centromere and measures 31.20  $\mu$ . The two arms being almost equal, arm ratio is 1:1.05 and relative length 6.29.

Chromosome XI: This being the smallest chromosome measures 28.00  $\mu$  in length. Long arm is 20.00  $\mu$  and while the short arm 8.00  $\mu$ . Relative length is 5.64 and arm ratio 1:2.50.

Table 1): Chromosome measurements at somatic metaphase of *I. MARINA* (Burm.) Merrill.

Group	No. of chromosome pairs	Arm length		Total length ( $\mu$ )	Relative length	Arm ratio	Type of chromosome
		Long ( $\mu$ )	Short ( $\mu$ )				
A	2	1.80	1.40	3.20	11.39	1:1.28	S
B	3	2.00	1.10	3.10	11.03	1:1.82	S
C	2*	1.70	0.80	2.50	8.90	1:2.12	AS
D	1	1.00	1.00	2.00	7.12	1:1.00	S
E	2	1.00	0.80	1.80	6.41	1:1.25	S
F	1	1.10	0.70	1.80	6.41	1:1.57	S

\* Nucleolar chromosome pairs

AS Asymmetrical

S Symmetrical

All the subsequent stages of meiosis viz., Diakinesis metaphase-I, Metaphase-II are normal and normal stainable pollen are obtained.

Out of the 330 bivalents in 30 pollen mother cells analysed to study the chiasma frequency, 90 bivalents showed one chiasma each, 231 bivalents possessed two chiasmata each; while 9 bivalents have three chiasmata each. Total chiasma is 543. Average chiasma per cell is 18.10 and average chiasma per bivalent is 1.76 (Table 7) (Fig. 32).

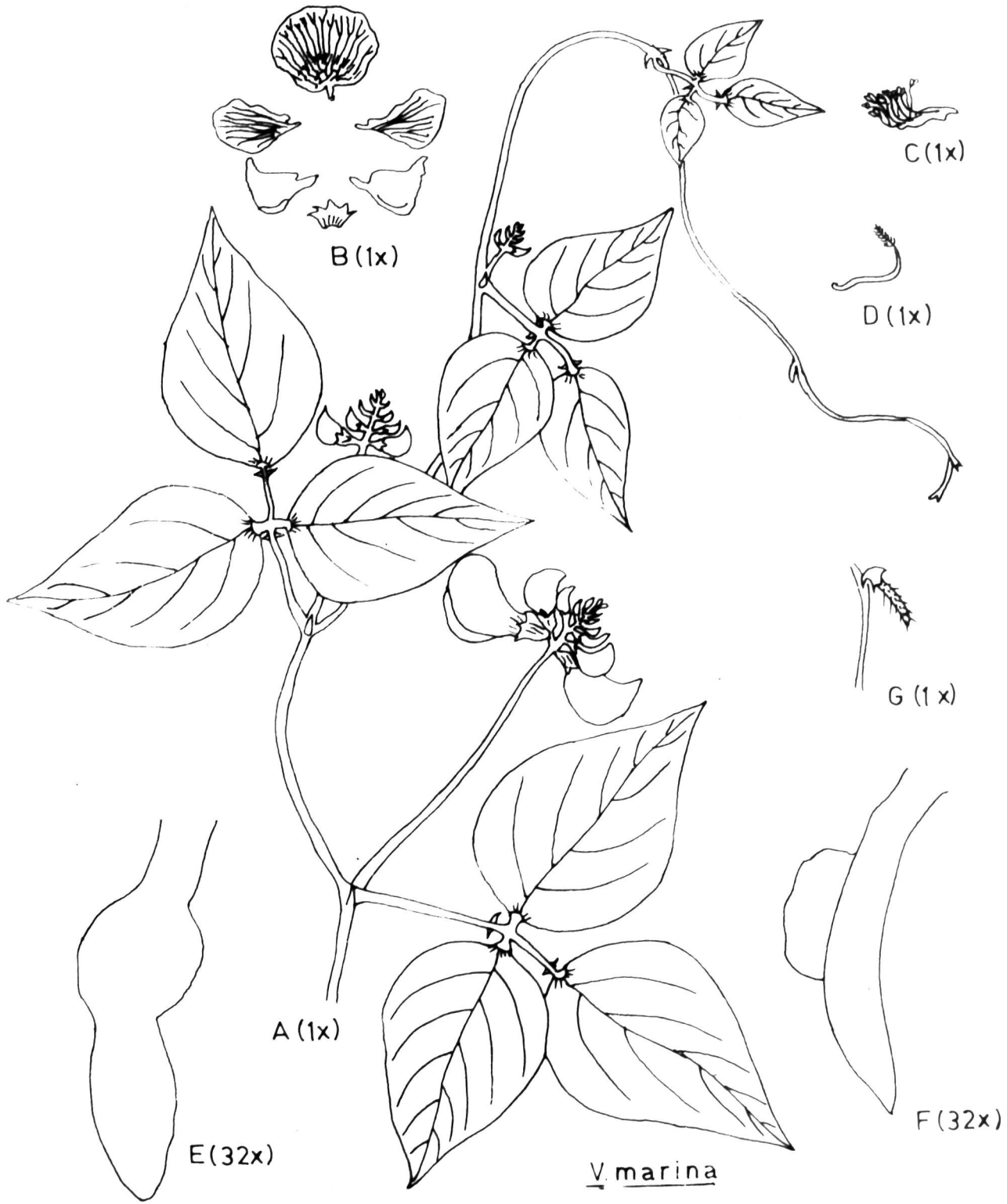
e) Mitotic chromosomes: The range in somatic chromosome length is  $3.2 \mu$  and  $1.8 \mu$ . The absolute length is  $56.20 \mu$ . The chromosomes have been grouped into 6 groups (Table 13) (Figs 26, 29).

Group A: Comprises two pairs of chromosomes with median primary constriction and measures  $3.2 \mu$ . Relative length and arm ratio are 11.39 and 1:1.26 respectively.

Group B: Has three pairs of submedian chromosomes measuring  $3.10 \mu$ , long arm being  $2.00 \mu$  and short arm  $1.10 \mu$ . Relative length is 11.03 and arm ratio 1:1.82.

Group C:  $2.50 \mu$  long this group has two pairs of chromosomes with satellites on the short arm which measures  $1.70 \mu$  and short arm  $0.80 \mu$ . Relative length and arm ratio are 8.90  $\mu$  and 1:2.12 respectively.

**Fig.27: Figure of morphological features of V.marina**



A (1x)

B (1x)

C (1x)

D (1x)

G (1x)

E (32x)

F (32x)

V. marina

Group D: Includes a pair of median chromosomes measuring 2.00  $\mu$ . Relative length is 7.12.

Group E: This has two pairs of chromosomes measuring 1.80  $\mu$  in length. The long arm measures 1.00  $\mu$  and the short arm 0.80  $\mu$ . Relative length and arm ratio of this median chromosome are 6.41 and 1:1.25 respectively.

Group F: 1.80  $\mu$  long this chromosome has a submedian primary constriction. Relative length and arm ratio are 6.41 and 1:1.57 respectively.

## **DISCUSSION**

## V. DISCUSSION

Results of the present study are discussed under the following heads:

1. External morphology
2. Pachytene chromosomes
3. Mitotic chromosomes
4. Phylogeny and species relationships

### 1. External morphology

Of the five species selected the three cultivated ones viz., V. unguiculata, V. mungo and V. radiatus are characterised by their erect plant type while the species V. cylindrica is bushy habit. However, V. marina differs from the other four species by having spreading and indeterminate plant type. V. marina can spread out over an area of about one square meter and is perennial in nature. V. unguiculata, V. mungo, V. radiatus and V. cylindrica, on the other hand, are annual in nature (Fig. 31).

Westphal (1974), has opined that not much work has been done with respect to the nature of germination, i.e., Epigeal or hypogeal, and has suggested that this might be an important taxonomic character and detailed studies should be taken up. Observations on the germination of seeds in the five species has shown that the two wild species viz., V. cylindrica and V. marina showed hypogeal germination while all the three cultivated species exhibited epigeal germination.

The first pair of leaves that emerge after germination of the seeds also exhibit inter-specific differences. The two wild species showed petiolated first foliage, while the three cultivated species, possessed sessile leaves. Earlier studies of Mackava (1955) and Tourneur (1958) on this character have pointed out that sessile nature of the first leaf is a characteristic feature of the genus Vigna while the petiolate nature is the characteristic of genus Phaseolus and Delichos.

In the present study, of the five species, three species viz., V. unguiculata, V. munge and V. radiatus, possess sessile leaves while the other two species viz., V. cylindrica and V. parina, bear petiolate first foliage. This should not mean that the latter two species do not belong to the genus Vigna as the other characters, of this species, agree very much with the genus Vigna. The variation for this character within the genus may be a case of evolutionary progress.

Based on the sessile nature of the first leaf which is a characteristic of the genus Vigna the two species P. munge and P. aureus have been regrouped under the genus Vigna. The observation and regrouping confirm to the earlier work of Mackava (1955) and Tourneur (1958).

As regards hairiness of plants, V. munge and V. radiatus possess profuse hair on them while the other species are

Fig. 28: Idiogram of chromosomes at pachytene stage

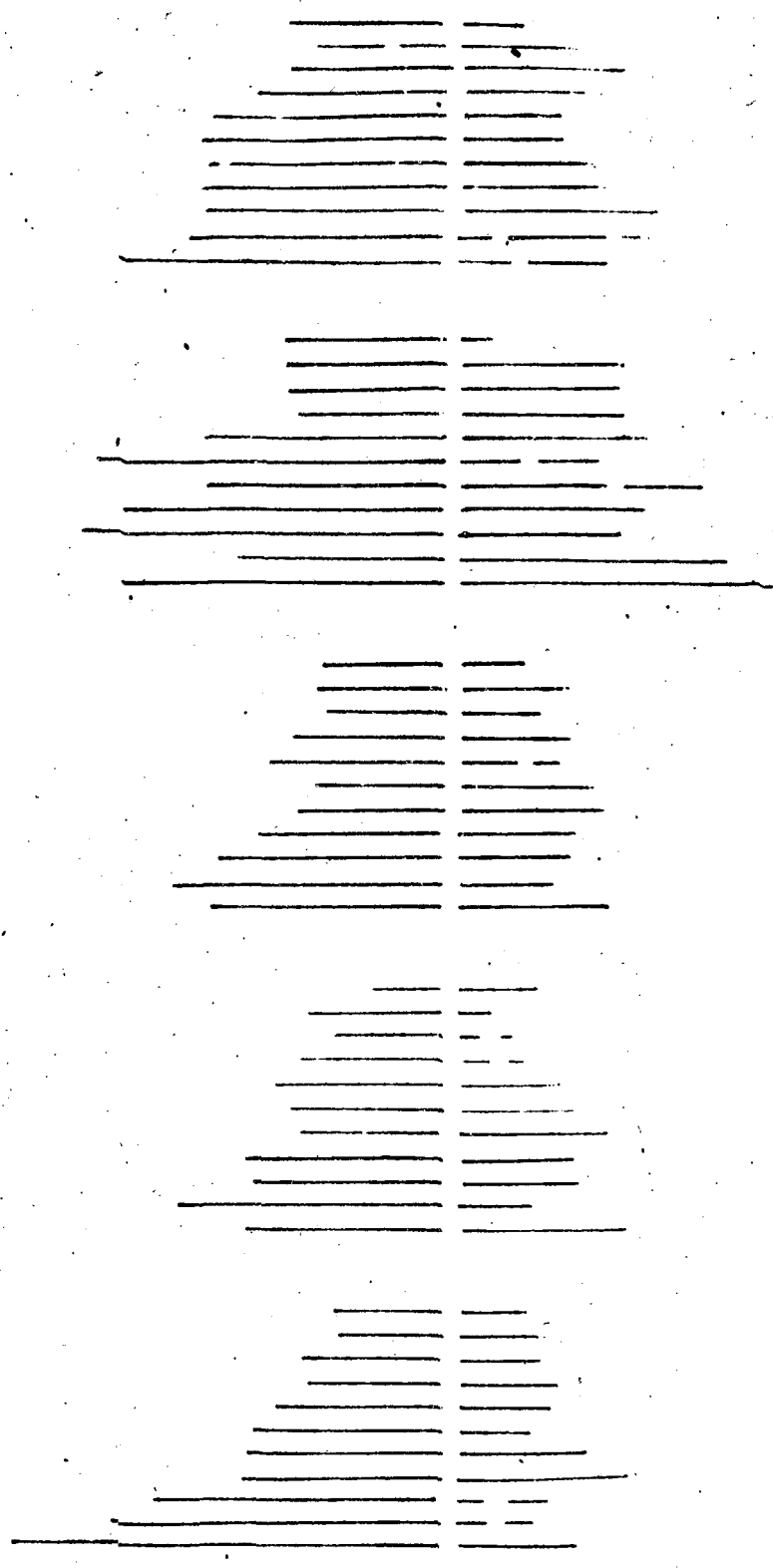
**I** ***Y-unguiculata***    **II** ***Y-sagax***    **III** ***Y-radiatus***    **IV** ***Y-cylindricus***  
**V** ***Y-marina***

V

IV

III

II



10%

Fig-28

glabrous. Though a lot of variation was observed regarding size of stipules among the five species they are present in all the species studied. The major difference has been that, it is appendaged in V. unguiculata, V. munge and V. radiata while it is not appendaged in V. marina and V. cylindrica. According to Wilczek (1954) stipules with appendage, that is, stipules extended below the point of insertion, is the character of the genus Vigna. In the present study stipules in, in V. marina and V. cylindrica are not extended below the point of insertion. This might again suggest that the stipule with appendage is an advanced character in the genus Vigna and thus generally present in cultivars.

The other morphological differences with regard to floral characters among the five species are flower colour, flower size, bracteoles, style and keel, apical appendage on stigma, nature and length of pods.

Flower size and colour: Though the flower colour in all the species studied is basically yellow slight variations have been observed. V. unguiculata possesses purple shaded standard petal and V. radiata has greenish yellow shaded standard petal. Accepting these two variations all other species exhibited yellow flowers. As for the size of the flower V. unguiculata and V. marina have large sized flowers, while the flowers of other three species are relatively small.

Fig. 29: Idiogram of somatic chromosomes

I *Y. magnicollata*    II *Y. munda*    III *Y. radiatus*    IV *Y. cylindrica*  
V *Y. marina*



V



IV



III



II



I

VI

**Style and keel:** In confirmity with the revised key proposed by Wilczek (1954), V.radiatus and V.munge possess style and keel making almost one complete turn - similar to the other three species included in the present study.

As regards style morphology all the five species studied possess an apical appendage, a beak like structure protruding beyond the stigma. This is similar to the type species of genus Vigna SAVI i.e. V.luteola, and not as in P.vulgaris, a type species of genus Phaseolus L.

**Pod length:** When pod length was observed V.unguiculata was found to have the longest pods, true to the direction in which the selection pressure has been applied for this economic character. All the other species have small pods ranging in length from 4-6 cms.

## 2. Pachytene chromosomes

Accurate and detailed information on chromosome morphology can be obtained by analysing mid-pachytene stage during meiosis. The condensation difference between cells is minimum at this stage and the position of the primary constriction can be easily identified. Pachytene analysis besides providing information on position of centromere, position of attachment of nucleolus, and nature of chromosome namely differentiated or

**Fig. 30: Comparative idiogram of individual chromosomes at pachytene stage**

**A. Y. unguiculata**

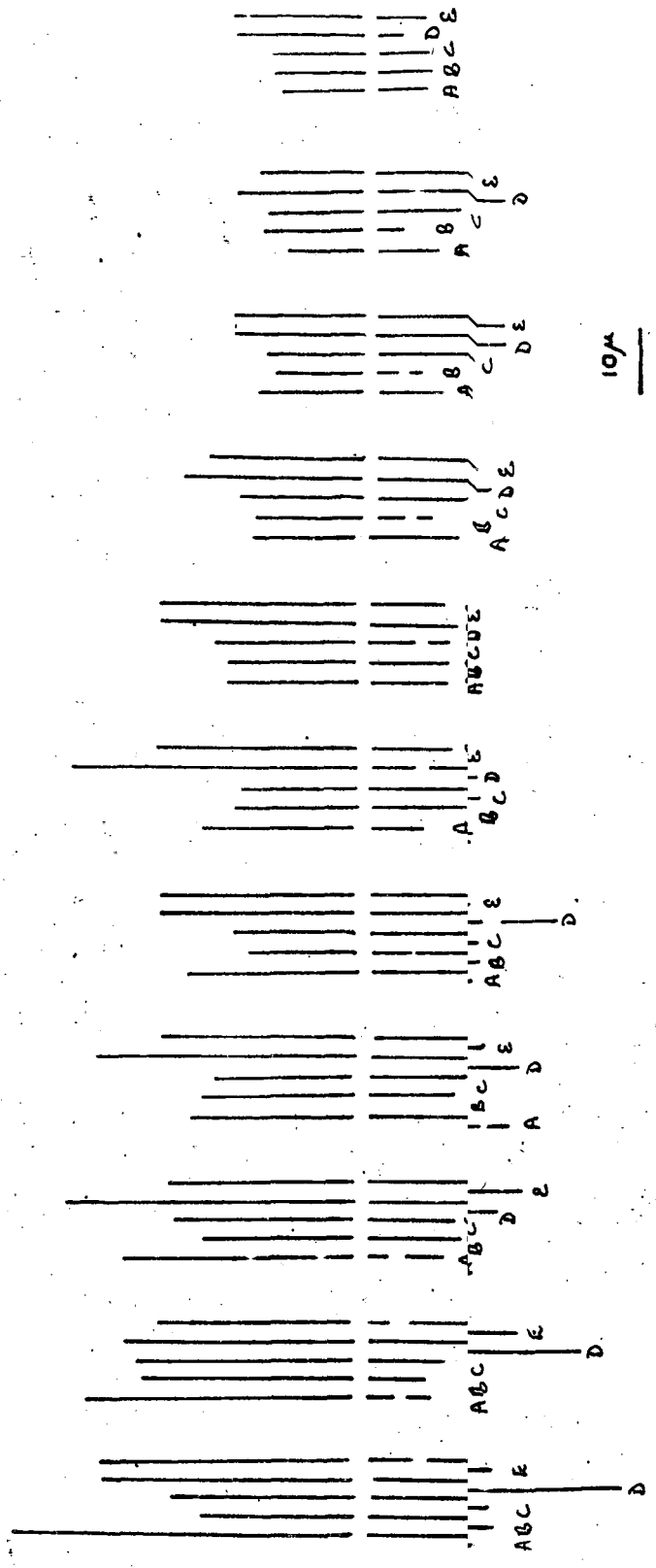
**B. Y. mungo**

**C. Y. radiatus**

**D. Y. cylindrica**

**E. Y. marina**

I      II      III      IV      V      VI      VII      VIII      IX      X      XI



10/A

Fig-34

undifferentiated, also provides us with information such as nature and distribution of chromatin material, which helps in identifying a species as advanced or primitive. This type of study was initiated with the pioneering work of McClintock (1929; 1931) in Zea mays. Since then the potential value of such an analysis and its wide application in solving of several cytogenetic and evolutionary problem has been realised (Venkateshwaralu, 1962).

It should however be cautioned that all plant species are not easily amenable for such studies. Success can be obtained, largely, in plants species which have low chromosome number and short sized chromosomes, but not in species with many and large chromosomes, because of the difficulty in getting well spread preparations. Further such a study cannot be taken up in plant species where chromosomes exhibit stickyness which is genetic in nature.

In the genus Vigna the haploid chromosome number has been only eleven, and the chromosomes are very short. These favourable factors have encouraged pachytene analysis in this genus (Krishnan and De (1965) in P. aurea; De and Krishnan (1967a) in P. unguis; and Mukharjee (1968) in V. sinensis).

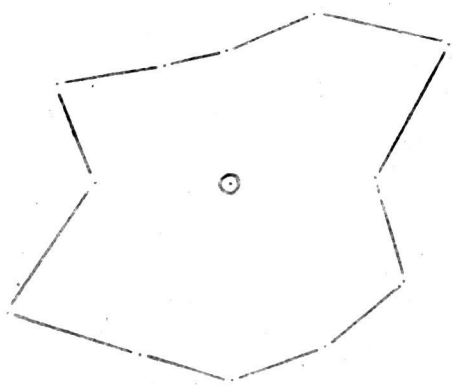
The pachytene chromosomes of all the five species exhibited the position of centromere very clearly and were also characterised by striking differential staining capacity.

Considering all the available information on pachytene analysis. Venkateshwaralu (1962) classified pachytene chromosomes into two broad types namely differentiated and undifferentiated. The former category is characterised by proximal darkly staining region and distally placed lightly staining region. The chromosomes of tomato (Barton, 1950), Plantago ovata (Hyde, 1963); Solanum (Coatechalk, 1954), Sorghum (Nagoon, 1960; 1961) and several other crop plant come under this category. The second category consists of chromosomes which stain uniformly or show chromomere size gradient from proximal to distal end. The pachytene chromosomes studied in Asperagus (Lima-de-Faria, 1952), Sorghum purpurio-sericeum (Reddy, 1958) and barley (Sarvella, et al., 1958) are of this type. The pachytene chromosomes of the five species in the present study may be grouped under the type "differentiated chromosomes". This classification of pachytene chromosomes has already been suggested by Krishnan and De (1965) in P. aureus and De and Krishnan (1967a) in P. unguo (Fig. 28).

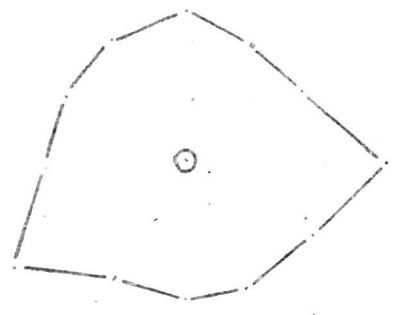
The total chromatin length being 434.40  $\mu$ , This species (P. unguiculata) consisted of the maximum number of asymmetrical chromosomes (having an arm ratio of more than 1:2). Of the four, the second chromosome was the most asymmetrical with an arm ratio of slightly more than 1:4. In this species two bivalents namely second and third appear to be in association with the nucleolus subterminally (Fig. 33).

**Fig.31: Polygraphs of the five species**

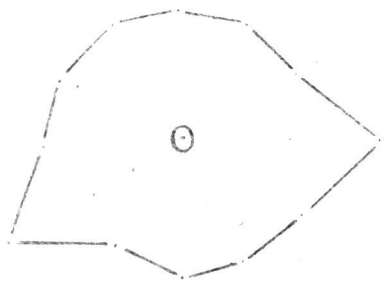
I V.unguiculata    II V.mungo    III V.radiatus    IV V.cylindrica  
V V.marina



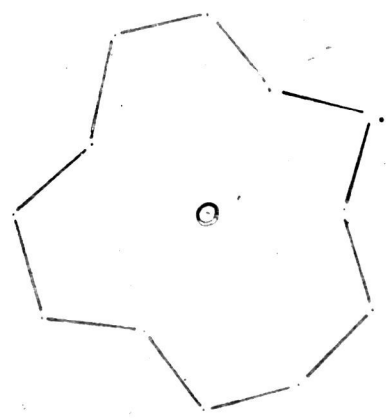
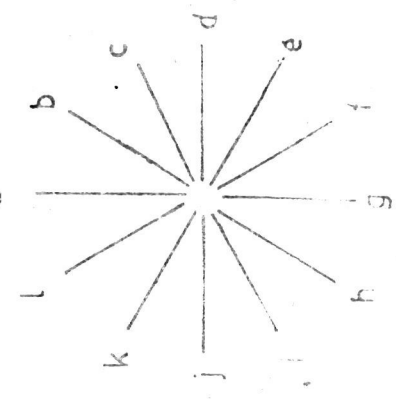
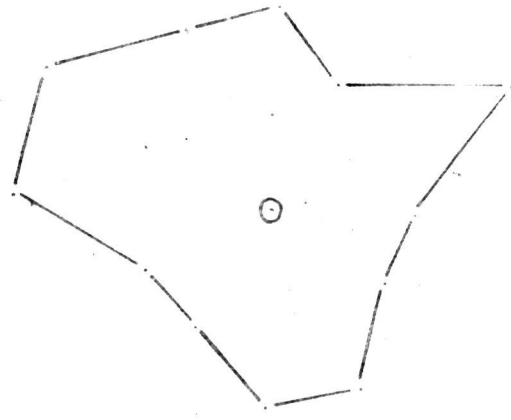
I.



II.



III.



IV

Fig- 31

In V.mungo also two bivalents have been found to be associated with the nucleolus (Eighth and Ninth). The complement of this species consisted of four asymmetrical bivalents and of these chromosome 10 was the most asymmetrical, with an arm ratio of 1:4.4. The total chromatin length at pachytene in this species was 357.60  $\mu$ . A similar observation has been made by De and Krishnan (1967a).

In V.radiatus (P.aureus) the complement consisted of two asymmetrical bivalents i.e., second and third. In this species only one bivalent with an arm ratio 1:1.77 and taking the seventh position in the order of length in the karyotype, was found to organise the nucleolus terminally on the short arm. Krishnan and De (1965) have studied the pachytene chromosome morphology in this species and have reported three asymmetrical type of bivalents i.e., second, third and eighth. However in the present study eighth chromosome was more of a median type with an arm ratio of 1:1.39.

In the species V.cylindrica the short<sup>est</sup> chromosome measured 24.00  $\mu$  and the longest is 80.00  $\mu$ . The overall length of the pachytene bivalents is 607.60  $\mu$ . It consists of three asymmetry type of chromosomes namely third, sixth and eleventh. The 11th pachytene bivalent is the most asymmetrical one with an arm ratio of 1:5. In this species also two pachytene bivalents, fifth and sixth appear to be in association with the nucleolus.

The complement of V. marina consists of four asymmetrical chromosomes. The shortest measures 28.0  $\mu$  and the longest 59.2  $\mu$ . The overall length of the pachytene bivalents is 496.00  $\mu$ . The nucleolus was found to be associated with two pachytene bivalents namely 1 and 2.

Considering the nucleolus organising chromosomes in all the species, it is evident that except in V. radiatus the nucleolus in the other species is associated with two pachytene bivalents. It is only in V. radiatus that only one pachytene bivalent is associated with nucleolus. A similar observation has been recorded by De and Krishnan (1967a) in P. mungo. The association of nucleolus with two bivalents leads to think in a different way about the basic number of the genus Vigna. It is generally accepted that the basic number of Vigna is 11. If eleven were to be the basic number then only one pachytene bivalent should have been in association with the nucleolus. But in the present study of the five species, in four out of five species, the nucleolus was found to be in association with two pachytene bivalents. This situation leads to a second thought about basic number and hence a basic number of 5 or 6 may be thought of for the genus Vigna. This is purely a suggestion, and until enough data is accumulated with respect to cytology of haploids, and genetics of certain qualitative characters, to substantiate this view point about the basic chromosome number no definite conclusion can be drawn (Fig. 30).

**Fig.32: Diagram of chiasma frequency and distribution  
at diakinesis in the five species.**

**I *Y.unguiculata*    II *Y.annua*    III *Y.radiata*    IV *Y.cylindrica*  
V *Y.marina***

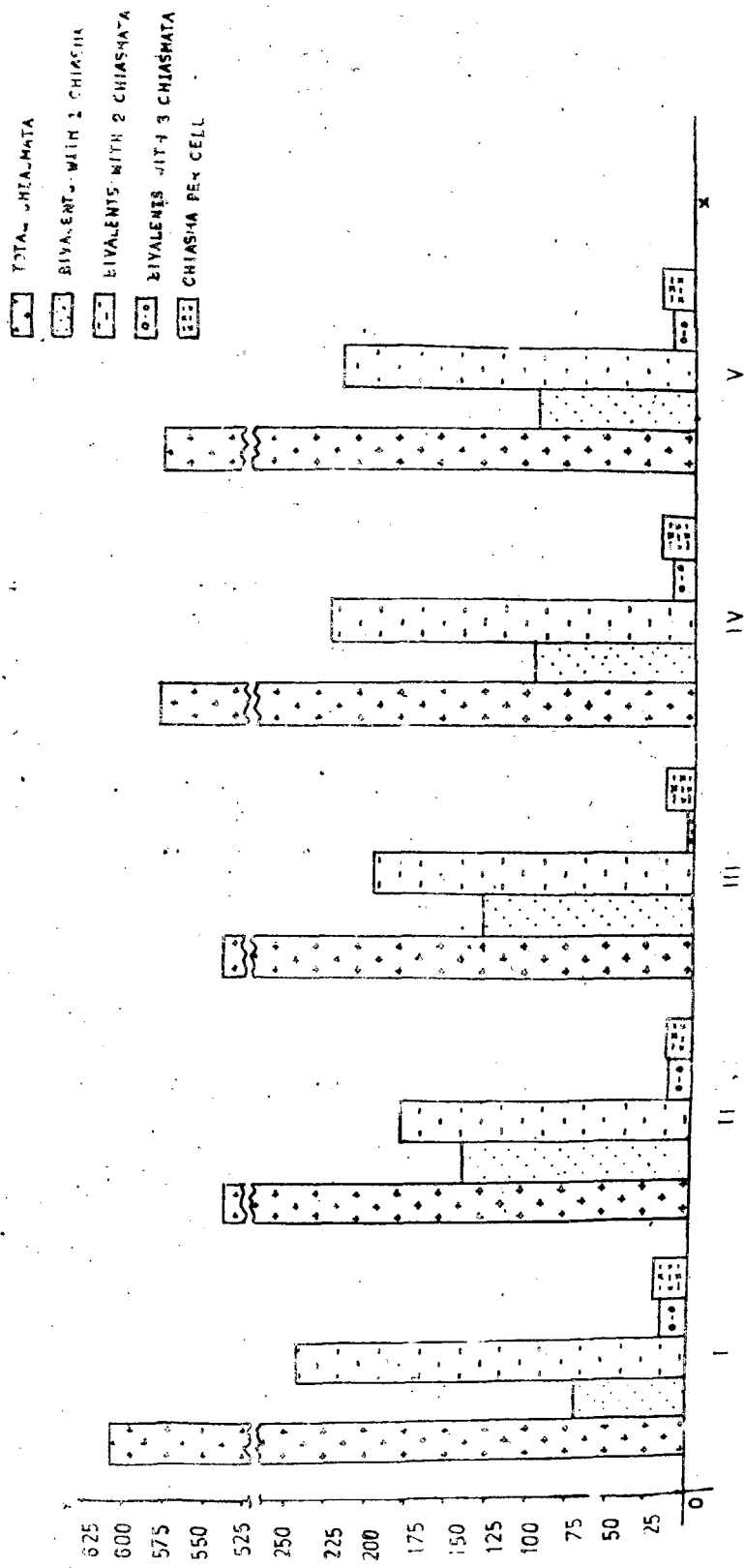


Fig - 3A

Differences amongst the idiogram of the five species is considerable. Taking into consideration the first chromosome of all the five species and observing the arm ratios. Y.gunago, Y.radiatus and Y.cylindrica are symmetry in nature while in Y.unguiculata and Y.marina it is asymmetry type. In none of the species except Y.marina, this chromosome was associated with nucleolus. Same trend holds good in chromosome two however, the second chromosome in Y.cylindrica and Y.marina is symmetrical and asymmetrical in other three species. Fourth and fifth chromosomes are symmetrical in all the five species. The sixth chromosome is symmetrical in Y.gunago and Y.radiatus but asymmetrical in the others. Seventh chromosome is symmetry in all species except Y.marina. Likewise, eighth, ninth and tenth chromosomes are asymmetrical in Y.gunago and symmetrical in all the other species. The shortest chromosome of the complement is asymmetrical in Y.cylindrica and Y.marina and symmetrical in the other three species (Tables 14, 15).

Levitzky, (1931) developed the concept of symmetry and asymmetry to describe the karyotype of a taxon. Symmetry karyo-type is one in which all the chromosomes are approximately of the same size and have median or submedian centromere. Asymmetrical karyotype is that which consists of chromosomes with median, subterminal or terminal centromere and possess great difference in relative size of individual chromosomes in the complement.

Table 14: Comparative chromosome lengths at pachytene in the five species of *Y. nana*

Chromosome No.	<i>Y. unipunctulata</i> ( $\mu$ )	<i>Y. fuzge</i> ( $\mu$ )	<i>Y. radiatus</i> ( $\mu$ )	<i>Y. cylindrica</i> ( $\mu$ )	<i>Y. parina</i> ( $\mu$ )
1	69.60	44.80	47.98	80.00	59.20
2	53.60	43.20	46.15	70.40	56.80
3	49.60	39.20	42.44	65.60	56.00
4	48.00	38.40	38.14	63.20	49.60
5	43.20	35.20	36.91	61.60	48.00
6	33.60	34.40	36.29	61.60	44.00
7	32.80	33.60	35.06	53.60	43.20
8	31.20	25.60	33.83	47.60	40.00
9	28.00	21.60	30.76	40.00	40.00
10	23.20	21.60	29.52	40.00	31.00
11	21.60	20.00	23.99	24.00	28.20
Total length	434.40	357.60	401.07	607.60	496.00

Stebbins (1958) employed the term symmetry and asymmetry. Based on the centromeric position he formulated four groups. The proportion of chromosomes with arm ratio less than 1/2 being - (0.00), (0.01-0.51), (0.51-0.99) and (1.00) in groups 1, 2, 3 and 4 respectively, and according to the variation between the shortest and longest chromosome in the complement, three groups were suggested, the ratio between the length of the shortest and longest chromosomes in the complement being, less than (1:2), (1:2 to 1:4), and more than (1:4) in the groups 'a', 'b' and 'c'. When both the criteria are used together (1, 2, 3, 4) x (a, b, c), 12 groups are obtained, the group 1a, represents the most symmetrical, karyotype, while group 4c represents the most asymmetrical karyotype.

Based on this classification it is evident that all the karyotypes of the five species studied fall in '2B'. Similar pattern of karyotype i.e., '2B' has been reported by Shambulingappa (1962) in almost all Eg-serghus. Stebbins (1971) has stated for Eg-serghus that '2B' type of karyotype is the end point in evolution and that further evolution should take place mostly by genic mutations. The same holds good for the genus Vigna.

The variation in the karyotype of the species at pachytene strongly suggest difficulty in transfer of genetic material

from one species to another, due to problems in crossing, or after crossing seed setting, or after seed setting, problem with  $F_1$  being sterile. This has been shown by Dana (1966a) in a cross between *V. mungo* and *P. radiatus* and De and Krishnan (1967b). Efforts to produce crosses between *V. marina* and other four species in this laboratory by the author have not been successful due to diploetic sterility, or abortion of the zygote.

Attempts at inducing crosses between *V. unguiculata* and *V. mungo* and also *V. radiatus* have not been done till now. Crosses with *V. marina* was undertaken as this wild species has resistance to septoria leaf spot and powdery mildew disease. Both of which is absent in *V. mungo*, *V. radiatus* and *V. unguiculata*.

### 3. Somatic chromosomes

Somatic chromosome number for several species of *Vigna* and *Phaseolus* have been reported by Karpachenko (1925).

Marshall (1969, 1970), has reported a total chromatin length of 35.20  $\mu$  in *V. unguiculata*, 31.44  $\mu$  in *V. radiatus*, 31.57 in *V. mungo* and the range of chromosome length is 1.20-2.20 in *V. unguiculata*, 0.96-2.27 in *V. radiatus* and 1.17-2.27 in *V. mungo*.

Sardhey (1978) has reported the total chromatin length as 34.40  $\mu$  in *P. aureus* with the shortest chromosome measuring

Table 15: Comparative chromosome lengths at somatic metaphase in the five species of *Vigna*

Chromosome No.	<i>V. unguiculata</i> ( $\mu$ )	<i>V. mungo</i> ( $\mu$ )	<i>V. radiatus</i> ( $\mu$ )	<i>V. cylindrica</i> ( $\mu$ )	<i>V. marina</i> ( $\mu$ )
1	2.60	2.20	3.30	3.00	3.20
2	2.30	2.10	2.40	2.40	3.20
3	2.30	2.10	2.00	2.40	3.10
4	1.70	2.10	2.00	2.00	3.10
5	1.70	1.70	2.00	2.00	3.10
6	1.70	1.70	1.80	2.00	2.50
7	1.70	1.40	1.60	1.80	2.50
8	1.40	1.40	1.60	1.80	2.00
9	1.40	1.40	1.40	1.80	1.80
10	1.40	1.40	1.40	1.80	1.80
11	1.20	1.10	1.40	1.50	1.80
Absolute length	19.40	18.60	20.90	22.50	28.10
Total	38.80	37.20	41.80	45.00	56.20

1.40  $\mu$  and the longest measuring 2.8  $\mu$ . In V. mungo the total chromatin length is 33.20  $\mu$ , range being 1.20  $\mu$  - 2.20  $\mu$ .

Sen and Bhowal (1962) have reported chromosome number in V. marina for the first time as  $2n=22$ , and the length of individual chromosome ranges from 1.6  $\mu$  to 3.7  $\mu$ .

Review of literature to-date has revealed that there is no prior report of chromosome number of the species V. cylindrica Skeels. So this is the first report of chromosome number of this species. The diploid chromosome number for this species is  $2n=22$ , the range in length is between 1.50  $\mu$  and 3.00  $\mu$ . Total chromatin length is 45.0  $\mu$  (Fig. 29).

#### 4. Phylogeny and species relationship

It may be seen from the consolidated idiogram of all the five species that the chromosome number of all the five species is same. The idiogram of the five species presents wide differences with respect to characters such as arm ratio, number of satellited chromosomes and overall length of the chromatin material. In V. marina the chromatin material is maximum (56.20  $\mu$ ) and is least in V. mungo (37.2  $\mu$ ). Further, if we consider the symmetry and asymmetry type of chromosomes in each of the species. V. mungo has a high number of asymmetry chromosomes (3 pairs) while V. marina has one pair only.

**Fig.33: Comparison of total chromatin length at pachytene and somatic metaphase in the five species**

**A *Y. maculata* B. *Y. masoni* C. *Y. radiatus* D. *Y. cylindrica*  
E. *Y. marina***

AT PACHYTENE



AT SOMATIC METAPHASE

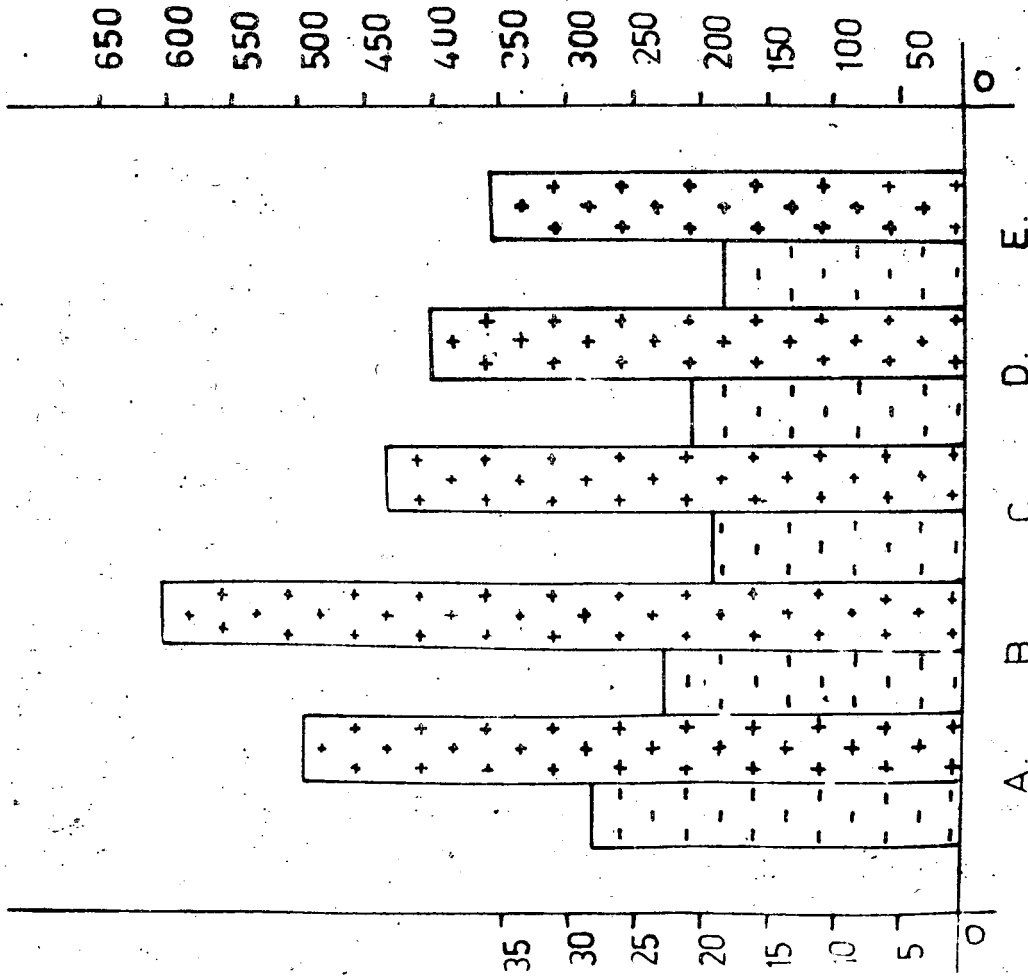


Fig - 33

A B C D E

Considering the overall length of the chromatin material and the number of asymmetry chromosomes. V.marina has the longest chromosomes 56.20  $\mu$ , followed by V.cylindrica (45.00  $\mu$ ), V.radiatus (41.80  $\mu$ ), V.unguiculata (38.80  $\mu$ ), and V.mungo (37.20  $\mu$ ). Three asymmetrical chromosomes were observed in V.mungo two each in V.unguiculata and V.cylindrica one each in V.radiatus and V.marina. This suggests that V.marina is the primitive form while V.mungo is evolved.

It may be also observed that total chromosome length shows a decreasing trend from V.cylindrica (607.60  $\mu$ ) to V.mungo (357.60  $\mu$ ) at pachytene and from V.marina (56.20  $\mu$ ) to V.mungo (37.20  $\mu$ ) at metaphase. This aspect clearly throws light on the evolutionary status of the five species and shows that V.cylindrica and V.marina are the wild relatives while V.mungo is the most evolved. Such a process has been shown to have played a role in the evolution of other genera such as Crepis (Bobcock and Cameron, 1934) and Phaseolus (Sarbhoy, 1978).

The fact that idiograms of the five species differ, considerably amongst themselves indicated that interspecific hybridization will be difficult.

Two chromosomes in four species namely V.unguiculata, V.mungo, V.cylindrica and V.marina are associated with the nucleolus while in V.radiatus only one pair of chromosome is

involved. The probable significance of the presence of two pairs of satellite chromosomes is already discussed in the previous pages.

Based on the present study, it can be concluded that speciation in Vigna is mainly due to the structural differences in chromosomes followed by natural selection. Thus reallocation of the two species P.mungo and P.aureus from the genus Phaseolus to Vigna based on the morphological characters may be substantiated to some extent by the pachytene analysis. The pachytene chromosomes in these two species are also differentiated and the nucleolus is organized terminally. However, more cytological information through pachytene analysis in the hybrids involving V.mungo and V.radiatus with the other Vigna species and also Phaseolus species should be sought before reaching a definite conclusion. Morphological observations alone cannot be taken into consideration for taxonomic consideration as there may be intra-generic differences that might have arisen as a result of genic mutations during the process of evolution.

## **SUMMARY**

## VI. SUMMARY

A detailed study of morphological features, particularly of those characters with diagnostic value, was undertaken in five species of the genus Vigna SAVI. viz., V. unguiculata, V. munge, V. radiatus, V. cylindrica, and V. marina. The five species exhibited differences with respect to germination; petiolate nature of first leaf; stipule extension below point of attachment to stem; extent of curvature of style and keel; presence of apical appendage and pod dehiscence.

The analysis of chromosomes at pachytene and later stages of meiosis was done in these species. The chromosomes were identified on the basis of relative length; centromeric position; arm ratio; total chromatin length; and presence of secondary constriction. The characteristic features of each of the eleven chromosomes in the haploid complement is described in detail with greater emphasis on similarities and differences among the species studied.

The nucleolus was found to be associated with two pairs of chromosomes in V. unguiculata, V. munge, V. cylindrica and V. marina, while in V. radiatus only one bivalent was found to be in association with the nucleolus. In all the five species studied nucleolus was found to be attached to the short arm.

Pairing of chromosomes was observed to start from the proximal region and proceed to the distal end and was perfectly normal in all the species studied.

Total chromatin length at pachytene was found to be as follows V. unguiculata 434.40  $\mu$ ; V. mungo 357.60  $\mu$ ; V. radiatus 401.07  $\mu$ ; V. cylindrica 607.60  $\mu$ ; V. marina 496.00  $\mu$ . The longest pachytene bivalent was observed in V. cylindrica (80.00  $\mu$ ) while shortest was in V. mungo (20.00  $\mu$ ).

Analysis of chiasma at diakinesis in 30 cells in each of the species studied gave the following results V. unguiculata had the maximum total chiasmata (607) while the least number (536) was observed in V. radiatus. The calculation of chiasmata per bivalent showed differences among the five species. The values were as follows: V. unguiculata 1.84; V. mungo 1.62; V. cylindrica 1.74; V. marina 1.73.

Total chromatin length at mitotic metaphase ranged from 56.20  $\mu$  in V. marina to 38.20  $\mu$  in V. mungo. Chromosome association with nucleolus at mitosis was similar to that observed in meiosis.

The results of the detailed karyomorphological studies carried out in the five species further substantiates the reclassification of Phaseolus mungo and P. aureus, under the genus Vigna as proposed by Wilczek (1956) and Hepper (1954).

Regarding the phylogenetic relationship - Comparison of chromatin length and symmetry of chromosomes among the five species indicated that V. marina as most primitive form while V. mungo and V. unguiculata are the highly evolved forms.

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