GENOMIC RELATIONSHIP IN *Vigna* SPECIES

By

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THESIS

submitted in partial fulfilment of the
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Department of Agricultural Botany
COLLEGE OF HORTICULTURE
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DECLARATION

I hereby declare that this thesis entitled "GENOMIC RELATIONSHIP IN *Vigna* SPECIES" is a bona fide record of work done by me during the course of research work and the thesis has not previously formed the basis for the award to me of any degree, diploma, associateship, fellowship or other similar title of any other University or Society.

College of Horticulture,
Vellanikkara,
7th June, 1986.

Neema V.P.

NEEMA V.P.

CERTIFICATE

Certified that this thesis is a record of research work done independently by Smt. Neema, V.P. under my guidance and supervision and that it has not previously formed the basis for the award of any degree, fellowship or associateship to her.

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We, the undersigned, members of the Advisory Committee of Smt. Neema, V.P., a candidate for the degree of Master of Science in Agriculture with major in Agricultural Botany, agree that the thesis entitled "Genomic relationship in Vigna species" may be submitted by Smt. Neema, V.P., in partial fulfilment of the requirements for the degree.

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CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF PLATES

Introduction

INTRODUCTION

Pulses which constitute a major group of crops of the legume family, form the chief source of protein in the vegetarian diet. India has the largest area in the world under grain legumes or pulse crops. It covers an area of 22.8 million hectares and yields about 13.1 million tonnes of grains.

Cowpea, the major pulse crop cultivated in Kerala, belongs to the genus *Vigna* of the tribe *Phaseoleae* under the family Leguminosae. Cultivation is confined to the uplands during the South West monsoon period (36 per cent) and to the fallow lands during the second (24 per cent) and during the third crop season.

Opinions seem to vary in respect of the taxonomy and nomenclature of the common cultivated cowpea. Piper (1912 and 1914) described cowpea as *Vigna sinensis* with three distinct subdivisions to the genus *Vigna*, namely, *Vigna sinensis* (cowpea), *Vigna sesquipedalis* (yardlong or asparagus bean) and *Vigna cylindrica* (catjang bean) and recognised these subdivisions as distinct species. However, Pavlov (1962) considered these subdivisions as subspecies of *Vigna sinensis* while Morse (1964) regarded them as only botanical varieties. Subsequently, based on the studies in the

Leguminosae-Papilionaceae for the 'Flora of Tropical East Africa' conducted by Verdcourt (1970), it has now been agreed upon that the common cultivated cowpea is Vigna unguiculata (L.) Walp. with five subspecies, of which Vigna unguiculata subsp unguiculata, Vigna unguiculata subsp. sesquipedalis and Vigna unguiculata subsp. cylindrica alone are cultivated. Their easily crossable nature, complete fertility of the F_1 hybrid, normal chromosome pairing in the F_1 hybrid etc. might have suggested the author to consider them as subspecies.

Pulse crops, in general have not been subjected for any extensive cytogenetic studies so far. Cowpea is no exception to this. Extensive diversity in morphology and maturity of pods are noticed in this crop. Plant types with both grain and vegetable characters having synchronisation in pod maturity will be of immense help to Kerala farmers. Cytological studies of different subspecies will give valuable information regarding the scope of genetic improvement of the crop.

The technique of genome analysis consists largely in the evaluation of species affinities by the degree of chromosome pairing at the F_1 meiosis of inter-specific crosses. The occasional pairing of non-homologous chromosomes is a disturbing feature in the

technique. Moreover, genomes may loose their individuality as a result of translocations between chromosomes of diverse genomes. Nevertheless, genome identifications provide a useful, albeit oversimplified picture of species relationships.

Studies on the morphology of chromosomes yield useful information on species differentiation and affinities. Chromosomal variability in relation to the diversity of life has been the outcome of evolution. Cytologists who compare the chromosomes of related species recognise five different kinds of variation such as variations in absolute chromosome size in staining properties, in chromosome morphology, in relative chromosome size and in chromosome numbers. When different groups of vascular plants are compared, the advanced genera belonging to highly specialised families have smaller chromosomes and lower nuclear DNA contents than primitive vascular plants. Chromosomal differences reflect more or less directly the genic content of the individual.

Karyotype is the morphological aspect of the chromosome complement as seen at mitotic metaphase. Differences in karyotype morphology reflect differences in gene arrangement, which can affect drastically the way in which genes can become segregated and recombined

in Mendelian heredity. Differences in chromosome number may reflect either differences in gene arrangement or gene duplication or both. In short, chromosomal differences reflect differences in the source of genetic variations, while morphological, physiological and biochemical differences reflect differences in the products of gene action, modified by environmental differences.

The present investigations will provide valuable basic information regarding the cytological properties of the subspecies under study. The cytological studies will give an insight to the genomic relationships of these subspecies which will have far reaching implications in the planning of successful breeding programmes. Hence, the present study was conducted with the following objectives.

1. To study the meiotic and mitotic behaviour of chromosomes of two subspecies of cowpea and their F_1 hybrid

2. To identify the crossability of the subspecies by conducting meiotic studies in the hybrid

3. To study the sterility in the hybrid of the subspecies of cowpea by studying the nature of pollen grains.

Review Of Literature

REVIEW OF LITERATURE

The genus *Vigna* belongs to the tribe *Phaseoleae* of the family *Leguminosae*. It is very closely related to two other genera of the tribe namely *Phaseolus* and *Dolichos*. About 186 species of *Vigna* have been identified, out of which only ten are commonly found in India. Information regarding the genomic relationships of the different taxomonic units can be achieved by detailed cytological investigations of the different species as well as their hybrids. However, not much cytological work seems to have been done in *Vigna* species. As such similar work done in other genera of the family Leguminosae is also included in this review in order to project the overall magnitude of the subject.

Origin

Vigna unguiculata (L.) Walp. commonly called Cowpea, black eye or southern pea is an important tropical and subtropical legume crop grown for both green and dry seed and also for forage. This is also referred to as *V. sinensis* by certain earlier authors. There is dispute in literature regarding the centre of origin of this crop. The tropical and subtropical genus of *Vigna* Savi is very diverse in form.

The West or Central African centre of origin for

Vigna sinensis was first proposed by Kornicke in 1885 (cited by Piper, 1913) after he had studied specimen collected in that area. Piper (1913, 1924) reversed his earlier support of the Indian Centre of Origin (Piper, 1912) after studying what he considered to be the wild form of V. sinensis which came from Africa. Two centres of origin, namely, India and Abyssinia were proposed by Vavilov (1951). Saunders (1959) is of the opinion that tropical Africa is the centre of origin because of the great diversity found in that area. According to Dana (1976) all the three cultivated forms have primary centre of origin in India and secondary centre in Africa and China. Out of about 186 species of Vigna only ten are endemic to India and 136 to Africa and together with some linguistic and historical evidence it has been suggested that cowpea originated in Central or West Africa rather than in India. He suggested chromosomal differentiation to have been contributed to evolution of cowpea, although the precise nature of chromosomal alterations is not known. All wild forms are climbers, the bushy growth habit being a cultivated trait. The wild types also have tuberous root and swollen stem with a tendency to perennation.

V. sinensis, V. catiang and V. sesquipedalis were considered as one species by Piper (1912). He concluded

that the wild African plant, V. sinensis with blakish scabrous pods and scabrous leaflets was the original wild form of our cultivated cowpea.

Taxonomy

V. unguiculata (L.) Walp. is a member of the family Papilionaceae, the largest of the three divisions of the Leguminosae. The general classification and nomenclature of the plant is however confused.

Based on the key of characteristics given by Linnaeus, Bentham and Hooker (1865) have listed 47 genera under Papilionaceae. Phaseolus L. and Vigna Savi were grouped under Papilionaceae by them.

Hooker (1879) stated 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.

Piper (1914) described the plant cowpea as V. sinensis and discarded V. unguiculata. He distinguished three varieties: 1) V. sinensis cowpea 2) V. sinensis var. cylindrica catjang and 3) V. sesquipedalis, asparagus bean.

The three cultivated types V. sinensis, V. catjang and V. sesquipedalis were not considered as distinct species by Sen and Bhowal (1960a), but were regarded as

members of a polymorphic species; (subspecies V. sinensis, V. catjang and V. sesquipedalis).

Faris (1965) made studies on origin and evolution of the cultivated forms of Vigna sinensis and reported four groups of Vigna species with worldwide distribution. Each group contained a number of closely related forms considered to be species by some texonomists but subspecies by others. These groups are 1) V. sinensis (L.) Savi, 2) V. luteola (Jacq) Benth, 3) V. vexillata (L.) Benth and 4) V. latea (A.) Grag.

There are three divisions of V. sinensis. These are designated as species by Piper (1912), subspecies by Pavlov, 1962 (cited by Sellschop, 1962) and as botanical varieties by Morse (1964) chiefly on the basis of seed and pod characteristics. The three divisions are var. sinensis, the common cultivated cowpea; var. sesquipedalis, the yardlong or asparagus bean and var. cylindrica, the catjang bean.

Verdcourt (1970) renamed cowpea as Vigna unguiculata, recognised five subspecies to it as shown below:

V. unguiculata subsp. dekindtiana, mensensis } wild

V. unguiculata subsp. *unguiculata*, *cylindrica*, *sesquipedalis* } cultivated

Chromosome number

The chromosome number of different species reported from time to time as given by Darlington and Wylie (1945) (cited by Sen and Bhowal 1960c) are *V. unguiculata* 2n = 22, *V. sinensis* 2n = 24 and *V. sesquipedalis* 2n = 24.

The cytogenetical study of *V. sinensis*, *V. sesquipedalis* and their hybrid by Floresca (1960) showed, a basic chromosome number of 12 giving 2n = 24. The somatic and meiotic studies revealed the same results. Normal behaviour of the chromosomes in the reduction division was observed in the hybrid, indicating a close affinity and homology of the parental genomes.

Faris (1964) studied the chromosome number of 192 cultivars of *Vigna sinensis* (L.) Savi including the cultivated forms var. *cylindrica* and var. *sesquipedalis* from 42 countries and reported 2n = 22. Earlier counts by other workers of 2n = 24 and n = 12 were not substantiated.

Kawakami (1930) stated n = 12 for all the three cultivated subspecies of *Vigna* namely *V. cylindrica*, *V. sinensis* and *V. unguiculata* and reported 2 n = 24

for V. cylindrica and V. sinensis. However, 2n was 22 for V. cylindrica and V. unguiculata and 24 for V. sinensis according to Karpenchenko (1925). Sen and Bhowal (1960c) reported 2n = 22 for all the three cultivated subspecies namely V. sinensis, V. catjang, V. sesquipedalis and for 11 wild species. Meiotic studies indicated the possibility of 11 as basic chromosome number for Vigna. A diploid number of 24 was listed for V. sinensis, V. sesquipedalis and their F_1 hybrid by Floresca et al. (1965).

Cytomorphological studies in five species of genus Vigna was conducted by Shashidhar (1981) and reported the somatic chromosome number as 2n = 22 and the meiotic number as n = 11.

Sen and Bhowal (1960b) reported that the chromosomes of all the subspecies were small ranging from 1.6 to 3.7 µm in length. Meiotic studies showed regular pairing of homologous chromosomes forming bivalents in diakinensis and metaphase and normal subsequent stages. Meiotic irregularities were noticed in the cultivated variety Poona of the subspecies V. catjang. Some cells showed more than two spindles in anaphase II and consequent separation of chromosomes in more than four poles.

A number of meiotic irregularities such as

nonpairing of chromosomes, early disjunction, and the presence of bridges and laggards with unequal distribution of chromosomes at anaphase and polyspory in the F_1 species of Phaseolus lunatus x P. polystachyus were reported by Dhaliwal and Pollard (1962). The meiotic behaviour of chromosomes was found to be normal in the material fixed from parent plants. A number of meiotic abnormalities were, however, recorded in the material fixed from the hybrid plants, under the same environmental conditions and method of preparation of slides. The irregularities were assumed to be due to other factors than the cytologic or genic causes. They attributed the cause to difference in the genetic composition or size of the chromosomes of the two genomes.

Studies on a diploid interspecific hybrid in Arachis by Raman and Kesavan (1962) showed congregation of the bivalents in two to three groups. Delayed disjunction of one to two bivalents caused bridges at anaphase I. Two nuclear bivalents were present.

Krishnan and Deepesh (1965) reported that the pachytene chromosome of Phaseolus aureus resembled the differentiated chromosomes of tomato. At pachytene, the chromosomes were characterised by heteropycnotic region exhibiting chromomere pattern near the centromere and light staining regions with no detectable

chromomere distal to them. The individual chromosomes could be identified on the basis of chromomere pattern, arm ratio and relative length. The somatic chromosomes could be grouped into six types based on their relative length, position of centromere and the presence or absence of secondary constriction. The relative length and position of secondary constriction of the two nucleolar chromosomes were not in agreement at pachytene and somatic metaphase stages. The diplotene separation is initiated both at the chromosome ends and near the centromere.

Studies on intranuclear distribution of chiasmata in Pisum sativum were carried out by Joshi and Chauhan (1973) and found that the chiasmata were found to be distributed in an uncorrelated manner among the different bivalents of a cell. This was attributed to the highly symmetrical karyotype possessed by Pisum sativum.

Goswami (1979) after detailed karyological studies in blackgram has reported the diploid chromosome number to be 22. Out of the total 11 pairs of chromosomes two were invariably with secondary constrictions. An average karyotype was drawn by utilising arithmetic mean of the individual chromosome of the whole sometic complement for all the varieties. The observation of TF per cent varying from 24.31 to 27.82 revealed a symmetrical karyotype for 19 varieties. The rest were

observed to be asymmetrical with TF per cent ranging from 28 to 38.23.

Raina and Verma (1979) studied a minimum of three cells in Crotalaria for arm ratio and other morphological details and prepared photoidiograms from photomicrographs by cutting out individual chromosomes and arranging them in descending order of their length and matching on the basis of morphology.

Results of meiotic studies of 16 species of Phaseolus reported by Sinha and Roy (1979) showed that the number of bivalents attached to the nucleolus at diakinensis was only one in most of the species. There was variation in the number of ring and rod bivalents among different species and their variation was attributed to structural difference in their karyotype or due to change in the nature of genes or set of genes controlling chiasmata frequency. The presence of univalents might be due to early disjunction in some of the partially homologous chromosomes as a consequence of which they were unable to form normal bivalents. Detailed mitotic analysis of 14 species of Phaseolus by them revealed the somatic chromosome number as 2n = 22. However, these species differed in the total chromatin length, relative length, F per cent and TF value of their chromosomes. In most of the species, the chromosomes were with median and submedian centromeres. In P. aureus, P. sublobatus and

P. radiatus, one pair of chromosomes each with secondary constrictions were recorded.

Ahmad and Godward (1980) reported 2n = 16 for Cicer arietinum with a single pair of satellited chromosomes. The knob like satellites have the tendency to stick together at mitotic anaphase forming bridges. However, these bridges were not permanent and were retracted into two daughter groups at late anaphase leaving no fragment behind. In meiosis all varieties showed very different diplotene and diakinetic stages which have proved impossible to fix stain or interpret.

Irregularities in the meiosis of the F_1 interspecific hybrid of Phaseolus vulgaris X P. coccineus, were reported by Hag et al.(1980). However, approximately 60 per cent of the plates showed 11 bivalents with no apparent irregularities. Abnormalities observed included multiple figures and univalents in metaphase I. In anaphase I irregular separation was quite frequent; Lagging chromosomes and dicentric bridges with acentric fragment were also seen. Both parental species produced pollen of high stainability, this was greatly depressed in the F_1 hybrid but increased in the F_2.

Cheng and Bassett (1980) studied the meiotic chromosomes of Phaseolus vulgaris and found that the banding pattern of each chromosomes was unique and could be used

to separate the chromosomes into long and short classes because of the differential condensation rates of chromatic and achromatic chromosome segments.

Cytomorphological studies conducted by Shashidhar (1981) in some species of genus Vigna Savi has revealed proper pairing of chromosomes at pachytene and normal behaviour of bivalents at different stages of meiosis resulting in high pollen fertility. However, at pachytene the nucleolus was found to be associated with two bivalents in V. unguiculata, V. mungo, V. cylindrica and V. marina where as one bivalent was associated with the nucleolus in V. radiata. The absolute length of all the chromosomes measured was found to be 434.40 µm; the longest 69.69 µm while the smallest being 21.60 µm. Two pairs of chromosomes associated with the nucleolus were 53.6 µm and 49.60 µm in length. Based on the number of asymmetrical chromosomes as well as the difference between the longest and shortest chromosome in the complement, the karyotype of the five species fell under 2B type. This indicated that these species had reached their end point in evolution and further evolution was possible only through gene manipulation.

Sharma and Gupta (1982) studied karyotypes in some pulse crops and found that in most of the cases the longest chromosome was less than twice the length of the

smallest. In no species studied the arm ratio ever exceeded two. Precocious separation of one or two bivalents at metaphase I was ocassionally observed by Machado et al. (1982) in the interspecific hybrid of V.radiata x V. umbellata.

In a study to assess the compatibility between V. radiata and V. umbellata Chowdhary and Chowdhary (1983) found that the pollen fertility was 2.6 per cent in the hybrids. No pod setting was observed. Meiosis in all the interspecific hybrids was irregular with quadrivalents, trivalents, bivalents and univalents being observed at metaphase I, bridges, laggards and fragments at anaphase I.

Prasad and Haida (1983) reported the regular persistence of nucleolus throughout mitosis in Phaseolus vulgaris.

Giemsa C banding analysis in Vigna was done by Chauhan and Bhadoria (1983) and found reduction in size and amount of heterochromatin material during evolution. The minor difference between karyotype and absence of polyploidy in different species indicated that structural changes had played an important role in speciation.

Cytological techniques

Thomas (1940) reported that it was difficult to make good acetocarmine preparations of plants with small chromosomes at meiosis, because the cytoplasm

readily took up the stain and this prevented the sharp differentiation between cytoplasm and chromosomes. The staining reaction was found to depend on four factors namely, constitution of the prefixative, duration of fixation and storage, strength of the stain and amount of iron introduced.

A simple propionocarmine PMC smear method for plants with small chromosomes was reported by Swaminathan et al (1954). The procedure was to fix the anthers in a mixture of three parts of absolute alcohol and one part of propionic acid saturated with ferric acetate. Fixation at low temperetures for 24 hours gave good results. The ferric acetate propionic acid component of the fixative was best prepared once in a week. Propionocarmine was used as the stain.

O'mara (1948) showed that the karyotypic analysis was aided by pretreeting the materials with certain chemicals prior to fixation. Pre-treatment before fixation had become a routine practice to prevent the clumping together of chromosomes. The use of chemicals like colchicine, 8 hydroxy quinoline, paradichlorobenzene, α bromonaphthalene or cold treatment were recommended. Success had been often shown to be due less to the particular fixative or stain used than to the choosing of good vigorous material at correct stage, with strongly

dividing root tips. Such pre-treatment helped to clarify the chromosome morphology by accentuating both primary and secondary constrictions, and by bringing about spreading of the chromosomes.

Root tips of Vigna sp. were treated in a saturated solution of paradichlorobenzene or 8 hydroy quinoline for about one to one and half hours at 12 to 14°C, washed in water and stained with two per cent acetoorcein, in the study conducted by Sen and Bhowal (1960).

Pretreatment of root tips with paradichlorobenzene for two to four hours at 10 to 14°C followed by fixation in acetic alcohol for two to six hours and stained with one per cent acetoorcein was the method used by Sarbhoy (1978).

Since in genus Vigna and Phaseolus, chromosomes were somewhat similar to tomato and sorghum, propiono-carmine method developed by Swaminathan et al. (1954) and successfully used in the study of chromosome analysis in sorghum by Shambulingappa (1962) seemed to be a better method.

Materials and Methods

MATERIALS AND METHODS

The investigations reported herein were undertaken in the Department of Agricultural Botany, College of Horticulture, Vellanikkara during the years 1983 - 1985.

A. Materials

Pure seeds of Vigna unguiculata subsp. unguiculata received from the Professor (Pulses), Tamil Nadu Agricultural University, Coimbatore and that of Vigna unguiculata subsp. sesquipedalis from Regional Agricultural Research Station, Kumarakom were made use of for the study.

B. Methods

Ten uniform seeds of the above subspecies were grown in pots during June - September 1984 adopting all the recommendations for cowpea contained in the Package of Practices Recommendations of Kerala Agricultural University, 1983.

1) Hybridisation and production of F_1 hybrids:

At the time of flowering, i.e. between 40 - 50 days after sowing, hybridisation of the two subspecies was effected adopting the following procedure.

Emasculation of the mature buds was carried out in

the previous evening, adopting the method described by Oliver (1910) and Hays and Gurber (1927) for cowpea. Selected flower bud was held in between the thumb and forefinger holding the keels upwards. A needle tip was run along the ridges, where the two edges of the standard united and thus the standard was forced to open. Standard halves on each side was held down using thumb and forefinger and the exposed keel was split open on one side. Using needle, tip of the keel was pushed underneath the thumb. Using a pair of fine pointed forceps, immature stamens were removed one by one to ensure that none was left behind. Other mature and unemasculated flowers and buds were removed from the inflorescence to avoid contamination. Emasculated flowers were protected using pollen proof butter paper bags. Pollination was done in the next morning between 6 and 7.30 a.m.

The number of crosses made and percentage of success were recorded.

ii) Raising of parents and F_1:

Ten uniform seeds of the two subspecies and their F_1 hybrid were grown in pots during October - January of 1984 - 1985 adopting all the recommendations for cowpea contained in the Package of Practices Recommendations of Kerala Agricultural University 1983.

iii) Morphological description of parents and F_1s:

During the vegetative and productive phases of the crop, observations on habit, height of the plant, duration in days (seed to seed), flower colour, days required for flowering commencement, number of primary branches, internodal length, pod length, number of seeds/pod, colour of the pod, colour of the seed, volume of seed, and shattering of the pod were recorded from both the parents and F_1. Based on the data so collected, the parents and their F_1 were described.

Utilising the above data critical morphological variations have been depicted in the form of a polygraph as suggested by Anderson, 1957 and Mehra, 1961. For plotting the polygraph all the 13 characteristics with 2 - 3 index values for each were utilised. The characters chosen and the corresponding range of variation and respective index values are given below:

Sl. No.	Characters	Variation	Index value for polygraph
1.	Habit	Erect	1
		Intermediate	2
		Indeterminate	3
2.	Plant height	85 - 95 cm	1
		175 - 185 cm	2
3.	Duration	80 - 90 days	1
		91 - 110 days	2

Sl. No.	Characters	Variation	Index value for polygraph
4.	Flower colour	Purple	1
		White	2
5.	Flowering commencement	40 - 45 days	1
		46 - 50 days	2
		51 - 55 days	3
6.	Number of primary branches	3	1
		4	2
		5 - 6	3
7.	Internodal length	14 - 15 cm	1
		16 - 17 cm	2
		18 - 19 cm	3
8.	Pod length	10 - 20 cm	1
		25 - 35 cm	2
		40 - 60 cm	3
9.	Seeds/pod	12 - 13	1
		18	2
		22	3
10.	Colour of the pod	Dark green	1
		Light green	2
11.	Seed colour	Light brown	1
		Dark brown	2
12.	Seed volume (10 seeds)	0.9 ml	1
		1.4 ml	2
		1.8 ml	3
13.	Shattering of the pod	Present	1
		Absent	2

The polygraph was constructed in the shape of a wheel with radiating spokes, the centre of the wheel being represented by a small circle. Each radiating spoke represented one character and the distance along the spoke from the centre representing the variation for that character. The tips of the radiating spokes were then joined to form a polygonal figure. Such polygraphs were drawn separately for the parents and their F_1 hybrids.

iv) Cytological studies:

This was confined to the two parents and their direct F_1 hybrid only.

Meiosis

The time of division varied greatly with season. So it was difficult to get the dividing cells in most of the buds fixed. The time of division ranged from 6 a.m. to 8 a.m. Two to four mm long buds were found to be the best. Those buds were fixed in 3:1 ethanol-propionic acid for 24 hours. Addition of a few drops of propionic acid saturated with ferric acetate to the above fixative gave better results. The buds were then transferred to 70 per cent ethanol and stored in refrigerator for further use. The anthers were dissected out using a dissection microscope, transferred to a slide and squashed in a drop of two per cent

propionocarmine. Judicious warming of the slide before and after squashing helped in better spreading of the chromosomes.

The meiotic chromosomes were measured using an ocular micrometer in Olympus research microscope in oil immersion using an eye-piece of 15X. The length of each chromosome corresponding to the ocular micrometer division was noted. The length of the chromosomes in micrometer was calculated by multiplying the ocular division with the standard value.

The number of univalents, bivalents and multivalents at metaphase I were noted for the two subspecies and F_1 hybrid. The pattern of chromosome segregation at anaphase was also noted for hybrids.

Photomicrographs of the meiotic metaphase I chromosomes were taken. Camera lucida drawings of the meiotic chromosomes of both the parent and the hybrid were also made.

Mitosis

For mitotic studies, actively dividing root tips were used. Seeds were kept in petridish after soaking for four to five hours. It was found to germinate the next day itself in the case of V. unguiculata subsp. unguiculata and the hybrid whereas it took two days in

V. unguiculata subsp. sesquipedalis. Healthy roots of about one cm were cut between 9.30 and 10 a.m. and pretreated in paradichlorobenzene for four to five hours at 15°C in B.O.D. incubator. After pretreatment, the roots were washed and fixed in 3:1 ethanolacetic acid mixture for 24 hours. It was transferred to 70 per cent ethanol and stored in refrigerator for further use.

Fixed roots were hydrolysed in one normal hydrochloric acid for 12 minutes and then washed in water. The roots were kept in Feulgen stain for 45 to 60 minutes in darkness for developing colour. The extreme tips of the roots which developed the characteristic magenta colour were cut and squashed in 0.5 per cent acetocarmine.

The somatic chromosomes were measured using an ocular micrometer in Leitz Orthoplan microscope in oil immersion using an eye-piece of 10X. The total length of the chromosomes as well as length of the long arm and short arm were measured separately. Using these, the following were calculated.

$$1)\quad RCL = \frac{\text{Length of the chromosome}}{\text{Length of largest chromosome}} \times 100$$

$$2)\quad TCL = \frac{\text{Length of the chromosome}}{\text{Total sum of the chromosome length}} \times 100$$

$$3)\quad F\% = \frac{\text{Short arm length}}{\text{Length of chromosome}} \times 100$$

$$4)\quad \text{Arm ratio} = \frac{\text{Long arm}}{\text{Short arm}}$$

$$5)\quad TF\% = \frac{\text{Total sum of short arm length}}{\text{Total sum of chromosome length}} \times 100$$

Photomicrograph and camera lucida drawings of the somatic metaphse I chromosomes were made. The slides were made permanent by passing them through acetic acid-butanol series.

v) Pollen studies:

Pollen grains of the two subspecies and their F_1 hybrid were stained with 1:1 acetocarmine glycerol. Completely stained pollen grains were considered as fertile and unstained as sterile. A minimum of 400 pollen grains were counted in each case. Fertility and sterility percentages were worked out following standard procedure. Pollen size was measured using an ocular micrometer in the lowpower (10X) of Olympus Research microscope.

Results

RESULTS

Observations on the behaviour of F_1 hybrid between *V. unguiculata* subsp. *unguiculata* and *V. unguiculata* subsp. *sesquipedalis* along with their parents with reference to the crossability of the two subspecies, chromosome number and pairing behaviour, abnormalities in chromosome separation, measurement of chromosomes in both meiosis and mitosis, pollen fertility and morphological descriptions based on thirteen parameters are presented in Tables 1 to 11.

Results of observations on the crossability of the two subspecies are presented in Table 1.

Out of 100 flower buds emasculated, 20 shed before pollination in the case of subsp. *unguiculata* and 30 in subsp. *sesquipedalis*. In the cross, when subsp. *unguiculata* was used as female parent, only 15 pods were set out of 80 flower buds pollinated. In the reciprocal cross, 10 pods were set out of 70 buds pollinated. All the pods set, remained intact till harvest. The percentage of success was nineteen in direct cross and fourteen in reciprocal cross. When subsp. *unguiculata* was used as the female parent, the average number of seeds per pod was 13, whereas in the reciprocal cross, the average number was 18 because of the increased

Table 1. Details of crosses effected in two subsp. of *Vigna unguiculata*

Female parent	Male parent	No. of flower buds		No. of pods		No. of seeds obtained	% of success
		Emascu-lated	Poli-nated	Set	Harve-sted		
Subsp. *unguiculata*	Subsp. *sesquipedalis*	100	80	15	15	195	18.75
Subsp. *sesquipedalis*	Subsp. *unguiculata*	100	70	10	10	180	14.28

pod length of the latter (Plates 1a and 1b). So the total number of seeds obtained were 195 and 180 from the direct and the reciprocal crosses respectively.

The morphological characteristics observed in the F_1 hybrids of both direct and reciprocal crosses between the two subspecies of Vigna unguiculata exhibited maternal influence with regard to most of the characters (Table 2; Plates 2 to 3).

The characters which showed maternal influence were height of the plant, duration, days required for flowering commencement, internodal length, pod length, number of seeds per pod, colour of the pod, seed colour and seed volume. However, with respect to habit of the plant and number of primary branches, they showed intermediate characters. In both the hybrids shattering of the pod was present which was the character possessed by subsp. unguiculata.

A polygraph was drawn based on the 13 morphological parameters (Fig. 1). In majority of the traits, the hybrids resembled their maternal parents.

Data on the pairing behaviour of chromosomes in the two subspecies and their F_1 hybrid showed that the chromosome number of subsp. unguiculata was 2n = 22, of subsp. sesquipedalis, 2n = 24 and that of the hybrid

Plate 1(a) Comparative pod length of the parents and their direct F_1 hybrid

1. Subsp. unguiculata
2. F_1 hybrid
3. Subsp. sesquipedalis

Plate 1(b) Comparative pod length of the parents and their reciprocal F_1 hybrid

1. Subsp. sesquipedalis
2. F_1 hybrid
3. Subsp. unguiculata

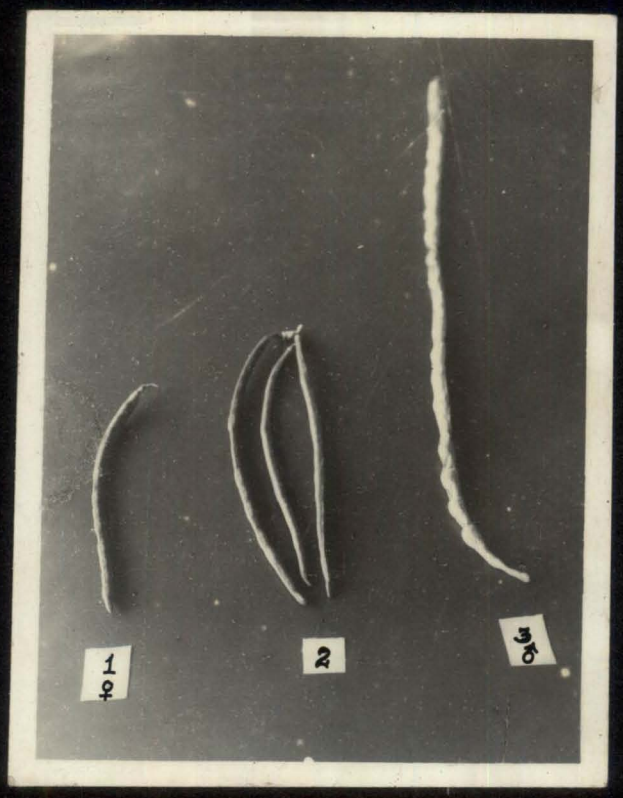


Plate 1(a) (x 0.11)

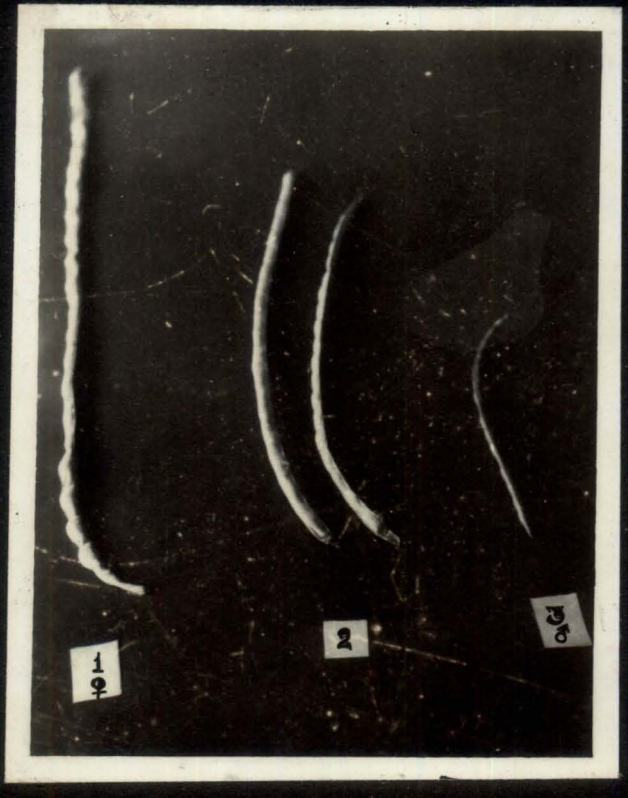


Plate 1(b) (x 0.11)

Table 2. Morphological characteristics of *Vigna unguiculata* subsp. *unguiculata*, *Vigna unguiculata* subsp. *sesquipedalis* and their direct and reciprocal F_1 hybrids

Sl. No.	Character	Subsp. *unguiculata*	Subsp. *sesquipedalis*	F_1 hybrid	
				unguiculata x *sesquipedalis*	*sesquipedalis* x *unguiculata*
1.	Habit	erect	indeterminate	intermediate	intermediate
2.	Height of the plant	66 cm	183 cm	92 cm	177 cm
3.	Duration	80 - 90 days	90 - 110 days	90 - 100 days	110 days
4.	Flower colour	purple	purple	purple	purple
5.	Flowering commencement	40 - 45 days	50 - 55 days	45 - 50 days	50 - 55 days
6.	No. of primary branches	6	3	4	4
7.	Internodal length	16 cm	19 cm	16 cm	18 cm
8.	Pod length	10 - 15 cm	40 - 60 cm	15 - 20 cm	25 - 35 cm
9.	Seeds per pod	12	22	13	18
10.	Colour of the pod	dark green	light green	dark green	light green
11.	Seed colour	light brown	dark brown	light brown	dark brown
12.	Seed volume(10 seeds)	0.9 ml	1.8 ml	0.9 ml	1.4 ml
13.	Shattering of the pod	present	absent	present	present

Plate 2 **Morphological differences in the two subspecies and their direct and reciprocal F_1 hybrids**

1. Subsp. *unguiculata*
2. Subsp. *sesquipedalis*
3. F_1 of *unguiculata* x *sesquipedalis*
4. F_1 of *sesquipedalis* x *unguiculata*

Plate 2 (x 0.05)

Plate 3(a) Variation in seed size of the two subspecies and their direct F_1 hybrid

1. Subsp. *unguiculata*
2. F_1 of *unguiculata* x *sesquipedalis*
3. Subsp. *sesquipedalis*

Plate 3(b) Variation in seed size of the two subspecies and their reciprocal F_1 hybrid

1. Subsp. *sesquipedalis*
2. F_1 of *sesquipedalis* x *unguiculata*
3. Subsp. *unguiculata*

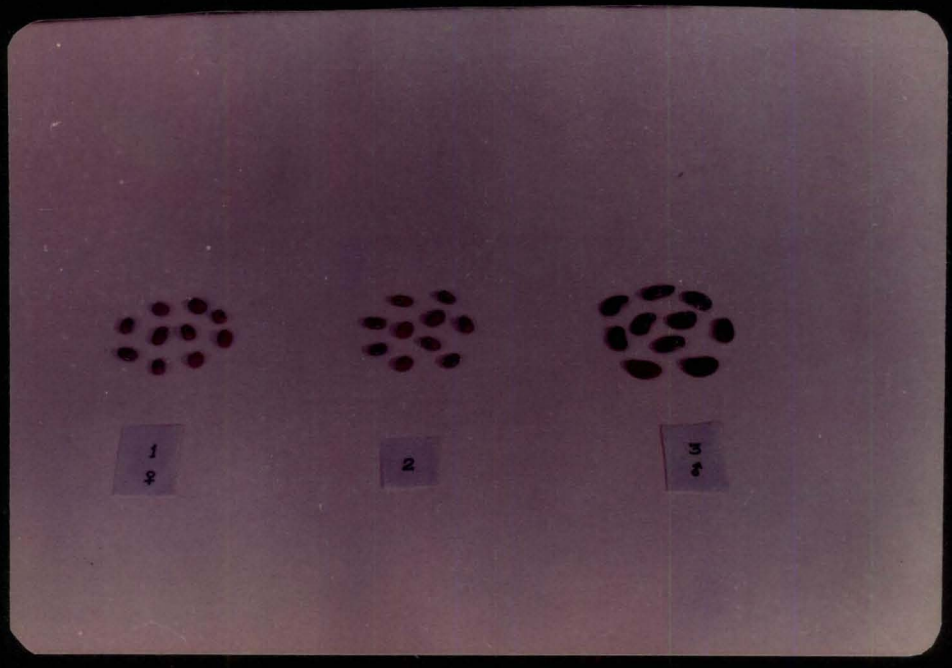


Plate 3(a) (x 0.5)

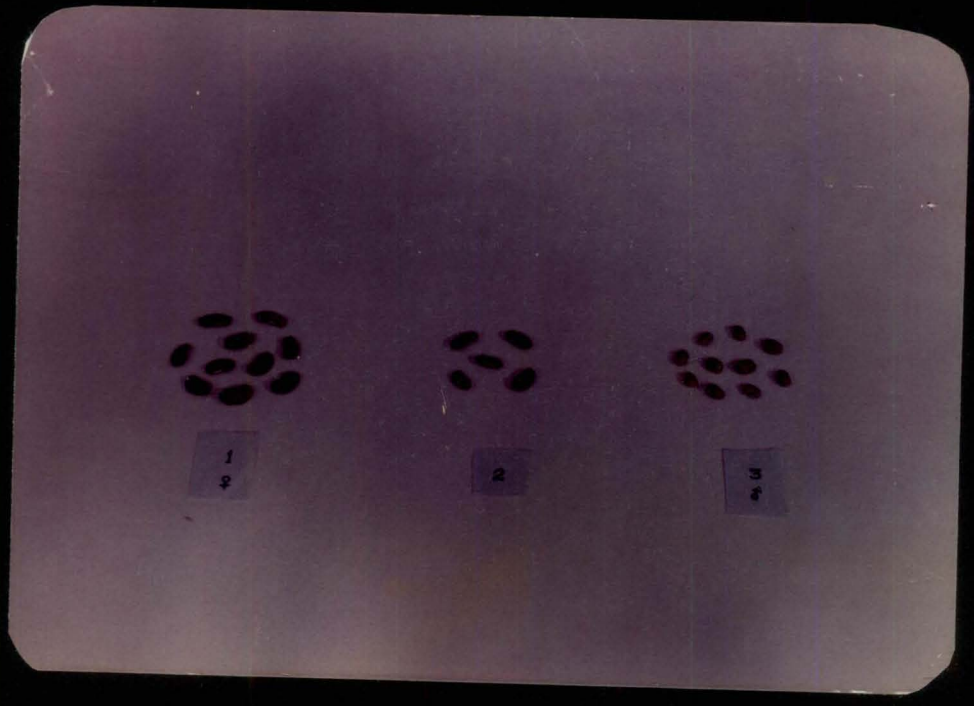
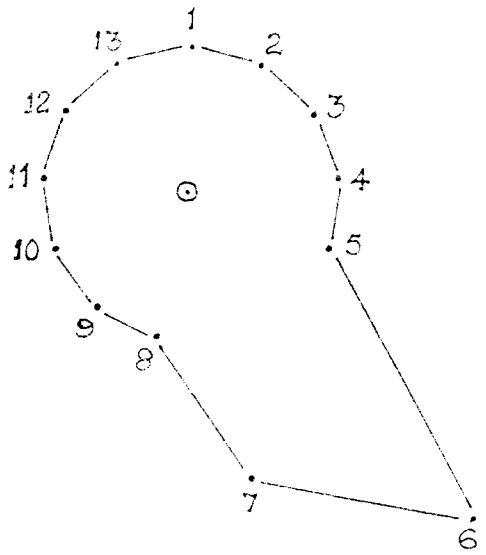


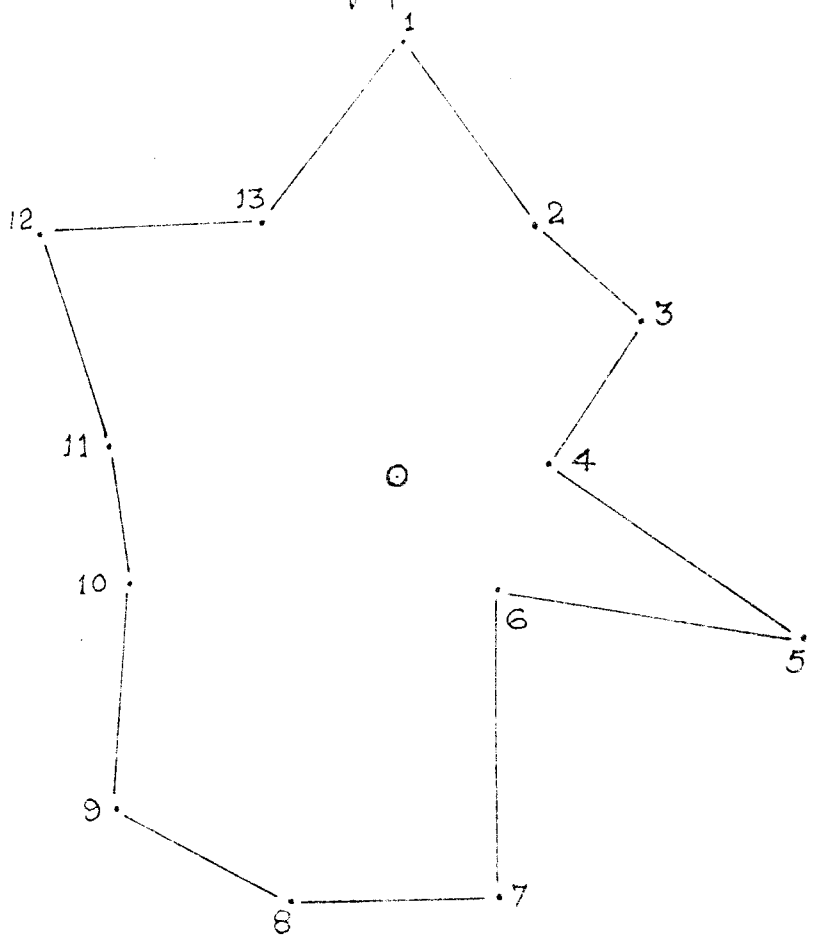
Plate 3(b) (x 0.5)

Fig 1 Polygraphs showing the morphological characteristics of the two subsp. of *Vigna unguiculata* and their F_1 hybrids

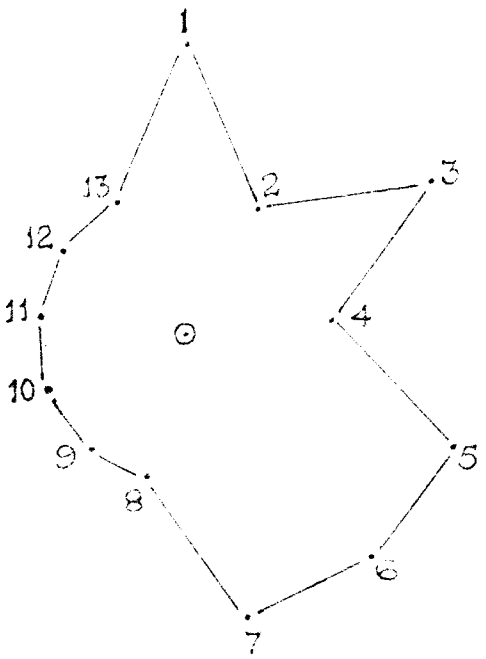
a) *unguiculata*



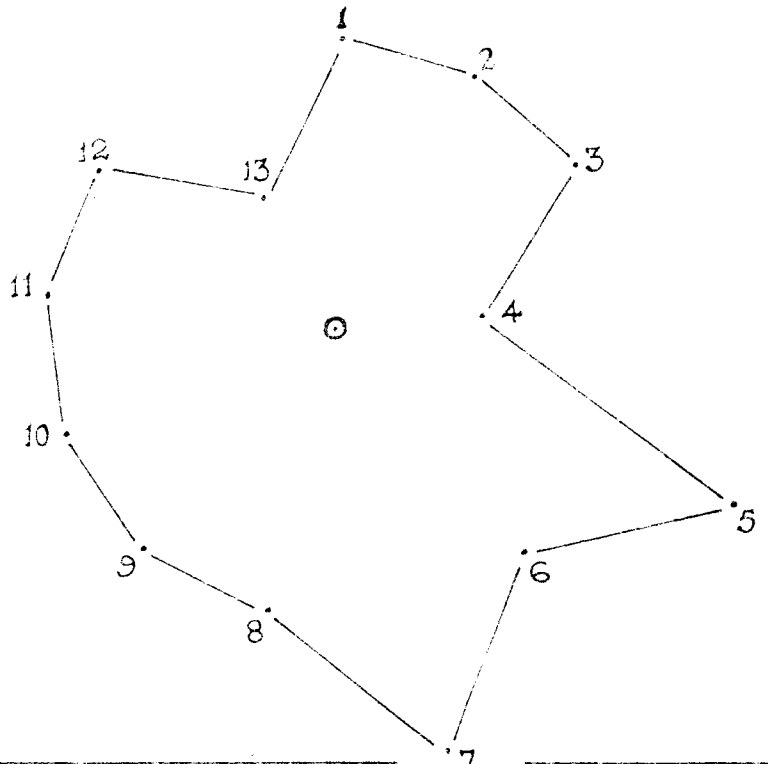
b) *sesquipedalis*



c) *unguiculata* × *sesquipedalis*



d) *sesquipedalis* × *unguiculata*



2n = 23 (Table 3). Camera lucida drawings of the metaphase I stage of the two subspecies and their hybrid are presented in Figures 2 to 4.

There were no univalents in the case of parents but were present in their F_1 hybrid.

Abnormalities observed in the movement of chromosomes in metaphase are expressed in Table 4.

In the case of subsp. unquiculata, out of 20 pollen mother cells studied, two cells showed precocious movement and in the F_1 hybrid, out of 15 pollen mother cells studied, only one cell showed precocious movement whereas in subsp. sesquipedalis no precocious movement was noticed. In both subsp. unquiculata and F_1 hybrid, only one chromosome had undergone precocious movement.

Abnormalities were observed in the separation of chromosomes in anaphase also (Table 5).

In anaphase I, both the parents and hybrid showed cells with laggards. In the case of subsp. unquiculata only one cell gave laggards whereas in subsp. sesquipedalis and hybrid, more than 10 cells showed laggards. In subsp. unquiculata, maximum number of lagged chromosomes was three, whereas in subsp. sesquipedalis and hybrid it was more than six. Frequency of cells showing laggards was more in the case of subsp.

Table 3. Pairing behaviour in the two subsp. of *Vigna unguiculata* and their F_1 hybrid

Subsp./F_1 hybrid	No. of pollen mother cells studied	No. per cell of		
		univalents	bivalents	multivalents
unguiculata	20	0	11	0
sesquipedalis	15	0	12	0
unguiculata x *sesquipedalis*	15	1	11	0

Fig. 2 Meiotic metaphase I chromosomes of *Vigna unguiculata* subsp. *unguiculata*

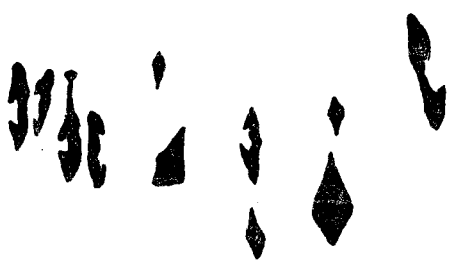


Fig. 3 Meiotic metaphase I chromosomes of *Vigna unguiculata* subsp. *sesquipedalis*

Fig. 4 Meiotic metaphase I chromosomes of the F_1 hybrid of the two subspecies, *unguiculata* × *sesquipedalis*

Table 4. Meiotic abnormalities during metaphase I in the two subsp. of *Vigna unguiculata* and their F_1 hybrid

Subsp./F_1 hybrid	No. of pollen mother cells studied	No. of cells with precocious movement of chromosomes in bivalent number				
		0	1	2	3	4
unguiculata	20	0	2	0	0	0
sesquipedalis	15	0	0	0	0	0
unguiculata x *sesquipedalis*	15	0	1	0	0	0

Table 5. Meiotic abnormalities during anaphase I in the two subsp. of *Vigna unguiculata* and their F_1 hybrid

Subsp./F_1 hybrid	No.of pollen mother cells studied	No. of cells showing laggards varying from							
		0	1	2	3	4	5	6	>6
unguiculata	20	19	0	0	1	0	0	0	0
sesquipedalis	21	7	0	1	3	3	2	0	5
unguiculata x *sesquipedalis*	21	5	1	3	4	2	2	0	4

sesquipedalis and hybrid. In subsp. unguiculata, out of 20 pollen mother cells studied, 19 showed regular telophase formation and only one cell was irregular or aberrant in telophase formation. In subsp. sesquipedalis, only 7 cells showed regular telophase formation. Abnormalities in cell division were more in subsp. sesquipedalis and in the hybrid.

Data on the length of bivalents of the two subspecies and their F_1 hybrid are presented in Table 6.

From the table it is seen that the length of the meiotic metaphase chromosomes was more in subsp. sesquipedalis. In subsp. unguiculata, the chromosome length varied from 2.08 to 4.27 µm, in subsp. sesquipedalis, from 2.26 to 4.96 µm and in the hybrid from 2.16 to 4.47 um. The total of the chromosome lengths was 32.24 µm in subsp. unguiculata, 40.98 µm in subsp. sesquipedalis and 36.71 in hybrid. Thus the hybrid had total chromosome length intermediate between the two parents.

Observations on the measurements of chromosomes at somatic metaphase I of subsp. unguiculata are shown in Table 7.

In subsp. unguiculata, the somatic chromosome number was 2n = 22 (Fig. 5). The length of somatic

Table 6. Length of bivalents (μm) of the two subsp. of Vigna unguiculata and their F_1 hybrid at meiotic metaphase I.

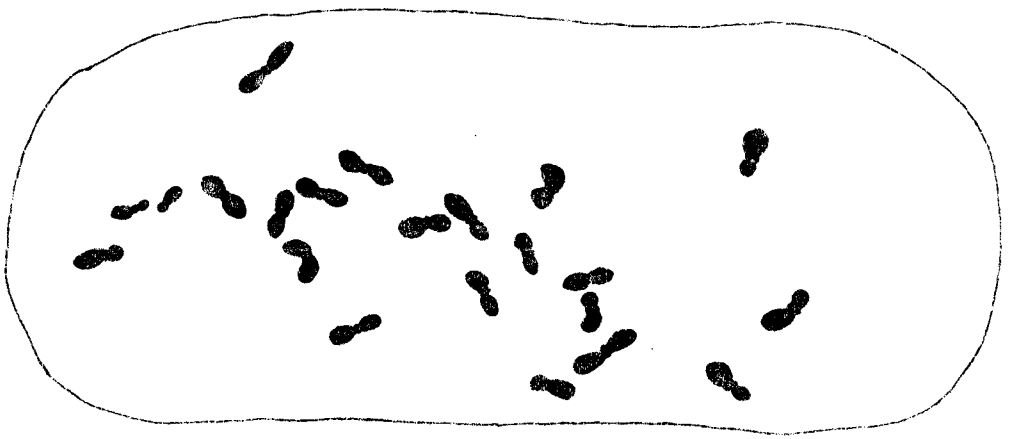
Chromosome pairs	unguiculata	sesquipedalis	unguiculata x sesquipedalis
1	4.27	4.96	4.47
2	3.72	4.75	3.78
3	3.41	4.10	3.78
4	3.22	3.88	3.34
5	3.13	3.67	3.24
6	2.91	3.45	3.09
7	2.50	3.24	2.97
8	2.43	3.02	2.82
9	2.34	2.80	2.49
10	2.23	2.59	2.35
11	2.08	2.26	2.22
12	-	2.26	2.16*
Total	32.24	40.98	36.71

* univalent

Table 7. Measurement of chromosomes at somatic metaphase of Vigna unguiculata subsp. unguiculata

Chromosome pairs	Total length (µm)	Arm length: Long arm (L) (µm)	Arm length: Short arm (S) (µm)	F %	TCL %	Relative chromosome length	Arm ratio (S : L)	Type of chromosome
1	0.1893	0.1018	0.0875	46.22	12.62	100	1 : 1.16	M •
2	0.1661	0.0866	0.0795	47.86	11.07	87.74	1 : 1.09	M
3	0.1536	0.0812	0.0723	47.07	10.24	81.14	1 : 1.12	M
4	0.1437	0.0786	0.0652	45.37	9.58	75.91	1 : 1.21	M
5	0.1393	0.0741	0.0652	46.80	9.29	73.58	1 : 1.14	M
6	0.1357	0.0750	0.0601	44.73	9.05	71.68	1 : 1.24	M
7	0.1348	0.0741	0.0607	45.03	8.99	71.20	1 : 1.22	M
8	0.1250	0.0670	0.0580	46.40	8.33	66.13	1 : 1.16	M
9	0.1152	0.0643	0.0509	44.18	7.68	60.86	1 : 1.26	SM
10	0.1000	0.0554	0.0446	44.60	6.67	52.83	1 : 1.24	M
11	0.0973	0.0536	0.0437	44.91	6.49	51.40	1 : 1.23	M

Fig. 5 Somatic metaphase chromosomes of *Vigna unguiculata* subsp. *unguiculata*



metaphase chromosomes ranged from 0.0973 to 0.1893 µm. The long arm length varied from 0.0536 to 0.1018 µm. The short arm length varied from 0.0437 to 0.0875 (Fig. 6a). The F % varied from 44.18 to 47.86. The highest value obtained for length of individual chromosomes expressed as percentage to total chromosome length was 12.62. This varied from 6.49 to 12.62 (Fig. 8a). The relative chromosome length for the smallest was 51.40 (Fig. 10a). The arm ratio varied from 1:1.09 to 1:1.26. All the chromosomes except one bivalent was found to be median, othersbeing submedian.

The somatic chromosome number in the subsp. sesequipedalis was 2n = 24 (Fig.7). The measurement of chromosomes of the somatic metaphase I of subsp. sesquipedalis showed that the length of somatic chromosomes ranged from 0.1143 to 0.2686 µm (Table 8).

The long arm length varied from 0.0571 to 0.1485 µm and the short arm length from 0.0571 to 0.1191 µm (Fig.6b). The F % ranged from 44.64 to 50.00. The length of individual chromosome expressed as percentage to total chromosome length varied from 5.83 to 13.70 (Fig. 8b). The relative chromosome length varied from 42.55 to 100 in subsp. sesquipedalis. The range of arm ratio was from 1:1 to 1:1.23. All the chromosomes were found to be median (Fig. 10b). The mitotic studies in the F_1 hybrid

Fig. 6 Idiograms of somatic chromosomes of the two subspecies and their F_1 hybrid

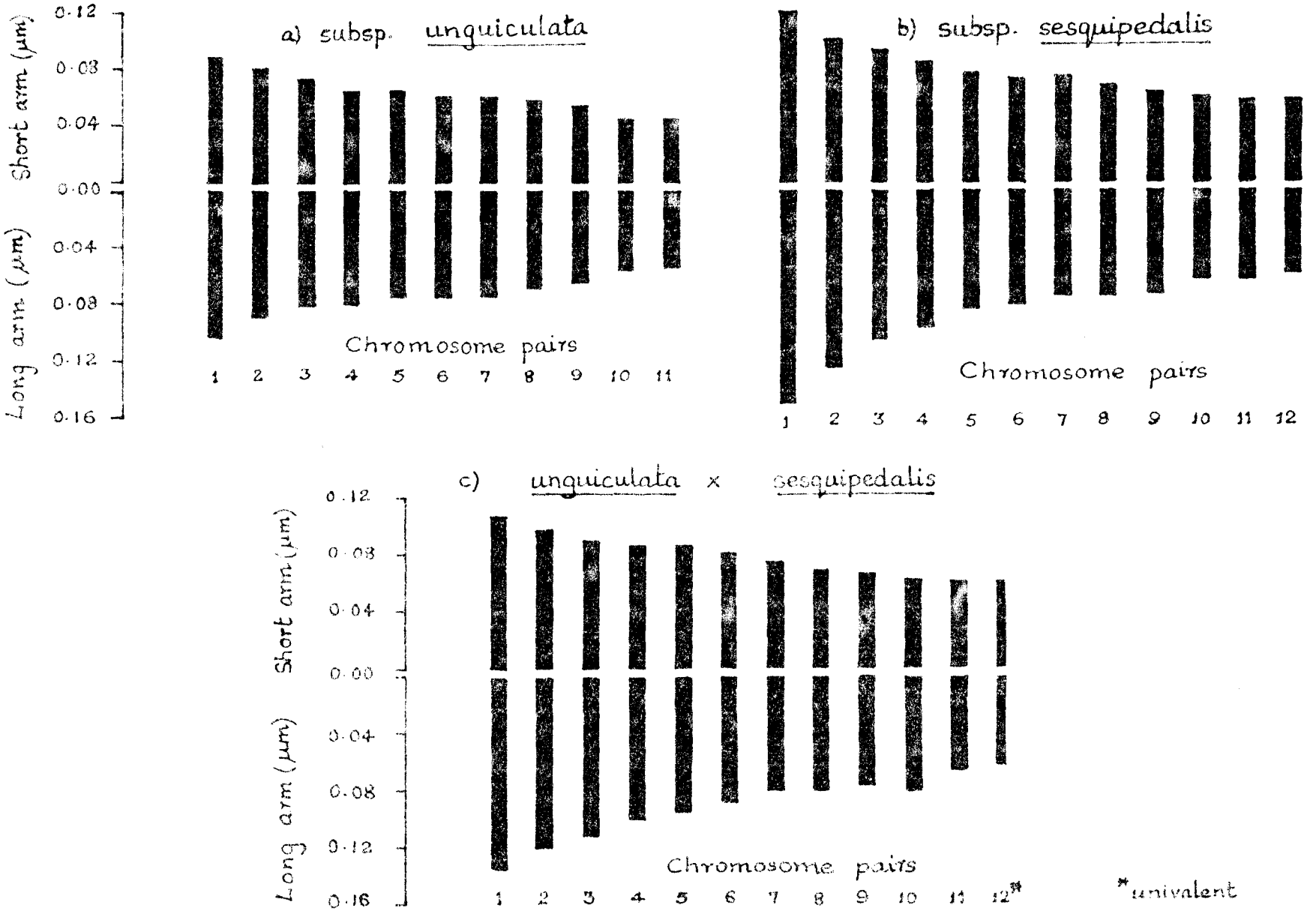


Fig. 7 Somatic metaphase chromosomes of Vigna unguiculata subsp. sesquipedalis

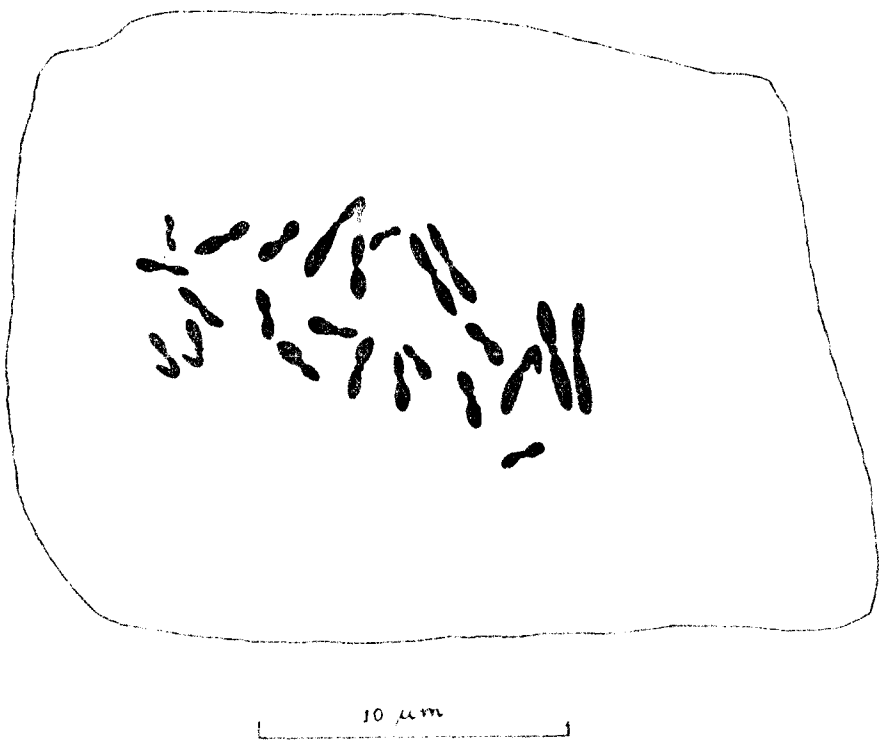


Fig. 8. % TCL at somatic metaphase of the two subspecies and their F_1 hybrid

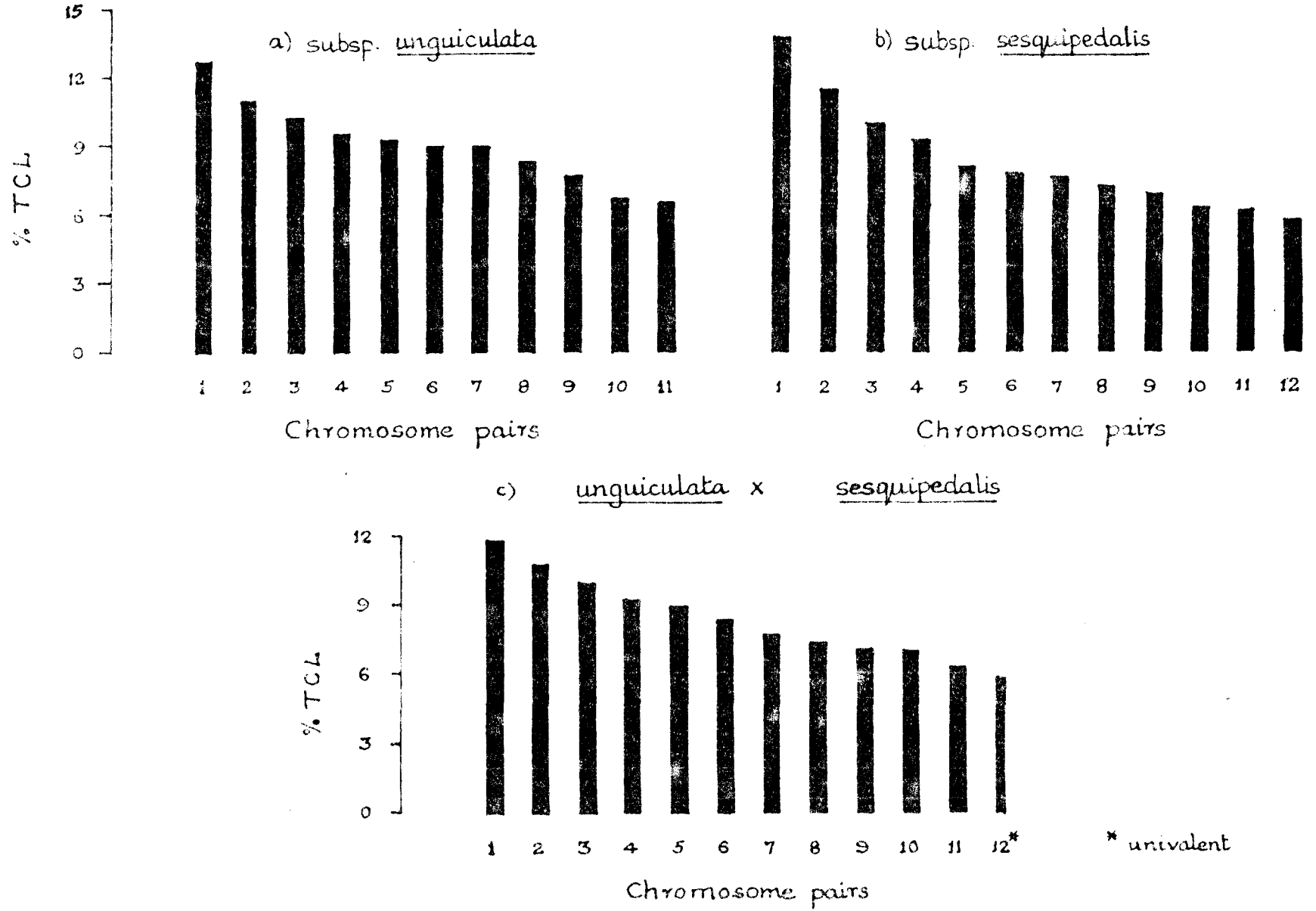


Fig. 10. Relative Chromosome Length (RCL) at somatic metaphase of the two subspecies and their F_1 hybrid

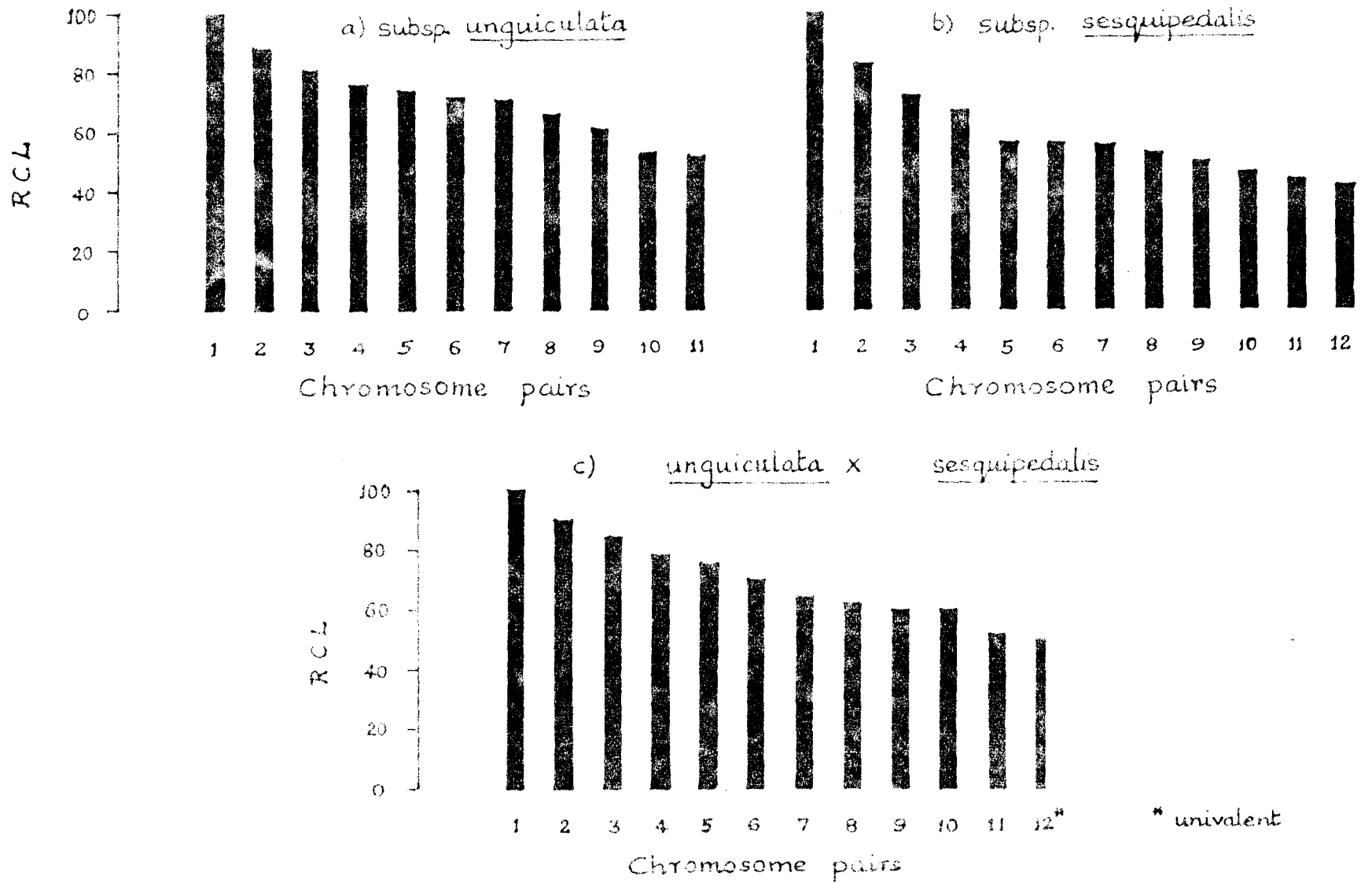


Table 8. Measurement of chromosomes at somatic metaphase of *Vigna unguiculata* subsp. *sesquipedalis*

Chromosome pairs	Total length (µm)	Arm length: Long arm (L) (µm)	Arm length: Short arm (S) (µm)	F %	TCL %	Relative chromosome length	Arm ratio (S : L)	Type of chromosome
1	0.2686	0.1485	0.1191	44.64	13.70	100	1 : 1.23	M
2	0.2228	0.1229	0.0999	44.84	11.37	82.95	1 : 1.23	M
3	0.1943	0.1029	0.0914	47.04	9.91	72.34	1 : 1.13	M
4	0.1800	0.0971	0.0829	46.06	9.18	67.01	1 : 1.17	M
5	0.1571	0.0829	0.0743	47.29	8.01	56.22	1 : 1.12	M
6	0.1514	0.0799	0.0714	47.16	7.72	56.37	1 : 1.12	M
7	0.1486	0.0743	0.0743	50.00	7.58	55.32	1 : 1.00	M
8	0.1429	0.0743	0.0686	48.00	7.29	53.20	1 : 1.08	M
9	0.1343	0.0714	0.0629	46.83	6.85	50.00	1 : 1.14	M
10	0.1229	0.0629	0.0600	48.82	6.27	45.76	1 : 1.05	M
11	0.1200	0.0629	0.0571	47.58	6.12	44.68	1 : 1.02	M
12	0.1143	0.0571	0.0571	49.95	5.83	42.55	1 : 1.00	M

revealed that the somatic chromosome number was 2n = 23 (Fig. 9).

Results on measurement of chromosomes at somatic metaphase of F_1 hybrid are presented in Table 9.

The length of chromosomes varied from 0.1200 to 0.2400 µm. The range for long arm was from 0.0600 to 0.1343 µm, and that of short arm from 0.0600 to 0.1057 µm (Fig. 6c). The F % varied from 44.04 to 50.00. The length of individual chromosome expressed as percentage to total chromosome length varied from 5.91 to 11.82 (Fig. 8c). The relative chromosome length for the smallest chromosome was 50 in the hybrid (Fig. 10c). Arm ratio ranged from 1:1 to 1:1.27. Two chromosomes were submedian and others median. The results also showed that the longest chromosome has twice the length of the smallest in hybrid.

Both the species and their hybrid have almost similar TF % (Table 10).

Data on pollen fertility and sterility of the two subspecies and their hybrid revealed that subsp. sesquipedalis has the highest pollen fertility of 96.28% (Table 11). In subsp. unguiculata the same was 91.06% and that for hybrid 91.09%. There was almost similar percentage sterility in subsp. unguiculata and hybrid.

Fig 9 Somatic metaphase chromosomes of the F_1 hybrid of the two subspecies, unguiculata x sesquipedalis

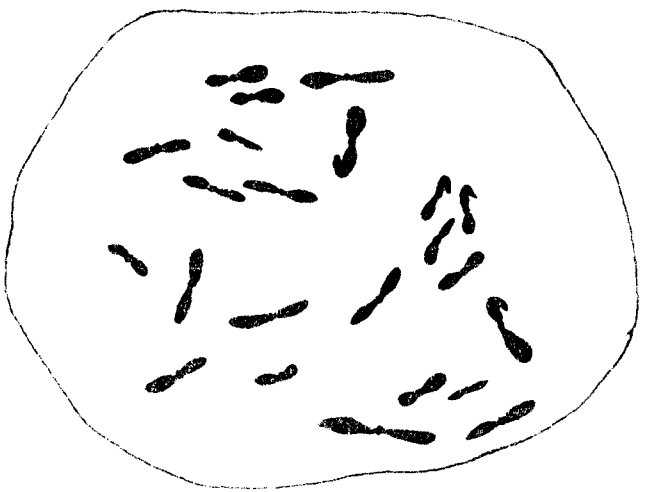


Table 9. Measurement of chromosomes at somatic metaphase of F_1 hybrid of two subsp. of Vigna unguiculata

Chromosome pairs	Total length (µm)	Arm length: Long arm (L) (µm)	Arm length: Short arm (S) (µm)	F %	TCL %	Relative chromosome length	Arm ratio (S : L)	Type of chromosome
1	0.2400	0.1343	0.1057	44.04	11.82	100	1 : 1.27	SM
2	0.2171	0.1200	0.0971	44.73	10.69	90.46	1 : 1.23	M
3	0.2900	0.1114	0.0886	44.30	9.85	83.83	1 : 1.25	M
4	0.1857	0.1000	0.0857	46.14	9.15	77.38	1 : 1.16	M
5	0.1800	0.0943	0.0857	47.61	8.87	75.00	1 : 1.10	M
6	0.1686	0.0886	0.0800	47.45	8.31	70.25	1 : 1.10	M
7	0.1543	0.0800	0.0743	48.15	7.60	64.30	1 : 1.08	M
8	0.1486	0.0800	0.0686	46.16	7.32	61.92	1 : 1.16	M
9	0.1429	0.0771	0.0657	45.98	7.04	59.54	1 : 1.17	M
10	0.1429	0.0800	0.0629	44.02	7.04	59.54	1 : 1.27	SM
11	0.1257	0.0657	0.0600	47.73	6.19	52.38	1 : 1.09	M
Univalent	0.1200	0.0600	0.0600	50.00	5.91	50.00	1 : 1.00	M

Table 10. TF percentage of the two subsp. of *Vigna unguiculata* and their F_1 hybrid

Subsp./F_1 hybrid	TF % {Total sum of short arm length / Total sum of chromosome length x 100}
unguiculata	45.89
sesquipedalis	46.93
unguiculata x *sesquipedalis*	46.02

Table 11. Pollen fertility and sterility (%) of two subsp. of *Vigna unguiculata* and their F_1 hybrid

Subsp./F_1 hybrid	Fertility(%)	Sterility(%)
unguiculata	91.06	8.94
sesquipedalis	96.28	3.72
unguiculata x *sesquipedalis*	91.09	8.91

Discussion

DISCUSSION

The evaluation of species affinities by the degree of chromosome pairing at F_1 meiosis of interspecific crosses is made by the technique of genome analysis. Some taxonomists express the view that chromosomal differences should be treated as just another morphological characters. However, if we know why the size, shape and number of chromosomes of some species differ from those of another, these chromosomal characters can tell us much more than differences in leaves, flowers and fruits. Genomic analysis can provide us with valuable information regarding the processes which have brought about evolutionary diversification and the direction in which evolution has taken place. The cytological studies of the species concerned provide the basic information for genome analysis.

The two subspecies of *Vigna unguiculata*, viz. *unguiculata* and *sesquipedalis* that have been studied during this investigation, exhibit distinct morphological variations with respect to plant and pod characteristics (Table 2; Plates 1 to 6). While the former grows up to an average height of 86 cm with a total duration of 80 to 90 days and flowers by 40 to 45 days, the latter attains a more luxuriant growth (183 cm) with 90 to 110 days total duration and flowers by 50 to 55 days.

The pod characteristics are much more distinctly different in them. The pods of subsp. sesquipedalis grown up to a length of 50 cm with more number (22 seeds per pod) of bold (volume 1.8 ml per 10 seeds) grains as against the smaller pods with less number of seeds in the case of subsp. unguiculata (Table 2).

Considering these clearcut variations, some of the earlier taxonomists have suggested that subsp. unguiculata and subsp. sesquipedalis should be considered as distinct species (Piper, 1912). However, the later authors have expressed the view that these two were not to be considered as distinct species but were to be given the status of subspecies belonging to a polymorphic species (Sen and Bhowal, 1960; Pavlov, 1962).

Since the best tool for finding out the relationships between these two subspecies is to find their affinities through hybridisation and study the behaviour of their F_1's direct as well as reciprocal crosses were tried in the present study. The results in Table 1 show that the success of these crosses were very low, 19 and fourteen in the case of subsp. unguiculata X subsp. sesquipedalis and its reciprocals respectively. A characteristic observation made during this hybridisation programme was that the shedding of flowers were noticed either before pollination or immediately after pollination (within 24

hours after pollination) which points to the possibility that almost all the sheddings were pre-fertilization. That post-fertilisation shed was almost not encountered in this programme. This is indicated by the fact that the number of pods set and harvested were same in both the crosses.

Thus, reduction in the percentage of success in crosses involving these two subspecies can not be considered solely due to the lack of affinity between them, but probably due to the mechanical injury caused to the buds during the process of emasculation. That the five subspecies of Vigna unguiculata are inter-fertile has been reported earlier by Faris (1965) while attempts to hybridise with other Vigna species, especially the wild progenitors, have failed.

While considering the morphological characteristics of F_1's in comparison to the parents, it has been observed that they have a general tendency of exhibiting resemblences to the maternal parents. This type of maternal inheritance is more pronounced with respect to plant height as well as pod and seed characteristics (Table 2). This behaviour is probably indicative of the plasmagenic influence of these traits. The shattering habits of pods seen in one of the parents (subsp. unguiculata) however, is found to be transmitted to the F_1 hybrids just like any other Mendelian dominant character.

In order to obtain more information regarding the genomic relationships of these subspecies, detailed cytological investigations were carried out. Meiotic studies were conducted in the two subspecies and their direct F_1 hybrid. Meiotic pairing of chromosomes were apparently normal in the parents and their hybrid. There were 11 bivalents in subsp. unguiculata and 12 in subsp. sesquipedalis (Table 3, Fig. 2 to 4). The hybrids were consistently showing 11 bivalents and one univalent. This observation points that the chromosome number in subsp. unguiculata is 2n = 22 and in subsp. sesquipedalis 2n = 24. However, according to the earlier reports, there is controversy regarding the chromosome number of these subspecies. Faris, (1965) has reported that the subsp. unguiculata has a chromosome number 2n = 2x = 22 while Karpechenko (1925), Darlington and Wylie (1945) and Sen and Bhowal (1960c) have contributed to the idea of Faris (1965) and reported the chromosome number of V. unguiculata as 2n = 22. Kawakami (1930) recorded a haploid number of 12 in this subspecies. In the subsp. sesquipedalis, the diploid number has been found to be 24 (Darlington and Wylie, 1945; Florescu, 1960). That the chromosome number in unguiculata and sesquipedalis to be 2n = 22 and 2n = 24 respectively was further confirmed through root tip mitotic studies during the present investigation (Fig. 5, 7 and 9).

The close pairing of homologous chromosomes at zygotene is a characteristic phenomenon noticed in all higher organisms. The most important and generally effective factor which determines the nature of chromosome pairing is the structural and chemical similarity known as homology. This type of pairing behaviour can be best studied during pachytene of meiosis. Therefore, to a cytologist interested in chromosomal relationships and evolution, pachytene analysis is the ideal method, provided the material is favourable for such a study. Analysis of chromosomal pairing can provide valuable indications of relationships between species if they are used carefully and with full recognition of all the factors involved (Stebbins, 1971).

With the above objectives in mind, the meiotic studies of chromosomes in the two subspecies, namely unguiculata and sesquipedalis as well as their direct F_1 hybrid have been carried out. During the present study, the method of pachytene analysis was not found to be much useful in these subspecies and their hybrid due to the difficulty in obtaining quality preparations of the different prophase stages of meiosis I and anaphase I of the mitotic division.

Meiotic pairing in both the subspecies and their

F_1 hybrid seems to be apparently normal as evidenced by the presence of regular bivalents during metephase I. A total of 11 bivalents in subsp. unguiculata, 12 in subsp. sesquipedalis and 11 bivalents along with a univalent in the F_1 hybrid could be observed in well spread metaphase plates (Fig. 2 to 4). However, in a few metaphase I preparations, precocious movement of the chromosomes of one bivalent was observed in the case of the subsp. unguiculata and its F_1 hybrid with the subsp. sesquipedalis. Two of the cells out of 20 studied in case of the former and one cell out of 15 studied in the latter exhibited this tendency (Table 4).

The most important and generally effective factor that determines the pairing behaviour of chromosomes during meiosis is the structural and chemical similarity known as homology. Each paternal chromosome associates with its counterpart among the maternal chromosome in this process making Mendelian segregation of genes possible. The degree of homology between chromosomes depends at least in part upon their similarity with respect to gene arrangements. In complements where homologous or homoeologous chromosomes may pair with each other, the homology between chromosomes in the same species can get reduced as a result of cryptic

structural changes leading to rearrangement of the genes. Even with perfect homology available, reduction in pairing and/or failure in chiasma formation have been observed in certain instances. Another factor that affects chromosome pairing is the cellular environment which prevails at meiosis. This can be affected by drastic alterations of the external environment such as heat or cold shocks or more generally by genes which presumably control the numerous enzymatic reactions required for meiosis. Individual recessive genes in homologous conditions are ascribed to be capable of causing the chromosomes to be unpaired at metaphase, either through failure of initial association at zygotene and pachytene, or through the failure of chiasma formation (Stebbins, 1971). However, such genes are greatly eliminated or reduced in frequency during evolution.

In the present study, desynapsis of only one of the bivalents in some of the metaphase preparations seems to be due to the reduced degree of affinity between the maternal and paternal chromosomes involved leading to improper pairing and/or reduction in the number of chiasma formed. That a good percentage of metaphases studied showed normal bivalent formation, indicates that the failure of chiasma formation may not be the reason for the

precocious movement of chromosomes in this bivalent. This phenomenon seems to be prevalent only in subsp. unguiculata and probably is carried to the F_1 hybrid by this parent.

During anaphase I, lagging chromosomes were found to be present in some of the cells studied (Table 5). In the normal situations, the chromosomes get disengaged from synapsis and move to the opposite poles to form the two daughter nuclei subsequently. However, one or more chromosomes may sometimes do not join in the process of movement and remain away from the group of chromosomes going to the poles. Such laggards may originate as a result of defective centromeres or due to an abnormal behaviour of the spindle fibres. Delay in chromosome disjunction also can lead to the phenomenon of chromosomes lagging towards the centre of the dividing cell during anaphase 1. Meiotic abnormalities in the interspecific hybrids of Phaseolus as well as Arachis have been reported earlier (Dhaliwal and Pollard, 1962; Raman and Kesavan, 1962). In Phaseolus the chromosomal irregularities have been ascribed to be not due to cytologic or gene causes but to either differences in the genetic composition or size of the chromosomes. A sizable proportion of abnormalities including quadrivalents, trivalents and univalents at metaphase I as well as bridges, laggards and fragments

at anaphase I have been reported earlier in the F_1 hybrid of Vigna radiata X Vigna umbelata (Chowdhury and Chowdhury, 1983).

Among the two subspecies of Vigna unguiculata studied during the present investigations, subsp. sesquipedalis (2n = 24) showed more number of laggards. A total of 12 cells out of 21 studied exhibited one to more than six lagging chromosomes in its anaphase I cells. The tendency was even higher for chromosomes in the F_1 hybrid. Only five cells out of 21 studied showed the normal disjunction in this case. However, this was comparatively very less in subsp. unguiculata. Almost all the cells (19/20) were normal with respect to anaphase movement. In an earlier report of the cytomorphological studies in some species of Vigna conducted by Shashidhar (1981) also the proper pairing of pachytene chromosomes and normal behaviour of bivalents at different stages of meiosis in subsp. unguiculata has been recorded. This observation points to the possibility that the tendency for some of the chromosomes to lag during the normal disjunction and movement is species specific and subsp. sesquipedalis is prone to this phenomenon. This has to be attributed probably to the inherent nature of the subspecies. This might have played a role in the evolution of species as well. This is suggested due to the fact

that the laggards many a times may be incapable of getting incorporated to any one of the deughter nuclei. Such a situation, most of the times, leads to the elimination of that particular chromosome from the complement. This type of chromosome elimination has played a great role in species evolution. The fact that tendency of a few chromosomes not to take part in the normal disjunction observed in the subsp. sequipedalis is carried over to the F_1 hybrid of this subspecies with subsp. unquiculata, also indicates its inherent nature.

The size of chromosomes and the total DNA content of the nucleus vary greatly from one group of plants to other. The total mass of chromosomes in a nucleus is closely correlated with its DNA content. With the objective of getting information regarding the size of chromosomes in the two subspecies and their F_1 hybrid, the length of bivalents during metaphase I of meiosis and somatic chromosomes in root tip mitotic cells have been measured (Table 6). A regular trend was observed in the lengths of bivalents from the largest to the shortest in both the subspecies and their hybrid. While the mean length of the longest bivalent in subsp. unquiculata was 4.27 µm and the shortest 2.08, the corresponding values in subsp. sesquipedalis were 4.96 and 2.26 respectively. The F_1 hybrid exhibited medium

values with respect to the bivalent lengths. In the hybrid, the univalent was the shortest among the complement. The total DNA content was more in subsp. sesquipedalis as evidenced by the higher value (40.90 µm) of total chromosome length in this subspecies. The total chromosome length in subsp. unguiculata was 32.24 and that in the hybrid 36.71 µm. The length of chromosomes during the meiotic divisions in the subspecies of Vigna unguiculata was measured by Sen and Bhowal (1960) and found that it ranged from 1.62 to 3.7 µm. According to Shashidhar (1981) the absolute length of pachytene chromosomes in the subspecies was found to be 434.40 µm, the longest chromosome being 69.69 µm and the shortest 21.60 µm.

A detailed study of the somatic chromosomes of the two subspecies and their F_1 hybrid has again revealed the gradual decrease in length from the longest pair to the shortest. In the present analysis, the chromosomes in general were very small ranging from 0.1893 µm in 0.0973 µm in subsp. sesquipedalis probably due to higher degree of condensation. The total length was more in the latter when compared with the former. The F_1 hybrid has the lengths ranging from 0.24 µm to 0.12 µm which is intermediate to the parental subspecies. The smallest chromosome was only about half of the largest as shown by their per cent TCL as well as RCL measurements (Table 7, 8 and 9).

A measurement of the lengths of long and short arms of individual chromosomes has shown that while all the chromosomes in subsp. sesquipedalis belonged to the median group (Arm ratio 1:1.25), in subsp. unguiculata one and in the F_1 hybrid two chromosomes were coming under the submedian group. In the mitotic study also, the shortest chromosome in the complement was found to lack a homologous counterpart in the F_1 hybrid. Presence of secondary constriction was not consistently observed probably due to the more condensed state of the chromosome. Taking data from the mitotic metaphase chromosomes, the idiograms of both the subspecies have been prepared taking the absolute values as well as the per cent TCL into consideration.(Fig.6). The F% values calculated in the two subspecies and their hybrids were found to range from 44.18 to 47.86, 44.64 to 50.00 and 44.02 to 50.00 in the case of subsp. unguiculata, subsp. sesquipedalis and their F_1 hybrid respectively. The TF% values (Table 10) of the two subspecies and their hybrid were 45.88, 46.93 and 46.02 respectively. This is indicative of the karyotypic similarities between them.

According to Stebbins (1971), chromosome pairing in a hybrid can be considered as a measure of evolutionary relationship. However, the question, to what extend does the closeness of pairing reflect the degree of genetic

relationship between two plants is not very simple. In the present analysis of the genomic relationship of the two subspecies of Vigna unguiculata namely, unguiculata (2n = 22) and sesquipedalis (2n = 24), the results of hybridisation have revealed a close relationship between them. So are the results of the chromosomal behaviour in the hybrid. In the metaphase I preparations of the hybrid studied, perfect pairing of the parental chromosomes to give rise to 11 bivalents and an unpaired univalent derived from one of the parents, subsp. sesquipedalis is regularly observed.

The meiotic anaphase I chromosomes did show certain abnormal behaviour in the hybrid. However, this lagging tendency of some of the chromosomes does not seem to be as a result of any incompatibility between the parental chromosomes but due to some of the inherent genic factors residing in one of the parents, subsp. sesquipedalis. This is evidenced by the lagging tendency of some of the chromosomes exhibited in the above subspecies. That these laggards in no wey effect the success of the F_1 hybrids, is evidenced by the observation that there is no apparent reduction in the pollen fertility of the F_1 hybrid. The pollen fertility of the F_1 hybrid was comparable to the parents.

The observation of TF% varying from 45.89 to 46.93 among the two parents as well as the hybrid, indicates the presence of a symmetrical karyotype for these groups. A similarity in karyotype is indicative of the similarity of the subspecies in the evolutionary path. That not much of karyotypic changes have taken place between the two subspecies during evolution is indicated by their symmetry in their karyotypes. The normal events of karyotypic evolution like deletions or additions, centric fusions, invessions etc. seem to have played practically no role in the evolution of these two subspecies.

That the two subspecies do exhibit morphological variations can probably be ascribed mainly to the fact that one of the subspecies, namely, sesquipedalis contains more of nuclear DNA. The higher amount of DNA has been suggested to contribute to the presence of a larger number of active genes and greater diversity of gene function eventhough its presence as a result of high amount of nonsense DNA or tandem duplication of genes cannot be ruled out.

Advanced genera belonging to highly specialised families are known to have smaller chromosomes and lower nuclear DNA, than primitive vascular plants (Stebbins, 1971). Considering this it can be assumed that the subsp. unguiculata with 2n = 22 is more advanced in comparison to

the other subspecies. Subsp. sesquipedalis (2n = 24) has longer chromosomes and thereby higher DNA content in comparison to subsp. unguiculata. Therefore, it can be tentatively suggested that the former had evolved from this subspecies during the course of evolution. Since both the subspecies possess homogeneous karyotypes without any substantial variation in their TF %, it can be assumed that evolutionary changes with respect to the morphology have not contributed much to the process of speciation. However, Mohan and Bhatia (1983) have suggested that structural changes might have played an important role in the speciation in Vigna. The present study indicates that the most probable event that might have led to the formation of a separate subspecies namely unguiculata from its ancestor sesquipedalis seems to be a chromosomal elimination leading to the reduction in chromosome number from 2n = 24 in the latter to 2n = 22 in the former. The tendency of some of the chromosomes to lag behind during the anaphase I movement observed in the present study in the subsp. sesquipedalis supports the above assumptions.

Detailed cytological studies including pachytene analysis can throw some more light to the information made available about the genomic relationships of these two subspecies.

Summary

SUMMARY

Investigations on the Genomic relationship in Vigna species were undertaken in the Department of Agricultural Botany, College of Horticulture, Vellanikkara during the years 1983 - 1985. Pure seeds of the two subspecies of Vigna unguiculata namely unguiculata and sesquipedalis were sown and crop raised. They were then crossed in direct and reciprocal combinations in September 1984 and the hybrid seeds collected. The F_1 hybrids along with their parents were raised in October - January, 1984 - 1985. Morphological description of both direct and reciprocal hybrids along with the two parents based on 13 parameters were made. Cytological investigations of the parents and direct hybrid were also conducted. The data so collected were processed. The important findings of the above investigations are summarised below.

1) The two subspecies were easily crossable. In both the subspecies there was high prefertilisation shed.

2) The chromosome number of subsp. unguiculata was 2n = 22, of subsp. sesquipedalis 2n = 24 and of F_1 hybrid 2n = 23.

3) Precocious movements of meiotic metaphase chromosomes were noticed in subsp. unguiculata and F_1 hybrid.

4) Both the parents and hybrid showed cells with laggards.

5) Length of meiotic metaphase chromosomes was more in subsp. sesquipedalis, lowest for subsp. unguiculata and intermediate for hybrid.

6) In subsp. unguiculata length of somatic metaphase chromosomes ranged from 0.0973 to 0.1893 µm.

7) In subsp. unguiculata all the chromosomes except one pair were found to be median, the other being submedian.

8) The length of somatic chromosomes ranged from 0.1143 to 0.2686 µm in subsp. sesquipedalis.

9) All the chromosomes were median in subsp. sesquipedalis.

10) The length of somatic chromosomes varied from 0.1200 to 0.2400 µm in the case of F_1 hybrid.

11) In the hybrid, two chromosomes were submedian and others median.

12) Both the sub species and their hybrid have almost similar TF % thereby indicating similarity of their karyotypes.

13) Pollen fertility was more in subsp. sesquipedalis.

14) Reciprocal difference was seen in the case of morphological characters like height of the plant, duration, number of primary branches, internodal length, seeds per pod, seed colour and seed volume. However, these differences were not seen traceable in the cytology of the hybrid.

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GENOMIC RELATIONSHIP IN *Vigna* SPECIES

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ABSTRACT OF A THESIS

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ABSTRACT

Investigations on the genomic relationship in *Vigna* species were undertaken in the Department of Agricultural Botany, College of Horticulture during 1983-1985. Pure seeds of the two subspecies of *Vigna unguiculata*, viz. *unguiculata* and *sesquipedalis* were sown and crop raised. Direct and reciprocal crosses were made. Morphological and cytological investigations were done.

It was found that the two subspecies were easily crossable. In both the subspecies there was high prefertilisation shed. The chromosome number of subsp. *unguiculata* was 2n = 22, of subsp. *sesquipedalis* 2n = 24 and of F_1 hybrid was 2n = 23. Both the parents and hybrid showed cells with laggards. Length of meiotic and somatic metaphase chromosomes was more in subsp. *sesquipedalis*, lowest in subsp. *unguiculata* and intermediate in their F_1 hybrid. Both the subspecies and their hybrid had almost similar TF % which gave an indication of similarity in their karyotypes. Pollen fertility was more in subsp. *sesquipedalis*. Reciprocal difference was seen in the case of morphological characters like height of the plant, number of primary branches, internodal length, seeds per pod, seed colour and seed volume. However, these differences were not seen traceable in the cytology of the hybrid.