KARYOMORPHOLOGY, POLLEN STERILITY AND SEEDSET IN VETIVER (*Vetiveria zizanioides* (Linn.) Nash.)

By

K. S. MINI

THESIS

Submitted in partial fulfilment of the

requirements for the degree of

Master of Science in Agriculture

Faculty of Agriculture

Kerala Agricultural University

Department of Agricultural Botany

COLLEGE OF HORTICULTURE

Vellanikkara, Trichur

1989

DECLARATION

I hereby declare that this thesis entitled ***'Karyomorphology, Pollen sterility and seedset in Vetiver (Vetiveria zizanioides (Linn) Nash.)'*** *is a bonafide 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.*

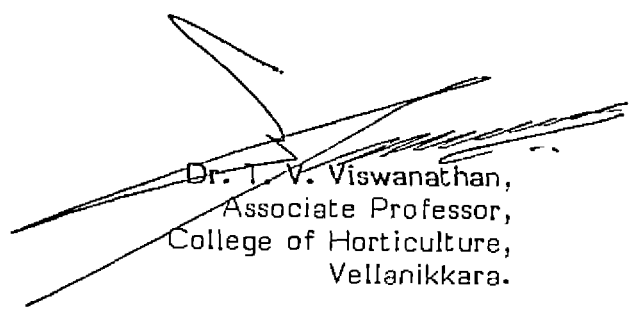
College of Horticulture,
Vellanikkara,
25-11-1989.

K. S. MINI

CERTIFICATE

Certified that this thesis is a record of research work done independently by Smt. K. S. Mini 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.

College of Horticulture,
Vellanikkara,
25-11-1989.



Dr. T. V. Viswanathan,
Associate Professor,
College of Horticulture,
Vellanikkara.

CERTIFICATE

We, the undersigned, members of the Advisory Committee of Smt. K.S. Mini, a candidate for the degree of Master of Science in Agriculture with major in Agricultural Botany, agree that the thesis entitled 'Karyomorphology, Pollen sterility and seedset in Vetiver [*Vetiveria zizanioides* (Linn) Nash.]' may be submitted by Smt. K. S. Mini, in partial fulfilment of the requirements for the degree.

CHAIRMAN :

Dr. T. V. Viswanathan

MEMBERS :

Dr. K. M. Narayanan Namboothiri

Sri V.K.G. Unnithan

Dr. V. K. Mallika

ACKNOWLEDGEMENTS

I wish to express my deep sense of gratitude and indebtedness to **Dr. T.V. Viswanathan**, Associate Professor, AICRP on Medicinal and Aromatic Plants, College of Horticulture, Vellanikkara for suggesting the field of investigation, guidance, inspiration and constant help throughout the period of study and in the preparation of this thesis.

I am grateful to **Dr. K.M.N. Namboodiri**, Professor and Head, Department of Agricultural Botany, College of Horticulture, Vellanikkara who is a member of my Adivisory Commitee for the advice and critical suggestions throughout the investigation and in the presentation of the thesis.

I am extremely thankful to **Dr. V.K. Mallika**, Associate Professor for acting as a member of my Advisory Committe and for the suggestions and constant help throughout the investigation, especially in carrying out the cytological studies and in taking photomicrographs.

I am also thankful to **Sri V.K.G. Unnithan**, Associate Professor, a member of my Advisory Committee, for the help and guidance extended particularly in analysing the data statistically.

Sincere thanks are also due to the member of the staff of the Department of Agricultural Botany and the Department of Plantation Crops for their wholehearted co-operation and assistance at various stage of this investigation.

I owe my gratitude to **Dr. A. Rajan**, Director, Centre for Excellence in Pathology, College of Veterinary and Animal Sciences, Mannuthy, for the help rendered in taking Photomicrographs. The co-operation extended by **Mr. Peter**, Farm Assistant, College of Veterinary and Animal Sciences, is also gratefully acknowledged.

I am also thankful to the staff and labourers of AICRP on Medicinal and Aromatic Plants, College of Horticulture, Vellikkara for their help throughout the experimental study.

I gratefully acknowledge the financial help extended by the Indian Council of Agricultural Research, Krishi Bhavan, New Delhi, for undertaking this work.

The moral support and constant encouragement extended by my parents, husband and friends are acknowledged with deepest sense of gratitude.

My sincere thanks to each and every one who have extended help and co-operation for the successful completion of this programme of study.

Vellanikkara,
25-11-1989.

K. S. Mini

CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF PLATES

Introduction

1. INTRODUCTION

Vetiveria zizanioides, commonly known as Vetiver, is an important aromatic oil yielding crop, native to India, Burma and Sri Lanka.

The genus, Vetiveria is a member of the tribe Andropogoneae, of family Poaceae. The reported species under this genus include Vetiveria zizanioides, V. lawsonii, V. filipes and V. nigritana.

Vetiver is cultivated in India, Java, Reunion, West Indies, parts of Africa and South America, mainly for its aromatic oil. In India vetiver is grown wild in North India and is cultivated in South India. In Kerala, its cultivation is practised in the coastal sandy soils of Trichur and Palghat district.

The aromatic roots of Vetiver yield the well known, "Oil of Vetiver", which has been highly valued by perfumery industry. The oil is used in pharmaceutical preparations and the roots in Ayurvedics medicines. The dried roots are used for making curtains, mats, handfans and baskets. Now, Vetiver is also used as an important soil and moisture conservation crop by planting on the contours.

The different plant types grown at various geographic loctions are known to vary with respect to oil content and oil quality. The relationship of these varitions with the karyomorphological features of these types has not been elucidated so far.

Comprehensive studies on the cytology of Vetiver, pollination characters and seed set pattern were scanty in literature. Hence, an investigation was undertaken with the specific objectives as follows

1. To work out the karyomorphology of Vetiver genotypes and to study the variability in karyotypes, if any.
2. To study the pollen sterility seen in Vetiver and to estimate its percentage.
3. To study the meiotic abnormalities in Vetiver in order to correlate it with pollen sterility.
4. To study the seed set pattern in the selected genotypes.

Review of Literature

2. REVIEW OF LITERATURE

The genus Vetiveria has been classified under the tribe Andropogoneae of the family Gramineae. Out of a number of species identified, only Vetiveria zizanioides has been found to yield the essential oil. A perusal of literature revealed only scanty reports on the cytological aspects of the above species.

2.1. Origin and distribution

Vetiveria zizanioides was reported to be a native of Tropical Asia (Bews, 1929) where as Ramanujam and Kumar (1964) opined Africa or Australia to be the centre of origin rather than India, since only two species were present in India.

According to Morris (1984) Vetiver had been known to Indians from the time of Vedas. During Moghul times French traders became aware of this fine aromatic grass and introduced it into Bourbon and to the New World Colonies of Louisiana and Haiti. India was thus concluded to be the true home of this crop.

While describing Vetiver as a native of Tropical Asia, Bews (1929) found it to be cultivated in Africa also. It was reported to occur in the tropics of old world and introduced into America. Vetiver had been described to be distributed in Burma, Ceylon, Java and

Tropical Africa (Ranga Achariyar, 1921). The cultivation of this crop was reported from Java, Malaya, Philippines, Japan, Reunion, Angola, Haiti, Brazil, Argentina, British Guiana, Jamaica and Mauritius by several workers (Guenther, 1950; Coveney and Pickering, 1956; Wildner, 1961). Celarier (1959) found Vetiveria filipes to be distributed in Australia. Bor (1960) found Vetiveria zizanioides in Burma, Ceylon, South East Asia and Tropical Africa.

The occurrence and distribution of Vetiver in India had been recorded by several workers (Ranga Achariyar, 1921; Guenther, 1950; Bor, 1960). Vetiver was found distributed throughout the plains and lower hills of India. (Ranga Achariyar, 1921). Its cultivation in Southern India was recorded by Guenther (1950). Bor (1960) described two species: Vetiveria zizanioides and Vetiveria lawsonii. The former was found distributed throughout India whereas the latter in Bombay and Madras states.

In an extensive study, Menon and Ittyachan (1945) found the plant to be growing wild in Punjab, Uttar Pradesh and Madras. In Central India, it was seen partially growing wild and partially cultivated. It was also found in Chota Nagpur, Bihar and Assam. Systematic cultivation of this crop was reported from certain pockets of Kerala, Madras, Mysore and Andhra Pradesh (Guenther, 1972).

In Kerala state, the cultivation was found principally from the villages of Chentrappinni, Peringanam, Koolimuttam, Andathode, Puniyoor, Chowghat and Ponnani (Virmani and Dutta, 1975).

The authors observed the cultivation in Trichur, Palghat, Kozhikode and Trivandrum districts. Wynad and its foot hills were also found to take up cultivation.

2.2. Species and Varieties

The attempts to classify Vetiver dates back to 1889 when Hackel included Vetiver in the genus Andropogon and named it as Andropogon squarrosus, but it was described as Vetiveria zizanioides by Linnaeus (Bor, 1960).

The genus Vetiveria was very closely related to Chrysopogon, but for the principal difference in having many jointed racemes (Celarier, 1959). The author has also described the intermediate forms such as Vetiveria fulvibarbis from Africa and Vetiveria filipes and allied species from Australia and based on studies on Vetiveria zizanioides, V. Lawsonii and V. filipes suggested that some forms such as Vetiveria zizanioides must be considered the most primitive and ancestral in the tribe Andropogoneae. The possibility that Vetiveria might contain some gene exchange between Vetiveria and Chrysopogon was pointed out by the above worker.

The genus Vetiveria was found to be comprised of atleast 10 species (Purseglove, 1975; Cobley and Steele, 1976). Out of these, Vetiveria zizanioides was the most common in Asia having aromaticity due to the presence of oil cells (Morris, 1984). The above author found that Vetiveria nigritana of Africa was also very similar, but was not known to be aromatic.

Reports on details of varieties were scanty in the literature. Studies (Sadgopal, 1960) had shown that the essential oil obtained from Vetiver of North India and South India provenance differed in respect of physical and biochemical characteristics. Ramanujam and Kumar (1964) conducted as preliminary survey and substantiated the classification as North Indian and South Indian types of Vetiver based on their morphologically distinctive features, though not complete Pillay (1967) supported this suggestion of Ramanujam and Kumar. This classification was indicated valid based on observations on seeding behaviour by Virmani and Dutta (1975), flowering characters by Morris (1984) and yield and quality of oil by Lavania (1985).

2.3. Morphology

Morphological description of Vetiver has been given by many workers (Ranga Achariyar, 1921; Singh & Sankhala, 1957; Bor, 1960; Pillay, 1967; Purseglove, 1975).

According to them, Vetiver was a densely tufted perennial grass with branching root stocks and spongy aromatic roots. Stem was leafy with equitant hard leaf sheath at the base, smooth and polished, solid and 2 to 3 1/2' high. Leaf sheath was smooth ,coriaceous, glabrous, keeled and compressed. Ligule was a short membrane. Leaf blade was narrowly linear, erect, strongly keeled and flat, acuminate, glabrous, pale green and 30-90 cm long with slender midrib. Panicle oblong, rachis stout, smooth, varying in length from 4 to 12". Whorls of branches 6 to 10 with upto 20 rays. Racemes were long and slender. Spikelets consisted of both sessile and pedicelled spikelets. Sessile spikelet was hermaphrodite and the other, staminate.

Bor (1960) had put forward the keys to identify species of Vetiveria. Vetiveria lawsonii had a row of marginal tubercles on the lower glume, joints and pedicel with a basal tuft of hairs and callus of sessile spikelet bearded. While in Vetiveria zizanioides, lower glume was muricate joints and pedicels glabrous and callus of sessile spikelet glabrous.

Ramanujam and Kumar (1964) conducted a preliminary survey on Vetiver genotypes. In their study some morphological characters were chosen and distinguished North Indian and South Indian types, based on them, though the distinction was not complete. North Indian types had large number of tillers and panicles, lower root production, lesser number of leaves on main tillers, yellowish brown roots of

lesser diameter, larger internodes and narrower leaves. While South Indian types had fewer tillers and panicles. larger root production, more leaves on main tillers, yellow thick roots, shorter internodes and wider leaves. They also reported that South Indian complex showed greater within complex variation. Pillay (1967) supported the suggestion by the above workers and reported that it might be necessary to recognize the existence of two morphologically differentiated complexes in Indian Vetiver.

2.4. Cytogenetics

Observations on cytological features of Vetiveria zizanioides were first made by Janaki Ammal (1945) from an Indian collection and described 20 somatic chromosomes. Similar findings have been reported by Celarier (1959); Mehra et al. (1962); Ramanujam and Kumar (1962); Larsen (1963) and Lavania (1985).

Similar studies on Vetiveria lawsonni from South India were also undertaken by Janaki Ammal (1945) and 2n was found to be 20, whereas one accession of Vetiveria filipes from Queensland was found to be 2n = 40 (Celaier, 1959).

Information on meiotic studies in Vetiveria zizanioides was scanty in the literature. Celarier (1959) studied the meiosis of V. zizanioides as well as V. filipes and reported that all accessions of the former species were essentially normally in meiotic behaviour and 10 bivalents

were usually seen at diakinesis and metaphase I. Occasionally tetravalents were observed which appeared to be more frequent in only one accession. A tendency of metaphase plates to clump was also observed, where as anaphase and telophase I were quite regular. In the case of one accession of V. filipes meiosis was essentially normal; with 20 bivalents, but there was extreme clumping of the chromosomes at metaphase. Sometimes cells with one tetravalent and 18 bivalents were observed. Anaphase I and telophase I were normal.

Celarier (1956) studied the cytology of Andropogon distachys (L) and reported meiotic irregularities like presence of univalents and multivalents at diakinesis and metaphase I stages and also, laggards and bridges at anaphase I and II and telophase I and II stages. Pollen fertility was found to be very low as expected from the cytological irregularities encountered.

Meiotic abnormalities associated with pollen sterility in Vetiveria zizanioides were also studied by Ramanujam and Kumar (1963). It appeared that univalent formation at diakinesis and metaphase I as well as lagging at anaphase resulting in the elimination of chromatin material were closely related with observed sterility.

Conspicuous meiotic abnormalities were also detected in some plants of the grass genus Dactylis during a cytogenetical study conducted

by Shah (1964). These plants could have originated due to break down of self incompatibility and an inbreeding depression could have associated with their abnormalities. Mehra and Ramanandan (1973) reported that grasses were in an active stage of evolution and hence numerous chromosomal changes like polyploidy, aneuploidy and structural rearrangement were exhibited in diverse species, many of which were eliminated during sex cell formation in the form of high pollen sterility. But such abnormal genotypes had a chance of limited propagation and survival through multiplication by vegetative means.

Similarly Sudharshan and Jagdish (1981) reported unusual meiotic behaviour and formation of 2n pollen in tetraploid Cymbopogon caesius (Nees.) Stapf. Verma and Sobti (1985) also conducted meiotic studies in six species of Cymbopogon Spreng. and their varieties. They reported presence of 10 bivalents in most of the PMCs of all the diploids at diakinesis - metaphase I stages and normal segregation in anaphase I & II stages indicating fairly regular meiosis except in those PMCs which had accessary chromosomes.

Although karyological studies were found to be scanty in Vetiveria sp., reports on related genera were available. Verma and Sobti (1982) conducted karyological studies in the genus Cymbopogon Spreng. and observed that karyotype evolution in closely related species in this genus appeared to be through structural differences. The authors

observed a general reduction in the total chromatin length during evolution. Further in 1985 they studied accessary chrmosomes in the same genus and found that B chromosomes present in both root tip meristematic cells as well as the PMCs had a direct effect on the total chromatin length and the chiasma frequency of A chromosome. In presence of the accessary chromosomes no effect on general morphology and oil content was noticed.

Detailed studies on nuclear DNA and karyomorphology in Vetiveria zizanioides conducted by Lavania (1985) revealed nuclear DNA amount in 20 different collections. The total chromatin length varied from 25.6μm to 38.7μm and 2C value from 2.02 to 2.56 picograms. The conclusion was that variation in karyological features might possibly account for qualitative and quantitative variation. Christopher (1986) found that most of the species of the tribe Andropogoneae showed the basic number 10. Lavania (1987) reported chromosome instability in lemongrass. The variation of chromosome number from 20 to 28 encountered from root tip suggested a gradual elimination of somatic chromosomes.

Induced polyploidy for attaining rapid genetic improvements in Vetiver was also reported. Lavania (1988) reported artificial auto-tetraploids produced by colchicine treatment in the important essential oil bearing vetiver (2n = 20). The raw tetraploids were stabilized

by selection for pure types in segregating vegetative progeny. The tetraploids were vigorous with thicker and longer roots. In terms of economic yield the tetraploids had the potential of producing 62.5% and 39.2% more oil over the diploid parent and check, respectively.

2.5. Flowering

2.5.1. Behaviour

The difference between the flowering behaviour of North Indian and South Indian types of vetiver was reported by Ramanujam and Kumar (1964). South Indian types flowered very late in the season in New Delhi. This was due to difference in photoperiodic requirement. This was also reported by Pillay (1967).

Purseglove (1975) reported that cultivated types of vetiver seldom flowered. Although Cobley and Steele (1976) also observed failure of some cultivated types to flower in an experimental study, most of them produced long terminal panicles of tender racemes. Sethi and Gupta (1980) observed that North Indian types were producing flowering shoots annually.

Morris (1984) classified Vetiver present in India as flowering type - characteristic of the wildling found in North India and non-flowering type - that found in commercial plantations of South India.

2.5.2. Inflorescence

Ranga Achariyar (1921) reported that Vetiver produced flowers in panicles which were conical and erect with branches varying in length from 4 to 12", where as singh and Sankhala (1957) described the panicle as oblongwith stout and smooth rachis, whorls of branches 6 to 10 with upto 20 rays, racemes 5, long and slender and spikelets in pairs.

Kumar (1963) reported a deviation in Vetiveria zizanioides. The occurence of perfect flowers in nearly all the pedicellate spikelets in a clone of the above species was noticed. This might be taken as the earliest stage in this line of differentiation.

Ramanujam and Kumar (1963) conducted correlation studies in Vetiver and presented that panicle length showed medium correlation with root yield. Significant parent progeny correlations were obtained for number of whorls in the panicle. Further, in 1964, the same authors observed difference in number of panicles between North Indian and South Indian types, as North Indian types produced larger number of panicles than others.

Purseglove (1975) described the inflorescene as 15-30 cm long, narrow with 2.5-5 cm long whorled branches, spikelets in pairs, narrow, acute appressed and awnless - one sessile and hermaphrodite and the other pedicelled and staminate.

2.5.3. Morphology of flowers

Morphology of flowers was described by many workers. Spikelets occur in pairs; one sessile and the other pedicellate. Sessile spikelets linear, lanceolate to almost linear, acute to sub acute, 4 to 4.5mm long, yellowish, olive, violet brown or purplish to almost black in colour. First glume was ovate-oblong, obscurely 2 to 4 nerved, acute and densely flat. Second glume was as long as the first glume, oblong, keeled, one-nerved and with minute prickles on the keel. The third glume was broadly oblong, hyaline and nerveless. Fourth glume was shorter than the third, linear oblong and paleate - palea about 2/3rd the length of the glume and lanceolate. Lodicules were two, quadrate and conspicuous; though small, styles and stigma short, stamens three with yellow anthers. Stigma white or purplish in colour. Pedicelled spikelets were similar to the sessile ones, but were slightly smaller and prickles less prominent. The fourth glume had no awn and had three stamens (Ranga Achariyar (1921). Bor (1960), Kumar (1963), Purseglove (1975), Singh and Sankhala (1957) and Sethi (1982) also described similar morphological features of flowers in vetiver.

Kumar (1963) reported the occurrence of perfect flowers in nearly all pedicellate spikelets in a clone of vetiver.

2.5.4. Floral biology

Studies on floral biology by Sethi and Gupta (1980) revealed that most of the materials in Vetiveria zizanioides selected were distinguished by marker genes such as a white chalkish bloom on stem, yellow anther colour and pink stigma in the material. Sethi (1982a) reported that none of the selfed inflorescences or isolated plants in Vetiver set seed. The author also reported that anthesis took place from top to bottom of the panicles and it would take 7 to 10 days for all the flowers to open.

2.6. Pollen studies

Information on pollen studies in vetiver was limited in the literature.

Celarier (1959) conducted cytological studies on Andropogon distachys and determined pollen fertility. The fertility was correlated with observed meiotic irregularities in the PMCs. It appeared that not more than 29 to 30 % of the pollen would be viable. This was expected from the cytological irregularities encountered.

Ramanujam and Kumar (1963) studied irregular meiosis associated with pollen sterility in Vetiveria zizanioides. Pollen fertility status of a large number of clones collected from different parts of India was calculated and study of microsporogenesis in some clones selected for different degrees of pollen sterility was conducted.

Varying degrees of pollen sterility were observed ranging from 20 to 100 %. The pollen sterility from male florets appeared to be greater than in anthers from hermaphrodite floret. It appeared that univalent formation at diakinesis and metaphase stages and lagging at anaphase resulting in the elimination of chromatin material was closely correlated with observed sterility.

Studies were conducted by Pillay (1967) to determine the mechanism of failure of self pollination in Vetiveria. Pollen germination and pollen tube growth were studied. It was observed that majority of pollen grains in the open pollinated styles had germinated (88 %) where as only 20 % of the pollen grains in the isolated styles had visibly germinated. Moreover, most of the germinated grains on the isolated styles had sent out only short tubes, while most of the pollen tubes on the open pollinated styles were much larger. It would appear that germination and growth of self pollen were somehow inhibited and the pollen tubes may not grow long enough to reach the ovule.

Pollen fertility was found to vary among the clones in both North Indian and South Indian types. Sethi (1982a)reported high pollen fertility in a clone studied.

2.7. Pollination and seedset

Preliminary studies (Kumar, 1962) had shown that most of the cultures of Vetiveria maintained at I.A.R.I. exhibited fairly high degree of pollen fertility. Such high pollen fertility could be compatible with self pollination and cross pollination as well as apomictic reproduction. Ramanujam and Kumar (1963) based on observations on irregular meiosis and pollen sterility, expected extensive cross pollination.

Pillay (1967) conducted studies on seed set pattern to determine whether self pollination or cross pollination was involved. None of the selfed inflorescences set seed eventhough open pollinated panicles of the same clones produced abundant seeds. The results of different approaches to the problem of seed setting under isolation would appear to rule out to a large extent any form of apomixis. On the basis of the results, it was concluded that Vetiveria zizanioides produced seeds through a sexual process and also that it was highly, if not completely, cross pollinated.

According to Sethi (1982 a) vetiver was a highly cross pollinated crop since none of the selfed inflorescences or isolated plants set seed while the open pollinated plants produced large amount of seed, although pollen in both the cases showed high fertility. Indicating its nature of obligatory cross pollination, it was also reported (Sethi, 1982b) that due to the presence of hermaphrodite florets

(84 %) and male florets (74 %) selfing did not take place in this taxon. That was considered to be the reason why materials obtained from wild population were frequently heterozygous.

Ramanujam and Kumar (1963) reported that some of the accessions, particularly those of South Indian origin, could only be maintained by vegetative methods as they did not produce seeds under controlled, hand pollinated conditions. Further, in 1963, the same authors reported that no seed set was seen in South Indian clones under New Delhi conditions, though varying proportions of pollen grains appeared to be fertile.

But, Pillay (1967) had disagreed with the above finding by reporting that South Indian types could also produce seedlings, even under New Delhi conditions, where these cultures flowered quite late.

According to Virmani and Dutta (1975) the one that was growing wild in North India was mainly the seeding type, while that of South India was non seeding. Akhila et al. (1981) had supported the above finding.

Materials and Methods

3. MATERIALS AND METHODS

The investigations reported herein were undertaken in the Department of Agricultural Botany, College of Horticulture, Vellanikkara, during 1987-88 and 1988-89 seasons. The weather data during the period (January-December, 1987 and '88) are given in Appendix-I.

A. Materials

Eleven cultivars of Vetiveria zizanioides containing North Indian and South Indian types and hybrid, received from the Medicinal and Aromatic Plant Research Station, Odakkali and maintained in the garden of AICRP on Medicinal and Aromatic Plants, College of Horticulture, Vellanikkara, were made use of for the study. The details of the cultivars are given in Table 1.

Table 1. Cultivars of Vetiver and their source

Sl. No.	ODV Nos.	Name of cultivar	Source
1	ODV-2	Shornur	South India
2	ODV-4	Irikkur	South India
3	ODV-10	Iritty	South India
4	ODV-3	Nilambur	South India
5	ODV-12	Hybrid	Indore
6	ODV-11	Thiruvampadi	South India
7	ODV-7	Moosanagar	North India
8	ODV-5	Akila	North India
9	ODV-6	Bharatpur	North India
10	ODV-8	Chalakkudy	South India
11	ODV-9	North Malabar	South India

B. Methods

1. Morphological characters

For recording morphological observations, the cultivars selected were raised in pots and normal cultural operations were followed. Observations on the following characters were recorded from 5 plants in each cultivar. The mean values were calculated and presented.

All morphological observations were made when the plants were six months old.

(i) Plant height: Height of plant was measured in cm from soil surface to the tip of the panicle or the tip of the largest leaf where panicles were not present.

(ii) Number of tillers per plant: Number of tillers in each plant was taken.

(iii) Number of leaves/main tiller: Number of leaves per main tiller was recorded from plants and the average worked out.

(iv) Length of leaves: Length of leaf was taken from the base to the tip of the leaf and presented in cm.

(v) Breadth of leaves: Breadth of lamina at the middle portion was taken and presented in mm.

(vi) Number of days taken for flowering: Days were counted from planting till flower emergence in all cultivars.

(vii) Number of inflorescences per plant: Number of inflorescences per plant was taken from every pot.

(viii) Length of inflorescence: Length of inflorescence in cm was recorded from base to the tip of inflorescence.

(ix) Number of racemes per inflorescence: Number of racemes per inflorescence was taken after emergence of panicle from boot leaf.

(x) Number of flowers per inflorescence: Number of hermaphrodite as well as staminate flowers per inflorescence was taken in each cultivar.

(xi) Colour of stigma: Colour of stigma was noted at the time of flower opening in all cultivars.

(xii) Yield (g/plant): Fresh weight of the roots per plant was recorded when the plants were 8 months old.

2. Cytogenetical studies

i) Mitosis

A modification of the procedure by Lavania (1985) was followed. This is described below:

Slips, collected from the growing plants, were planted in sand. Young and actively growing roots were excised after two days. Only the tip was taken. The outer covering (Including epiblemma and cortical cells) of the root tip was scraped. The remaining root tip tissue was pretreated in saturated aqueous solution of 8-hydroxyquinoline

for three hours at 12 to 14°C for metaphase arrest. Such pretreated root tips were fixed for 48 hours in 6:3:1 Carnoy's fixative and later on hydrolysed in IN HCl at 60°C for 14-16 minutes. The root tips were stained with Feulgen stain for 1 to 2 hours in darkness. Magenta coloured root tip portion was squashed in 1 % acetocarmine. The chromosome measurements were taken from camera lucida drawings of five well spread cells with cell wall intact and chromosomes properly contracted. Photomicrographs were taken using orthoplan microscope.

Observations

Data were recorded from temporary slides only, since excessive staining of cytoplasm reduced the clarity of preparations in permanent mounts. Karyomorphological measurements were recorded from five cells at metaphase stage in each cultivar.

Measurements of length of long arm (L), short arm (S) and satellite of individul chromosomes were tabulated and homologous pairs were identified. All measurements were converted into microns and arm ratios (L/S) for each of the homologous pair were calculated.

For the purpose of idiograms, the chromosomes were designated as 1 to 10, in decreasing order of size and increasing order ofasymmetry.

The chromosomes were classified into the following groups based on the position of the centromere and presence or absence

of satellites (Giorgi and Bozzinii, 1969).

SAT - Satellited chromosome - The arm ratio of which is calculated leaving out the satellite length.

M - Median chromosome; the arm ratio of which is between 1.00 and 1.25.

SM - Submedian chromosome; the arm ratio of which is between 1.26 and 1.75.

ST -Subterminal chromosome; the arm ratio of which is more than 1.75.

The Total Chromatin Length (TCL) of the haploid complement and the Relative Chromosome Length (RCL) which is expressed as the percentage of individual chromosome length over the total length of the haploid complement were estimated. Average Chromosome Length (ACL) in each variety was also calculated.

Categorisation of karyotype asymmetry of somatic complement has been made according to the method of Stebbins (1958) who classified the karyotype under 12 classes (1 A to 4 C) taking into account both the position of centromere in the chromosome and the degree of difference between the largest and smallest chromosome of a complement (Table 2). Total Form percentage (TF %) was also calculated following the formula given by Huziwara (1962).

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

Table 2. Stebbin's classification (1958)

Largest chromosome length / Smallest chromosome length	Proportion of chromosomes with arm ratio more than 2:1			
	0.00	0.01-0.5	0.5-0.99	1.00
2 : 1	1a	2a	3a	4a
2 : 1 - 4 : 1	1b	2b	3b	4b
4 : 1	1c	2c	3c	4c

ii) Meiosis

a. Procedure

For the study of meiotic patterns, immature panicles still inside the boot sheath, were collected, seperated and fixed in alcoholacetic acid (3 : 1) for 24 hours. The time of fixation was standardised as 8 to 9.30 AM. After fixation, the spikelets were washed and stored in 70% alcohol. The spikelets were taken out from alcohol and the immature anthers were dissected out using foreceps and needle. They were squashed in 1% acetocarmine and smears were made. Excess stain was removed by pressing through blotting paper. Then they were observed through the microscope after making the slide semipermanent.

b. Observations

The following observations were taken for each cultivar.

i) Mean univalents/bivalents/multivalents per PMC at diaknesis and metaphase I stages.

ii) Mean chiasmata per PMC.

iii) Mean chiasmata per bivalent.

iv) Percentage of cells with normal segregation at anaphase and telophase stages.

v) Percentage of cells with bridges and laggards at anaphase and telephase stages.

Photomicrographs were taken in Orthoplan microscope.

3. Pollen studies

i) Pollen fertility

Fertility of pollen was assessed on the basis of stainability of pollen grains in acetocarmine-glycerine mixture. Pollen grains were extracted from fully matured anthers just before anthesis using needle and stined in a drop of aceto-carmine-glycerine mixture on a clean slide and kept aside for one hour. All the pollen grains that were stained were counted as fertile. Five hundred pollen grains per cultivar were scored. Pollen sterility was expressed as percentage.

ii) Pollen size

Pollen diameter was measured using an ocular micrometer after calibration. Mean size and range were calculated.

Photomicrographs of pollen grains showing fertile and sterile ones as well as range in size were also taken.

4. Studies on pollination and seed set

i) Selfing

Inflorescences in each variety were covered using cloth bag before any of the florets opened. The bag was closed at the base of the inflorescence and maintained in position by proper support for the tillers. When the dehiscence was completed all along the panicle, usually within a fortnight, the bag was removed and the inflorescence was exposed to natural condition to facilitate proper development of seeds. When the panicle started maturing from the top, the inflorescence was rebagged so that the falling fluffs could be collected in the bag. When the whole panicle was matured it was harvested with the bag intact. The fluffs so obtained were threshed and kept in labelled envelope. The fluff was examined for the presence of seeds.

ii) Crossing

Crossing was attempted between cultivars having maximum pollen fertility and that having maximum pollen sterility. The pots containing selected parents were placed side by side so that the inflorescence from both parents could be put together in a pollination bag to permit interpollination. Wherever the parents differed in height at the time of crossing suitable adjustments were made (by placing bricks below the pots) to make their heights almost same. Tillers of parents which

synchronised in flowering as judged by the emergence of panicle, were chosen. They were carefully introduced into a cloth bag. Just as in selfing, the bags were closed at the bottom. After the completion of anthesis on both the panicles, the bag was removed. When the panicle started maturing from the top the inflorescences were bagged seperately. After harvesting, the fluff was threshed and put in labelled envelope. The fluff was examined for the presence of seed.

iii) Open pollination

Panicles were allowed to open pollinate. After the panicle completed anthesis and started maturation, that was bagged. At the time of maturation, the whole panicle, was harvested and threshed. The fluff was examined for the seeds.

Number of filled seeds per panicle in each case was counted and recorded.

iv) Germination test

Open pollinated seeds were subjected to germination test in the laboratory. Hundred seeds from each variety were counted. Petri-dishes were filled with sand. The seeds were sown in sand and watered daily. Observation on the number of seeds germinated was carried out daily for one month. The values were subjected to chi-square analysis.

Results

4. RESULTS

4.1. Morphological characters of cultivars

The observations on morphological characters of eleven cultivars of vetiver are presented in Table 3. These included both vegetative and floral characters viz. plant height (cm), number of tillers per plant, number of leaves per main tiller, length of leaves (cm), breadth of leaves (mm), days of appearance of inflorescences, number of inflorescences per plant, length of inflorescence (cm), number of racemes per inflorescence, number of flowers per inflorescence, colour of stigma and root yield (g/plant). It was observed that no gross morphological character could be used in differentiating North Indian and South Indian types of Vetiver. But cultivars differed greatly with respect to some characters like number of tillers per plant, days for appearance of flowers, number of inflorescences per plant (Plates 1 and 2) and root yield. The three North Indian types behaved similarly except for some characters in the case of ODV-5. Two of the South Indian cultivars viz. ODV-8 and ODV-9 showed many characters similar to North Indian types. The behaviour of ODV-12 and the rest of the South Indian types were almost similar.

4.2. Cytogenetical studies

4.2.1. Mitosis

The karyomorphology of eleven cultivars of vetiver was analysed in the present study. All the collections possessed 20 chromosomes

Table 3. Morphological characters in different cultivars of vetiver

Characters	ODV-2	ODV-4	ODV-10	ODV-3	ODV-12	ODV-11	ODV-7	ODV-5	ODV-6	ODV-8	ODV-9
Plant height (cm)	168.00	128.75	169.16	138.33	135.00	170.00	158.00	145.00	153.00	165.00	146.67
No. of tillers per plant	35.00	21.50	27.25	31.00	33.75	26.25	21.00	23.00	23.75	20.34	21.50
No. of leaves/main tiller	7.00	8.00	7.30	7.50	6.30	7.30	6.50	6.30	7.50	6.30	7.30
Length of leaves (cm)	61.80	69.40	69.60	66.50	68.67	83.80	65.40	66.40	71.03	62.00	75.10
Breadth of leaves (mm)	5.53	4.80	5.90	5.80	4.92	7.75	5.55	6.20	5.80	4.70	6.40
Days for emergence of inflorescence	180.00	165.00	150.00	150.00	240.00	215.00	30.00	60.00	60.00	30.00	90.00
No. of inflorescence per plant	1.60	4.00	2.00	2.00	1.00	5.00	7.00	4.00	5.00	4.00	7.50
Length of inflorescence (cm)	37.20	27.92	39.56	26.00	28.50	29.33	30.90	33.00	25.50	33.33	25.72
No. of racemes/ inflorescence	81.60	58.83	113.89	66.20	52.25	97.56	96.50	102.28	56.20	115.56	75.40
No. of flowers/ inflorescence *	595.20 + 679.90	318.00 + 387.50	822.22 + 936.67	436.40 + 511.20	387.50 + 436.25	692.78 + 784.44	589.00 + 677.00	824.00 + 908.00	365.00 + 443.70	905.00 + 1004.40	443.31 + 512.78
Colour of stigma	White	Light purple	Purple	Purple	Light purple	White	White	Purple	Light purple	Light purple	Light purple
Root yield/plant (g)	102.50	176.00	113.50	165.50	169.00	133.00	91.00	124.00	99.00	98.00	83.00

* Hermaphrodite flowers + staminate flowers

Plate 1. Cultivars of Vetiveria zizanioides : Morphological features.

1,2,3 & 4 : South Indian cultivars

5 : Hybrid

6 : South Indian cultivar

7,8 & 9 : North Indian cultivars

10 & 11 : South Indian cultivars

Plate 2.A. Cultivars of Vetiveria zizanioides : Early flowering.

7,8 & 9 : North Indian cultivars

10 & 11 : South Indian cultivars

Plate 2.B. Cultivars of Vetiveria zizanioides : Late flowering.

1,2,3 &4 : South Indian cultivars

5 : : Hybrid

6 : South Indian cultivar


7
8
9
10
11
A


1
2
3
4
5
6
B

in the root tip cells. The morphological characteristics of individual chromosomes with respect to length of long arm, short arm and satellite, arm ratio, type of chromosome, total chromatin length and relative chromosome length are documented in Tables 4 to 17. The idiograms of chromosomes drawn in the decreasing order of length are presented in Fig. 1.

A. Karyotypes of different cultivars.

a. ODV-2

The details on karyomorphology of ODV-2 are given in Table-4 and Fig. 1. The chromosome complement in this cultivar was characterised by three pairs of median and seven pairs of submedian chromosomes. The length of individual chromosomes ranged from 1.75μm to 4.1μm and the total chromatin length of the haploid complement was 29.4μm. The relative lengths of chromosomes ranged from 5.95 per cent to 13.95 per cent. The average arm ratio was found to be 1:1.403.

b. ODV-4

The karyotype analysis of this cultivar is given in Table 5, Fig1 and Plate 3 A. Out of the ten pairs of chromosomes, three were belonging to median and seven to submedian type in this cultivar. The individual lengths of chromosomes ranged 1.8μm to 3.8μm. The

Table 4. Karyotype analysis in ODV-2 (2n = 20)

Chromosome numbers	Long arm (L) (μm)	Short arm (S) (μm)	Total length (μm)	Arm ratio S:L	Type	Relative Chromosome Length (%)
1	2.10	2.00	4.10	1:1.05	M	13.95
2	2.50	1.50	4.00	1:1.67	SM	13.61
3	2.00	1.63	3.63	1:1.23	M	12.35
4	2.00	1.63	3.63	1:1.23	M	12.35
5	1.90	1.13	3.03	1:1.68	SM	10.31
6	1.50	1.20	2.70	1:1.25	SM	9.18
7	1.50	1.00	2.50	1:1.50	SM	8.50
8	1.30	1.00	2.30	1:1.30	SM	7.80
9	1.13	0.63	1.76	1:1.70	SM	5.99
10	1.00	0.75	1.75	1:1.33	SM	5.95

M - median SM - submedian

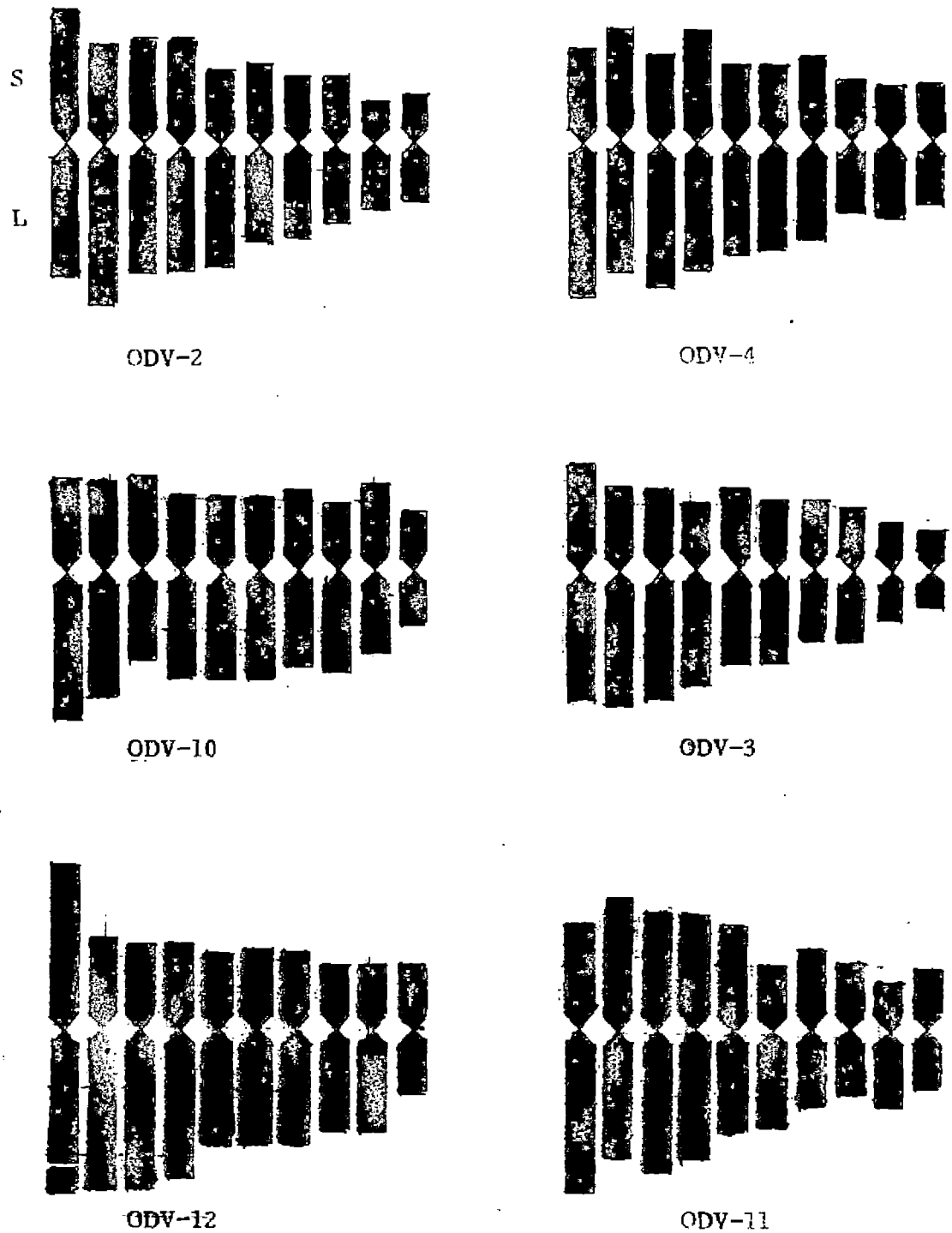
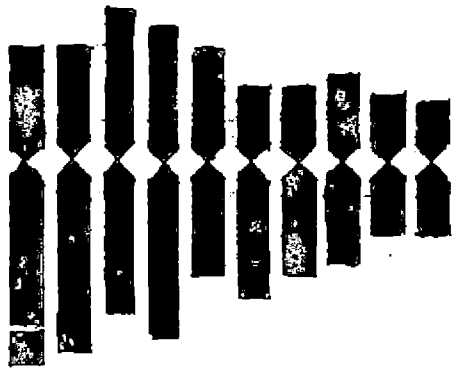
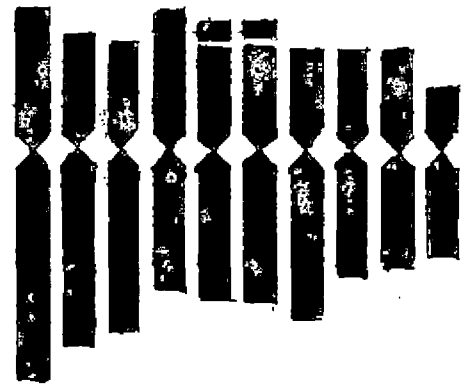


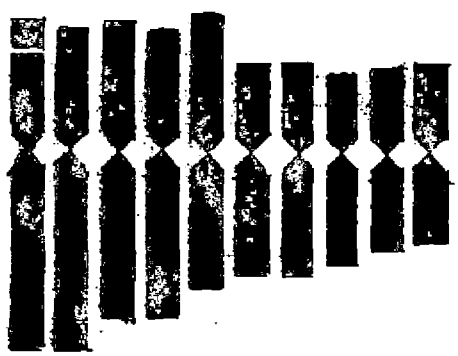
Fig.1 Idiograms of different Vetiver Cultivars



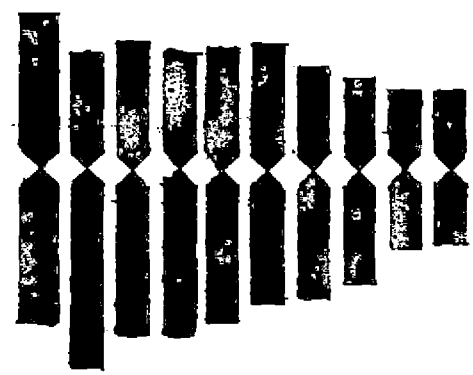
ODV-7



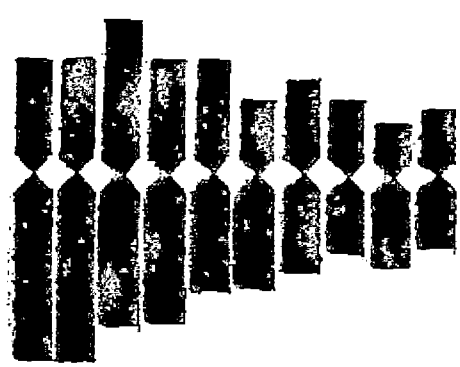
ODV-5



ODV-6



ODV-8



ODV-9

Fig.1 Idiograms of Different Vetiver Cultivars

Table 5. Karyotype analysis in ODV-4 (2n = 20)

Chromosome number	Long arm (μm)	Short arm (μm)	Total length (μm)	Arm ratio S:L	Type	Relative Chromosome Length (%)
1	2.40	1.40	3.80	1:1.71	SM	12.79
2	2.00	1.70	3.70	1:1.17	M	12.46
3	2.30	1.30	3.60	1:1.70	SM	12.12
4	2.00	1.60	3.60	1:1.50	SM	12.12
5	1.80	1.20	3.00	1:1.50	SM	10.10
6	1.70	1.10	2.80	1:1.55	SM	9.43
7	1.50	1.30	2.80	1:1.15	M	9.43
8	1.60	1.00	2.60	1:1.60	SM	8.75
9	1.20	0.80	2.00	1:1.50	SM	6.73
10	1.00	0.80	1.80	1:1.00	M	6.06

M - median SM - submedian

Plate 3. Mitotic chromosomnes in some cultivars of Vetiveria zizanioides. (2n = 20)

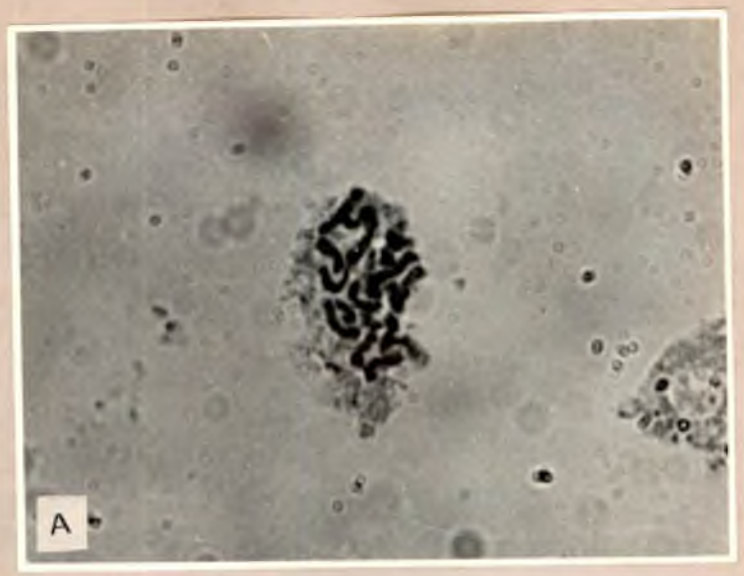
A : ODV-4(×1000)

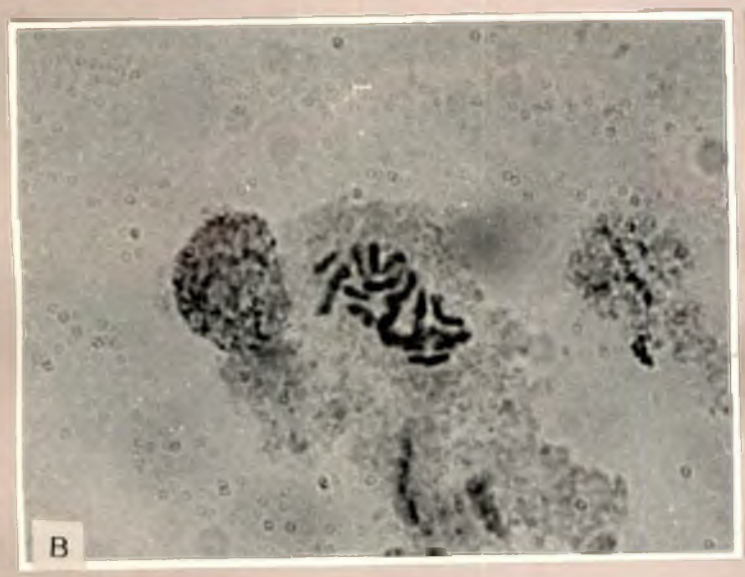
B : ODV-3(×1000)

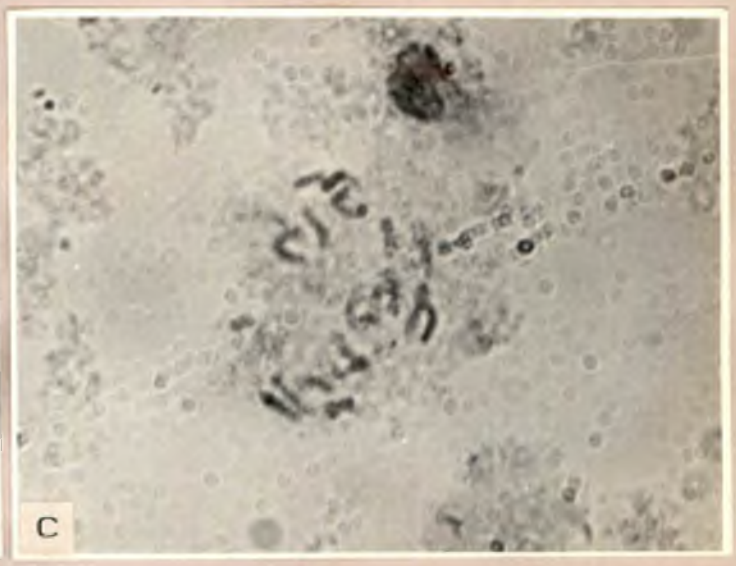
C : ODV-7(×1000)

D : ODV-6(×1000)

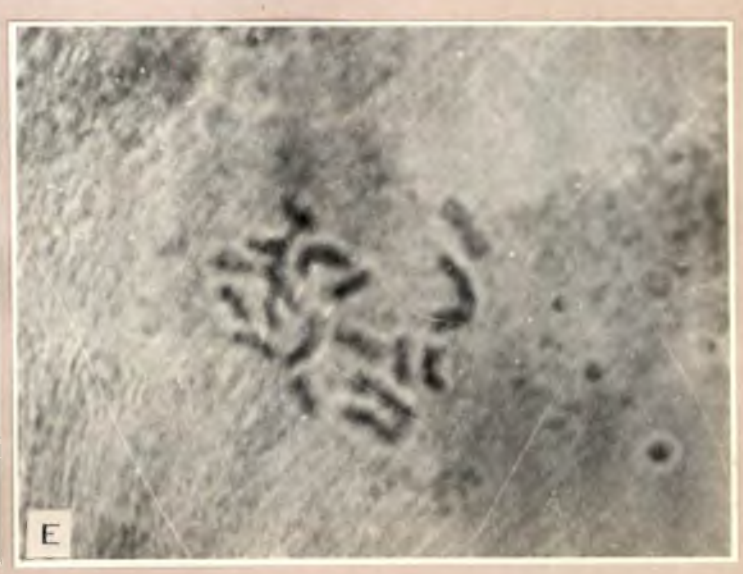
E : ODV-8(×1000)


A


B


C


D


E

relative lengths of chromosomes ranged from 6.06 per cent to 12.79 per cent. The average arm ratio was 1:1.44.

c. ODV-10

The absolute length of individual chromosomes ranged from 1.8μm to 3.7μm. The tot' chromatin length of haploid complement was 27.3μm (Table 6. Fig 1). Out of the ten pairs, four have median and six have submedian centromers. The relative chromosome length ranged from 6.59 per cent to 13.55 per cent. The average arm ratio was 1:1.38.

d. ODV-3

The karyomorphology of ODV-3 is presented in Table 7 and idiogram in Fig 1 :: (' Plate 3B). It was having four pairs of median, five pairs of submedian and one pair of sub terminal chromosomes. The chromosomes ranged in size from 1.21μm to 3.63μm. The total chromatin length was 25.04μm and the Relative Chromosome Length ranged from 4.83% to 14.49%. The average arm ratio was 1:1.41.

e. ODV-12

The chromosomes ranged in size from 2μm to 5μm with a mean total chromatin length of 32.2μm (Table 8 Fig 1). The complement was also characterised by two median, seven submedian and one subterminal pairs of chromosomes. The relative chromosome length ranged from 6.21 per cent to 15.33 per cent. The average arm ratio

Table 6. Karyotype analysis in ODV-10 (2n = 20)

Chromosome number	Long arm (L) (μm)	Short arm (S) (μm)	Total length (μm)	Arm ratio S:L	Type	Relative Chromosome length (%)
1	2.30	1.40	3.70	1:1.64	SM	13.55
2	2.00	1.30	3.30	1:1.54	SM	12.09
3	1.40	1.40	2.80	1:1.00	M	10.26
4	1.70	1.10	2.80	1:1.55	SM	10.26
5	1.70	1.10	2.80	1:1.55	SM	10.26
6	1.70	1.10	2.80	1:1.55	SM	10.26
7	1.50	1.20	2.70	1:1.25	M	9.89
8	1.60	1.00	2.60	1:1.60	SM	9.52
9	1.30	1.20	2.50	1:1.08	M	9.16
10	0.90	0.90	1.80	1:1.00	M	6.59

M - median SM - submedian

Table 7. Karyotype analysis in ODV-3 (2n = 20)

Chromosome number	Long arm (L) (μm)	Short arm (S) (μm)	Total length (μm)	Arm ratio S:L	Type	Relative Chromosome length (%)
1	2.00	1.63	3.63	1:1.23	M	14.49
2	2.10	1.20	3.30	1:1.70	SM	13.18
3	2.00	1.20	3.20	1:1.67	SM	12.78
4	1.80	1.00	2.80	1:1.80	ST	11.18
5	1.50	1.20	2.70	1:1.25	SM	10.78
6	1.50	1.00	2.50	1:1.50	SM	9.98
7	1.20	1.00	2.20	1:1.20	M	8.79
8	1.20	0.80	2.00	1:1.50	SM	7.99
9	0.80	0.70	1.50	1:1.14	M	5.99
10	0.63	0.58	1.21	1:1.09	M	4.83

M - median SM - submedian ST - subterminal

Table 8. Karyotype analysis in ODV-12 (2n = 20)

Chromosome number	Long arm (L) (μm)	Short arm (S) (μm)	Total length (μm)	Arm ratio S:L	Type	Relative Chromosome length (%)
1	2.50	2.50	5.00(sc)	1:1.00	M	15.53
2	2.50	1.40	3.90	1:1.70	SM	12.11
3	2.50	1.30	3.80	1:1.92	ST	11.80
4	2.30	1.30	3.60	1:1.70	SM	11.18
5	1.80	1.10	2.90	1:1.64	SM	9.01
6	1.80	1.10	2.90	1:1.64	SM	9.01
7	1.80	1.10	2.90	1:1.64	SM	9.01
8	1.60	1.00	2.60	1:1.60	SM	8.07
9	1.60	1.00	2.60	1:1.60	SM	8.07
10	1.00	1.00	2.00	1:1.00	M	6.21

M - median SM - submedian ST - subterminal SC - Secondary constriction

was 1:1.54.

f. ODV-11

The karyotype analysis of this cultivar is presented in Table 9 and Fig, 1. It has shown the variation in length from 1.8μm to 4.1μm between different pairs. The total chromatin length of the haploid complement was 29.6μm. This cultivar also showed six pairs of median and four pairs of submedian chromosomes in its complement. The Relative Chromosome Length ranged from 6.08% to 13.85%. The average arm ratio was 1:1.22.

g. ODV-7

The length of individual chromosomes in this North Indian cultivar ranged from 1.75μm to 4.1μm with a total chromatin length of 30.15μm for the haploid complement. The classification of chromosomes according to arm ratio has shown six pairs of median and four pairs of submedian chromosomes in the complement (Table 10. Fig 1 and Plate 3C). The Relative Chromosome Length ranged from 5.8 per cent to 13.59 per cent and the average arm ratio was 1:1.32.

h. ODV-5

The karyotype of this North Indian cultivar was characterised by three pairs of median and seven pairs of submedian chromosomes. The length of chromosomes varied from 2.2μm to 4.8μm and the total chromatin length was 34.9μm (Table 11, Fig 1). The average arm ratio was 1:1.49. The cultivar has shown two pairs of satellited

Table 9. Karyotype analysis in ODV-11 (2n = 20)

Chromosome number	Long arm (L) (μm)	Short arm (S) (μm)	Total length (μm)	Arm ratio S:L	Type	Relative Chromosome Length (%)
1	2.50	1.60	4.10	1:1.56	SM	13.85
2	2.00	2.00	4.00	1:1.00	M	13.51
3	2.20	1.80	4.00	1:1.22	M	13.51
4	2.20	1.50	3.70	1:1.46	SM	12.50
5	1.60	1.60	3.20	1:1.00	M	10.81
6	1.50	1.00	2.50	1:1.50	SM	8.45
7	1.20	1.20	2.40	1:1.00	M	8.10
8	1.00	1.00	2.00	1:1.00	M	6.76
9	1.20	0.70	1.90	1:1.71	SM	6.42
10	0.90	0.90	1.80	1:1.00	M	6.08

M - median SM - submedian

Table 10. Karyotype analysis in ODV-7 (2n = 20)

Chromosome number	Long arm (L) (μm)	Short arm (S) (μm)	Total length (μm)	Arm ratio S:L	Type	Relative Chromosome Length (%)
1	2.60 (sc)	1.50	4.10	1:1.73	SM	13.59
2	2.50	1.50	4.00	1:1.67	SM	13.27
3	2.00	2.00	4.00	1:1.00	M	13.27
4	2.00	1.75	3.75	1:1.14	M	12.44
5	1.50	1.50	3.00	1:1.00	M	9.95
6	1.75	1.00	2.75	1:1.70	SM	9.12
7	1.50	1.00	2.50	1:1.50	SM	8.29
8	1.25	1.25	2.50	1:1.00	M	8.29
9	0.90	0.90	1.80	1:1.00	M	5.97
10	0.90	0.85	1.75	1:1.06	M	5.80

M - median SM - submedian sc - Secondary Constriction

Table 11. Karyotype analysis in ODV-5 (2n = 20)

Chromosome number	Long arm (L) (μm)	Short arm (S) +Satellite (μm)	Total length (μm)	Arm ratio S:L	Type	Relative Chromosome Length (%)
1	3.00	1.80	4.80	1:1.67	SM	13.75
2	2.60	1.50	4.10	1:1.73	SM	11.75
3	2.40	1.40	3.80	1:1.71	SM	10.89
4	1.80	1.80	3.60	1:1.00	M	10.32
5	2.00	1.30+0.30	3.60	1:1.54	SAT	10.32
6	2.00	1.30+0.30	3.60	1:1.54	SAT	10.32
7	2.20	1.30	3.50	1:1.69	SM	10.02
8	1.60	1.30	2.90	1:1.22	M	8.30
9	1.50	1.30	2.80	1:1.13	M	8.02
10	1.40	0.80	2.20	1:1.70	SM	6.30

M - median SM - submedian SAT - satellited chromosome

chromosomes. The Relative Chromosome Length ranged from 6.3 to 13.75 per cent.

i. ODV-6

The karyotype analysis of this North Indian cultivar, presented in Table 12, Fig 1 and Plate 3D, showed that the length of chromosomes ranged from 2.4μm to 4.3μm with a total chromatin length of 32.7μm for the haploid complement. It has seven pairs of median and three pairs of submedian chromosomes according to arm ratio grouping. The average arm ratio was found to be 1:1.35. The Relative Chromosome Length ranged from 7.34 to 13.15 per cent.

j. ODV-8

The length of chromosomes in this cultivar ranged from 2μm to 4μm with a total haploid chromatin length of 31.85μm ; (Table 13., Fig 1., Plate 3E). The complement included five pairs of median, four pairs of submedian and one pair of subterminal chromosomes. The Relative Chromosome Length ranged from 6.28 per cent to 12.56 per cent. The average arm ration was 1:1.28.

k. ODV-9

The karyotype of this cultivar was characterised by six pairs of median and four pairs of submedian chromosome which varied in length from 1.8μm to 4μm (Table 14, Fig 1) and the total chromatin

Table 12. Karyotype analysis in ODV-6 (2n = 20)

Chromosome number	Long arm (L) (μm)	Short arm (S) (μm)	Total length (μm)	Arm ratio S:L	Type	Relative Chromosome Length (%)
1	2.60	1.70(sc)	4.30	1:1.53	SM	13.15
2	2.60	1.60	4.20	1:1.63	SM	12.84
3	2.20	1.70	3.90	1:1.18	M	11.93
4	2.20	1.60	3.80	1:1.25	M	11.62
5	1.80	1.80	3.60	1:1.00	M	11.01
6	1.60	1.20	2.80	1:1.30	M	8.56
7	1.60	1.20	2.80	1:1.30	M	8.56
8	1.50	1.00	2.50	1:1.50	SM	7.65
9	1.30	1.10	2.40	1:1.18	M	7.34
10	1.20	1.20	2.40	1:1.00	M	7.34

M - median SM - submedian sc - Secondary constriction

Table 13. Karyotype analysis in ODV-8 (2n = 20)

Chromosome number	Long arm (L) (μm)	Short arm (S) (μm)	Total length (μm)	Arm ratio S:L	Type	Relative Chromosome Length (%)
1	2.00	2.00	4.00	1:1.00	M	12.56
2	2.60	1.40	4.00	1:1.85	ST	12.56
3	2.20	1.60	3.80	1:1.38	SM	11.93
4	2.20	1.50	3.70	1:1.47	SM	11.62
5	2.00	1.50	3.50	1:1.33	M	10.99
6	1.75	1.60	3.40	1:1.09	M	10.52
7	1.75	1.25	3.00	1:1.40	SM	9.42
8	1.50	1.10	2.60	1:1.36	SM	8.16
9	1.00	1.00	2.00	1:1.00	M	6.28
10	1.00	1.00	2.00	1:1.00	M	6.28

M - median SM - submedian ST - subterminal

Table 14. Karyotype analysis in ODV-9 (2n = 20)

Chromosome number	Long arm (L) (μm)	Short arm (S) (μm)	Total length (μm)	Arm ratio S:L	Type	Relative Chromosome Length (%)
1	2.50	1.50	4.00	1:1.67	SM	13.70
2	2.50	1.50	4.00	1:1.67	SM	13.70
3	2.00	2.00	4.00	1:1.00	M	13.70
4	2.00	1.50	3.50	1:1.23	M	11.99
5	1.50	1.50	3.00	1:1.00	M	10.27
6	1.50	1.00	2.50	1:1.50	SM	8.56
7	1.25	1.25	2.50	1:1.00	M	8.56
8	1.00	1.00	2.00	1:1.00	M	6.85
9	1.20	0.70	1.90	1:1.71	SM	6.51
10	0.90	0.90	1.80	1:1.00	M	6.16

M - median SM - submedian

length of the haploid complement was 29.2µm. The Relative Chromosome Length ranged from 6.16 per cent to 13.7 per cent. The average arm ratio was 1:1.29.

The karyotype differences are summarised in Table 15. It was seen that the total chromatin length among the cultivars ranged from 25.04µm for ODV-3 to 34.9µm for ODV-5 (Fig 2). The maximum difference of chromosome length was observed in ODV-12 (2µm to 5µm) and minimum in ODV-10 (1.8µm to 3.7µm).

B. Classification of karyotype according to Stebbin's classification

The classification of karyotype according to the degree of asymmetry was also worked out for the eleven cultivars using Stebbin's classification and is presented in Table 16. In general vetiver cultivars belonged to the symmetrical groups 1 a or 1 b. However, the slightly asymmetrical nature of karyotypes (between these two) as represented in the class 1 b was observed in ten out of the eleven cultivars. The cultivar ODV-6 belonged to 1 a group. These ten cultivars belonging to 1 b group had ratios of largest/smallest chromosomes between 2:1 and 4:1. None of the chromosomes had arm ratio more than 2:1. On the other hand ODV-6 was having largest/smallest chromosome ratio less than 2:1 and no chromosomes with arm ratio more than 2:1.

Table 15. Variation in length of chromosomes in different cultivars

Sl. No.	Cultivar	Range in chromosome length (μm)	Average chromosome length (μm)	Total chromosome length (μm)	Average arm ratio (S:L)
1	ODV-2	1.75 - 4.10	2.94	29.40	1:1.40
2	ODV-4	1.80 - 3.80	2.97	29.70	1:1.44
3	ODV-10	1.80 - 3.70	2.73	27.30	1:1.38
4	ODV-3	1.21 - 3.63	2.50	25.04	1:1.41
5	ODV-12	2.00 - 5.00	3.22	32.20	1:1.54
6	ODV-11	1.80 - 4.10	2.96	29.60	1:1.22
7	ODV-7	1.75 - 4.10	3.01	30.15	1:1.32
8	ODV-5	2.20 - 4.80	3.49	34.90	1:1.49
9	ODV-6	2.40 - 4.30	3.27	32.70	1:1.35
10	ODV-8	2.00 - 4.00	3.18	31.80	1:1.28
11	ODV-9	1.80 - 4.00	2.92	29.20	1:1.29

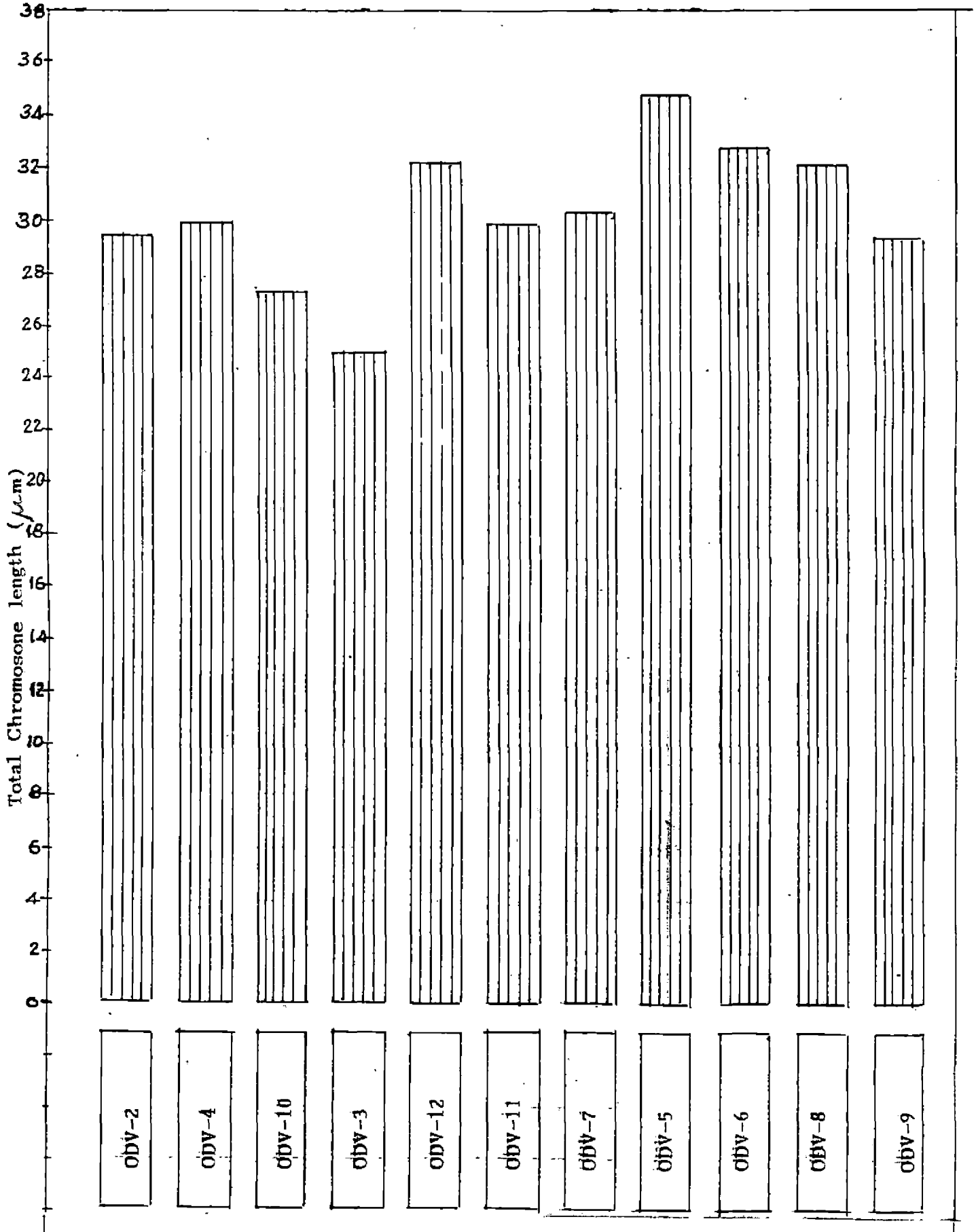


Fig. 2. Total Chromatin (Chromosome) length in different cultivars of Vetiver

Table 16. Karyotype symmetry/asymmetry in different cultivars

Sl. No.	Cultivar	Proportion of chromosomes with arm ratio more than 2:1	largest/smallest chromosome ratio	Stebbin's classification	Total form % (TF%) (Huziwara,1962)
1	ODV-2	0.00	2.34:1	1b	42.41
2	ODV-4	0.00	2.11:1	1b	41.08
3	ODV-10	0.00	2.05:1	1b	42.88
4	ODV-3	0.00	3.00:1	1b	41.17
5	ODV-12	0.00	2.50:1	1b	39.75
6	ODV-11	0.00	2.28:1	1b	44.93
7	ODV-7	0.00	2.28:1	1b	43.94
8	ODV-5	0.00	2.18:1	1b	41.26
9	ODV-6	0.00	1.91:1	1a	43.12
10	ODV-8	0.00	2.00:1	1b	43.80
11	ODV-9	0.00	2.22:1	1b	44.01

The total form percentage (TF%) of different cultivars are furnished in Table 16. The South Indian type, ODV-11 showed the highest TF% value among the cultivars, (44.93%). This was followed by ODV-8 and ODV-9 (43.8 & 44.01%). The rest of the South Indian types, viz. ODV-2, 3, 4 and 10 showed TF% values as 42.4, 41.17, 41.08 and 42.88 respectively. The North Indian types, ODV-6 and ODV-7 showed TF% values, 43.8 and 43.94, repectively. This was followed by ODV-5 (41.26%). The hybrid, ODV-12, showed the lowest TF% value (39.75%).

C. Variation in the number of median, submedian and subterminal chromosomes

The number of median, submedian and subterminal chromosomes in different cultivars are presented in Table 17. Cultivars, ODV-3,8 and 12 showed a pair of subterminal chromosomes. Cultivar, ODV-6, was having the highest number of median chromosomes (7 pairs). Cultivrs ODV-7, 8, 9 and 11 showed more median chromosomes than submedian chromosome (6, 5, 6 and 6 pairs of median chromosomes respectively). ODV-2, 4, 5 and 12 showed highest number of submedian chromosomes (7 pairs each). ODV-3 and ODV-10 showed more number of submedian chromosomes (5 and 6 pairs, respectively) than median chromosomes (4 pairs, each).

Table 17. Type of chromosomes in different cultivars of vetiver

Sl. No.	Cultivar	2n	Type of chromosome		
			Median	submedian	Subterminal
1	ODV-2	20	3	7	--
2	ODV-4	20	3	7	--
3	ODV-10	20	4	6	--
4	ODV-3	20	4	5	1
5	ODV-12	20	2	7	1
6	ODV-11	20	6	4	--
7	ODV-7	20	6	4	--
8	ODV-5	20	3	7	--
9	ODV-6	20	7	3	--
10	ODV-8	20	5	4	1
11	ODV-9	20	6	4	--

4.2.2. Meiosis

All the cultivars, both North Indian and South Indian types, flowered within one year after planting. Pollen Mother Cells were examined for different meiotic stages and abnormalities, wherever observed were recorded. Mean number of univalents, bivalents and multivalents per PMC, mean number of rod and ring bivalents per PMC,mean chiasmata per PMC and mean chiasmata per bivalent are presented in Table 18 and 19 and Fig 3. Percentage of normal cells and cells with laggards and bridges at anaphase and telophase stages were calculated in each cultivarand are presented in Table 20.

The South Indian cultivar, ODV-2 showed 8.2 bivalents per PMC having a range of 4 to 10. The mean frequency of univalents and multivalents were 1.24 (range 0 to 6) and 0.64 (range 0 to 3) per PMC, respectively. Out of the bivalents, 6.08 were rods and 2.12 were rings. This cultivar showed 11.5 chiasmata per PMC and 1.24 chiasmata per bivalent. Out of 27 PMCs observed at anaphase and telophase stages, 70.37% were normal and the rest showed bridges and laggards.

ODV-4, another South Indian cultivar, showed maximum number of univalents per PMC compared to other cultivars ie. 6.36 univalents per PMC and it ranged from 0 to 16. Mean bivalents and multivalents per PMC were 6.28 (range 2 to 10) and 0.32 (range 0 to 1) per bivalent , respectively. Out of these bivalents, 4.5 were rods and 1.7 were rings.

Table 18. Chromosome configuration at metaphase I during meiosis in different cultivars of Vetiver

Sl. No.	Cultivar	No. of univalents/P M C. Range	Mean ± SE	No. of bivalents/P M C. Range	Mean ± SE	No. of multivalents/P M C. Range	Mean ± SE
1	ODV-2	0-6	1.24 ± 0.31	4-10	8.20 ± 0.31	0-3	0.64 ± 0.16
2	ODV-4	0-16	6.36 ± 0.89	2-10	6.28 ± 0.45	0-1	0.32 ± 0.09
3	ODV-10	0-8	3.25 ± 0.86	4-9	6.38 ± 0.50	0-2	1.00 ± 0.18
4	ODV-3	0-6	2.06 ± 0.43	6-10	8.13 ± 0.18	0-2	0.38 ± 0.09
5	ODV-12	0-6	1.48 ± 0.40	3-10	8.20 ± 0.38	0-3	0.52 ± 0.15
6	ODV-11	0-10	2.88 ± 0.71	5-9	7.38 ± 0.29	0-1	0.50 ± 0.12
7	ODV-7	0-6	0.78 ± 0.22	4-10	8.72 ± 0.27	0-3	0.47 ± 0.13
8	ODV-5	0-4	0.81 ± 0.23	5-10	7.96 ± 0.28	0-3	0.85 ± 0.15
9	ODV-6	0-3	0.60 ± 0.10	5-10	8.16 ± 0.33	1-2	0.76 ± 0.15
10	ODV-8	0-6	1.23 ± 0.30	2-10	8.04 ± 0.41	0-3	0.69 ± 0.16
11	ODV-9	0-8	2.56 ± 0.54	3-10	7.40 ± 0.33	0-3	0.68 ± 0.16

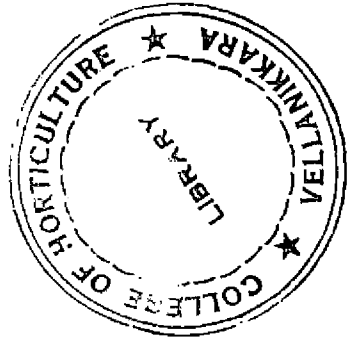


Table 19. Configuration of bivalents during diakinesis of meiosis and chiasma frequency in different cultivars of Vetiver

Sl. No.	Cultivars	No. of Bivalents/P.M.C.				Total chiasmata/P.M.C.	Chiasmata/bivalent
		Rods		Rings			
		Range	Mean ± SE	Range	Mean ± SE	Mean ± SE	Mean ± SE
1	ODV-2	3-9	6.08 ± 0.31	0-5	2.12 ± 0.28	11.5 ± 0.32	1.24 ± 0.03
2	ODV-4	2-7	4.50 ± 0.29	0-6	1.73 ± 0.37	8.5 ± 0.83	1.21 ± 0.04
3	ODV-10	4-6	5.00 ± 0.25	0-5	1.63 ± 0.50	10.25 ± 0.65	1.22 ± 0.05
4	ODV-3	1-8	4.75 ± 0.42	0-8	3.44 ± 0.41	12.38 ± 0.54	1.39 ± 0.05
5	ODV-12	2-8	4.88 ± 0.30	0-5	3.32 ± 0.33	12.56 ± 0.44	1.39 ± 0.04
6	ODV-11	3-7	4.69 ± 0.36	1-6	2.56 ± 0.32	10.94 ± 0.62	1.35 ± 0.04
7	ODV-7	1-9	5.69 ± 0.33	0-8	3.03 ± 0.33	12.79 ± 0.49	1.42 ± 0.09
8	ODV-5	1-8	5.00 ± 0.39	0-8	3.80 ± 0.41	12.69 ± 0.58	1.37 ± 0.05
9	ODV-6	3-10	5.52 ± 0.42	0-7	2.56 ± 0.35	12.16 ± 0.41	1.32 ± 0.04
10	ODV-8	1-9	4.97 ± 0.44	1-9	3.27 ± 0.47	12.58 ± 0.69	1.39 ± 0.05
11	ODV-9	3-7	4.80 ± 0.27	0-5	2.56 ± 0.32	11.28 ± 0.46	1.32 ± 0.04

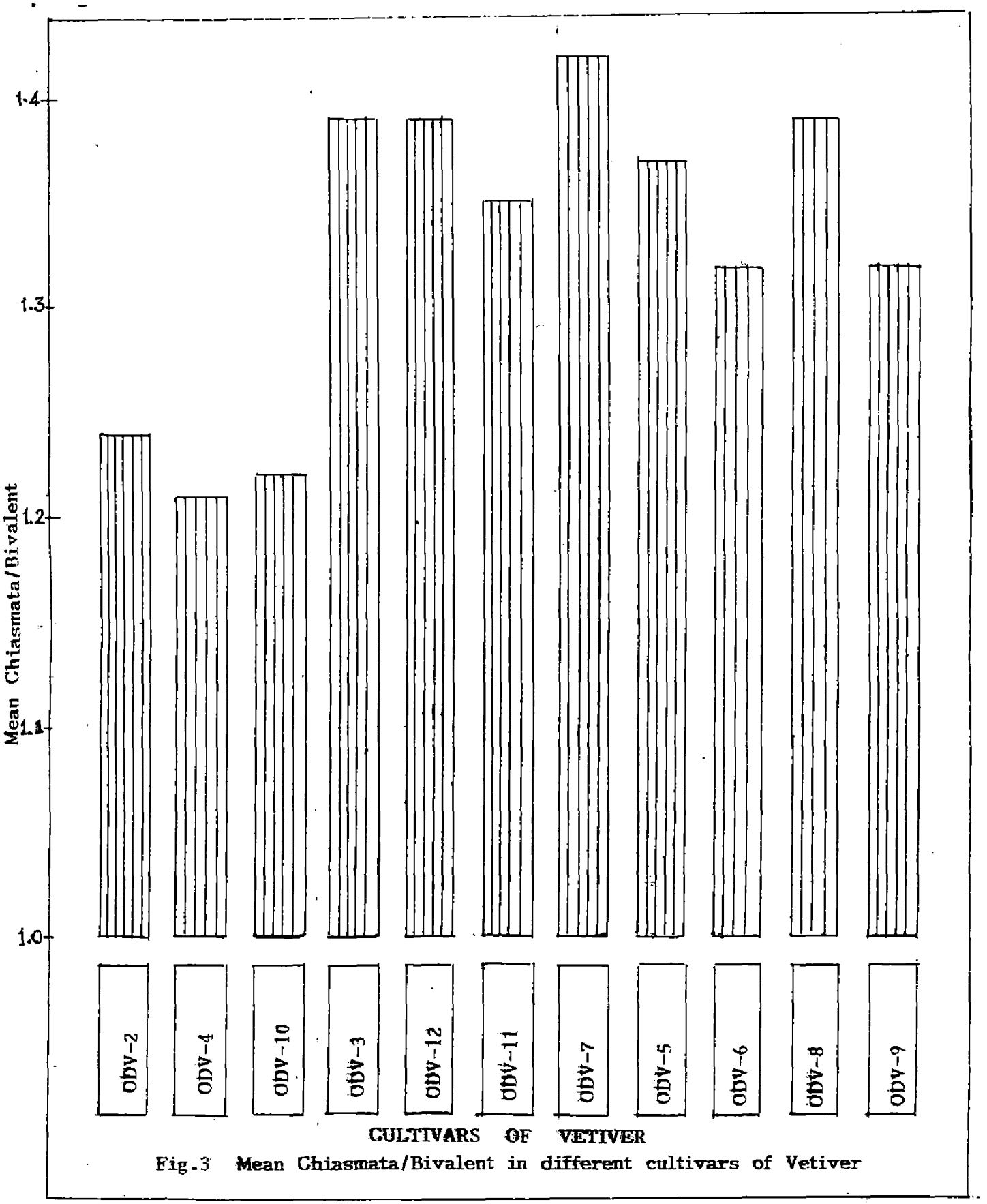


Fig.3 Mean Chiasmata/Bivalent in different cultivars of Vetiver

Table 20. Number of cells showing abnormalities at anaphase I and telophase I in different cultivars

Sl. No.	Cultivar	Total No. of PMCs	Normal Cells		Abnormal cells													
					1		2		3		4		1		2		3	
					Cells with laggards								Cells with bridges					
			No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
1	ODV-2	27	19	70.37	2	7.40	3	11.00	-	-	-	-	1	3.70	-	-	-	-
2	ODV-4	36	15	41.67	7	19.40	4	11.00	-	-	-	-	8	22.00	2	5.50	-	-
3	ODV-10	10	8	80.00	1	10.00	1	10.00	-	-	-	-	-	-	-	-	-	-
4	ODV-3	13	10	76.92	2	15.38	-	-	-	-	-	-	1	7.60	-	-	-	-
5	ODV-12	18	15	83.33	2	11.00	1	5.50	-	-	-	-	-	-	-	-	-	-
6	ODV-11	17	13	76.47	2	11.70	2	11.70	-	-	-	-	-	-	-	-	-	-
7	ODV-7	10	7	70.00	1	10.00	-	-	-	-	-	-	1	10.00	-	-	1	10.0
8	ODV-5	46	36	78.26	4	8.60	1	21.70	-	-	3	6.50	2	4.30	-	-	-	-
9	ODV-6	31	26	83.87	3	9.68	1	3.23	-	-	-	-	1	3.23	-	-	-	-
10	ODV-8	31	20	64.52	5	16.12	2	6.45	-	-	-	-	4	12.90	-	-	-	-
11	ODV-9	28	23	82.14	3	10.70	2	7.10	-	-	-	-	-	-	-	-	-	-

Chiasma frequency was the lowest ie. 8.5 chiasmata per PMC and 1.21 chiasmata per bivalent. Out of 36 PMCs examined at anaphase and telophase stages, only 41.67 per cent showed normal segregation. About 30 per cent of the cells showed laggards, (Plate 5E, 5F) and another 27 per cent showed bridges. (Pates 6A,6B).

In the case of ODV-10, mean number of univalents, bivalents and multivalents per PMC were 3.25 (range 0 to 8), 6.38 (range 4 to 9) and 1.00 (range 0 to 2) respectively. Five of the bivalents were rods and the rest, rings. Total chiasmata per bivalent was 1.22 and that per PMC was 10.25. About 80 per cent of PMCs observed at anaphase and telophase were normal and the rest showed laggards. No bridges were observed.

ODV-3, a South Indian cultivar, showed 8.13 bivalents (range 6 to 10), 2.06 univalents (range 0 to 6) and 0.38 multivalents (range 0 to 2) per PMC. Number of rod and ring bivalents were 4.75 and 3.44 per PMC respectively. On an average, 12.38 chiasmata per PMC and 1.4 chiasmata per bivalent were recorded. Percentage of normal cells at anaphase and telophase stages was 77 (Plate 4F). Out of the remaining, 11.38 per cent showed laggards and another 7.6 per cent, bridges.

In the case of ODV-12, frequency of bivalents was 8.2 per PMC (range 3 to 10) and that of univalents and multivalents was 1.48 (range 0 to 6) and 0.52 (range 0 to 3), respectively. There were 4.88 rod bivalents and 3.3 ring bivalents per PMC. Total chiasmata per PMC was 12.56

Plate 4. Meiotic division in some cultivars of Vetiveria zizanioides : Normal stages observed.

A : ODV - 6 : Diakinesis showing 10 II (X1000)

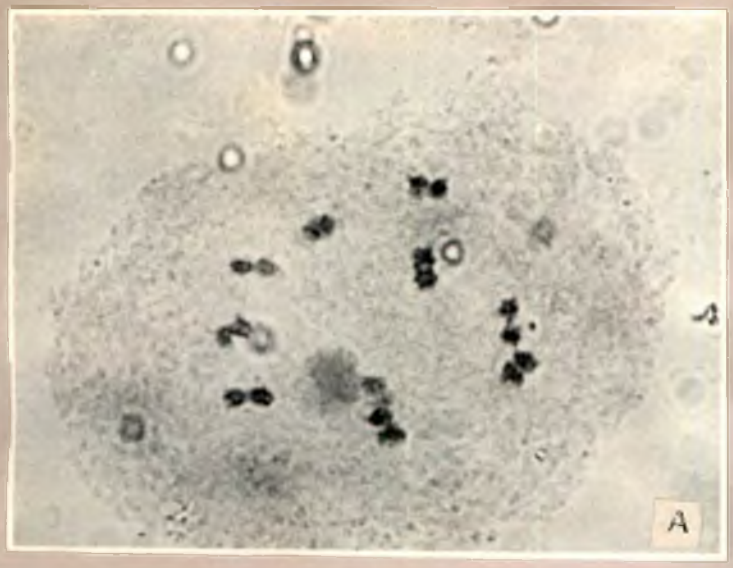
B : ODV - 8 : Diakinesis showing 10 II (X1000)

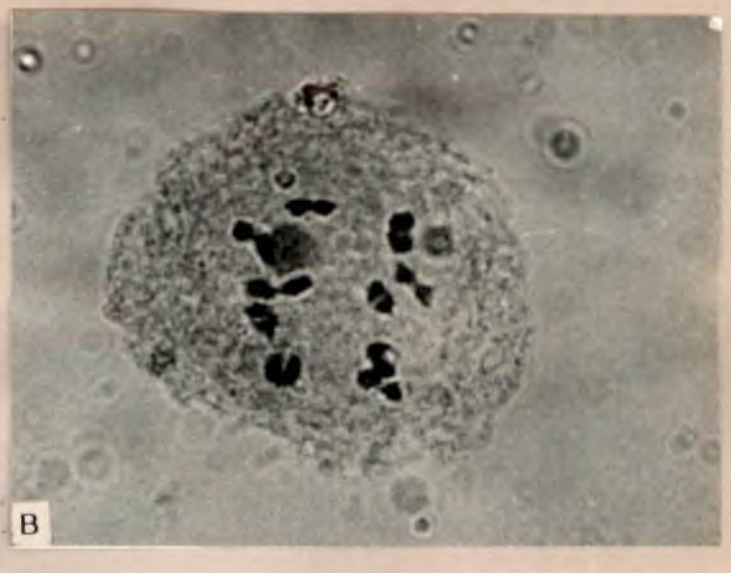
C : ODV - 7 : Metaphase I showing 10 II (X1000)

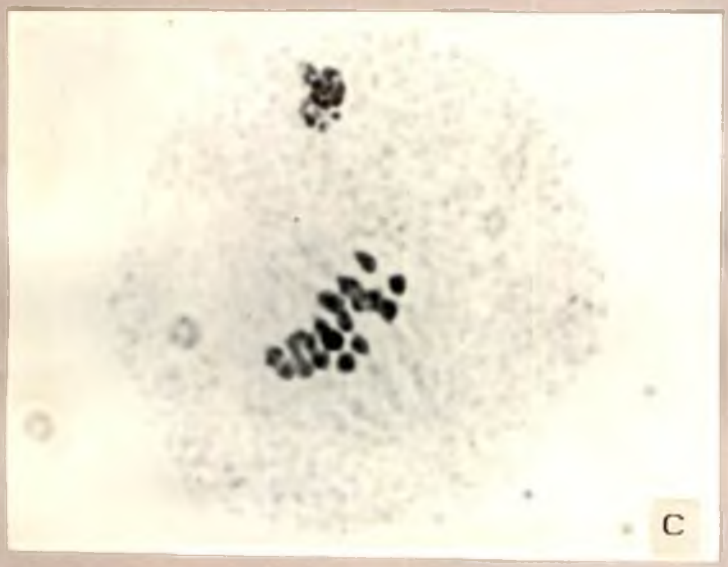
D : ODV - 6 : Anaphase I showing equal seperation (X1000)

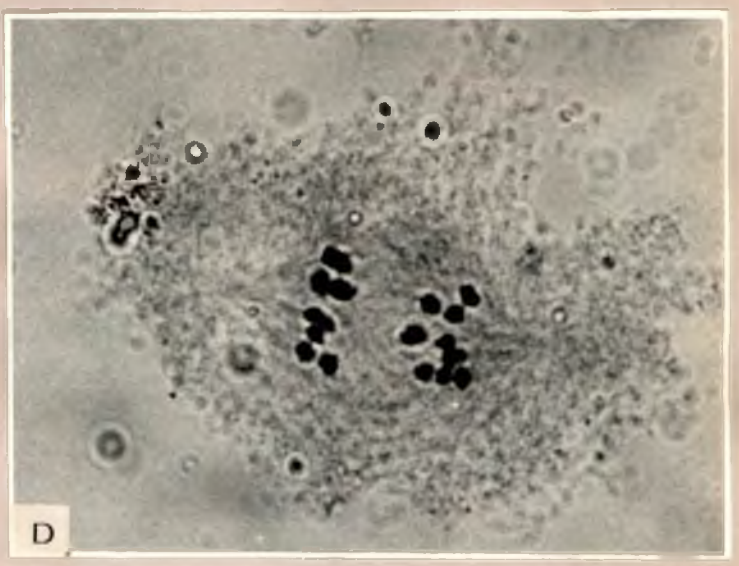
E : ODV - 12 : Late anaphase I (X1000)

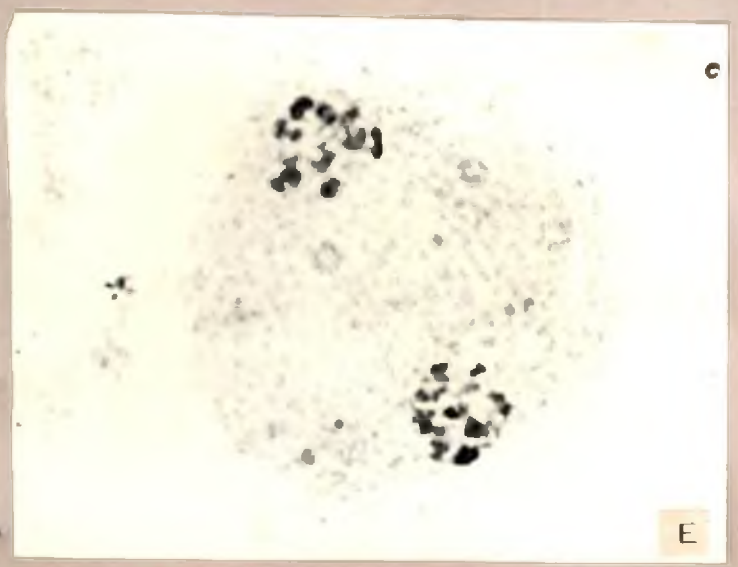
F : ODV - 3 : Late anaphase I (X1000)


A


B


C


D


E

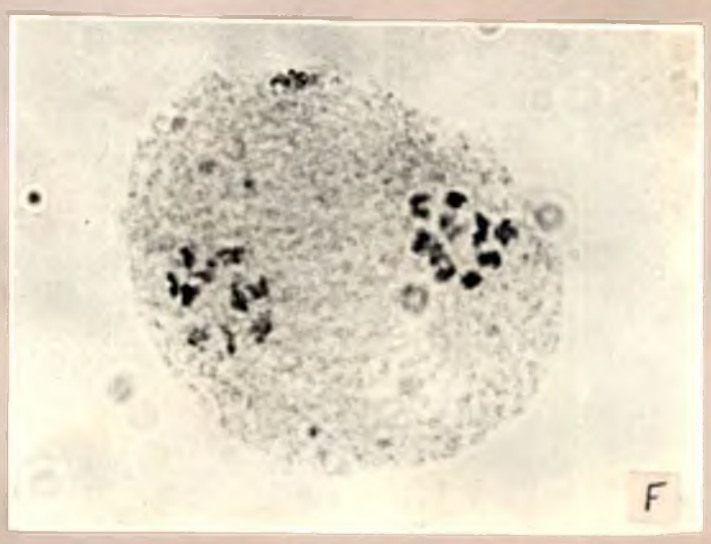

F

Plate 5 : Meiotic division in some cultivars of Vetiveria zizanioides : Abnormal stages observed.

A : ODV - 7 : Diakinesis showing 2 IV + 5 II + 2 I (×1000)

B : ODV - 8 : Diakinesis showing 2 IV + 6 II (×1000)

C : ODV - 7 : Anaphase I showing bridges (×1000)

D : ODV - 7 : Anaphase I showing 2 laggards (×1000)

E : ODV - 4 : Anaphase I showing 1 laggard (×1000)

F : ODV - 4 : Late anaphase I showing 2 laggards (×1000)

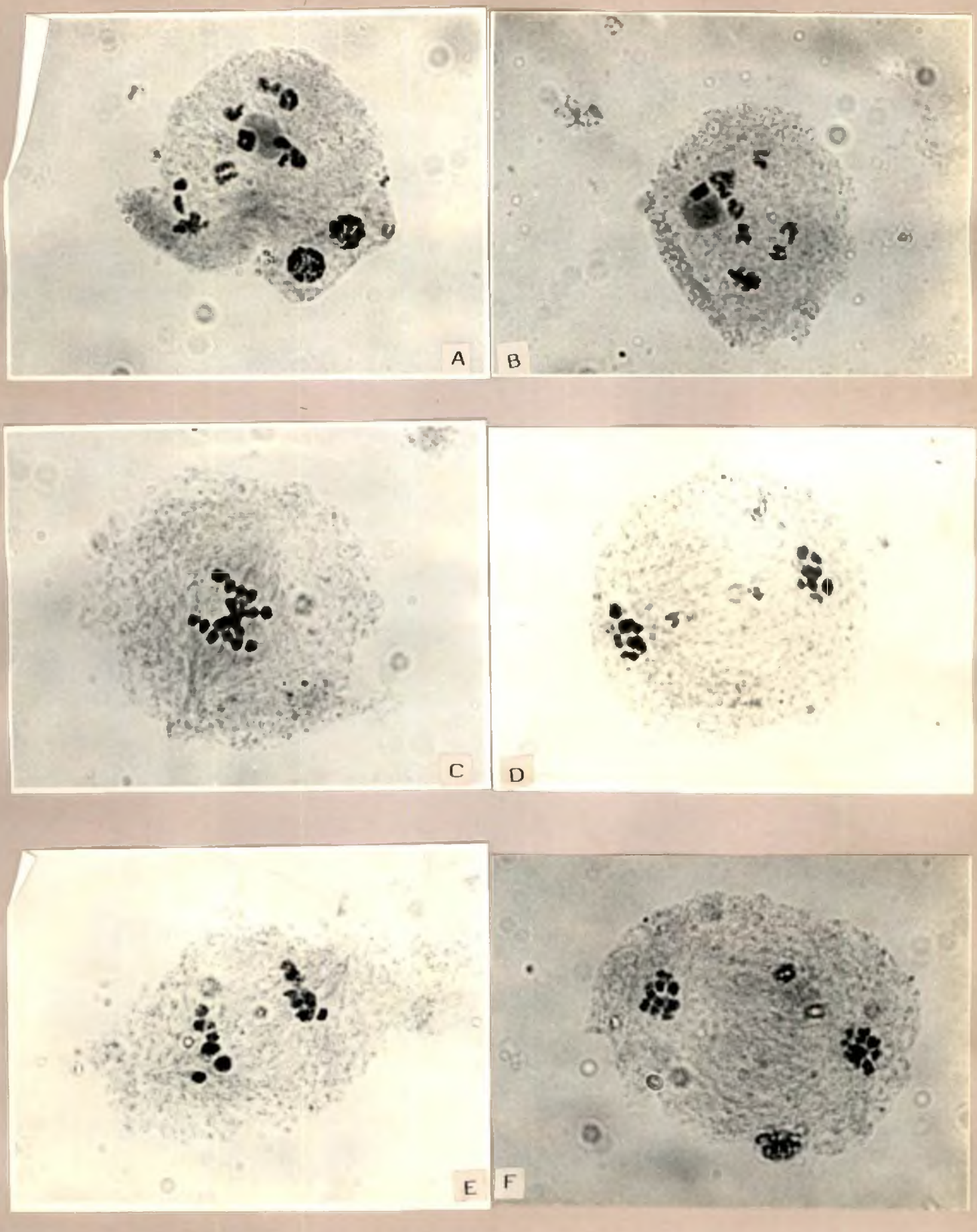
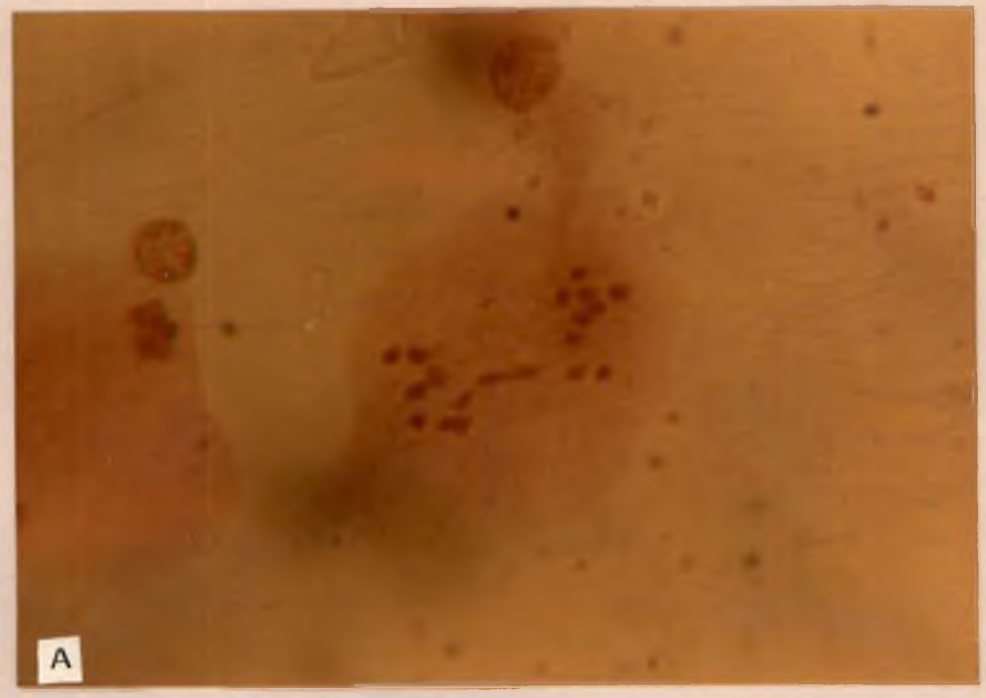
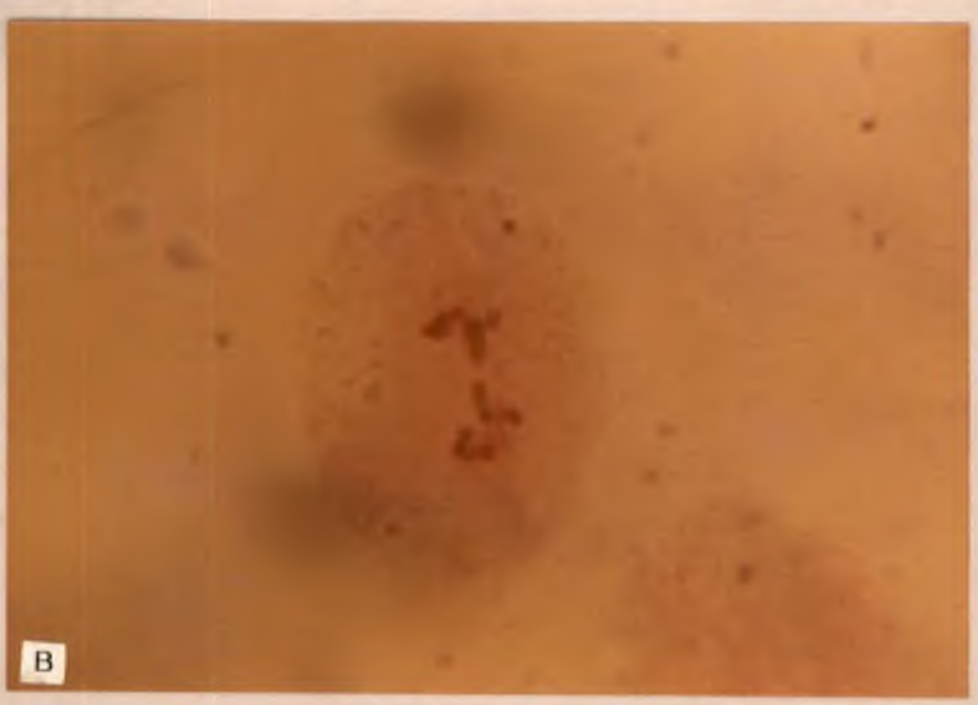

A
B
C
D
E
F

Plate 6. Abnormal meiotic division in ODV-4

A : Anaphase I with one lagging bivalent (×1000)

B : Anaphase I with bridge (just seperated)(×1000)


A


B

and that per bivalent was 6.39. It was found that 77.78 per cent of the cells examined showed normal segregation during anaphase-I (Plate4E) and the rest showed laggards. No bridges were observed.

Cultivar ODV-11 showed 7.38 bivalents (range 5 to 9), 2.88 univalents (range 0 to 10) and 0.5 multivalent (range 0 to 1) per PMC. The bivalents included rings (2.56/PMC) and rods (4.69 per PMC). The mean chiasma frequencies were 10.94 per PMC and 1.35 per bivalent. At anaphase I, bridges were not observed, but 23.4 per cent of the cells showed laggards. It was found that 76.47 per cent of the PMCs showed normal segregation.

North Indian cultivar, ODV-7, showed fairly high frequency of bivalents (8.72 /PMC and a range of 4 to 10) compared to other cultivars (Plate4C). Mean number of univalents and multivalents per PMC were 0.78 (Plate 5A) (range 0 to 6) and 0.47 (range 0 to 3) respectively. Mean rod bivalents per PMC was 3.03. Chiasma frequency was the highest in this cultivar (12.79 chiasmata per PMC and 1.42 chiasmata per bivalent). Seventy per cent of cells examined at anaphase and telophase stages showed normal segregation. Ten per cent showed laggards (Plate 5D) and 20 per cent, bridges (Plate 5C)

In another North Indian cultivar, ODV-5, a bivalent frequency of 7.96 per PMC (range 5 to 10) was noticed. Mean univalents per PMC was 0.81 (range 0 to 4) and mean multivalents per PMC was 0.85 (range

0 to 3). Frequency of rod bivalents was 5 per PMC and that of rings was 3.8 per PMC. Chiasma frequency was 12.69 per PMC and 1.37 per bivalent. Out of 46 cells examined for abnormalities at anaphase and telophase, 78.26 per cent of the cells showed normal segregation and 17.27 per cent of the PMCs showed laggards and 4.3% showed bridges.

ODV-6, a cultivar from North India; showed fairly high number of bivalents (Plate 4A) (8.16 per PMC and a range of 5 to 10), followed by multivalents (0.76 per cell and a range of 0 to 2) and univalents (0.6 per cell and a range of 0 to 3). Frequency of rod bivalents was higher (5.52 per cell) than that of rings (2.56 per cell). Mean chiasmata per PMC was 12.16 and that per bivalent was 1.32. Normal anaphase I was noticed in 83.87 per cent of the PMCs (Plate 4D) where as 12.91 per cent showed laggards and 3.23 per cent showed bridges.

In the case of ODV-8, mean bivalents per PMC (Plate 4B) 8.04 (range 2 to 10) and univalents and multivalents per PMC were 1.23 (range 0 to 16) and 0.69 (range 0 to 3), respectively. Frequency of rod bivalents was 4.97 per cell and that of rings 3.27 per cell. Chiasma frequency was found to be 12.58 per PMC and 1.39 per bivalent. Bridges and laggards were recorded in 12.9 per cent and 22.57 per cent of the PMCs, respectively.

ODV-9, a South Indian cultivar, showed 7.4 bivalents (range 3 to10), 2.56 univalents (range 0 to 8) and 0.68 multivalents (range 0 to 3) per PMC.

Out of the bivalents, frequency of rod bivalents was 4.8 per PMC and that of rings was 2.56 per PMC. Chiasma frequency was 11.28 per PMC and 1.32 per bivalent. Out of 28 cells examined, 82.14 per cent showed normal segregation at anaphase and telophase stages and the rest showed laggards. No bridges were observed.

4.2.3. Pollen fertility and size

The morphological features of pollen grains of Vetiver cultivars are presented in Table 21 and Plates 7A and 7B. Polen fertility among the cultivars as assessed by stainability in acetocarmine ranged from 27.54% in ODV-4 to 85.63 per cent in ODV-9 (Table 21, Plate 7A, 7B). The percentage of fertile pollen in ODV-6 was 84.85 which was followed by ODV-12 and ODV-11 (82.55 and 82.18 per cent respectively). Pollen fertility of ODV-8, 10, 7, 3, 2 and 5 were 80.82, 79.31, 78.14, 76.06, 75.67 and 72.21 per cent respectively. ODV-4 showed the lowest pollen fertility ie. 27.54 per cent. The pollen size also varied widely in the case of ODV-4 ie. 28.8μm to 48μm (Plate 7A). The other cultivars showed a range in pollen size which were almost similar. The mean diameter of pollen grains ranged from 28.8μm in ODV-5 to 32.4μm in ODV-2.

4.2.4. Seed set pattern

Seeds were collected from open pollinated as well as selfed panicles in all the cultivars selected for the present study. The number of well filled seeds obtained upon open pollination as well as selfing were counted

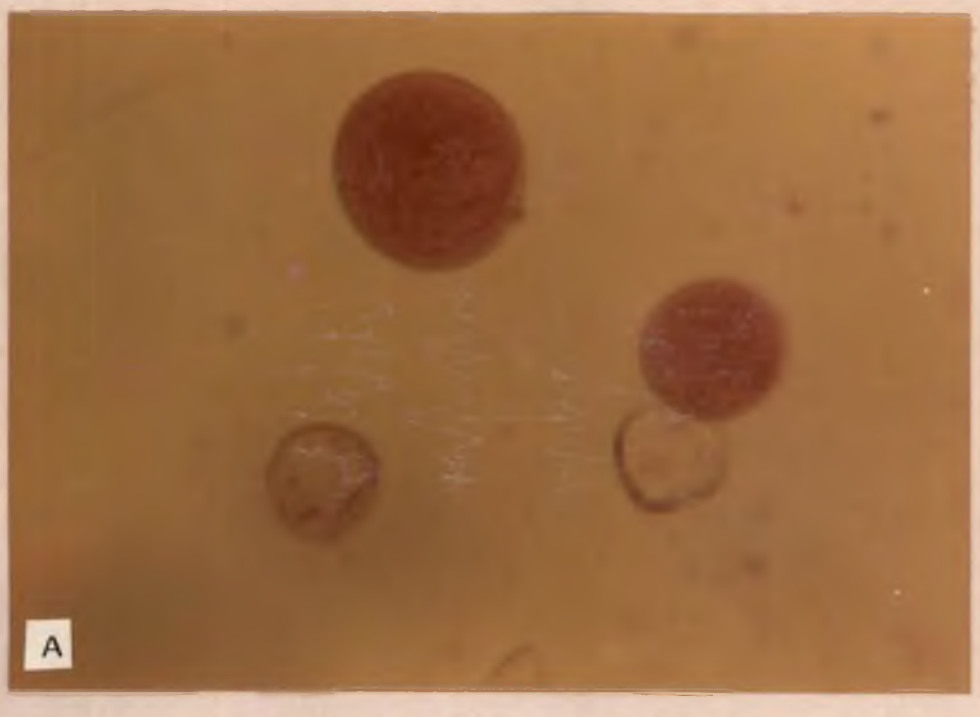
Table 21. Pollen fertility and size of pollen in different cultivars

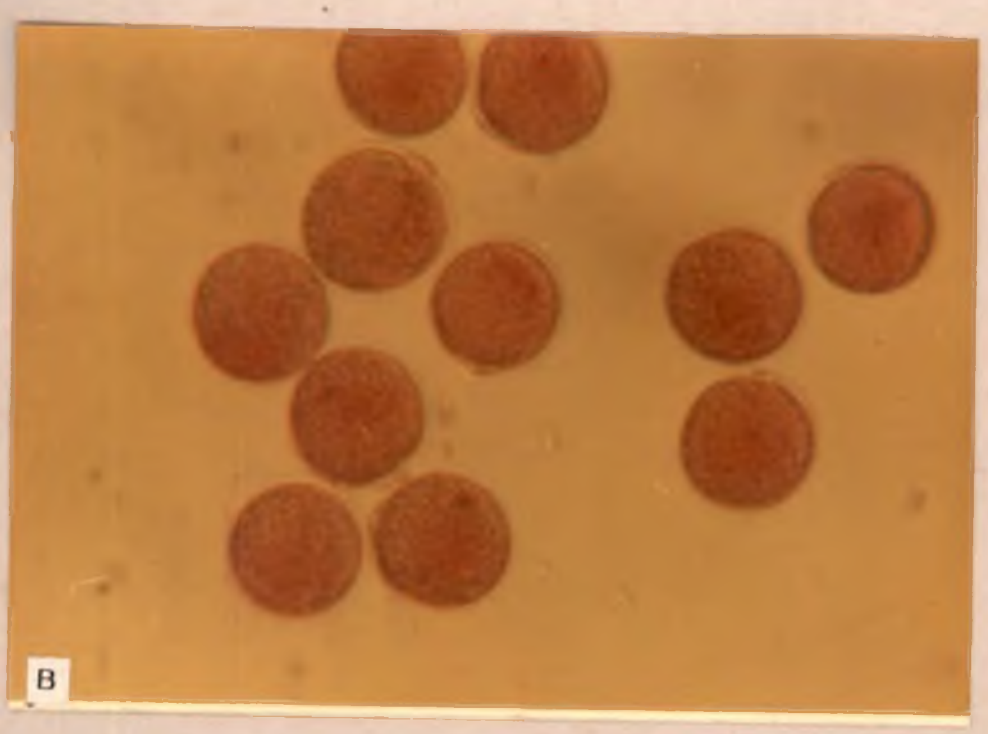
Sl. No.	Cultivar	Pollen fertility %	Pollen diameter	
			Range (μm)	Mean (μm)
1	ODV-2	75.67	28.80 to 36.00	32.40
2	ODV-4	27.54	28.80 to 48.00	32.20
3	ODV-10	79.31	28.80 to 33.60	31.20
4	ODV-3	76.06	28.80 to 36.00	31.20
5	ODV-12	82.55	28.80 to 30.40	30.00
6	ODV-11	82.18	28.80 to 33.60	28.97
7	ODV-7	78.14	24.00 to 33.60	28.99
8	ODV-5	72.21	24.00 to 33.60	28.80
9	ODV-6	84.85	24.00 to 36.00	30.00
10	ODV-8	80.82	28.80 to 33.10	31.20
11	ODV-9	85.63	28.80 to 33.60	29.52

Plate 7. Pollen size and Pollen fertility

A : ODV-4: High size variation and low pollen fertility (×400)

B : ODV-9: Low size variation and high pollen fertility (×400)


A


B

and the mean values are presented in Table 22.

Under open pollinated condition all cultivars set seed. The number of panicles from which seeds were collected and the average number of seeds per panicle are given in Table 22. Cultivar ODV-10 (South Indian) produced lowest number of seeds (12 per panicle) ODV-4 (South Indian) also produced less seeds ie. 23 per panicle. Cultivrs ODV-5 (North Indian), 6 (North Indian) and 12 (Hybrid) produced fairly high amount of seed compared to ODV-10 and ODV-4 ie. 35 in ODV-5 and 12, 37 in ODV-6. ODV-3 (South Indian) produced 45 seeds per panicle. Cultivars ODV-2, 8 and 11 (all South Indian) produced seeds at the rate of 60, 56 and 57 per panicle, respectively. ODV-9 produced 83 seeds per panicle which is very high compared to other South Indian cultivrs. North Indian cultivars ODV-7 produced the largest amount of seeds upon open pollination ie. 98 seeds/panicle.

Seed set under selfed condition was very low in most of the cultivrs. Cultivars ODV-2, 3, 4 and 8 which were all South Indian produced no seed at all upon selfing. A few seeds were obtained from ODV-5 (North Indian), ODV-6 (North Indian), ODV-10 (South Indian) and ODV-12 (hybrid) ie. 2 from ODV-5, 3 each from ODV-6, 10 and 12. ODV-9 (South Indian) produced 5 seeds per panicle and ODV-7 (North Indian) produced 10 seeds per panicle upon selfing.

Crossing was attempted between ODV-4, cultivar having low pollen fertility of 27.54 per cent and ODV-6, cultivar having high pollen fertility

Table 22. Seed set upon open pollination as well as selfing in different cultivars

Sl.No.	Cultivar	Open pollination		Selfing	
		No. of panicle	No. of seeds/panicle	No. of panicles	No. of seeds/panicle
1	ODV-2	4	60	3	0
2	ODV-4	3	23	4	0
3	ODV-10	4	12	2	2
4	ODV-3	4	45	4	0
5	ODV-12	4	35	4	3
6	ODV-11	5	57	3	0
7	ODV-7	5	98	3	10
8	ODV-5	6	35	4	3
9	ODV-6	4	37	4	3
10	ODV-8	5	56	3	0
11	ODV-9	4	83	4	5

Table 23. Seed germination in different cultivars of vetiver

Cultivars	No. of seeds germinated	No. of seeds not germinated
ODV-4	3	97
ODV-3	4	96
ODV-12	4	96
ODV-8	6	94
ODV-6	9	91
ODV-10	11	89
ODV-9	11	89
ODV-5	12	88
ODV-7	14	86
ODV-11	14	86
ODV-2	15	85

of 84.85 per cent. The attempt failed in many cases due to heavy wind. However, in one cross, the crossed panicle of ODV-4 with ODV-6 produced 58 seeds. The crossed panicle of ODV-6 with ODV-4 produced 10 seeds. Crossing could not be attempted to make with the remaining parents.

Germination test was also conducted by taking 100 seeds each from all cultivars. Number of seeds germinated in each case are presented in Table 23. The cultivars were arranged in the increasing order of seed germination. The values were subjected to repeated Chi-Square analysis. The analysis revealed that the first eight cultivars were coming under one homogenous group. (Significant at 5% level). The 2nd to the last cultivar were coming under another homogenous grop. Hence it can be inferred that seed germination in the cultivar ODV-4 was significantly low compared to the cultivars ODV-7, ODV-11 and ODV-2.

Discussion

5. DISCUSSION

Morphology, karyomorphology, meiosis, pollen sterility and seed set in the selected genotypes of Vetiveria zizanioides have been studied and the results are discussed in this chapter.

5.1. Morphological studies

The members of the species Vetiveria zizanioides have been classified into two groups viz. North Indian type and South Indian type, based on their geographical distribution by a number of workers (Sadgopal, 1960; Ramanujam and Kumar, 1964; Pillay, 1967; Virmani and Dutta,1975; Morris, 1984 and Lavania, 1985). In thepresent study, an attempt was made to understand the morphological characters and identify any typical features which could be of significance in differentiating these two types.

Morphological characters of seven South Indian cultivars, three North Indian cultivars and one hybrid were recorded. On comparison, although, no clear cut morphological character could be projected as one with which North Indian and South Indian types could be identified, variation with respect to number of inflorescence/plant, days for appearance of flowers and root yield per plant, was observed.

All North Indian types studied flowered earlier, produced more inflorescences per plant and yielded less roots than South Indian types. But, some of the South Indian types (two out of seven cultivars - viz.

ODV-8 and ODV-9) showed these characters on par with the North Indian types. Similar type of differences in flowering between North Indian and South Indian types have been reported by Ramanujam and Kumar(1964) and later by Pillay (1967). The present observations are in agreement with the findings of the above worker. Thus a preliminary conclusion may be drawn from these observations that the profuse flowering habit could be used as a tool in identifying North Indian types.

5.2. Karyomorphology

For the present study of karyomorphology of Vetiver genotypes, 11 cultivars, consisting of seven South Indian, three North Indian and one hybrid, were included. Somatic chromosome number, size of individual chromosome and types of chromosomes, whether median, submedian or subterminal, based on arm ratios were determined. The somatic chromosome number in all the cultivars was found to be the same ie. 2n = 20. Hence numerical variation in the evolution of different cultivars of Vetiver would not have occurred. This finding is in agreement with the reports of earlier workers who counted chromosomes from pollen mother cells (Janaki Ammal, 1945, Celarier, 1959, Mehra et al., 1962, Ramanujam and Kumar, 1963 and Larsen, 1963) and also with that of Lavania (1985) who made studies on mitotic chromosomes of Vetiver for the first time. The results presently obtained indicated that the ecotypes grown in different geographical areas possessed no variation in somatic chromosome number.

When the total haploid chromatin content was taken, the cultivrs showed much difference. The total chromatin length was highest in ODV-5, a North Indian cultivar and the lowest in ODV-3, a South Indian cultivar. It was found that, in general, North Indian types possessed higher total chromatin content than South Indian types. Stebbins (1950, 1974) held the view that these differences would have occurred through structural alterations of chromosomes.

Based on arm ratios, the individual chromosomes of all the cultivars were classified as either median, submedian or subterminal types. The North Indian types, ODV-6 and ODV-7, were having the highest number of median chromosomes. One South Indian type and the hybrid showed one pair of subterminal chromosomes each. All the South Indian types, except ODV-9 and ODV-11 showed more submedian chromosomes. This observation was found to be in agreement with the findings of Lavania (1985), according to whom these karyotypic changes would have occurred through gross chromosomal mutations such as translocations inversions and deletions. Such changes are quite possible in vetiver, which may possibly account for variation in genic expression of oil content and composition. At this stage, it would be immature to classify one of the two categories as the evolved type and the other as the primitive one, based on chromosome architecture alone. But, in general, it can be stated that the species Vetiveria zizanioides as a whole, could be considered as one to belong to the primitive species of angiosperms.

This inference was arrived at based on the classification of V. zizaniodes as one with a symmetrical karyotype (Stebbins, 1958).

The comparison of TF percentage data obtained from all the cultivars did not indicate much variation. This also strengthened the view that the karyotypes were nearly symmetrical in all the cases. Lavania (1985) reported the same with respect to the karyotypes of cultivars of Vetiver. However, variations were noticable with respect to size of chromosomes. In general, chromosomes of South Indian type were shorter and had narrow range in length. This finding also is in good agreement with the reports of Lavania (1985).

It is known that North Indian and South Indian types of Vetiver differ greatly with respect to oil content and composition. Karyological features presently observed also exhibited considerable variation in the different Vetiver types. The probability that the structural alterations of chromosomes might affect the threshold of genes in the altered types has been pointed out by Lavania (1985). Such an alteration in gene dosage and location might possibly account for variation in genic expression of oil content and composition. Hence, it is felt that further detailed studies incorporating more North Indian and South Indian types are necessary to critically examine whether the karyological features are having any direct bearing on oil content and composition.

5.3. Meiotic studies

The analysis of microsporogenesis was carried out in all the 11 cultivars of Vetiver. The cultivars were compared at metaphase I stage for the mean number of univalents, bivalents and multivalents per PMC.

It was noted that all the cultivars produced multivalents and univalents in addition to bivalents. The North Indian cultivars showed high frequency of bivalents as well as multivalents, but very few univalents. One cultivar, which was South Indian (ODV-4) showed the highest mean frequency of univalents. The presence of univalents at metaphase I has been variously attributed to failure of chromosomes to pair at zygotene or early terminalisation of chiasmata due to small size of chromosomes (Ratnambal, 1979). The finding that the presence of univalents and multivalents in addition to bivalents in Vetiver cultivars is in agreement with the findings of Ramanujam and Kumar (1963) who reported high frequency of univalents and some multivalents in the clones studied. But Celarier (1959) after studying the microsporogenesis of Vetiveria zizanioides made a conclusion that meiosis was normal in all the accessions studied and ten bivalents were usually observed at diakinesis and metaphase I stages. Groupings of four chromosomes in some accessions were also reported.

In this study the chiasma frequency per PMC of Vetiver cultivars was determined to be ranging from 8.5 to 12.79. Chiasma frequency

increased as the mean frequency of bivalents per PMC increased. The cultivar that showed highest frequency of univalents per PMC and lowest bivalents per PMC, was found to have the lowest chiasma frequency per PMC.

Abnormalities of varying degrees were also observed as bridges and laggards at anaphase I stage of meiosis in all the 11 cultivars. The cultivar, ODV-4, showed the highest percentage of PMCs with abnormal anaphase I and telophase I stages. This cultivar also showed the highest frequency of univalents per PMC. These might be the reason for the production of sterile pollengrains by the cultivar. The cultivars having low percentage of PMCs with meiotic abnormalities, produced only less number of sterile pollengrains. There seems to exist a direct relationship between meiotic abnormalities and pollen sterility in Vetiver too. This finding is in agreement with the findings of Ramanujam and Kumar (1963), that univalent formation at diakinesis and metaphase and lagging at anaphase, resulting in the elimination of chromatin material, was closely correlated with the observed sterility. One of the reasons for the formation of sterile pollengrain might be presence of laggards and bridges which as a result of unequal seperation might give rise to microspores with deficient or duplicate chromosome segment. The situation cited above may be true in the case of vetiver also. This is indicated by the presence of pollengrains with different sizes in the cultivar that showed highest percentage of abnormalities.

5.4. Pollen studies

In the present investigation, pollen size and pollen sterility of all the cultivars were studied. Cultivr, ODV-4, a South Indian type, showed lowest percentage of pollen fertility ie. 27.54. This cultivar also showed widest range of pollen diameter from 28.8 µm to 48µm. Cytological observations of the meiotic irregularities of this cultivar revealed a high proportion of univalents compared to bivalents and multivalents. These univalents might have lagged behind during anaphase seperation and remained as laggards. There would have been an unequal distribution of chromosomes, resulting in variation in size of pollengrains and also high pollen sterility (Jain, 1959). Other cultivars showed fairly high percentage of pollen fertility. Meiotic irregularities were comparatively less in the PMCs of these cultivars. The range in diameter of pollengrains was not as wide as that of ODV-4.

In the present study, the range in pollen sterility was from 14.37 per cent to 72.46 per cent. Ramanujam and Kumar (1963) reported pollen sterility ranging from 2 to 100 per cent in vertiver clones. The presence of univalents at diakinesis and metaphase I and lagging at anaphase were considered as reasons for the observed pollen sterility. The finding at present was adequately supported by the findings of Ramanujam and Kumar (1963). A direct relationship between cytological irregularities during meiosis and pollen sterility was also observed in Andropogon distachys by Celarier (1956).

The vegetative propagation by slips can be also one of the reasons for high pollen sterility in certain cultivrs, since the sexual system is not of much significance in propagation. It can be concluded that sterility is present in some vetiver cultivars and this may be the reason for the failure of seed production in them grown in different regions.

5.5. Seed set pattern

A few workers, based on seed set studies, held the view that North Indian types produced seeds while South Indian types did not. On analysis of seed set pattern in Vetiver, Ramanujam and Kumar (1963) found that some of the South Indian clones never produced seeds in the experimental cultivation. This observation was further supported by Virmani and Dutta (1975) and Akhila (1981) and considered North Indian types to be the seeding type, while South Indian type, nonseeding. In this investigation seed set pattern was analysed with a view to assess the acceptability of this concept as a general rule.

5.5.1. Open pollinated condition

The data obtained under open pollinated cndition revealed that all cultivars, irrespective of their type, produced seeds, though the number of seeds per panicle varied among them. The cultivar that produced maximum number of seeds was a North Indian type. At the same time there were many South Indian cultivars which produced fairly good quantity of seeds, though some yielded only less seeds. These

observations are in agreement with the report of Pillay (1967) that South Indian types could also produce seeds. From the present observations, it could be inferred that the practice of classifying North Indian types as seeding type and South Indian type as non seeding type is not exactly correct.

5.5.2. Selfpollinated condition

It was noticed that seed set was very low in most of the cultivars upon selfing and some cultivars produced no seed at all. While selfing in both North Indian and South Indian geographical races, there was a drastic reduction in the quantity of seeds produced. Sethi(1980) found that seedset was absent in selfed inflorescences or isolated plants of Vetiver, and hence considered it to be a cross pollinated crop. Pillay(1967) also noticed absence of seed set under selfed condition. Further studies on pollen germination and pollen tube growth by Pillay (1967) revealed that germination and growth of self pollen were some how inhibited and the pollen tube might not reach the ovule to effect fertilization. The results of the present study strongly supported the views of Pillay (1967). There must have been some incomapatibility mechanism, which is strongly exhibited by the South Indian clones than North Indian clones as seed set was completely absent in some of the South Indian clones. Further studies are very much needed to make reliable conclusions in this aspect.

5.5.3. Crossing

Crossing was undertaken between cultivars having low pollen fertility (ODV-4) and that having high pollen fertility (ODV-6). After crossing, ODV-4 produced fairly high amount of seeds(ie. 58 seeds/panicle) than ODV-6 (ie. 10 seeds/panicle). Seeds obtained from ODV-4 could beconsidered exclusively from crossing since selfing of ODV-4 did not produce any seed. The pollengrains of ODV-6 were shown to have a substantially high order of fertility (84.85%). This high pollen fertility of ODV-6 adequately explains the high seed yield/panicle in ODV-4 in crosses.

The features were slightly different in the case of ODV-6. The pollengrains of ODV-4 were found to be of very low degree of fertility (27.54%), and this might partially explain the recorded low seed yield/panicle in the case of ODV-6. But, while discussing this aspect, it should also be considered that, in ODV-6 self pollination could as well result in seed set. The present pollination studies did not provide any suggestive clue for determining the exact mode of pollination encountered. The seed set observed might be resulted from crossing, selfing or posssibly both combined. Hence, further studies under more controlled conditors are to be undertaken to resolve these aspects.

5.5.4. Seed germination

Informations on seed germination characters were found to be extremely scanty in the literature. In the present study, germination

test was conducted in all the cultivars by taking open pollinated seeds. Germination was low in all the cultivars, one cultivar, ie. ODV-4 showed significantly low germination percentage compared to cultivars ODV-2, 7 and 11. The seed yield and germination percentage did not hold any relationship in any of the cultivars except in ODV-4, wherein both the parameters recorded very low values.

Pillay (1967) in his preliminary studies on Vetiver had indicated that under cultivated conditons the propagation was mainly through slips and under natural wild conditions it was through seeds. The above author also observed that in North India the population was mostly growing wild, where as in South India it was being maintained by cultivation. Under cultivated condition seeds do not play much of a significant role in plant propagation. The cultivars used in the present study were all vegetatively propagated, and this might be a possible reason for the observed low seed germination rate.

Summary

6. SUMMARY

Detailed investigations were carried out on *Vetiveria zizanioides*, in order to study and compare the plant morphology, karyomorphology, pollen sterility and seed-set pattern in eleven cultivars viz. Seven South Indian, three North Indian and one hybrid vetiver.

1. On evaluation of morphological characters, no clear cut features were identified that could differentiate North Indian and South Indian types. However, appreciable differences existed with respect to flowering and root yield. Flowering was profuse in North Indian type while it was shy in South Indian. Root yield was more in South Indian than in North Indian type.

2. Karyomorphological observations confirmed the presence of a constant somatic chromosome number (2n = 20) in all the cultivars of Vetiver.

3. In general, all the cultivars had a symmetrical karyotype, indicating their primitivity.

4. The size of chromosomes, position of centromere and total chromatin length varied among cultivars.

5. The studies on meiotic features revealed that all cultivars contained univalents and multivalents in addition to bivalents in the PMCs.

6. Among all, one South Indian Cultivr (ODV-4) was identified to have high frequency of univalents per PMC. This particular cultivar

also revealed a higher rate of occurrence of laggards and bridges.

7. The meiotic abnormalities were found to have a direct relationship with pollen sterility.

8. The pollen fertility in the cultivars ranged from 27.54% to 85.63%.

9. In ODV-4 the pollen size was prominantly different. This cultivar also revealed high meiotic abnormalities as well as pollen sterility.

10. The observations on the pattern of seed set proved that under open pollinated condition, all the cultivars, including North Indian and South Indian types, produced seeds, though the number of seeds per panicle varied among them.

11. Under selfed condition, only a few seeds were produced by most of the cultivars, while some produced no seed. These observtions indicated prevelance of some self incompatibility system in Vetiver, which warrents further studies including more entries from North Indian and South Indian types.

References

REFERENCES

Akhila, A., Nigam, M.C. and Virmani, O.P. 1981. Vetiver oil - A review of Chemistry. *CROMAP* 3 (3):195-211.

Bews, J.W. 1929. *The World's grasses.* Longmans, Green and Co., London pp.246.

Bor, N.L. 1960. *The grasses of Burma, Ceylon, India and Pakistan.* Pergamon Press, Oxford, London. pp.767.

Celarier, R.P. 1956. Cytology of *Andropogon distachyus* L. BULLETIN OF THE TORREY BOTANICAL CLUB. 83(3):183-191.

Celarier, R.P. 1959. Cytotaxonomy of the Andropogoneae IV. Sub tribe sorgheae. *Cytologia* 24:285-303.

Christopher, J. 1986. Karyomorphological studies on members of the tribe Andropogoneae, Sub tribe Rotthoellineae. *Cytologia* 51(1): 43-50.

Cobley, L.S. and Steele,W.M. 1976. *An introduction to the botany of Tropical crops.* 2nd ed. The English Language Book Society and Longman, pp.256.

* Coveney, R.D. and Pickering, G.B. 1956. Vetiver roots from British Guiana. *Colon. Pl. Anim. Prod.* 6(1):63-66.

Giorgi, S. and Bozzini, A. 1969. Karyotype analysis of *T. turgidum* (L) and related tetraploid wheats. *Caryologia.* 22:249-259.

Guenther, E. 1950. *The Essential Oils. Vol. IV.* Van Nostrand Company, Inc., New York. 156-180.

Guenther, E. 1972. *The Essential Oils. Volume IV.* Rober E Krieger Publishing Co. Inc., New York. 159-180.

Huziwara, Y. 1962. Chromosomal evolution in the subtribe Asterinae. *Evolution* 13:188-193.

* Jain, S.K. 1959. Male Sterility in Flowering Plants. *Biblio Genet.* 18 : 101-166.

Janaki Ammal, E.K. 1945. In Darlington, C.D. and Janaki Ammal, E.K. 1945. *Chromosome atlas of cultivated plants.* George Allen & Unwin Ltd. London. pp.397.

Kumar, S. 1962. *Studies on morphological and genetic variability in Vetiveria zizanioides (Linn.) Nash.* Unpublished thesis submitted in partial fulfilment of the requirement of the M.Sc. degree of the Post Graduate School, Indian Agricultural Research Institute, New Delhi-12.

Kumar, S. 1963. A taxonomically interesting deviation in *Vetiveria zizanioides. Sci & Cult.* 20: 152.

* Larsen, K. 1963. Studies in the flora of Thailand. 14 Cytological studies in vascular plants of Thailand. *Dansk Bot. Ark.* 20: 221-275.

Lavania, U.C. 1985. Nuclear DNA and karyomorphological studies in Vetiver (*Vetiveria zizanioides* L. Nash.) *Cytologia.* 50(1): 177-185.

Lavania, U.C. 1987. Chromosomal instability in lemongrass. *Cymbopogon flexuosus* (Steud. Wats) *Genetica*. 72(3):211-215.

Lavania, U.C. 1988. Enhanced productivity of the essential oil in the artificial autopolyploid of Vetiver *(Vetiveria zizanioides* L. Nash). *Euphytica*. 38:271-276.

Mehra, P.N. and Remanandan, P. 1973. Cytological investigations on W. Himalayan Pooideae. *Cytologica* 38:237-258.

Mehra, K.L., Subramanyan, K.N. and Swaminathan, M.S. 1962. Cytogenetical studies in some members of Andropogoneae. *Jour. Indian Bot. Soc.* 41:491-503.

Menon, A.K. and Ittyachan, C.T. 1945. *Survey of Indian vetiver (khus) and its oil.* Council of Scientific and Industrial Research, New Delhi. pp.45.

Morris, E.T. 1984. Vetiver - Gift of India. *Pafai J.* 6(4):31-34.

Pillay, P.N.C. 1967. *Studies on Vetiveria zizanioides (Linn.) Nash. with special reference to analysis of geographical complexes and genetic architecture of quantitative characters in Indian Vetiver.* Thesis submitted to the Faculty of Post Graduate School of the IARI, New Delhi in partial fulfilment of the requirement for the degree of Doctor of Philosophy.

Purseglove, J.W. 1975. *Tropical Crops - Monocotyledons.* The English Language Book Society and Longman, London. pp.607.

Ramanujam, S. and Kumar, S. 1963. Irregular meiosis associated with pollen sterility in *Vetiveria zizanioides* (Linn.) Nash. *Cytologia* 28:242-247.

Ramanujam, S. and Kumar, S. 1964. Metroglyph analysis of geographical complexes in Indian Vetiver. *India J. Genel. Pl. breed.* 24(2):144-149.

Ranga Achariyar, R.B.K. 1921. *A handbook of some South Indian grasses.* M/s. Bishen Singh Mahendra Pal Singh,23-A, Dehradun. pp.257.

* Ratnambal, M.J. 1979. *Cytogenetical studies in ginger (Zingiber officinale* Rosc.). Ph.D. thesis, University of Bombay, India. pp.145.

Sadgopal, 1960. Exploratory studies in the development of essential oils and their constituents in aromatic oils. Part-3. Oil of Vetiver. *S o a p . Perfum. Cosm.* 298-310.

Sethi, K.L. 1982a. Development of high root and oil yielding hybrid clones in Vetiver. *Proc. of National Seminar on Medicinal and Aromatic Plants.* 147-149.

Sethi, K.L. 1982b. Breeding and cultivation of new KHAS hybrid clones. *Indian Perfumer.* 26:54-61.

Sethi, K.L. and Gupta, R. 1980. Breeding of high essential oil content in Khas *(Vetiveria zizanioides)* roots. *Indian Perfumer.*24(2):72-78.

Shah, S.S. 1964. A study of plants with meiotic abnormalities in *D a c t y l i s glomerata.* Sub. sp. *lusitanica. Cytologia,* 29:48-53.

Singh, B. and Sankhala, K.J. 1957. From Khas Roots to Root Khas. *The Indian Forester.* 83(5):302-306.

Stebbins, G.L. 1950. *Variation and evolution in Plants.* Columbia Univ. Press, New York. pp.645.

* Stebbins, G.L. 1958. Longivity, habitat and release of genetic variability in higher plants. *Cold Spring Harb Symp. Quant Biol.* 23:365-378.

Stebbins, G.L. 1974. *Variation and evolution in flowering Plants.* Edward Arnold (Publishers) Ltd., London. pp.399.

Sudharshan, M.R. and Jagdish Chandra, K.S. 1981. Unusual meiotic behaviour in the formation of 2n pollen in tetraploid *Cymbopogon caesius* (Nees) Stapf. *Cytologia.* 46:117-123.

Verma, V. and Sobti, S.N. 1982. Karyological studies in the genus *Cymbopogon Spreng.* I. *Nucleus.* 25(3):165-171,

Verma, V. and Sobti, S.N. 1985. Cytogenetical studies in the Genus *Cymbopogon Spreng* II. Meiotic studies in six species and their varieties. *Cytologia,* 50:351-359.

Virmani, O.P. and Datta, S.C. 1975. *Vetiveria zizanioides* (Linn.) Nash. *Indian Perfumer.* 19(1):35-54.

Wildner, P. 1961. Vetiver oil from Reunion. *Indian Perfumer.* 4(1):31-34.

---

* Originals not seen.

Appendices

APPENDIX - I

Whether data during the period (January - December, 1987 & 1988)

Month & Year	Total rainfall	Sunshine hours		Temperature		Mean relative humidity %
		Total	Mean	Mean X	Mean N	
January 1987	0	299.0	9.6	33.2	22.7	52
February 1987	0	285.0	10.1	35.0	22.4	52
March 1987	0	305.0	10.2	36.4	22.2	55
April 1987	131.3	236.0	7.8	36.2	25.3	64
May 1987	95.0	279.0	9.0	36.1	24.7	66
June 1987	837.7	126.0	4.2	30.7	23.7	83
July 1987	336.5	176.0	5.7	30.3	23.5	84
August 1987	388.4	113.5	3.7	29.6	23.5	87
September 1987	174.0	222.9	7.4	31.5	23.9	79
October 1987	280.4	193.3	6.2	31.9	23.9	79
November 1987	224.4	200.8	6.7	31.6	22.8	77
December 1987	64.6	250.4	8.1	31.6	23.3	77
January 1988	0	321.4	10.4	32.4	22.0	56
Februay 1988	7.8	290.0	10.0	35.8	23.1	56
March 1988	37.9	282.0	9.1	35.7	24.4	67

(Contd .....)

APPENDIX - I (Contd .....)

(1)		(2)	(3)	(4)	(5)	(6)	(7)
April	1988	145.4	265.0	8.8	35.1	24.3	70
May	1988	242.6	191.1	6.2	33.7	25.4	76
June	1988	632.1	125.9	4.2	30.0	23.7	86
July	1988	545.0	93.1	3.0	29.0	23.2	88
August	1988	507.8	114.9	3.7	29.2	24.3	86
September	1988	700.0	153.5	5.1	29.9	23.2	85
October	1988	116.6	218.7	7.1	31.7	23.3	78
November	1988	11.0	236.3	7.9	32.6	22.9	68
December	1988	14.9	259.6	9.0	32.6	22.3	57

KARYOMORPHOLOGY, POLLEN STERILITY AND SEEDSET IN VETIVER (*Vetiveria zizanioides* (Linn.) Nash.)

By

K. S. MINI

ABSTRACT OF A THESIS

Submitted in partial fulfilment of the

requirements for the degree of

Master of Science in Agriculture

Faculty of Agriculture

Kerala Agricultural University

Department of Agricultural Botany

COLLEGE OF HORTICULTURE

Vellanikkara, Trichur

1989

ABSTRACT

Investigations on karyomorphology, pollen sterility and seedset in Vetiveria zizanioides were undertaken using eleven cultivars of Vetiver, including North Indian type, South Indian type and one hybrid.

The observations on plant morphology indicated no clearcut morphological features employable for exact identification of North Indian and South Indian types of Vetiver. The somatic chromosome number was observed constant in all cultivars ie. 2n = 20. However, the different cultivars differed cytologically with respect to chromosomal characters like size and shape, total chromatin content and meiotic configurations during different stages of division. Presence of meiotic abnormalities like bridges and laggards were observed in all cultivars with highest frequency in ODV - 4. This cultivar also showed high percentage of pollen sterility. A direct relationship between meiotic abnormalities and pollen sterility was noticed. Studies on seedset pattern of different cultivars revealed very low set, mostly nil upon selfing, while all the cultivars produced fairly high quantity of seeds upon open pollination.