PACHYTENE ANALYSIS, INTERSPECIFIC HYBRIDIZATION AND RESPONSE TO CHROMOSOMAL DOUBLING IN TWO STEROID-BEARING *Solanum* SPECIES — *Solanum viarum* Dunal. and *Solanum mammosum* Linn.

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DIVISION OF HORTICULTURE
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Dedicated to Ever Loving

Memory of Beloved Sister

LAKSHMI

Division of Horticulture
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C E R T I F I C A T E

This is to certify that the thesis entitled "PACHYTENE ANALYSIS, INTERSPECIFIC HYBRIDIZATION AND RESPONSE TO CHROMOSOMAL DOUBLING IN TWO STEROID-BEARING *Solanum* SPECIES - *Solanum viarum* Dunal and *Solanum mammosum* Linn" submitted by Mr.D.L. Maheswar, for the degree of DOCTOR OF PHILOSOPHY in HORTICULTURE of the University of Agricultural Sciences, Bangalore, is a record of *bona-fide* research work done by him during the period of his study in this University, under my guidance and supervision, and the thesis has not previously formed the basis for the award of any degree, diploma, associateship, fellowship or other similar titles.

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INTRODUCTION

I. INTRODUCTION

The discovery of the dramatic effects of cortisone in the year 1949 (Hench *et al.*, 1949) heralded a new chapter in the history of modern medicine. The steroids find varied uses as corticosteroids (regulating carbohydrate and electrolyte metabolism), Oestrogens and progestogens (female hormones exerting control on mensural cycle and pregnancy), androgens (male hormones), anabolic agents, cardiotonic glycosides etc. Though animal sources played a key role in the early periods, vegetable sources located subsequently paved way for the wide use of steroids. Presently nearly 50 per cent of the world's requirements of steroids come from *Dioscorea* especially the three species, *D.deltoidea*, *D.composita* and *D.floribunda*. Steroid-bearing *Solanum* species such as *S.viarum* Dunal (Syn. *S.khasianum* var. *chatterjeanum*), *S.laciniatum* and *S.aviculare* are also commercially used as a source of steroid drugs since solasodine - a glycoalkaloid present in these species, offer a good starting material for the synthesis of steroid hormones (Schreiber, 1968; Bakshi and Hamied, 1971).

Among the steroid-bearing *Solanum* species, *S.viarum* has been commercially exploited in India and is found to be amenable for successful cultivation over a wide range of agroclimatic conditions (cf. Sharma and Verghese, 1980).

Unlike the diosgenin-bearing *Dioscorea* species which are generally long duration crops requiring special cultural practices, *S.viarum* is a short duration crop that can be cultivated with ease even in marginal lands. But to be a competitive alternate source of steroids, commercial varieties of *S.viarum* should possess higher contents and yields of solasodine and field resistance to wilt caused by *Fusarium oxysporum* (Bordoloi *et al.*, 1971).

In the context of enhancing solasodine content in berries, induced autotetraploidy holds promise since autotetraploids are reported to possess higher content of solasodine besides reasonably high level of seed fertility (Ammal and Bhatt, 1971; Bhatt, 1977). A detailed study of the meiosis of autotetraploids in *S.viarum* has not been reported so far, even though a knowledge of meiosis in autotetraploids is essential for understanding the causes underlying sterility and prospects of securing true breeding tetraploid lines in successive generations.

For incorporation of wilt resistance in *S.viarum* cultivars, interspecific hybridisation merits consideration as a prospective means of genetic improvement since related steroid bearing *Solanum* species - *S.mammosum*, and *S.incanum* exhibit field resistance to wilt disease (Telek *et al.*, 1977 and Pal, 1949) and interspecific gene transfer has played an

important role in the genetic upgrading of several species of the genus Solanum. Efforts to hybridize non-steroid bearing Solanum species with S.viarum have proved so far unsuccessful (Krishnappa and Chennaveeraiah, 1968; Rao, 1979). The steroid-bearing S.mammosum Linn. exhibits field resistance to wilt and could prove an useful source of genes conferring resistance to wilt for incorporation into S.viarum. Further, both S.viarum and S.mammosum are apparently closely related taxonomically as they have been assigned to the same subgenus, Leptostemonum and section, Acanthophora (Pearce and Lester, 1979). Thus the prospects of securing viable interspecific hybrid involving these two species seems quite bright.

For pursuing the above programme on autotetraploidy and interspecific hybridization, a knowledge of cytogenetical background of S.viarum and S.mammosum is essential. Information on the karyology and chromosomal homology of S.viarum and S.mammosum is an essential prerequisite for successful implementation of interspecific hybridization programme involving these two species. Considering the small size of mitotic chromosomes in Solanum species, in general, these studies can be profitably pursued at pachytene stage. Pachytene karyology of S.viarum has been reported by Pingle and Dnyansagar (1976). However, certain unusual features such as absence of nucleolus in most of the pachytene nuclei and the presence of terminal or

intercalary dark-staining segments in the light-staining regions of the arms in some of the chromosomes have been observed by these workers. These observations have not been reported at pachytene stage in any of the several Solanum species investigated so far (Gottschalk, 1954; Rao, 1972 and 1975). In view of this, a reinvestigation of the pachytene karyology of S.viarum was felt imperative.

An implicit need for conducting detailed cytogenetic studies in S.mammosum was also felt since this species is considered to be a potential commercial source of steroids (Telek et al., 1977) and has performed satisfactorily under Indian conditions (Gandhi Ram and Kaul, 1980). Further S.mammosum differs from S.viarum and other spinous Solanum species by virtue of its lower chromosome number of 2n=22, as against 2n=24 in the others and the available cytogenetic information on S.mammosum is restricted to determination of chromosome number (Madhavadian, 1968; Moore, 1971). In elucidating the taxonomic affinity between these two steroid-bearing Solanum species differing in chromosome number, a comparative study of leaf flavonoids appeared to be relevant as this character has been recently recognised to be of value in estimating species relationship in Solanaceae (Harborne and Swain, 1979). An assessment of response to chromosomal doubling

in S.sammoeum seems justified as autotetraploidy had proved an useful avenue for genetic improvement of S.viarum.

Considering the above, in the present investigation the following studies were undertaken:

i) re-investigation of pachytene karyology of S.viarum in the context of providing chromosomal basis for genetic studies and future breeding programme,

ii) induction of autotetraploids in S.viarum and detailed study of meiosis in autotetraploids for understanding causes underlying sterility and

iii) a comprehensive study to assess the interrelationships of the S.viarum and S.sammoeum based on cytomorphology, crossability, response to chromosomal doubling and distribution of leaf flavonoids to arrive at a possible crop amelioration strategy involving these two species.

REVIEW OF LITERATURE

II. REVIEW OF LITERATURE

The review of pertinent literature is covered under five major heads, viz., (i) steroid-bearing Solanum species (ii) leaf flavonoids in Solanaceae (iii) pachytene chromosomes (iv) interspecific hybridization in medicinal plants and (v) induced tetraploidy in medicinal plants.

2.1. STEROID-BEARING SOLANUM SPECIES

The literature on species of economic importance, taxonomy, cytology, interspecific hybridization and also autotetraploidy in steroid-bearing Solanum species is reviewed here.

2.1.1. Species of economic importance

Steroid-bearing species of Solanum are commercially exploited as the source of solasodine and related glyco-alkaloids. Solanum viarum, (Syn. S.khasianum var. chatterjeanum) is the exclusive species used by steroid industry in India (Maiti et al., 1964; Maiti et al., 1979; Gadwal, 1977a and b). According to Bradley et al. (1979) besides S.viarum, S.laciniatum, S.aviculare and S.marginatum are the most favoured plant species in the steroidal industry for the production of solasodine in Australian regions. According to Telek et al. (1977) S.mammosum can be a commercially potential source for solasodine

production. Among the indigenous species, S.incanum and S.indicum are also considered as potential source of solasodine (Ammal and Viswanathan, 1974). In most of the above species, berries contain solasodine, whereas, in S.laciniatum and S.aviculare, the leaves constitute the major source of solasodine.

2.1.2. Taxonomic status

Among the steroid-bearing Solanum species, S.aviculare and S.laciniatum belong to the same sub-genus and section Archae-Solanum (Bradley et al., 1979). The other species namely, S.viarum, S.mammosum, S.incanum, S.indicum and S.marginatum belong to the sub-genus Leptostemonum (cf. Pearce and Lester, 1979, and Randell and Symon, 1976). S.viarum and S.mammosum belong to the section Acanthophora. S.incanum and S.marginatum belong to the section Melongena while S.indicum to Oliganthus.

Certain extent of taxonomic confusion persisted until recently in respect of S.viarum. Babu and Hepper (1979) have recently reevaluated and concluded that the correct name for S.khasianum var. chatterjeanum. Sen Gupta is S.viarum. Owing to the earlier taxonomic confusion in respect of this taxon earlier reports on S.khasianum are treated as referring to S.viarum.

2.1.3. Cytology

2.1.3.1. Chromosome number: The chromosome numbers of the steroid-bearing Solanum species have been determined as: 2n=24 for S.mammosum (Nasrallah and Hopp, 1963) and 2n=22 for S.mammosum (Madhavadian, 1968; Moore, 1971). 2n=24 for S.viarum (Krishna Mitra, 1966; Zutshi, 1972; Krishnappa and Chennaveeraiah, 1976), S.incanum (Meige, 1962; Zutshi, 1974), S.indicum (Bhadrui, 1933; Meige, 1962; Bezbaruah and Bezbaruah, 1963; Zutshi, 1972), S.marginatum (Vilmorin and Simonet, 1927; Heiser, 1963; Madhavadian, 1968; Krishnappa and Channaveeraiah, 1971), 2n=48 (Vilmorin and Simonet, 1927, 1928) and 2n=92 (Baylis, 1954, 1963; Stray et al., 1962) for S.laciniatum; and 2n=46 (Baylis, 1954 and 1963; Stary et al., 1962; Bezbaruah and Bezbaruah, 1963), 2n=48 (Vilmorin and Simonet, 1927 and 1928; Gottschalk, 1954), and 2n=96 (Baylis , 1954 and 1863; Hardas and Joshi, 1954; Bezbaruah, 1965; Vilmorin and Simonet, 1927 and 1928; Gottschalk, 1954; Storchova, 1957, c.f. Federov 1974) for S.aviculare.

2.1.3.2. Somatic karyology: Karyomorphological studies using mitotic chromosomes of the root tip cells have been reported for S.khasianum var. chatterjeanum (Syn. S.viarum)(Krishna Mitra, 1966), S.khasianum (Zutshi, 1972; Krishnappa and Cheenaveeraiah, 1976), S.incanum (Zutshi, 1972; Narasimha Rao et al., 1980), S.indicum (Zutshi, 1972; Narasimha Rao et al., 1980), S.marginatum (Krishnappa and Chennaveeraiah, 1976).

In S.khasianum var. chatterjeanum (Syn. S.viarum) the somatic chromosome ranged in length from 1.6 /u to 2.8 /u (Krishna Mitra, 1966). Six chromosomal types (A to F) have been distinguished. The chromosomal pairs belonging to type 'B' measuring 2.5 /u in length was found to bear secondary constriction.

In S.khasianum, Zutshi (1974) recorded chromosome length variations from 1.99 /u to 3.3 /u. A pair of satellite chromosomes with sub-median centromere measuring 2.66 /u was observed. Among the five types recognised by her, no distinction has been made in respect of the centromere positions in two of the groups. The somatic chromosomes of S.khasianum have been classified under median (2.5 /u to 4.0 /u) and short (less than 2.0 /u) chromosome groups by Krishnappa and Chennaveeraiah (1976). Unlike Zutshi (1974), Krishnappa and Chennaveeraiah (1976) reported two pairs of satellited chromosomes and total absence of median centromere chromosomes. However, Narasimha rao et al. (1980) reported the presence of one median chromosome in the haploid complement of S.khasianum and a distinct absence of secondary constricted chromosomes in whole of the complement.

In Solanum incanum according to Zutshi (1972) the mitotic chromosomes ranged from 1.32 /u to 4.00 /u in length. Eight chromosomal types were recognised and a pair of satellited chromosomes with sub-median centromere was observed. But

Narasimha Rao et al. (1980) reported presence of five median and seven sub-median chromosomes. The somatic chromosomes of S.indicum were shorter than S.incanum and ranged in length from 1.33 /u to 2.66 /u. Two pairs of chromosomes bearing secondary constriction with median and sub-median centromeres were reported (Zutshi, 1972). S.indicum possessed a maximum number of eight chromosomes with median constrictions (Narasimha Rao et al., 1980). The somatic chromosomes of S.marginatum were reported to consist of two pairs of satellited chromosomes (Krishnappa and Chennaveeraiah, 1976). Only one pair of median centromere bearing chromosomes has been reported. The total chromatin length of this species is reported to be longer (33.9 /u) than that of S.khasianum (26.9 /u).

Based on the Karyotype analysis of some of the non-tuberous Solanum species including steroid-bearing ones, Narasimha Rao et al. (1980) opined that the study would not offer any scope for evaluation of phylogenetic relationships between the species. The study of chromosome morphology in Solanum species, at best can have only a phyletic significance as a commentary upon the validity of conclusions based upon evidence from external morphology and other cytogenetical data.

2.1.3.3. Pachytene karyology: Pachytene analysis among steroid-bearing Solanum species is reported for S.viarum only (Pingle and Dnyansagar, 1976). The differentiated pachytene chromosomes

are reported to vary in length from 9.4 /u to 34.2 /u. In the identification of entire haploid complement content, the distribution of heterochromatin was employed as the major criterion. Three of the chromosomes were reported to be metacentric. The conspicuous absence of nucleolusat pachytene was considered to be a specific character of this species. In one of the armsof chromosome I and IV and both arms of chromosome IX the light-staining regions are flanked on either side by dark staining regions. Based on morphological similarity of pachytene chromosomes, Pingle and Dnyansagar (1976) have suggested a basic number of n=10 for this species.

2.1.3.4. Accessory chromosomes: The presence of accessary chromosomes have been reported in S.indicum, S.khasianum (Syn. S.viarum) and S.viarum (Chennaveeraiah and Krishnappa, 1965; Dnyansagar and Dhanraj, 1972). In S.indicum, Chennaveeraiah and Krishnappa (1965) reported the presence of an extra chromosome in root tip cells of one collection and two fragment chromosomes in two other collections. In the latter case, one of the collections was found to have accessary chromosomes of unequal length and the other of equal length. The detection of accessary chromosomes in pollen mother cells of two more collectionsis also reported. These chromosomes occurred either unpaired or paired with chromosomes of the normal complement in trivalent and pentavalent configuration.

In S.khasianum an accessory chromosome occurred in PMC of one of the three collections examined by Chennaveeraiah and Krishnappa (1965) and was not found to pair with chromosomes of normal complement. In S.viarum Dnyansagar and Dhanraj (1972) found one or two B-chromosomes at meiosis in 20.6 per cent of the 121 plants analysed by them. The B-chromosomes did not pair with normal chromosomes or between themselves when two such chromosomes were present. Pollen fertility and seed setting was found to be reduced in plants with B-chromosomes. Dnyansagar and Pingle (1976) concluded that the presence of B-chromosomes in the plants of S.viarum may contribute to the increase in their solasodine content either directly due to the polygenic nature of these chromosomes or indirectly by decreasing the seed weight and increasing leaf weight.

2.1.4. Interspecific hybridization

Of the nearly 2000 species of Solanum, about a hundred are tuber-bearing and the rest are non-tuberiferous (cf. Rao, 1972). The steroid-bearing species belong to non-tuber bearing members of the genus. A large number of inter-specific hybrids have been reported among them (cf. Rao, 1979). Interspecific hybridization in steroid-bearing Solanum species offers a good prospects of transferring yield attributes,

hardiness and disease and pest resistance from wild or related taxa.

In the present review only those interspecific hybrids involving steroid-bearing species as one of the parents are considered. These include the hybrids between steroid-bearing species on the one hand and few of the edible or other non-tuberiferous Solanum species on the other.

2.1.4.1. Crossability relationship: Steroid-bearing species of the genus Solanum are characterized by certain peculiarities with regards to crossability among its species. Barriers to crossability appear to have developed to various degrees at different levels. These include complete failure of fruitset, parthenocarpic fruit set, production of shrunken or aborted seeds, production of well-developed but non-germinable seeds and seedling mortality (cf. Rao, 1979 and cf. Omidigi, 1979).

Stylar heteromorphism is reported in steroid-bearing Solanum species (Murthy and Abraham, 1975; Bakshi, 1979 and Rao and Kumar, 1980). In the context of relating the role of differences in stylar length with effective fruit set various flower types in spinous Solanum species were screened (Rao and Rao, 1977b). It was found that flowers with styles exerted well beyond the connate anthers had eight nucleate

embryosacs and were only functional (Rao and Rao, 1977b; Murthy and Abraham, 1975). Rao and Rao (1977b) mentioned the relation of abortive development of embryosac to stylar heteromorphism.

The results of the interspecific hybridization involving steroid-bearing Solanum species as parents or as parent with other non-tuberous Solanum species can be classified into seven following categories.

2.1.4.1.1. Fertile hybrids: In this category successful crosses resulting in fertile hybrids are included. Among them are reciprocal crosses between S.gilo and S.indicum (Nasrallah and Hopp, 1963 and Rao, 1979) between S.indicum and S.melongena (Chopde and Wanjari, 1973) Magoon et al., 1962a; Jaipurkar et al., 1973; Rao and Kumar, 1980; Rao, 1979; and Lakshmi et al., 1981) (Chopde and Wanjari, 1973) between S.insanum and S.melongena (Magoon et al., 1962a; Zutshi, 1966; Rao, 1968; Siddiqui and Khan, 1978; Lakshmi et al., 1981; Rao, 1979; and S.insanum x interrifolium, S.insanum x S.indicum (Rao, 1979) and S.aviculare x S.laciniatum (Motvenkova, 1968).

2.1.4.1.2. Partially fertile hybrids: Successful interspecific crosses yielding partially fertile hybrids are: reciprocal crosses between S.indicum and S.melongena (Rajashekaran, 1968, 1969 and 1970; Krishnappa and Chennaveeraiah, 1965; Rao, 1968;

Rangaswamy and Kadambavana Sundaram, 1973, 1974; and Nasrallah and Hopp, 1963); between S.indicum and S.incanum (Zutshi, 1966; Rao, 1968) and between S.incanum and S.integrifolium (Rao, 1968; and Khan et al., 1975); S.incanum as seed parent with S.melongena (Baksh, 1979), S.gilo and S.surattense (Rao, 1968) and as pollen parent with S.macrocarpon (Omidiji, 1975), S.indicum as seed parent with S.surattense (Raju et al., 1981), S.gilo (Rao, 1968).

2.1.4.1.3. Sterile hybrids: Sterile hybrids recovered from interspecific hybridisation include: reciprocal crosses between S.indicum and S.melongena (Lakshmi et al., 1981; Nasrallah and Hopp, 1963), between S.indicum and S.surattense (Rao and Rao, 1981; Mittal, 1950; Rajashekaran, 1968; Rao, 1968; Magoon et al., 1962a), S.indicum x S.torvum (Kirti and Rao, 1981), S.incanum x S.melongena (Lakshmi et al., 1981), S.indicum x S.gilo (Rangaswamy, 1973); S.gilo (Rangaswamy, 1973) and S.incanum as seed parent with S.melongena (Lakshmi et al., 1981) and S.surattense (Swaminathan, 1949 and Mittal, 1950).

2.1.4.1.4. Mortality of hybrid seedlings: Mortality of hybrid seedlings has been reported in crosses involving S.indicum as seed parent with S.incanum and S.sundaramianum (Rao, 1979) and between S.aculeatissimum and S.khasianum (Krishnappa and Chennaveeraiah, 1965).

2.1.4.1.5. Formation of partially developed and/or aborted seeds: Crosses leading to the formation of partially developed and/or aborted seeds are reported in cross combinations with S.indicum being seed parent with S.aculatissimum (Rao, 1979) and S.surattense (Rao, 1979 and Rao and Rao, 1981) and S.incanum as seed parent with S.macrocarpon and S.nigrum (Omidiji, 1975) and S.khasianum x S.indicum (Krishnappa and Chennaveeraiah, 1965) and S.melongena x S.khasianum (Rao, 1979).

2.1.4.1.6. Formation of parthenocarpic fruits: Reciprocal crosses involving S.melongena and S.khasianum (Baksh and Iqbal, 1979); and S.melongena and S.mammosum (Baksh and Iqbal, 1979); and S.melongena x S.incanum (Baksh, 1979), S.mammosum x S.incanum (Baksh and Iqbal, 1979) and Rao, 1979); and S.sisymbrifolium with S.incanum and S.khasianum (Rao, 1979) have produced only parthenocarpic fruits.

2.1.4.1.7. Cross incompatibility: Cross incompatibility leading to no success in crossability has been reported in the following parental combinations.

Reciprocal crosses between S.melongena and S.mammosum (Nasrallah and Hopp, 1963; Baksh and Iqbal, 1979), S.incanum and S.khasianum as seed parents with S.succosumianum, S.surattense (Syn. S.xanthocarpon), S.sisymbiifolium (Rao, 1979); S.indicum as seed parent with S.mammosum (Nasrallah and Hopp, 1963),

S.khasianum and S.sisymbrifolium (Rao, 1979); S.melongena as seed parent with S.indicum (Swaminathan, 1949; Mittal, 1950; Bhaduri, 1951; Rajashekaran, 1970; Rao, 1979), with S.khasianum (Krishnappa and Chennaveeraiah, 1965) its reciprocal (Rao, 1979) and with S.incanum (Bakshi and Iqbal, 1979 and Lakshmi et al., 1981); S.melongena var. incanum as seed parent with S.indicum (Rajashekaran, 1969; Rao, 1979) and S.khasianum (Rao, 1979); S.incanum x S.khasianum; S.khasianum x S.gilo; S.khasianum x S.integrifolium and its reciprocal (Rao, 1979); S.gilo x S.indicum (Rao, 1968 and Rao, 1979); S.torvum x S.indicum (Krishnappa and Chennaveeraiah, 1965); S.sisymbrifolium x S.indicum (Rao, 1979); and S.indicum with S.melongena and S.macrocarpon (Omidiji, 1975).

The above categorised results of interspecific hybridisation involving steroid-bearing and/or non-tuberous Solanum species when reviewed in terms of crossability behaviour of individual species revealed conflicting results. These results are dealt in the following pages taking important steroid-bearing Solanum species individually into consideration.

2.1.4.1.8. S.incanum: Reciprocal crosses between S.incanum and S.melongena have been reported to produce fertile hybrids (Swaminathan, 1949; Mittal, 1950; Magoon et al., 1962a; Zutshi, 1966; Rao, 1968; Siddiqui and Khan, 1978; Rao, 1979)

or resulted in no fruit set (Omidiji, 1975; Baksh and Iqbal, 1979; Lakshmi et al., 1981). Crosses between S.incanum x S.melongena have also been reported to give rise to partially fertile hybrid, while its reciprocal combination, produced parthenocarpic fruits (Baksh, 1979). S.incanum as pistilate parent with S.indicum, is reported to produce fertile hybrid (Rao, 1979) or partially fertile hybrid (Rao, 1968). In the reciprocal cross, partially fertile hybrid (Zutshi, 1966) or hybrid seedling mortality (Rao, 1979) have been reported.

The reciprocal cross between S.incanum and S.integrifolium resulted in a partially fertile hybrid (Rao, 1968; Khan et al., 1975). But, Rao (1979) reported the production of a fertile hybrid when S.incanum was used as a pistillate parent. S.macrocarpon as pistillate parent in crosses with S.incanum produced a partially fertile hybrid (Omidiji, 1975), while use of S.incanum as pistillate parent, resulted in either aborted seed formation or total incompatibility (Omidiji, 1975).

The results of crosses involving S.incanum as pistillate parent with other Solanum species include; the production of partially fertile hybrid with S.gilo (Rao, 1968) and S.aureitense (Swaminathan, 1949); sterile hybrids with S.aureitense (Mittal, 1950); formation of aborted seeds with

S.nigrum (Omidiji, 1975); incompatibility with S.aureitense, S.anguivi, S.khasianum and S.sisymbrifolium (Rao, 1979). As pollen parent S.incanum was reported to produce only parthenocarpic fruits with S.khasianum (Baksh and Iqbal, 1979; Rao, 1979), S.mammosum (Baksh and Iqbal, 1979) and S.sisymbrifolium (Rao, 1979).

2.1.4.1.9. S.indicum: In S.indicum, fertile interspecific hybrids have been obtained in reciprocal crosses with S.gilo (Nasrallah and Hopp, 1963; Rao, 1979) and with S.melongena (Magoon et al., 1962a; Chopde and Wanjari, 1973; Jaipurkar et al., 1973; Rao and Kumar, 1980 and Rao, 1979; Lakshmi et al., 1981). On the contrary, S.indicum and S.melongena crosses have been reported variously as incompatible (Swaminathan, 1949; Mittal, 1950; Bhaduri, 1951; Rao, 1968; Rangaswamy, 1973; Rao, 1979) or cross compatible but resulting in partially fertile hybrid (Rao, 1968; Krishnappa and Chennaveeraiah, 1965; Rajashekaran, 1968 and 1970; Rangaswamy and Kadambavanasundaram, 1973 and 1974), and sterile hybrid (Nasrallah and Hopp, 1963; and Lakshmi et al., 1981). Similarly, in case of S.indicum and S.gilo cross, a sterile hybrid with S.indicum as pistillate parent, and cross incompatibility in reciprocal combination have been reported (Rangaswamy, 1973). Crosses with S.incanum as pistillate parent have been reported to result in fertile hybrids (Rao, 1979). The reciprocal combination is reported

to yield a partially fertile hybrid (Zutshi, 1966; Rao, 1968) or a hybrid showing seedling mortality (Rao, 1979). In crosses with S.torvum, S.indicum as pistillate parent produced a sterile hybrid (Kirti and Rao, 1981), while the reciprocal combination revealed cross incompatibility (Krishnappa and Chennaveeraiah, 1965; and Kirti and Rao, 1981). Similarly, in crosses with S.surattense (Syn. S.xanthocarpon), S.indicum as seed parent is reported to produce aborted seeds (Rao, 1979; and Rao and Rao, 1981), partially fertile hybrids (Raju et al., 1981) and a sterile hybrid (Rao and Rao, 1981). The reciprocal combination has been reported to give rise to sterile hybrid (Magoon et al., 1962a; Mittal, 1950; Rajashekaran, 1968; Rao, 1968). S.indicum x S.integrifolium cross resulted in partially fertile hybrid (Rao, 1968). Formation of aborted seeds in the cross S.khasianum x S.indicum has been reported by Krishnappa and Chennaveeraiah (1965). Incompatibility has been reported between S.indicum and S.khasianum, S.sisymbrifolium (Rao, 1979) and S.[illegible] (Nasrallah and Hopp, 1963).

2.1.4.1.10. S.khasianum: Reciprocal crosses involving S.khasianum and S.melongena has been attempted by a number of workers. Crosses with S.melongena as pistillate parent was reported as incompatible (Krishnappa and Chennaveeraiah, 1965), and produced either parthenocarpic fruits (Baksh and Iqbal, 1979)

or aborted seeds (Rao, 1979). Recently, by adopting nutrient culture for the naked embryos resulting from S.khasianum ex S.melongena cross, a partially fertile hybrid has been developed (Sharma et al., 1981). Crosses involving S.khasianum as pistillate parent with S.melongena are reported as failure (Rao, 1979 and Sharma et al., 1981) or to produce parthenocarpic fruits (Baksh and Iqbal, 1979). Reciprocal crosses have been attempted between S.khasianum and S.incanum but without success. S.khasianum as pistillate parent was reported to produce parthenocarpic fruits (Baksh and Iqbal, 1979 and Rao, 1979), while no fruit set was obtained when S.indicum was used as pistillate parent (Rao, 1979). Similarly, results of reciprocal crosses between S.indicum and S.khasianum indicate formation of aborted seeds with S.khasianum as seed parent (Krishnappa and Chennaveeraiah, 1965) or a total failure with S.indicum as seed parent (Rao, 1979). Cross incompatibility was reported for crosses involving S.khasianum and S.sisymbrifolium (Rao, 1979). Failure in crossability has also been reported when S.khasianum was used as pistillate parent in crosses with S.torvum, S.gilo, S.xanthocarpon and S.sisymbrifolium (Rao, 1979).

2.1.4.1.11. S.mammosum: Reciprocal crosses involving S.melongena and S.mammosum, former as pistillate parents, are reported to

produce parthenocarpic fruits (Baksh and Iqbal, 1979) or total failure in fruit set (Nasrallah and Hopp, 1963; Baksh and Iqbal, 1979). S.mammosum as pistillate parent in crosses with S.incanum produced parthenocarpic fruits (Baksh and Iqbal, 1979 and Rao, 1979). As pollen parent, S.indicum did not effect fruit set in crosses with S.melongena (Nasrallah and Hopp , 1963).

Thus the above observations on crossability of species seem to support the opinion expressed by Rao (1979) that the information obtained by crossability studies alone cannot offer any clue to the cytogenetic relationship of the species involved and their systematics, eventhough the ability of the species to cross with one another to give rise to viable offsprings is normally accepted as one of the principles used in assessing relationships.

2.1.5. Response to chromosome doubling

Among the steroid-bearing Solanum species response to chromosomal doubling (autotetraploidy) has been studied in S.xiarum (Syn. S.khasianum) and S.indicum.

2.1.5.1. S.xiarum: Induced autotetraploids of S.xiarum (but reported as S.khasianum) was first reported by Ammal and Bhatt (1971). The material used was collected from Dehra Dun probably from wild stand. Autotetraploids were obtained from

seedlings treated with 0.1 to 0.2 per cent aqueous colchicine solution. Bhatt (1977) reported further studies using C_1, C_2 and C_3 generations of these tetraploids. Induction of autotetraploids in a curved spine mutant developed by Bhatt (1972) using one per cent colchicine was also reported (Bhatt, 1975). Bhatt and Noble (1978) compared glyco-alkaloid/Solasodine contents and agronomical characters of spiny tetraploid, mutant tetraploid and the progenitor of the latter.

Ammal and Bhatt (1970) observed that the spiny tetraploid could be easily distinguished by its dark and thick foliage and lesser lobing of leaves. Besides, decrease in spine number, delayed flowering (about a month), smaller size and lesser weight of fruits were also reported to characterise tetraploids from diploids. The authors observed 'the presence of 3 to 5 fruits at some nodes instead of the usual single fruit indicating development of more than one hermophrodite flowers'. The general superiority of autotetraploids over diploids in the ratio of male flowers to hermophorodite flowers was confirmed by Bhatt (1975) from the study of advanced generation of mutant colchiploids. Based on the comparative study of these spiny tetraploids belonging to C_1, C_2 and C_3 generations, Bhatt (1977) reported that the proportion of male flowers to hermophrodite flowers was 10:6 in tetraploids compared to 10:3 of diploids resulting in increased fruit

number i.e., 236 in tetraploids against 140 in diploid. This was attributed to altered hormonal pattern incidental to tetraploidy.

Based on her study of the variation in number of spines on leaves in C_2 and C_3 generation plants of curved spine mutant tetraploids, Bhatt (1975) surmised a probable association of spinelessness with sterility. Notwithstanding the above observation, marked improvement in pollen fertility and fruit set over C_2 lines could be obtained by her in C_3 generation lines, thereby indicating scope for improvement in advanced generations of polyploids. Similar behaviour in respect of spinelessness and sterility was reported later by Bhatt (1977) in her study of C_1, C_2 and C_3 generationsof autotetraploids of wild (spiny) type induced earlier by Ammal and Bhatt (1971). Large variability was observed in C_1 tetraploid plants for size and number of spines on leaves, flower abscission and plant height. Spineless plants recorded high flower abscission that could be controlled by an application of NAA which, however, did not promote fruit set. Bhatt (1977) also observed increase in plant height, pollen fertility and fruit yield in C_3 generation over C_1 and C_2.

Bhatt (1977) observed an increase in the total number of fruits per plant from 140 in diploids to 236 in spiny tetraploids and also total fruit yield per plant from 700 g in

diploids to 760 g in spiny tetraploids. The increment in yield was attributed to increase in the number of nodes and fruits per node. In a comparative study, Bhatt and Heble (1978) noted the inferiority of spiny tetraploid over mutant tetraploid in average height, length of branches, period of flowering, number of fruits, fruit yield per plant and average weight of fruit. A wide variability for weight of fruits per plant was discerned among mutant tetraploids which offered scope for selection. The active fruiting period was found to be shortened (22-130 days) in mutant tetraploid as compared to mutant diploid (77-148 days).

Ammal and Bhatt (1971) reported the glyco-alkaloid content of six diploids and four tetraploids. The variation for this character in diploid ranged from 2.8 to 4.8 per cent and among tetraploids from 1.4 to 7.6 per cent. Two of the tetraploid lines showed as high as 7.4 and 7.6 per cent glyco-alkaloid. In mutant tetraploids, Bhatt (1975) recorded 8.0±2.5 per cent solasodine content in dried berries of C_3 generation plants while the corresponding values for diploid was 6.92±0.25 per cent. Bhatt and Heble (1978) also recorded an increased glyco-alkaloid content of 8.09 per cent in spiny tetraploid and 7.72 per cent in spiny mutant tetraploids compared to 4.38 per cent and 4.75 per cent in diploid wild type and diploid curved spine mutant types, respectively.

Likewise, average solasodine content in spiny tetraploid was 2.8 per cent and in curved spine mutant tetraploid was 2.5 per cent compared to 1.78 and 2.02 per cent in diploid wild type and diploid curved spine mutant, respectively.

According to Bhatt and Heble (1978) the projected solasodine yield per hectare in the tetraploid was estimated at 80 kg against 60 kg in diploid. The superiority in the performance of the mutant tetraploid was ascribed to cumulative effects of colchicine and radiation.

2.1.5.1.1. Cytology: Meiosis has been studied in autotetraploids. Chromosomal associations at metaphase-I/diakinesis stages have been reported in raw autotetraploids (Ammal and Bhatt, 1971) and in C_2 and C_3 generation plants of S.xiarum (Bhatt, 1977). Chromosomal associations in raw autotetraploids (C_1) consisted of quadrivalents (2.22 to 6.18), trivalents (0.02 to 0.14), bivalents (11.34 to 19.42) and univalents (0.02 to 0.14)(Ammal and Bhatt, 1971). Quadrivalents formation was considerably reduced to 1.4 in C_3 generation as against 3.8 for C_1 tetraploids, associated with an increase in bivalent frequency (3 to 22 per cell)(Bhatt, 1977). However, a detailed study of various meiotic stages has not been reported.

2.1.5.2. S.indicum: Autotetraploids were also induced in S.indicum (Rajashekaran, 1970). Morphologically, autotetraploids

did not show any increase in plant height but had larger flowers than diploids. Pollen stainability was 70.2 per cent but was associated with low seed set of three seeds per fruit as against 51 seeds per fruit in diploids.

2.1.5.2.1. Cytology: Meiosis in induced autotetraploids of S.indicum was characterized by precocious movement, of chromosomes at metaphase-I, irregularities such as disjunction and occurrence of laggards and chromatid bridges at anaphase-I and micronuclei formation at quadret stage (Rajashekaran, 1970).

2.2. LEAF FLAVONOIDS IN SOLANACEAE

Flavonoids have been widely used as markers in systematic studies in a number of taxa (Harborne, 1975). Richness and structural diversity in flavonoids have been reported for families such as Ericaceae (Harborne and Williams, 1975), Primulaceae (Harborne, 1968) and Leguminosae (Harborne, 1971). But in Solanaceae the structural diversity in flavonoids is limited (Harborne and Swain, 1979). Among the flavonoids present in Solanaceae the most conspicuous are the anthocyanins, which impart purple colours in flowers and fruits and flavonol glycosides which occur in both flowers and leaves. The leaf flavonoids namely flavanol and flavhone glycosides have been studied in 26 species belonging to 14 genera by Bate-Smith (1962) and 32 species from 24 genera in Solanaceae (cf. Harborne and Swain, 1979). The tuberous Solanum species have been

extensively studied (Harborne, 1964). These studies have revealed the relative frequency of flavones unlike in the genus Capsicum where flavones are the major constituents than flavonols.

According to Hawkes (1956), the flavonol glycosides in wild Solanum species have indicated a fair correlation with classification of species into series. Thus, there appears to be a considerable potential for data on flavonoids in resolving systematics and phylogeny of the genus Solanum. However, the leaf flavonoids of non-tuber bearing Solanum species have not received attention. The study of leaf flavonoids in spinous Solanum, therefore, appears to be imperative in the context of elucidating interrelationships of various systematic categories of this group.

2.3. PACHYTENE CHROMOSOMES

The utility of pachytene stage for karyological studies has been realised since the pioneering work of McClintock (1929) in Zea mays. McClintock identified all the ten chromosomes of the haploid complement at this stage. Since then pachytene stage has been studied in a number of plants on the context of karyological and homological investigations. The present review covers the major criteria employed in identification

and description of pachytene chromosomes and the use of pachytene analysis in cytogenetic and phylogenetic investigations.

The pachytene chromosomes may show differential stainability along their length or may stain uniformly and are accordingly referred to as differentiated or undifferentiated chromosomes, respectively(Venkateshwarlu, 1962). The differentiated chromosomes have proximal densely staining regions and distal light-staining regions. Differentiated chromosomes are observed in Lycopersicon (Barton, 1950), Plantago (Hyde, 1953), Solanum (Rao, 1972 and 1975), Eu-sorghum (Magoon and Shambulingappa, 1961) and several other plant species. The plants like, Zea mays (McClintok, 1929), Cynodon (Oureshy, 1963) and Hordeum vulgare (Sarvella, et al., 1958) have un-differentiated chromosomes. Both differentiated and undifferentiated chromosomes are known to occur in the same genus, eg. Sorghum (Garber, 1947; Magoon and Shambulingappa, 1962a and 1962b) and within races of the same species, eg. Coix lachrymajobi (Venkateshwarlu, 1960 and 1961).

2.3.1. Pachytene chromosomes in cytogenetical studies

The study of pachytene chromosomes had contributed much to the cytogenetical investigations of Zea mays and Lycopersicon esculentum.

Since the pioneering work of McClintock (1929) on Zea mays, pachytene chromosome studies have been extensively used in obtaining cytological basis for genetical studies. The assignment of the ten linkage groups to specific chromosomes as identified at pachytene, was accomplished using initially primary trisomics (McClintock and Hill, 1931) and later translocations and deficiencies. A further step towards the location of genes on the chromosomes has also been achieved using deficiencies (McClintock, 1933, 1944; Stadler, 1933; Rhoades, 1935), and duplications (Rhoades, 1936). Creighton and McClintock (1931) demonstrated cytological evidences for genetic cross-over. Using both translocation and inversion, McClintock (1931, 1933) and Creighton and McClintock (1931) obtained cytological proof of chromatid cross-over.

McClintock (1943) and Morgan (1950) also demonstrated higher frequencies of cross-over in the distal regions of the chromosomes. It has also been concluded from pachytene analyses that late pairing and frequent non-homologous pairing lead to reduction in cross-over at translocation points (McClintock, 1933, 1934; Burnham, 1932, 1934). McClintock (1944) also brought about the genetical effect of deficiency for small terminal segments of chromosome detectable only at pachytene stage.

Tomato is another plant, in which pachytene chromosome study has made way for the integration of informations from

cytology and genetics. Following the identification of the entire complement of pachytene by Barton (1950), the eleven of the twelve linkage groups have been assigned to the respective chromosomes (Rick and Barton, 1954; Rick et al., 1964). Using the satellite as cytological marker and genetical data, Moens and Butler (1963) determined the position of centromere of chromosome II in the genetic map. In another study, using the same cytological marker in tetraploid material, Moens (1964) observed good correlation between data based on cytological behaviour of chromosome II and genetic segregation, bringing out thereby the importance of meiotic behaviour of chromosomes in genetic segregation of ratios of autotetraploids. Rick et al. (1964) have shown the non-random distribution of mutant genes in the chromosomes, the predominant localization of genes in the achromatic regions and irregular distribution of mutant genes in these regions. As in maize, induced-deficiencies have been used to locate genes on the chromosomes (Khush and Rick, 1963). Using similar methods in tomato, Khush et al.(1964) have demonstrated for the first time the location of a gene 'nv' in the heterochromatic region of chromosome XI the heterochromatic regions are believed to be void of active macrogenes.

The study of pachytene chromosomes of spinous Solanum species have recently received attention (Rao, 1972 and 1975). Comparative pachytene analysis have confirmed the divergence

between S.surattense and S.torvum as established earlier by Rajashekaran (1969) on taxonomic and crossability grounds.

2.4. INTERSPECIFIC HYBRIDIZATION IN MEDICINAL PLANTS

The wild and distantly related plant species are of great importance in serving as a source of genes for pests and disease resistance, for higher yields, quality attributes and adaptability. Thus, interspecific hybridization enables in the transference of desirable characters, exploitation of hybrid vigour, allopolyploidization, genetic engineering and to study the phylogenetic and evolutionary aspects in delineating the species relationship. Earlier records on the application of interspecific hybridization in practical plant breeding improvement could be traced out from 18th century (Briggs and Knowles, 1967). Such a significant role of interspecific hybridization has been realized in medicinal plants also and pertinant literature is reviewed in this chapter.

2.4.1. Dioscorea species

The new world species of D.floribunda, D.composita and D.friedrichsthalli have been reported to produce partially fertile hybrids when crossed in all possible combinations (Martin and Cabanillas, 1966). These workers have earlier reported the occurrence of wild hybrid between D.composita and D.floribunda (Martin and Cabanillas, 1963).

Attempts have also been made to cross old world diploid species, D.deltoidea (2n=20) and three tetraploid (2n=40) new world species, namely D.floribunda, D.composita and D.friedrichsthalli (Rao et al., 1975). But no success was achieved by way of recovery of viable interspecific hybrid.

2.4.2. Digitalis species

Calcandi et al. (1961) demonstrated the presence of digoxin in the interspecific hybrids between D.lanata x D.purpurea and D.purpurea x D.lutea. Interspecific hybrids of D.lanata and D.purpurea were found to contain digoxin within the range found in the D.lanata parents (Mihalea and Silva, 1972). Kennedy (1978) recovered viable interspecific hybrids by crossing D.lanata and D.grandiflora which contained glycoside-digoxin levels comparable to that of D.lanata.

2.4.3. Datura species

Romeike (1959) recovered a viable interspecific hybrid by crossing D.ferox and D.stramonium. D.ferox was small, virus susceptible plant possessing minute amounts of alkaloid especially higher amounts of hyoscine. On the contrary D.stramonium was vigorous growing, non-susceptible to virus and possessed ten times higher the content of hyoscyomine than D.ferox, but not hyoscine. The resulting interspecific hybrid had higher alkaloid level of D.stramonium with an alkaloid yield of

700 mg per plant against 100 mg per plant in D.ferox with hyoscine content of over 90 per cent than D.ferox.

2.4.4. Papaver species

Semi-fertile hybrids were obtained by hybridising diploid P.somniferum and tetraploid P.setigerum (Grover, 1979). Likewise, Jonsson and Loof (1973) hybridised tetraploid P.somniferum and P.orientale. The resulting interspecific hybrid showed wide variation for vigour and morphology with poor pollen production. Further, advancing the interspecific hybrid to subsequent generations, the hybrid possessed markedly higher content of codeine with lower morphine content than P.somniferum.

2.5. INDUCED TETRAPLOIDY IN MEDICINAL PLANTS

The simple and effective colchicine method by Blakeslee and Avery (1937) for chromosomal doubling has been used extensively in the production of autotetraploid in a large number of plants.

Autotetraploidy is reported to be generally associated with an increase in plant height, thicker stem, lesser number of branches, thicker and broader but fewer leaves and increased fruit size with lesser seeds (Stebbins, 1971) and chemical principles like vitamins (cf. Ramanujam and Parthasarathy, 1953) and alkaloids in Solanaceous crops (Steinegger, 1955).

Since several of the medicinal plants are grown for their active principles present in roots, leaf and/or stem, the possible role of autotetraploid breeding in genetic improvement of these crops attracted attention. The production and evaluation of autotetraploidy has been attempted in a number of medicinal plants (cf. Ammal, 1965). The characteristic feature of the induced polyploids in certain important medicinal plants is reviewed here.

2.5.1. Catharanthus roseus

The plant is reckoned for its root alkaloids, reserpine, ajmalicine and serpentine which possess hypotensive properties and for the alkaloids in leaves, viz., Vincristine and vinblastine possessing anti-neoplastic properties.

Induction of tetraploidy using colchicine in this crop was reported by Fursato (1940). This was followed by Cross and Johnson (1941), Schnell (1941) and Eigsti and Schnell (1943). Since its recognition as a medicinal plant of commerce, successful induction of tetraploidy was reported by Ammal and Bezarbaush (1965) and Dnyansagar and Sudhakaran (1970a and b) following seed or seedling treatment.

Autotetraploids were characterised by their broader leaves, larger stomata, larger and wider corolla, larger pollen

size and lowered pollen fertility and seed fertility (Ammal and Bezarbuah, 1963; Dnyansagar and Sudhakaran, 1970a and b, and Mohan Kumar, 1980).

The increase in the content of root alkaloids in autotetraploids over diploids has been reported by Dnyansagar and Sudhakaran (1970a and b). But Mohan Kumar (1980) based on the comparision of total alkaloid content in roots of diploid pregenitors and their corresponding tetraploids, concluded that increase in total alkaloid content following chromosomal doubling occurred only when diploids with low alkaloids in roots were used. The induced autotetraploids of diploids with higher total alkaloid content possessed lower content of alkaloids in their roots.

2.5.1.1. Cytology: Detailed cytology of autotetraploids has been studied by Dnyansagar and Sudhakaran (1970a and b). Cytological behaviour of autotetraploids was characterized by the formation of varied frequencies of quadrivalents and univalents, non-orientation and irregular distribution of chromosomes at the equator, lagging chromosomes and tetrads with extra groups of micronuclei.

2.5.2. Rauvolfia serpentina

The roots of Rauvolfia serpentina contain reserpine and ajmalicine besides other alkaloids which are used in the preparation of hypotensive drugs.

Ammal (1962) obtained autotetraploids by colchicine treatment of seeds, seedlings and cuttings. Dnyansagar and Torne (1972) reported that seed treatment with 0.5 per cent colchicine for 10 hours proved effective in the induction of autotetraploid. Tapedar (1963) induced colchiploids by treating the growing tip of seedlings with 0.5 per cent colchicine for 9 hours duration.

The morphological characteristics of autotetraploids that distinguish diploids as reported by these authors include; reduced plant height and internodal length, slow growth of shortened and thickened branches, thick and dark green coloured leaves, fewer and larger sized stomata, delayed flowering, reduced number of flowers per inflorescence, increased flower size, larger sized pollen grains with slightly changed morphology and reduced pollen fertility with a reduced number of fruits and seeds (Ammal, 1962; Tapedar, 1963; Bhaduri and Biswas, 1965 and Dnyansagar and Torne, 1972).

Induced autotetraploids had higher fresh weight of roots (50.9 to 56.7 g/plant) compared to diploids (36.8 to 41.8 g/plant)(Dnyansagar and Torne, 1972). Bhaduri and Biswas (1965) also reported the distinctly higher root yield of tetraploid plants over diploids of the same age.

Ammal (1962) reported higher total alkaloid content of 2.28 per cent in tetraploids as against 1.54 per cent in

diploids. The higher alkaloid content of tetraploid root was also confirmed by her in another report where tetraploids recorded 2.28 per cent of total alkaloid against 1.65 per cent of diploids (Ammal, 1963). Dnyansagar and Torne (1972) reported an increase of 11 per cent in total alkaloids in roots of tetraploid over diploid. Bhaduri and Biswas (1965) also observed an increase in roots alkaloids in autotetraploids (1.925%) as compared to diploids (1.79%). But the reserpine content did not indicate proportionate increase. Seed fertility in tetraploids was 12 per cent as compared to 48 per cent in diploids and thus was lower in autotetraploids (Bhaduri and Biswas, 1965).

2.5.2.1. Cytology: Tapedar (1963) and Dnyansagar and Torne (1972) have made detailed studies on the meiosis of autotetraploids R.serpentina. The salient features include: formation of quadrivalents (4.8 per cell) and precocious movement of chromosomes at metaphase-I, faulty spindle formation leading to absence of complete orientation of chromosomes; at anaphase-I, absence of active polar movement, occurrence of laggards and bridges; precocious movement of bivalents and random seperation leading to formation of equal and unequal groups at metaphase-II, formation of supernumerary spored quadrets or polyods and instances of cytomixis at telophase-II (Dnyansagar and Torne, 1972 and Tapedar, 1963).

2.5.3. Dioscorea species

The roots of D.floribunda are used as a source of diosgenin. Diosgenin serves as a raw material for the preparation of steroidal drugs used extensively in contraceptive pills, cortisones, anabolic agents and several others.

Ammal and Singh (1962) induced tetraploidy in D.floribunda by injecting 0.4 to 1 per cent of aqueous colchicine solution to the dormant rhizomes. Induced autotetraploids had thicker leaves, bigger stomata and slower rate of growth than diploids. Autotetraploids have also been obtained from both seed and seedling treatment with 0.25 per cent of colchicine (Murthy, 1977). Autotetraploids reported to exhibit robust growth, longer and thicker leaves, larger petiole, larger stomata, more number of shoots, larger stem diameter, longer internodes, increased number of flowers and larger sized pollen grains. The pollen fertility in tetraploids was found to be comparable to diploids and ranged from 80 to 100 per cent.

2.5.3.1. Cytology: Meiotic studies revealed presence of univalents, bivalent, trivalents with a predominance of bivalents (25.6 per cell) and quadrivalents (4.8 per cell) at metaphase-I. Inspite of the presence of univalents and multivalents no chromosomal irregularities were observed at any stage resulting in normal meiosis (Murthy, 1977).

2.5.4. Datura species

Dried leaves, flowering tops and seeds of the plant possess an alkaloid, hyoscyamine, used in formulating drugs for antispasmodic and bronchitis conditions.

Tetraploids in D.metal were induced by soaking seeds with 0.8 per cent aqueous solution of colchicine (Ammal and Zutshi, 1970), in D.stramonium by treating the growing points with 0.2 per cent aqueous solution of colchicine (Botnarenko, 1971) and by treating the germinating seeds (Dzhurmanski and Yankulov, 1978) and in D.innoxia by seed treatment of organic solvent-Ethyl alcohol (Singh and Kaul, 1967; and Khosla and Singh, 1974).

Induced autotetraploids in different species of the genus Datura were characterised by increased vigour and plant height (Singh and Kaul, 1967), increased petiole length, increased size of dark green thick leaves, larger leaf weight and larger sized stomata (Dzharmanski and Yankulov, 1978 and Botanararko, 1971; Solomon and Crane, 1970; Raicu et al., 1962; Yankulov and Alipur, 1975; Ammal and Zutshi, 1970), increased flower size, pollen grain size, smaller sized fruits with fewer and larger seeds per fruit (Ammal and Zutshi, 1970; Solomon and Crane, 1970; Botnarenko, 1971; Dzharmanski and Yankulov, 1978) and increased dry matter content (Dzhurmanski and Yankulov, 1978). Tetraploids in D.innoxia are reported

to have better adaptability to changed environmental conditions than their diploid counterparts (Dzhurmanski and Yankulov, 1981). Rawson (1944) reported that tetraploids of D.stramonium and D.tatula contained higher total alkaloids.

Similar substantial increment in total alkaloid content from 0.1 to 0.2 per cent in diploids to 0.2 to 0.7 per cent in autotetraploids have been reported for D.metel (Ammal and Zutshi, 1970). In D.stramonium, alkaloidal content of diploid was 0.35 per cent while in tetraploids 0.58 per cent (Botmarenko, 1971). In D.ferox, autotetraploids recorded an increase of total alkaloid upto 65 to 66 per cent over diploids (Dzhurmanski and Yankulov, 1978) with a large increase in the percentage of atropine and scopolamine (Solomon and Crane, 1970; Dzhurmanski and Yankulov, 1978 and 1981). In D.innoxia, induced tetraploids had increased number of seeds per capsule and higher total alkaloid content per plant (Singh and Kaul, 1967).

Information pertaining to the cytological behaviour of these autotetraploids is not available.

2.5.5. Papaver species

In P.somniferum latex extracted from the capsule possess the alkaloids - morphine and codeine which are widely used in the formulation of tranquilizers and analgesics.

In *P.somniferum* autotetraploids were induced following seed treatment with 0.4 per cent colchicine (Kaul *et al.*, 1979). Autotetraploids showed reduction in plant height, stomatal size, pollen fertility, capsule size and delayed flowering. But leaf breadth, seed size and weight were increased (Andereev, 1963; Czabajska, 1965; Kiskeri *et al.*, 1977; Kaul *et al.*, 1979). According to Andereev (1963) and Czabajska (1965) alkaloid content was increased in autotetraploids over diploids. But Kaul *et al.* (1979) reported that the polyploids were inferior to the diploids in alkaloid content.

2.5.5.1. Cytology: Kaul *et al.* (1979) reported the meiosis in autotetraploid *P.somniferum*. Chromosomal associations consisted of quadrivalents, trivalents and bivalents. Irregular distribution of chromosomes at anaphase-I was also reported by these workers.

MATERIAL AND METHODS

III. MATERIAL AND METHODS

3.1. MATERIALS

The materials used for the study included two accessions of *Solanum viarum* Dunal. (Syn. *S.khasianum* var. *Chatterjeeanum*), and *Solanum mammosum*. Both the species are steroid bearing and are reported to be native of South America (Miller, 1969 and Babu and Hepper, 1979) but have naturalised in our country.

Considering the prevailing confusion on the taxonomic nomenclature of *S.viarum* both the accessions used for the study were checked up for distinguishing morphological characters delineating *S.viarum* (Syn. *S.khasianum* var. *Chatterjeeanum*) and *S.khasianum* (Syn. *S.myriacanthum*) as reported by Babu and Hepper (1979). In the accession used, leaves were sinuately lobed with soft pilose indumentum and denser aggregation of white coloured glandular hairs. Flowers were characterised by prickly calyx having white coloured petals enclosing creamy white coloured anthers and the ovary was pubescent. Thus in the exomorphic characters the material conformed to *S.viarum* (Fig.1)

The accession of *S.mammosum* used in the study was originally obtained from Keralaand the generations raised at the Institute were found to be generally uniform for several exomorphic characters. The plants produced large pyriform

Plate 1. Legend for photograph

Fig.1. Flowering twigs with fruit of *S.mammosum* (P_1) and *S.viarum* (P_2)

PLATE.1

FIG.1

shaped mammilliform fruits characteristic of the species and showed vigorous vegetative growth (Fig.1).

3.2. METHODS

3.2.1. Hybridization

Under this programme S.viarum and S.mammosum were reciprocally crossed. In the female parent the flower buds possessing longer style which were due to open on the same day at later hours or on the following day were selected for emasculation. The choice of such buds in case of S.mammosum was restricted to the axillary bud, while in S.viarum upto three buds per cluster. For emasculation, the petals of the unopened flower buds were carefully seperated with the help of pointed tweezers and the five undehisced anthers were carefully removed by snapping the filaments just below the anthers without injuring the anthers. The exposed stylesof emasculated flower buds were pollinated with adequate quantity of pollen collected from the dehisced anthers of the male parent scooped on to the smooth edge of the tweezer and carefully brought on to the stigma. After pollination, other buds in the inflorescence were removed. The pollinated flower buds were bagged and labelled. The bags were removed after 10 to 12 days.

3.2.2. Induction of Autotetraploids

For induction of tetraploids, 0.25 per cent aqueous solution of colchicine was used. Diploid seedlings of S.viarum and S.mammosum with fully expanded cotyledonary leaves were selected for the colchicine treatment. Colchicine was applied on to a cotton wad placed on the growing tip between the first pair of cotyledonary leaves. Colchicine treatment was given for five hours a day for three days. During treatment the seedlings were covered with plastic cages to check evaporation losses.

After 10 to 12 days of treatment, the treated seedlings were transplanted into polythene bags filled with the mixture of soil and FYM (Farm Yard Manure) in the ratio of 1:3. They were field planted after 3 to 4 weeks. For the detection of autotetraploid sectors, besides leaf thickness, pollen fertility and size were used as criteria. Individual branches at flowering were screened for these characters. After initial screening, cytological confirmation was made from the chromosomal counts in the pollen mother cells.

3.2.3. Grafting

Reciprocal grafting was attempted between diploids of S.viarum and S.mammosum primarily to assess the effects of grafting on crossability of the two species and to study the

effect of stock on the solasodine content of berries of the scion. Reciprocal grafts were made in diploid seedlings raised in polythene bags of about 10 cm x 10 cm size filled with the mixture of soil and FYM mixture in the ratio of 1:2. Healthy and vigorous seedlings of 4 to 5 weeks of age having comparable stem thickness were selected to serve as scion and/or stock for wedge grafting. In parent seedlings used as stock, at an height of 5 to 8 cm below the growing apex an horizontal sharp cut was given. To the resulting stump, a vertical incision of about 1.5 cm long was given at the centre, to accommodate a similar sized wedge-shaped scion secured from the seedlings of the other species. The scion was secured firmly with the help of polythene strip until the union of grafted portion. The grafts were labelled and protected under partial shade in the nursery until 'graftake' and on the onset of normal growth in the scion the plastic strip was removed. The grafted plants were field planted with suitable estakes to prevent the possible damages due to wind and rain.

3.2.4. Vegetative propagation

Vegetative propagation was resorted for multiplication of parental species (Fig.2), F_1 interspecific hybrid (Fig.3) and autotetraploids. Single nodes measuring 3 to 5 cm in length were used as propagules. Cuttings were dipped in one per cent

Plate 2. Legend for photographs

Fig.2. Vegetatively propagated cuttings of S.mammosum (P_1) and S.yiarum (P_2)

Fig.3. Vegetatively propagated cutting of F_1 hybrid ($P_1 \times P_2$)

PLATE.2

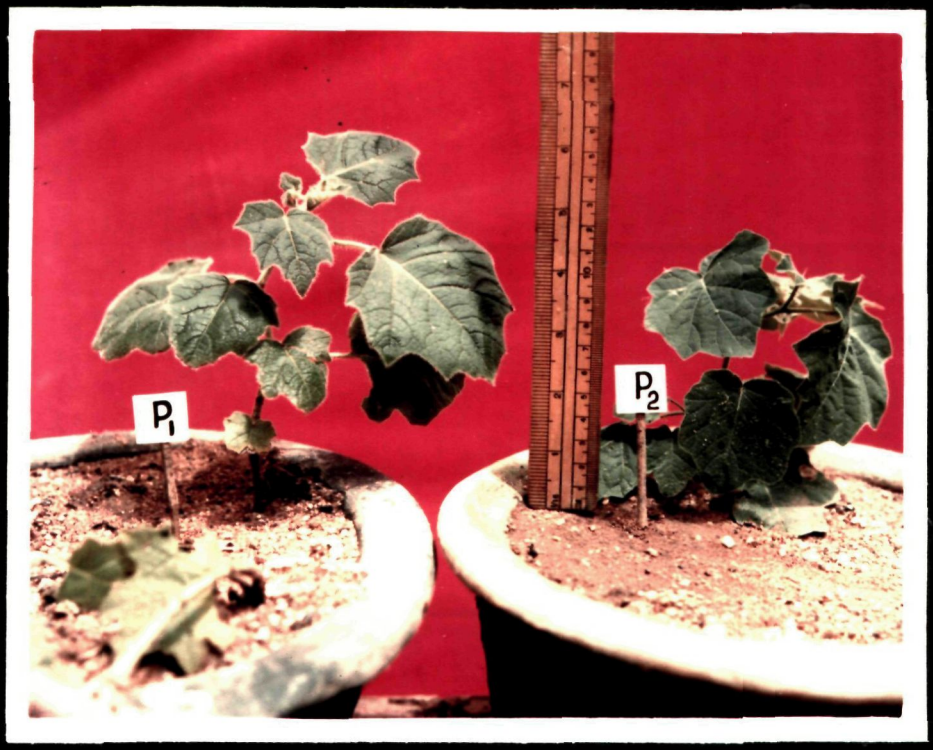


FIG.2

FIG.3

solution of the systemic fungicide, 'Blitox' for 3 to 4 minutes. After prophylactic treatment, the basal cut end was dipped in 2500 ppm of IBA solution for 15 to 20 seconds and later in Seredex-B powder. After treatment cuttings were planted in seed pans filled with medium coarse sand and rooted in mist chamber. Rooted cuttings were transplanted after about 3 to 4 weeks to polythene bags filled with the mixture of soil and FYM in the ratio of 1:2. Cuttings were allowed a months time for establishment in polythene bags prior to transplantation to 30 cm diameter pots.

3.2.5. Cytological studies

3.2.5.1. Fixation of buds and slide preparation: For meiotic analysis, flower buds of appropriate sizes were fixed in the morning hours between 9.30 a.m. to 12 noon in Carnoy's fixative consisting of 6 parts of ethyl alcohol, 3 parts of chloroform and 1 part of acetic acid. The acetic acid component was saturated with ferric acetate which served as mordant. Meiotic preparations were made generally after 24 hours of fixing and upto two weeks of fixation for the latter purpose the buds were stored in the fixative. PMCs were smeared in one per cent acetocarmine.

Destaining was effected using 45 per cent acetic acid. Desirable spread and differential staining of chromosomes was

obtained by application of pressure on the cover slip over several folds of blotting paper after warming. This process was repeated until desirable spread and differentiation of chromosomes was secured. Slides were temporarily sealed with paraffin wax. Camera lucida drawings and photomicrographs were made from temporary preparations. The slides were made permanent after removal of paraffin sealing and seperation of cover slip was achieved by inverting the slide in a petri dish containing normal butyl alchohol. Following seperation, the cover slip it was remounted on the slide using BDH-mounting medium.

3.2.5.2. Pollen fertility: Pollen fertility was assessed based on the pollen stainability in one per cent acetocarmine stain. Stained pollen grains were scored as fertile and unstained ones as sterile. The values were expressed in percentage.

3.2.5.3. Study of pachytene chromosomes: For this study, Camera lucida drawings were made from the temporary preparations at a table magnification of X1750. Measurements of the length of the light-staining and dark-staining regions in the arms were made with the help of thread.

3.2.5.4. Arm length and total length: In S.viarum, light stained and dark stained regions of the differentiated

chromosomes were separately measured. In calculating the total length centromere region was excluded.

3.2.5.5. Arm ratio: The arm ratio was expressed as the ratio of short arm to long arm of a chromosome. Chromosomes having the arm ratio values from 0.75 and above were classified as median, and from 0.25 to 0.74 as sub-median and less than 0.24 as sub-terminal.

3.2.5.6. Photomicrography: Photomicrographs were exposed using olympus PM-60 camera adopted on Olympus microscope with 15X eye piece under oil immersion (X100). Positives were made at suitable magnifications.

3.2.6. Leaf flavonoid analysis

The thin layer chromatography method described by Dass and Nyborn (1967) was used for the study of leaf flavonoids. Leaf flavonoid analysis was carried out in diploids of S.viarum, S.[redacted] and their interspecific hybrid.

Dried leaves of S.viarum, S.[redacted] and F_1 hybrid were powdered separately and 0.2 g of the sample was used for solvent extraction with 2.0 ml of acidic methanol (1% HCl in Methanol). 18.5 g of Cellulose powder (MN-300) was dissolved in 135 CC of distilled water and the slurry was coated on the glass plates

for a thickness of about 0.35 mm. Three per cent formic acid was used in first direction and amyl alcohol, acetic acid and water in proportion of 10:6:5 was used in the second direction. The flavonoid spots were intensified with chromatogenic spray containing one per cent sodium hydroxide and one per cent aluminium chloride in methanol. A qualitative comparison of flavonoids was made based on the presence or absence of spots.

3.2.7. Solasodine analysis

The solasodine content of berry samples was estimated by adopting the method described by Crusena et al. (1965). The values are expressed in percentage.

3.2.8. Statistical analysis

Comparision of mean values for characters in diploids, autotetraploids and interspecific hybrids were done using students 't' test (Panse and Sukhatme, 1967).

EXPERIMENTAL RESULTS

IV. EXPERIMENTAL RESULTS

4.1. CYTOLOGY

4.1.1. Solanum viarum

4.1.1.1. Meiosis

Meiosis was studied commencing from pachytene stage onwards.

4.1.1.1.1. Pachytene: At pachytene, the chromosome complement resolved itself into haploid set of 12 bivalents exhibiting complete pairing. Though several bivalents could be traced from end to end in a number of PMCs, in only one PMC all the 12 bivalents could be analysed (Figs.4 and 4a). As a result, the computation of cytological values for chromosomes of the haploid complement was based on variable number of bivalents ranging from a minimum of six in chromosome III to a maximum of 16 in chromosomes VII and IX.

All the bivalents belonged to differentiated group characterised by darkly stained heterochromatic regions located on either side of the centromere and light-stained euchromatic regions at the distal ends. The distal light-stained regions was absent in the short arm of three bivalents. These included two non-nucleolar chromosomes and the nucleolus-associated bivalent. The relative contribution of light-stained

Plate 3. Legend for photographs

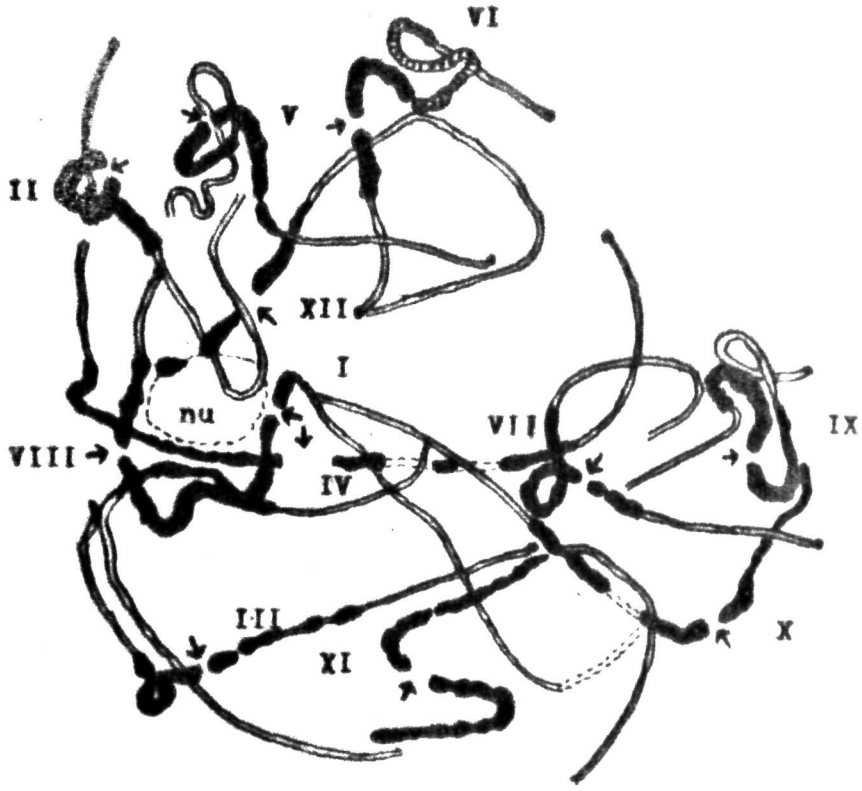
Fig.4. A PMC at pachytene with well spread bivalents x 3000

PLATE.3

FIG.4

Explanatory diagram for figure in Plate 3

Fig.#a. A PMC at pachytene with well spread bivalents x 3000



Explanatory diagram for figure in Plate 3.

and dark-stained regions to the arms and to the chromosome varied. The darkly stained regions in many bivalents could be resolved into distinct chromomeres which aided in the identification of individual bivalents. The light-stained regions were found to be more amenable to stretching and by virtue of their poor stainability posed difficulty in tracing them along their length. Some of the bivalents were found to possess telochromomeres at the distal end of the arms.

Based on the position of centromere, the bivalents could be categorized into four median (arm ratio 0.75 and above), seven sub-median (arm ratio of 0.25 to 0.74) and one sub-terminal, (arm ratio of 0.24 and less) chromosome represented by a nucleolar chromosome.

The pachytene bivalents ranged in length from 22.35 μ to 58.14 μ. In the identification of bivalents in addition to total length, arm ratio, differences in the length of dark-and light-stained regions of the arms as well as the chromomere pattern, nucleolar association, absence of distal light-staining regions and chromomeres were taken into consideration. Data on the above cytological values of the 12 bivalents are summarized in Table 1. Individual bivalents were numbered from I to XII in the descending order of their length. The diagnostic characteristics of chromosomes is given below:

Table 1. Cytological values of pachytene chromosomes of Solanum viarum Dunal (2n=24)

Chromosome number	Length of Short Arm(μ)			Length of Long Arm(μ)			Length of Chromosome(μ)			Arm ratio (SA/LA)	No.of bivalents analyzed
	DSR	LSR	Total	DSR	LSR	Total	DSR	LSR	Total		
I	5.31±1.02	14.18±1.30	20.49±0.91	11.36±1.57	26.29±1.27	37.65±1.24	17.67±1.49	40.47±1.30	58.14±2.01	0.55±0.10	8
II	9.94±0.98	14.25±1.37	24.19±1.04	6.33±0.58	21.78±1.61	28.11±1.27	16.27±0.66	36.03±2.04	52.30±1.98	0.86±0.05	9
III	4.65±0.36	10.35±1.00	15.00±0.92	8.26±1.16	18.33±0.65	26.59±1.06	12.91±1.39	28.68±1.35	41.59±1.93	0.56±0.02	6
IV	8.34±0.77	7.69±0.78	16.03±1.09	9.17±0.71	10.41±0.95	19.58±1.35	17.51±1.11	18.10±1.43	35.61±2.37	0.82±0.02	11
V	8.74±0.68	7.74±0.75	16.48±1.26	9.31±0.66	9.45±0.83	18.76±1.64	18.05±1.32	17.19±1.49	35.24±2.43	0.87±0.05	12
VI	7.75±0.24	8.78±0.56	12.03±0.67	9.40±0.64	13.37±0.60	22.77±1.80	12.65±0.64	22.15±0.84	34.80±1.35	0.53±0.05	10
VII	3.38±0.53	6.09±0.88	9.47±0.97	12.18±0.93	9.16±0.99	21.34±0.98	17.56±1.01	15.25±1.44	32.81±1.68	0.54±0.04	16
VIII	4.48±0.72	10.50±0.78	14.98±0.90	9.69±0.96	7.48±0.68	17.18±1.70	14.17±1.66	17.99±0.99	32.16±2.20	0.88±0.08	7
IX	5.50±0.44	6.13±0.67	11.63±0.73	9.48±0.65	10.72±0.90	20.20±1.20	14.98±0.86	16.85±1.31	31.83±1.74	0.60±0.02	16
X	11.80±0.46	-	11.80±0.46	[illegible]	[illegible]	[illegible]	19.04±1.86	11.80±1.02	30.84±1.86	0.61±0.08	10
XI	7.93±0.61	-	7.93±0.61	10.07±0.87	9.26±1.29	19.33±1.85	18.00±1.29	9.26±1.29	27.26±2.28	0.43±0.14	12
XII*	3.54±0.18	-	3.54±0.18	9.69±0.97	9.12±0.46	18.81±1.55	13.23±0.92	9.12±0.56	22.35±1.64	0.19±0.02	12

DSR=Dark-stained region; LSR=Light-stained region; *Nucleolar associated chromosome; SA=Short arm; LA=Long arm; ±Standard error.

Chromosome I: It is the longest chromosome of the complement with an average length of 58.14 /u (Figs.5 and 5a). It has sub-median centromere and an average arm ratio of 0.55. The distinct feature that helps in identifying this chromosome, is the presence of long light-stained regions in both the arms accounting for nearly two third of the chromosomal length. In both the arms light stained region is nearly twice the length of dark-stained region. The light-stained region of both the arms terminate in telochromomeres. The presence of long light-stained region in this chromosome proved a serious deterrant in the analysis of the chromosome woven in well spread cells. As a result only PMCs wherein the bivalents occurred totally separated from other bivalents were considered for securing the data on cytological values.

Chromosome II: This is the longest of the four median chromosomes (52.30 /u) the other chromosomes being chromosomes IV, V and VIII. In addition to its total length, the presence of longer light-stained region as compared to dark-stained regions in the chromosome offers a ready criterion for its identification. This chromosome like chromosome VIII has unequal distribution of dark-staining regions in two arms.

Chromosome III: This is a sub-median chromosome measuring an average length of 41.59 /u. It is the second longest chromosome

of the sub-median group. The total light-staining region of this chromosome exceeds that of dark-staining region and thus this chromosome bears similarity to two other sub-median chromosomes viz., I and VI. It can be distinguished from chromosome No.I by virtue of its shorter length. The distinction of chromosome III from chromosome VI is reliably made on the distribution of light-staining region in the two arms.

Chromosome IV: This is one of the four median chromosome of the complement with a mean length of 35.61 /u. This chromosome, like V (and unlike VIII and II), has equal lengths of dark-staining regions in the arms. The diagnostic characteristic of the chromosome is the presence of distinct medium sized chromomeres in one of the arms. Telechromomeres occurs in both the arms.

Chromosome V: This is one of the four median chromosomes measuring an average length of 35.24 /u. The total dark-stained region of this chromosome equals that of the light-stained region, a diagnostic feature it shares with two other median chromosomes, viz., VIII and IV. This chromosome is further similar to chromosome IV in the equal distribution of proximal dark-stained region in the two arms. However, the diagnostic feature of this chromosome is the presence of macrochromomeres in both the arms in the proximal segment with

a gradual decrease in size of the chromomeres towards distal ends. Telochromomeres are present in both the arms.

Chromosome VI: It belongs to the sub-median group and measures an average, 34.80 /u. Like chromosome I and III, the total light-staining region of this chromosome exceeds that of dark-staining region. Chromosome VI is shorter than I and III and is distinguished from the latter chromosomes by the lower ratio of light-staining region in the arms.

Chromosome VII: This is a sub-median chromosome (arm ratio 0.54) measuring 32.18 /u on an average. Like chromosome IX, this chromosome is characterised by the presence of equal lengths of dark-and light-stained regions. The dark-stained segments of this chromosome, as in chromosome IX, are unequally distributed in the arms with the longer segments located in the long arm. The occurence of two distinct segments differing in their stability in the dark-stained regions of the long arm aids in its consistent identification. These two segments include a deep stained segment proximal to the centromere and a distal segment characterized by relatively light-stained chromomeres adjoining it. Telochromomeres are present in both the arms.

Chromosome VIII: It is one of the four median chromosomes and measures an average length of 32.16 /u. Like chromosome V and IV it is recognised by the presence of nearly equal lengths of

dark-and light-staining regions. The major criterion for distinguishing chromosome VIII from V and IV is the presence of unequal segments of dark-stained regions in the arms. Chromosome VIII is also characterized by the presence of large chromomeres in both the arms.

Chromosome IX: It is one of the two sub-median chromosomes (31.83 /u) the other being chromosome VII characterized by nearly equal lengths of dark-and light-staining regions in the chromosome (Figs.6 and 6a). The short arm is characterized by a distinct telochromomere and presence of three or four distinct macrochromomeres. The dark-staining regions of the long arm exceeds that of short arm. The absence of relatively light-stained chromomeres in the long arm distinguishes this chromosome from VII.

Chromosome X: Like chromosome XI, this chromosome also possesses heteropycnotic short arm. This arm can be resolved into a proximal region consisting of deeply stained segments and relatively light stained distal regions. The latter, unlike in chromosome XI, is destitute of any dark-staining chromomere. A further characteristic that render its consistent distinction from chromosome XI, is the presence of relatively smaller chromomeres in the long arm. The chromosome measures 30.84 /u in length.

Chromosome XI: This is one of the two non-nucleolar bivalent the other being chromosome X characterized by the presence of heteropycnotic short arm, which however, can be delineated into a proximal deeply stained region and a distal relatively light-stained region. The latter is characterized by two distinct chromomeres which enable its distinction from chromosome X. Additionally, chromosome XI differs from chromosome X by the presence of macrochromomere containing segment in the long arm. In chromosome X the chromomeres of the long arm are relatively smaller than their counter parts in the short arm. This chromosome measures 27.26 /u in length.

Chromosome XII: This is the shortest chromosome of the complement (22.35 /u). Its readily recognised by its nucleolar association (Figs.7, 7a, 8 and 8a). The nucleolar organiser region is located sub-terminally in the short arm which is totally devoid of distal light-stained region. The long arm consists of equal length of dark-and light-staining regions. This chromosome recorded the lowest arm ratio (0.19). In a number of PMCs the distal regionsoof the short arm of this chromosome was unpaired. A telochromomere was present in the long arm.

An idiogram was constructed incorporating all the above diagnostic characters including length of dark-stained and light-stained regions and arm ratio (Fig.9).

Plate 4. Legend for photomicrographs of pachytene stage in *S.viarum* x 3000
(→ indicates centromere; nu=nucleolus)

Fig.5. Chromosome-I

Fig.6. Chromosome-IX

Fig.7. Chromosome-XII

Fig.8. Chromosome-XII

PLATE.4

FIG.5

FIG.6

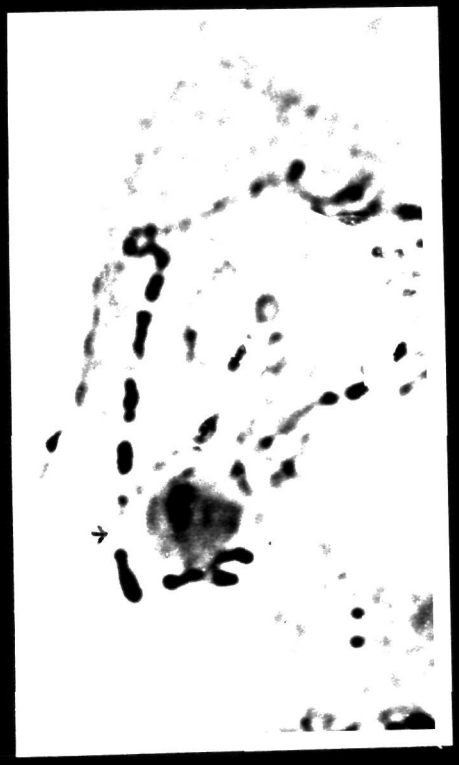


FIG.7

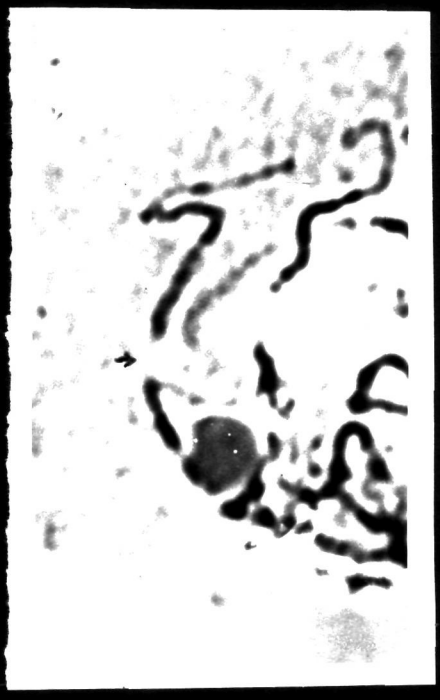


FIG.8

Explanatory diagrams for figures in Plate 4

Fig.5a. Chromosome-I

Fig.6a. Chromosome-IX

Fig.7a. Chromosome-XII

Fig.8a. Chromosome-XII

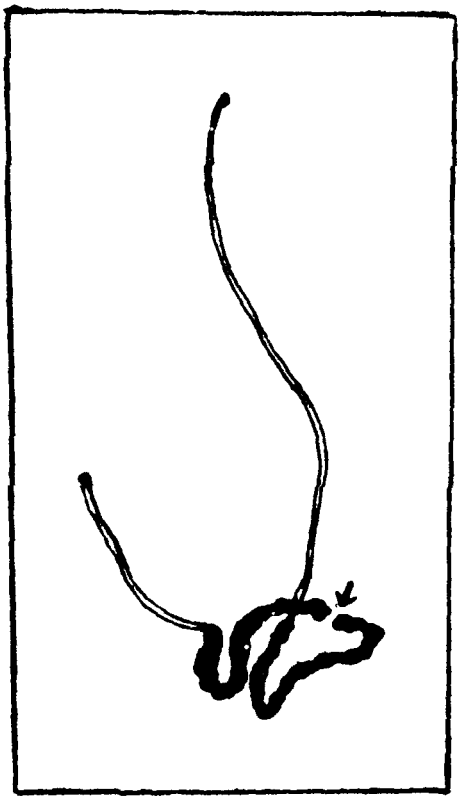


FIG.5a

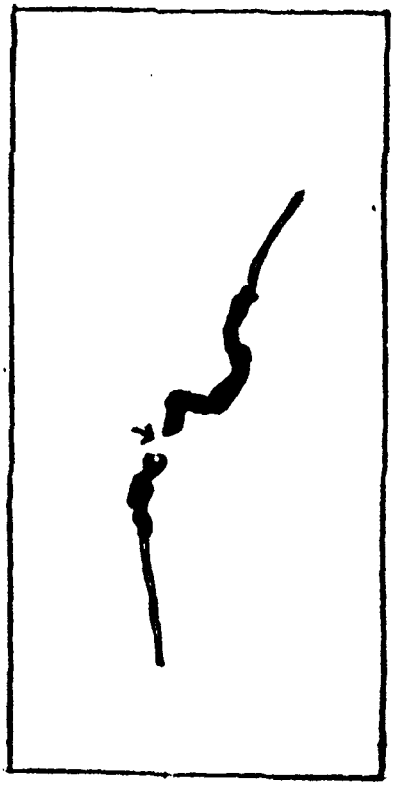


FIG.6a

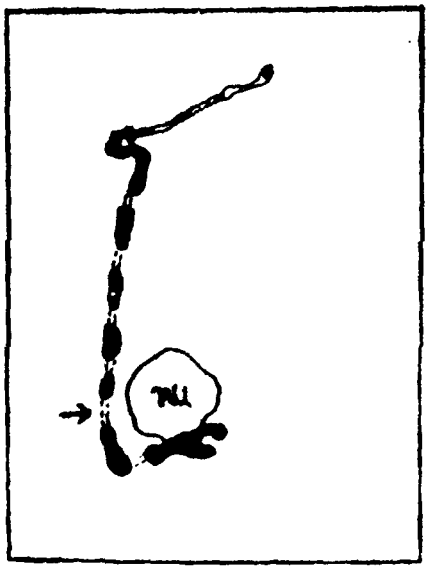


FIG.7a

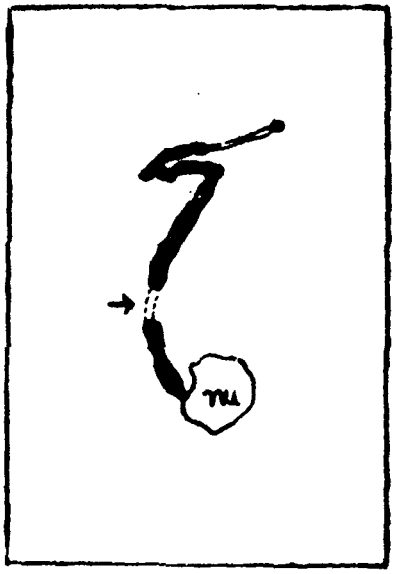


FIG.8a

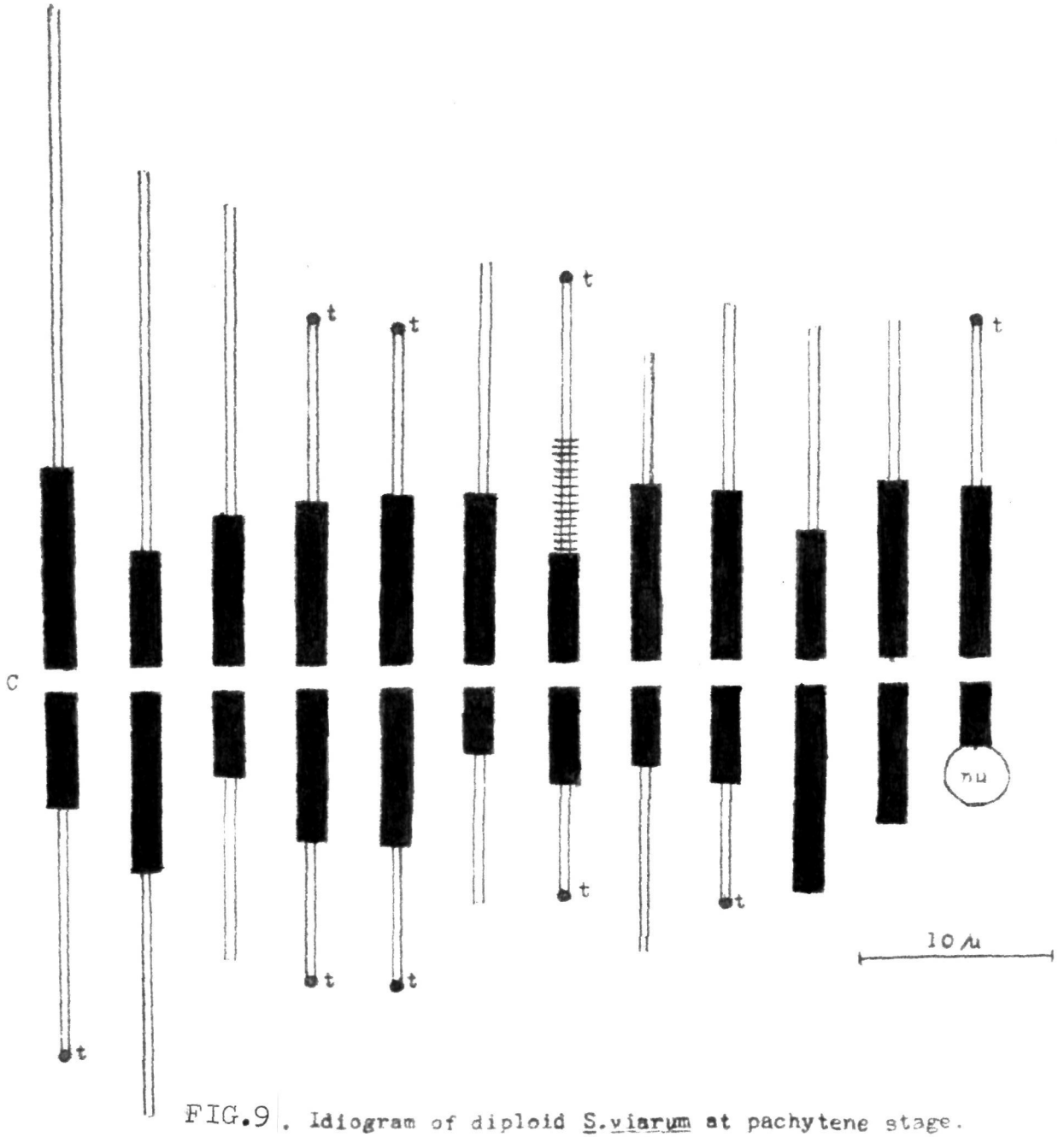


FIG.9. Idiogram of diploid S.viarum at pachytene stage.

4.1.1.1.2. Diakinesis: Twelve bivalents were observed at this stage. One bivalent was found to be associated with the nucleolus in all the PMCs observed.

4.1.1.1.3. Metaphase I: Chromosomal association at this stage consisted of bivalents only (Figs.10 and 10a). A maximum number of two chiasma per bivalent was observed per cell. Mean number of chiasma per cell was 18.5 and per paired bivalent was 1.5 (Table 2).

4.1.1.1.4. Later stages: Subsequent stages of meiosis were normal. At anaphase-I, disjunction of chromosomes was normal without the formation of bridge or laggards. Distribution of the chromosomes to the poles was equal at telophase-I and metaphase-II (Fig.11). At microspore tetrad stage, four microspores were observed.

Pollen fertility was 86.2 per cent. The mean diameter of the pollen grain was 27.2 μ (Fig.12) and the range was 24 μ to 32 μ.

4.1.1.2. Mitosis

In the meiotic preparation, somatic cells at mitotic prophase stage were observed. The presence of differentially stained segments were evident in the arms of some of the chromosomes (Fig.13).

Table 2. Chiasma frequency in bivalents at metaphase-I stage in diploid Solanum viarum (2n=24)

Number of chiasmata			Total chiasma per cell	Frequency (number)
2	1	0		
10	2	-	22	3
9	3	-	21	3
9	2	1	20	1
8	4	-	20	6
8	3	1	19	1
7	5	-	19	2
6	6	-	18	5
6	5	1	17	1
5	7	-	17	2
5	6	1	16	1
4	8	-	16	4
4	7	1	15	1
3	9	-	15	2
3-10	2-9	0-1	15-22 Range	Total 32
6.66	5.19	-	18.50 Mean	

Plate 5. Legend for photomicrographs of meiotic stages in diploid *S. virgatum*

Fig.10. Metaphase-I showing 12 bivalents x 2500

Fig.11. Metaphase-II showing 12+12 chromosome distribution to daughter nuclei x 2000

Fig.12. Pollen grains x 1000

Fig.13. Mitotic prophase of anther wall cells x 1000
→ indicates distal light-staining region

PLATE.5

FIG.10

FIG.11

FIG.12

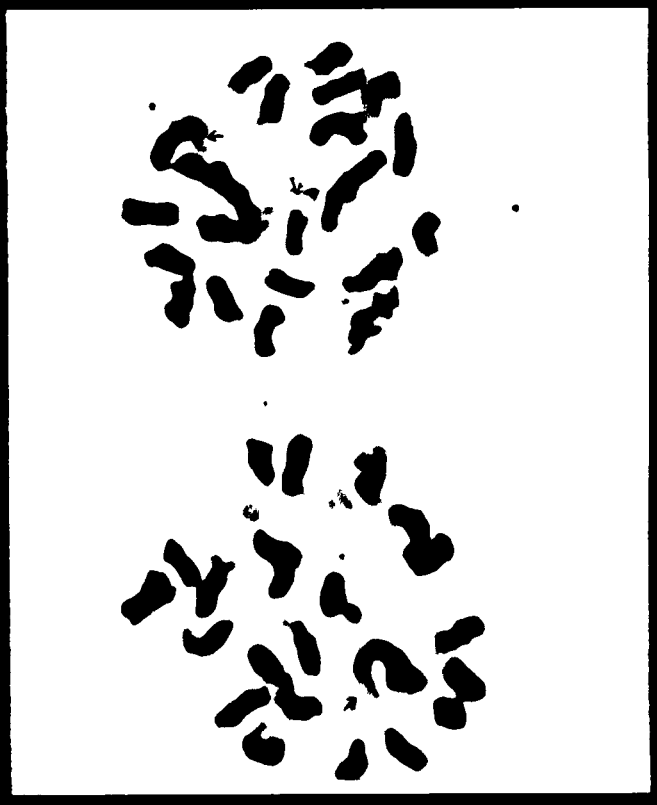


FIG.13

Explanatory diagram for figure in Plate 5

Fig.10a. Metaphase showing 12 bivalents

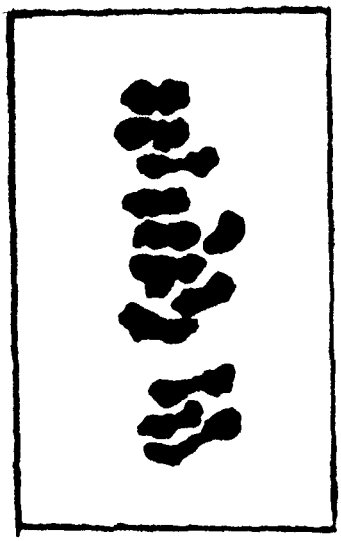


FIG. 10a

4.1.2. Solanum nemoscum

4.1.2.1. Meiosis

The course of meiosis was studied commencing from pachytene. The haploid chromosome number of n=11 was confirmed at metaphase-I.

4.1.2.1.1. Pachytene: The pachytene chromosomes were undifferentiated and possessed nearly uniform stained segments/ chromomeres along their entire length (Fig.14). A nucleolus-associated bivalent with interstitial nucleolar organizing region was observed (Fig.15). The bivalents were readily stained but were not amenable for spreading. No bivalent could be traced from end to end. The centromere in some bivalents could be delineated by the presence of deeply stained regions on either side.

4.1.2.1.2. Later stages: At diakinesis, a bivalent was found associated with the nucleolus. At diakinesis and metaphase-I (Fig.16), chromosomal associations consisted of eleven bivalents. At anaphase-I (Fig.17) and anaphase-II, normal disjunction of chromosomes to the poles was observed in all the PMCs examined. At telophase-I and telophase-II, two and four groups of nuclei respectively were formed. Four microspores were observed in majority of the PMCs at tetrad stage.

Plate 6. Legend for photograph

Fig.14. Pachytene stage in diploid *S.mammosum* x 3000

PLATE.6

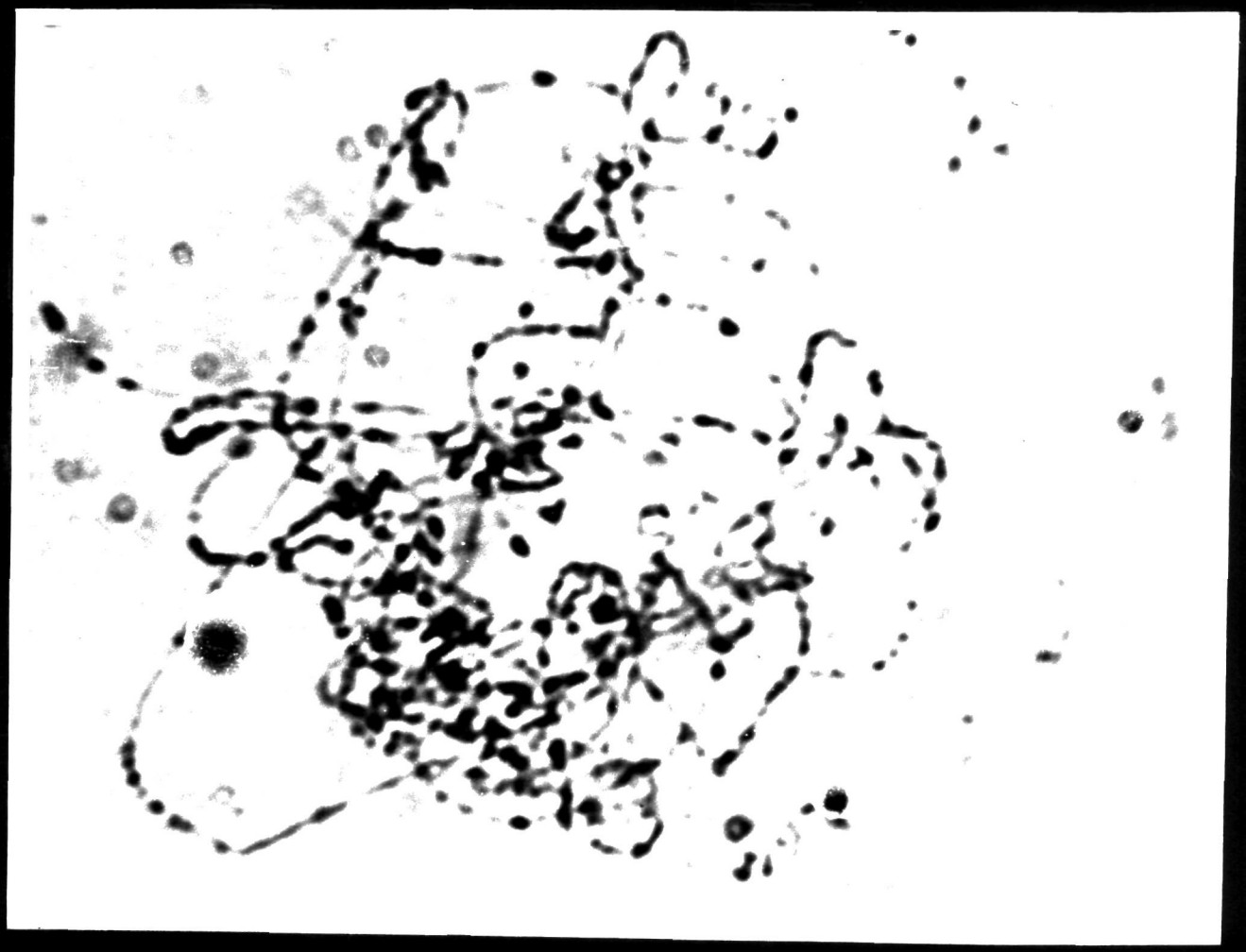


FIG. 14

Plate 7. Legend for photomicrographs of meiotic stages in diploid S.mammosum

Fig.15. Nucleolus associated bivalent x 3000 (nu = nucleolus)

Fig.16. Metaphase-I showing 11 bivalent x 2500

Fig.17. Anaphase-I showing normal chromosomal disjunction x 2000

PLATE.7

FIG. 15

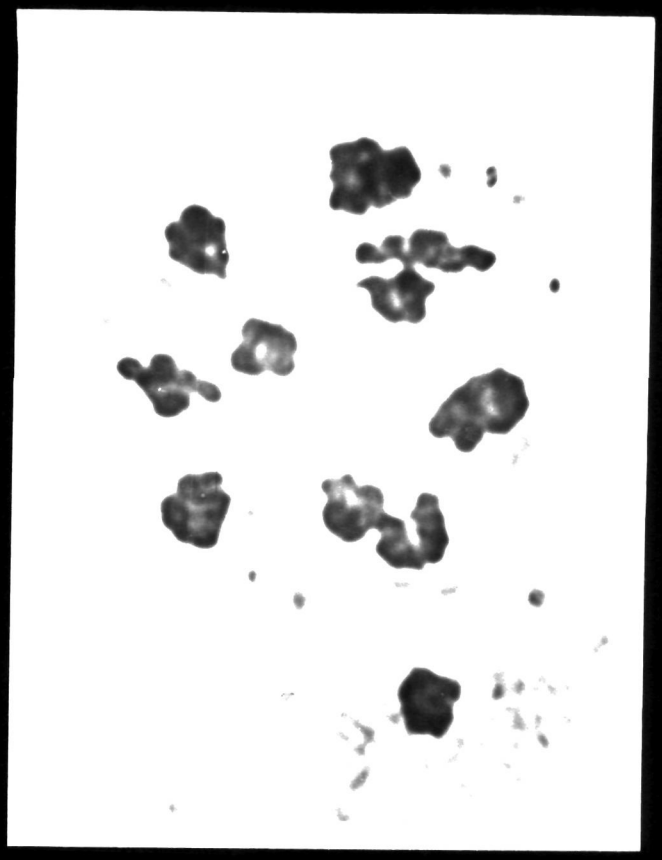


FIG. 16

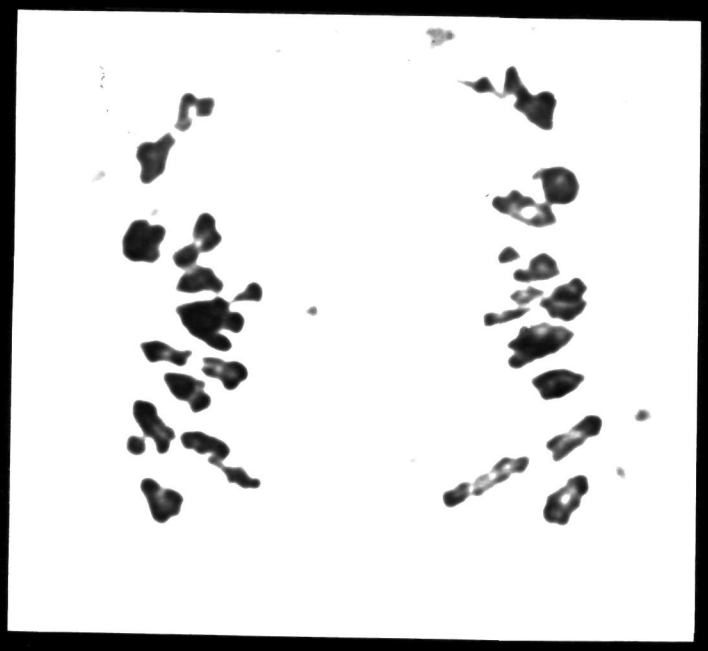


FIG. 17

The average pollen fertility was 87.2 per cent and the mean pollen diameter, is 32.6 μ (Fig.18).

4.1.2.2. Mitosis

As in S.viarum, somatic cells at mitotic prophase (Fig.19) and mitotic metaphase (Fig.20) were encountered in meiotic preparations. The chromosomal counts at both stages confirmed 2n=22. At prophase, no differentially stained chromosomes were observed. The presence of two nucleolus associated chromosomes was observed (Figs.19 and 21). A visual comparision of the mitotic chromosomes at pachytene stage in S.viarum and S.mammosum revealed the larger size of the latter.

4.2. AUTOTETRAPLOIDY

4.2.1. Solanum viarum

4.2.1.1. Morphology

The study of morphological characters and meiosis was conducted at C_2 generations. The autotetraploids were characterised by thick dark green leaves (Fig.22).

Comparision of the mean values of fourteen characters of diploid and autotetraploid S.viarum were made using 't' test. However, mean values of characters such as specific leaf weight,

Plate 8. Legend for photomicrographs of meiotic stages in diploid *S.*

Fig.18. Pollen grains x 1000

Fig.19. Mitotic prophase in anther cell wall showing two nucleolus associated chromosome x 2500

Fig.20. Mitotic chromosomes of metaphase in anther cell x 2500

Fig.21. Nucleolus associated chromosomes at mitotic prophase in anther cells x 3000

PLATE.8

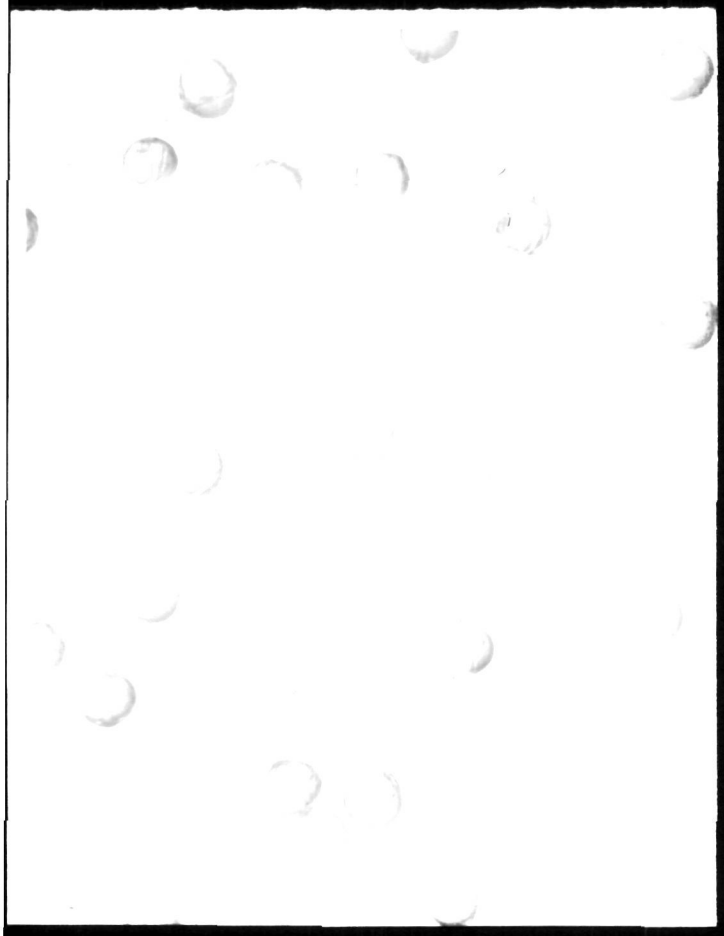


FIG. 18

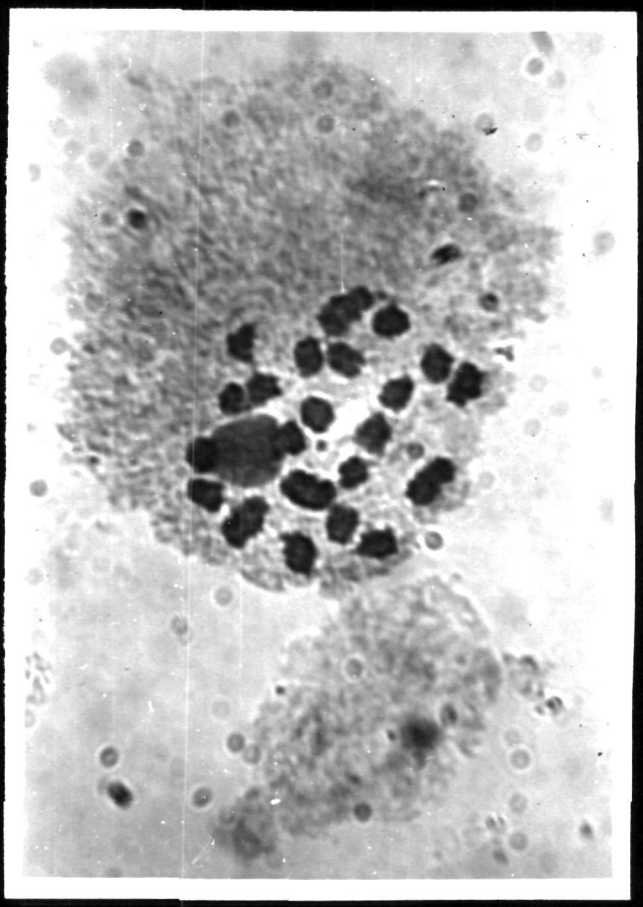


FIG. 19

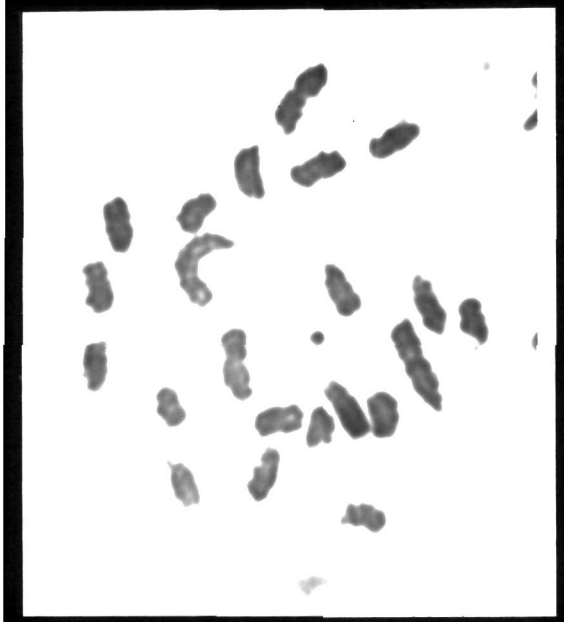


FIG.20

FIG.21

percentage of exerted, medium and short styled flowers, pollen fertility and pollen size in tetraploids were compared for their percentage increase or decrease over diploid values. The data are presented in Table 3.

Among the characters for which mean values were compared for significance of differences, mean value of diploid was higher than autotetraploid for petiole length, fruit diameter (Fig.23), fruit weight and average number of seeds per fruit (Fig.24). Autotetraploids recorded higher mean values over diploids for leaf length, leaf breadth, number of spines on dorsal and ventral leaf surfaces, length and breadth of petal, length of anther and 100 seed weight. Diploids and tetraploids were on par for length of exerted style.

Specific leaf weight and pollen size, tetraploid registered 40.9 and 41.7 per cent increase, respectively, over diploids. Pollen fertility recorded reduction from 86.2 per cent in diploids to 54.3 per cent in tetraploids. Diploids had higher number of exerted styled and short styled flowers than tetraploids. However, medium styled flowers were observed in autotetraploids only and accounted to 11.94 per cent of the total number of flowers produced.

4.2.1.2. Meiosis

Meiosis in PMCs was studied commencing from diakinesis onwards. The chromosome number of the autotetraploid plants was determined as 2n=48 at metaphase-I.

Table 3. Comparision of means of morphological characters in diploids and auto-tetraploids of Solanum viarum

Character	Diploid	Tetraploid	't' value/% value
1. Leaf length(cm)	8.6±0.2(41)	11.4±1.6(43)	52.1*
2. Leaf breadth(cm)	9.5±0.2(41)	14.0±1.8(43)	14.7*
3. Petiole length(cm)	6.3±0.8(25)	5.1±0.9(25)	4.8*
4. Specific leaf weight	0.0220	0.0310	+40.9
5. Spine number(leaf)			
a) Dorsal	5.5±1.1(25)	10.6±1.5(40)	14.4*
b) Ventral	7.8±1.1(25)	13.4±1.9(40)	13.4*
6. No.of flowers/inflorescence	3.2±0.9(25)	3.6±2.3(116)	0.8 NS
7. Petal length(mm)	10.5±0.8(16)	15.2±0.3(14)	17.6*
8. Petal breadth(mm)	2.0±0.2(16)	3.7±0.3(13)	17.5*
9. Length of anther(mm)	7.0±0.2(13)	7.5±0.5(33)	3.3*
10. Flowers with			
a) Exerted style(%)	96.6	86.6	-10.3
b) Medium style(%)	-	11.9	-40.8
c) Short style(%)	3.4	2.0	-40.8
11. Length of exerted style(mm)	8.5±0.8(22)	9.1±0.3(14)	1.1 NS
12. Fruit diameter(mm)	28.8±0.3(32)	22.4±0.5(56)	23.1*
13. Fruit weight(g)	8.9±1.1(55)	6.5±0.7(22)	9.6*
14. No.of seeds per fruit	438.1±16.7(11)	54.6±8.4(65)	118.7*
15. 100 seeds weight(mg)	256.6±32.1(15)	495.1±3.5(18)	31.4*
16. Pollen fertility(%)	86.2	54.3	-37.1
17. Pollen diameter(μ)	27.2	38.5	+41.7

NS=Non-significant; *Significant at 1 per cent; ±Standard error.
Values indicated within the parenthesis refer to number of observations.

Plate 9. Legend for photographs

Fig.22. Established autotetraploid plant of S.viarum

Fig.23. Comparision of mature berries of diploid (2n) and autotetraploid (4n) S.viarum

Fig.24. Comparision of longitudinal section of diploid (2n) and autotetraploid (4n) fruits of S.viarum

PLATE 9

FIG.22

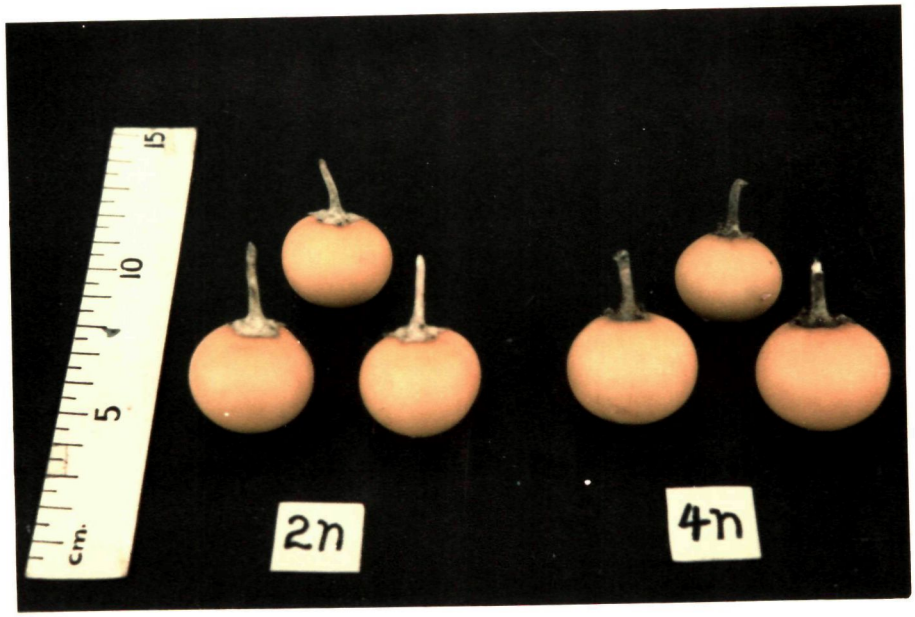


FIG.23

FIG.24

4.2.1.2.1. Diakinesis: The study of chromosome configuration was restricted to nucleolar chromosome only. A total of 31 PMCs were examined. Two bivalents were associated with nucleolus.

4.2.1.2.2. Metaphase-I: The chromosome association at this stage is summarized in Table 4. In 95.4 per cent of the PMCs total pairing of chromosomes was observed (Figs.25 to 28). Incomplete pairing consisting of 46 paired chromosomes and 47 paired chromosomes (Fig.29) occurred in 3.2 and 1.6 per cent of the PMCs examined, respectively. Though chromosomal configuration consisted of quadrivalents, trivalents, bivalents and univalents, quadrivalents and bivalents were predominent (Figs.25 to 28 and 25a to 28a). Quadrivalents ranging from four to ten were found in all the PMCs examined. PMCs with seven and eight (Fig.27) quadrivalents were more frequent and accounted, respectively, for 25.0 and 26.6 per cent of the PMCs examined. Quadrivalents were mostly of closed ring type. One (Fig.29) or two trivalents occurred in 3.2 per cent of the PMCs. Bivalents ranged from 4 to 16. In 51.6 per cent of the PMCs examined, 8 or 10 bivalents (Figs.27 and 29) were observed. One or two univalents were recorded in 4.7 per cent of PMCs examined.

Precocious movement of one to three chromosomes towards either one or both poles occurred in 18.5 per cent of the PMCs at metaphase-I (Table 5). In 13.1 per cent of the PMCs movement

Table 4. Chromosome association at metaphase-I in PMCs of autotetraploid Solanum viarum (2n=48)

IV	III	II	I	Total No.of paired chromosomes	Frequency	
					Number	Percentage
4	-	16	-	48	1	1.6
5	-	14	-	48	12	18.7
6	-	12	-	48	9	14.1
7	-	10	-	48	16	25.0
8	-	8	-	48	17	26.6
9	-	6	-	48	5	7.8
10	-	4	-	48	1	1.6
6	1	10	1	47	1	1.6
7	-	9	2	46	1	1.6
8	2	4	2	46	1	1.6
4-10	0-2	4-16	0-2	Range	Total 64	
6.91	0.5	10.08	0.08	Mean/cell		

Table 5. Precocious movement of chromosomes to poles at metaphase-I in PMCs of autotetraploid Solanum viarum

Frequency	Normal	Number of chromosomes in poles					Total
	0-0	0-1	1-1	2-0	2-1	3-0	
Number	181	18	9	9	6	2	222
Percentage	(81.54)	(8.10)	(2.70)	(4.05)	(2.70)	(0.90)	-

Table 6. Chromosomal distribution to daughter nuclei at metaphase-II in PMCs of autotetraploid Solanum viarum

Chromosomal distribution	Frequency	
	Number	Percentage
24-24	128	94.12
23-25	8	5.88

Plate 10. Legend for photomicrographs of metaphase-I stages in autotetraploid S.viarum x 2500

Fig.25. Metaphase-I showing $10^{IV}+4^{II}$ association

Fig.26. Metaphase-I showing $9^{IV}+6^{II}$ association

Fig.27. Metaphase-I showing $8^{IV}+8^{II}$ association

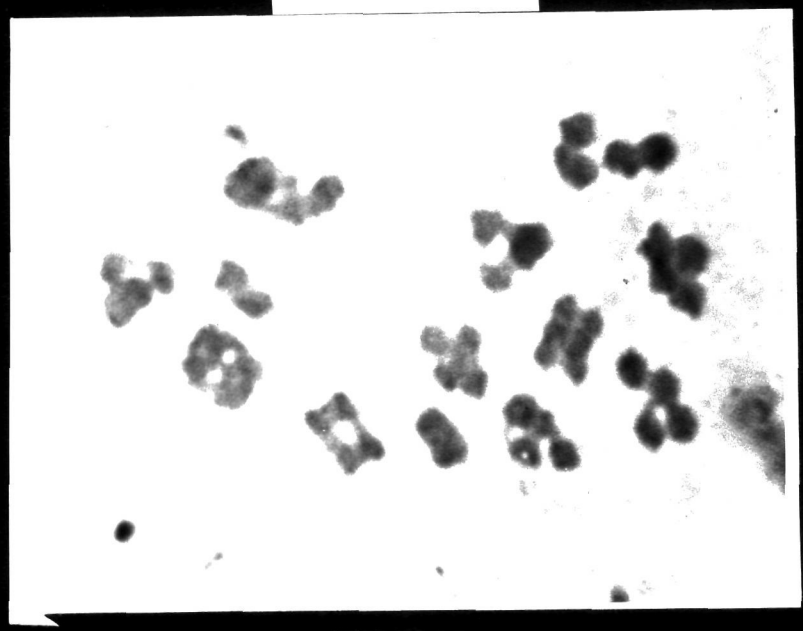


FIG.25

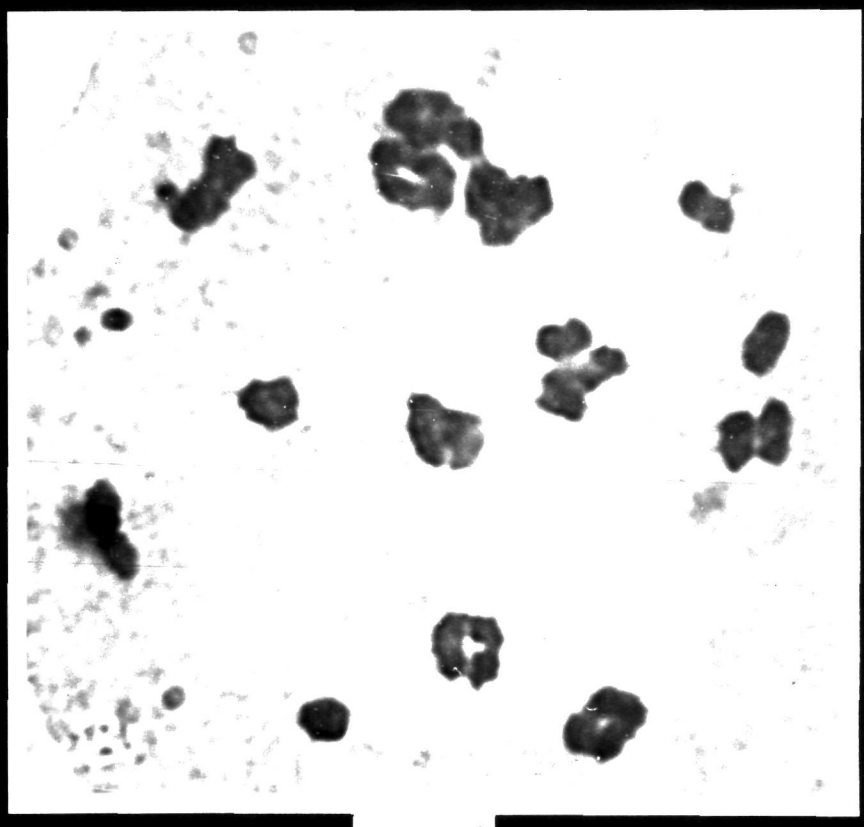


FIG.26

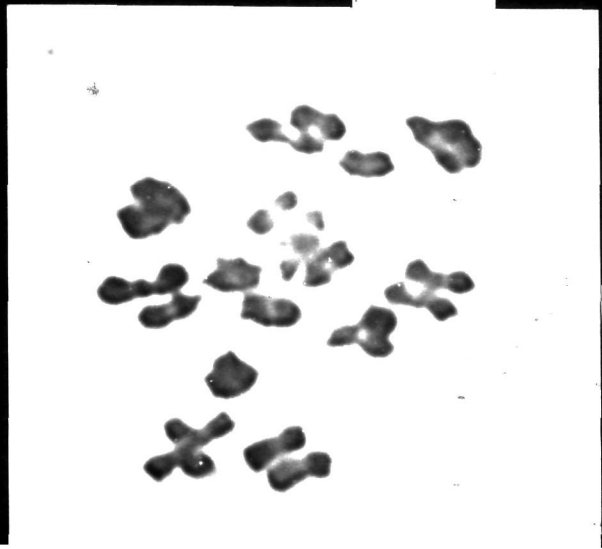


FIG.27

Explanatory diagrams for figures in Plate 10
(IV - in stripes; II - in shade)

Fig.25a. Metaphase-I showing $10^{IV}+4^{II}$

Fig.26a. Metaphase-I showing $9^{IV}+6^{II}$

Fig.27a. Metaphase-I showing $8^{IV}+8^{II}$

FIG.25a

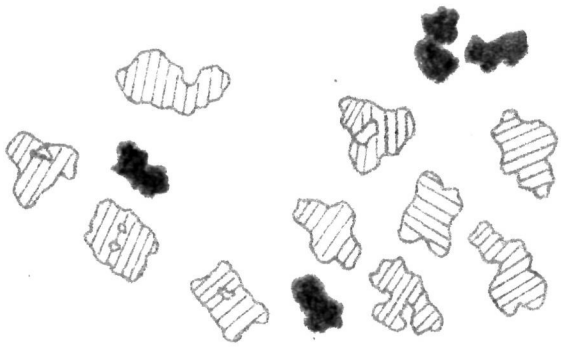


FIG.26a

FIG.27a

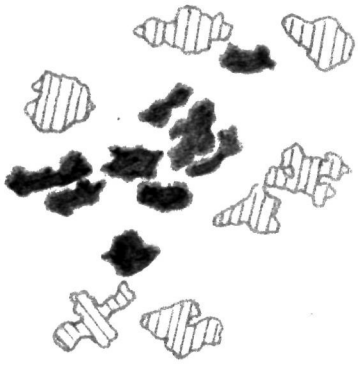


Plate 11. Legend for photomicrographs of metaphase-I stages in autotetraploid S.viarum x 2500

Fig.28. Metaphase-I showing $6^{IV}+12^{II}$ association

Fig.29. Metaphase-I showing $6^{IV}+1^{III}+10^{II}+1^{I}$ association

Fig.30. Metaphase-I showing precocious movement of chromosomes to both poles

PLATE 11

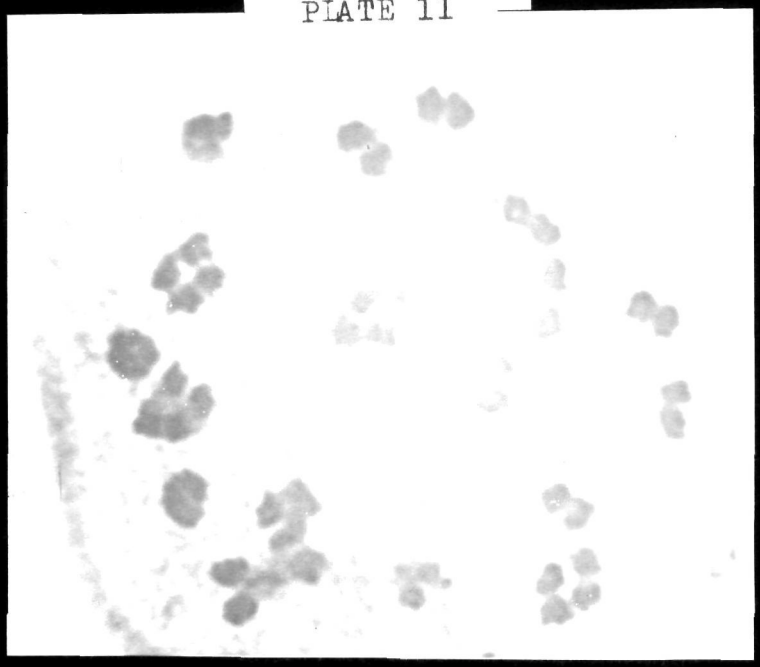


FIG.28

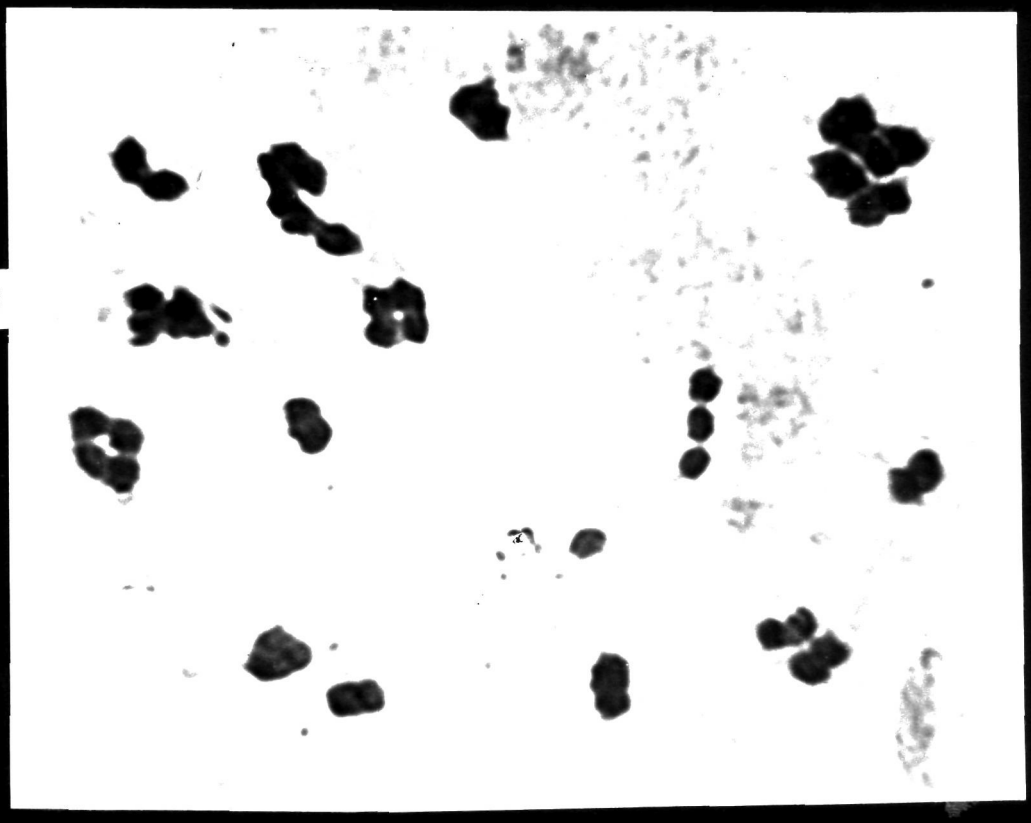


FIG.29

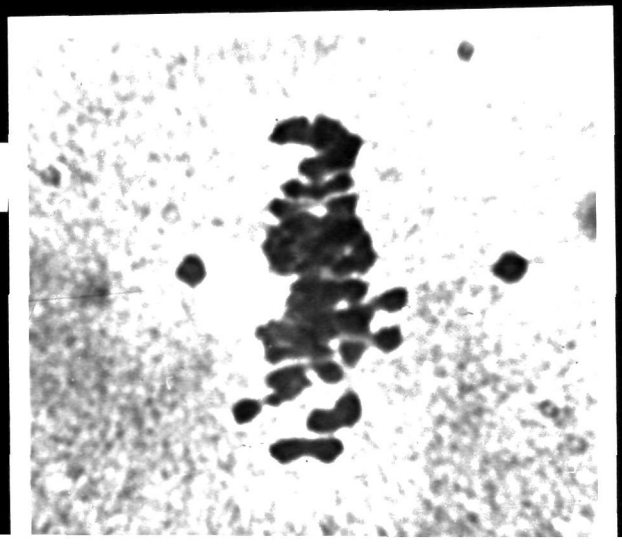


FIG.30

Explanatory diagrams for figures in Plate 11
(IV and III - in stripes; II - in shade; I - in outline)

Fig.28a. Metaphase-I showing $6^{IV}+12^{II}$

Fig.29a. Metaphase-I showing $6^{IV}+1^{III}+10^{II}+1^{I}$

FIG.28a

FIG.29a

of one to three univalents occurred towards one of the poles. In 5.4 per cent of the PMCs such movement occurred to both poles (Fig.30) involving at least one univalent to each pole.

4.2.1.2.3. Metaphase-II: The distribution of chromosomes to the daughter nuclei at the end of first meiotic division was studied at this stage. In 94.1 per cent PMCs equal number of chromosomes (24+24) were observed (Figs.31 and 31a) in the daughter nuclei (Table 6). However, in 5.88 per cent PMCs unequal chromosome distribution of 23+25 (Figs.32 and 32a) was observed. Two nuclei were observed in 98.5 per cent of the PMCs (Table 7).

Telophase-II: The number of daughter nuclei was also studied at telophase-II (Table 7).

Four nuclei were observed in 90.91 per cent of the 176 PMCs examined. In the remaining five (2.27%), six (5.68%) and seven (1.14%) chromosomal groups were observed.

4.2.1.2.4. Pollen fertility and size: The average pollen fertility of the autotetraploid was 54.3 per cent. Pollen grains ranged from 36.0/u to 44.0/u in diameter with a mean diameter of 38.5/u (Fig.33).

4.2.2. Solanum [illegible]

C_1 generation plants were studied for morphological and cytological characters.

Table 7. Frequency of PMCs with different number of daughter nuclei at metaphase-II and telophase-II stages in autotetraploid S.viarum (2n=48)

Stage	Number of nuclei						
	2	3	4	5	6	7	Total
Metaphase-II							
Number	136	2	-	-	-	-	138
Percentage	(98.55)	(1.45)	-	-	-	-	-
Telophase-II							
Number	-	-	160	4	10	2	176
Percentage	-	-	(90.91)	(2.27)	(5.68)	(1.14)	-

Plate 12. Legend for photomicrographs of metaphase-II stage in autotetraploid *S.virgum*

Fig.31. Metaphase-II showing 24+24 chromosomal distribution x 2000

Fig.32. Metaphase-II showing 23+25 chromosomal distribution x 2000

Fig.33. Pollen grains x 1000

PIATE 12

FIG.31

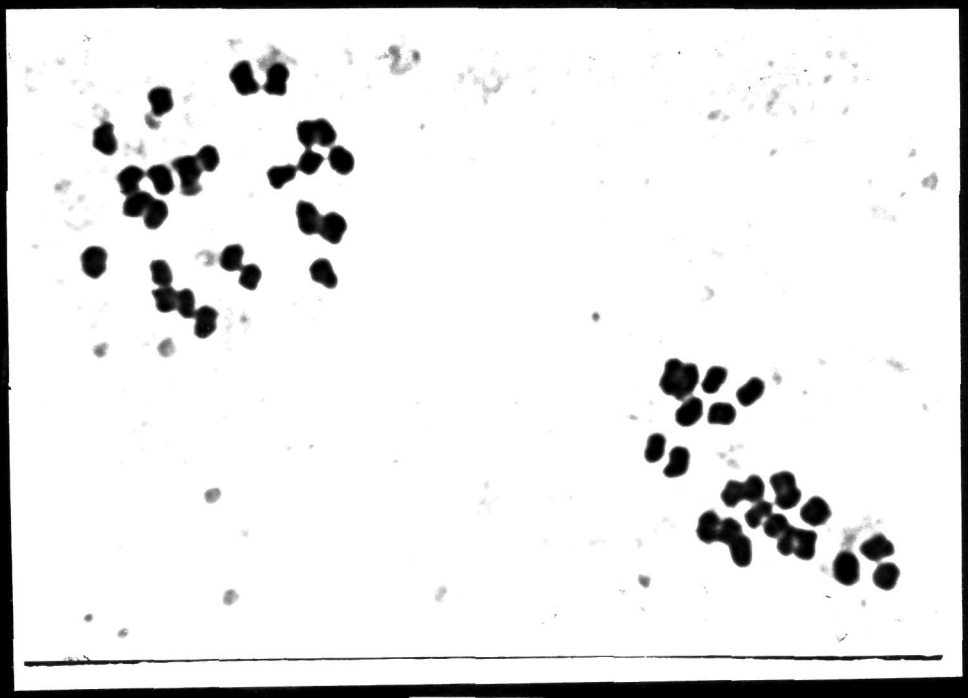


FIG.32

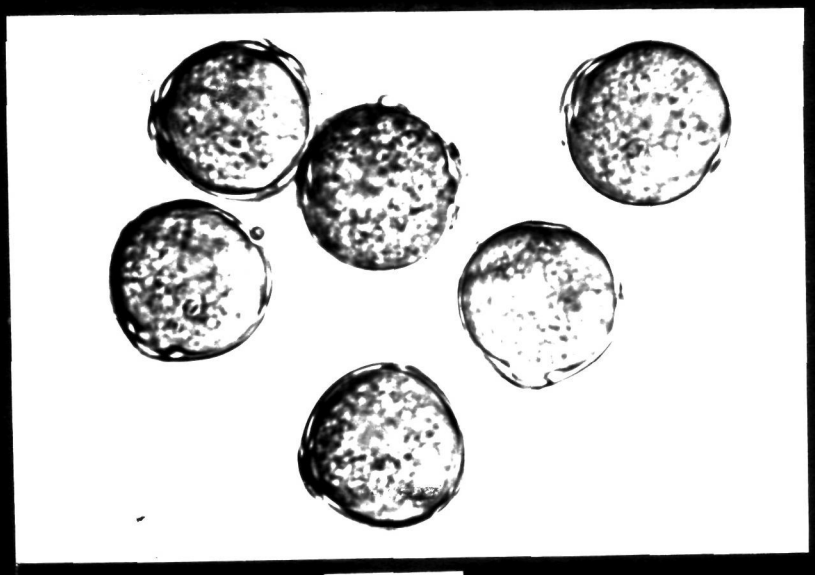


FIG.33

Explanatory diagrams for figures in Plate 12

Fig.31a. Metaphase-II showing 24+24 chromosomal distribution

Fig.32a. Metaphase-II showing 23+25 chromosomal distribution

FIG.31a

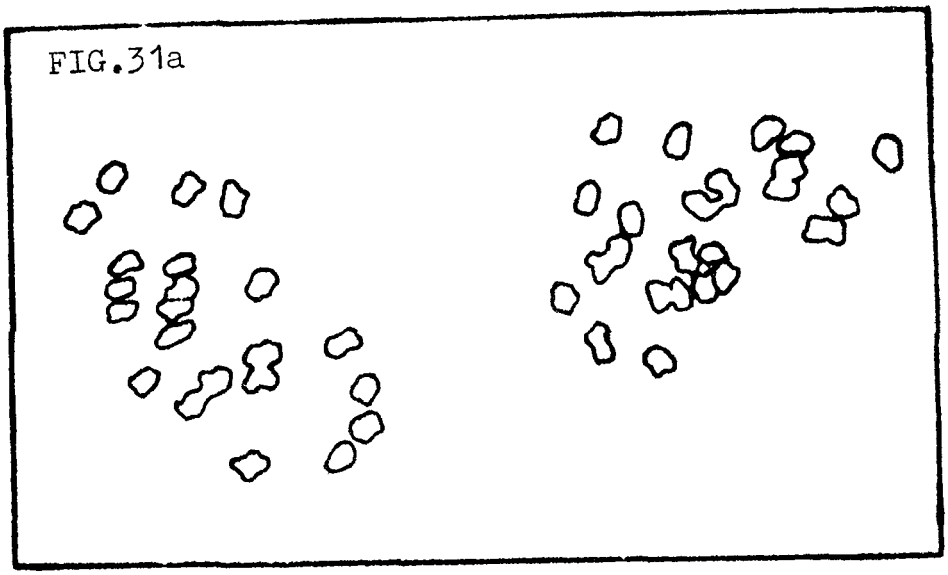
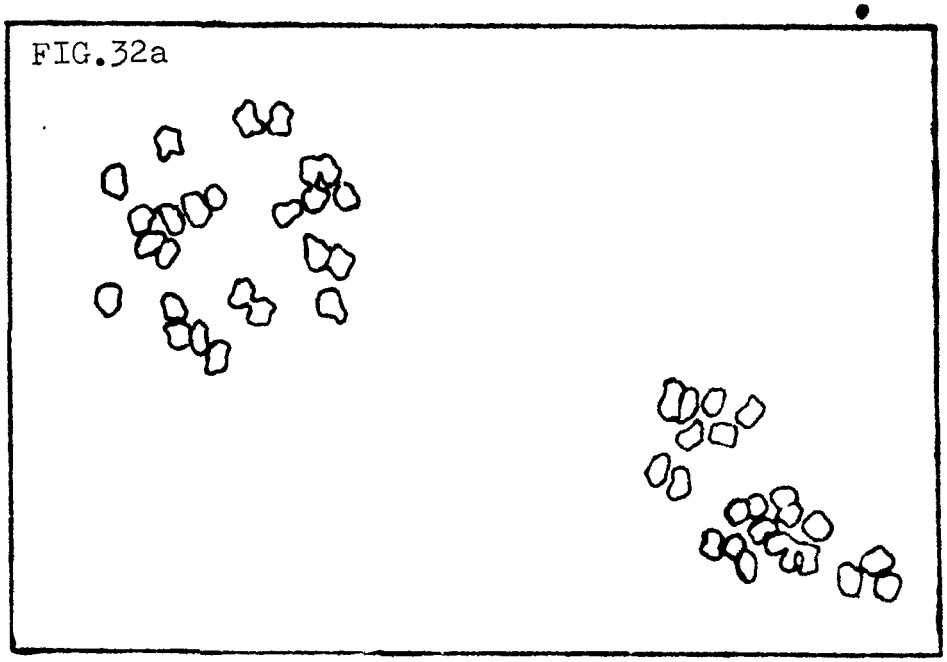


FIG.32a



4.2.2.1. Morphology

Autotetraploids showed thick dark green leaves (Fig.34). The mean values of characters of diploid and induced autotetraploid S.mammosum are presented in Table 8, alongwith 't' values. Characters such as specific leaf weight, percentage of heterostyled flowers, pollen fertility and pollen size were not statistically analysed for the differences of the mean values.

Among the characters for which mean values were compared for significance of differences, the mean values of diploid were found to be higher than those of tetraploids for number of flowers per inflorescence, petal length, length of anther, fruit diameter, fresh weight of fruits, number of seeds per fruit and 100 seed weight (Figs.35 to 38). The mean values of autotetraploids for leaf length, leaf breadth, petiole length, spine number on ventral leaf surface; and petal breadth was higher than those of diploids. Diploids and tetraploids were on par for spine number on dorsal leaf surface and length of exerted styled flowers.

In specific leaf weight, percentage of exerted styled, medium styled flowers as well as pollen size the respective mean values of tetraploids were 15.3, 126.3, 116.9 and 26.51 per cent higher over those of diploids. Diploids had higher

Table 8. Comparision of means of morphological characters in diploids and autotetraploids of Solanum nigrescens

Character	Diploid	Tetraploid	't' value/% value
1. Leaf length(cm)	12.0±0.3(25)	12.8±0.9(15)	4.0*
2. Leaf breadth(cm)	12.6±0.4(25)	15.8±1.2(15)	12.4*
3. Petiole length(cm)	6.7±0.9(25)	7.9±0.9(25)	4.6*
4. Specific leaf weight	0.0196	0.0226	+15.3
5. Spine number(leaf)			
a) Dorsal	15.8±2.1(20)	16.5±2.0(24)	1.2^{NS}
b) Ventral	6.0±1.9(20)	7.6±1.6(24)	2.9*
6. No.of flowers/inflorescence	6.6±1.2(25)	1.7±2.2(71)	2.8*
7. Petal length(mm)	19.4±1.3(31)	15.8±1.2(25)	10.6*
8. Petal breadth(mm)	4.1±1.7(11)	10.5±0.8(20)	14.6*
9. Length of anther(mm)	13.5±0.7(20)	10.8±1.7(15)	49.0*
10. Flowers with			
a) Exerted style(%)	7.4	16.8	+126.3
b) Medium style(%)	11.2	24.2	+116.9
c) Short style(%)	81.5	58.9	-27.7
11. Length of exerted style(mm)	10.7±0.7(20)	10.8±1.9(20)	2.1^{NS}
12. Fruit diameter(mm)	47.3±1.7(14)	22.4±0.5(56)	2.9*
13. Fruit weight(g)	28.6±2.5(38)	4.2±0.4(55)	71.9*
14. No.of seeds per fruit	273.4±17.9(33)	38.0±3.2(55)	93.8*
15. 100 seeds weight(mg)	1014.6±45.0(13)	390.3±97.0(3)	4.7
16. Pollen fertility(%)	87.3	44.7	-48.7
17. Pollen diameter(μ)	32.6	41.3	+26.5

NS=Non-significant; *Significant at 1 per cent; ±Standard error.
Values indicated within the parenthesis refer to the number of observations.

Plate 13. Legend for photographs of autotetraploid S.[redacted]

Fig.34. Established plant

Fig.35. Comparision of diploid (2n) and autotetraploid (4n) flowers

Fig.36. Comparision of diploid (2n) and autotetraploid (4n) fruits

PLATE 13

FIG.34

FIG.35

FIG.36

Plate 14. Legend for photographs of autotetraploid *S.mammosum*

Fig.37. Comparision of longitudinal section of diploid (2n) and autotetraploid (4n) fruits

Fig.38. Comparision of diploid (2n) and autotetraploid (4n) seeds

PLATE. 14

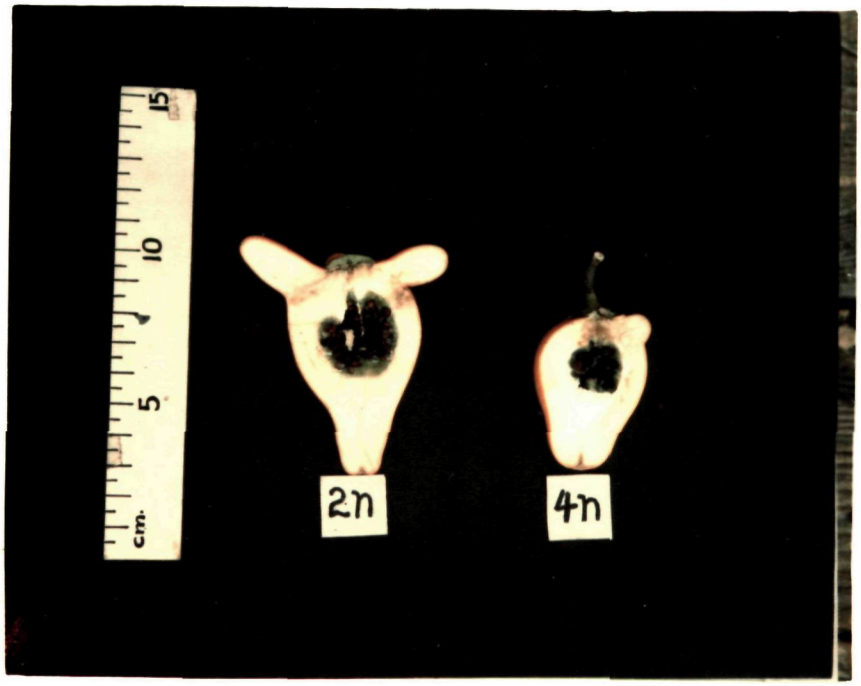


FIG. 37

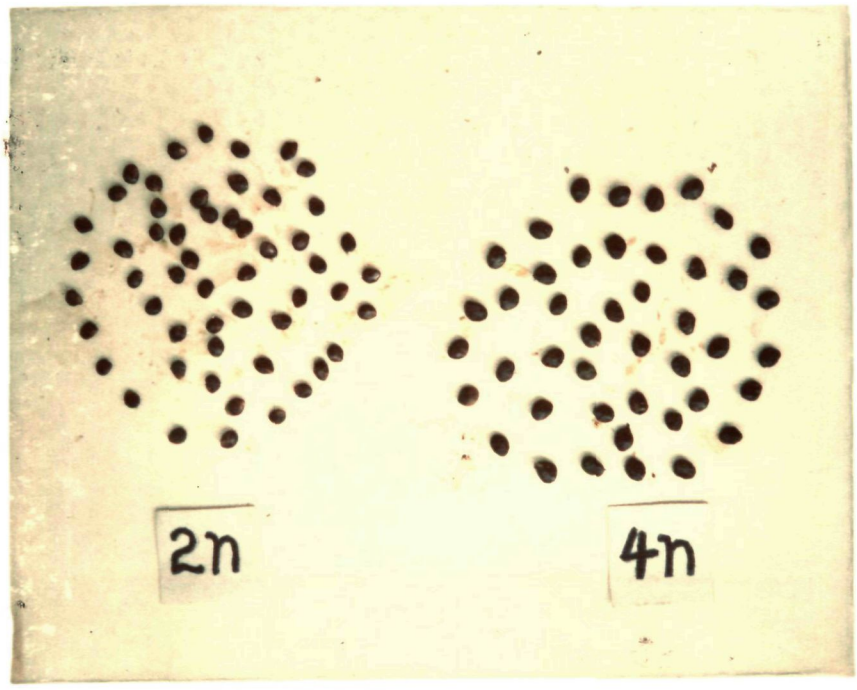


FIG. 38

percentage of short styled flowers (27.7) and pollen fertility (48.7) than tetraploids.

4.2.2.2. Meiosis

The course of meiosis was studied from metaphase-I onwards.

4.2.2.2.1. Metaphase-I: At metaphase-I, chromosomes association consisted of quadrivalents, trivalents, bivalents and univalents (Table 9). Complete pairing of chromosomes was observed in 63.0 per cent PMCs. Incomplete pairing consisting of 43, 42 and 40 paired chromosomes occurred in 23.9, 10.9 and 2.2 per cent of the PMCs respectively.

Quadrivalents ranging from three to nine per cell occurred in all the PMCs examined. Formation of seven quadrivalents (Figs.39 and 39a) was most frequent and accounted for 32.6 per cent of the PMCs examined. The occurrence of five and eight quadrivalents was recorded in 19.5 per cent of the PMCs. Trivalents occurred in 30.4 per cent of the PMCs examined and ranged in number from 1 to 4. The occurrence of a single trivalent (Figs.40, 40a, 41 and 41a) was most frequent and was recorded in 23.9 per cent of the PMCs examined. In 4.3 per cent of the PMCs two trivalents were observed, while four trivalents in 2.2 per cent PMCs. Bivalents ranged

Table 9. Chromosome association at metaphase-I in PMCs of autotetraploid S.mammosum (2n=44)

IV	III	II	I	Total No.of paired chromosomes	Frequency Number	Frequency Percentage
5	1	10	1	43	2	4.35
6	1	8	1	43	1	2.17
9	-	4	-	44	2	4.35
6	-	10	-	44	5	10.87
5	-	11	2	42	1	2.17
8	-	5	2	42	1	2.17
7	-	8	-	44	11	23.91
7	1	6	1	43	4	8.70
9	-	3	2	42	1	2.17
8	-	6	-	44	4	8.70
6	2	6	2	42	1	2.17
5	-	12	-	44	6	13.04
8	1	4	1	43	4	8.70
3	2	12	2	42	1	2.17
4	-	14	-	44	1	2.17
3	4	8	4	40	1	2.17
3-9	0-4	3-14	0-4	Range	Total	46
6.54	0.41	8.02	0.54	Mean/cell		

Plate 15. Legend for photomicrographs of metaphase-I stage in autotetraploid S.mammosum x 2500

Fig.39. Metaphase-I showing $7^{IV}+8^{II}$ chromosomal association

Fig.40. Metaphase-I showing $7^{IV}+1^{III}+6^{II}+1^{I}$ chromosomal association

Fig.41. Metaphase-I showing $4^{IV}+1^{III}+12^{II}+1^{I}$ chromosomal association

PLATE. 15

FIG. 39

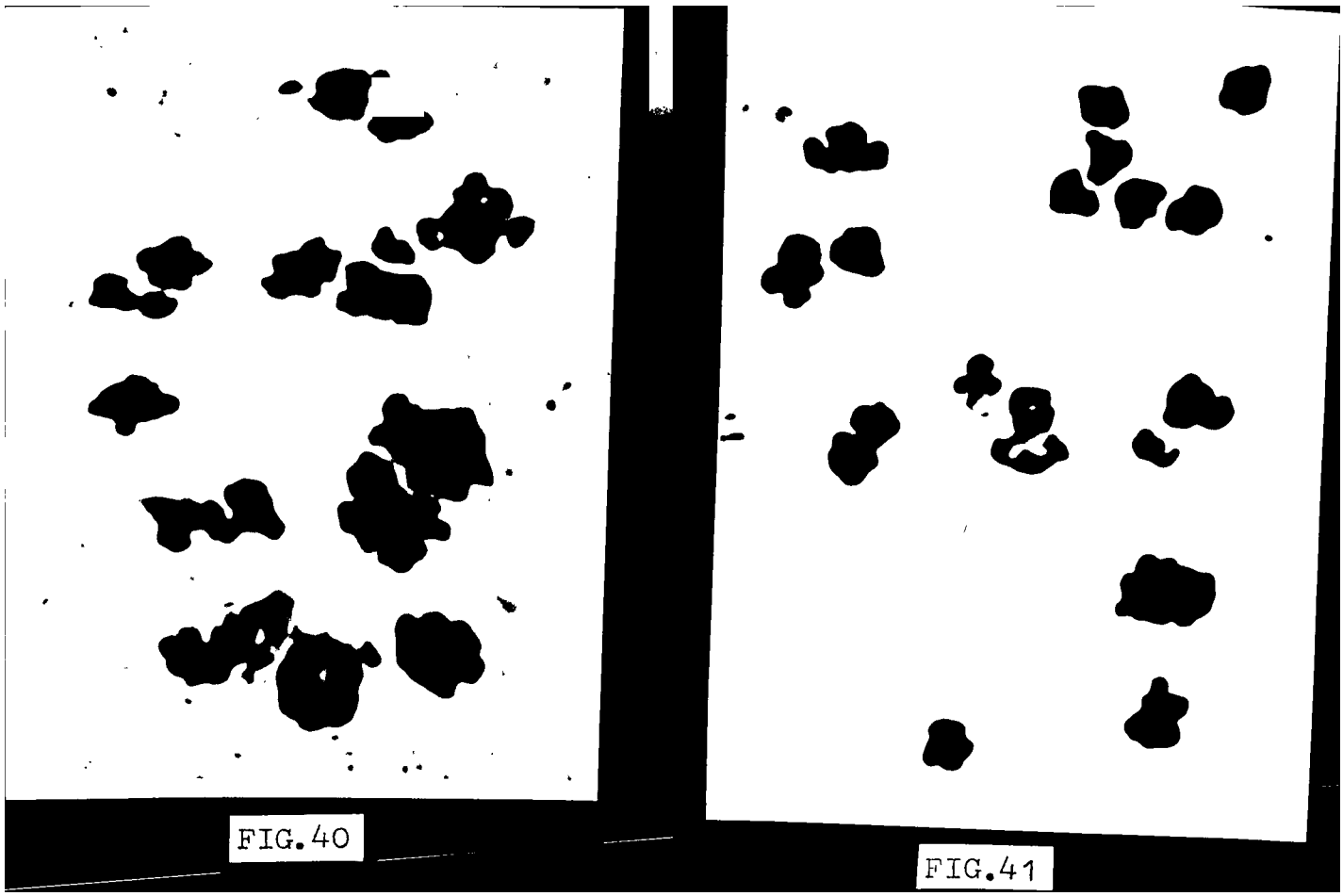


FIG.40

FIG.41

Explanatory diagrams for figures in Plate 15
(IV and III in stripes; II - in shade; I - in outline)

Fig.39a. Metaphase-I showing $7^{IV}+8^{II}$

Fig.40a. Metaphase-I showing $7^{IV}+1^{III}+6^{II}+1^{I}$

Fig.41a. Metaphase-I showing $4^{IV}+1^{III}+12^{II}+1^{I}$

FIG.39a

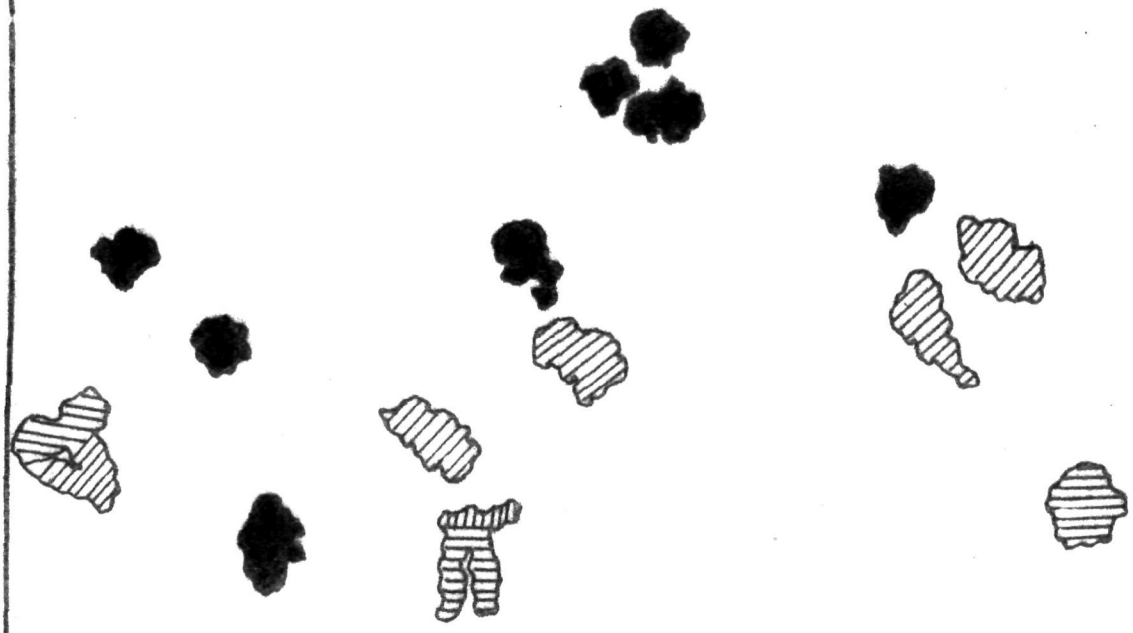


FIG.40a

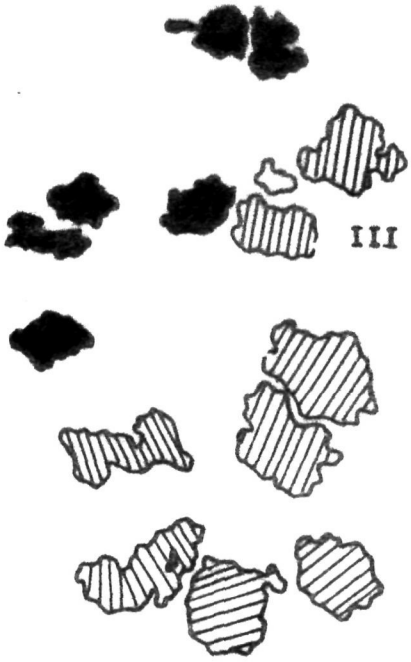
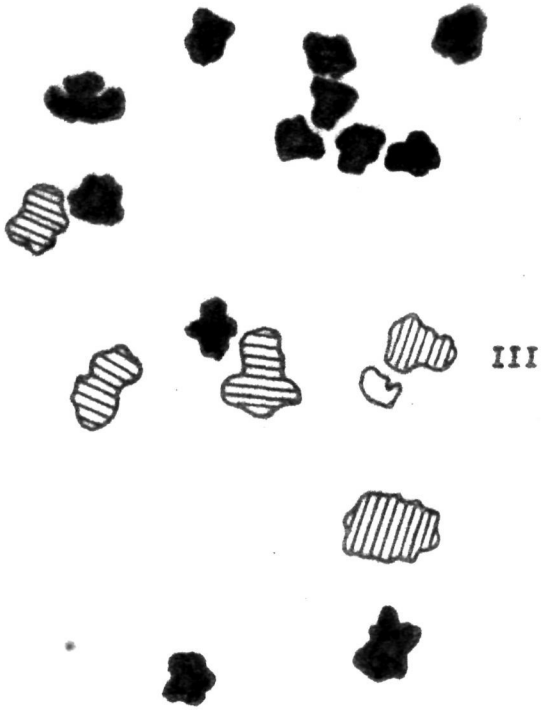


FIG.41a



from 3 to 14 (Figs.42 and 42a) per cell. The occurrence of 8 bivalents per cell was more frequent and accounted for 28.3 per cent PMCs examined. Univalents ranged from 0 to 4 and were observed in 36.96 per cent of the PMCs. The number of univalents in PMCs corresponded to the number of trivalents present and in cells where trivalents were absent two univalents were observed.

Precocious movement of chromosomes was observed in 44.2 per cent PMCs at metaphase-I and involved movement of upto six chromosomes (Table 10). Precocious movement of chromosomes to either pole involved equal number of chromosomes in is 5.92 per cent of the PMCs. In 28.0 per cent of the PMCs such movement involved one chromosome and in 10.3 per cent PMCs unequal number of chromosomes to poles.

4.2.2.2.2. Anaphase-I: At anaphase-I, 3.23 per cent PMCs showed chromosomal bridges (Table 11) and in 20.99 per cent PMCs, presence of one or two (Fig.43) lagging chromosomes (Table 12).

4.2.2.2.3. Later stages: At prophase-II, metaphase-II and anaphase-II due to persistence of chromosomal association, chromosomal distribution to the daughter nuclei could not be determined. This was evident from the presence of lesser number of chromosomal bodies in the PMCs at metaphase-II stage than the expected normal number of 44 (Figs.44 and 45). The

Table 10. Precocious movement of chromosomes to poles at metaphase-I in PMCs of autotetraploid Solanum mammosum (2n=44)

Frequency	Normal	Number of chromosomes in poles								Total
	0-0	0-1	1-1	1-2	2-0	2-2	3-0	3-1	3-3	
Number	179	90	12	6	15	6	11	1	1	321
Percentage	(55.76)	(28.04)	(3.74)	(1.87)	(4.67)	(1.87)	(3.43)	(0.31)	(0.31)	-

Table 11. Occurrence of chromosomal bridges at anaphase-I in PMCs of autotetraploid Solanum mammosum

Frequency	PMCs		Total
	With bridges	Without bridges	
Number	5	150	155
Percentage	(3.23)	(96.77)	-

Table 12. Occurrence of laggards at anaphase-I in PMCs of autotetraploid Solanum mammosum

Frequency	Number of laggards			Total
	0	1	2	
Number	64	10	7	81
Percentage	(79.01)	(12.35)	(8.64)	-

Plate 16. Legend for photomicrographs of meiotic stages in autotetraploid S.mammosum

Fig.42. Metaphase-I showing $4^{IV}+14^{II}$ chromosomal association x 2500

Fig.43. Anaphase-I showing lagging chromosomes x 2000

Fig.44. Metaphase-II showing persistance of chromosomal association resulting in less than 44 chromosomal bodies (16+13) x 2000

PLATE 16

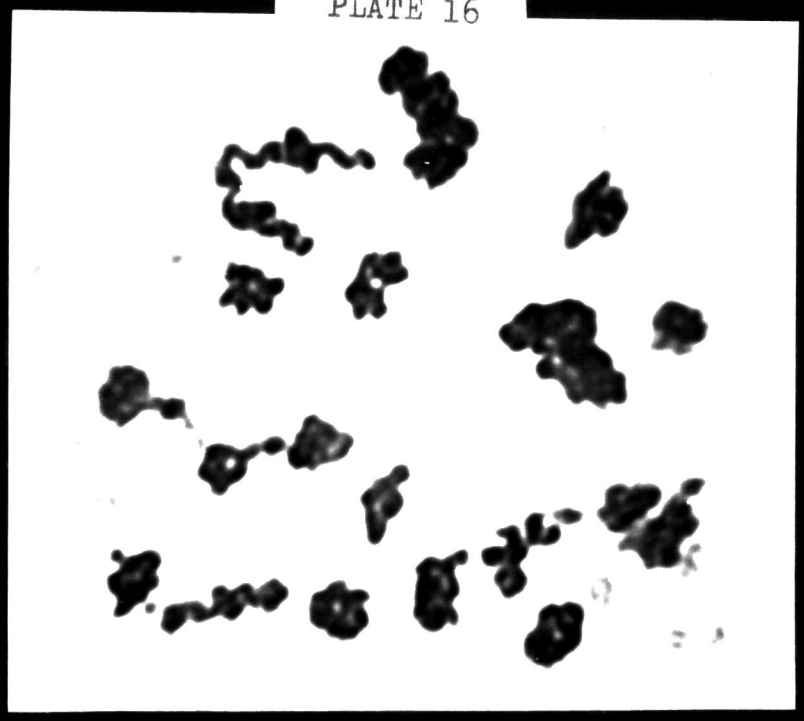


FIG.42

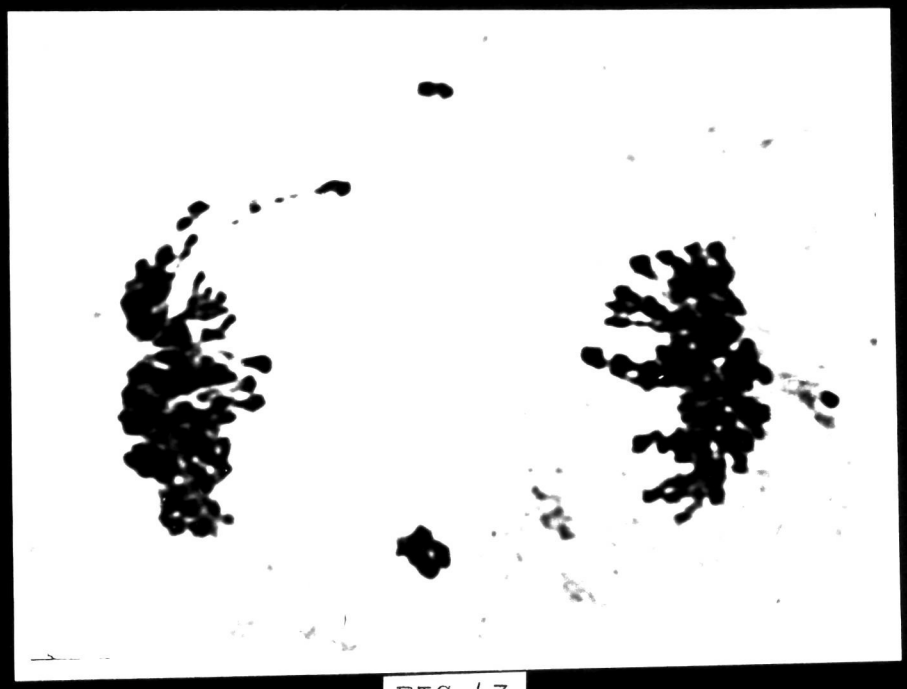
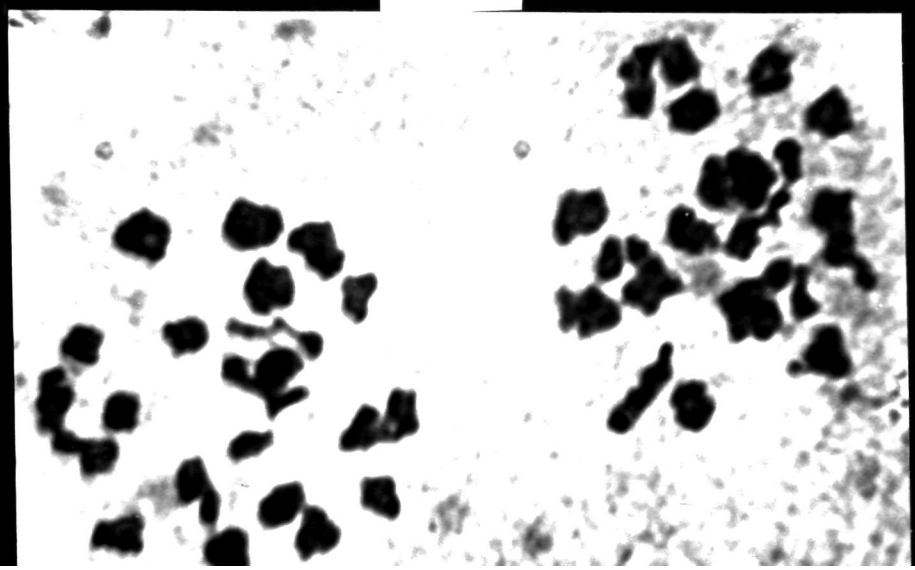


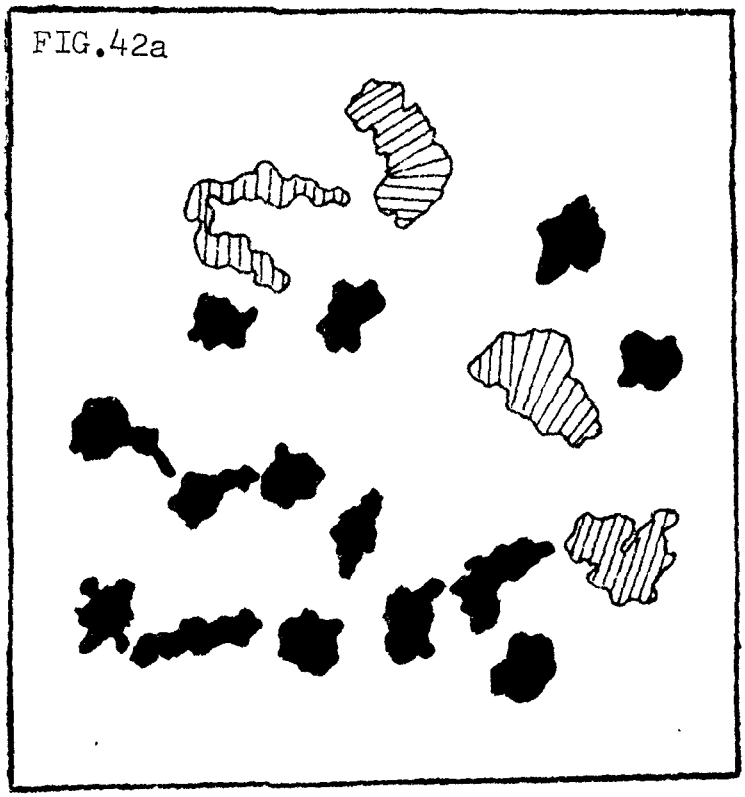
FIG.43



Explanatory diagrams for figure in Plate 16

Fig.42a. Metaphase-I showing $4^{IV}+14^{II}$

FIG.42a



total number of such bodies in both the poles of the PMCs ranged from 19 to 34 (Table 13) and the mean 26.15 per PMC. Occurrence of 25 bodies per cell accounted for the maximum frequency of 18.2 per cent. In 68 PMCs at metaphase-II stage a good spread of chromosomes was observed in one of the nuclei and the data on the number of chromosomal bodies were recorded. Here again, as against the expected 22 chromosomal bodies only 10 to 16 bodies were observed with the mean of 11.1 per PMC (Table 14).

Formation of two daughter nuclei was observed at anaphase-I and telophase-I and an extra group comprising of five nuclei (3.72%) was observed at telophase-II (Fig.46; Table 15).

Among the 225 PMCs examined at anaphase-II, one or two lagging chromosomes occurred in 1.78 and 1.33 per cent of the PMCs, respectively, and in 1.33 per cent of the PMCs, bridge formation was observed.

4.2.2.2.4. Microspore tetrad stage: At microspore tetrad stage, 50.28 per cent of the PMCs had four microspores (Fig.47) and 8.94, 36.31 and 4.47 per cent of the PMCs had three (Fig.48), five (Fig.49) and six microspores, respectively (Table 16).

4.2.2.2.5. Pollen fertility and size: Average pollen fertility in autotetraploid S.[illegible] was 44.75 per cent. Pollen grains

Table 13. Number of chromosomal bodies in daughter nuclei at metaphase-II in PMCs of autotetraploid S. [redacted] (2n=44)

Chromosomal bodies in nuclei			Frequency	
I	II	Total	Number	Percentage
11	8	19	1	3.03
11	9	20	1	3.03
14	8	22	1	3.03
12	10	22	1	3.03
11	11	22	1	3.03
13	9	22	1	3.03
12	11	23	2	6.06
12	12	24	2	6.06
14	10	24	1	3.03
13	11	24	1	3.03
13	12	25	2	6.06
15	10	25	1	3.03
14	11	25	3	9.09
14	12	26	1	3.03
13	13	26	1	3.03
15	12	27	1	3.03
16	12	28	1	3.03
15	13	28	2	6.06
16	13	29	1	3.03
15	14	29	1	3.03
16	14	30	2	6.06
16	15	31	1	3.03
17	15	32	1	3.03
18	15	33	2	6.06
18	16	34	1	3.03
		Total	33	

Table 14. Number of chromosomal bodies in one of the nuclei at metaphase-II in PMCs of auto-tetraploid S.mammosum (2n=44)

Number of chromosomal bodies	Frequency	
	Number	Percentage
10	1	1.47
11	8	11.76
12	13	19.12
13	17	25.00
14	9	13.24
15	13	19.12
16	7	10.29
Total	68	

Table 15. Frequency of PMCs with different chromosomal groups/nuclei at various meiotic stages in autotetraploid Solanum mammosum (2n=44)

Stage	Number of nuclei				Total
	2	3	4	5	
Anaphase-I					
Number	150	-	-	-	150
Percentage	(100.00)	-	-	-	
Telophase-I					
Number	204	-	-	-	204
Percentage	(100.00)	-	-	-	
Telophase-II					
Number	-	-	207	8	215
Percentage	-	-	(96.28)	(3.72)	

Table 16. Frequency of microspores at tetrad stage in PMCs of autotetraploid Solanum mammosum

Frequency	Number of microspores				Total
	3	4	5	6	
Number	16	90	65	8	179
Percentage	(8.94)	(50.28)	(36.31)	(4.47)	-

Plate 17. Legend for photomicrographs of meiotic stages in autotetraploid S.gammosum

Fig.45. Metophase-II showing persistance of chromosomal association resulting in less than 44 chromosomal bodies (10+8) x 2000

Fig.46. Telophase-II showing five daughter nuclei x 2000

Fig.47. Microspore tetrad with four spores x 2000

Fig.48. Microspore tetrad with three spores x 2000

Fig.49. Microspore tetrad with five spores x 2000

PLATE. 17

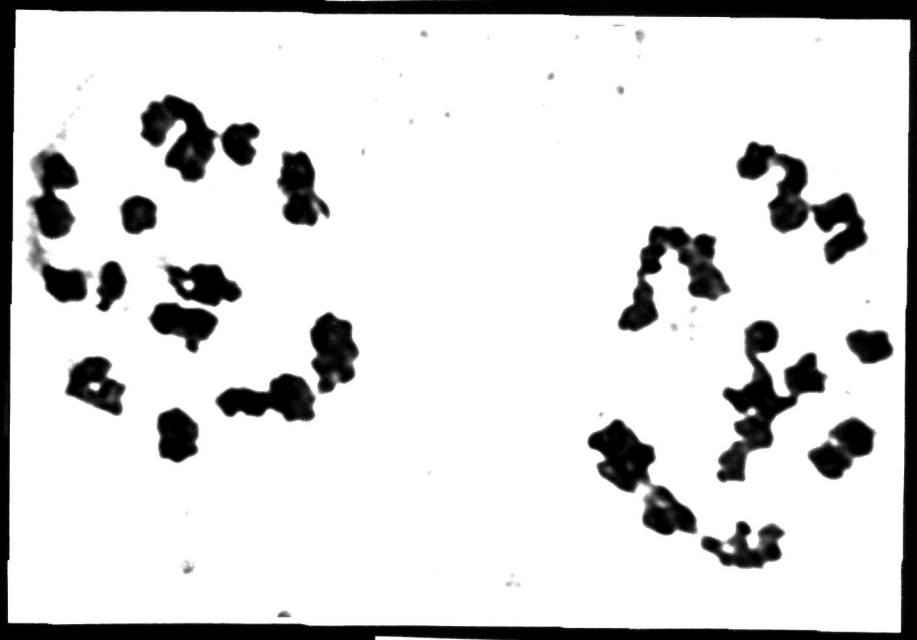


FIG.45

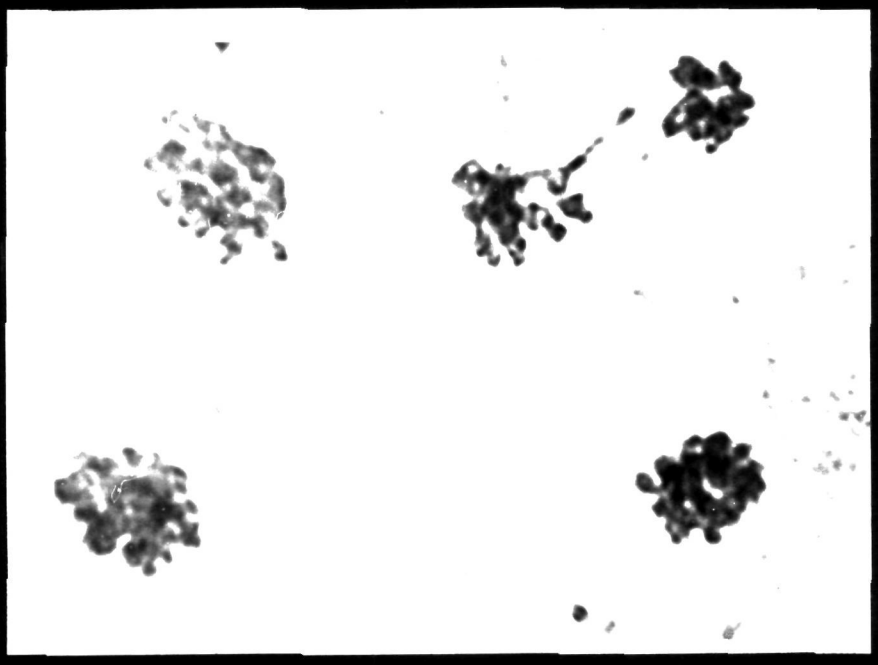


FIG.46

FIG.47

FIG.48

FIG.49

diameter ranged from 36.0/u to 44.0/u and the mean diameter was 41.28/u.

4.3. INTERSPECIFIC HYBRIDIZATION

The results of artificial hybridisation between S.xiarum and S.[redacted] is reported here.

4.3.1. Crossability

Interspecific hybridisation between Solanum xiarum and S.[redacted] was carried out initially using diploids. Following successful induction of autotetraploids using colchicine in both the species in the present study (Sec.4.2). The induced autotetraploids were also used for interspecific hybridisation. The results of the crosses are summarised in Table 17.

Diploids of both S.xiarum and S.[redacted] were crossed reciprocally. In 350 pollinations carried out using S.xiarum as female parent fruit set was not observed. Crossed flowers dropped off within 8 to 10 days. Out of 500 pollinations made with S.[redacted] as pistillate parent, a large number of berries were set. However, only 25 of them reached maturity. From these berries both apparently mature and immature seeds numbering 335 and 873, respectively, were obtained. A large number of seedlings raised from these seeds were affected by

Table 17. Results of controlled pollinations involving S.viarum (SV) and S.mammosum (SM)

Cross	No.of flowers pollinated	No.of fruits set	Percentage fruit set	No.of matured fruits harvested	No.of seeds obtained	No.of seedlings survived	Remarks
Diploid-Diploid							
Normal plants							
SV x SM	350	Nil	Nil	Nil	Nil	Nil	-
SM x SV	500	-	20	25	1108	1	-
Grafted plants							
SM/SV x SV	120	Nil	-	-	-	-	-
Diploid(2n)-Autotetraploid(4n)							
2nSV x 4nSM	34	1	3	-	-	-	Pre-mature fruit dropping
4nSM x 2nSV	19	Nil	-	-	-	-	-
2nSM x 4nSV	68	20	29	18	169	Nil	-
4nSV x 2nSM	55	16	29	23	2	Nil	Smaller sized parthenocarpic fruits
Autotetraploid-Autotetraploid							
SV x SM	72	26	36	22	Nil	Nil	-
SM x SV	48	Nil	-	-	-	-	-

SM/SV = S.mammosum as scion on S.viarum stock.

disease resulting in mortality. Among the surviving seedlings one of the seedlings was found to be of hybrid origin, while the rest from selfing.

Crosses between diploid S.mammosum and autotetraploid S.viarum resulted in fruit set. Though a large number of seeds were obtained, none of the seeds germinated. The reciprocal cross yielded mostly parthenocarpic fruits and the two seeds obtained from this cross did not germinate.

Crosses between diploid S.viarum and autotetraploid S.mammosum resulted in 3.0 per cent of fruit set when S.viarum was used as female parent. But the fruits were shed prematurely. The reciprocal cross was not attended by fruit set.

Attempt to hybridise autotetraploids of both species was unsuccessful. In these crosses where S.viarum was used as pistillate parent, a number of parthenocarpic fruits were obtained. But in the reciprocal cross fruit set did not occur.

Reciprocal grafts involving S.viarum and S.mammosum were also assessed. The grafted plants with S.mammosum as scion and S.viarum as stock were evaluated for the inter-specific crossability. Fruit set was not observed in any of the 120 flowers pollinated with the pollen of S.viarum.

4.3.2. Comparative morphology

The characters of S.mammosum, S.viarum and the interspecific F_1 hybrid of S.mammosum x S.viarum are presented in Table 18. The mean values of characters of parents and hybrid were compared based on 't' values. Characters such as, specific leaf weight, percentage of exerted styled and short styled flowers, pollen fertility, pollen size were compared in terms of percentage increase or decrease over one or the other parents and/or hybrid. The mean values of other characters of parents and hybrid are compared among each other.

Parental differences were recorded for a number of characters. These included characters such as leaf length, leaf breadth, spine number of dorsal leaf surface, number of flowers per inflorescence, length of petal, breadth of petal, length of anther, fresh weight of fruits, fruit diameter, and 100 seed weight for which S.mammosum recorded higher values. In addition, pollen diameter was higher in S.mammosum. On the other hand, S.viarum showed higher mean value over S.mammosum for characters such as spine number on leaf ventral surface, percentage of flowers with exerted style, and number of seeds per fruit. Both the species were on par with respect to length of petiole.

Interspecific hybrid (Figs.50 to 52) differed from both the parents (Figs.53 to 57) in a number of qualitative and

Table 18. Comparision of qualitative and quantitative characters of S.viarum (SV), S.mammosum (SM) and F_1 interspecific hybrid of SM x SV

Character	S.mammosum	F_1 (SM x SV)	S.viarum	't' value for comparision of F_1 vs SM	F_1 vs SV	SM vs SV
1. Leaf length(cm)	12.0±0.3(25)	7.5±1.1(25)	8.6±0.2(41)	20.4*	6.8*	53.0*
2. Leaf breadth(cm)	12.6±0.4(25)	6.6±0.9(25)	9.5±0.2(41)	28.6*	18.2*	42.7*
3. Leaf hairiness	Densely pilose	Intermediate	Moderately pubescent			
4. Petiole length(cm)	6.7±0.9(25)	3.7±0.8(25)	6.3±0.8(25)	12.6*	11.2*	1.9 NS
5. Spine number(leaf) a) Dorsal	15.8±0.2(20)	9.9±2.1(36)	5.5±1.1(25)	10.0*	9.7*	20.5*
b) Ventral	6.0±1.9(20)	4.8±1.9(36)	7.8±1.1(25)	2.2*	6.9*	3.8*
6. Specific leaf weight	0.0195	0.0142	0.0219			
7. No.of flowers per inflorescence	6.5±1.2(25)	7.4±2.5(107)	3.4±[illegible]	82.6*	8.3*	10.6*
8. Petal colour	Deep blue	Light blue	Creamy-white			
9. Petal length(mm)	19.4±1.3(31)	16.2±1.2(30)	10.5±0.8(16)	10.2*	15.2*	25.3*
10. Petal breadth(mm)	4.[illegible]±1.7(31)	[illegible].6±0.8(30)	2.0±0.2(16)	1.2 NS	12.0*	4.9*
11. Anther colour	Yellowish	Yellowish-white	Creamy-white			
12. Anther length(mm)	13.5±0.7(20)	5.7±0.8(30)	7.0±0.2(13)	49.0*	6.0*	43.2*
13. Flowers with a) Exerted style(%)	7.4	88.5	96.6			
b) Medium style(%)	11.2	-	-			
c) Short style(%)	81.5	5.5	3.4			
14. Length in exerted style(mm)	10.7±0.7(20)	9.0±1.5(11)	8.5±0.8(22)	12.1*	1.3 NS	11.5*
15. Pollen fertility(%)	87.3	3.5	86.2			
16. Pollen diameter(µ)	32.6	26.4	27.2			
17. Fruit shape	Oblong-ovoid with prominent nipple	Sharply ovoid with a slight point	Spherical			
18. Fresh weight of fruit(g)	28.6±2.5(38)	0.63	8.9±1.1(55)	-	-	24.5*
19. Fruit colour a) Immature	Greenish	Light-green	Light-green with white irregular stripes			
b) Mature	Yellowish-orange	Light-yellowish	Yellow			
20. Fruit diameter(mm)	47.4±1.7(14)	1.1	28.8±0.3(32)	-	-	58.5*
21. No.of enations per fruit	3.8±1.1(38)	Absent	Absent			
22. No.of seeds per fruit	273.4±17.9(33)	Absent	438.1±16.7(11)	-	-	26.8*
23. 100 seed weight(mg)	1014.6±45.0(13)	Absent	256.7±32.1(15)	-	-	31.8*
24. Seed coat colour	Dark-blackish	-	Light-brown			
25. Field reaction to root wilt	Resistant	Tolerant	Susceptible			
26. Field reaction to virus	Highly susceptible	Susceptible	Susceptible			

*Significant at 1 per cent; NS=Non-significant; ±Standard error; Values indicated within the parenthesis refer to number of observations.

Plate 18. Legard for photographs of F_1 interspecific hybrid (SM x SV)
(Note: SK=S.khasianum Syn. S.viarum)

Fig.50. An established plant showing virus susceptibility

Fig.51. Vegetatively propagated healthy plant

Fig.52. Twig of the plant showing parthanocarpically developed fruit

PLATE. 18

FIG.50

FIG.51

FIG.52

Plate 19. Legend for photographs of diploid S.mammosum

Fig.53. An established plant

Fig.54. A seedling (P_1)

PLATE. 19

FIG.53

FIG.54.

Plate 20. Legend for photographs of diploid S.viarum (Syn. S.khasianum)

Fig.55. An established plant

Fig.56. A seedling (P_2)

PLATE.20

FIG.55

FIG.56

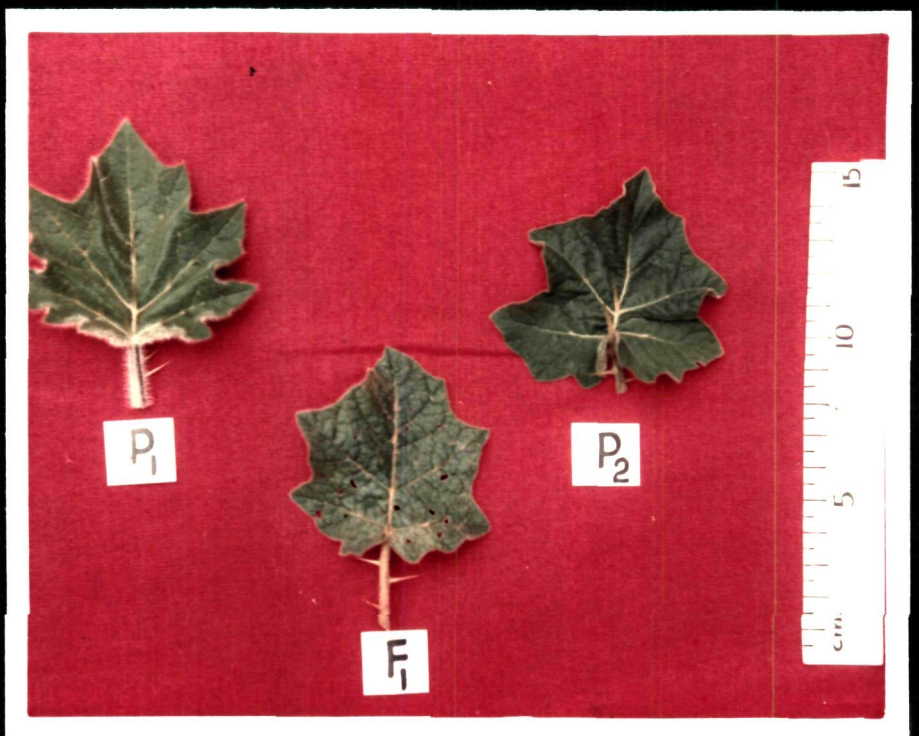
Plate 21. Legend for photographs of parents (P_1 and P_2) and interspecific hybrid

Fig.57. Comparision of mature fruits in S.mammosum (P_1) and S.viarum (P_2)

Fig.58. Comparision of leaf and petiole hairiness in parents - S.mammosum (P_1) and S.viarum (P_2) and their hybrid (F_1)

PLATE.21

FIG.57



quantitative characters. In qualitative characters like leaf hairiness (Fig.58), petal colour (Fig.59), anther colour (Fig.59), fruit shape (Fig.61) and fruit colour the hybrid was intermediate to the parents.

Interspecific hybrid recorded lower mean values than both the parents for characters such as leaf length, leaf breadth, petiole length, spines on ventral surface of leaf, specific leaf weight, petal length, anther length, pollen fertility, fresh weight of fruit and fruit diameter. Additionally, in S.mammosum, number of dorsal spines, petal length, stylar length and pollen diameter were more than that of hybrid. Mean values of interspecific hybrid was higher for number of flowers per cluster (Fig.60), breadth of petal, and number of spine on dorsal surface than that of pollen parent S.viarum. Stylar length and pollen diameter in S.viarum and F_1 hybrid were comparable. Fruit set in the F_1 hybrid was comparable to pollen parent where more than one fruit was observed per cluster (Fig.62). The female parent generally had a single fruit per cluster. Unlike the parents, the F_1 hybrid did not set seed (Fig.63). Seed size and colour differed in the parents (Fig.64).

4.3.3. Leaf flavonoids

A comparision of the parents and their hybrid was also attempted based on the distribution of leaf flavonoids. A total

Plate 22. Legend for photographs of parents (P_1 and P_2) and interspecific hybrid

Fig.59. Comparision of petal and anther colour in parents - S.mammosum (P_1) and S.viarum (P_2) and their hybrid (F_1)

Fig.60. Comparision of number of flowers per inflorescence in parents and their hybrid

PLATE.22

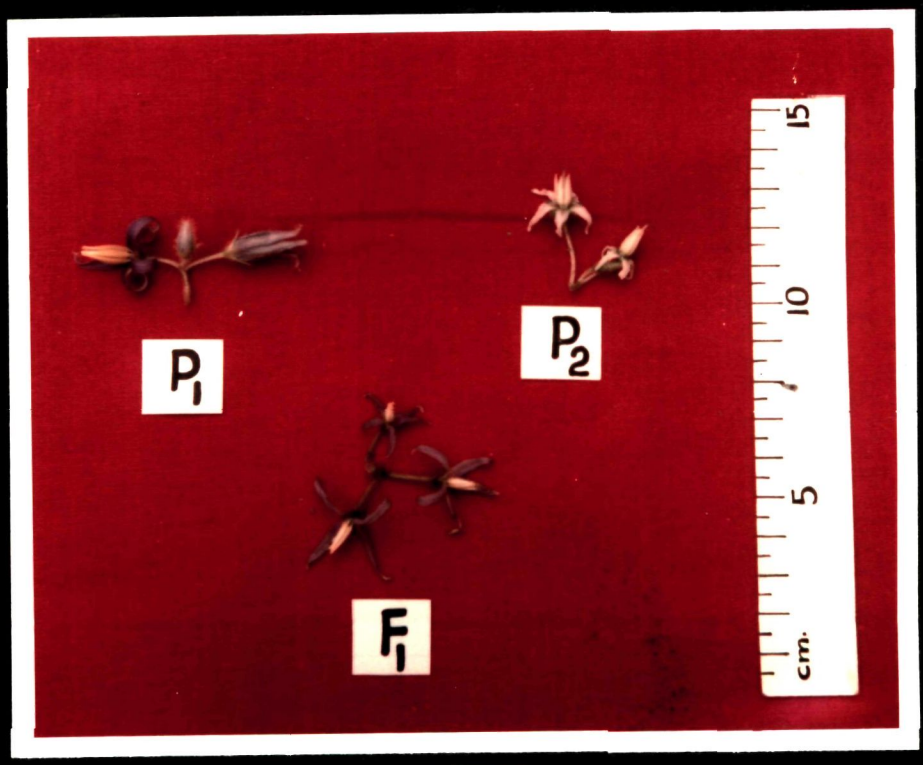


FIG.59

FIG.60

Plate 23. Legend for photographs

Fig.61. Comparision of size and stage of matured fruits in parents and hybrid

Fig.62. Comparision of fruits per cluster in parents and hybrid

PLATE.23

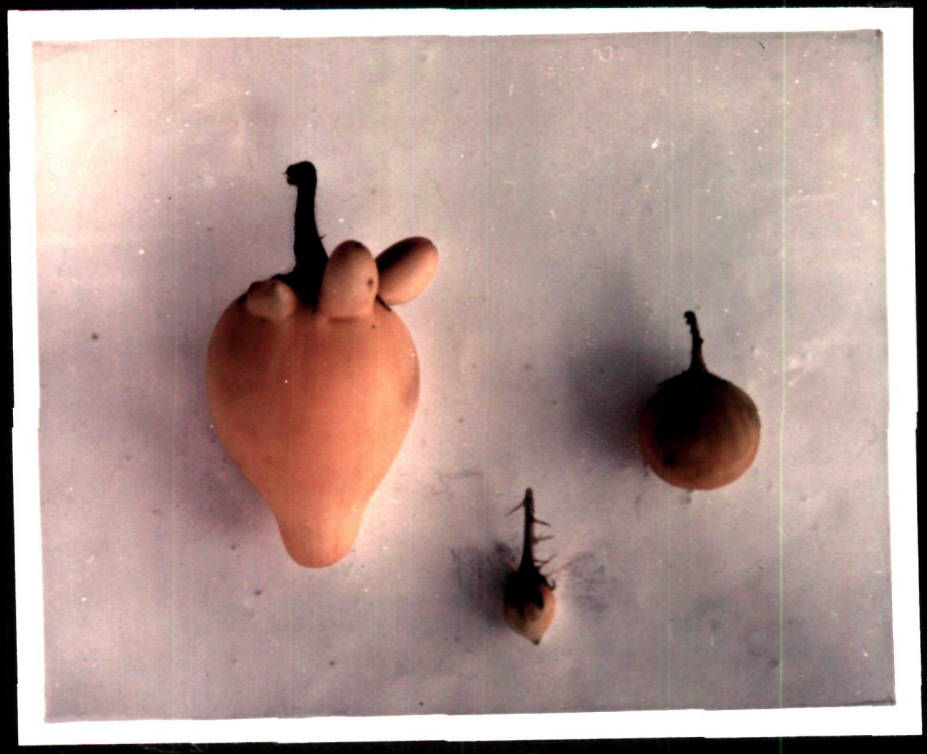


FIG.61

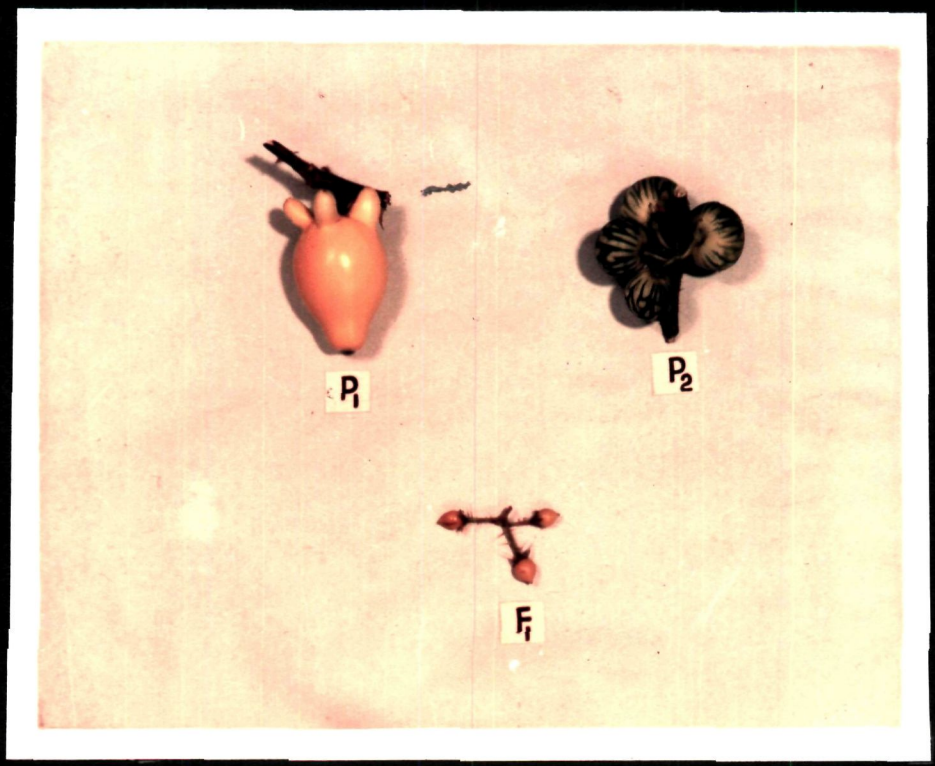


FIG.62

Plate 24. Legend for photographs
(Note: SK-S.khasianum Syn. S.viarum)

Fig.63. Comparision of longitudinal section of matured fruits in parents (SM & SV) and hybrid (F_1)

Fig.64. Comparision of seed colour and size in parents (SM & SV)

PLATE. 24

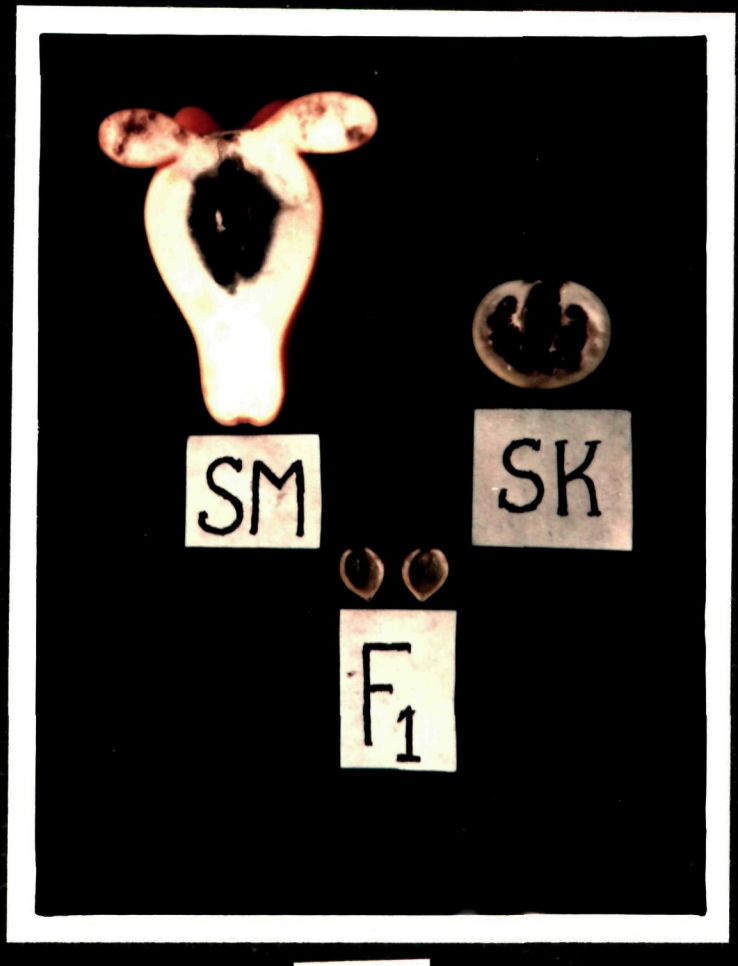


FIG. 63

FIG. 64

of 25 distinct spots were identified on the chromatogenic plate in parents and hybrids (Fig.65). Among these 15 spots were present in S.[redacted], 23 in S.viarum and 13 in F_1 hybrid (Table 19; Fig.66). Spots number 1 to 4, 6, 10, 14, 18 and 24 were present only in Solanum viarum. Spots present exclusively in Solanum [redacted] were 23 and 25. F_1 hybrid did not show the presence of any exclusive spots. The parents shared in common 13 spots viz., 5, 8, 9, 11, 12, 13, 15, 16, 17, 19, 20, 21 and 22 which were also present in F_1 hybrid. Spot No.7 was present in F_1 and male parent (Solanum viarum).

4.3.4. Meiosis in hybrid

Meiosis in the interspecific hybrid between diploids S.[redacted] (2n=22) and S.viarum (2n=24) was studied commencing from pachytene onwards.

4.3.4.1. Chromosome number

The chromosome number of the interspecific hybrid was determined as 2n=23 (Figs.67 and 67a) at anaphase-I.

4.3.4.2. Pachytene

Nuclei at this stage exhibited paired and unpaired chromosomes (Fig.68). Adequate spreading of chromosomes could not be obtained and hence the study of pairing behaviour of chromosomes has to be confined to isolated chromosomes or segments.

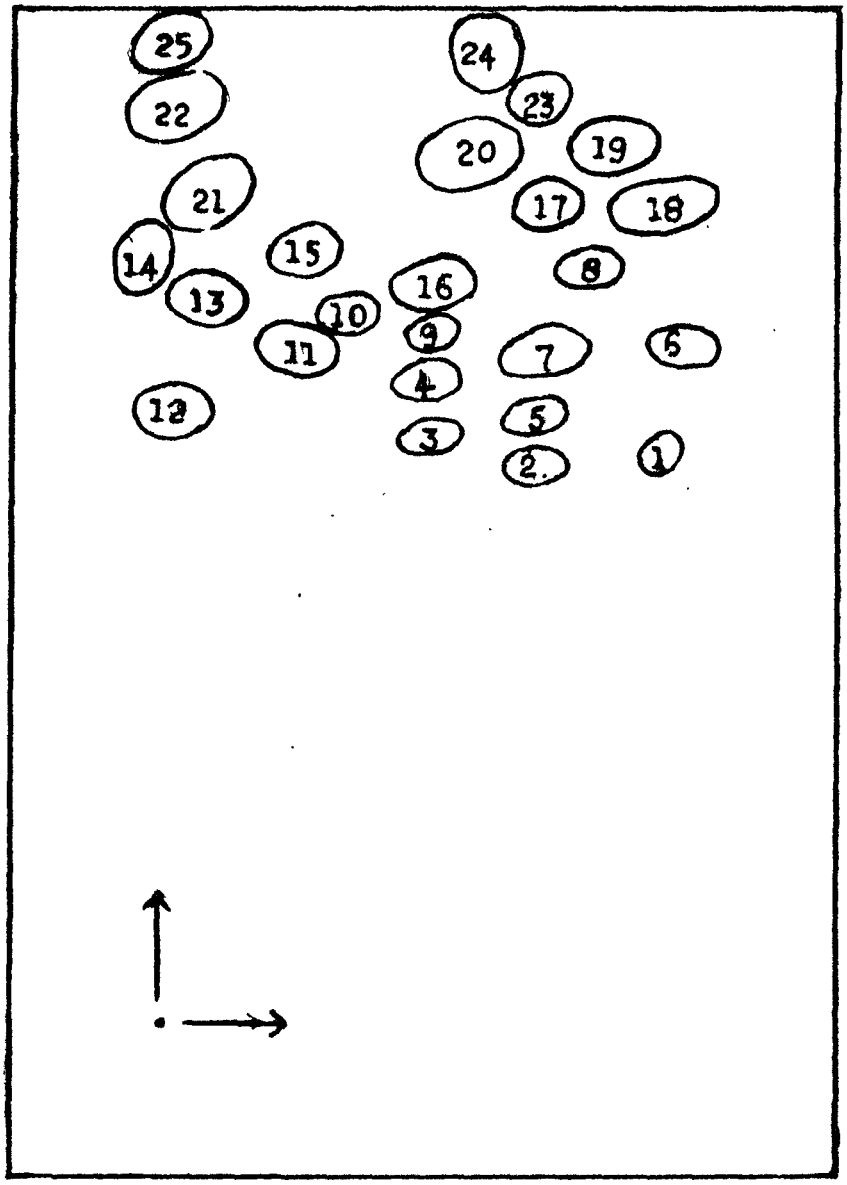


FIG.65 . Master plate showing the distribution of leaf flavonoids.

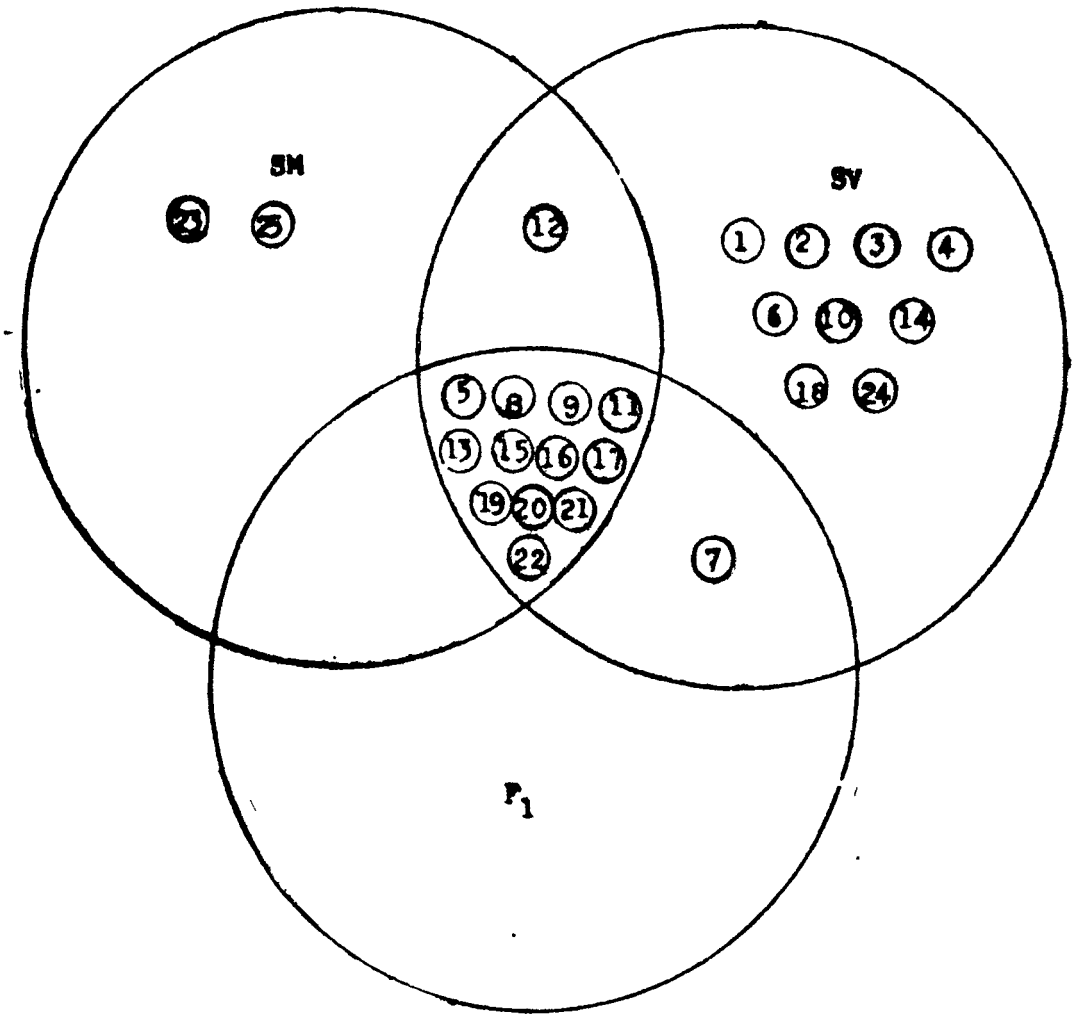


FIG.66 . Venn-diagram indicating distribution of leaf flavanoids in parents (SM and SV) and interspecific hybrid (F_1).

Plate 25. Legend for photomicrographs of meiotic stages in F_1 interspecific hybrid (SM x SV)

Fig.67. Anaphase-I showing 23 chromosomal bodies x 2500

Fig.68. A portion of pachytene nuclei showing paired and unpaired chromosomal segments x 3000

Fig.69. Nucleolar chromosome of *S.virgum* in association with the nucleolus of F_1 hybrid x 3000

PLATE.25

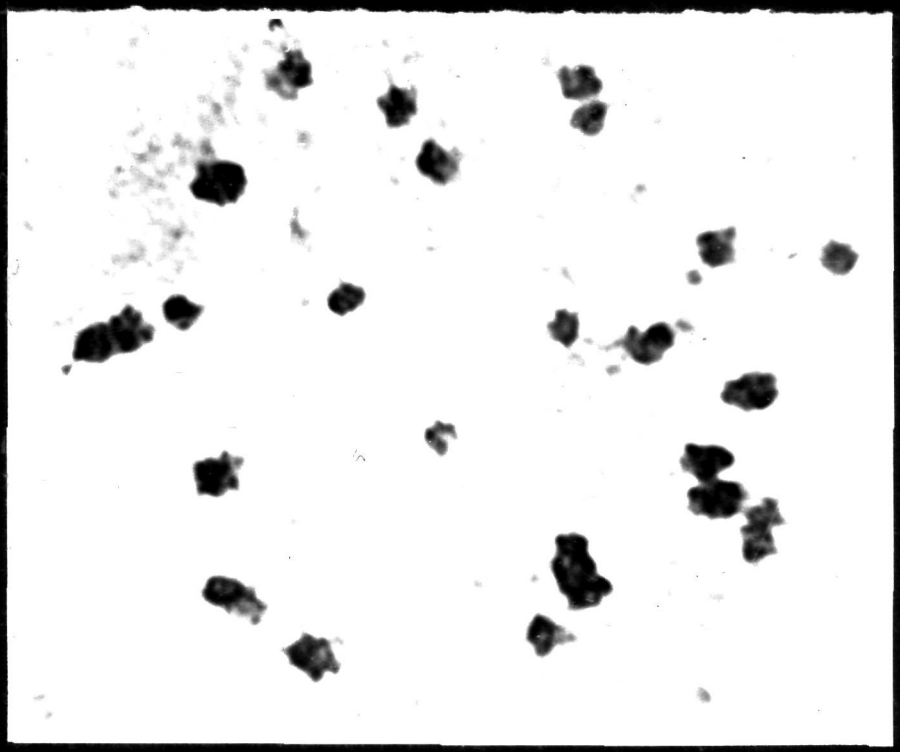


FIG.67

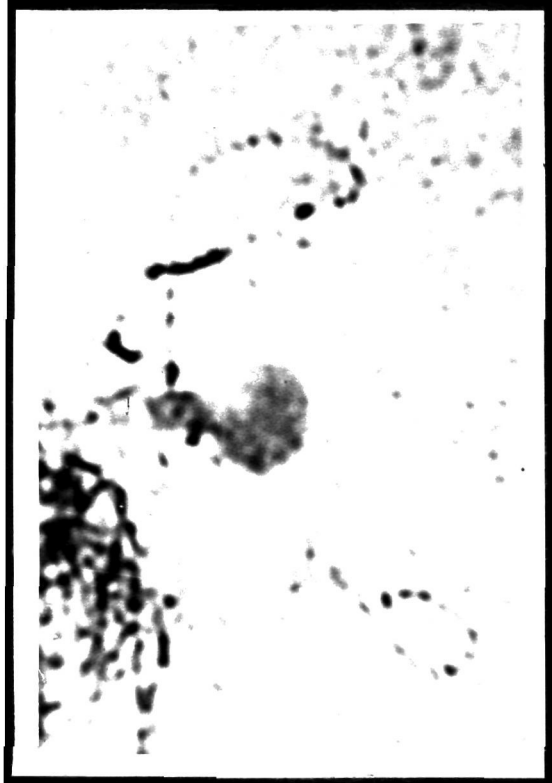


FIG.69

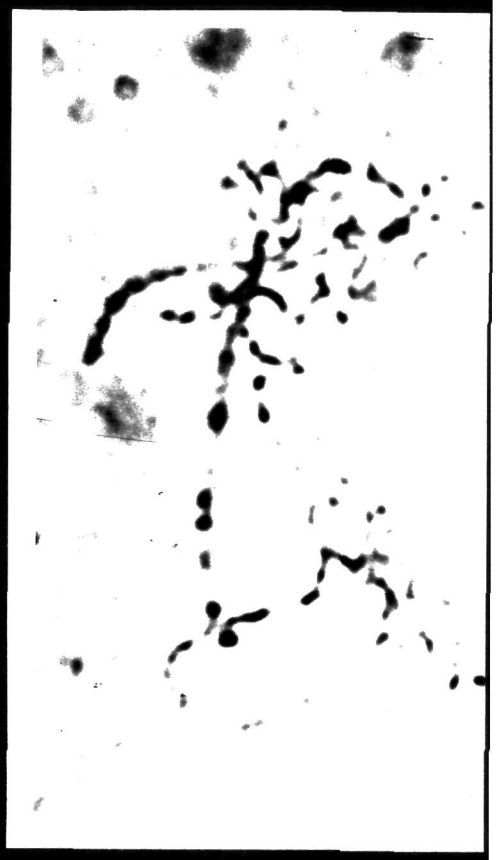


FIG.68

Explanatory diagrams for figures in Plate 25

Fig.67a. Anaphase-I showing 23 chromosomal bodies

Fig.69a. Association of nucleolar chromosome of *S.viarum* with that of F_1 hybrid

FIG.67a

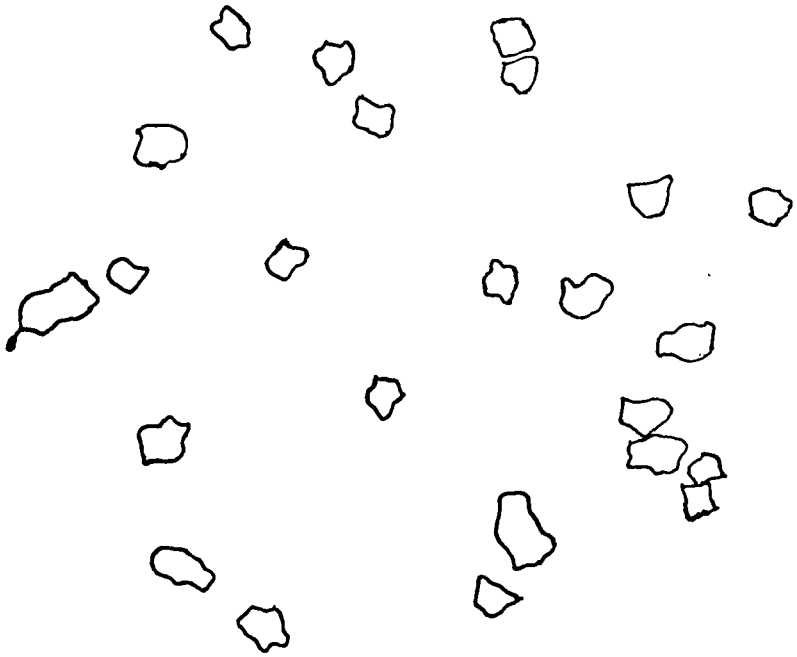
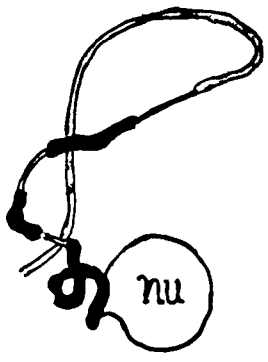


FIG.69a



In resolving the parental origin of the chromosomes, the differences in the stainability behaviour of the chromosomes of the parental species was taken as the major criterion. The pachytene chromosomes of the S.viarum (the male parent) was characterized by the differential staining of proximal and distal segments of arm and S.[redacted] (the female parent) by uniformly stained segments.

Cytological evidence for hybridity could be adduced at pachytene stage by the presence of nucleolar chromosomes of S.viarum which was associated with the nucleolus but as univalent (Fig.69 and 69a). Univalent configuration of a S.viarum chromosome bearing telochromomere was recorded (Fig.70). A telochromomere - bearing chromosome of S.viarum in bivalent association could be distinguished but its partner could not be identified (Fig.71).

Pairing behaviour of S.[redacted] chromosome were incompletely traced in a number of nuclei at pachytene and included both terminal (Figs.72 to 74) and interstitial paired segments (Fig.75). Heteromorphic chromosomal association showing interstitial pairing failure were also incompletely traced (Figs.76 to 78). Chromosomal segments showing sub-terminal unpaired segments were also traced (Figs.79 and 80). A chromosomal segment bearing terminal dumb-bell shaped

Plate 26. Legend for photomicrographs of pachytene stage in F_1 interspecific hybrid (SM x SV) x 3000

Fig.70. Telochromomere bearing univalent of S.viarum

Fig.71. Telochromomere bearing chromosome of S.viarum in bivalent association with an unidentified partner

Figs.72 to 74. Bivalent association in F_1 interspecific hybrid involving terminal chromosomal segment of S.mammosum

Fig.75. Interstitial segment of a paired bivalent of S.mammosum

Fig.76. Heteromorphic chromosomal association represented by interstitial pairing failure. → indicates unpaired region

PLATE.26

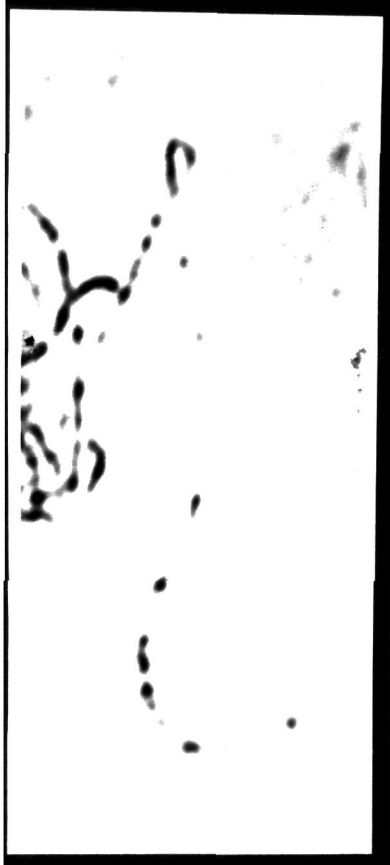


FIG.70

FIG.71

FIG.72

FIG.74

FIG.75

FIG.73

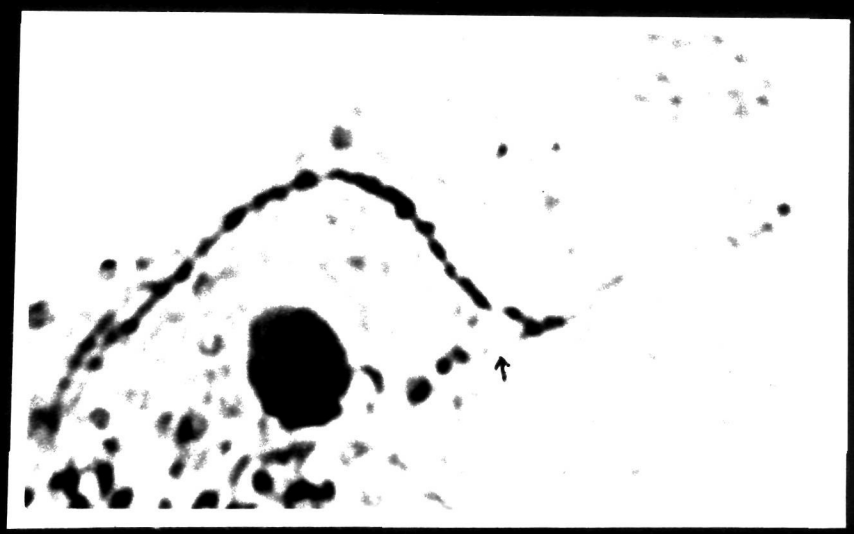


FIG.76

Plate 27. Legend for photomicrographs at pachytene stage in F_1 interspecific hybrid (SM x SV) x 3000

Figs.77-78. Metromorphic chromosomal association represented by interstitial pairing failure. → indicates unpaired region

Figs.79-80. Sub-terminal unpaired segments. → indicates unpaired region

Figs.81 to 83. Dumb-bell shaped dark-stained segment (indicated by: →)

PLATE.27

FIG.77

FIG.78

FIG.79

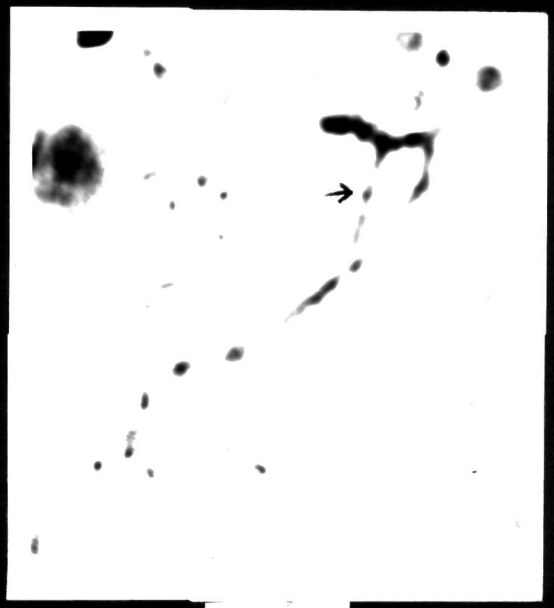


FIG.80

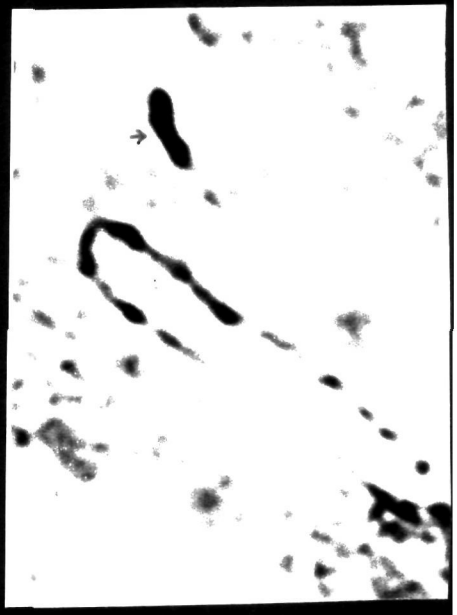


FIG.81

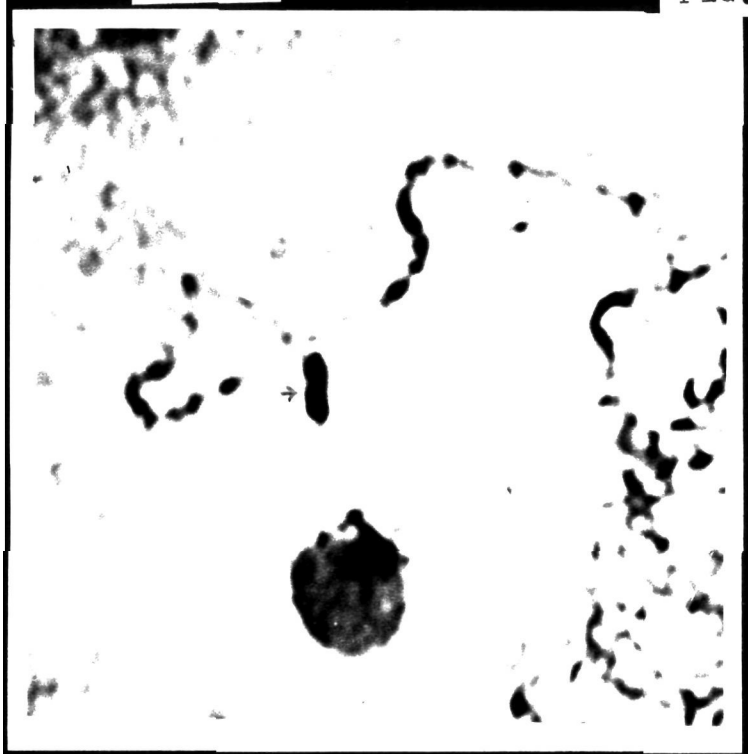


FIG.82

FIG.83

dark-staining region was frequently observed in the PMCs at this stage (Figs.81 to 83).

4.3.4.3. Diakinesis

Observations at this stage was restricted to nucleolus associated chromosome. Among the 50 PMCs examined, 90 per cent had a single nucleolus-associated bivalent, and the rest had two bivalents.

4.3.4.4. Metaphase-I

Chromosomal association consisted of quadrivalents, trivalents, bivalents and univalents (Table 20). A total of 38 types of chromosomal associations were discerned in 73 PMCs examined. The number of paired chromosomes in these cells ranged from 6 to 22 per cell (Fig.84). The maximum pairing involving 22 chromosomes was observed in 5.5 per cent of the PMCs and consisted of $1^{IV}+2^{III}+6^{II}+1^{I}$ (2.7%), $1^{IV}+9^{II}+1^{I}$ (1.4%) and $11^{II}+1^{I}$ (1.4%). PMCs with 16 and 17 paired chromosomes were more frequent and each accounted for 13.7 per cent of the PMCs examined. Though one (Fig.85) or two quadrivalents occurred in 68.4 per cent PMCs and the presence of a single quadrivalent was frequent and was detected in 58.8 per cent PMCs. Trivalents ranging from one to three occurred in 64.2 per cent of the PMCs. The frequency of PMCs with

Table 20. Chromosomal association at metaphase I in PMCs of F_1 hybrid of S.mammosum (2n=22) and S.viarum (2n=24)

Sl. No.	IV	III	II	I	No.of paired chromosomes	Multivalents per cell	Frequency (Number)
1	2	2	2	5	18	4	1
2	2	2	1	7	16	4	1
3	2	1	5	2	21	3	2
4	2	1	4	4	19	3	1
5	2	1	2	8	15	3	1
6	2	0	6	3	20	2	1
7	1	3	3	4	19	4	1
8	1	2	6	1	22	3	2
9	1	2	5	3	20	3	1
10	1	2	4	5	18	3	2
11	1	2	3	7	16	3	1
12	1	2	1	11	12	3	1
13	1	1	7	2	21	2	4
14	1	1	6	4	19	2	2
15	1	1	5	6	17	2	5
16	1	1	4	8	15	2	4
17	1	1	3	10	13	2	5
18	1	1	2	12	11	2	1
19	1	0	9	1	22	1	1
20	1	0	8	3	20	1	1
21	1	0	7	5	18	1	2
22	1	0	6	7	16	1	3
23	1	0	5	9	14	1	3
24	1	0	4	11	12	1	1
25	1	0	3	13	10	1	2
26	1	0	2	15	8	1	1
27	0	3	4	6	17	3	2
28	0	2	5	7	16	2	2
29	0	1	7	6	17	1	3
30	0	1	6	8	15	1	1
31	0	1	5	10	13	1	2
32	0	1	4	12	11	1	2
33	0	0	11	1	22	-	1
34	0	0	8	7	16	-	3
35	0	0	7	9	14	-	1
36	0	0	6	11	12	-	4
37	0	0	5	13	10	-	1
38	0	0	3	17	6	-	1
	0-2	0-3	1-11	1-17	Range	Total	73
	0.78	0.88	5.05	7.14	Mean		

Plate 28. Legend for photomicrographs at metaphase-I stage in F_1 interspecific hybrid (SM x SV) x 3000

Fig.84. Metaphase-I showing maximum number of paired chromosomes in an association of 1IV+1III+7II+2I

Fig.85. Metaphase-I showing $1^{IV}+5^{II}+9^{I}$ association

Fig.86. Metaphase-I showing $1^{III}+5^{II}+10^{I}$ association

PLATE 28

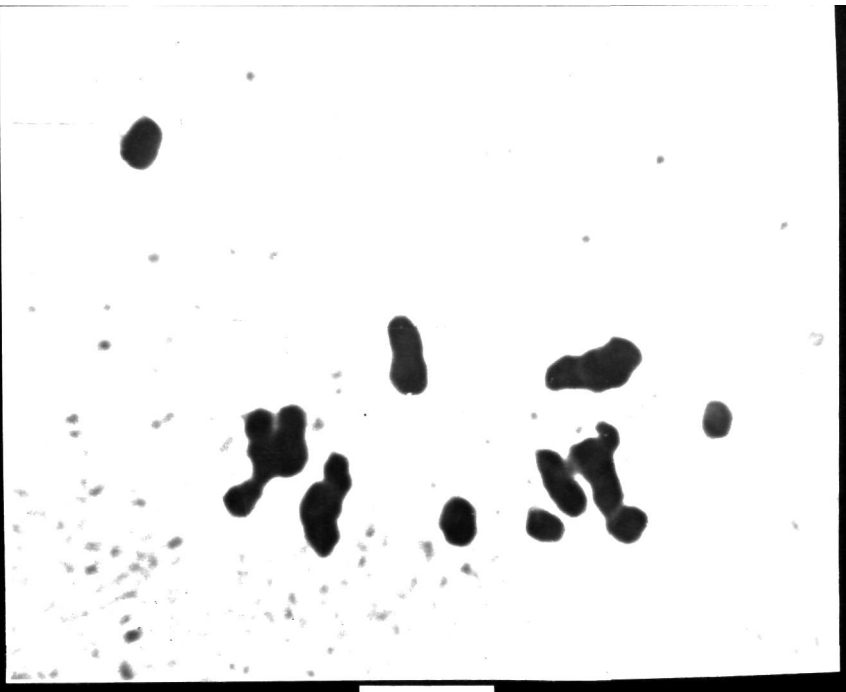


FIG.84

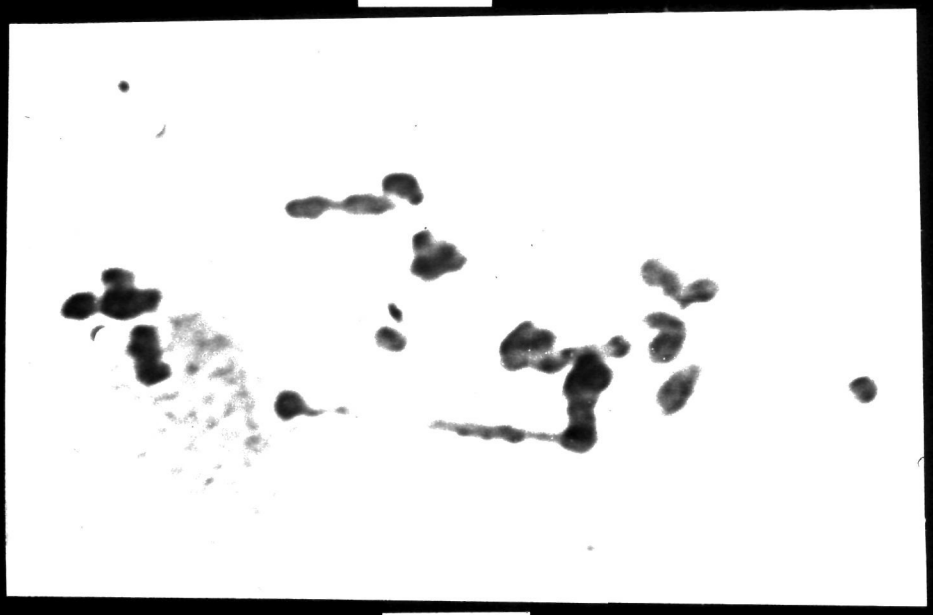


FIG.85

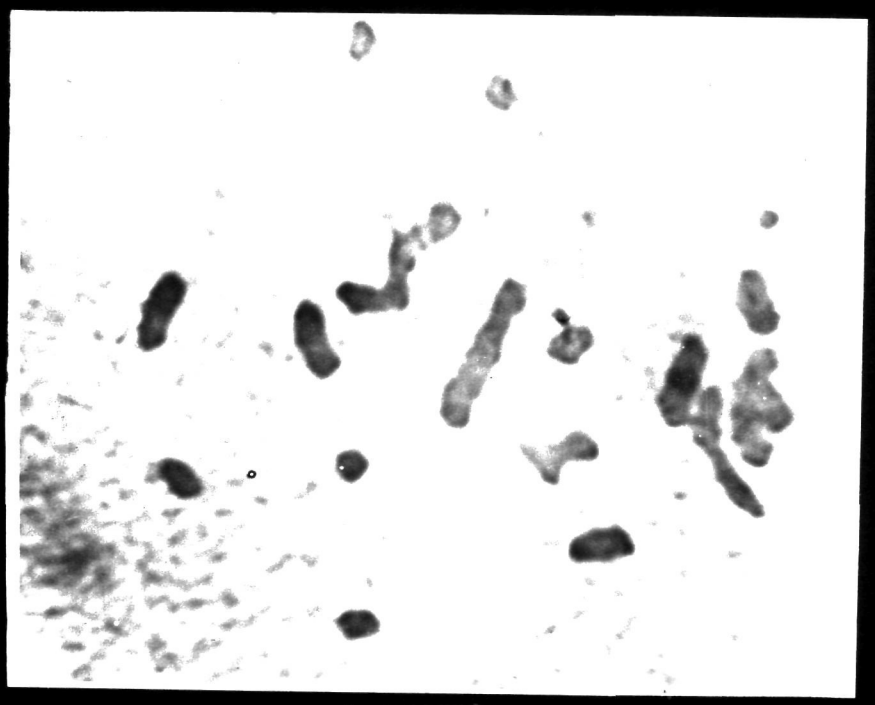


FIG.86

Explanatory diagrams of figures in Plate 28
(IV and III - in stripes; II - in shade; I - in outline)

Fig.84a. Metaphase-I showing $1^{IV}+1^{III}+7^{II}+2^{I}$

Fig.85a. Metaphase-I showing $1^{IV}+5^{II}+9^{I}$

Fig.86a. Metaphase-I showing $1^{III}+5^{II}+10^{I}$

FIG.84a

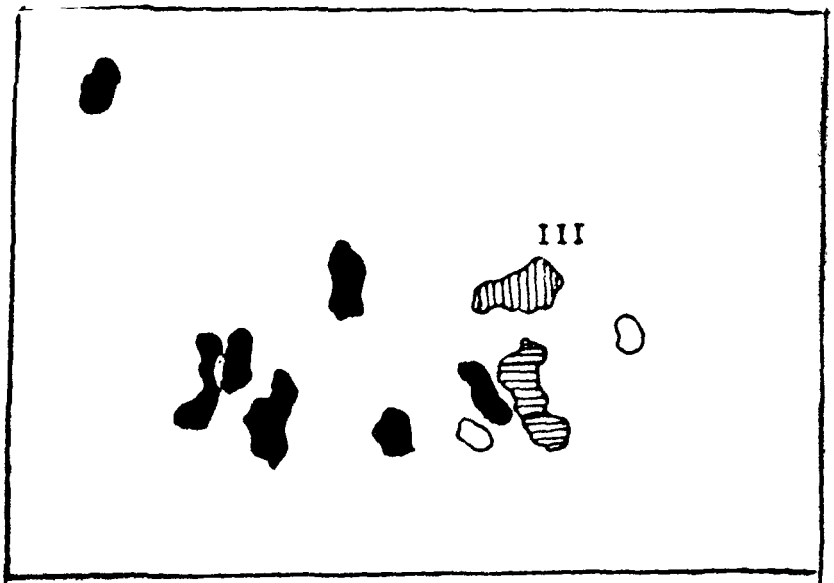


FIG.85a

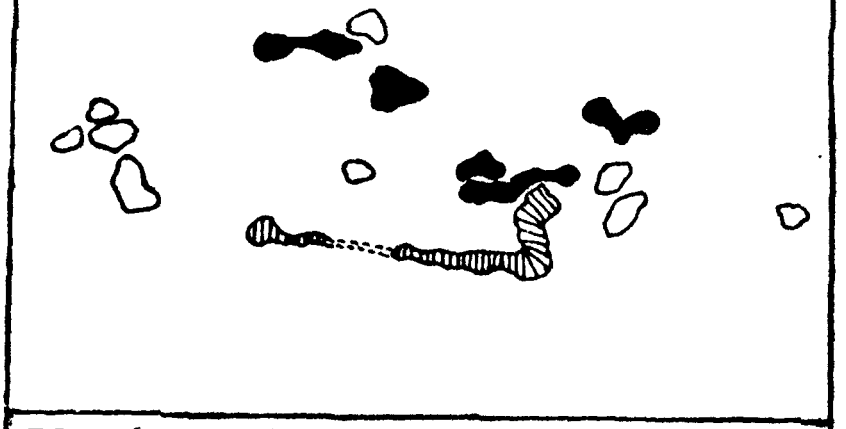
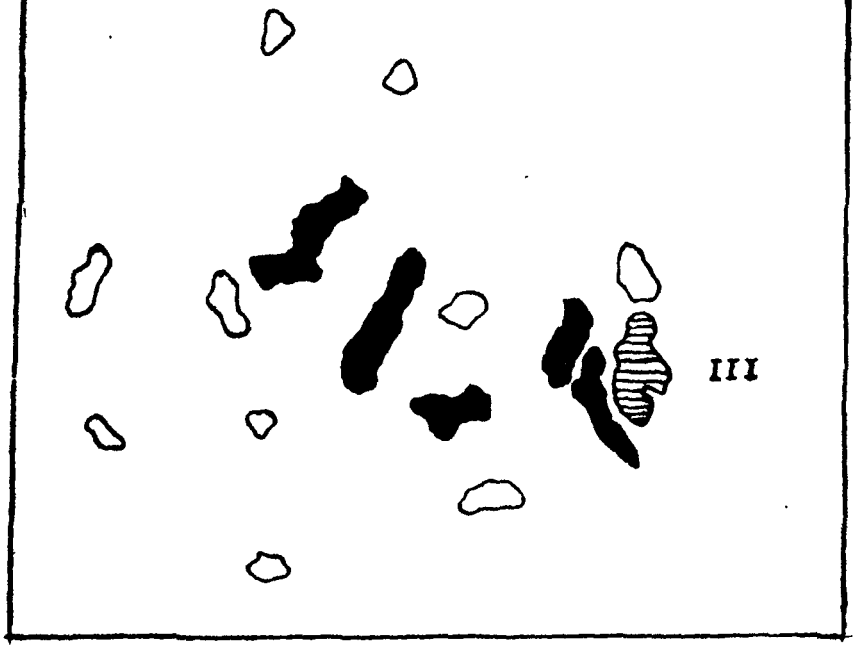


FIG.86a



one (Fig.86), two (Fig.87) and three trivalents (Fig.88) was 45.2, 15.1 and 4.1 per cent, respectively. Bivalents were present in all the cells examined and ranged from 1 to 11 per cell (Fig.86). The occurrence of three to seven bivalents was observed in 83.6 per cent PMCs. Four (Fig.88), five (Figs.85 to 87) and six bivalents was observed in 16.4, 21.9 and 17.8 per cent PMCs, respectively. Heteromorphic bivalents were observed in 52.10 per cent of the PMCs and involved a maximum number of two bivalents per cell (Figs.85, 86 to 89). Univalents occurred in all the PMCs and their number ranged from 1 to 17 per cell (Figs.84 to 89). The occurrence of six (13.7%)(Fig.88) and seven (13.7%)(Figs.87 and 89) univalents was more frequent than others.

Precocious movement of chromosomes during metaphase-I occurred in all the PMCs examined and involved one or both the poles (Fig.90) - in 39.7 and 60.2 per cent PMCs, respectively (Table 21). The number of chromosomes involved in precocious movement ranged from one to 14. The more frequently observed number of chromosomes involved in precocious movement were one (23.1%), two (19.2%), three (10.25%), five (11.5%) and eight (10.25%). In 23.1 per cent PMCs, the number of chromosomes involved in precocious movement to the poles was equal and in the rest, unequal.

Table 21. Precocious movement of chromosomes during metaphase-I in PMCs of the F_1 hybrid between S.mammosum (2n=22) and S.viarum (2n=24)

Number of chromosomes involved	Pole-I	Pole-II	Frequency	
			Number	Percentage
1	1	0	18	23.08
2	1	1	6	7.69
2	2	0	9	11.54
3	3	0	2	2.56
3	1	2	6	7.69
4	4	0	2	2.56
4	1	3	2	2.56
4	2	2	2	2.56
5	1	4	1	1.28
5	3	2	8	10.26
6	4	2	2	2.56
6	3	3	2	2.56
7	4	3	7	8.97
8	4	4	7	8.97
8	5	3	1	1.28
10	6	4	1	1.28
13	6	7	1	1.28
14	7	7	1	1.28
		Total	78	

Plate 29. Legend for photomicrographs at metaphase I stages in F_1 interspecific hybrid (SM x SV) x 3000

(→ indicates heteromorphic bivalents)

Fig.87. Metaphase-I showing $2^{III}+5^{II}+7^{I}$ association

Fig.88. Metaphase-I showing $3^{III}+4^{II}+6^{I}$ association

Fig.89. Metaphase-I showing $8^{II}+7^{I}$ association

PLATE.29

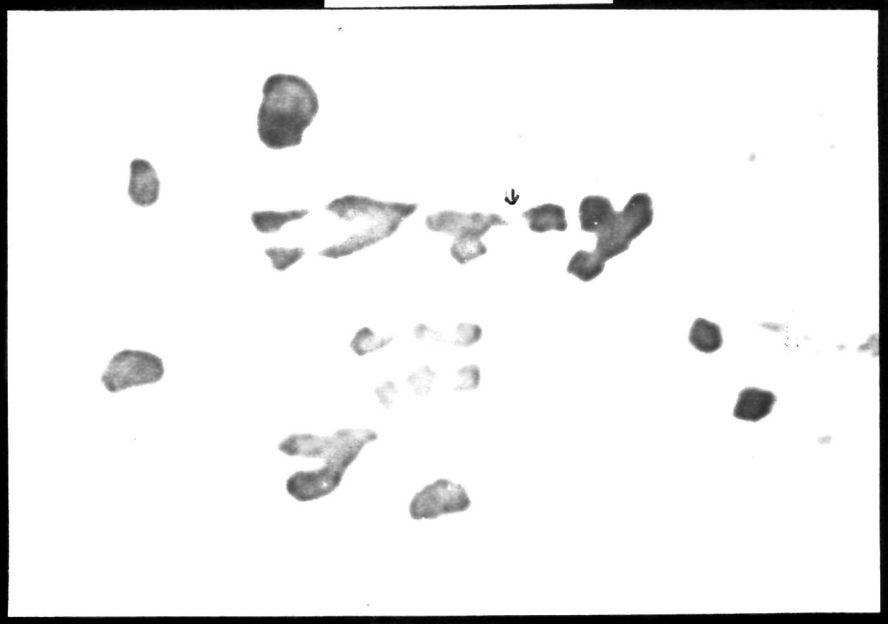


FIG.87

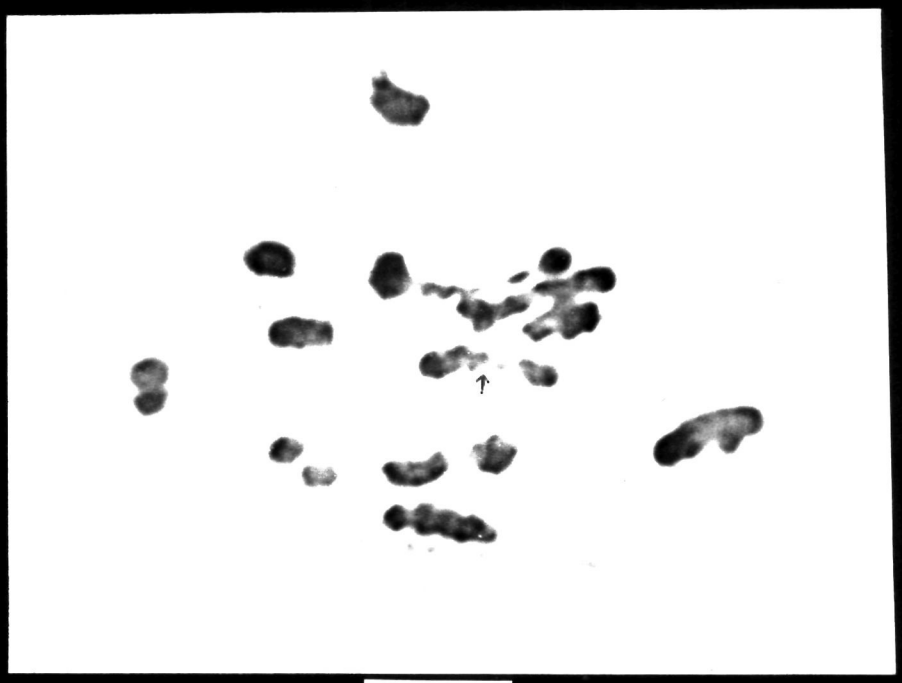


FIG.88

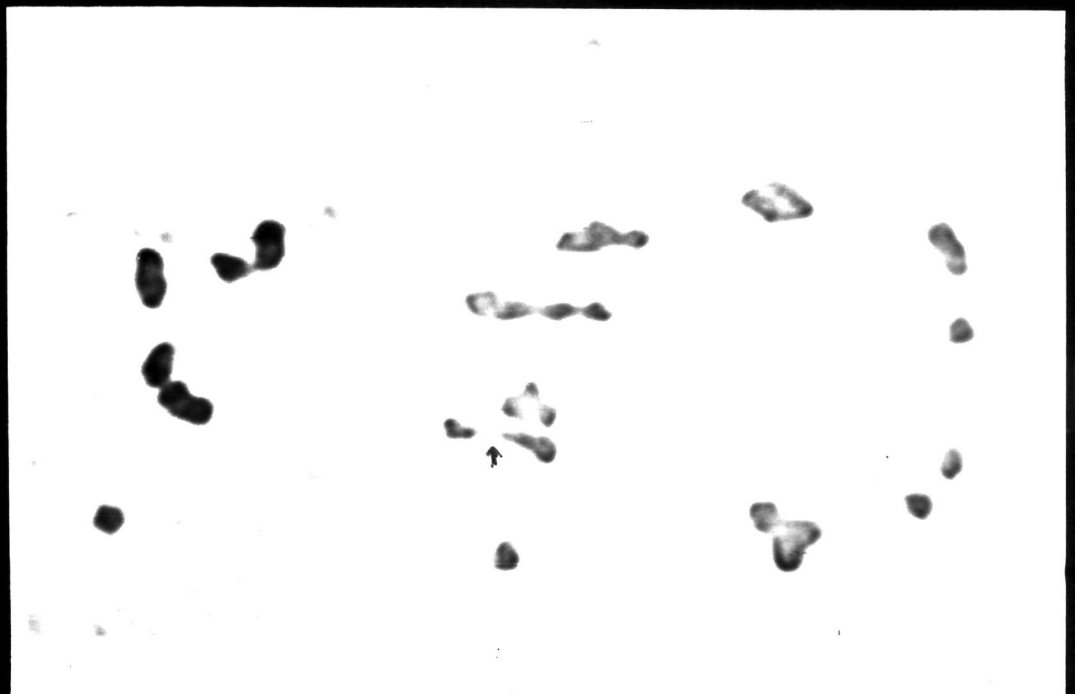


FIG.89

Explanatory diagrams for figures in Plate 29
(IV and III - in stripes, II - in shade, I - in outline)

Fig.87a. Metaphase-I showing $2^{III}+5^{II}+7^{I}$

Fig.88a. Metaphase-I showing $3^{III}+4^{II}+6^{I}$

Fig.89a. Metaphase-I showing $8^{II}+7^{I}$

FIG.87a

FIG.88a

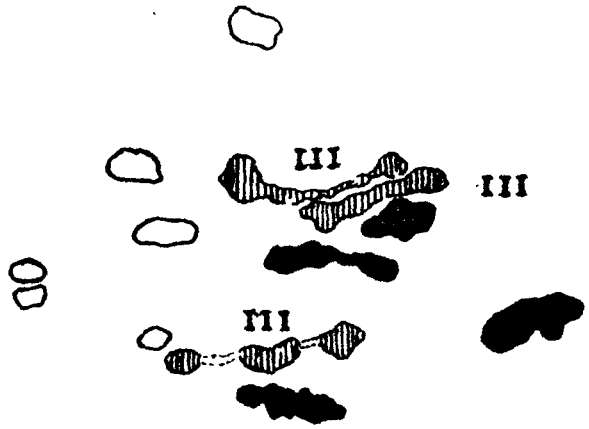
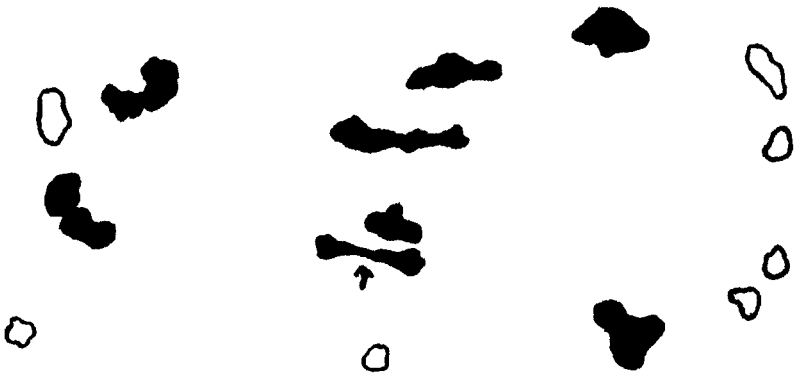


FIG.89a



4.3.4.5. Anaphase-I

The presence of lagging chromosomes (Table 22; Fig.91), bridges (Table 23) and varying groups of nuclei were observed at this stage. Lagging chromosomes ranging from one to five occurred in 61.3 per cent of the PMCs. Occurrence of a single lagging chromosome was most frequent (34.4%). Formation of bridges and extra group of nuclei was observed in 25.32 per cent and 32.98 per cent of the PMCs examined, respectively.

4.3.4.6. Telophase-I

Apart from the occurrence of two nuclei in 69.7 per cent, three (Fig.92) and four nuclei occurred in 21.4 and 8.9 per cent of PMCs examined, respectively (Table 24).

4.3.4.7. Prophase-II

Occurrence of two and three daughter nuclei was observed in 80.0 and 20.0 per cent of the PMCs examined, respectively (Table 24).

4.3.4.8. Metaphase-II

At this stage the number of chromosomal groups ranged from two to four (Table 24). The percentage frequency of PMCs having two, three (Fig.93) and four chromosomal groups were 68.2, 29.7 and 1.65, respectively. Chromosomal counts

Table 22. Frequency of laggards at anaphase-I and anaphase-II stages in PMCs of the F_1 hybrid between S.[redacted] (2n=22) and S.[redacted] (2n=24)

Stage	PMCs with lagging chromosomes								Total
	0	1	2	3	4	5	6	7	
Anaphase-I									
Number	112	97	51	20	1	1	-	-	282
Percentage	(39.72)	(34.40)	(18.10)	((7.10)	(0.35)	(0.35)	-	-	
Anaphase-II									
Number	-	18	23	11	6	5	1	1	65
Percentage	-	(27.70)	(35.40)	(16.90)	(9.20)	(7.70)	(1.50)	(1.50)	

Table 23. Occurrence of chromosomal bridge during anaphase-I and anaphase-II stages in PMCs of the F_1 hybrid between S.[redacted] (2n=22) and S.[redacted] (2n=24)

Stage	PMCs		Total
	With bridge	Without bridge	
Anaphase-I			
Number	39	115	154
Percentage	(25.32)	(74.68)	
Anaphase-II			
Number	24	9	33
Percentage	(72.70)	(27.30)	

Table 24. Frequency of PMCs with different number of nucleoli at various stages of F_1 hybrid of S.[illegible] (2n=22) and S.viarum (2n=24)

Stage	Number of nucleoli					Total
	2	3	4	5	6	
Anaphase-I						
Number	63	27	4	-	-	94
Percentage	(67.02)	(28.72)	(4.26)	-	-	
Telophase-I						
Number	101	31	13	-	-	145
Percentage	(69.66(	(21.38)	(8.97)	-	-	
Prophase-II						
Number	28	7	-	-	-	35
Percentage	(80.00)	(20.00)	-	-	-	
Metaphase-II						
Number	124	54	3	-	-	181
Percentage	(68.20)	(29.70)	(1.65)	-	-	
Anaphase-II						
Number	9	11	42	3	1	66
Percentage	(13.60)	(16.60)	(63.40)	(4.53)	(1.51)	
Telophase-II						
Number	-	39	87	3	-	129
Percentage	-	(30.20)	(67.40)	(2.30)	-	

Plate 30. Legend for photomicrographs of meiotic stages in F_1 interspecific hybrid (SM x SV)

Fig.90. Metaphase-I showing precocious movement of chromosomes to both poles x 2500

Fig.91. Anaphase-I showing the occurrence of laggards x 2000

Fig.92. Telophase-I showing the formation of three daughter nuclei x 2000

PLATE.30

FIG.90

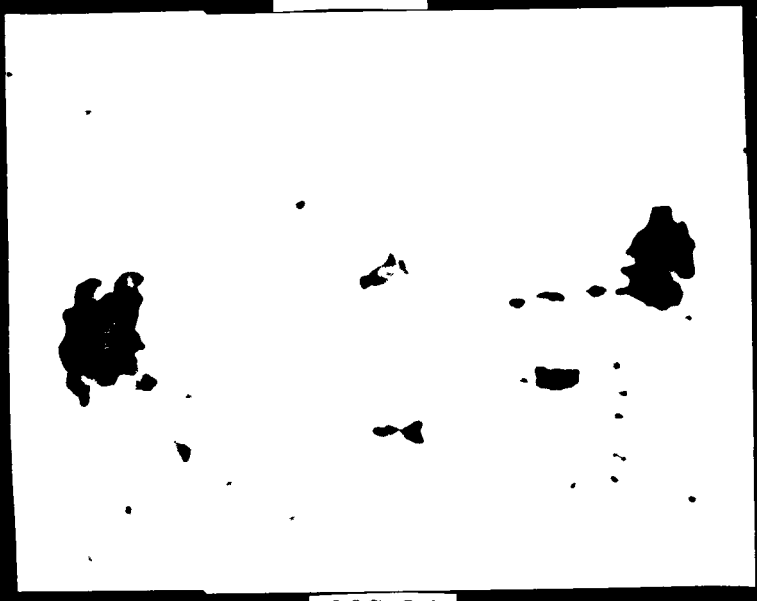


FIG.91

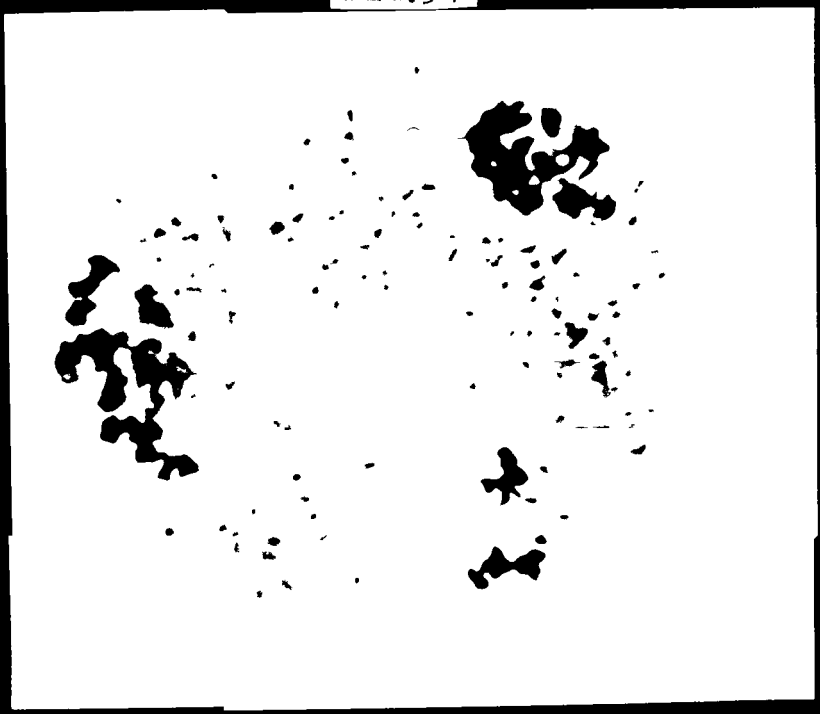


FIG.92

Plate 31. Legend for photomicrographs of meiotic stages in F_1 interspecific hybrid (SM x SV)

Fig.93. Metaphase-II showing three chromosomal groups x 2000

Fig.94. Telophase-II showing three daughter nuclei x 2000

PLATE.31

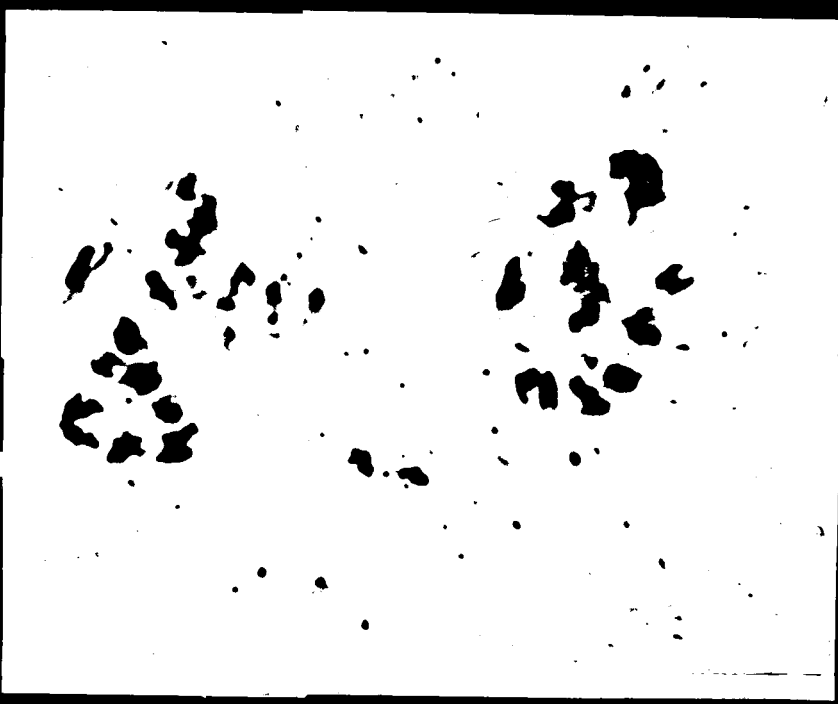


FIG.93

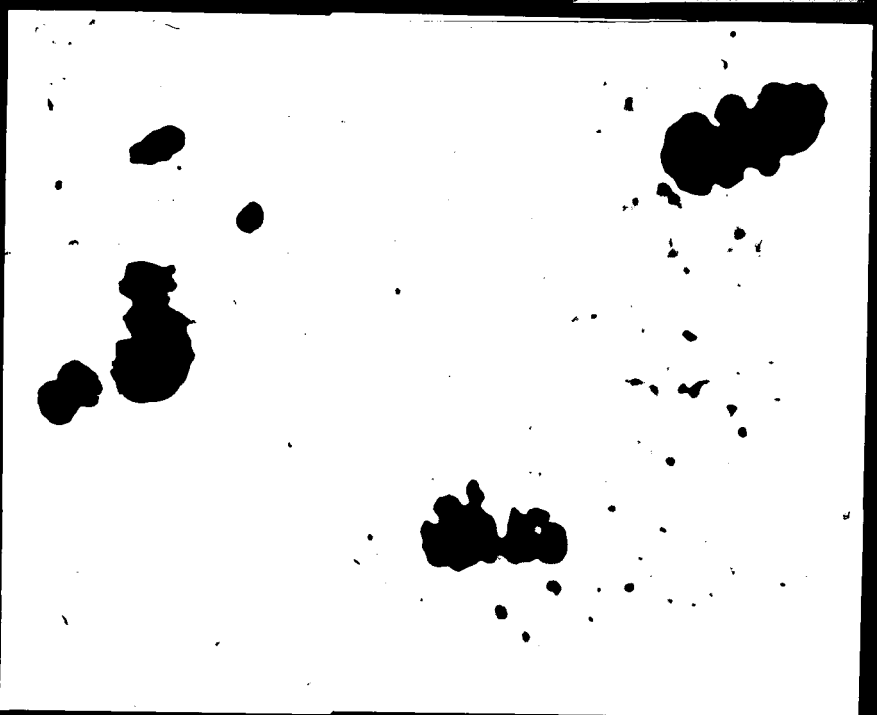


FIG.94

in these groups was made in 39 PMCs (Table 25). In PMCs with two chromosomal groups, the number of chromosomes in groups were unequal and the differences in chromosome number involved were one, three or five. In PMCs where three chromosomal groups (Fig. 93) were detected the smallest group had one, two or three chromosomes. Chromosome distribution in the remaining two groups was equal in some of the PMCs and in the others unequal. In the latter, the differences involved upto nine chromosomes. PMCs showing four chromosomal groups were fewer and two of the chromosomal groups contained the majority of the chromosomes.

4.3.4.9. Anaphase-II

At this stage 63.4 per cent of the PMCs showed four chromosomal groups while the rest had two (13.6), three (16.6%), five (4.53%) and six (1.51%) chromosomal groups (Table 24). Chromosomal counts in the daughter nuclei was made in 29 PMCs at Anaphase-II (Table 26). The number of chromosomes in the daughter nuclei of the PMCs at this stage showed a wide variation and ranged from 7 to 21 (Table 27). Among them daughter nuclei having 9, 10, 11, 12 and 13 chromosomes were more frequent and occurred in 11.9, 11.1, 17.6, 19.3 and 12.7 per cent of daughter nuclei. The presence of lagging chromosomes (Table 24) and formation of bridges (Table 25) were

Table 25. Chromosome number of daughter nuclei at metaphase-II in PMCs of F_1 hybrid between S.[redacted] (2n=22) and S.viarum (2n=24)

Chromosome number	Frequency	
	Number	Percentage
14-9	2	5.13
13-10	4	10.26
12-11	7	17.95
14-7-2	1	2.56
12-9-2	5	12.82
12-10-1	2	5.13
11-11-1	6	15.38
10-10-2	5	12.82
11-9-3	1	2.56
10-10-3	3	7.69
11-10-1-1	2	5.13
9-7-4-3	1	2.56
Total	39	

Table 26. Chromosome distribution to different poles at anaphase-II of the F_1 hybrid of S.[redacted] (2n=22) and S.[redacted] (2n=24)

Sl. No.	Pole I	Pole II	Pole III	Pole IV	Pole V	Total	Frequency (number)
1	7	9	9	21	-	46	1
2	8	7	13	18	-	46	1
3	8	10	13	15	-	46	2
4	9	9	12	16	-	46	1
5	7	12	13	14	-	46	2
6	8	11	12	15	-	46	1
7	8	11	13	14	-	46	1
8	8	11	11	16	-	46	1
9	10	9	11	16	-	46	1
10	9	10	15	12	-	46	1
11	9	11	12	14	-	46	2
12	9	11	13	13	-	46	1
13	14	12	10	10	-	46	2
14	14	11	12	9	-	46	1
15	12	13	13	8	-	46	1
16	13	12	11	10	-	46	1
17	14	11	12	9	-	46	1
18	12	9	12	13	-	46	1
19	10	11	11	14	-	46	1
20	11	11	11	13	-	46	1
21	12	12	12	10	-	46	1
22	11	11	12	12	-	46	1
23	12	11	12	11	-	46	1
24	13	10	13	10	-	46	-
25	9	9	8	12	7	46	1
							Total 29

Table 27. Projected frequency of chromosomal number in gametes based on chromosomal counts in 29 PMCs at anaphase-II in F_1 hybrid of SM x SV

Number of chromosomes	Frequency	
	Number	Percentage
7	5	5.95
8	8	6.80
9	14	11.90
10	13	11.05
11	20	17.00
12	23	19.55
13	15	12.75
14	10	8.50
15	4	3.40
16	3	2.55
18	1	0.85
21	1	0.85
Total	117	

also observed at this stage. Occurrence of lagging chromosomes was common in all the PMCs observed, with the most frequent being two (35.4%), one (27.7%) and three (16.5%) per cell. Formation of bridges was observed in 72.7 per cent of the PMCs examined.

4.3.4.10. Telophase-II

In 67.4 per cent of the PMCs, four daughter nuclei were observed and in 30.2 per cent and 2.30 per cent of the PMCs three (Fig.94) and five daughter nuclei, respectively, were observed (Table 24).

4.3.4.11. Microspore tetrad stage

The number of microspores, the product of meiosis, ranged from two to nine (Figs.95 to 97)(Table 28). PMCs with four and five microspores were most frequent and occurred in 31.6 and 35.3 per cent PMCs, respectively.

4.3.4.12. Pollen fertility

Pollen fertility as estimated based on stainability was low, 3.5% (Fig.98). Fertile pollen grains had a mean diameter of 26.40 μ.

4.4. SOLASODINE CONTENT

The solasodine content in berries of *S.viarum*, *S.* [illegible], their reciprocal grafts and induced autotetraploid of *S.viarum* was determined (Table 29).

Table 28. Number of microspores in PMCs at tetrad stage in F_1 hybrid of S.mammeanum (2n=22) and S.viarum (2n=24)

Frequency	Number of microspores								Total
	2	3	4	5	6	7	8	9	
Number	6	28	60	67	21	4	3	1	190
Percentage	(3.16)	(14.74)	(31.58)	(35.26)	(11.05)	(2.11)	(1.58)	(0.53)	-

Plate 32. Legend for photomicrographs of meiotic stages in F_1 interspecific hybrid (SM x SV)

Fig.95. Microspore tetrad with two spores x 2000

Fig.96. Microspore tetrad with three spores x 2000

Fig.97. Microspore tetrad with nine spores x 2000

Fig.98. Sterile pollen grains x 1000

PLATE.32

FIG.95

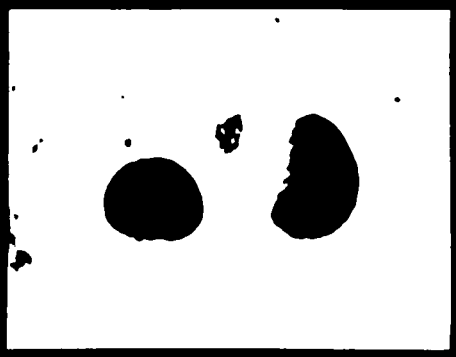


FIG.96

FIG.97

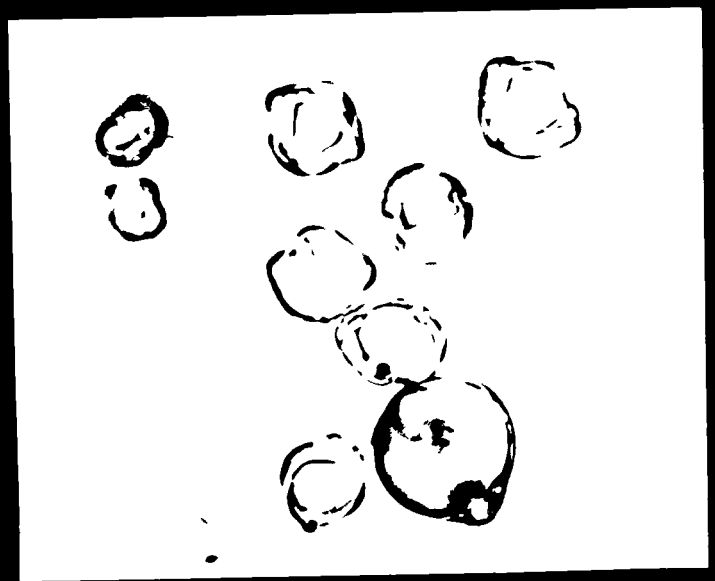


FIG.98

Table 29. Solasodine content (%) in dried berries of S.xiarum and S.mammosum

Material	Solasodine content (%)
1. Diploid	
S.xiarum	1.83±0.27
S.mammosum	1.20±0.13
2. Grafts scion	
S.xiarum	2.16
S.mammosum	1.54
3. Autotetraploid	
S.xiarum	2.72±0.29

4.4.1. Diploid S.viarum and S.mammosum

The solasodine content in berries of S.viarum was found to be higher than that of S.mammosum.

4.4.2. Reciprocal grafts

Reciprocal grafts were made using seedlings of S.viarum and S.mammosum. Both species grew well on the stock of other. But S.mammosum scion was affected by virus resulting in premature mortality. However, five berries were harvested before the plant died. The berries were smaller in size and had fewer number of seeds (3.18 per berry as against 273.36 in ungrafted control). S.viarum as scion on S.mammosum showed vigorous growth. But in the grafted plants berry size as well as seed number was reduced. Solasodine content in grafted plants were higher than ungrafted control plants. S.mammosum as scion the solasodine content was 1.54 per cent as against 1.20 per cent in ungrafted plants.

4.4.3. Autotetraploids

In the berries of autotetraploid S.viarum, the solasodine content was 2.72 per cent and thus was higher than that of diploids.

DISCUSSION

V. DISCUSSION

The thesis presents data on pachytene karyology of the commercially important steroid-bearing S.viarum Dunal and its relationship with another steroid-bearing Solanum species, S.mammosum which is also considered to be a potential source of Solasodine (Telek et al., 1977). The evaluation of the inter-relationship between the two species based on data on chromosome morphology at pachytene, chromosome number, crossability, cytomorphic behaviour of interspecific hybrid, response to chromosomal doubling and study of leaf flavonoids. These informations besides providing the basic cytogenetical edifice for future breeding programme in S.viarum and S.mammosum have also brought out interesting information for the first time, on the interrelationship of two steroid-bearing species of the new world.

Karyomorphology of S.viarum Dunal

In S.viarum karyological studies have been reported by earlier workers both at mitotic metaphase (Krishna Mitra, 1966; Zutshi, 1972) and at pachytene (Dnyansagar and Pingle, 1978). The chromosome number has been determined as 2n=24 by these workers. The somatic chromosomes are reported to range in length from 1.6 µ to 2.8 µ by Krishna Mitra (1966) and

1.9 /u to 3.3 /u by Zutshi (1972). Thus, the smaller size of the mitotic chromosomes was confirmed by these reports. The number of median and sub-median chromosomes in the haploid complement has been reported as four and eight, respectively (Krishna Mitra, 1966). The presence of a single satellite chromosome in the haploid complement has been reported both by Krishna Mitra (1966) and Zutshi (1972). The length of satellite chromosome as reported by Krishna Mitra (1966) and Zutshi (1972) being 2.5 /u and 2.6 /u respectively, is in close agreement.

However, some conflicting observations on the karyology of somatic chromosomes have been made. Rao et al. (1980) reported absence of satellited chromosomes while Krishnappa and Chennaveeraiah (1977) reported presence of two pairs of satellited chromosomes. Neither of these observations could be confirmed in the present investigation where both pachytene and mitotic prophase stages have been examined. On the other hand, presence of a single nucleolar associated chromosome as reported by Krishna Mitra (1966) and Zutshi (1972) could be confirmed. Karyomorphological studies at somatic metaphase could only enable identification of six (Krishna Mitra, 1966) or five chromosomal types (Zutshi, 1972) but not the entire haploid chromosomal complement. The small size of the chromosomes and the inherent limitation of this stage imposed by the paucity of criterion could be identified as the major bottleneck in

karyological studies conducted at root tip mitosis in this species.

The small size of the chromosomes which generally poses a limitation in karyological studies at somatic metaphase is considered to be an advantage when karyological studies are conducted at pachytene (c.f. Venkateshwarlu, 1962). This observation seems to be substantiated by pachytene karyological studies in S.xiarum conducted by Pingle and Dnyansagar (1976). These authors have reported successful identification of the entire haploid complement using distribution of heterochromatin as a major criterion. The length of pachytene chromosomes in this species is reported to vary from 9.4 /u to 34.2 /u. Three median chromosomes have been recognised in the complement while the rest of the chromosomes are reported to be sub-median.

Pingle and Dnyansagar (1976) have reported certain peculiar features at pachytene stage in this species. The absence of nucleolus in most of the PMCs at this stage with the exception of one cell is one of these observations. The absence of nucleolus at pachytene stage has not been generally reported in Solanum species or in any of the crop plants investigated so far (c.f. Venkateshwarlu, 1962). Another such observation relates to the length of range of pachytene chromosomes of S.xiarum which is reported to vary from 9.3 /u to 34.2 /u as against 28.8 /u to 41.8 /u in S.torvum and 22.4 /u

to 44.2 /u in S.surattense. In prespect of the pattern of distribution of dark and light-staining region in the various chromosomes Pingle and Dnyansagar (1976) recorded departures not encountered in other spinous Solanum species such as S.torvum and S.surattense. This pertains to the presence of terminal and interstitial dark-staining regions interposed in the light-staining regions of five chromosomes of the haploid complement. No supporting photomicrograph has been presented by these workers. In view of these discrepencies, in the present study re-investigation of pachytene karyology of S.viarum was taken up.

The reinvestigation of pachytene karyology of S.viarum in the present study has broughtout certain interesting departures from the observations of Pingle and Dnyansagar (1976) in this species. These include the differences in the range of pachytene chromosome length which was 22.4 /u to 58.1 /u as recorded in the present investigation as against 9.4 /u to 34.2 /u as reported by Pingle and Dnyansagar (1976). The former values are comparable to those reported for S.torvum (28.8 to 41.8 /u) and S.surattense (22.4 to 41.2 /u). In the present investigation contrary to the report of Pingle and Dnyansagar (1978) the occurrence of single nucleolus was consistently observed in all the PMCs examined at pachytene. Likewise, presence of single terminally associated nucleolus to the chromosome has been reported in other non-tuberiferous and/or steroid-bearing Solanum species like S.torvum (Rao, 1972), S.surattense

(Rao, 1975), S.integrifolium (Kirti and Rao, 1977) and S.imdicum (Kirti and Rao, 1978). The present study also could not confirm the report of Pingle and Dnyansagar (1976) on the presence of five bivalents with intercalary or terminal dark-staining regions interposed in the light-staining region at pachytene. The pachytene karyology of S.viarum as reported in the present study conform to the general morphology of differentiated pachytene chromosomes of other tuberous and non-tuberous Solanum species and of the taxa possessing differentiated pachytene chromosome. The number of median and submedian chromosomes in the haploid complement of S.viarum was found to be four and eight respectively, in contrast to three and nine reported by Pingle and Dnyansagar (1976). Occurrence of four median chromosomes has been observed in S.surattense (Rao, 1975) and S.imdicum (Kirti and Rao, 1978). Thus, the reinvestigation of the pachytene karyology in the present study has brought out glaring discrepencies in the earlier investigation of Pingle and Dnyansagar (1978) and the general agreement in the chromosomal morphology of pachytene chromosomes of S.viarum with those of other Solanum species and taxa possessing differentiated pachytene chromosomes.

The present study has rendered the feasibility of identification of the entire haploid complement at pachytene

based on the extent and distribution of light-and dark-stained regions in the arms, relative proportion of dark and light stained regions in the chromosome, chromomere pattern, nucleolar association and relative length. Thus, the basis for future intensive cytogenetical studies has been laid out which would enable pursuit of such studies as identification of primary trisomics in this species in the content of assigning linkage groups to chromosomes and also comparative karyological and homological studies involving S.xiarum and other Solanum species.

Basic number: Pingle and Dnyansagar (1966) have postulated a lower basic number of n=10, for the species S.xiarum Dunal and for that matter for the genus Solanum in general, based on similarity in the morphology of four of the pachytene chromosomes of the haploid complement. The present study also brings out similarity in general morphology of pachytene chromosomes. These similarities pertain to position of centromere and distribution of dark-staining region in the chromosomal arms. Of the four median chromosomes IV, V, VIII and IX, chromosome IV and V bear close similarity to each other and chromosome VIII to IX. Similarly, the sub-median chromosomes X and XI, characterised by the presence of heteropycnotic short arms, bear close similarity. Thus, based on the comparative morphology of the chromosomes of the haploid complement, a lower basic number of n=9 can be postulated for S.xiarum.

Interrelationship between S.viarum and S.mammosum: Both S.viarum and S.mammosum belong to the spinous group (c.f. Rao, 1972). Both the species have been assigned to the same sub-genus Leptostemonum (cf. Randell and Symon, 1976 and Pearce and Lester, 1979) and the section Acanthophora. Thus, taxonomically, the two species are held to be closely related. The present investigation has rendered possible a closer look into the interrelationships of the two species from the point of view of the chromosome number, chromosome morphology, crossability, distribution of leaf flavonoids, chromosomal homology and response to chromosomal doubling (autotetraploidy) and reciprocal grafting. All these aspects are individually considered in the following paragraphs.

Comparative karyological studies in S.viarum and S.mammosum: The two species differ in the basic number. S.viarum has a basic number of n=12 against n=11 for S.mammosum. It is interesting to note that with the exception of S.mammosum all the Solanum species are based on n=12 (cf. Federov, 1974). Thus, S.mammosum stands out as a singular species in this genus by virtue of its chromosome number. A comparative study of general morphology of pachytene chromosomes of the two species as well as other Solanum species reveal that unlike the differentiated pachytene chromosomes in S.viarum and other Solanum species, the pachytene

chromosomes of S.mammosum is undifferentiated. Thus, in the morphology of pachytene chromosomes also, S.mammosum stands out as an exception in the genus Solanum.

A comparision of somatic karyology of the two species is excluded due to paucity of published information in S.mammosum. However, the present study, enables a visual comparision of mitotic metaphase/prophase chromosomes from anther wall cells of both the species. A comparision of chromosome size reveal that the somatic chromosomes of S.mammosum are larger than those of S.xiarum (Figs.13 and 20). The evidences for the larger size of the chromosomes of S.mammosum as compared to S.xiarum can also be had from a comparative study of anaphase-I stage of both the species as well as in the F_1 interspecific hybrid. In the F_1 hybrid, the presence of 11 large and 12 small chromosomes can be discerned (Fig.67). Further, the overall cell size in these two species as reflected by the size of the pollen grains showed that the diameter of pollen grain in S.mammosum is 20 per cent more than that of S.xiarum. It will be interesting in this context to note that S.xiarum has an additional chromosome in its haploid complement and even so has smaller pollen. Ramanujam and Parthasarathy (1953) have attributed the increased pollen size of the autotetraploids to the higher chromatin content.

Drawing an analogy of the above situation with that of the two species under discussion, the larger pollen size in S.sanmosum can be considered to reflect the higher chromatic content in S.sanmosum. Thus, strong indirect evidences can be advanced in favour of the larger size of the chromosome of S.sanmosum over those of S.viarum.

Both the species are characterized by the presence of single nucleolar associated chromosome at this stage. But the presence of interstitial nucleolar organizer at pachytene in S.sanmosum (Fig. 15) in contrast to the sub-terminal location of nucleolar-organizing region in S.viarum brings out additional karyological difference. Thus, even a casual comparision of the general morphology of pachytene morphology of the species bespeak of a wide diversity between the two species. It is, however, not justified to decide on the affinity of the two species based exclusively on the differences on the pachytene chromosome morphology. A parallel case is reported in sorghum, where the Eu-sorghums have differentiated pachytene chromosomes (Magoon and Shambulingappa, 1960), whereas para-sorghums have undifferentiated pachytene chromosomes (Garber, 1947; Magoon and Shambulingappa, 1962). Even so, the species belonging to these two groups are accommodated in same genus. Further, occurrence of differentiated and

undifferentiated chromosomes have been reported among races of Coix lachrymajobi (Venkateshwarlu, 1960 and 1961). But the difference in the stainability of pachytene chromosomes of S.viarum and S.mammosum viewed in the context of the differences in chromosome size and number could only be considered as indicative of their distant relationship.

Crossability: In the present investigation the assessment of crossability between the two species, has been based on comprehensive programme of artificial crosses involving reciprocally both the species and their autotetraploids both within and across ploidy levels. The number of pollinations made between the two species reciprocally at diploid level has been relatively much larger and have indicated unequivocally the unidirectional nature of cross with success attending only when diploid S.mammosum is used as female parent. Even so, the extent of fruit set is only 20 per cent and the number of mature fruits harvested is as low as five per cent. Though a large number of seeds have been harvested which included an assortment of bold and immatured ones, only one of the seedlings proved to be of hybrid origin. In contrast, when S.viarum is used as female parent, no fruit set occurred. The diploid S.mammosum also proved to be a successful female parent in as far as fruit set is concerned in crosses with autotetraploid S.viarum as

evident from 20 per cent fruit set. The diploid S.viarum recorded about 3 per cent fruit set in crosses with tetraploid S.mammosum but no mature fruits could be harvested due to premature fruit dropping. Autotetraploid S.viarum as female parent in crosses with diploid S.mammosum gave 29 per cent fruit set and also small sized parthenocarpic fruits. Parthenocarpically developed fruits as observed in the present investigation were also observed by Omidiji (1975) and Sharma et al. (1980) in non-tuberiferous Solanum species. Crosses between autotetraploids of the two species resulted in 36 per cent fruit set when auto-tetraploid S.viarum was used as female parent but resulted in no fruit set when autotetraploid S.mammosum was used as female parent. It is interesting that the nature of crossability seems to be reversed on chromosome doubling. The few crosses attempted with autotetraploid S.mammosum as female parent and diploid S.viarum as male parent resulted in no fruit set. Thus, different levels of cross compatibility was recorded in the present study involving these two species at diploid and tetraploid levels.

It will however be premature to conclude on the crossability relationship of S.viarum and S.mammosum based on the results of present study. This will be evident from the fact that in the genus Solanum crossability relationships reported

by different workers for a set of species was found to be highly conflicting (cf. Rao, 1979) even distantly related species such as tuber-bearing S.tuberosum and non-tuber bearing S.pinnatisectum held to be incompatible could be successfully crossed (Hermsen and Taylor, 1979). In the light of the above, as Rao (1979) has rightly observed, crossability in this genus as by itself cannot be taken as a criterion for close relationship or affinity of taxa.

Confirmity of hybrid origin

The thesis offers conclusive and direct evidences for the successful production of interspecific hybrid from controlled crosses between S.mammosum and S.viarum. The hybrid origin of the seedling resulting from cross between S.mammosum x S.viarum has been confirmed after a consideration of a number of morphological and cytological characters. The hybrid resembled the male parent in its leaf length, leaf breadth and colour of the matured fruit. The characters shared by the F_1-interspecific hybrid and female parent include, the fruit shape and susceptibility to virus. The hybrid was intermediate to the parents in respect of hairiness on the leaf especially on petiole, petal colour, anther colour, and fruit colour (Figs.58, 59 and 61).

The cytological proof of the hybridity was obtained at anaphase-I stage where 23 chromosomal bodies could be clearly identified. These included 11 larger chromosomes and 12 relatively smaller chromosomes (Fig.67).

Further, direct proof for the hybrid origin could be adduced from the examination of pachytene nuclei of the hybrid. Both S.sanguineum and S.viarum parental chromosomes could be identified by virtue of their differences in the pachytene chromosome morphology. The S.viarum chromosomes identified included the nucleolar organising chromosome which was observed as an univalent in association with the hybrid nucleolus. A univalent at pachytene with telochromomere bears morphological similarity to S.viarum chromosome (Fig.69). The presence of S.sanguineum chromosomes showing partial and complete pairing were also detected in hybrid nuclei at pachytene.

Further, confirmation of hybrid origin of the plant could be secured from the study of leaf flavonoids in the parents and hybrid, which showed that parental differences for leaf flavonoids and the presence of 12 spots in F_1 hybrid which were also shared by the parents. One of the exclusive spot of the male parent was also present in the F_1 hybrid.

Chromosomal homology

The present investigation presents for the first time information on the homology between the chromosomes of the two

species. In tracing the homology of the two species, the study of pachytene stage has also been utilised exploiting the basic differences, in the stainability behaviour of the parental chromosomes. As pointed out earlier, the pachytene chromosome of S.viarum conforms to the general morphology of pachytene chromosomes encountered in the genus Solanum and related genera with proximal dark staining regions flanking the centromere and light-staining regions distal to them. In contrast, the pachytene chromosomes of the S.[redacted] showed the presence of chromomeres along its entire length. The study of pachytene chromosomes in the F_1 hybrid was more of explorative nature largely due to the fact that adequate spreading could not be ensured in most of the nuclei examined. No instance of pairing between the chromosomes of the parental species could be discerned. However, evidences for autosyndetic pairing was secured in an instance where a telochromomere - bearing chromosomes of S.viarum and a non-telochromomere bearing chromosome of S.viarum were involved in a bivalent association (Figs.70 and 71). Besides, a number of instances of incompletely paired heteromorphic segments involving S.[redacted] chromosomes could be distinguished. These included both terminal and interstitial segments. Thus, the study of pachytene nuclei seem to indicate the occurrence of autosyndetic pairing in the hybrid nuclei and lack of homology between chromosomes of parental species.

The chromosome association in the F_1 hybrid at metaphase-I included multivalents such as quadrivalents and trivalents besides bivalents, and univalents. The number of paired chromosomes found to vary widely from 6 to 22 per cell of the possible 23 chromosomes. Quadrivalents occurred in nearly 68.4 per cent cells and presence of single quadrivalent occurred in 58.8 per cent of PMCs. Similarly, the occurrence of single trivalent is frequently observed (45.1 per cent cells), while two and three trivalents occurred in 15 and 4.5 per cent of the PMCs, respectively. The maximum number of multivalents were found to be four involving proportionate change in the frequency of quadrivalents and trivalents per cell. Thus, the maximum number of quadrivalents was limited to two while the maximum number of trivalents was limited to three. It is interesting that the maximum of four trivalents were not detected in cells which lacked in quadrivalent association. The participation of S.sammosum chromosomes in the trivalent and quadrivalent association seems likely considering the size of the chromosomes involved in this association. However, the involvement of S.viarum chromosomes in this configuration is not categorically demonstratable. However, instances of autosyndetic pairing of S.viarum chromosomes could be resolved based on the relative size of the bivalent (Figs.670 and 71). Similar heteromorphic bivalent resulting from association

of S.mammosum chromosomes could also be adduced based on relative size consideration. The occurrence of autosyndetic pairing involving both the genomes as verified at pachytene and as adduced at metaphase-I stages could be interpreted as evidences for distant relationship between the two species.

The occurrence of multivalents has been reported in a number of interspecific hybrids involving spinous Solanums. These include S.aureitense x S.trilobatum (Rao and Rao, 1977a), S.indicum x S.insanum (Zutshi, 1966b), S.indicum x S.torvum (Kirti and Rao, 1981). The multivalents in these instances included trivalents and quadrivalents with the maximum of upto three (Kirti and Rao, 1981). The occurrence of multivalents has been explained in those instances as evidences for chromosomal repatterning. Thus, chromosomal repatterning seems to have played an important role in species differentiation. The role of cryptic structural hybridity has been considered as a major factor underlying species differentiation has been held by several workers (Rajashekaran, 1971a, b and c; Rao and Rao, 1977a; Wanjari, 1976). Their conclusion is based on the fact that despite in normal meiosis high sterility has been encountered in those interspecific hybrids. However, in these interspecific hybrids of S.mammosum x S.viarum reported in the present study the occurrence of autosyndetic pairing could be clearly demonstrated at pachytene. This fact has

also to be borne in mind in the interpretation of chromosomal homology based on the study of meiotic metaphase-I stages where parental chromosomes cannot be identified. The demonstration of autosyndetic pairing in the present study can also lend support to the possible lower basic number for the Solanum species as suggested based on similarities in chromosomal morphology.

Sterility in the hybrid: The F_1 hybrid between S.[redacted] and S.xiarum proved to be highly pollen sterile to the extent of 97 per cent. Though fruit set was observed but the fruits were found to be parthenocarpic. The cytological basis for the high pollen sterility could be enquired into taking into consideration the data available on the meiosis in the PMCs. At pachytene, unpaired chromosomes and incompletely paired chromosomes were encountered showing the role of pairing failure in the occurrence of univalents at metaphase-I. In all the 73 PMCs examined at metaphase-I univalents occurred from one to 17 per cell the occurrence of six and seven univalents being most frequent (13.7 per cent of PMCs). The mean of univalents was 7.1 per cell. The preponderence of univalents at this stage was also reflected in the precocious movement of upto 14 chromosomes with the most frequent number being one, two, three, five and eight. The distribution of such chromosomes was unequal in 76.9 per cent of the PMCs.

Further, abnormalities were also recorded at later stages and these included, presence of lagging chromosomes, bridges, groups of nuclei at anaphase-I, presence of three or five nuclei in 30.9 per cent of the PMCs at telophase-I, three nuclei in 20 per cent of the PMCs and three or four chromosomal groups in 31.4 per cent of the PMCs at metaphase-II. At the later stages the number of chromosomes in the daughter nuclei was unequal with the differences in chromosome number ranging from one to five. At anaphase-II, 36.6 per cent of PMCs were abnormal in respect of number of chromosomal groups. Lagging chromosomes occurred in all the PMCs and bridges were found in 72.7 per cent of the PMCs. Likewise, 32.5 per cent of PMCs at telophase-II, were abnormal in respect of number of daughter nuclei and the presence of four microspores at tetrad stage was observed only in 31.6 per cent of the PMCs scored. Thus, the occurrence of chromosomal abnormalities at all stages could account for the high pollen sterility in the F_1 hybrid.

The high pollen sterility in the interspecific hybrid of S.mammosum x S.viarum seems to be a unusual feature in interspecific hybrids of non-tuberiferous and/or spineus Solanum species. A nearly total pollen sterility has been reported in number of interspecific hybrids viz., S.zuccagnianum x S.melongena (Rajashekaran and Sivanasubramanian (1971b and c),

S.indicum x S.melongena (Rajasnekaran, 1970 and Rangaswamy and Kadambavanasundaram, 1974). S.indicum x S.incanum (Zutshi, 1966b) and S.indicum x S.torvum (Kirti and Rao, 1981). S.xanthocarpum x S.melongena (Rajashekaran, 1971b) and in others. In these hybrids cryptic structural hybridity has been ascribed for their high pollen sterility encountered in the species. In some of the species hybrids chromosomal repatterning resulting in genic imbalance in gametes hase also been attributed for the high pollen sterility (Rangaswamy and Kadambavana Sundaram, 1974, and Rao and Rao, 1977a).

Response to chromosomal doubling

In the present investigation, autotetraploids have been induced both in S.viarum and S.mammosum. The induction of autotetraploids in S.mammosum is being reported for the first time but the production of autotetraploids in S.viarum has been reported by earlier workers (Ammal and Bhatt, 1971 and Bhatt, 1972). However, the detailed meiosis has not been reported in respect of S.viarum autotetraploids. In the present investigation this aspect has been paid due tattention.

The response to chromosomal doubling in both the species was similar in respect of increase over diploids for leaf length, leaf breadth, specific leaf weight, number of ventral

spines on leaf, petal breadth and pollen size. Characters for which both the autotetraploids showed lower values included petal length, percentage of short styled flowers, fresh weight of fruits, number of seeds per fruit, and pollen fertility. Both the tetraploids were similar with respect to length of exerted style.

But differential response of the autotetraploids of both the species was evident for a number of characters. Petiole length was considerably reduced in S.viarum whereas it was increased in S.mammosum. S.viarum autotetraploids possessed higher number of spines on the dorsal surface of the leaves than diploids, while diploid and autotetraploid S.mammosum were on par. Unlike the S.mammosum autotetraploids which had fewer flowers per cluster as compared to the diploids, autotetraploids of S.viarum were on par with the diploid progenitors. In anther length autotetraploid recorded an increase while in S.mammosum autotetraploids showed a decrease. The length of exerted style was decreased in S.viarum autotetraploids while in S.mammosum autotetraploids it was increased. Fruits of autotetraploid S.viarum were larger in diameter while considerable decrease in fruit diameter was observed in S.mammosum autotetraploids. In two major characters, such as number of flowers per cluster and fruit diameter the inferiority of

autotetraploids of S.mammosum over those of S.xiarum was evident. The reduction in the number of flowers per cluster has to be traced to physiological causes that determine their production. But since fruit diameter is directly related to the number of seeds, the lowered seed set has to be enquired into from cytological stand point.

The present study has confirmed that autotetraploidy in S.xiarum resulted in an increase in the percentage Solasodine content over proginator diploids as reported earlier by Ammal and Bhatt (1977a and b), Bhatt and Heble (1978). Similar beneficial effect of autotetraploidy was reported for Rauvolfia serpentina (Ammal, 1962), Datura species (Singh and Kaul, 1967 and Jankulov and Alipur, 1975) in Catharanthus roseus (Dnyansagar and Sudhakaran, 1970 and Mohankumar, 1980) and in several other medicinal plants (Ammal, 1963).

The course of meiosis in autotetraploids of S.xiarum has been studied commencing from diakinesis. At this stage, the nucleolus associated chromosomes were found to be associated as two bivalents. Metaphase-I stage was characterised by total chromosomal pairing in 96 per cent of the PMCs. The chromosomal associations consisted predominantly of bivalents and quadrivalents. Precocious movement of chromosomes was observed only in 18.5 per cent of the PMCs. Chromosome

distribution to daughter nuclei was also nearly normal with 91.4 per cent of the PMCs showing the distribution of 24+24 at metaphase-II stage. At telophase-II, 90.9 per cent PMCs contained four nuclei. Even so, the pollen fertility was low being 52 per cent.

In contrast at metaphase-I in autotetraploid S.[redacted] total pairing was observed only in 63.04(44) per cent of the PMCs. Precocious movement of chromosomes occurred in 44.2 per cent of the PMCs and involved into six chromosomes. At anaphase-I, abnormalities such as chromosomal bridges and lagging chromosomes occurred in 24.2 per cent PMCs. Persistence of chromosomal association was found at prophase II, metaphase-II and anaphase-II, as a result of the chromosomal distribution to the daughter nuclei at the end of first division could not be determined. However, evidences for unequal distribution of chromosomes to daughter nuclei could be gleaned at Metaphase-II stage where the numbers of chromosomal bodies in the two nuclei were unequal. The number of nuclei at the end of first division was found to be two in all the PMCs examined at anaphase-I and telophase-I. At microspore tetrad stage 50.3 per cent of the PMCs had four daughter nuclei, and pollen fertility was found to be 44.8 per cent.

Thus, in the autotetraploids of S.vigarum and S.[redacted] though the end products of meiosis in PMCs namely the pollen

grains, showed comparable percentage of stainable pollen, the study of the course of meiosis in these autotetraploids reveal that in S.viarum autotetraploid pollen grains receive the full diploid number of chromosomes while in those of autotetraploid S.xanthocarpum, the pollen grains possibly receive variable number of chromosomes. The consequences of such differences in the meiotic behaviour is apparently reflected in the differences in the fruit set. The autotetraploid S.viarum set fruits more freely than those of S.xanthocarpum and with the parity in the number of berries per node with diploid progenitors. The overall fruit set in S.viarum diploids and autotetraploids are comparable. On the other hand, S.xanthocarpum autotetraploids with the high degree of unbalanced gamete formation at the end of meiosis and a lower frequency of flowers per cluster in comparision to those of diploids, is characterised by low fruit set. Thus, in the two species, sharp differences in the response to chromosomal doubling were demonstratable both in morphological and cytological characters. Thus, unlike in S.xanthocarpum, in S.viarum a fruitful programme on autotetraploid breeding could be possibly achieved.

Thus, the balance sheet of similarities and dissimilarities characterizing the two species seems to be in favour of a distant relationship between the two species than seems warranted by the present taxonomic treatment of assigning them

to the same sub-genus and section. The characteristics that bespeak of a distant relationship of the two species include; differences in chromosome number, accompanied by gross differences in the stainability of pachytene chromosomes, size differences in the chromosome of the parent, the unidirectional but low cross compatibility, the poor growth and low viability of interspecific hybrid, the direct evidences for autosyndetic pairing in the F_1 hybrid, an abnormal meiosis resulting in as high as 96.5 per cent of non-stainable pollen formation, the basic differences in the pattern of distribution of leaf flavonoids and contrasting response to chromosomal doubling. Thus, the two species seem to be widely different to permit transfer of genes through recombination.

The studies on reciprocal grafts, in the present investigation has shown the influence of stock on scion by way of increased solasodine content in the scion berries. Such an influence of stock in increasing the levels of solanaceous alkaloids has been observed in Solanaceous species pointing out the role of roots as the site of alkaloid synthesis (Wilson, 1953).

SUMMARY

VI. SUMMARY

The thesis embodies results of intensive karyological studies at pachytene in Solanum viarum Dunal. (Syn. S.khasianum var. chatterjeeanum), a commercially important steroid bearing Solanum species, cytomorphic study of induced autotetraploids in S.viarum and interrelationship of S.viarum with S.xanthocarpum Linn. a potentially valuable steroid bearing Solanum species, based on a comparative pachytene chromosome morphology, crossability, cytomorphic behaviour of interspecific hybrid, response to chromosomal doubling and distribution of leaf flavonoids.

The chromosome number of S.viarum was confirmed as n=12. Pachytene chromosomal analysis of S.viarum led to identification and characterization of the entire haploid complement. The pachytene bivalents showed differential stainability and conform in general morphology to those of other Solanum species. The length of pachytene bivalents ranged from 22.4 /u to 58.1 /u. Four of them have median centromere and the rest sub-median centromere. The only nucleolar associated bivalent with sub-terminal nucleolar organizer was found to be the shortest chromosome fof the complement and was found in constant association with the nucleolus. Based on the morphological similarity of pachytene bivalents a lower basic number of n=9 instead of n=12 for S.viarum Dunal has been proposed.

The haploid chromosome number of S.mammosum was confirmed as n=11. Unlike the differentiated pachytene chromosomes of S.viarum, pachytene chromosomes of S.mammosum were characterised by distribution of uniformly stained chromomeres along its entire length. A nucleolar associated bivalent with interstitial nucleolar organizer was present. Detailed pachytene karyology in this species could not be conducted due to poor spreading of bivalents.

Visual comparison of chromosomal size in the two species at pachytene and metaphase-I revealed that the chromosomes of S.mammosum are larger in size than those of S.viarum.

Response to chromosomal doubling in these two species has been studied. The thesis presents for the first time the induction and cytomorphological study of autotetraploids in S.mammosum and a detailed study of meiosis in autotetraploid of S.viarum. Comparative evaluation of diploid and autotetraploids of these two species indicated similarities and differences in certain morphological characters. Similar response to the autotetraploidy was recorded in both the species for morphological characters like leaf length, leaf breadth, specific leaf weight, spine number on ventral surface of leaf, petal breadth, petal length, percentage of short styled flowers, length of exerted style, fresh weight of fruits,

number of seeds per fruit, pollen size and pollen fertility. Differential response was observed for petiole length, spine number on the dorsal surface of leaf, flowers per cluster, anther length, length of exerted style and fruit diameter.

The detailed study of the course of meiosis in autotetraploids of S.viarum Dunal (4n=48) and S.mammosum (4n=44) have revealed the formation of laggards and bridges, persistence of chromosome association, formation of extra number of daughter nuclei at different stages including microspore tetrad stage which apparently contribute to the reduction in the pollen fertility. But the extent of such abnormalities vary widely in the autotetraploids of the two species. In S.viarum, the percentage of PMCs exhibiting such abnormalities ranged from 1.14 to 18.5 per cent whereas in S.mammosum autotetraploid from 2.30 to 44.24 per cent.

The crossability relationship of the two species has been assessed by reciprocal artificial hybridization of diploids, reciprocal grafts and autotetraploids. Unidirectional crossability of diploids of S.mammosum as female parent with diploid S.viarum was observed. The thesis presents for the first time the recovery of an interspecific hybrid with S.mammosum as pistillate parent. Morphological comparision of the interspecific hybrid with the parents is reported. A

detailed cytological analysis of the interspecific hybrid at pachytene and other stages has been made. Pachytene analysis in the interspecific hybrid has brought out direct cytological evidences for hybridity. Meiotic abnormalities such as occurrence of univalents, formation of multivalents, precocious movement of chromosomes, occurrence of laggards and bridges, unequal distribution of chromosomes to the poles and formation of extra groups of daughter nuclei appear to contribute to the higher pollen sterility (96.5 per cent) and poor viability of the hybrid.

Leaf flavonoid distribution in the parents and hybrids gave a supporting evidence for confirmation of hybridity, besides indicating divergence for this character in the two species.

Crosses among and between diploid and autotetraploids in S.viarum and S.[illegible] proved unsuccessful but led to the formation of parthenocarpically developed fruits.

Solasodine content in dried berries of S.viarum (1.63%) was found to be higher than those of S.[illegible] diploids (1.20%) but autotetraploids of S.viarum contained higher percentage of solasodine (2.16%) than diploids. In reciprocal grafts between the diploid species of S.viarum and S.[illegible] increase in solasodine accumulation in the scion berries over ungrafted control was observed.

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