Morphological, cytological and genetical studies on *Camellia* and related genera

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CERTIFICATE

This is to certify that the thesis entitled "**MORPHOLOGICAL, CYTOLOGICAL AND GENETICAL STUDIES ON *Camellia* AND RELATED GENERA**" submitted to the Faculty of Agriculture, Assam Agricultural University, in fulfilment for the degree of **DOCTOR OF PHILOSOPHY (Agriculture)** in **TEA HUSBANDRY & TECHNOLOGY** is a record of research work carried out by **SHRI HIMADRI BHATTACHARJEE** under our personal supervision and guidance.

All help received by him have been duly acknowledged.

No part of this thesis has been reproduced elsewhere for any degree.

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Dedicated to

My

Beloved parents

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ABSTRACT

For better understanding of the morphological, cytological, genetical and breeding behaviour of nine species of *Camellia viz.*, *C. assamica, C. cambodiensis, C. drupifera, C. irrawadiensis, C. japonica, C. kissi, C. rosaeflora, C. sasanqua*, *C. sinensis*, F_1 of *C. sinensis* X *C. japonica* and three species of related genera- *Eurya japonica*, *Gordonia excelsa* and *Pyrenaria berringtoniaefolia*, an investigation was undertaken with the following objectives :

i. to study the pollen morphology, pollen viability and stigma receptivity of *Camellia* and related genera,

ii. to study the comparative performance of certain morphological and anatomical characters of the parents and their interspecific and intergeneric hybrids,

iii. to study self- and cross- incompatibility in *Camellia* and related genera and their mechanism, and

iv. to investigate the cytological status of some of the *Camellia* species and their F_1 hybrids.

Scanning electron microscopic observation of the pollen grains of various species revealed that the sizes (length and width) ranged from 20.00 μm to 65.42 μm and 14.41 to 41.50 μm, respectively. The shape of the grains was prolate to sub-prolate, trizonocolporate. The length : width ratio ranged from 1.11 to 1.96. The surface topography varied from papillate to verrucose with ridges and perforations. Various species of *Camellia* and related genera and their ploidy levels could be identified and characterised depending on the length, width and length : width ratio and surface topography or exine pattern.

For storage of pollen grains, among the different combinations of temperature (0, 5 and 20°C) and relative humidity (0, 35 and 65%) tried, the viability retained for more than 200 days at 0°C and 35 per cent relative humidity. In most of the species, the best combination was observed to be 0°C and 35 per cent relative humidity. A few species *viz.*, *C. rosaeflora*, *G. excelsa* and *P. berringtoniaefolia* lost viability sooner than other species when stored at similar condition of temperature and relative humidity.

A total of 13,195 flower buds were artificially (hand) pollinated during 1993-94 and 1994-95 involving 154 combinations including reciprocal crosses, which resulted in 529 interspecific and intergeneric hybrids representing 58 compatible combinations. However, only 209 hybrids representing 31 combinations survived for more than 18 months. In general, the interspecific and

intergeneric hybrids were weak, slow in growth habit and a few of them died within a few days after germination to about 6 months. The percentage of fruit-set ranged from 1.77 to 46.65, higher percentage was recorded among the tea species with similar ploidy levels (diploid).

Studies on receptivity of stigma revealed that in general, the species of *Camellia* were protogynous and stigma became receptive from 24 hours prior to anthesis and remained so up to a few hours after anthesis They were best receptive in the morning hours from 8-30 a.m. to 11-30 a.m. in all the species investigated.

Results of success in selfed flowers indicated that there was considerable variation among the species with regard to fruit-set. Highest (10.00%) percentage of fruit set was recorded in *C. drupifera*, while *C. rosaeflora, C. irrawadiensis* and F_1 hybrid between *C. sinensis* and *C. japonica* were completely self-sterile. Among the tea species, Cambod tea, *C. cambodiensis* had more prominent self incompatibility as compared to *C. assamica* and *C. sinensis*. The seeds resulted from selfing were aborted or partially developed. The germination of these seeds were abnormal, in some cases there were no development of plumule. In a few other cases, where the seeds germinated normally but the seedling were very stunted with deformed leaf and were merely surviving.

Anatomical observation of the self-pollinated styles to ascertain the mechanism of self-incompatibility revealed that the rate of growth of pollen tube was slower as compared to crossed style and there was deposition of callose tissue at the tip of the pollen tube. As a result, there was no fertilization. The incompatibility system operating in all the species of *Camellia* and related genera were gametophytically controlled, multiple alleles of 'S' genes and certain physiological factors also played important role in the mechanism.

Morphological observation of the interspecific and intergeneric hybrids showed that there was wide variation and the characters like the size of leaf, length of internodes were intermediate between the parents. However, in a few hybrids dominance of maternal parent over the pollen parent was also observed. In most of the hybrids, the anatomical characters like the frequency and sizes of stomata and sclereids approached the mid-parental value. Overlapping of the characters was also observed when there was only slight differences between the parents.

Cytological observation to ascertain the chromosome number of the interspecific and intergeneric hybrids showed that except a few aneuploids, generally they had chromosome number equal to the summation of gametic chromosome numbers of both the parents. Cell division in most of the hybrids was normal with normal disjunction of chromosomes at anaphase.

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Introduction

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INTRODUCTION

The genus *Camellia*, along with eight other genera *viz.*, *Franklinia, Gordonia, Laplacea, Pyrenaria, Schima, Stewartia, Tutcheria* and *Yunnanea* belong to the family *Theaceae*, tribe *Gordonieae* (Sealy, 1958). However, recently, Chang and Bartholomew (1985) included three more genera - *Eurya, Ternstroemia* and *Adinandra* into the family. *Camellia*, the largest genus in the family comprising over 200 species, is economically the most important as the source of most widely accepted beverage of the world-tea, as ornamentals and for production of oil from the seeds of certain species.

Tea was used as a medicinal drink in the Shung Dynasty as early as 2737 B.C. in China. After the Zhou dynasty (1000 B.C.) it was used as a beverage. For many centuries the Chinese cultivated various species of *Camellia* for their decorative value. Around A.D. 593, the knowledge of tea reached Japan, a country outside China. In Japan also, two species, *C. sasanqua* and *C. japonica* were cultivated for centuries. The first *Camellia* to be known to the Western world was the tea plant in the 16th century. The aborigines of North East India knew about tea from time immemorial. They knew of it first as a vegetable food in the form of a *miang*, letpet or pickled tea. A Chinese legend mentioned that the first tea plants were introduced to China from India by Gan Lu, a

Buddhist monk in the later part of Han dynasty during A.D. 25-221. The first mention about the drinking of tea in India dates back to 1662 when Mandelslo discussed drinking of tea as a medicinal drink in India, which he visited around 1640. It was during 1780, the European brought a few Chinese tea seeds from Canton to Calcutta. However, the commercial cultivation of tea started only after the discovery of indigenous tea plants during 1823 in Assam by Major Robert Bruce. Seeds were the only source of propagating tea during the early years of tea cultivation, whether introduced or indigenous. So, there were wide variation with respect to the morphology as well as yield and quality of tea produced. The first scientific attempt to select improved tea in North East India was made by Stiefelhagen bothers during 1860's.

Tea is a cross-pollinated population resulting from various sources of seed freely interbred resulting in a highly heterogeneous hybrid forms extending from extremely Chinery plants to those of Assam origin. Tea plant is broadly classified as Assam, China and Cambod considering the three varietal status among the cultivated population. Recently, all the three forms have been given the specific ranks and scientific nomenclature as : *Camellia assamica* for Assam tea, *C. sinensis* for China tea and *C. cambodiensis* for Cambod tea.

Various species of *Camellia* and related genera have been collected and conserved at Tocklai Experimental Station, Jorhat. It is unclear as to whether or not existing tea populations have resulted from free hybridization among the three main taxa. Species introgression from other *Camellia* species are also involved (Wood and Barua, 1958; Cannel *et al.*, 1977). Other species of *Camellia* suspected to have contributed to the ea genetic pool by hybridization include *C. irrawadiensis* (Wood and Barua, 1958) and ossibly *C. lutescens* (Sharma and Venkataramani, 1974). Wight and Barua (1939) and Barua (1963) suspected that the unique flavour in Darjeeling tea might have come from some related species of tea.

Although, tea populations are the product of natural hybridization, attempts ave rarely been made to produce artificial interspecific hybrids (Banerjee, 1992). Neither there was any systematic approach to study the characteristics and breeding behaviour of various species endemic to this region and the species introduced from other countries (Bezbaruah, 1971a), which might be due to complexities in the origin and taxonomic classification, variety-species controversy and long juvenility of tea. However, information regarding the characteristics of various related species of a particular crop, their wild relatives, the extent of relationship between species is of prime importance for commercial exploitation, for incorporation of desirable genes into the cultivated species or taxa and also from the taxonomic, evolutionary and breeding points of view.

With this background, the present investigation was undertaken to study the morphological, anatomical and genetical behaviour of various species of *Camellia* and related genera and to broaden the genetic base of the tea plant with the following objectives :

1. To study the pollen morphology, pollen viability and stigma receptivity of *Camellia* and related genera.

2. To study the comparative performance of certain morphological and anatomical characters of the parents and their inter-specific and intergeneric hybrids.
3. To study self-and cross-incompatibility in *Camellia* and related genera and their mechanism, and
4. To investigate the cytological status of some of the *Camellia* species and their F_1 hybrids.

CHAPTER - II

Review of Literature

CHAPTER – II

REVIEW OF LITERATURE

2.1 POLLEN MORPHOLOGY

Modern pollen analysis dates back to 1916, when E.J. Lennart von Post wrote his paper on fossil pollen of Swedish bogs. J. Fruh was one of the pioneers of pollen analysis and as early as 1885, enumerated the pollen types of most of the common trees and herbs. G. Lagerheim (1860-1926), an eminent microscopist and one of the most versatile micropalaeontologists has been regarded as the "father of modern pollen analysis" (Erdtman, 1943).

Pollen grains are very useful material for distinguishing various closely related species, varieties or even clones (Fogle, 1977a; 1977b; Thakur and Thakur, 1970; Martens and Fretz, 1980). Characteristics of pollen grains such as shape, size, exine pattern, ridges and pores or surface sculpture are generally considered as taxonomic indices and have been utilised to clarify the relationship among families, genera and species (Matsuta *et al.*, 1986; Maas 1977; Birks and Peglar, 1980;Nowicke and Skvarla, 1979; Smit, 1973; Tseng, 1971). Scanning electron microscope (SEM) offers a new tool for such studies. Exine pattern of pollen grains is genetically so stable that it could be used for species identification (Stanley and Linskens, 1974; Kozaki and Hirai, 1986) and determining the phylogenetic relationship at both specific and generic levels (Maas, 1977). Goldy *et al.* (1984) also reported that the

SEM studies on pollen morphology have proven to be a useful plant identification tool for taxonomists and horticulturists. Characterisation of the cultivars and clones of various species on the basis of morphological characters like vegetative, floral and fruit characteristics and plant growth habit is extremely difficult. Those species could be characterised on the basis of either pollen morphology alone or in combination with the morphological characters. In general, pollen examination holds promise as an aid to the species and clonal characterisation and SEM offers a new tool for such studies. Recupero and Russo (1980) observed the pollen grains of some *Citrus* species at two ploidy levels and suggested that the ektexine sculptures could be a useful character for taxonomy. Kozaki and Hirai (1981) and Ye *et al.* (1981) also showed specific differences among *Citrus* species.

Fogle (1977a, 1977b) studied the ultrastructure of pollen grains of 21 clones of peach (*Prunus persica*), 11 each of nectarine (*P. persica* var. *nectarina*) and sweet cherry (*P. avium*), two of pear (*P. communis*), 12 of European plum (*P. domestica*), 10 of apple (*Malus domestica*) and one each of sour cherry (*P. cerasus*), Japanese plum (*P. salicina*), apricot (*P. armenica*). He (Fogle, 1977a; 1977b) observed the differences in the pollen grains size and in the conformation, coarseness and depth of ridges and striae of the exine. Thakur and Thakur (1970) reported that the cultivars of three *Prunus* species could not be distinguished depending on the size of the pollen grains but the ridges on the exine afforded an infalliable key for identification. Micromorphology of pollen has been utilized by many authors (Nowicke and Skvarla, 1979; Argue, 1980; Walker and Walker, 1980) for taxonomic purposes. Micromorphological differences in the size, form and profile of the meshes and in the thickness of the muri has also been reported in *Avena* (Gornall, 1977), 72 grape cultivars (Ahmedullah, 1983; Tompa and Kozma; 1978), 18 pear, *Pyrus* species (Matsuta et al., 1982; Westwood and Challice, 1978), 9 olive, *Olea europaea* cultivars (Paccini and Vosa, 1979) and 8 crabapple, *Malus* species and their hybrids (Martens and Fretz, 1980). Neither chemistry nor descriptive botany alone could provide taxonomy of *Pyrus* consistent with the geographic distribution of the species. Challice and Westwood (1973) combined 29 chemical characteristics with 22 botanical ones in a computerised taxonomic model. The combination of pollen and anther features was unique for each species indicating their value for taxonomic identification (Westwood and Challice, 1978). Pollen samples from three selections of blueberry (*Vaccinium* spp.), four species of *Vitis*, two cultivars of raspberry (*Rubus* spp.) and several species and ploidy levels of strawberry (*Fragaria* spp.), *Duchesnea* and *Potentilla* were observed by Maas (1977). Ridges, perforations and nanoprocesses on the exine and micromorphological characters in four cultivars of Japanese pear (*Pyrus serotina* var. *culta*) were investigated (Matsuta *et al.*, 1982) and a numerical approach was used to estimate the morphological differences quantitatively.

Taxonomists and paleobotanists have recently recognised the importance of pollen development and morphology in clarifying the classification of plants (Martens and Fretz, 1980). Parthasarathy (1970) reviewed the pollen characteristics of five palm species and observed definitive differences among them. Smit (1973) classified pollen types based on exine sculpture of several species of *Quercus* and related pollen type. Both external sculpturing and internal structure of the pollen grains of 160 species in the tribe *Lactuceae* were examined and correlated the evolutionary advances with morphological features (Tomb, 1975). Nowicke (1975) studied the pollen morphology under light microscope and SEM to support taxonomical grouping of 190 species in the order *Centrospermae*. Pollen morphology of more than 70 species representing 39 genera belonging to the orders *Myrtales* (Patel *et al.*, 1983; 1984) and *Euphorbiales* (Cronquist, 1981; Nowicke, 1984; Nowicke and Skvarla, 1984) in the family *Thymelaeaceae* have been studied. The pollen grains of three closely related genera *Aetoxylon*, *Amyxa* and *Gonystylus* were compared with that of the genera in the family *Thymelaeceae* and distinct variation in the exine structure supported the treatment of 3 genera as a separate family *Gonystylaceae*, allied to the *Thymelaeaceae* (Nowicke *et al.*, 1985).

The study of the pollen morphology of parents and their hybrids provides clues not only for the nature of hybridity at such micromorphological levels as the pollen walls but also offers information on the relative roles of sporophytic and gametophytic complements on the controversial topic, namely genetic control of exine pattern (Mamatha Rao *et al.*, 1979). To establish a taxonomic relationship (Goldy *et al.*, 1984) characterised the pollen of ten *Vaccinium* species, ten inter-specific hybrids and three colchicine induced polyploids. They (Goldy *et al.*, 1984) determined the intra-and inter-specific differences comparing hybrids and parental species, studying the effects of polyploid induction and providing information about inheritance and taxonomic relationship. Mamatha Rao *et al.* (1979) studied the pollen morphology of *Cotyledon grandiflora* and *Echeveria* spp. and their hybrids.

However, a few authors were not successful in characterizing various taxa depending on the pollen morphology. Grant (1972) was unable to distinguish between subspecies of *Zea mays* on the basis of wall sulpturing alone.

Various authors (Wellensiek, 1938; Erdtman, 1952; Venkataramani, 1970; Visser and Tillekeratne, 1958; Bezbaruah, 1971a) investigated the morphology of the pollen grains of tea under light microscope. Bezbaruah (1971a) reported that morphologically pollen grains of all the three varieties of tea, (China, Assam and Cambod) were similar – triangular, tricolporate and binucleate. Erdtman (1952), Ikuse (1956) and Keng (1962) also reported only a very slight differences of pollen in the whole family *Theaceae*. Ackerman and Kondo (1980) observed the size and variability of the pollen grains of 30 collections representing 24 *Camellia* species with different levels of ploidy and varieties. The diameter of the pollen grains was more closely related to the chromosome number among species within a subgeneric section than between different sections. Highly developed cultivars were more highly heterozygous than their wild relatives. It was also suggested that pollen size, diameter and percentage of good pollen could serve as an indicator of hybridity among *Camellia* species (Ackerman and Kondo, 1980).

Reports on the study of the pollen grains of tea and related genera under SEM are scanty. Shu and Chen (1996) studied the pollen grains of 15 germplasm of tea from China. The size and the length:breadth values decreased in the course of evolution. The surface ornamentation varied form smooth to coarse, while the aperature evolved from colpate to the rhombus, the oval and the rectangle (Shu and Chen, 1996).

2.2 STORAGE AND VIABILITY OF POLLEN GRAINS

The practice of storing pollen grains dates back to early years of human civilization. Storage and transportation of date palm (*Phoenix dactylifera*) pollen were among the earliest concerned with pollen viability. As early as 2000 B.C. , in the Hammurabi period, male inflorescences of date palm (*P. dactylifera*) were an important commodity of commerce and the male flowers were stored in a dark dry place for prolonging fertilizing capacity (Zirkle, 1935). However, systematic research on pollen storage started at the end of the 19th century, when the observation on the pollen viability of about eighty species stored at low humidity were reported (Stanley and Linskens, 1974).

Attempts are being made by plant breeders to cross different varieties, species or even genera with a view to produce newer and improved types of plants better suited to human requirements. When two parents flower at different times or grow under different geographical and ecological regions they impose most important barrier for hybridization (Harrington, 1970). The most efficient technique to overcome this barrier, imposed by time and space, is the use of stored pollen. The importance of pollen storage has been recognised since long and a large number of investigators have attempted to store pollen grains of many taxa, particularly of the crop species (Shivanna and Johri, 1985).

The data on longevity of pllen grains of 231 species belonging to 175 genera and 23 families at air dry condition has been compiled by Holman and Brubaker (1926). Vasil (1962) studied the effect of humidity, temperature and diluents on the longevity of the pollen grains of *Brassica nigra, Solanum melongana, S. tuberosum, Arachis hypogea, Gossypium herbaceum, Pennisetum typhoideum, Capsicum annum, Triticum aestivum* and *Zea mays* and concluded that near freezing temperatures and 0-40 per cent relative humidity (R.H.) proved to be the best for pollen storage. King (1965) listed 60 species, whose pollen could be stored either at controlled temperature alone, or at controlled humidity, or by adjusting both the factors. The optimum range of temperature for storage of pollen grains of most of the species laid between –10°C to 10°C as the metabolic activities were minimum. Pollen grains of *Pyrus communis* could be successfully stored for nine years at -17°C and 5% R.H. (Ushirozawa and Shibukawa, 1951). The effects of various storage conditions on *in vitro* germination of 24 genera and 36 species have been summarised by Stanley and Linskens (1974).

Under natural conditions, the viability of the pollen grains varied considerably from species to species. Some species retained viability for a few weeks, while some others, lost viability minutes after dehiscence like grasses. However, in the laboratory, by manipulating the environmental factors like temperature, relative humidity, pressure and surrounding atmosphere using freeze-drying or by dissolving in organic solvents, viability could be extended even for a number of years. Pollen grains of four species of *Pinus* maintained germinability after 15 years of storage at 5°C and at 10% R.H. (Stanley and Poostach, 1962). Retention of viability was correlated with the cytology of pollen – three-celled pollen generally retained viability for over a day, while two-celled pollen retained viability for longer periods (Brewbaker, 1957). Shivanna and Johri (1985) suggested three causes for the loss of pollen viability – deficiency of respiratory substrates, loss of membrane permeability and inactivation of enzymes and growth hormones.

Pollen, a non-chlorophyllus tissue, contains enzymes to metabolize external and internal substrates essential for tube growth (Stanley and Linskens, 1974). They shed under dehydrated condition and continue to respire or metabolism. The degree of humidity is the most important single factor affecting viability during storage.

Various reports on storage of homogeneous pollen at different controlled humidities (Visser, 1955; Lanner, 1962) indicated that viability of the pollen was indirectly related with R.H. during storage. Excepting a few like *Gramineae*, longevity of most species was best at a R.H. between 6 and 60 per cent (Stanley and Linskens, 1974), as at reduced humidity, the respiratory activity was decreased (Shivanna and Johri, 1985). Temperature also plays an important role on the viability of pollen during storage. McGuire (1952) reported that the viability could be extended considerably at a temperature of about 0°C. However, other investigators (Nebel and Ruffle, 1937; Nebel, 1939; Stone *et al.* 1943; Duffield and Callaham, 1959) studying on various species concluded that viability can be extended upto 3 years or longer for some pollen by storage at 0°C or -15°C with R.H. between 10 and 50 per cent. At ultra-low temperatures of liquid gases, the metabolic activities virtually stops, which resulted in viability for unlimited period. Bredemann *et al.* (1948) calculated that *Lupinus* pollen preserved at -180°C should remain unchanged and live for about 1 million years. Literature on pollen storage has been reviewed by Visser (1955); Brewbaker (1957), Johri and Vasil (1961), Linskens (1964).

For testing the viability and germinability of the pollen grains, various tests have been developed (Stanley and Linskens, 1974; Shivanna and Johri, 1985). However, each test had some limitations and most of them did not give uniform results. Pollen grains could be stained with various non-vital stains such as acetocarmine, iodine solution and aniline blue, which stain the contents of vegetative cell. Such staining are useful in identifying the fertile pollen from plants of hybrid origin (Alexander, 1969, 1980). However, Heslop-Harrison *et al.* (1984) concluded that non-vital staining is not reliable for viability tests. In some species, viable grains could be easily detected by the physical appearance - shape, size and exine colour (Jovancevic, 1962) but in some other, such as in the *Graminae* family physical appearance may not change greatly.

The most common and reliable test of viability of the pollen grains is by drawing small representative sample and by germinating them in suitable medium *in vitro*. This test is rapid, reasonably simple, fully quantitative (Shivanna and Rangaswami, 1993) and in various species, percentage of *in vitro* germination could well be correlated with the ability to set fruits and seed following *in vivo* pollination (Visser, 1955; Akihama *et al.*, 1978; Janssen and Hermsen, 1980).

Visser and Tillekeratne (1958) conducted an experiment on storage of pollen grains of tea at 0°C and at various ranges of relative humidities and concluded that the tea pollen could be stored for more than 3 months at 40 per cent R.H. with germination percentage of above 60 per cent. However, Tomo *et al.* (1955) observed a R.H. of 60 per cent to be optimum when the pollen grains of tea were successfully stored at 5-7°C for one month. Venkataramani (1970) investigated the size, fertility and germination of pollen in some tea clones of South India. Bezbaruah (1971a) also reported a successful storage of pollen grains of three types of tea (China, Assam and Cambod) for more than 3 months at 0°C. Wellensiek (1938), Visser and Tillekaratne (1958), Bezbaruah (1971a) observed that the pollen grains of tea germinated equally well in water and in sucrose solutions at concentrations upto 25 per cent by "hanging drop" method. However, for regular observation of germinability of pollen grains, a sucrose solution with a concentration of 10 per cent was advocated (Visser and Tillekeratne, 1958; Bezbaruah, 1971a; Mohanan and Sharma, 1981). For long term storage of germplasm of tea, Amma and Watanabe (1983) reported upto 73 per cent germination of freeze dried tea pollen packed in nitrogen gas or in sealed film cases at –80°C for more than five years. They (Amma and Watanabe, 1983) also used the stored pollen for hybridization, which resulted in normal hybrid seeds with high germination percentage.

Almost all groups of plant growth substances have been reported in pollen grains of various species (Stanley and Linskens, 1974). Haeckel (1951) reported the activity of amylases and phosphatases was reduced with the storage of pollen grains of several taxa. The enzyme activities were detected indirectly by the volume of CO_2 released by pollen as a result of respirationon glutamic acid and pyruvic acid in various species including *C. japonica* and tea (Okunuki, 1939). Tea pollen contains a coloured methanol - extractable complex of three flavonoids, pollenin *a*, *b* and *c*, all related by the common aglycon pollenitin, 3, 5, 8, 4 - tetrahydroxy-7-methoxyflavon (Sakamoto, 1969). The pollenins differ by their associated sugars. Pollenin *a* is complexed with 3-rhamnoglucose, *b* with glucose and *c*, with an unidentified sugar.

Most flavones and flavnonols combine to form yellow-coloured pollen. A kaempferol-3-0-rhamnodiglucoside, first detected in tea leaves was finally isolated and the relationship of the sugars was determined by Sosa and Percheron (1970) form the ethanol extract *of Populus yunnanensis* pollen. Iwanami (1972) developed the technique of storing pollen in organic solvents which eliminate the problem of maintaining a specific relative humidity. The method was easy and has promise, particularly for sticky pollen grains. Iwanami (1972) and Iwanami and Nakamura (1972) reported successful storage of pollen grains of various species including *C. japonica* and *C. sasanqua* in acetone, benzene, ethanol, petroleum ether and chloroform at 5°C for three days. The pollen grains germinated well and pollen tubes were significantly longer than in controls (Iwanami, 1972).

2.3 INTERSPECIFIC, INTERGENERIC HYBRIDIZATION

Man uses 3,000 or more plant species for food, fuel and fibre and cultivates some 150 species commercially, prominent among those are wheat, rice, corn, potato, barley, sweet potato, cassava, grape, soybean, oat, sorghum, sugarcane, millet, banana, tomato, sugarbeet, rye, yam orange, coconut, coton, apple, peanut, watermelon (Harlan, 1976a) and tea. Wild races and species of these crops have been exploited most often as sources of biotic and abiotic stress resistance, to widen adaptation, to provide alternate cytoplasm and develop cytoplasmic male sterility systems, to improve quality, alter modes of reproduction and increase yield (Harlan, 1976b). Incorporation of desired genes from wild species into cultivated varieties has played an important role in plant breeding for increasing yield, quality and resistance to various biotic and abiotic stresses. This is a multidisciplinary effort involving botany, taxonomy, cytology, genetics, ecology, plant breeding and biochemistry (Stalker, 1980). Obtaining hybrids between cultivated and wild species often requires a great effort. Although interspecific hybridization has been attempted in all these crops, the transfer of desirable genes from wild species into commercial varieties has had varying success. Utilization of wild species germplasm to improve a crop species depend on understanding of gene centres, centre of diversity, species relationship, modes of reproduction, the extent to which the crop can be changed genetically without reducing the economic value, the number of genes controlling the trait to be introgressed, methods to overcome undesirable linkage groups, ease and power of screening procedures and the amount of effort that can be devoted to the problem.

The first recorded interspecific hybrid was made in 1717 between carnation (*Dianthus caryophyllus*) and sweet william (*D. barbatus*) by Thomas Fairchild (Allard, 1960). Since then, thousands of interspecific crosses were attempted primarily because of curiosity. However, the list of wild species and incorporation of desirable genes from them to the cultivated species has been ever increasing. Genes for various traits are being identified in wild species unknown to man a few decades ago and modern technologies are developed to transfer those traits into cultivars. Some crops could not maintain commercial status without genetic support of their wild relatives. Many of our important crop species are allopolyploids e.g. wheat, oats, sugarcane, cotton etc. which evolved through distant hybridization followed by spontaneous chromosome doubling of the F_1 hybrids (Singh, 1983).

Price (1963) reported that all modern sugarcane varieties are developed from interspecific hybrids and they contain three to five species in their pedigree. James (1972) also reported that the sugarcane industry depends almost entirely on complex chromosome inter-specific hybrid derivatives incorporating wild relatives. The cultivated species of wheat, *Triticum aestivum,* which is allo-hexaploid, contains the genomes of three different species (Stalker, 1980) *viz.*, *T. monococcum, T. searsii* (*Aegilops speltoides*) and *T. tauschi* (*A. squarrosa*). Modern cultivars of octoploid strawberries are marvelous synthetic mixtures of wild races of *Fragaria virginiana* from Canada and Virginia and *F. chiloensis* form California and Chile (Wilhelm, 1974). Most varieties of potato, *Solanum tuberosum* have two or more wild species in their pedigree (Hougas and Ross, 1956).

A number of new species (allopolyploid) have been created utilizing inter-specific and inter-generic hybridization involving wild species. To exploit the possibilities of producing a cereal, combining the baking and yielding qualities of wheat (*Triticum* sp.) and hardiness of rye (*Secale cereale*), the two genera were crossed by Rimpau during 1890 and *Triticale* resulted. Many workers attempted intergeneric hybridization, between radish, *Raphonus sativus* and *Brassica* crops, however, hybridization was more successful between *R. sativus x B. derecea* and *B. campestris* x *R. sativus*. The hybrids were named *Raphanobrassica* (Karpechenko, 1927) and *Brassicoraphanus* (Terasawa and Shimotomai, 1928) respectively.

Another area of promise in wide hybridization is the production of haploids. In barley and potato, haploids have been produced by interspecific hybridization. Kasha and Kao (1970) reported successful production of haploids from

Hordeum vulgare x *H. bulbosum*. Van Breukelen *et al.* (1975) produced haploids in *Solanum tuberosum* when *S. phureja* was used as pollen parent. Bains *et al.* (1995) rescued haploid regenerated plants of wheat resulting from wheat x maize crosses.

Wild races and species have been exploited most often as sources of disease, insect and nematode resistance. They have been used for this purpose in almost every crop grown by man (Harlan, 1976b). During 1840's, to save the potato crop from devastating late blight infestation, breeders used Mexican hexaploid species, *S. demissum*. Besides, late blight, *S. demissum* carries genes conditioning resistant to leaf roll and virus X (Ross, 1966) and the most common germplasm resource for potato improvement. Different accessions of *S. acule, S. andigena, S. phureja, S. stoloniferum, S. vernei, S. spegazzinii* carry the major sources of biotic and abiotic stress resistance. The work on the utilization of wild taxa for the improvement of potato cultivars has been reviewed by Ross (1986). Resistance to several diseases has been incorporated into cultivated wheat from closely related species of *Triticum, Aegilops* and *Agropyron.* In addition to serving as a source of genes for disease resistance wild species have been used as a source for improved winter hardiness, lodging resistance conditioned by short stature, and cytoplasmic male sterility (Sears, 1969; 1981). From the cross of *Nicotiana goodspedii* and *N. tabacum* the cultivars resistant to *Peronospora tabacina*, *Erysiphe cichoracearum*, tobacco mosaic virus and *Phytophthora* were developed (Palakarcheva *et al.*, 1986; Kalloo and Chowdhury, 1992). Wildfire and blackfire resistant hybrids were developed (Clayton, 1947) using *N. longiflora* while, *N. plumbiginifolia* was used as a source of blank shank resistance in flue-cured tobacco. To produce improved quality of tobacco with low nicotine content Palakarcheva and Edreva (1972) hybridized tobacco with *N. debneyi*. Shintaku *et al.* (1985) reported successful production of viable hybrid plants by pollinating *N. repanda* with irradiated pollen of *N. tabacum*. In tomato, (*Lycopersicon esculentum*) resistance to tomato leaf curl virus was transferred from *L. pimpinellifolium*. Resistance to early blight caused by *Alternaria* solani has been bred into *L. esculentum* from *L. hirsutum f. glabratum* and *L. pimpinellifolium* (Kalloo, 1991). Several lines with high soluble solid content resulted from the crosses involving *L. chmielewiskii* (Rick, 1974).

Sugarcane has probably been hybridized with species of more genera than any other taxon. Grassl (1977) listed eight genera in which intergeneric hybrids have been made with *Saccharum* including *Eccoilopus, Erianthus, Miscanthidium, Miscanthus, Narenga, Ripidium, Sclerostachya* and *Sorghum*. Gupta *et al.* (1976) reported a two-fold increase in grain yield in the *Saccharam-Sorghum* intergeneric hybrid-derivatives. Wild relatives of rice have been utilized to generate genetic variability and to develop resistant varieties. Resistance to grassy stunt virus was incorporated in cultivated rice, *Oryza sativa* from *O. nivara* (Khush, 1977). All modern varieties of sugarcane are hybrids between *S. officinarum* and wild species of the genus.

Wild species of *Gossypium* possess several desirable characters such as fibre quality, resistance to disease, insects and environmental stresses (Prentice, 1972). Interspecific hybridization has also been used to increase the genetic diversity of cotton (Arutyunova *et al.*, 1988; Kalloo and Chowdhury, 1992). Amin (1985) reported that the aphid fecundity was reduced in three wild species of groundnut

(*Arachis hypogaea*) *viz., A. villosa, A. chacoens and A. glabrata.* In the genus *Avena, A. sterilis* has been found to be the most promising source for various desirable characteristics and has been utilized for resistant to rust, mildew, for high protein content and yield (Kalloo and Chowdhury, 1992). The tuber yields of interspecific hybrids increased when cultivated potato was crossed with wild species (Tarn and Tai, 1977). The yield of several other interspecific hybrids of *Vigna, Zea, Arachis* was also more than the recurrent parent. Wild coffees are resistant to rust and *Hemileia vastatrix*, and saved the industry from devastation by witches broom (Harlan, 1976b).

Camellia L. along with eight other genera, namely *Stuartia*, L., *Gordonia* Ellis, *Laplacea* H.B.K., *Schima* Bl., *Franklinia* Marshall, *Pyrenaria* Bl., *Tutcheria* Dunn and *Yunnanea* H.H. Hu belong to the family *Theaceae* Mirb. and tribe *Gordonieae* DC. (Sealy, 1958). Recently, three other genera, *Eurya*, *Ternstroemia* and *Adinandra* have also been included in the family (Chang, 1985). *Camellia* is the most important and the largest genus in the family. *Camellias* are important for the source of tea, the most popular beverage of the world, as ornamentals and for the oil extracted from the seeds of certain species. Sealy reported 82 species under the genus *Camellia* while, Chang and Bartholomew (1985) reported over 200 species. The number of species is increasing day by day with the discovery and identification of newer species under the genus. Tan *et al.* (1989) described 17 new *Camellia* species and one new variety of *C. sinensis*, 11 of which are still in use for preparation of green tea in Yunnan Province in China.

Tea has a long history of cultivation, which spread from South West China to other parts of Asia along with the migratory people. Owing to its self-incompatible nature, various closely and distantly related species growing in the same geographical location, hybridized naturally and a highly heterogeneous population resulted (Kingdon-Ward, 1950). In Yunnan and neighbouring provinces in China, various species of *Camellia* grow naturally and during hundreds of years of cultivation, it appears that intra- and inter-specific hybridization have occurred freely under natural condition with some related species growing in the vicinity and introgression of genes has taken place considerably. In the cultivated tea population, there exists interspecific hybrids involving not only three tea species viz., *C. sinensis, C. assamica* and *C. cambodiensis* but also a number of non-tea *Camellias* (Wight and Barua, 1957; Wood and Barua, 1958; Roberts *et al.*, 1958; Barua, 1965; Bezbaruah 1975b, 1987). A plant collected by Kingdon-Ward from Mt. Victoria, Burma was reported to be a natural interspecific hybrid between tea, *C. assamica* and a closely related species, *C. irrawadiensis* (Wood and Barua, 1958). Morphological, anatomical and biochemical investigation have revealed that species hybrids involving some non-tea *Camellias* also exist in cultivated tea population (Barua, 1965; Wu, *et al.*, 1970). The numerous hybrids that apparently have resulted from free intercrossing are often referred to as of Assam, China or Cambod origin depending on their morphological proximity to the main taxa (Banerjee, 1992).

As per the records, first artificial hybridization in tea was attempted in N.E. India by Sir George Watt during 1880 to 1900, which resulted in the popular 'Rajghur' variety by crossing light leaf Assam *jat* and dark-leaf Manipuri *jat*. Nature and extent of fertilization between different varieties of tea plant was studied in Georgia, Russia (Bakhtadze, 1932). Intra-specific or inter-varietal hybridization in tea in North East India was started during 1937 (Wight, 1938) but due to intervening war years, tea breeding programme virtually commenced in an organized way from 1946 (Barua, 1963). Breeding programmes were carried out to identify potential characters and their inheritance pattern, to create desirable variation and to study the cross-compatibility of individual bushes within and between varieties (Bezbaruah and Saikia, 1977). Wight and Gilchrist (1961) were of the opinion that the characteristic odour (flavour) of tea in general and Darjeeling tea in particular has been contributed by other taxa like *C. irrawadiensis or C. taliensis* or even more distantly related species.

Although tea populations are the products of natural hybridization, attempts have rarely been made to produce artiticial interspecific hybrids (Banerjee, 1992) involving tea. In 1948, an attempt was made to hybridize tea (*C. assamica*) with a related species, *C. irrawadiensis* Barua, which is devoid of caffeine and do not produce tea of commerce. About 300 plants were obtained from crosses with three clones of Assam tea (Wight and Barua, 1957). The species hybrids were vigorous and found to inherit caffeine and produced acceptable but low quality tea. Selected hybrid plants from *C. assamica* X *C. irrawadiensis* were back crossed with high quality Assam-China hybrid clone (TV 1). From the back-crossed progeny, one high yielding, high quality clone, TV 24 was released for the tea industry in N.E. India (Bezbaruah, 1987). Reciprocal crosses and the crosses with F_1 progeny as female parent were not successful (Wight and Barua, 1957). To investigate the relationship among the species and to explore the possibility of introducing some of the desirable genomes to tea, a few interspecific crosses involving *C. caudata, C. irrawadiensis, C. japonica* and *C. kissi* were attempted (Bezbaruah and Saikia, 1977). Out of those crosses, only tea (*C. assamica*) with *C. japonica* and *C. irrawadiensis* gave outstanding results. *C. caudata* and *C. kissi* were also found to be compatible with tea but all the crosses with *C. sasanqua* failed. However, reciprocal crosses were unsuccessful. Barua (1989) concluded that the crossability of tea with other species of *Camellia* has opened the possibility of incorporating some desirable character in the genomic configuration of tea.

In Japan, a natural interspecific hybrid between *C. japonica and C. sinensis* was identified and named `Robiraki' (Hagiya and Kinoe, 1981) and `Chatsubaki' (Takeda, 1990). Another interspecific hybrid between *C. sasanqua* and *C. sinensis*, called `Sazancha' (Fuchinoue, 1975) is commercially cultivated and produce good quality of green tea in Japan. Presence of caffeine and a glycoside `sasanquin' from the clones of `sazancha' confirmed their hybrid origin (Nagata and Sakai, 1981). *C. wabisuke* was considered (Kitamura, 1970) as a hybrid between *C. sinensis* and *C. japonica*, which was supported by the karyotypic analysis of Kato and Simura (1971). However, Nagata and Sakai (1981) studying the biochemical content (theanine and caffeine) doubted the progenitors of *C. wabisuke* are *C. sinensis* and *C. japonica*.

Besides three tea species and their numerous hybrids, *C. japonica* is the most popular among all the *Camellias*, which has magnificent flower. Successful production of hybrid plants from the crosses between *C. japonica* X *C. sinensis* was reported by Ackerman (1971) and Kato and Simura (1978). Bezbaruah and Gogoi (1972) and Ackerman (1971) reported to obtain a single plant each from the reciprocal cross. Takeda (1990) observed that the hybrids had high level of resistance to the most common and damaging diseases of tea in Japan, grey blight (*Pestalotia longiseta*), tea anthracnose (*Gloesporium theae-sinensis*) and to cold damage in winter. He (Takeda, 1990) reviewed the hybridization done on *Camellia* and concluded that interspecific crosses in *Camellia* have been done mainly to improve the flower of ornamental *Camellias*. As tea flowers are small and incospicuous, they were rarely been used in the hybridization programme. Various species used successfully were *C. japonica, C. irrawadiensis, C. taliensis, C. kissi* and *C. caudata*. Many attempts to cross *C. japonica* with *C. chrysantha* have been failed on account of seed abortion (Hagiya, 1986; Hwang *et al.*, 1990) Xia (1984) attempted hybridization using pollen from *C. chrysantha* onto *C. japonica, C. pitardii* var. *yunnanica, C. reticulata* and *C. saluensis*. Seedlings were produced from crosses on to *C. pitardii* var. *yunnanica* and *C. reticulata*, however, only *C. chrysantha* x *C. reticulata* hybrids survived. Ikeda (1993) crossed four cultivars and lines of tea, *C. sinensis* with *C. oleifera* and *C. brevistyla* to produce homozygous lines.

During the last two hundred years of cultivation, at least 5000 *Camellia* varieties resulting from combination between plant of one species (nearly all *japonica* or *sasanqua*) have come into commercial use. However, there are relatively few interspecific combinations or hybrids (Peer, 1958). In the Western hemisphere, the first successful artificial hybridization in *Camellia* was done during 1920's by a British ameteur, Mr J.C. Williams between two ornamental species, *C. saluensis* Stapf ex Bean and *C. japonica* L. (Smith, 1949) and was taxonomically named as *C. x williamsi*. Since then, numerous hybrids have been raised, named, registered and many of them are being cultivated commercially and as a result, introgression after artificial hybridization in *Camellia* also might have occurred (Kondo, 1977). Until 1960's only sporadic attention was given to interspecific hybridization in *Camellia* in U.S.A. Extensive breeding programme of wide crosses involving interspecific and intergeneric hybridization was initiated to develop wider genetic variation and to investigate the compatibility relationship in the genus *Camellia* particularly with the ornamental species (Ackerman, 1971). Twenty species of *Camellia* were used as parents, which resulted in 459 hybrids from 106 combinations. Ten species were crossed with tea, *C. sinensis* (Ackerman, 1970, 1971, 1973). In some of the combinations, embryo rescue technique was used to grow the partially developed or aborted embryo. Genetic variation within the species of *C. japonica* has been exploited to a considerable extent (Anderson, 1961; Savige, 1967). However, since 1977, after a devastating series of severe winter druing 1976-77, breeding programme was concentrated on the development of inter-specific hybrids with greater hardiness (Ackerman, 1984). Takeda (1990) reported that the interspecific hybrids between *C. japonica* x *C. sinensis* inherited hardiness similar to the hardiest parent, *C. japonica*. For obtaining cold-hardy plants in U.S.A., Ackerman (1989) attempted interspecific hybridization involving *C. oleifera* and *C. kissi*. Eight drought tolerant biclonal seed cultivars were released in N.E. India by selection from a population of intra-and inter-specific hybrids of tea (Singh and Handique, 1991).

First inter-generic hybridization in the family *Theaceae* was attempted by Wight and Barua (1939). They attempted to cross *Schima wallichi* Chois and *Pyrenaria berringtoniaefolia* Seem with tea, *C. sinensis*. Ackerman (1971) was successful in obtaining intergeneric hybrids involving *Camellia*, and *Tutcheria* and doubtful hybrids between *Franklinia* and *Camellia.* Orton (1977) reported to obtain hybrids between *Franklinia* and *Gordonia*, while Ackerman and Williams (1982) obtained two intergeneric hybrids between *C. japonica* x *F. alatamala* and *C. sasanqua* X *F. alatamaha.*

2.4 FLORAL BIOLOGY

The vegetative structures are more variable as compared to floral or reproductive structures in tea. Therefore, the later were used more reliably for identification and classification of various taxa and selecting breeding material. Sharma *et al.* (1981) reported that a complete understanding of the biology of flower in different tea clones would make the choice of pairs of clones for controlled hybridization easier and the timing and techniques employed in hybridization programme more appropriate. Several authors (Cohen Stuart, 1916; Bakhtadze, 1931, 1932; De Haan, 1939; Wellensiek, 1938; , Wight, 1938; Wight and Barua, 1939; Wight, 1962; Wu, 1963; Amma and Harada, 1955; Barua, 1963; Kapil and Bala Sethi, 1963; Barua, 1970; Bezbaruah, 1975c; Sharma *et al.*, 1981) studied the growth and development of flower bud, morphological characteristics, fertilization, embryology and fruit-set in tea.

Geographical location determines the time of flowering of the tea plant (Barua, 1989). Wight (1939) observed that the tea plant in North-East India (24-28° N) flower profusely during October to February. Barua (1970) obsrved that there was an acropetal succession of flowers determined by the phasic activity of the apical bud. The main crop of flowers started blooming from late September to early October and continued until late January to early February. He also observed that in spite of considerable time lag between anthesis, the fruits produced, matured and dehisced at the same time during October to November. Although the peak flowering time did not usually extend beyond December. There was considerable variation between different seed trees of each race of tea in flowering time and occurrence of the peak.

In Cambod tea, the flowering started early and continued for a longer period, the Assam tea had shorter flowering season and the China tea occupied an intermediate position (Bezbaruah, 1975c). However, in South India, (10° N) Sharma *et al.* (1981) observed that tea seed trees started flowering towards the end of December and continued to flower upto the end of March, with the peak flowering period during February. In Russia (Bakhtadze, 1931) and in Taiwan (Wu, 1967) also the peak flowering period of December to January coincided almost with that of North-East India. The main flowering season in Kenya was May to September, while in Malawi (16°S), tea flowered from February to June (Barua, 1989). In Sri Lanka, situated at a latitude of 6°N, tea flowers all the seasons, however, the most productive period being December to March (Elliot and Whitehead, 1926). There was no flowering peak in Java (Cohen Stuart, 1916a).

Wu (1963) studied various morphological characters of tea flower namely, colour of pedical, size of sepal, number of outer stamen, length of style and splitting percentage of the style arm in *sinensis* and *assamica* and concluded that all the characteristics were significantly different in both the varieties. Wight (1962) also classified the three basic types of tea depending on the length to which the styler arm was united. In China tea, the arms were free for greater part of their length or to the base geniculate. In Assam tea, they were united for greater part of their length, the free part short, more or less horizontal. While, in Cambod tea, styler arms were free for about half their length, linear and ascending. Barua (1965) studied the detailed reproductive structure of *Camellia irrawadiensis*.

Kapil and Bala Sethi (1963) observed the development of male and female gametophytes in tea. The flowers were solitary, axillary, bisexual and actinomorphic. The pollen grains shed at the two-celled stage. The ovules were anatropous, bitegminal and tenuinuclear. They (Kapil and Bala Sethi, 1963) reported that the development of the female gametophyte was of the *Alluim* type but not of *Polygonum* or *Adoxa* type as reported by Cohen Stuart (1916a). Cavara (1899) found that an 8-nucleate embryo-sac arose from micropylar megaspore of the tetrad while Cohen Stuart (1916a) recorded a tetrasporic embryosac. However, Kapil and Bala Sethi (1963) observed the development of the female gametophyte as bisporic, which was in conformation with the findings of Fagerlind (1939). Wu (1960) recorded the development of the embryo as of solanad type. Bala Sethi (1965) studied the events taking place after fertilization in tea and observed that there was only three antipodal cells and there was no increase in their number. Cavara (1899) reported that the division of the primary endosperm nucleus commenced when the male nucleus penetrated the egg, which indicated that triple fusion preceded syngamy. Bala Sethi's observation revealed that syngamy and triple fusion took place simulataneously, and the pollen tube persisted upto filamentous stage of the pro-embryo. The first as well as a few subsequent division of the primary endosperm nucleus were nuclear and the endosperm became cellular only at a later stage.

From the anatomical observation of the floral parts, Wu (1963) suggested that tea flower should be classified as central placenta instead of parietal placenta type. Takahashi and Yanase (1958) studied the differentiation, morphology and anatomy of flower buds of tea. Development of flower was more speedy on higher temperature and the first and fourth flushes needed longer time to differentiate flower buds than the second and third flushes. The flower development was promoted under short day, but the photoperiodic response of the tea flower differentiation was weak and mainly depended upon the thermal induction. The flower development of the tea plant was not inhibited by the low light intensity or by low carbohydrate content that was brought about by low light intensity.

Bakhtadze (1932) in the U.S.S.R., Wellensiek (1938) in Java, Wight (1938) in North East India and Wu (1967) in Taiwan reported that the tea plant showed an appreciable degree of self sterility and invariably set a better crop of seed with pollen from antoher bush. Bakhtadze (1931, 1932) also found that the plants resulting from self-pollination were inferior to those resulting from cross pollination, besides which, the self-fertilized seed showed a marked reduction in germinating capacity. Insects such as bees and wasps would carry pollen from bush to bush, but as the tea plant in the plains of North-East India flowers at a time of lessened insect activity and at a time when bees probably migrate to the hills as a result the cross transfer of pollen by insects was not very effective (Wight, 1938, 1939; Bakhtadze, 1932; Bezbaruah 1975c). Amma and Harada (1955) observed that only non-viable, dried up pollen grains were carried by wind which hardly could produce good fruit in Japan. Natural cross-pollination was difficult as the pollen grains of tea was heavy and sticky in nature and occurred mostly in clumps - a condition not favourable for carriage of pollen grains by wind (Bezbaruah, 1975c). The chances of pollination by insects were further lessened by the removal of corolla and stamens (Wight, 1939 and Visser, 1969). Degree of self-incompatibility was generally more prominent in the China and Assam varieties than in Cambod (Bezbaruah and Saikia, 1977). Tomo *et al.* (1956), Sebastiampillai (1961), Wu (1967) observed variable degree of self fertilization in production of seeds naturally. Pollination mechanism in tea had been studied by various authors (Bakhtadze, 1932) in the U.S.S.R., Wight (1938, 1939) in North East India, Wellensiek (1938) in Java and Wu (1967) in Taiwan. Self-incompatibilty in tea vary from clone to clone. Wight (1938) and Harler (1956) reported only 2 per cent fruit set in tea under natural condition and an average of 14 per cent from artificial pollination. Bezbaruah (1975c) also observed an average fruit set of 13 per cent in the seed orchard and more than 60 per cent in certain crosses.

The extent of fruit-drop and percentage fruit-set in various clones was observed by Bezbaruah (1975c) in North East India and *Sharma et al.* (1981) and Satyanarayana and Sharma (1981b) in South India. The drop of fruits occurred in three distinct waves - the first drop of the intensity of 70-80 per cent took place during or shortly after flowering, the second drop of 10-16 per cent occurred during March-April and the third, of about 1 to 6 per cent took place during May-June (Bezbaruah, 1975c).

The cause of incompatibility in tea has not yet been fully elucidated (Barua, 1989). Simura and Osone (1956) observed that the pollen tubes in selfed styles grew much slowly than that of crossed flower. Tomo *et al.* (1955) and Fuchinoue and Fuchinoue (1966) suggested that the cause of self-incompatibility resides in the base of the style where pollen tube growth was inhibited. However, Bezbaruah (1971b) studied the pollen tube growth in different combinations by artificially pollinating them. He could not differentiate the extent of tube growth in selfed and crossed flowers to the micropylar end of the ovary. Ackerman (1971, 1973) studied the compatibility relationship among the species in the genus *Camellia*. From the detailed study of the mechanism of incompatibility in tea, Bezbaruah (1971b) concluded that the cause of incompatibility was due to failure of syngamy or triple fusion or breakdown of the zygote following fertilization. Rogers (1975) reported that the self-incompatibility in tea was of multiple allele gametophytic system. The genetic explanation of the incompatibility prevailed in tea was given by Fuchinoue (1979), who explained to be composed of five oppositional alleles S_1 to S_5. The incompatibility was caused by the mutual relation between genotypes of pollen and that of style, i.e. when the genotype of pollen was common to that of style, no fertilization took place–called genetic reaction. Self-incompatibility in tea was observed by Tomo *et al.* (1956) with Japanese varieties, Simura (1956) with Chinese varieties and Indian hybrids and by Wu (1967) with Taiwan varieties and Manipuri varieties. While Fuchinoue (1979) studied the incompatibility system operating in tea from physiological aspects.

2.5 MORPHOLOGY, ANATOMY AND CYTOLOGY

2.5.1 Morphology

Morphological features are the phenotypic expression of genes which play an important role in the taxonomic classification of a species or variety of plant and are used more extensively than any other parameter. Tea plant was also identified and later classified depending on the morphology. Tea plant was identified by Linnaeus (1752) from an illustration by Kaempfer (1712). Various botanists (Watt, 1898; Cohen Stuart, 1916a; Pasquier 1924; Kitamura, 1950; Sealy, 1958; Barua, 1963; Wight, 1958; 1962; Satyanarayana and Sharma, 1981; Amma, 1986) classified various taxa of tea mainly on the basis of morphological features of leaf and flower. Wickremaratne (1981) used leaf length, width, base angle, apical angle, petiole length, internodal length, internodal girth, leaf pose (angle) to identify twelve tea clones of Sri Lanka. Wight (1953) used two characters of leaf surface matt or glossy and leaf pose - erect, dependent or intermediate as an agrotype index. Other morphological characters of leaf used for classification of tea include - size, colour and texture (Visser, 1969) while size, shape, colour, texture, patina and pose, type of apex and venation, length of internode, size and pubescence of bud (Richards and Sebastiampillai, 1964), shape, size and area of leaf (Pethiyagoda and Rajedram, 1965; Krishnapillai and Pethiyagoda, 1978). Leaf colour (light or dark) and serration on leaf margin by Eden (1976). Floral characters like hairiness of ovary and stigmatic features were used for classification by Richards and Sebastiampillai (1964). However, Stebbins and Ladyard (1967) reported that the vegetative characters have more plasticity and are generally less reliable as diagnostic criteria. In tea, there are hardly any characters which show discontinuous variation depending on which individual genotypes may be separated clearly and certainly into discrete groups. Vegetative characters also give reliable criteria as there were also differences in floral characters (Wight, 1939).

Morphological variability in the tea population is the most important features for selecting elite planting materials for plant improvement in tea till today (Visser and Kehl, 1958; Venkataramani, 1966; Bezbaruah (1975b). They are judged mainly by morphological characters and to a lesser extent by biochemical parameters (Venkataramani, 1970; Satyanarayana and Sharma, 1981). Tea taxonomy is still a challenge and it continues to be an area of less priority in tea research programmes, possibly due to complexities involved. However, information on taxonomic characters, genetic diversity and biogeography of *Camellia* may still help in identifying genotypes with high productive potentiality to improve the existing genetic base of commercially grown tea (Banerjee, 1992). Most authentic and accepted reports of classification of *Camellia* (Sealy, 1958; Chang and Barthelomew, 1985) are also based on various morphological features.

Leaf area is an important factor influencing the metabolism of a plant, the determination of which aids in predicting the productivity of a cultivar particularly in a leaf crop like tea (Satyanarayana and Rao, 1981). Positive association of yield and total leaf area in tea has been reported in Russia (Memedov, 1961); Japan (Toya, 1966); South India (Satyanarayana and Rao, 1981; Satyanarayana and Sharma, 1981) and in North East India for China and China hybrid population (Bezbaruah, 1968b). Satyanarayana and Sharma (1981) observed various biometric parameters like serration along leaf margin, leaf area, shoot per unit area and the pruning weights for their positive association with yield. However, no single criterion could be applied in isolation for yield prediction in tea, a combination of simple criteria, more importantly, total leaf area, as determined on the basis of total leaf dry matter, density and weight of shoots and pruning weights in conjunction with spread of the bush is likely to form a reliable basis for yield prediction.

Wight and Barua (1954) observed the morphological features of pubescence to be positively correlated with quality, while Wight and Gilchrist (1961) established the same relationship with the strength of tea produced. The relationship between the canopy architecture with the biomass productivity of various *jats* of tea was studied by Hadfield (1968, 1974). He analysed the mean leaf angle from vertical, the angle formed by the foliar halves and the leaf area index of mature bush. Significant correlation between the degree of pubescence on the under surface of young leaves with the quality and strength of black tea manufactured was studied by Wight and Barua (1954), Venkataramani and Padmanabhan (1964), Wu (1964) and Wu *et al.* (1958) while Wellensiek (1947), Venkataramani and Padmanabhan (1964) and Wight *et al.* (1963) correlated leaf colour with the quality of tea produced. The inheritance pattern of leaf size in intervarietal crosses were studied by Wellensiek (1940, 1947) in Java, Wu and Shyu (1966) in Taiwan and Bezbaruah (1973) in Assam. Eden (1941) and Barua (1965) were of the opinion that bush area and plucking point density were the most reliable criteria for yield capabilities in tea. Bezbaruah (1973) studied the inheritance pattern of various characters and concluded that apart from leaf characters, a number of other important morphological characters also appear to be inherited in a polygenic and quantitative manner. Quality of made tea of the F_1 hybrids generally approached the mid-parental value and no dominance or interactions were observed by Bezbaruah(1971a, 1973). Wu and Shyu (1966) and Bezbaruah (1973) reported absence of any significant difference in yield and quality of reciprocal hybrids from crosses between morphologically dissimilar varieties.

Seed grown tea population are extremely heterogeneous. The extent of variability in tea was studied by Wellensiek (1934) in Indonesia, Tubbs (1938) and Visser (1969) in Sri Lanka and Wight (1939) in North East India. Most of these variations (67%) are environmental while remaining 33 per cent are of genetical nautre (Tubbs, 1938; Eden, 1941; Hasselo, 1964; Bezbaruah, 1974; Kulasegaram, 1980). Kulasegaram (1980) correlated the morphological characters of young tea seedlings with the size and yield of the same plants at maturity. Kato and Simura (1978) observed the morphological features of leaf and flower of the F_1 hybrids in the genus *Camellia*. Rashid *et al.* (1985) in Bangladesh studied the morphology, anatomy, cytology and some other non-paramatric characters of the parents (TV1 and TRI clone) and their hybrids. Various non-parametric characters involved leaf pose, colour, shape, texture, apex and leaf patina. In Assam, Sarmah and Bezbaruah (1984) observed the growth and vigour of the triploid progenies on the basis of trunk diameter, plant height, number of branches, number of leaves, leaf area, leaf angle and shoot weight. Morphological and anatomical studies on open pollinated triploid tea progenies revealed a wide range of phenotypic variation and also of anatomical characters like the frequency and size of stomata and sclereid (Chaudhuri and Bezbaruah, 1985). The variation in the tea bushes may occur in the outward morphological attributes such as growth habit, branching pattern, foliar and floral characters or the bushes may vary in their internal characters governing yield, quality, flavour etc. These variations might be due to free natural cross pollination among the indigenous as well as introduced tea and non-tea relatives (Bezbaruah, 1967).

2.5.2 Anatomy

2.5.2.1 Stomata

Stomata are pores which are bordered by a pair of inflatable guard cells whose movements effect variation in the size of the pore. Stomatal movements are resulted from osmotically-regulated variations in guard cell volume. They are characteristic structures of chlorophyllous epidermal tissues in gymnosperms, angiosperms and of ferns. The prime function of stomata is to permit gaseous exchange which is necessary for photosynthesis (Stevens and Martin, 1979). Stomata are well recognised as one of the histomorphological characters of systematic importance. Though the stomata were noticed by the earlier workers (Nees, 1847; Clarke, 1884-85) of the last century, Vesque (1881, 1889) first observed that the cells surrounding the guard cells of stomata showed a tendency to form a constant pattern for a particular taxa and even families and classified them into four morphological types. He (Vesque, 1889) first employed the stomata in the plant systematics and stated that one can easily define the ontogeny by the structure of adult stoma. Later, various authors (Pant, 1965; Van Cotthem, 1970; Fryns-Claessens and Van Cotthem, 1973) classified the stomatal types, mainly depending upon the number and forms of subsidiary cells.

Various tribes of the family *Umbelliferae* (Guyot, 1971) and *Papillionaceae* (Shah, 1968) were observed on the phylogenetic and systematic value. Pant and Banerji (1965) studied the ontogenetics of the stomata of *Passifloraceae*, *Cucurbitaceae* and *Caricaceae*. The variations in the structure and development of the stomata in 26 species of *Rubiaceae* were investigated by Bir *et al.* (1971).

Mazumdar and Bezbaruah (1978) studied the size and distribution of stomata at different positions in the leaves of twenty TV series (TV1 to TV20) clones. However, they (Mazumdar and Bezbaruah, 1978) could not correlate drought tolerance of the clones with either size or density of the stomata as established by Grice (1972) and Basu (1975). Saikia and Dey (1987) studied the length, breadth, size and frequency of stomata in two clones of tea. They found a highly significant positive correlation between length and breadth of stomata. Comparative foliar anatomical studies of four South Indian clonal teas under scanning electron microscope were made by Raman and Cheng (1981). By analysing the various anatomical structure of green leaves, Wu (1968) correlated the quality of made tea with the anatomy of leaves. Like in other crops, in tea also, stomata play a great role in water use efficiency (Dey, 1978). Grice (1972) and Basu (1975) observed differences in the performance of clones in tea plantation under droughty situations. Raman and Cheng (1981) observed that the drought resistant tea clones in South India possessed thicker cuticle on the adaxial epidermis and most of the stomata ont he abasial surface remained closed as compared to drought susceptible clones. In tea, size and frequency of stomata appeared to be an individual character rather than varietal (Mazumdar and Bezbaruah 1978). However, ploidy level greatly influenced the size of stomata, tetraploid plants possessed bigger stomata than the diploid or triploid (Chaudhuri and Bezbaruah, 1985). Karasawa (1932) observed that the size of stomata in the leaves of triploid plant was considerably larger than those of diploid species. Rashid *et al.* (1985) utilized the size and density of stomata and guard cells for identification of hybrids between diploid (TV 1) and tetraploid (TRI) tea clone along with other morphological and cytological attributes. Chen and Ye (1984) and Ahmed and Singh (1993) observed a good positive correlation between stomatal chloroplast number in each guard cell and ploidy level in tea.

2.5.2.2 Sclereids

Sclereids or stone cells are the plant cells with strongly thickened walls, normally there are no living contents inside the cavity of these cells called lumen. There are minute holes on the walls known as pit-canals. Sclereids develop in many parts of a plant such as in leaves, stems and roots, in the flesh of succulent fruits, in the coverings of seeds and nuts and so on. They have elongated main body disposed almost at right angles to the mid-rib of the leaf, slightly branched, with claw-like extensions at the lower end and usually a disc at the upper end remain attached to the upper epidemis of the leaf (Barua, 1963, 1965).

The first treatise on the comparative morphology and function of sclereids in seed plants was written by C.J. Wijnaendts Franken in 1890. He investigated the sclereids of fourteen genera of gymnosperms and representatives of fourteen families of angiosperm including *Ternstroemiaceae*. In *Camellia*, he investigated the sclereid morphology of *C. japonica, C. sasanqua, Thea viridis* and *Thea bohea*. The structure and development of sclereids in the petiole of *C. japonica* in more detail was studied by Foster (1944). For comparative purpose, he (Foster, 1944) also included the leaves of *C. reticulata* and *C. sinensis*. Wight (1958) and Wight and Gilchrist (1961) studied the phloem index of the leaf petiole as reliable index and correlated with the quality of tea. Wight (1958) also reported quantitative inheritance of the calcium-oxalate crystal frequency (phloem index) of the leaf petiole in *Camellia*. However, Green (1971) observed that the phloem index varied considerably with the environmental condition. Shape, size and branching pattern of sclereids were used in taxonomical and phylogenetic classification of seed plants (Arzee, 1953; Rao, 1957).

Wight and Barua (1957) studied the frequency of pellucid punctation in the leaves of *C. sinensis* and *C. irrawadiensis*, their hybrids and the hybrids between Assam variety and China variety of *C. sinensis*. While, Barua and Wight (1958) examined the sclereids in the leaves of *C. sinensis, C. irrawadiensis, C. assamica, C. taliensis* and hybrids between *C. assamica* and *C. irrawadiensis* from the taxonomic point of view. Barua and Dutta (1959) and Barua (1963) studied the morphology and distribution of sclereids in the lamina of three basic types of tea, China, Assam and Cambod. Barua (1965) also studied the shape, size and structure of sclereids, responsible for pellucid dots or punctations having taxonomic significance (Masters, 1844). Bezbaruah (1975a) used morphology of sclereids for identification of the F_1 hybrids between *C. sinensis* var. *assamica* and *C. irrawadiensis*. Bera *et al.* (1994)

studied various morphological parameters of sclereids for characterization and identification of ten clones released by Tocklai. They (Bera *et al.*, 1994) concluded that length, breadth and the ratio between length and breadth are important for identification, while, the number of spicule was important for characterization of a clone of tea.

2.5.3 Cytology

Cytology contributes to a great extent in solving taxonomic problems and determining the evolutionary path among plant species. Characteristics of chromosome such as basic number, form and size, number and size of primary constriction or centromere, secondary constrictions, absolute size of the chromosome, distribution of euchromatin and heterochromatin region, centromere index etc. are considered as the basic criteria for differentiation of a species.

For improving any crop, detailed cytogenetical studies is inevitable. When the question of improvement comes to tea, the immediate requirement of the industry is the improvement in yield and cup quality. Unlike annual crop plants, breeding of perennial crop with long life cycle is difficult and time consuming process, particularly for tea which is of out-breeding nature and most of the desirable characters are complex and polygenic. It is practically impossible to proceed with breeding schemes including hybridization, induced mutation and polyploidy breeding or other modern techniques for improvement like recombinant DNA technology, production of transgenic plants etc. without its basic cytogenetical knowledge on the existing germplasm. However, comparatively less attention has been paid to the basic cytology and hereditary mechanism of this economically important crop.

Most of the cytological investigations carried out on tea (Morinaga *et al.*, 1929; Simura, 1935; Subba Rao 1938; Patterson *et al.*, 1950; Janaki-Ammal, 1952; Simura and Inaba, 1952, 1953; Longley and Tourje, 1959, 1960; Bezbaruah 1968a, 1971a, 1975a; Ackerman, 1971; Kondo, 1977a, 1977b; Dutta and Chowdhury, 1975; Jayasurya and Govindarajulu, 1975; Goswami and Sharma, 1979; Cherian and Stephen, 1981) till recently, showed remarkably constant number, 2n = 30 in all the cases except for a few natural triploid reported by Karasawa (1932, 1935); Simura (1935), Janaki-Ammal (1952); Bezbaruah (1971a); Venkataramani and Sharma (1974); Kapanadze and Eliseev (1975) and Kondo (1977b). Despite severe injuries to which tea plant has been subjected by pruning and plucking throughout its long history of cultivation, it is remarkable that the cytological behaviour has remained virtually unchanged, which might be attributed to deliberate elimination of polyploids wherever and whenever they occurred, by the conservative tea drinkers of China and Japan as the quality of tea produced from polyploids was inferior to the diploid teas (Bezbaruah, 1971a). He (Bezbaruah, 1971a) reported that the clones belonging to the three races of tea (diploid) did not reveal any major differences in their karyotype. Although, some minor differences exist, none of the minor differences could be correlated with any morphological features of the plants. In tea, there was no significant difference in the gross morphology of the chromosomes of different species, which were mostly primitive and sub-median in nature except the number of secondary constricted as well as satellited chromosomes present in some of the clonal lines. The chromosomes were in general short in length but showed size difference in the complements, though the differences were not very much pronounced. There was a gradual decrease in size from longest (3.44 μm) to the shortest one (1.28 μm), but with a very little difference in terms of actual length, between two adjacent chromosomes. Bezbaruah (1971a) classified the tea chromosomes morphologically into three classes (A to C) basing on the relative lengths, position of the centromere and presence or absence of secondary constrictions. However, Dutta and Chowdhury (1975) classified the chromosomes into ten classes (A to J) on the basis of their relative size (3.71 μm to 1.50 μm) and position of primary and secondary constrictions. Close morphological similarities of the tea chromosomes suggested that the observed differences in growth and form of plants of the different races of tea were the results of mutative changes of the genes or cryptic gene mutation (Kondo, 1977; Chaudhuri, 1979).

From the critical observation on the tea genomes, Chaudhuri (1983) concluded - "The absence of sharp morphological differentiation between the genomes of different *Camellia* taxa which are mostly primitive and sub-median in nature facilitate in enormous genetic introgression during the entire course of evolution of tea and thus structural heterozygosity and hybrid heterozygosity have been kept floating as evidenced from the meiotic configurations. Preferential pairing of chromosomes in metaphase I of the first meiotic division and the existance of cryptic structural differences and their significance in chromosomes and genetic sterility, biochemical differences, introgression and secondary hybridization, reversability of polyploidization, interphase nuclear volume, differential gene replication in heterochromatic regions and their effects on phenotypic expression was also observed".

Bezbaruah (1971a) studied the chromosome numbers of different species and genera in the family *Theaceae* as shown in Table 2.1.

Table 2.1 Chromosome numbers in *Camellia* and allied genera

Sl. No.	Species	Chromosome number, 2n =	Previous findings
1.	*Camellia sinensis*		
	var. *assamica*	30, 45	30 (Janaki-Ammal, 1952)
	var. *sinensis*	30	30 (-do-)
2.	*C. kissi*	30	30 (-do-)
3.	*C. caudata*	30	-
4.	*C. irrawadiensis*	30	-
5.	*C. japonica*	30, 45	30, 45 (-do-)
6.	*C. rosaeflora*	45	-
7.	*C. sasanqua*	90	90 (-do-)
8.	*Camellia* spp.	30, 45	-
9.	*Gordonia excelsa*	30	-
10.	*Pyrenaria berringtoniaefolia*	30	-
11.	*Schima wallichi*	36	-

After, Bezbaruah, 1971b.

The triploid tea plants have 2n = 45 chromosomes in their chromosome complement. Triploids were reported to be common in ornamental *Camellias* and also in cultivated tea (Janaki-Ammal, 1952). Karasawa (1932) reported a wild plant to be autotriploid plant with fifteen trivalent chromosomes. Most of the triploids were suspected to have arisen from the union of haploid with an unreduced diploid gamete. Investigation on the karyotype of triploid tea variety Sundaram by Jayasurya and Govindarajulu (1975) supported this view.

Morphologically, the triploids have been observed to be more vigorous and hardier as compared to normal diploids. Simura (1935, 1956) found the triploids to be more cold tolerant under conditions prevailing in Japan. Sarmah (1984) observed that the triploids derived from tetraploid X diploid crosses to be good rooter and gave higher yield. However, they varied greatly in their chemical constituents, which might be attributed to their parental influence. Quality of made tea from triploids was comparable with that of clones like TV1 (Sarmah, 1984). Genetic variation or mutation of diploid tea plants into polyploids can, therefore be expected to improve vigour and hardiness. So, popularity of the triploids are increasing day-by-day. A detailed karyomorphological study was undertaken by Chaudhuri (1979) on the open pollinated progenies of triploid tea which ranged from diploid-aneuploid to tetraploid and rare pentaploids. Triploid mother was allogenomic and showed mostly trivalents in pollen mother cells. Detailed analysis of chromosome configuration in the pollen mother cells with that of karyotypic studies of diploid and polyploids had exhibited wide range of association like bi-, tri-, quadri-, penta-, and even hexavalents. Unusual pairing like partial, ring and chain type were observed. All those complex configurations indicated structural heterozygosity and duplicate loci for genes in other words "cryptic structural hybridity" in chromosomes in triploid *Camellia* for allosynthetic pairing (Kondo, 1977b, 1978; Chaudhuri, 1993).

The ploidy level in the genus *Camellia* range upto hexaploid level (Longley and Tourje, 1959; Ackerman, 1971, 1973; Kondo, 1977b). Most of the species belonging to higher ploidy level i.e. pentaploid (2n = 75) and hexaploid (2n = 90) are in the ornamental *Camellia* viz., *C. japonica, C. reticulata, C. pitardii, C. rusticana, C. sasanqua* and their hybrids. Cytological observation by Bezbaruah (1975d) showed that in *C. sinensis* the ploidy level ranged from diploid to pentaploid (2n = 75).

Cytological studies of the tetraploid teas showed that in general, there were 28 pairs of homologous chromosomes and 4 univalents or single chromosome. The average chromosome length ranged from 1.63 µm to 3.00 µm in one of the stocks (398/4), while in another (398/2), the length ranged from 1.40 µm to 3.00 µm. Except the satellited chromosomes, the rest of the chromosomes were characterised in having only sub-median primary constriction in each stock. The gradation was not uniform and a number of similar chromosomes were observed in both the stocks (Sarmah, 1984).

Kondo (1977b, 1977c) studied the meiotic behaviour of hexaploid species (2n = 90) and varieties of *Camellia* and also their hybrids. Among parental species, 45 regular bivalents were invariably observed in the hexaploids which explained the complex origin of the plants. The hybrids studied by him include – *C. reticulata* X *C. pitardii, C. pitardii* X *C. fraterna, C. sasanqua* X *C. oleifera, C. sasanqua* X *C. reticulata, C. reticulata* X *C. fraterna* and *C. pitardii* X *C. sasanqua.*

In general, the hybrids showed 40 bivalents with a few quadrivalents and occassional univalents. Among the higher configurations, quadrivalents were most frequent. Hexavalents were reported to occur most frequently in one case, while in another, univalents were frequently met with. Higher configuration, like trivalent, quadrivalent, pentavalent and hexavalents in the hexaploids were also not uncommon. Laggards and chromosomal bridges were also reported in a few plants. Various chromosomal association in hexaploid *Camellias* and their hybrids were explained for segmental allopolyploidy, in which large number of homologous segments might have distributed throughout the genomes (Kondo, 1977c). On the origin of such polyploidy, Janaki-Ammal (1952) and Ackerman (1971) proposed that "segmental allopolyploidy" might have originated during the course of evolution in diploid level and continued in the polyploids through inter-crossing among them.

Cherian and Stephen (1981) observed that the meiosis in Assam and hybrid teas was regular with 15 bivalents at diakinesis and metaphase I and the segregation was also regular. However, the hybrids showed several meiotic abnormalities in the pachytene chromosomes suggesting that they might have differences in gene content or arrangements from their parental species. Unpaired segments were also found to co-exist with regions of higher association of chromosome perhaps caused by stickiness.

Choudhuri (1979) observed general association of the chromosomes at diakinesis which indicated a common origin of all the three varieties of diploid teas. In the fomation of microspore nuclei also there were variation with four haploid nuclei formed in natural fashion in some pollen mother cells, while others showed more or less number of microspore nuclei. Supernumerary division resulting in eight microspore nuclei was also observed in hybrid tea (Cherian and Stephen, 1981).

Kondo (1979) analysed the karyotype of 30 clones of *C. sinensis* var. *sinensis* and five clones of *C. irrawadiensis* and observed some degree of interspecific variation in the karyotypes. In *C. sinensis,* there were satellite chromosomes while in *C. irrawadiensis,* no satellite chromosome was observed (Kato and Simura, 1971). All the clones of both the species showed heteromorphology and heteromorphic chromosomes. Karyotypic characteristics of diploid clones of *C. sinensis* had also been reported by Ackerman (1971), Bezbaruah (1971b), Kato and Simura (1971) and Kondo (1977a). So, sat-chromosomes are not of significance for *C. sinensis* var. *sinensis*. In *C. japonica,* wide range of morphological variability in the cultuvars might be directly correlated with their wider range of karyotypic variation (Kondo, 1977a). Cytological study of a triploid tea (Bezbaruah, 1975d) revealed that the arm length of the individual chromosome showed that most of them had median or nearly median primary constriction, except for two sub-terminal pairs. Secondary constrictions could not be located in any of the chromosome. As regards to chromosomes with secondary constriction, there was a similar form in *C. sinensis, C. wabisuke* and *C. japonica.* In sat-chromosomes, they were partially identical in *C. sinensis* and *C. wabisuke* and also in *C. japonica* and *C. sinensis* (Kato and Simura, 1971).

Ackerman (1971, 1973) reported that among the cultivated species in the family *Theaceae*,, the largest number of taxa were either diploid (2n = 30) or hexaploid (2n = 90) and less frequently were triploid (2n = 45), tetraploid (2n = 60) and penaploid (2n = 75). In addition, a heptaploid (2*n* = 105) inter-generic hybrid between *Tutcheria virgata* (Koidz) Nakai X *Camellia miyagii* (Koidz) Makino and Nemoto has also been reported (Ackerman, 1973). Aneuploidy has been considered a rarity among *Camellias*, only reported in interspecific and intergeneric hybrids. Though, aneuploidy in tea has been reported by Bezbaruah (1971b, 1976), Ackerman (1971, 1973), Chaudhuri and Bezbaruah (1985). Ackerman (1991) observed a spontaneous translocated chromosome approximately 50 per cent longer than the next longest chromosome in *C. japonica*, `Kuro-tsubaki' and several of its hybrids. The results were also correlated with the intense black-red pigmentation of the variety and some of its hybrids apparently originated from an unequal reciprocal translocation between homologous chromosome resulting in one chromosome with a deficiency and one with a duplication.

There are reports of the occurrence of natural polyploids which has a special significance in a crop like tea (Katsuo, 1966; Bezbaruah, 1971a; Amma, 1974; Sebastiampillai, 1976). However, commercial exploitation of the polyploids has not been reported in tea except for the planting in South India of a natural triploid high yielding clone `Sundaram' (Venkataramani, 1969; Jayasurya and Govindarajulu, 1971) and `TV 29' in North East India and in Kenya (Wachira, 1991). Rashid *et al.* (1985) studied the F_1 plants derived from the cross between diploid and tetraploid, all of which showed variability in their morphogenetic attributes.

CHAPTER - III

Materials and Methods

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MATERIALS AND METHODS

3.1 MATERIALS

Various species of *Camellia* and related genera collected and maintained at Tocklai Experimental Station, situated at 26°47' N latitude, 94°12' E longitude and 78 meters above mean sea level were used in the present investigation. Detailed description of all the species, their source of collection etc. have been presented in Table 3.1.

Table 3.1. Detailed description of the species used in the present investigation

Name of the species	Sub-generic section	Chromosome number, 2n=	Year of introduction	Source
Assam (Naga) tea, ***Camellia** assamica* **(Ma**sters) Wight	Thea	30	1918	Raised from seeds collected by A.C. Tunstall from supposedly 'wild' source in the Naga hills at 26.45°N latitude and 94.40°E longitude
Cambod tea *C. cambodiensis* **King**don Ward= *C. assamica* ssp. *lasiocalyx* (**Planchon** ex Watt) **Wight**	Thea	30	1918	Raised during 1923 by H. R. Cooper from open pollinated seeds obtained from former Indo China
C. drupifera Lour	Dubiae	30	1955	Collected by R.I. Macalpine from Dooars, North East India

3.1. Continued …..

Name of the species	Sub-generic section	Chromo-some number, 2n=	Year of introduction	Source
C. japonica	Camellia	30	1956	Introduced from Tokai Kinki Experimental Station, Japan
C. kissi Wall.	Paracamellia	30	1950	Collected by Dr. W. Wight from Meghalaya
C. rosaeflora Hook	Theopsis	45		Introduced from Sri Lanka
C. sasanqua Thunb.	Paracamellia	90		Introduced from Japan
China tea, *C. sinensis* (L.) O. Kuntze	Thea	30	1914	A China hybrid stock collected from an area of tea in the plains of Assam planted with seeds imported from China
F_1 hybrids of *C. sinensis* L. X *C. japonica* L.		45	1972	Clone obtained from hybrid plant obtained from *C. sinensis* (4x) X *C. japonica* (2x) by Bezbaruah and Gogoi (1972)
Eurya japonica			1950	Clones raised from plants collected by Dr. W. Wight from Shillong
Gordonia excelsa Blume		30	1918	Collected by A.C. Tunstall from Meghalaya
Pyrenaria berringtoniaefolia Seem.		30		Collected from Assam
Tetraploid tea	Thea	60		Open pollinated plant from a triploid plant obtained from the open pollinated seeds of a plant collected from Indo China
Triploid clone (TV 29) Cambod type	Thea	45	1990	Clone raised from the progeny of tetraploid plant, which is a second generation open pollinated progeny from a triploid obtained from a plant from Indo China

3.2 METHODS

3.2.1 Pollen Morphology

For studying the morphology of the pollen grains under scanning Electron Microscope (SEM), fully matured unopen flower buds from previously bagged branches were collected in the evening and the following morning they were taken to the laboratory of Regional Sophisticated Instrumentation Center, North Eastern Hill University, Shillong for observation. Air dried pollen grains were placed on adhesive tape adhered to metal stub and gold coated *in vacuo* by the usual technique (Fogle, 1977a, 1977b) prior to viewing under JEOL J&M 35 CF. Japan Scanning Electron Microscope. A magnification of 540 x to 2000 x for studying the whole pollen and 6600 x to 10,000 x for the surface topography were used. Photomicrographs of the representative pollen grains and sections of exine were taken with the help of camera attached to the electron microscope.

3.2.2 Pollen Storages and viability Studies

Branches with young immature flower buds were bagged with muslin cloth bags to reduce contamination. Just opened flower buds were collected in the morning in polythene bags and rushed to the laboratory. Kaurov (1957) reported that pollen grains collected in the early morning germinated better than that collected at other times of the day. The reason for such deviations have not been associated, they probably were related to metabolic transitions and moisture stress (Stanley and Linskens, 1974). In the morning hours, the activities of insects and wind also lesser than later hours, so the chances of adulteration were also less. In the laboratory, the buds were exposed to either sun or under a table lamp for an hour when the anthers started to dehisce. By gently tapping the flowers, the pollen grains were collected on a glossy butter paper, then they were transferred to an aluminium foil and kept for 2 to 3 hours in a dry dust free area and thereafter, pollen grains were desiccated for 6 to 8 hours putting over silica gel. Finally, the grains were transferred to previously autoclaved, clean, dry glass vials with lid. Collection was avoided on cloudy or foggy days.

The glass vials containing pollen grains of each species were kept in desiccator maintaining at three relative humidities : (i) 0 per cent by calcium chloride (anhydrous), (ii) 34 per cent by saturated solution of magnesium chloride and (iii) 65 per cent by saturated solution of ammonium nitrate (Vasil, 1962). The desiccators were then kept in refrigerator to maintain at three temperatures - 0°C, 5°C and 20°C.

For routine observation, the pollen grains were taken out very carefully at weekly intervals. All possible precautionary measures were taken while storing and drawing representative samples so that microbial (fungal and bacterial infection could be avoided. Glass vials were opened for shortest possible time in aseptic condition in the desiccator and refrigerator. The viability was tested by staining with 0.5 per cent acetocarmine and germinability was tested by germinating them in 10 per cent sucrose solution by "hanging drop" method (Bezbaruah, 1971a). Two replicates of 200 grains each were counted per treatment per observation. Normal, well stained filled pollen grains were counted as viable, while the unstained and shrunken were counted as non-viable or non-fertile. The testing of viability was stopped when the pollen grains failed to germinate in three successive observations.

3.2.3. Artificial Pollination

Healthy flowering branches were chosen for artificial (hand) pollination. To avoid contamination, the open flowers and immature fruits were removed. The immure unopen flower buds were bagged with muslin cloth. The flower buds were emasculated 24 hours before anthesis. Emasculation was done by carefully cutting off one third of the petals from top and then the stanens below the anther were removed with the help of a fine pointed scissors and forceps. Care was taken so that the style was not injured. Emasculation was done in the morning just before pollination or artificial transfer of pollen over the stigma. Fully developed unopen flower buds which will open the following morning were ideal for emasculation and artificial pollination.

Pollen from the desired pollen parent was collected from previously bagged flowers, to avoid chances of contamination. Just opened flower buds were collected on dry and sunny morning and pollen grains were collected in a dust free place on a clear dry butter paper. After collection, they were stored in previously washed and dried glass vials and kept in refrigerator.

Soon after emasculation, the pollen grains were either dusted with the help of a camel hair brush or the stamens collected from the selected pollen parent were gently touched over the stigmatic surface. After pollination, the branches with pollinated flower buds were bagged with muslin cloth bags. The bags were retained for 2 days after pollination.

Following the procedure described above, artificial pollination was done in all possible combinations including reciprocal crosses during 1993-94 and 1994-95 for interspecific and intergeneric hybridization using all the species (Table 3.1).

3.2.4 Receptivity of stigma, self and cross-incompatibility and their mechanism

(i) Receptivity of stigma

To determine the receptivity of stigma, five flower buds in each case were emasculated and pollinated at one hour intervals starting from 72 hours prior to and continued till 48 hours after anthesis. Changes in the flower buds, mainly the ovaries were observed on 15th and 30th day after pollination done at 72, 48 and 24 hours prior to and on the day of anthesis.

(ii) Self- and cross-incompatibility and their mechanism

For studying the self incompatibility, depending on the availability, 100 to 400 flower buds of all the species were artificially selfed and the number of seed-set, their germinability and the resulted plants were observed.

For cross-incompatibility, five flower buds in each combination were pollinated during 1995-96 where seed-set could not be achieved as a result of artificial pollination during 1993-94 and 1994-95.

To ascertain the mechanism of self- and cross-incompatibility anatomical observations were made to study the nature and length of pollen tube growth in the style, following the technique of Alexander (1987).

3.2.5 Foliar and plant morphology

(i) Foliar Morphology

Various morphological characters on leaf length, breadth (maximum). length: breadth ratio, were measured using Leaf Area Meter (CI 202) Eijkelkamp. Agrisearch equipment The Netherlands. In all the parameters, the average of 20 leaves in case of parents and 5 leaves in case of hybrid were taken due to non-availability of sufficient number of leaves in the hybrids. Observation on other morphological features, like number of serration, anthocyanin pigmentation and leaf angle were made either with single measurement with scale and slide callipers or visually and were compared with the parents.

(ii) Plant Morphology

Various morphological attributes of the interspecific and inter-generic hybrids include plant height, number of leaf, internodal length and grith at collar taken and compared with those of the parents at three monthly intervals.

3.2.6 Anatomy of leaf

(i) Stomata

For observing the size and frequency of stomata in parental species and their hybrids small discs were punched with a cork borer from the middle of the leaves. They were gently boiled in a dilute solution of KOH for about 5 minutes. The discs were washed with distilled water and the lower epidermis from the discs were peeled off with the help of a needle and forceps, stained with Heaths (1947) reagent. Ten leaves were examined for each species. However, for the hybrid, the average of three positions *viz.*, tip, middle and base of the leaves were taken. Frequency of stomata was counted under low magnification while the length and breadth of each stomata were measured under high magnification.

For counting the number of stomatal chloroplasts in guard cell, the abaxial epidermal layer was peeled off and stained with a mixture of iodine and potassium iodide solution and mounted in dilute glycerol as described by Choudhuri and Burrow (1975).

(ii) *Sclereid :*

For sclereid morphology, pieces of about 1 sq.cm. from the middle of fifth leaf from the terminal bud were boiled in 35 per cent sodium hydroxide (NaOH) solution for a few minutes. Then, the pieces were washed with distilled water until the water was clear. A few drops of safranin was added and warmed and excess of stain was removed with 1 N HCl and washed thoroughly in water. The pieces were macerated on a clear dry slide on 2-3 drops of glycerine. The number of sclereid were counted under microscope and camera lucida drawing made to study their morphology (Barua and Dutta, 1959; Chaudhuri and Bezbaruah, 1985; Chen and Ye, 1989).

3.2.7. Cytological Study

Cytological status of *Camellia japonica* and *C. sasanqua*, introduced recently and *E. japonica* were studied following the technique of Bezbaruah (1968a). Somatic chromosomes were observed from the leaf tip squashes. After collection, the leaf tips were pre-treated in a saturated solution of p-dichlorobenzene for 3 hours at 4°C. Then, they were washed in distilled water and fixed in 1:3:6 mixture of propionic acid, chloroform and ethanol for 10 hours. Hydrolysis and staining were done in a solution containing 1 N HCl, 1 part and 2 per cent orcein in 45 per cent propionic acid, 9 parts, warmed over a small flame for 10-15 minutes, after which the tips were squashed in a drop of 1 per cent carmine in 45 per cent propionic acid on a clean slide under a coverslip.

However, for hybrids, sufficient number of vegetative buds for leaf tip squashes were not available so cytological studies were made using the root tips. Root tips were collected when the hybrid seedlings (about 10 cm tall) were transferred from germinating sleves to bigger sleeves. After collection, they were washed thoroughly with tap water in a watch glass and transferred to a glass vial containing 0.2 per cent aqueous solution of colchicine and kept for 2 hours at 20°C for pre-fixation. Root tips were then fixed for 8-10 hours in 3:1 ratio of 95 per cent ethyl alcohol and glacial acetic acid at 0°C. After fixation, they were hydrolysed in 1 N HCl at 60°C for 10 minutes and then stained in acetocarmine for about half an hour. Squashes were made on clean, dry slide, covered with coverslip and temporarily sealed with transparent nail varnish and observed under microscope.

CHAPTER - IV

Experimental Findings

CHAPTER – IV

EXPERIMENTAL FINDINGS

4.1 MORPHOLOGY AND SURFACE TOPOGRAPHY OF POLLEN GRAINS

Details of the morphological characteristics of the pollen grains of the materials studied like dimension of length, width, length to width ratio, and length of germinal furrows have been presented in Table 4.1. Specific descriptions of various taxa on gross morphology and utlrastructure of the exine have been presented below.

(i) ***Camellia assamica*** : The polar length of the pollen grains ranged from 43.75 to 50.50 µm with an average of 48.65 µm, while the width ranged from 26.13 to 32.04 µm. the average was found to be 30.05 µm. The size was observed to be the largest among the diploid tea species. The length to breadth ratio was 1.57 to 1.69, which indicated the shape of prolate. The shape as observed under 2200 x magnification was elliptical with tapering ends (Plate 1A), rarely grains with fattened ends were also observed. One or two longitudinal germinal furrow (s) were observed in equatorial view, which were not of equal length and thickness even in the same pollen. They ranged from 34.92 to 43.04 µm with an average of 38.72 µm. The aperature was of banded model. From the polar view, the grains were found to be tricolpate, circular to semi - angular with the furrows intruding

Table 4.1 **Size and shape of pollen grains of the species of *Camellia* and related genera observed under Scanning Electron Microscope**

Sl. No.	Species	Average length (µm)	Range (µm)	Average width (µm)	Range (µm)	Average length : width	Range	Average length of germinal, furrows (µm)	Range (µm)
1.	*C. assamica*	48.65	43.75-50.50	30.05	26.13-32.04	1.64	1.57-1.69	38.72	34.92-43.04
2.	*C. cambodiensis*	45.45	40.22-46.89	27.18	25.68-29.78	1.60	1.43-1.77	36.36	32.68-38.97
3.	*C. drupifera*	39.52	37.00-42.25	22.58	20.50-25.00	1.75	1.66-1.91	32.59	28.36-35.00
4.	*C. irrawadiensis*	54.00	51.08-56.95	31.00	29.68-33.80	1.73	1.69-1.78	48.50	45.18-50.50
5.	*C. japonica*	53.50	49.17-56.30	28.83	25.83-29.95	1.89	1.82-1.92	43.75	39.17-48.50
6.	*C. kissi*	37.14	31.20-39.44	23.94	22.22-26.85	1.46	1.22-1.77	28.11	22.80-33.33
7.	*C. rosaeflora*	22.22	20.00-25.33	11.44	12.00-16.67	1.57	1.20-1.94	17.55	16.17-20.00
8.	*C. sasanqua*	48.33	33.32-54.49	31.11	24.91-41.25	1.56	1.30-1.74	36.59	34.70-40.03
9.	*C. sinensis*	37.69	34.88-44.19	24.09	24.42-28.50	1.39	1.23-1.58	27.00	23.65-30.07
10.	F_1 of *C. sinensis* X *C. japonica*	32.50	28.73-34.56	25.08	21.68-27.93	1.23	1.16-1.29	24.68	20.45-28.93
11.	*Gordonia excelsa*	56.80	50.50-60.13	32.15	29.55-35.35	1.78	1.66-1.96	51.18	47.33-54.58
12.	*Pyrenaria berringtoniaefolia*	54.68	49.68-63.28	37.45	29.60-40.19	1.28	1.20-1.34	49.56	45.83-56.26
13.	Tetraploid tea	59.67	56.94-65.42	41.50	38.36-46.78	1.24	1.14-1.38	32.25	27.56-33.33
14.	Triploid tea (TV 29)	54.68	49.68-63.28	37.45	29.60-40.19	1.31	1.11-1.48	27.83	21.66-32.92

PLATE – 1

Scanning Electron Microphotogrophs

A. Whole pollen grain of *C. assamica,* X 2,200

B. Surface topography, X 8,600

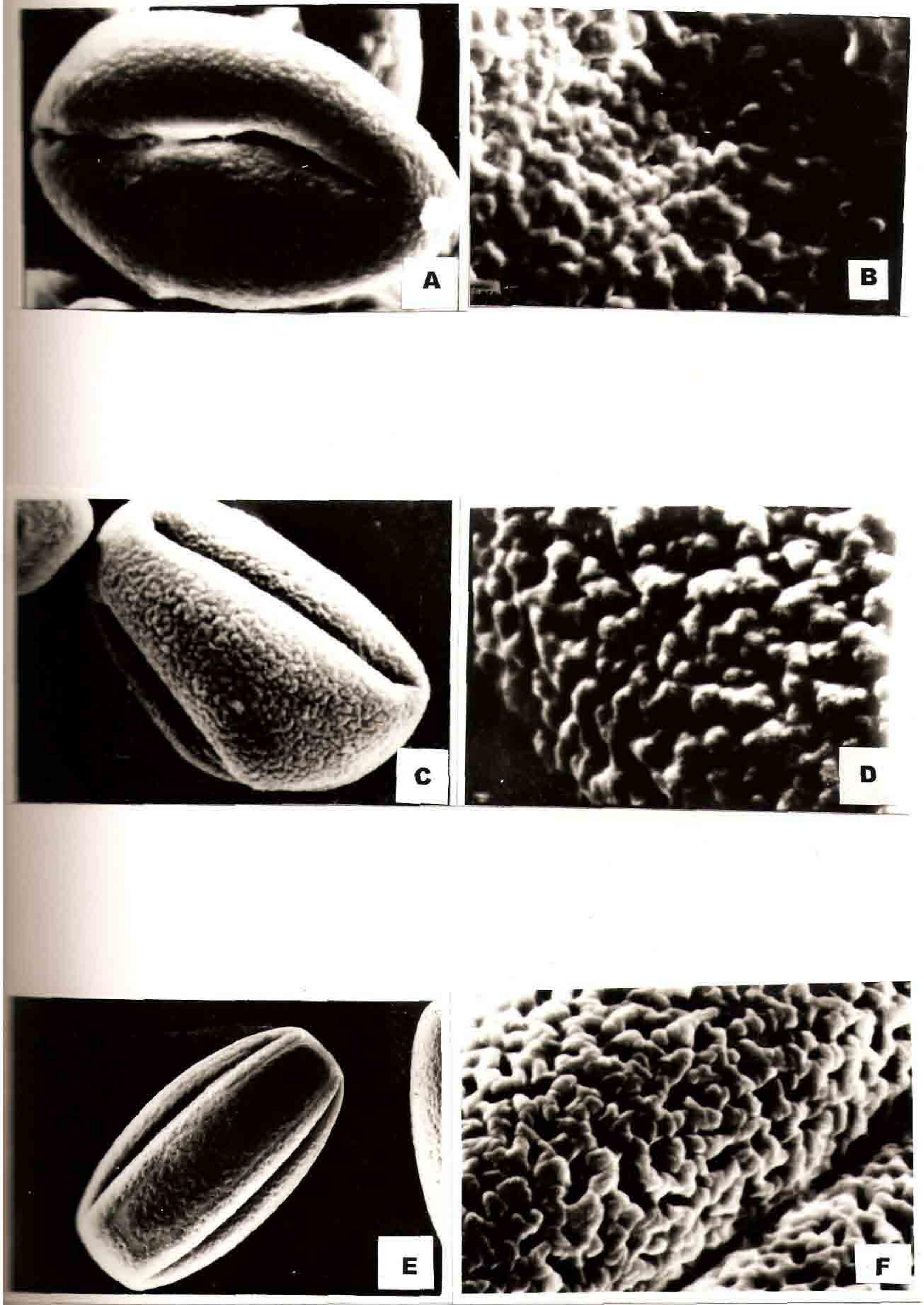
C. Whole pollen grain of *C. cambodiensis*, X 2,000

D. Surface topography, X 10,000

E. Whole pollen grain of *C. drupifera,* X 2,000

F. Surface topography, X 7,800

PLATE 1



or sometimes open at the angles. For studying surface topography of the pollen grains, a portion of the surface was magnified at 8,600 x (Plate 1B). The outer exine layer was observed to be rough, irregularly verru cose, appearing like the surface of an orange rind to nearly papillate in areas. The surface was more distinct in the inter colporate region. The frequency of the perforations was very less and their shape was irregular.

(ii) ***C. cambodiensis*** **:** The average length of the pollen grains was observed to be 45.45 µm with a range from 40.22 to 46.89 µm. The width ranged from 25.68 to 29.78 µm, the average being 27.18 µm. The size was observed to be smaller than *C. assamica.* The ratio between the polar length to width varied from 1.43 to 1.77 (average 1.60) which also revealed a shape of prolate. However, the shape, as observed under SEM was of elliptic with tapering ends to depressed oval (Plate 1C). The germinal furrows ranged from 32.68 to 38.97 µm, the average length of the pores was oberved to be 36.36 µm., which was also smaller than *C. assamica* but larger than *C. sinensis.* The aperature was of narrow split model, in some pollen grains they appeared to be wide and had more depth. Though the depth of germinal furrows could not be measured but, in equatorial view one or two furrows could be seen. The polar view revealed three furrows per grain-trizonocolpate.

PLATE – 2

Scanning Electron Microphotographs

A. Whole pollen grain of *C. irrawadiensis,* X 2,000

B. Surface topography, X 10,000

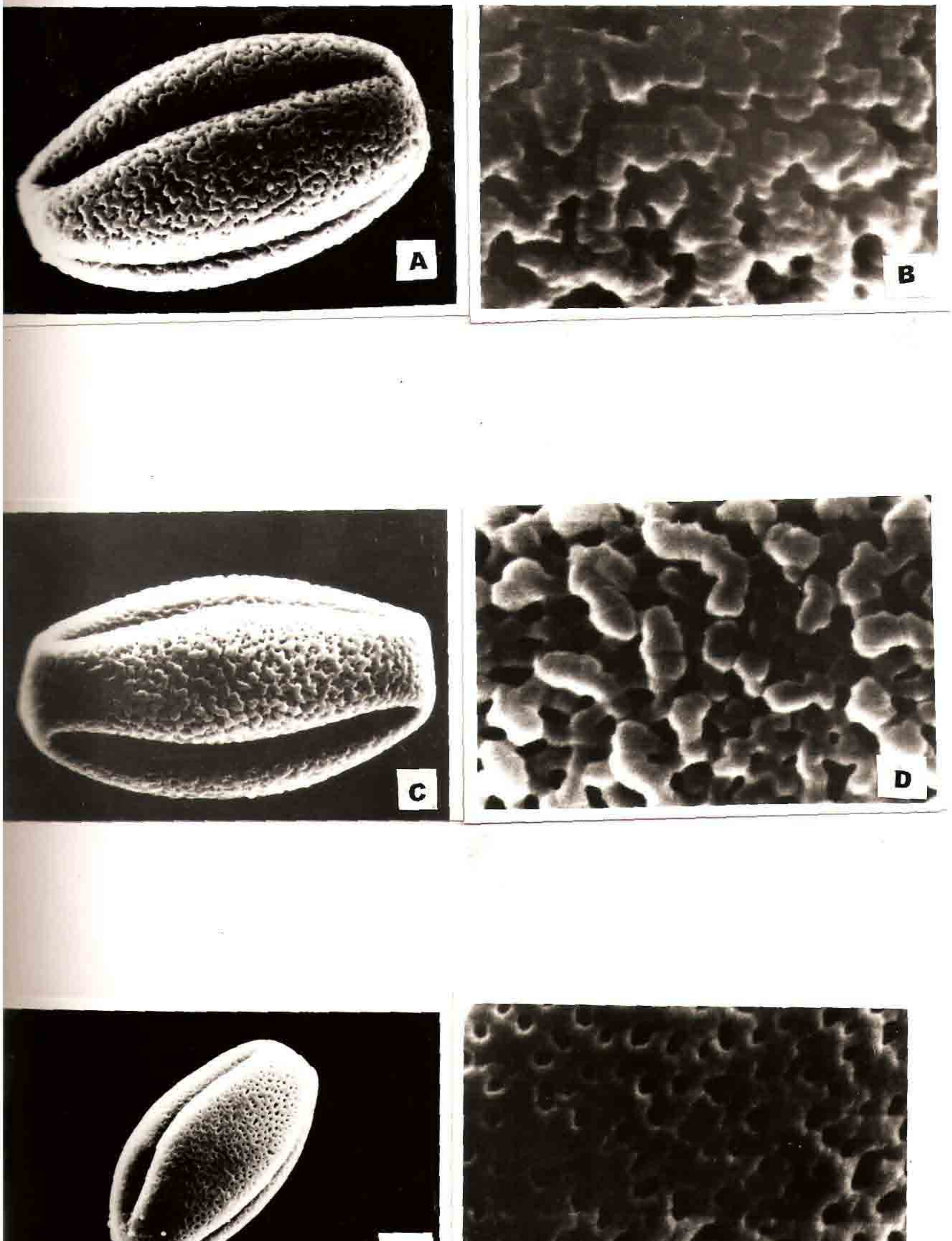
C. Whole pollen grain of *C. japonica*, X 2,000

D. Surface topography, X 10,000

E. Whole pollen grain of *C. kissi,* X 1,800

F. Surface topography, X 10,000

PLATE 2



Surface ornamentation was more rough than C. *assamica*, irregalarly verrucose to nearly papillate in areas (Plate 1D). The muri were more distinct in intercolporate region. The frequency of lamina was also less.

(iii) ***C. drupifera*** **:** The length of the pollen grains of this species ranged from 37.00 to 42.25 μm, the average length measured to be 39.52 μm. The average width was 22.58 μm, although it varied from 20.50 to 25.00 μm. The pollen grains as seen at a magnification of 2000 x (Plate 1E) were elliptic with tapering or flattened ends to depressed oval. The length to width ratio ranged between 1.66 to 1.91 which indicated a shape of prolate. The gross morphology of the pollen grains was almost similar to the tea species. The lenght of germinal furrows ranged from 28.36 to 35.50 μm with an average length of 32.59 μm. The aperature was of narrow split model, while in a few grains, they were banded.

The exine pattern as observed under a magnification of 7,800 x (Plate 1F) depicted sharp differences in surface toprgraphy from other species. In this species, the exine was verrucose to thin reticulate type, the muri were more distinct. The frequency of perforations was more, they were circular to irregular in shape as a few were fused together. The outer exine was more distinct near the longitudinal germinal furrows.

(iv) ***C. irrawadiensis*** **P.K. Barua :** The size of the pollen grains ranged from 51.08 to 56.95 μm x 29.68 to 33.80 μm with an average size of 54.00 μm x 31.00 μm. The shape was ovserved to be elliptical, prolate, length to breadth ratio ranged from 1.69 to 1.78 with an average of 1.73 (Plate 2A). The germinal furrows were very distinct, three in number, trizonocolpate. The surfaee ornamentation (Plate 2B) was very distinct particularly in the intercolporate regions, ridges were absent but the muri were distinct, 2 to 7 perforations fused together resulting in irregular shape. The length of germinal furrows ranged between 45.18 to 50.50 µm. The species could very easily be ideintified with the help of exine pattern. The polar view represented a circular to semi-angular shape with the furrows intruding at an angle of 120°.

(v) ***C. japonica* L.** : The length of the pollen grains ranged from 49.17 to 56.30 µm, while the width ranged from 25.83 to 29.95 µm, largest of all the *Camellia* species studied. The length : width ratio ranged from 1.82 to 1.92, which was also larger. The shape of the pollen grains was elliptical to depressed oval (Plate 2C). The inter colporate region was more wide spread at the equatorial region than near the poles. Micro-morphology of the surface sculpture (Plate 2D) revealed that the perforations and nanoprocesses were distinct. The muri were distantly spaced at the intercolporate region. Lumina were fused together and formed a channel like appearance. However, near the germinal furrows the perforations were distinct but irregularly shaped.

(vi) ***C. kissi*** : The average size of the pollen grains was observed to be 37.14 µm x 23.94 µm. Though the length and width ranged from 31.20 to 39.44 µm and 22.22 to 26.85 µm respectively. The length : width ratio ranged from 1.22 to 1.77, the

PLATE – 3

Scanning Electron Microphotographs

A. Whole pollen grain of *C. rosaeflora,* X 1,500

B. Surface topography, X 10,000

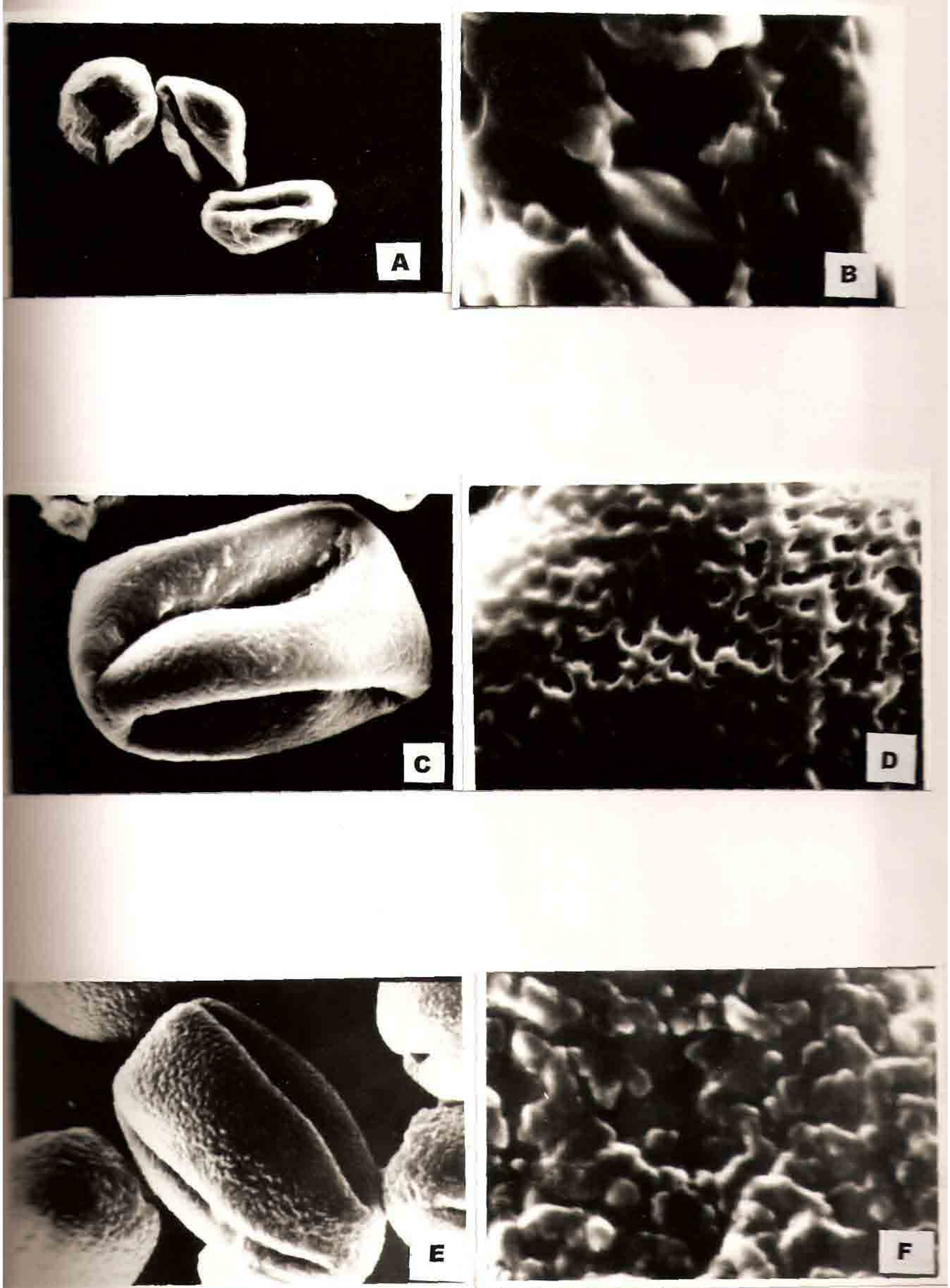
C. Whole pollen grain of *C. sasanqua*, X 1,800

D. Surface topography, X 9,400

E. Whole pollen grain of *C. sinensisi,* X 1,800

F. Surface topography, X 10,000

PLATE 3



average being 1.46. The shape of the pollen grains was prolate (Plate 2E), trizonocolpate. The germinal furrows were of narrow split model, the distance between them was maximum at the equatorial region. Two farrows could be seen at the equatorial view. The shape of the pollen grains when observed from polar view was semi-angular to circular with furrows intruding while in some grains they were open. Exine sculpture was rough reticulate with distinct but irregularly shaped perforations (Plate 2F). The frequency of lumina was very high. In areas, the perforations were fused together.

(vii) ***C. rosaeflora*** **:** The average length of the pollen grains was observed to be 22.22 µm, while they ranged from 20.00 to 25.33 µm. The average width was found to be 14.44 µm with a range of 12.00 to 16.67 µm. The size of the pollen grains was the smallest among all the species investigated in this study. The length to width ratio ranged from 1.20 to 1.94 with an average value of 1.57. Though, the shape of the pollen grains as observed from the length : width ratio was prolate, (Plate 3A), they were completely different from other *Camellia* species. The pollen grains were shrunken and poorly filled. The germinal furrows were smaller, concave type and wider than other species. The exine sculpturing was also different, certain areas had somewhat psilate surface without distinct nanoprocesses and perforations (Plate 3B). The inter-colporate region was slightly concave with a few ridges. From these observations, it could be easily concluded that the species was a triploid, the viability and germinability of these pollen grains were very poor.

(viii) *C. sasanqua* : In gross morphology, (Plate 3C) the pollen grains were different fram other species. The length varied from 33.32 to 54.59 µm with an average of 48.33 µm, while the width ranged from 24.91 to 41.25 µm. The average width was found to be 31.11 µm. There were considerable variations in shape. The shape of the pollen grains ranged from elliptical, depressed oval to rectangular, though from the length to width ratio (1.30 to 1.74) the shape varied from sub-prolate to prolate. Three longitudinal germinal furrows were seen in each grain, however, they differed in their type or model of split. In some grains, the aperature was of narrow split model, while in others, they were of banded model (Plate 3C). The distance between germinal furrows also varied – at one pole they were at maximum distance, while at the othe,r the distance was minimum.

The surface topography of exine was thin reticulate type with irregular shaped perforations (Plate 3D) which might resulted because of fusion of a few perforations together. The surface was verrucose with distinct papillate in the inter-colporate regions. Ridges were absent. The muri were sparsely spaced.

(ix) *C. sinensis* **L. (O. Kuntze)** : The average length and width of pollen grains of China tea was found to be 37.69 µm and 24.09 µm respectively. The ranges of length and width were 34.88 to 44.19 µm and 21.42 to 28.50 µm respectively. The size of the pollen grains of China tea was the smallest among all the diploid tea species. The length to width ratio was also the least (average 1.39, ranged from 1.23 to 1.58). The pollen grains were prolate trizonocolpate with unequal germinal furrows (Plate 3E). In polar view, they were circular to semi angular with the furrows intruding to open. The length of germinal furrows varied from 23.65 to 30.07 μm with an average value of 27.00 μm.

The surface sculpture of exine (Plate 3F) was fine reticulate with the muri fused together giving an irregular shaped perforations. Ridges were not found, In some inter-colporate areas the surface was verrucose, however, the muri were sparsely spaced in other areas.

(x) **F_1 of *C. sinensis* X *C. japonica*.** : The size of the pollen grains of this hybrid plants varied from 28.73 to 34.56 μm in length (32.50 μm average) and 21.68 to 27.93 μm in width (25.08 μm average), which was smaller than both the parents. Length to width ratio observed was also the smallest (ranged from 1.16 to 1.29 average 1.23). The shape of the pollen grains was sub-prolate with three longitudinal germinal furrows (Plate 4A). The germinal furrows were smaller, with an average size of 24.68 μm, though they ranged between 20.45 to 28.93 μm. In gross morphology, the pollen grains were distinguishable from other species.

The ultrastructure of the surface revealed the presence of longitudinal and continuous ridges along the entire surface. The ridges were apparently equally spaced without whirling. Verrucose type sexine was also present in some areas. (Plate 4B). The germinal furrows were also irregularly shaped. Perforations were very rare. From surface ornamentation also it could be identified as hybrid.

PLATE – 4

Scanning Electron Microphotographs

A. Whole pollen grain of F_1 hybrid of *C.* sinensis X C japonica X 2,000

B. Surface topography, X 10,000

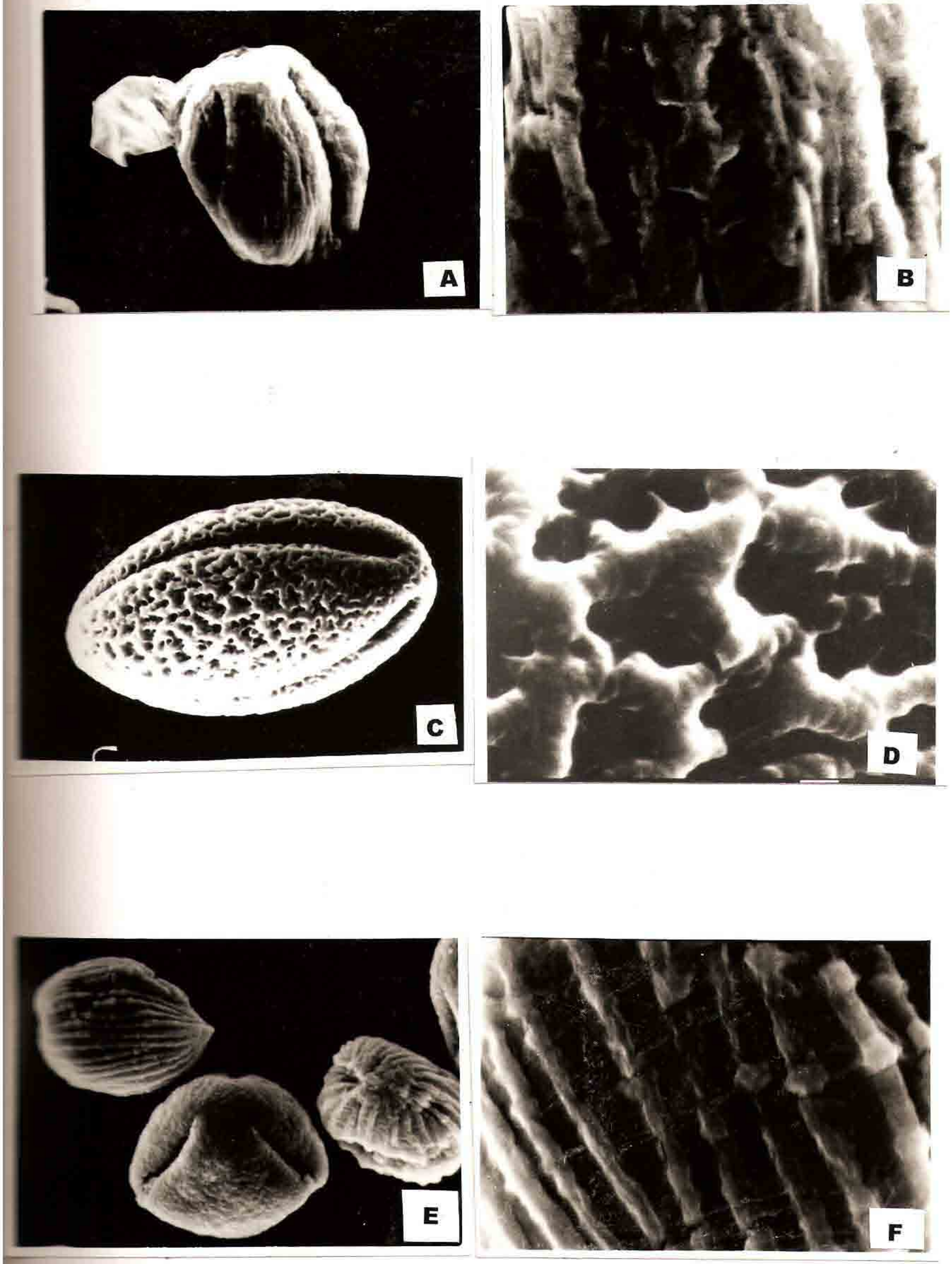
C. Whole pollen grain of *G. excelsa*, X 1,800

D. Surface topography, X 10,000

E. Whole pollen grain of *P. berringtoniaefolia,* X 1,500

F. Surface topography, X 7,200

PLATE 4



(xi) ***Gordonia excelsa*** : The average length of the pollen grains of this species was observed to be 56.80 µm, which was the longest among all the diploid species investigated. The length ranged from 50.50 µm to 60.13 µm. Similarly, the average width was also found to be maximum, 32.15 µm with a range of 29.55 to 35.55. Shape of pollen grains as observed under SEM was typically elliptical, (Plate 4C) unlike *Camellia* species, particularly the polar ends, which were not depressed. Length to width ratio ranged from 1.66 to 1.96 (average 1.78), which indicated the shape to be prolate. Length of the germinal furrows ranged from 47.33 to 54.58 µm. The average length was found to be 51.18 µm. The germinal furrows were parallel to the periphery of the pollen grain. They were at maximum distance at the equatorial position, while convergent towards the poles. The aperature was of narrow split model with almost uniform splitting.

The examination of the ultrastructure at 10,000x revealed that the exine pattern was rough reticulate with distinct nanoprocesses and collared perforations (Plate 4D). The muri were so fused that the perforations were very large and of irregular shape.

(xii) ***P. berringtoniaefolia*** : The length and breadth of the pollen grains of this species ranged from 49.68 to 63.28 µm (average 54.68 µm) and from 29.60 to 40.19 µm (average 37.45 µm), respectively. The shape of the pollen grain were also variable (Plate 4E), the shape ranged from subprolate (length to width ratio 1.20) to prolate (ratio 1.34). The grains were tricolopate with the species specific

PLATE – 5

Scanning Electron Microphotographs

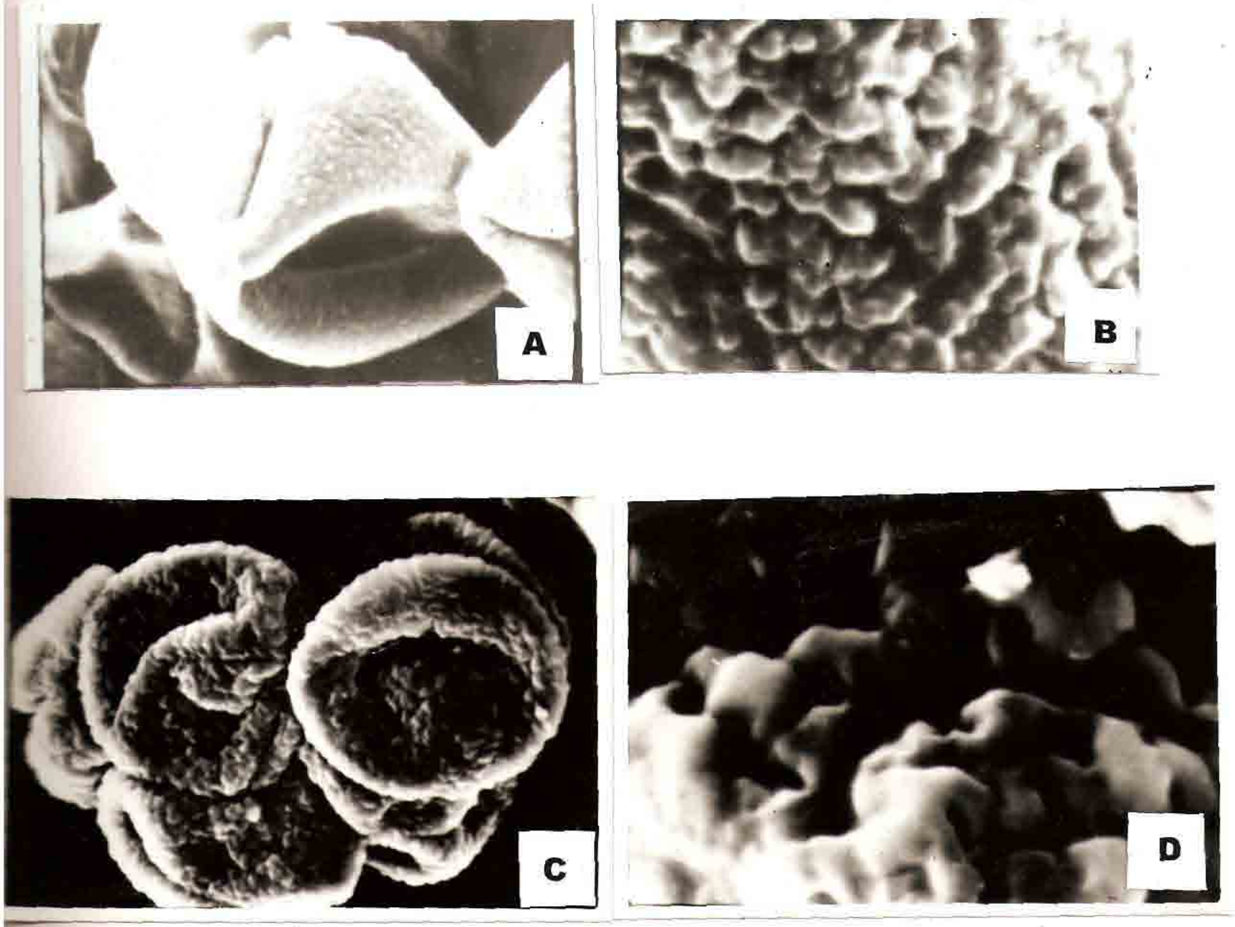
A. Whole pollen grain of tetraploid tea, X 1,800

B. Surface topography, X 10,000

C. Whole pollen grain of triploid tea, X 1,600

D. Surface topography, X 10,000

PLATE 5



germinal furrows. In the equatorial view, two furrows formed a V-shaped structure with the convergent point at one polar end. The exine pattern was observed to be ridged longitudinally. The ridges were also convergent at the polar end. At equatorial region they were almost parallel. On high magnification (Plate 4F) the ridges had finger like projections with joints at unequal lengths. Perforations could not be seen.

(xiii) **Tetraploid tea :** The size ranged from 56.94 to 65.42 μm in length and 38.36 to 46.78 μm in width. The average size of the pollen grain was observed to be 59.67 μm X 41.50 μm. The pollen grains of this species were the largest of all the species studied. The length to width ratio ranged between 1.14 to 1.38, with an average value of 1.24, which indicated the shape to be spheroidal to prolate. The germinal furrows were typical to this species, they formed a V-shaped structure from the polar end and divergent towards the other pole (Plate 5A). The furrows were comparatively short, however, had more depth. Average length of the furrows was found to be 32.25 μm, even smaller than those of Assam and Cambod tea. In most of the grains, a depressed or concave area was present in the intercolporate region.

The surface features were fine reticulate, verrucate, papillate, with less number of perforations (Plate 5B). The perforations were of irregular shape. Exine pattern was similar to other tea species.

(xiv). **Triploid tea :** Average length of the pollen grain was observed to be 54.68 µm, while it varied from 49.68 to 63.28 µm. Average width was 37.45 µm, which ranged from 29.60 to 40.19 µm. The pollen grains were of irregular shape (Plate 5C), however, from the length to width ratio they varied from spheroidal (1.11) to prolate (1.48). Germinal furrows were also irregular – in some grains they were straight while in some others, they were semi-circular. Surface topography (Plate 5D) was rough reticulate. The pollen grains were shrivelled and poorly filled. The muri were present, however, the frequency of perforations was less.

4.2 STORAGE AND VIABILITY OF POLLEN GRAINS

Viability of pollen grains (in days), of nine species of *Camellia*, one interspecific hybrid, two species of related genera *viz.*, *Gordonia* and *Pyrenaria* and two taxa of polyploid teas stored at three levels of relative humidities (0, 35 and 65%) and at three temperatures (0°C, 5°C and 20°C) have been presented in Tables 4.2 to 4.4. Viability of the freshly collected pollen grains of various species studied ranged from 78.2 to 96.0 per cent. It was observed to be maximum (96.0%) for *C. assamica* and *C. sinensis* and minimum (78.2%) in case of triploid tea (Table 4.2). Among the *Camellia* species, *C. rosaeflora* had the minimum percentage of viable pollen (83.3%) followed by F_1 of *C. sinensis* X *C. japonica*. Cambod tea, *C. cambodiensis* had 94.3 per cent good viable pollen, while in tetraploid tea, the viability was observed to be 91.3 per cent. In the two species of related genera investigated, *G. excelsa* and *P. berringtoniaefolia* had 89.0 and 91.8 per cent viable pollen, respectively.

Table 4.2 Viability of the pollen grains stored at 0°C and various levels of relative humidity

Sl. No.	Species	Viability at the time of storage, %	Viability, in days		
			Relative humidity, %		
			0	35	65
1.	*C. assamica*	96.0	91	182	63
2.	*C. cambodiensis*	94.3	77	161	56
3.	*C. drupifera*	93.5	77	161	63
4.	*C. irrawadiensis*	92.0	77	119	49
5.	*C. japonica*	88.0	126	168	91
6.	*C. kissi*	93.0	91	175	77
7.	*C. rosaeflora*	83.3	70	91	49
8.	*C. sasanqua*	92.5	119	161	119
9.	*C. sinensis*	96.0	105	203	56
10.	F_1 of *C. sinensis* X *C. japonica*	80.5	49	119	49
11.	*Gordonia excelsa*	89.0	63	119	42
12.	*Pyrenaria berringtoniaefolia*	91.8	63	105	49
13.	Tetraploid tea	91.3	119	175	91
14.	Triploid tea (TV 29)	78.2	112	175	77

When stored at 0°C and 0 per cent relative humidity (R.H.), the pollen grains of *C. japonica* retained viability for 126 days followed by tetraploid tea and *C. sasanqua* (119 days). Under the similar storage conditions, the pollen grains of *G. excelsa* and *P. berringtoniaefolia* remained viable for 63 days. At 35 per cent relative humidity, the China tea pollen grains remained viable for 203 days, which was found to be the maximum of all the treatments at 0°C. Pollens of *C. assamica* retained viability for 182 days followed by *C. kissi* and both the polyploid (tetraploid and triploid) teas (175 days). Longevity of the pollen grains was shortest (91 dyas) in *C. rosaeflora*. At higher level of relative humidity (65%), the species behaved differently. Maximum days of viability (119 days) could be observed in *C. sasanqua*, while it was minimum (42 days) in *G. excelsa*.

It was clear from Table 4.3 that the pollen grains of *C. japonica*, when stored at 0°C and 0 per cent relative humidity remained viable for maximum number of days (112 days) and that of *P. herringtoniaefolia* was only for 42 days. In case of the tea species, Assam tea and tetraploid tea behaved similarly, both the taxa had good viable pollens for 105 days along with *C. kissi*. Cambod tea and China tea retained their viability for 91 days and 77 days, respectively. At 35 per cent relative humidity, the longevity of the pollen grains of *C. kissi* was found to be for 161 days followed by *C. assamica*, *C. japonica* and *C. sasanqua* (147 days). In general, the viability of the pollen grains at 65 per cent relative humidity was less than either 0 per cent or 35 per cent relative humidity. At lower (0%) relative humidity, most of the grains when approached zero, were shrivelled, might be due to dehydration. On the other hand, at higher humidity storage, there was contamination and infestation of bacteria, fungi and algae.

Table 4.3 Viability of pollen grains stored at 5°C and various levels of relative humidities

Sl. No.	Species	Viability, in days		
		Relative humidity, %		
		0	35	65
1.	*C. assamica*	105	147	77
2.	*C. cambodiensis*	91	133	70
3.	*C. drupifera*	98	112	56
4.	*C. irrawadiensis*	63	105	35
5.	*C. japonica*	112	147	91
6.	*C. kissi*	105	161	63
7.	*C. rosaeflora*	77	98	35
8.	*C. sasanqua*	91	147	63
9.	*C. sinensis*	77	133	63
10.	F_1 of *C. sinensis* X *C. japonica*	63	105	42
11.	*Gordonia excelsa*	49	91	28
12.	*Pyrenaria berringtoniaefolia*	42	112	42
13.	Tetraploid tea	105	119	56
14.	Triploid tea (TV 29)	98	119	49

Viability of the pollen grains stored at 20°C and three levels of R.Hs i.e., 0, 35 and 65 per cent has been presented in Table 4.4. It was observed that the pollen grains lost viability within ten days of storage at 20°C. Most of the pollen grains were infested with microbial contamination, more particularly at higher level of relative humidity. The range of viability of all the species was from three to ten days. The viability was found to be slightly more at lower (35%) relative humidity.

Table 4.4 Viability of the pollen grains stored at 20°C and various levels of relative humidities

Sl. No.	Species	Viability, in days		
		Relative humidity, %		
		0	35	65
1.	*C. assamica*	5	6	8
2.	*C. cambodiensis*	5	5	3
3.	*C. drupifera*	6	4	4
4.	*C. irrawadiensis*	5	5	5
5.	*C. japonica*	8	10	6
6.	*C. kissi*	5	5	4
7.	*C. rosaeflora*	3	3	3
8.	*C. sasanqua*	6	5	3
9.	*C. sinensis*	6	8	5
10.	F_1 of *C. sinensis* X *C. japonica*	6	8	10
11.	*Gordonia excelsa*	7	7	5
12.	*Pyrenaria berringtoniaefolia*	6	6	5
13.	Tetraploid tea	5	5	4
14.	Triploid tea (TV 29)	6	8	5

4.3 INTERSPECIFIC AND INTERGENERIC HYBRIDIZATION

4.3.1 Interspecific hybridization

Artificial (hand) pollination among the species of *Camellia* and related genera listed in Table 3.1 was done in all possible combinations. A total of 7086 flower buds involving 136 combinations were artificially pollinated during 1993-94, while during 1994-95 a total of 6109 flower buds representing 151 combinations were carried out. Details of the successful crosses–number of flower buds pollinated, development of the fruits as observed at the three monthly intervals and number of capsule harvested have been shown in Table 4.5 (1993-94) and Table 4.7 (1994-95). Artificial pollination was considered to be successful when hybrid plants were obtained from the resulting seeds. Total number of capsule harvested, seeds obtained, their nature, number of seeds germinated and the number of hybrid plants obtained during 1993-94 and 1994-95 have been presented in Table 4.6 and Table 4.8, respectively. The average per cent of fruit-set during both the years has been shown in Table 4.9. Various combinations did not produce seeds while certain others did produce seeds but failed to germinate or died just after germination considered to be unsuccessful, have been listed in Table 4.9.1. (Appendix).

Camellia and most of its allied genera required about ten months from flowering to physiological maturity and ripening of fruits. Throughout this period fruit-drop occurred. So, observations on retaining and maturity of the fruits were taken at

Table 4.5 Successful interspecific and intergeneric crosses involving *Camellia* and related genera for the year 1993-94

Sl. No	Interspecific / Intergeneric crosses	No. of flowers pollinated	No. of capsule in			No. of seed capsule harvested
			Feb.	May.	Aug.	
1.	*C. assamica* X *C. cambodiensis*	80	59	42	37	34 (42.50)*
2.	Reciprocal	81	41	33	25	23 (28.40)
3.	*C. assamica* X *C. drupifera*	78	19	13	5	3 (3.85)
4.	Reciprocal	83	20	11	9	6 (7.23)
5.	*C. assamica* X *C. irrawadiensis*	61	21	11	7	3 (4.92)
6.	Reciprocal	29	15	9	5	2 (6.90)
7.	*C. assamica* X *C. kissi*	57	23	13	5	4 (7.02)
8.	*C. assamica* X *C. sinensis*	66	41	34	30	28 (36.84)
9.	Reciprocal	92	44	36	32	32 (34.78)
10.	*C. cambodiensis* X *C. drupifera*	95	21	12	8	5 (5.26)
11.	Reciprocal	76	16	8	4	3 (3.95)
12.	*C. cambodiensis* X *C. irrawadiensis*	72	20	9	5	2 (2.78)
13.	Reciprocal	25	11	7	4	1 (4.00)
14.	*C. cambodiensis* X *C. kissi*	61	23	11	7	3 (4.92)
15.	*C. cambodiensis* X *C. sinensis*	70	29	18	14	14 (20.00)
16.	Reciprocal	78	42	30	26	25 (32.05)
17.	*C. cambodiensis* X *P. berringtoniaefolia*	101	23	17	3	2 (1.98)
18.	Reciprocal	86	11	6	4	1 (1.16)
19.	*C. drupifera* X *C. kissi*	53	16	7	3	3 (5.66)
20.	Reciprocal	33	14	5	3	3 (9.09)
21.	*C. drupifera* X *C. sinensis*	80	16	15	7	5 (6.25)

Table 4.5. (Continued) ……..

Sl. No	Interspecific / Intergeneric crosses	No. of flowers pollinated	No. of capsule in			No. of seed capsule harvested
			Feb.	May.	Aug.	
22.	Reciprocal	87	27	13	8	6 (6.90)
23.	*C. irrawadiensis* X *C. sinensis*	30	10	5	2	1 (3.33)
24.	Reciprocal	48	13	9	4	3 (6.25)
25.	*C. japonica* X *C. drupifera*	56	19	5	3	3 (5.36)
26.	*C. japonica* X *C. kissi*	50	26	12	5	2 (4.00)
27.	*C. japonica* X *C. sinensis*	66	19	10	3	2 (3.03)
28.	*C. kissi* X *C. sinensis*	46	23	8	4	3 (6.52)
29.	Reciprocal	89	22	11	5	2 (2.25)
30.	*C. rosaeflora* X *C. sinensis*	58	7	4	2	2 (3.45)
31.	*C. sasanqua* X *C. sinensis*	73	20	10	7	4 (5.48)
32.	*C. sasanqua* X Tetraploid tea	72	17	12	7	3 (4.17)
33.	*Gordonia excelsa* X *C. assamica*	45	9	7	3	3 (6.67)
34.	*G. excelsa* X *C. cambodiensis*	50	8	7	3	1 (2.00)
35.	*G. excelsa* X *C. sinensis*	42	11	5	3	3 (7.14)
36.	*P. berringtoniaefolia* X *C. assamica*	77		11	7	2 (2.60)
37.	*P. berringtoniaefolia* X *C. sinensis*	71		8	3	1 (1.41)
38.	Tetraploid tea X *C. assamica*	82	37	29	25	23 (28.05)
39.	Tetraploid tea X *C. cambodiensis*	77	37	25	18	18 (23.38)
40.	Tetraploid tea X *C. drupifera*	76	20	17	13	9 (11.84)
41.	Tetraploid tea X *C. irrawadiensis*	89	19	9	5	2 (2.25)
42.	Tetraploid tea X *C. kissi*	105	22	15	6	4 (3.81)
43.	Tetraploid tea X *C. sinensis*	64	41	35	32	31 (48.44)

* figures in parentheses indicate per cent values

Table 4.6 Details of interspecific and intergeneric hybrid seeds and plants for the year 1993-94

Sl. No	Interspecific / Intergeneric crosses	Number of				
		Capsule harvested	Total seeds	Sinker seeds	Seeds germinated	**Hybrid plants**
1.	*C. assamica* X *C. cambodiensis*	34	57	49	43	**37**
2.	Reciprocal	23	40	35	29	**22**
3.	*C. assamica* X *C. drupifera*	3	5	4	4	**4**
4.	Reciprocal	6	10	7	6	**3**
5.	*C. assamica* X *C. irrawadiensis*	3	5	5	5	**3**
6.	Reciprocal	2	5	4	4	**4**
7.	*C. assamica* X *C. kissi*	4	7	5	5	**5**
8.	*C. assamica* X *C. sinensis*	28	44	39	30	**26**
9.	Reciprocal	32	55	47	42	**37**
10.	*C. cambodiensis* X *C. drupifera*	5	10	8	8	**5**
11.	Reciprocal	3	5	3	3	**3**
12.	*C. cambodiensis* X *C. irrawadiensis*	2	5	3	3	**2**
13.	Reciprocal	1	3	1	1	**1**
14.	*C. cambodiensis* X *C. kissi*	3	5	3	2	**2**
15.	*C. cambodiensis* X *C. sinensis*	14	25	23	17	**13**
16.	Reciprocal	25	43	37	33	**28**
17.	*C. cambodiensis* X *P. berringtoniaefolia*	2	4	3	3	**3**
18.	Reciprocal	1	6	5	3	**3**
19.	*C. drupifera* X *C. kissi*	3	7	4	3	**3**
20.	Reciprocal	3	3	3	3	**2**
21.	*C. drupifera* X *C. sinensis*	5	10	7	5	**4**
22.	Reciprocal	6	10	8	8	**6**

Table 4.6. (Continued) ....

Sl. No	Interspecific / Intergeneric crosses	Number of				
		Capsule harvested	Total seeds	Sinker seeds	Seeds germin-ated	Hybrid plants
23.	*C. irrawadiensis* X *C. sinensis*	1	3	2	1	1
24.	Reciprocal	3	5	5	4	3
25.	*C. japonica* X *C. drupifera*	3	5	4	4	2
26.	*C. japonica* X *C. kissi*	2	3	2	2	1
27.	*C. japonica* X *C. sinensis*	2	3	2	2	2
28.	*C. kissi* X *C. sinensis*	3	3	3	2	2
29.	*Reciprocal*	2	3	2	2	2
30.	*C. rosaeflora* X *C. sinensis*	2	3	2	2	2
31.	*C. sasanqua* X *C. sinensis*	4	5	4	3	3
32.	*C. sasanqua* X Tetraploid tea	3	3	3	2	1
33.	*Gordonia excelsa* X *C. assamica*	3	21	19	15	4
34.	*G. excelsa* X *C. cambodiensis*	1	6		3	2
35.	*G. excelsa* X *C. sinensis*	3	24		10	2
36.	*P. berringtoniaefolia* X *C. assamica*	2	15	11	11	8
37.	*P. berringtoniaefolia* X *C. sinensis*	1	8	8	6	4
38.	Tetraploid tea X *C. assamica*	23	28	25	19	14
39.	Tetraploid tea X *C. cambodiensis*	18	23	20	18	11
40.	Tetraploid tea X *C. drupifera*	9	14	13	12	6
41.	Tetraploid tea X *C. irrawadiensis*	2	5	4	4	2
42.	Tetraploid tea X *C. kissi*	4	6	5	5	4
43.	Tetraploid tea X *C. sinensis*	31	41	35	32	30

Table 4.7 Successful interspecific and intergeneric crosses involving *Camellia* and related genera for the year 1994-95

Sl. No	Interspecific / Intergeneric crosses	No. of flowers pollinated	No. of capsule in			No. of seed capsule harvested
			Feb.	May.	Aug.	
1.	*C. assamica* X *C. cambodiensis*	63	48	39	35	32 (50.79)*
2.	Reciprocal	74	28	21	19	17 (22.97)
3.	*C. assamica* X *C. drupifera*	49	7	3	1	1 (2.04)
4.	Reciprocal	62	13	8	5	4 (6.45)
5.	*C. assamica* X *C. irrawadiensis*	40	11	6	4	2 (5.00)
6.	Reciprocal	43	17	13	6	1 (2.32)
7.	*C. assamica* X *C. japonica*	43	17	8	7	5 (11.63)
8.	*C. assamica* X C. *kissi*	34	19	13	7	7 (20.59)
9.	Reciprocal	62	9	6	4	3 (4.84)
10.	*C. assamica* X *C. sinensis*	63	35	26	23	20 (31.75)
11.	Reciprocal	73	40	36	30	28 (38.36)
12.	*C. assamica* X *P. berringtoniaefolia*	23	-	5	2	2 (8.70)
13.	Reciprocal	39	-	7	5	3 (7.69)
14.	*C. cambodiensis* X *C. drupifera*	52	14	7	3	3 (7.69)
15.	Reciprocal	60	10	7	7	7 (11.67)
16.	*C. cambodiensis* X *C. kissi*	53	21	6	6	5 (9.43)
17.	*C. cambodiensis* X *C. sasanqua*	51	15	13	10	9 (17.65)
18.	Reciprocal	54	4	3	1	1 (1.85)
19.	*C. cambodiensis* X *C. sinensis*	62	27	23	19	14 (22.58)
20.	Reciprocal	64	30	22	19	17 (26.56)
21.	*C. cambodiensis* X *G. excelsa*	40	8	5	3	3 (7.50)
22.	*C. cambodiensis* X *P. berringtoniaefolia*	17	-	3	2	1 (5.88)
23.	Reciprocal	32	-	4	2	2 (6.25)
24.	*C. drupifera* X *C. irrawadiensis*	53	13	9	6	5 (9.43)
25.	*C. drupifera* X *C. kissi*	61	17	12	10	10 (16.39)
26.	Reciprocal	44	9	6	5	5 (11.36)

Table 4.7 (Continued) ....

Sl. No	Interspecific / Intergeneric crosses	No. of flowers pollinated	No. of capsule in			No. of seed capsule harvested
			Feb.	May.	Aug.	
27.	*C. drupifera* X *C. sinensis*	48	11	9	5	5 (10.42)
28.	Reciprocal	40	7	5	5	5 (12.50)
29.	*C. drupifera* X *G. excelsa*	43	5	2	1	1 (2.33)
30.	*C. japonica* X *C. drupifera*	17	3	1	1	1 (5.88)
31.	*C. japonica* X *C. sinensis*	28	3	2	2	2 (7.14)
32.	Reciprocal	64	10	5	3	3 (4.69)
33.	*C. kissi* X *C. sinensis*	44	2	2	2	2 (4.55)
34.	Reciprocal	50	10	5	5	4 (8.00)
35.	*C. rosaeflora* X *C. sasanqua*	31	2	2	1	1 (3.23)
36.	*C. sasanqua* X *C. assamica*	37	8	6	3	3 (8.11)
37.	*C. sasanqua* X *C. drupifera*	42	5	5	4	2 (4.76)
38.	*C. sasanqua* X *C. kissi*	60	4	4	4	4 (6.67)
39.	*C. sasanqua* X *C. sinensis*	55	7	3	2	2 (3.64)
40.	Reciprocal	80	13	7	5	5 (6.25)
41.	*C. sasanqua* X Tetraploid tea	64	7	3	3	3 (4.69)
42.	*C. sinensis* X *C. irrawadiensis*	67	5	1	1	1 (1.49)
43.	*C. sinensis* X *P. berringtoniaefolia*	53	-	8	4	3 (5.66)
44.	Reciprocal	47	-	1	1	1 (2.13)
45.	*G. excelsa* X *C. assamica*	79	4	4	2	1 (1.27)
46.	*G. excelsa* X *C. sinensis*	64	6	3	3	3 (4.69)
47.	Tetraploid tea X *C. assamica*	55	33	21	16	14 (25.45)
48.	Tetraploid tea X *C. cambodiensis*	52	37	19	11	11 (21.15)
49.	Tetraploid tea X *C. drupifera*	59	9	6	5	5 (8.47)
50.	Tetraploid tea X *C. irrawadiensis*	42	17	9	2	2 (4.76)
51.	Tetraploid tea X *C. japonica*	38	5	3	2	2 (5.26)
52.	Tetraploid tea X *C. kissi*	35	8	4	2	2 (5.71)
53.	Tetraploid tea X *C. sinensis*	48	30	24	20	20 (41.67)

* Figures in parentheses indicate per cent values.

Table 4.8 Details of interspecific and intergeneric hybrid seeds and plants for the year 1994-95

Sl. No	Interspecific / Intergeneric crosses	Number of				
		Capsule harvested	Total seeds	Sinker seeds	Seeds germin-ated	Hybrid plants
1.	*C. assamica* X *C. cambodiensis*	32	53	46	34	29
2.	Reciprocal	17	24	21	17	13
3.	*C. assamica* X *C. drupifera*	1	2	2	1	1
4.	Reciprocal	4	5	4	3	2
5.	*C. assamica* X *C. irrawadiensis*	2	3	3	3	2
6.	Reciprocal	1	3	1	1	0
7.	*C. assamica* X *C. japonica*	5	8	7	6	5
8.	*C. assamica* X *C. kissi*	7	11	10	9	9
9.	Reciprocal	3	3	2	2	0
10.	*C. assamica* X *C. sinensis*	20	25	20	18	17
11.	Reciprocal	28	36	30	25	17
12.	*C. assamica* X *C. berringtoniaefolia*	2	3	3	2	1
13.	Reciprocal	3	21	20	13	8
14.	*C. cambodiensis* X *C. drupifera*	3	5	4	4	2
15.	Reciprocal	7	10	6	3	1
16.	*C. cambodiensis* X *C. kissi*	5	8	7	4	2
17.	*C. cambodiensis* X *C. sasanqua*	9	13	11	10	7
18.	Reciprocal	1	2	2	1	0
19.	*C. cambodiensis* X *C. sinensis*	14	20	15	14	13
20.	Reciprocal	17	25	23	20	19
21.	*C. cambodiensis* X *G. excelsa*	3	4	2	2	0
22.	*C. cambodiensis* X *C. berringtoniaefolia*	1	2	2	2	1
23.	Reciprocal	2	13	13	10	6
24.	*C. drupifera* X *C. irrawadiensis*	5	7	4	3	3
25.	*C. drupifera* X *C. kissi*	10	13	7	5	3
26.	Reciprocal	5	4	4	3	1

Table 4.8 (Continued) ....

Sl. No	Interspecific / Intergeneric crosses	Number of				
		Capsule harvested	Total seeds	Sinker seeds	Seeds germin-ated	Hybrid plants
27.	*C. drupifera* X *C. sinensis*	5	9	4	4	1
28.	Reciprocal	1	2	2	1	0
29.	*C. drupifera* X *G. excelsa*	1	1	1	1	0
30.	*C. japonica* X *C. drupifera*	1	2	1	0	0
31.	*C. japonica* X *C. sinensis*	2	3	2	1	0
32.	Reciprocal	3	4	2	1	1
33.	*C. kissi* X *C. sinensis*	2	2	1	1	0
34.	Reciprocal	4	5	4	3	1
35.	*C. rosaeflora* X *C. sasanqua*	1	2	1	1	1
36.	*C. sasanqua* X *C. assamica*	3	4	2	1	1
37.	*C. sasanqua* X *C. drupifera*	2	2	1	1	0
38.	*C. sasanqua* X *C. kissi*	4	5	3	2	1
39.	*C. sasanqua* X *C. sinensis*	2	2	1	1	0
40.	Reciprocal	5	4	3	2	1
41.	*C. sasanqua* X Tetraploid tea	3	4	3	2	1
42.	*C. sinensis* X *C. irrawadiensis*	1	2	2	1	1
43.	*C. sinensis* X *C. berringtoniaefolia*	3	4	3	2	2
44.	Reciprocal	1	5	4	3	1
45.	*G. excelsa* X *C. assamica*	1	9	-	3	2
46.	*G. excelsa* X *C. sinensis*	3	21	-	8	3
47.	Tetraploid tea X *C. assamica*	14	23	21	15	13
48.	Tetraploid tea X *C. cambodiensis*	11	15	10	6	4
49.	Tetraploid tea X *C. drupifera*	5	6	4	4	2
50.	Tetraploid tea X *C. irrawadiensis*	2	2	2	1	1
51.	Tetraploid tea X *C. japonica*	2	2	2	2	1
52.	Tetraploid tea X *C. kissi*	2	3	3	3	1
53.	Tetraploid tea X *C. sinensis*	20	16	15	12	9

Table 4.9. Per cent fruit-set in interspecific and intergeneric crosses during 1993-94, 1994-95 and their average

Sl. No	Cross	Fruit set, %		
		1993-94	1994-95	Average
1	*C. assamica* X *C. cambodiensis*	32.05	26.56	29.31
2.	Reciprocal	28.40	22.97	25.69
3.	*C. assamica* X *C. drupifera*	3.85	2.04	2.95
4.	Reciprocal	6.45	7.00	6.84
5.	*C. assamica* X *C. irrawadiensis*	0.92	5.00	4.96
6.	Reciprocal	6.90	2.32	4.61
7.	*C. assamica* X *C. japonica*	-	11.63	11.63
8.	*C. assamica* X *C. kissi*	7.02	20.59	13.81
9.	Reciprocal	-	4.84	4.84
10.	*C. assamica* X *C. sinensis*	36.84	31.75	34.30
11.	Reciprocal	42.50	50.79	46.65
12.	*C. assamica* X *P. berringtoniaefolia*	-	8.70	8.70
13.	Reciprocal	2.60	7.69	5.15
14.	*C. cambodiensis* X *C. drupifera*	5.26	5.77	5.52
15.	Reciprocal	3.95	11.67	7.81
16.	*C. cambodiensis* X *C. irrawadiensis*	2.78	-	2.78
17.	Reciprocal	4.00	-	4.00
18.	*C. cambodiensis* X *C. kissi*	4.92	9.43	7.18
19.	*C. cambodiensis* X *C. sinensis*	20.00	22.58	21.29
20.	Reciprocal	34.78	38.36	36.57
21.	*C. cambodiensis* X *G. excelsa*	-	7.50	7.50
22.	Reciprocal	2.00	-	2.00
23.	*C. cambodiensis* X *P. berringtoniaefolia*	1.98	5.88	3.93
24.	Reciprocal	1.16	6.25	3.71
25.	*C. drupifera* X *C. irrawadiensis*	-	9.43	9.43
26.	*C. drupifera* X *C. kissi*	5.66	16.39	11.03
27.	Reciprocal	9.09	11.36	10.23
28.	*C. drupifera* X *C. sinensis*	6.25	10.42	8.34
29.	Reciprocal	6.90	12.50	9.70
30.	*C. drupifera* X *G. excelsa*	-	2.33	2.33
31.	*C. irrawadiensis* X *C. sinensis*	3.33	-	3.33

Table 4.9. (Continued) ....

Sl. No	Cross	Fruit set, %		
		1993-94	1994-95	Average
32.	Reciprocal	6.25	1.49	3.87
33.	*C. japonica* X *C. drupifera*	5.36	5.88	5.62
34.	*C. japonica* X *C. kissi*	4.00	-	4.00
35.	*C. japonica* X *C. sinensis*	3.03	7.14	5.09
36.	Reciprocal	-	4.69	4.69
37.	*C. kissi* X *C. sinensis*	6.52	4.55	5.54
38.	Reciprocal	2.25	8.00	5.13
39.	*C. rosaeflora* X *C. sasanqua*	-	3.23	3.23
40.	*C. rosaeflora* X *C. sinensis*	3.45	-	3.45
41.	*C. sasanqua* X *C. assamica*	-	1.85	1.85
42.	*C. sasanqua* X *C. cambodiensis*	-	1.85	1.85
43.	*C. sasanqua* X *C. drupifera*	-	4.76	4.76
44.	*C. sasanqua* X *C. kissi*	-	6.67	6.67
45.	*C. sasanqua* X *C. sinensis*	53.48	3.64	4.56
46.	Reciprocal	-	6.25	6.25
47.	*C. sasanqua* X Tetraploid tea	4.17	4.69	4.43
48.	*C. sinensis* X *P. berringtoniaefolia*	-	5.66	5.66
49.	Reciprocal	1.41	2.13	1.77
50.	*G. excelsa* X *C. assamica*	6.67	1.27	3.97
51.	*G. excelsa* X *C. sinensis*	7.14	5.69	5.92
52.	Tetraploid tea X *C. assamica*	28.05	25.45	26.75
53.	Tetraploid tea X *C. cambodiensis*	23.38	21.15	22.27
54.	Tetraploid tea X *C. drupifera*	11.84	8.47	10.16
55.	Tetraploid tea X *C. irrawadiensis*	2.25	4.76	3.51
56.	Tetraploid tea X *C. japonica*	-	5.26	5.26
57.	Tetraploid tea X *C. kissi*	3.81	5.71	4.76
58.	Tetraploid tea X *C. sinensis*	32.81	41.67	37.24

PLATE – 6

Photographs of interspecific hybrids

A. *C. assamica* X *C. drupifera*

B. *C. assamica* X *C. irrawadiensis*

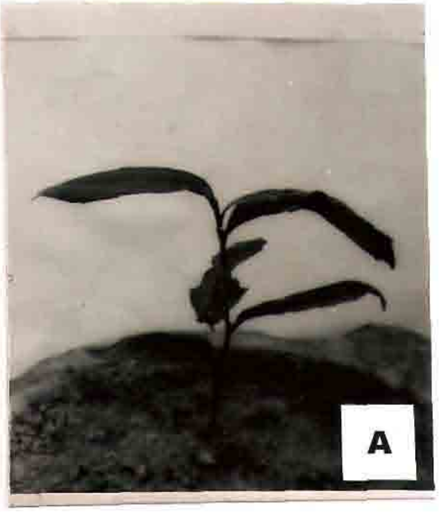
C. *C. cambodiensis* X *C. drupifere*

D. *C. cambodiensis* X *C. irrawadiensis*

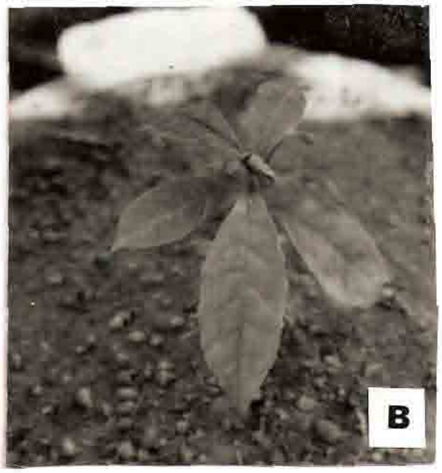
E. *C. drupifera* X *C. assamica*

F. *C. drupifera* X *C. cambodiensis*

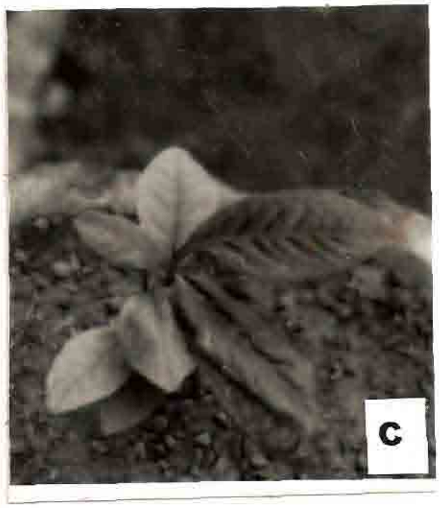
PLATE 6



A



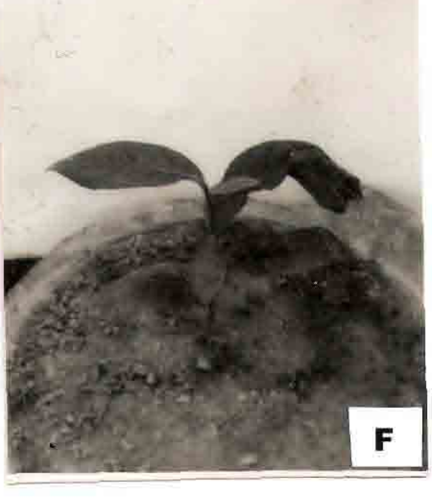
B



C

D

E



F

three monthly intervals. In general, first recognizable morphological sign of development of fruit after pollination could be observed after about 30 days when the ovary was surrounded by persistent sepal and the dried stigmatic lobe still remained attached. However, first sign of fertilization did not reflect the success of hybridization at maturity because of fruit drop.

Out of 136 combinations including reciprocals attempted during 1993-94 (Table 4.5), only 43 combinations were observed to be compatible from which 330 capsules (11.30%) out of 2920 pollinations were harvested. In all the cases, observations were made at three monthly intervals from pollination. In February, except four combinations, 952 flowers showed development of fruit (32.60%), which reduced to 614 (21.03%) in May. However, only 401 capsules physiologically matured and were retained in August representing a success of 13.73 per cent.

In the individual combinations, crosses involving tea species showed a success up to 42.50 per cent than non-tea species. Among the three tea species, China tea, *C. sinensis* was found to be better female parent than Assam tea, *C. assamica* and Cambod tea, *C. cambodiensis*. Among the crosses involving Assam tea as the seed bearer and non-tea species as pollen parent, *C. kissi* was observed to be the best with a fruit set of 7.02 per cent. However, in reciprocal crosses with Assam tea as pollen parent, *C. drupifera* was better seed parent than *C. irrawadiensis* and *C. kissi*. With *C. irrawadiensis* as a pollen parent, all the three tea species produced seeds (2.78 to 6.25%).

PLATE – 7

Photographs of interspecific hybrids

A. *C. drupifera* X *C. irrawadiensis*

B. *C. drupifera* X *C. kissi*

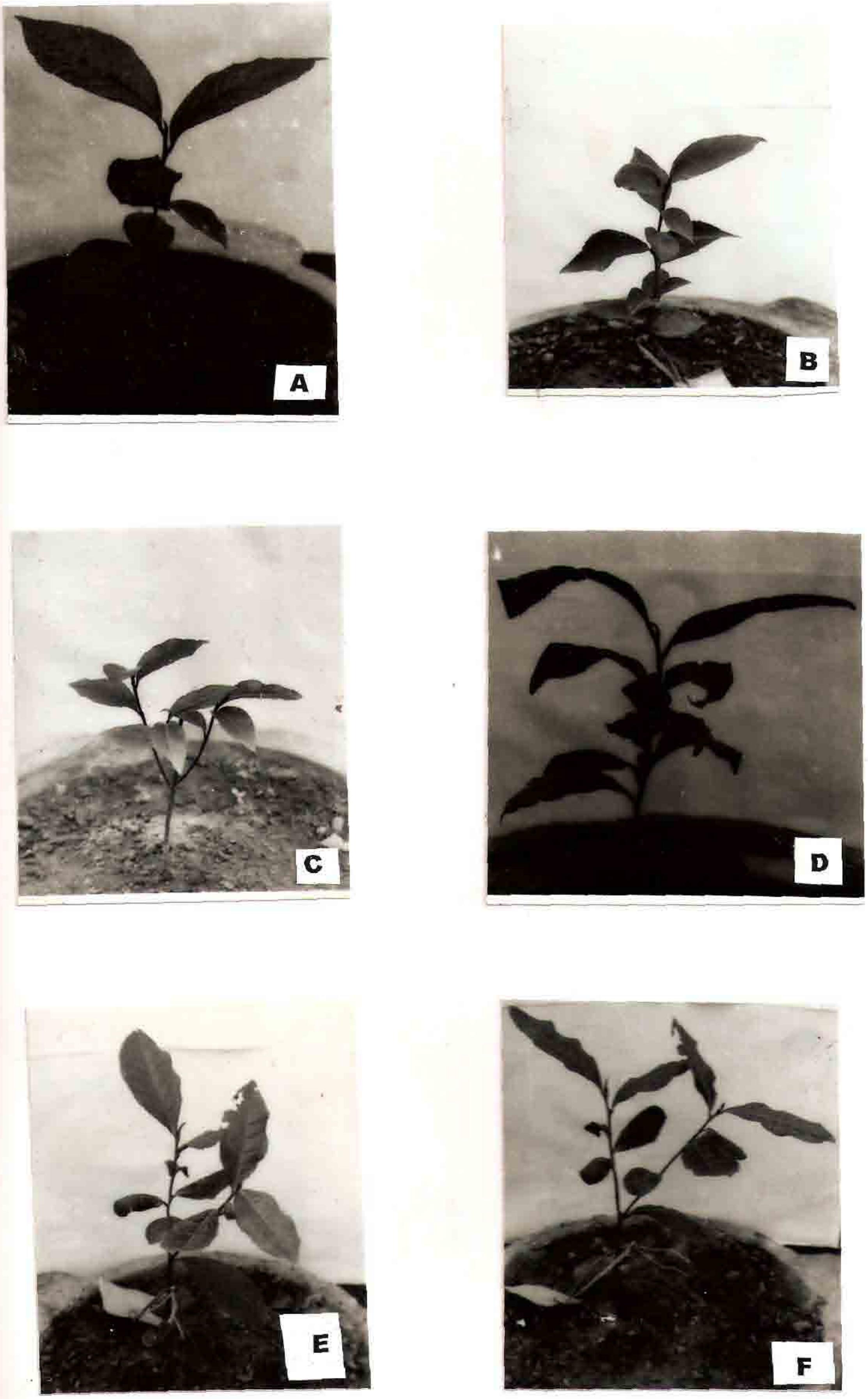
C. *C. drupifera* X *C. sinensis*

D. *C. irrawadiensis* X *C. assamica*

E. *C. irrawadiensis* X *C. cambodiensis*

F. *C. irrawadiensis* X *C. sinensis*

PLATE 7



In the reciprocal crosses, *C. assamica* proved to be better seed parent (6.90%) than *C. cambodiensis* (4.00%) and *C. sinensis* (3.33%). This was the first successful report to obtain hybrids using *C. irrawadiensis* as female parent. During 1993-94, five species were found to be compatible with *C. assamica* as seed parent. All of which except *C. kissi* were compatible with *C. assamica* in reciprocal crosses. However, during 1994-95 two more species, *C. japonica* and *P. herringtoniaefolia* along with reciprocal cross with *C. kissi* were successful (Table 4.7). Reciprocal crosses with *C. japonica* as female parent did not produce any seed.

Out of twelve species tried as male parent on to *C. cambodiensis* as seed parent only five species of *Camellia* and two species of related genera *viz.*, *Gordonia excelsa* and *Pyrenaria berringtoniaefolia* yielded seeds. All of them except *C. kissi* were successful in reciprocal crosses. With *C. cambodiensis* also, *C. kissi* was found to be better pollen parent than other species.

Besides three tea species, *C. irrawadiensis, C. kissi* and *Gordonia excelsa* were found to be compatible with *C. drupifera* as female parent. However, only *C. kissi* was successful in reciprocal cross, with an average fruit-set of 11.03 per cent and 10.23 per cent, respectively.

All the three tea species were observed to produce hybrid plants from seeds obtained from interspecific crosses with *C. irrawadiensis* as female parent. *C. assamica* was slightly better (4.61%) than *C. cambodiensis* (4.00%) and *C. sinensis* (3.33%). All

PLATE – 8

Photographs of interspecific hybrids

A. *C. japonica* X *C. sinensis*

B. *C. kissi* X *C. drupifera*

C. *C. kissi* X *C. sinensis*

D. *C. sasanqua* X *C. drupifera*

E. *C. sasanqua* X *C. kissi*

F. *C. sasanqua* X *C. sinensis*

PLATE 8

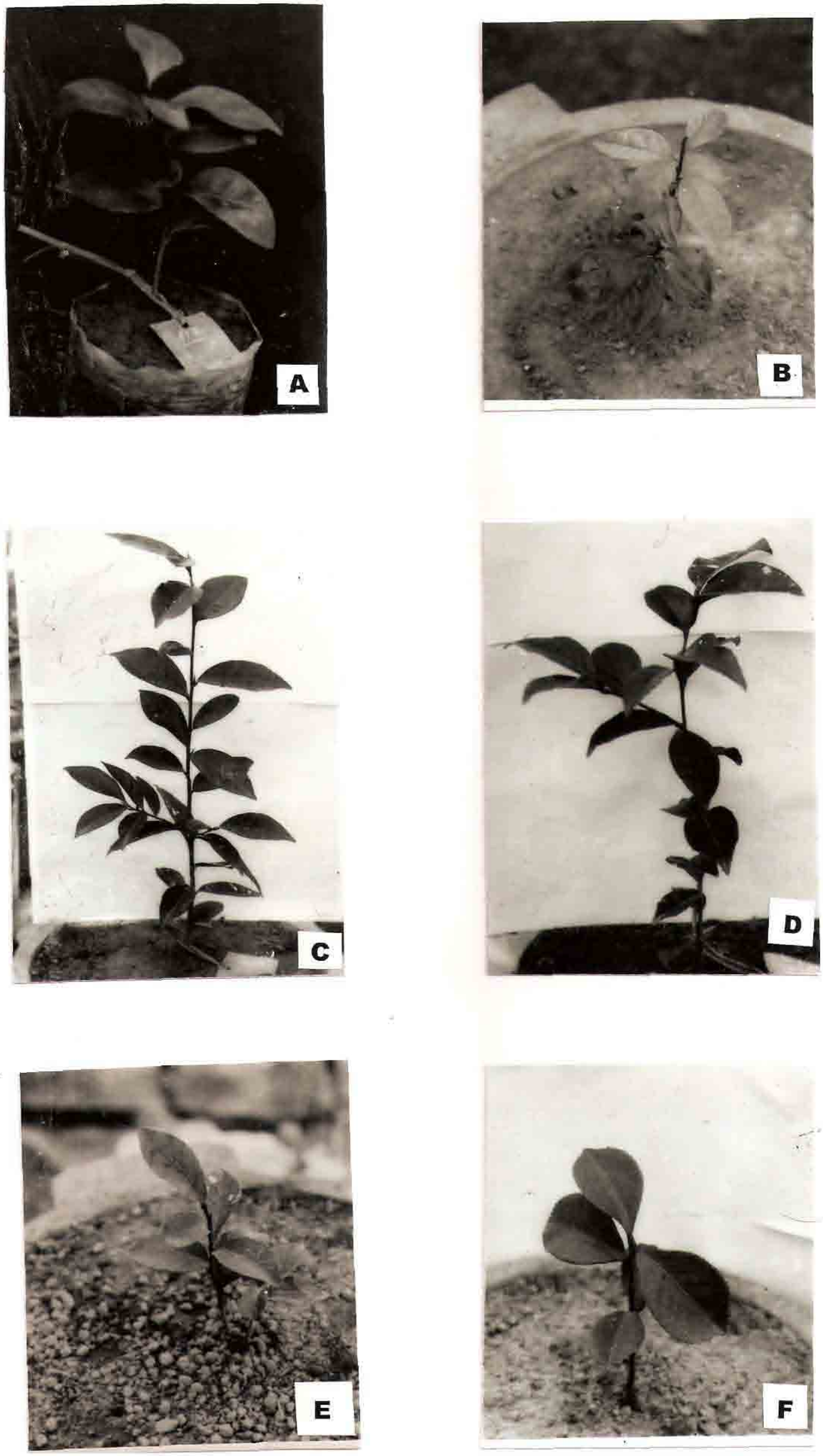


PLATE – 9

Photographs of interspecific hybrids

A. *C. sinensis* X *C. drupifera*

B. *C. sinensis* X *C. kissi*

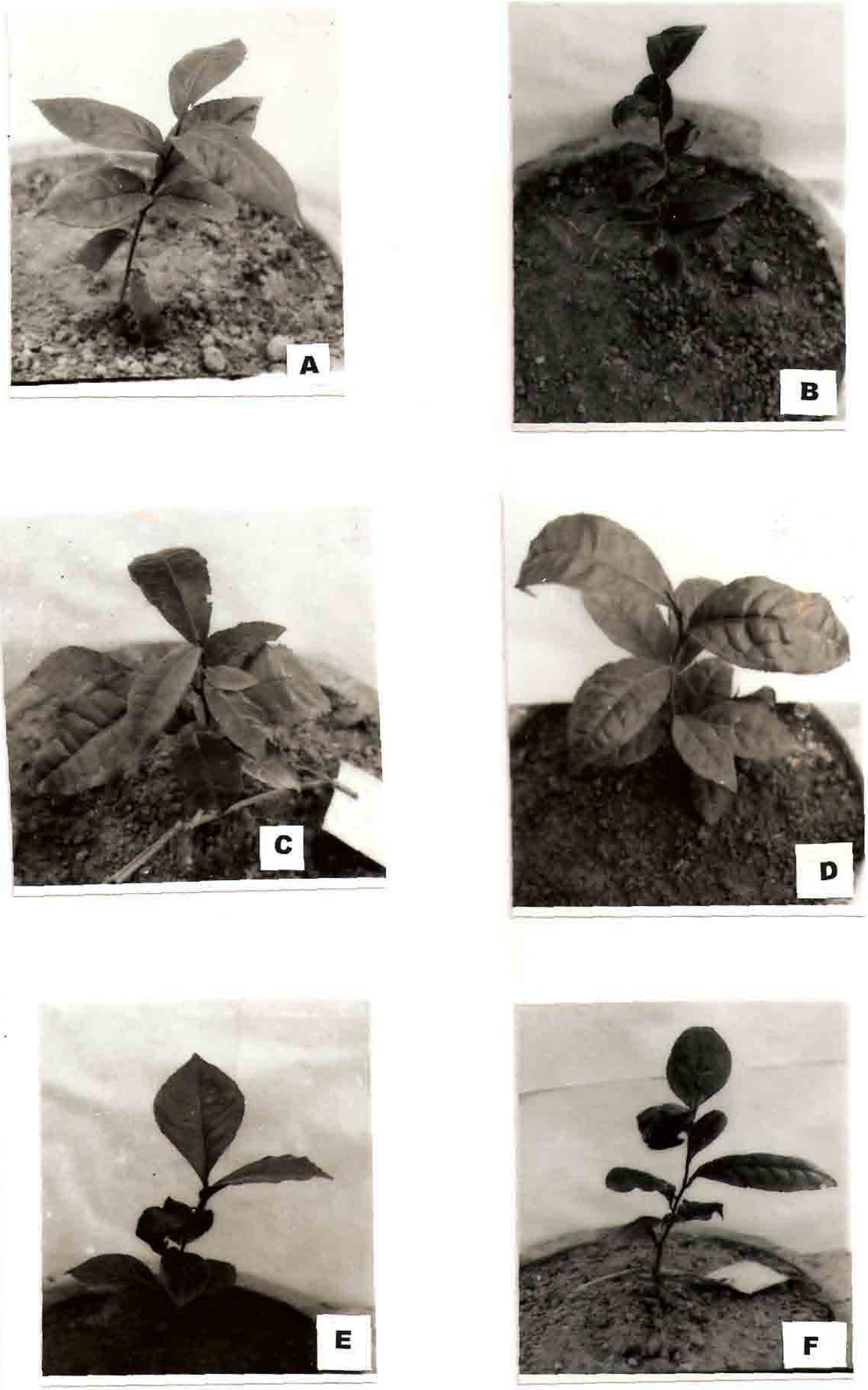
C. Tetraploid tea X *C. assamica*

D. Tetraploid tea X *C. cambodiensis*

E. Tetraploid tea X *C. drupifera*

F. Tetraploid tea X *C. irrawadiensis*

PLATE 9



other species of *Camellia, Gardonia* and *Pyrenaria* were incompatible with *C. irrawadiensis*. When *C. irrawadiensis* was used as pollen parent, *C. drupifera* and tetraploid strain of *C. sinensis* along with three tea species yielded seed.

C. drupifera, C. kissi and *C. sinensis* pollen when pollinated on *C. japonica* produced hybrid seeds. Two other tea species, *C. assamica* and *C. cambodiensis* failed to fertilize *C. japonica*. *C. japonica* was also very poor male parent. The pollen grains of *C. japonica* could fertilize and produced seeds only when crossed with *C. assamica* (11.63%), *C. sinensis* (4.69%) and tetraploid tea species (3.51%).

In the case of *C. kissi*, two tea species, *C. assamica* and *C. sinensis* and only one non-tea species. *C. drupifera* were successful in producing hybrid seeds. All these species produced seeds in forward and reciprocal crosses. Other species, which yielded seed when pollinated with the pollen grains of *C. kissi* were *C. sasanqua, C. cambodiensis, C. japonica* and tetraploid taxon of tea.

C. rosaeflora, which was a triploid species, produced seeds only when pollinated with *C. sinensis* (3.45%) and *C. sasanqua* (3.23%).

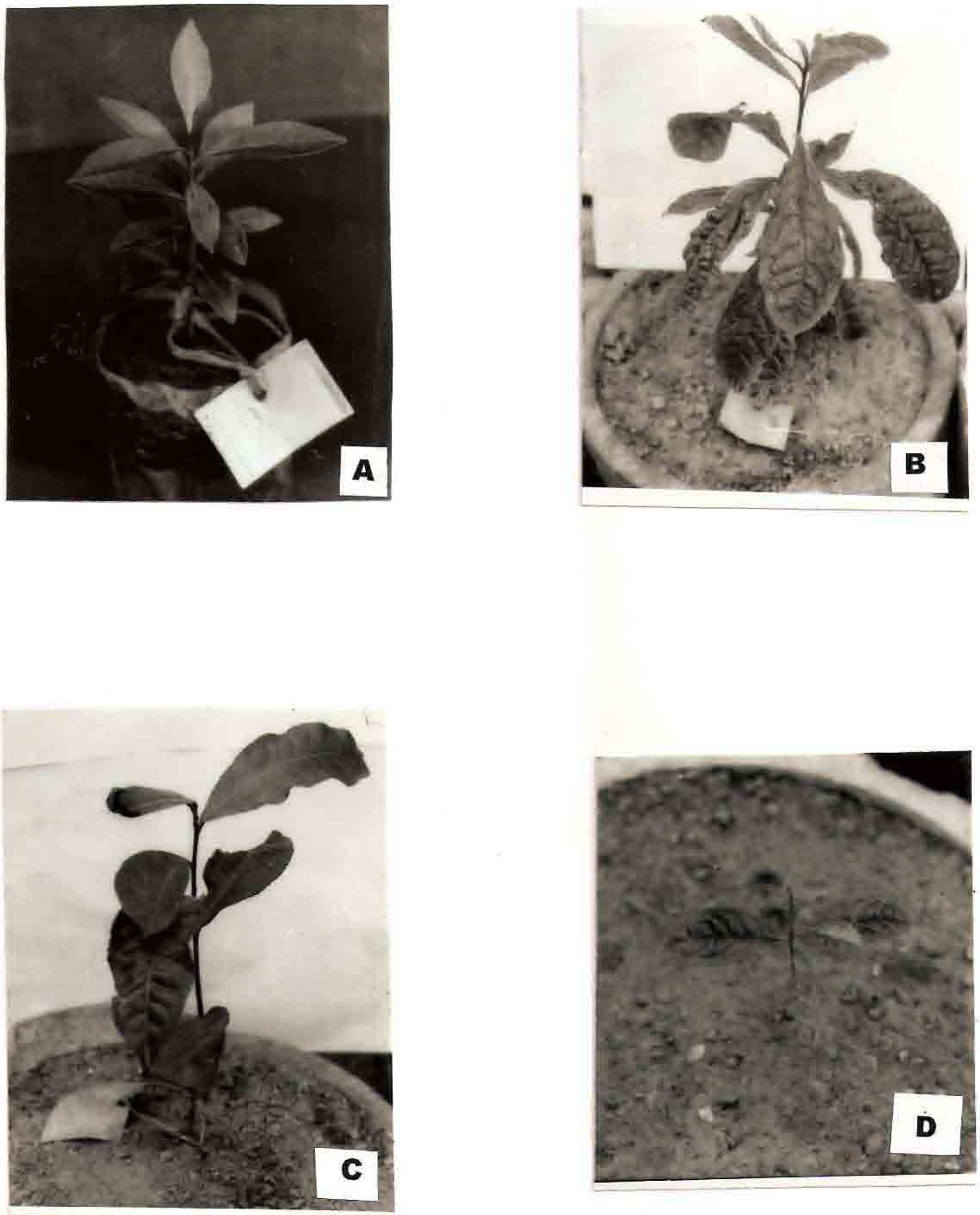
Besides all the three species of tea, *viz., C. assamica, C. cambodiensis* and *C. sinensis, C. drupifera* and *C. kissi* were found to be compatible with *C. sasanqua* as seed parent. However, crosses with only *C. sinensis* were successful in both the directions. Among the tea species, Assam tea, *C. assamica* set 8.11 per cent of fruits while Cambod tea, *C. cambodiensis* and China tea, *C. sinensis* produced 1.85 per cent and 3.64 per cent

PLATE – 10

Photographs of intergeneric hybrids

- A. *G. excelsa* X *C. assamica*
- B. *P. berringtoniaefolia* X *C. assamica*
- C. *P. berringtoniaefolia* X *C. cambodiensis*
- D. *P. berringtoniaefolia* X *C. sinensis*

PLATE 10



of fruit-set, respectively, when *C. sasanqua* was used as seed parent. Other two species, *viz.*, *C. drupifera* and *C. kissi* yielded 4.76 per cent and 6.67 per cent fruits, respectively.

As seed parent, *C. sinensis* was observed to be the best among all the species studied. It combined well and produced hybrid seeds with *C. assamica* (46.65%), *C. cambodiensis* (36.57%), *C. drupifera* (9.70%), *C. irrawadiensis* (3.87%), *C. japonica* (4.69%), *C. kissi* (5.13%), *C. sasanqua* (6.25%) and only one species of related genera *P. berringtoniaefolia* (5.66%).

In general, it was observed that the compatibility was usually greater among the species within a subgenus as compared to the species between two subgenera.

4.3.2 INTERGENERIC HYBRIDIZATION

Intergeneric hybridization was attempted among the species of *Camellia, Gordonia* and *Pyrenaria* (Tables 4.5 to 4.9). *Gordonia excelsa* was found to be compatible with all the three tea species only when it was used as female parent. In reciprocal crosses, only *C. cambodiensis* when pollinated with the pollen grains of *G. excelsa* produced (7.50%) seed capsule. *Pyrenaria berringtoniaefolia* produced hybrid seed with all the three tea species *viz.*, *C. assamica* (5.15%), *C. cambodiensis* (3.71%) and *C. sinensis* (1.77%). Reciprocal crosses were also successful. When used as male or pollen parent on to *C. assamica, P. berringtoniaefolia* produced 8.70 per cent fruit, while as a seed bearer the later produced 7.69 per cent fruit. These results indicated that *P.*

herringtoniaefolia was more closely related to *C. assamica* than to *C. cambodiensis* (3.93%) and *C. sinensis* (5.66%).

4.4 RECEPTIVITY OF STIGMA

To find out the duration for which the stigma of various species of *Camellia* and related genera remain most receptive, all the species, except *C. sinensis* which was pollinated with *C. assamica*, were pollinated with the pollen grains of China tea, C. *sinensis* which was found to be more compatible then other species. Preliminary observation made on the stage of flower bud reflected from the size, colour and retention of ovary after pollination revealed that the germination of pollen grains on the stigmatic surface and elongation of pollen tube was better from 3rd day prior to anthesis to the day of anthesis and pollination done from 8.30 a.m. to 1.00 p.m. when pollination was done earlier than 3rd day before anthesis, all flower buds dropped off along with the corolla and stamens. Therefore, observations were made on the number of ovaries retained and continued to develop after 15 and 30 days after pollination done on 20 flower buds from 3 days prior to anthesis to the day of anthesis from 9.00 a.m. to 11.30 a.m. The results have been presented in Table 4 .10.

It was observed that only a few ovaries were retained and continued to enlarge in size on 15th and 30th day after pollination, done on the third day prior to anthesis. 60 to 100 per cent of the pistil withered and dropped off along with the corolla and stamens. The percentage drop of the pistils reduced in almost all the species when pollinated on the second day prior to anthesis. On the other hand, when pollination was

Table 4.10. Observation on developing ovaries for stigmatic receptivity

Sl. No	Species	Days after pollination	No. of ovaries retained			
			Pollinated on (days before anthesis)			
			3	2	1	0
1.	*C. assamica*	15	3 (15)*	4 (20)	14 (70)	17 (85)
		30	0	1 (5)	10 (50)	13 (65)
2.	*C. cambodiensis*	15	0	2 (10)	13 (65)	14 (70)
		30	0	0	11 (55)	11 (55)
3.	*C. drupifera*	15	3 (15)	5 (25)	11 (55)	12 (60)
		30	(5)	1 (5)	10 (50)	8 (40)
4.	*C. irrawadiensis*	15	0	0	6 (30)	7 (35)
		30	0	0	2 (10)	2 (10)
5.	*C. japonica*	15	4 (20)	3 (15)	8 (40)	6 (30)
		30	0	1 (5)	2 (10)	4 (20)
6.	*C. kissi*	15	2 (10)	3 (15	10 (50)	11 (55)
		30	0	2 (10)	7 (35)	9 (45)
7.	*C. rosaeflora*	15	0	0	2 (10)	1 (5)
		30	0	0	0	0
8.	*C. sasanqua*	15	5 (25)	7 (35)	12 (60)	13 (65)
		30	3 (15)	5 (25)	10 (50)	8 (40)
9.	*C. sinensis*	15	5 (25)	6 (30)	15 (75)	16 (80)
		30	3 (15)	4 (20)	13 (65)	12 (60)
10.	*C. sineisis* X *C. japonica*	15	0	0	0	0
		30	0	0	0	0
11.	*G. excelsa*	15	0	1 (5)	2 (10)	3 (15)
		30	0	0	2 (10)	3 (15)
12.	*P. berringtoniaefolia*	15	0	4 (20)	4 (20)	5 (25)
		30	0	2 (10)	2 (10)	2 (10)
13.	Tetraploid tea	15	4 (20)	8 (40)	17 (85)	16 (80)
		30	2 (10)	6 (30)	12 (60)	11 (55)

* all the species except *C. sinensis* which was pollinated with *C. assamica,* were pollinated with *C. sinensis* pollen.

done on one day prior to anthesis, the retention of pistil increased considerably. Up to 85 per cent of the ovaries retained in case of tetraploid tea, while all the 20 pollinated buds dropped off on *C. rosaeflora* and the F1 hybrids of *C. sinensis* X *C. japonica*. In other tea species, the retention percentage of developing ovaries varied from 50 on the 30th day after pollination on *C. assamica* to 75 per cent on the 15th day after pollination on *C. sinensis*. Among the non-tea species, maximum number (55%) of growing ovaries were recorded on *C. drupifera* on the 15th day after pollination and minimum number (only 10%) was recorded on *C. irrawadiensis*, *C. japonica*, *C. rasaeflora*, *G. excelsa* and *P. herringtoniaefolia*. The percentage of retention of developing ovaries in all the species except two sterile species was maximum when pollinated on the day of anthesis. Microscopic observation of the free hand sections of the style just above the ovary when they dropped off after 24, 48 or 72 hours after pollination revealed that the pollen tube failed to grow up to that point.

From the forgoing results it could be concluded that all the species were protogynous and the receptivity of stigma was at their peak from 8.30 a.m. to 11.30 a.m. on 24 hours prior to and on the day of anthesis. For obtaining hybrid seeds, the female flowers should be pollinated with maximum percentage of viable pollen grains after emasculating them on those days only. Otherwise either the pollen grains would not germinate or the germination and pollen tube growth would be so slow that the flower wither and fall off before they enter the embryo-sac and fertilize the egg cell.

4.5 SELF-INCOMPATIBILITY AND ITS MECHANISM

In order to study the mechanism of self-incompatibility in various species of *Camellia* and related genera, all the species under investigation were artificially selfed. A total of 400 previously bagged flower buds from each of the ten species, where sufficient number of blooms were available, were artificially pollinated on the day of anthesis with the pollen grains from bagged flowers of the same plant which had anthesis on the previous day, and immediately bagged. In other species, 200 flower buds in the case of *C. drupifera* and 100 each in *C. irrawadiensis, C. japonica* and *C. kissi* could be selfed. Details of the number of capsule harvested, number of seeds obtained, sinker seeds, number of seeds germinated and progenies survived for more than three months have been shown in Table 4.11.

There was considerable variation among the species in fruit-set and maturity of fruits resulting from selfing (Table 4.11). Highest percentage (10.00%) of fruit-set was obtained in *C. drupifera*, followed by *C. sinensis* (7.75%), whereas, a few species, *viz.*, *C. irrawadiensis*, *C. rosaeflora* and F_1 hybrids of *C. sinensis* X *C. japonica* were completely self-sterile. Among the three tea species, *C. cambodiensis* had more prominent self-incompatibility (2.25% fruit-set) as compared to *C. assamica* (5.00%) and *C. sinensis* (7.75%). In the case of tetraploid tea, out of a total of 400 flower buds artificially selfed, only 14 fruits, representing a success percentage of 3.50 per cent matured. Among the non-tea *Camellia* species, *C. japonica, C. kissi* and *C. sasanqua* yielded a fruit-set of 2.00, 3.00 and 4.25 per cent, respectively. Among the species of three related genera, *Eurya* did not set any fruit as the flowers were imperfect or pistillate flower without any sign of stamen. This species was found to be dioecious. While other two species, *Gordonia* and *Pyrenaria*, set 1.25 and 1.00 per cent fruits, respectively.

Table 4.11. Details of artificial selfing in various species of *Camellia* and related genera

Sl. No	Species	No. of flower buds pollinated	No. of capsule harvested	Number of			
				Total seeds	Sinker seeds	Seeds geminated	Plants
1.	*C. assamica*	400	20 (5.00)*	27	11	8	5
2.	*C. cambodiensis*	400	9 (2.25)	20	8	6	3
3.	*C. drupifera*	200	20 (10.00)	23	9	3	2
4.	*C. irrawadiensis*	100	0	0	0	0	0
5.	*C. japonica*	100	2 (2.00)	2	0	0	0
6.	*C. kissi*	100	3 (3.00)	3	2	2	0
7.	*C. rosaeflora*	400	0	0	0	0	0
8.	*C. sasanqua*	400	17 (4.25)	26	10	4	1
9.	*C. sinensis*	400	31 (7.75)	57	27	16	9
10.	*C. sinensis* X *C. japonica*	400	0	0	0	0	0
11.	*Gordonia excelsa*	400	5 (1.25)	34	7	3	0
12.	*Pyrenaria berringtoniaefolia*	400	4 (1.00)	21	10	6	2
13.	Tetraploid tea	400	14 (3.50)	29	17	10	8
	Total	4,500	125 (2.78)	242	101	58	30

* Figures in parentheses indicate per cent values.

Most of the seeds obtained by selfing were either partially developed or empty, without any cotyledon and embryo, so they were floaters. In some cases, the sinker seeds were also deformed, which reduced the germinability (23.97%) to a considerable extent. In total, 30 plantlets were obtained from the seeds resulting from selfing. Again, most of the seedlings were very stunted. The leaves and internodes were extremely small. However, there were considerable variation and segregation of characters among the seedlings or selfed progeny of a particular species. A few seedlings resulted from *C. drupifera*, *C. cambodiensis*, *G. excelsa* and *P. berringtoniaefolia* were merely surviving up to three months, while others died just a few weeks after germination.

Anatomical observation to ascertain the nature of incompatibility mechanism revealed that the pollen grains of all the species germinated normally on the selfed stigma. In general, germination and the rate of elongation of the pollen tubes in the style canal was slower than the crossed pollen tubes (Table 4.12). After traversing up to half to two third of the length of the style (0.73 to 6.2 mm), the tip of the pollen tubes of *C. irrawadiensis*, *C. rosaeflora* and F_1 of *C. sinensis* X *C. japonica*, became swollen like a bulb and further elongation was stopped. The pollen tubes did not enter the embryo-sac. In some cases, they did not enter the micropyler end and could not fertilize the egg nucleus. In some other, the growth was irregular and deposition of callose tissues was observed, which also resulted in the failure of the tube along with the tube nucleus to enter and fertilize the ovary. However, the ovary did not wither and drop off. In *C. japonica* and *C. sasanqua*, they enlarged like fertilized ovaries, which might result in the seeds without embryo and cotyledon or there might be development of seeds apomictically.

Table 4.12. Length of pollen tube in the style canal after 18 hours of artificial selfing

Sl. No.	Species	Length of pollen tube, mm					
		Replication					
		I	II	III	IV	V	Average
1.	*C. assamica*	8.9	10.0	8.6	9.0	8.5	9.0
2.	*C. cambodiensis*	9.3*	10.1	9.3	9.1	8.7	9.3
3.	*C. drupifera*	4.9	4.6	3.8	5.2	4.7	4.64
4.	*C. irrawadiensis*	8.8	8.4	6.9*	9.0	7.9	8.2
5.	*C. japonica*	10.4*	11.3	9.7	14.1	12.7	11.64
6.	*C. kissi*	4.3	3.9	4.7	4.6	4.8	4.46
7.	*C. rosaeflora*	7.3	8.1*	7.3	7.6	6.9	7.44
8.	*C. sasanqua*	8.2	8.0	8.0	7.5	7.8	7.9
9.	*C. sinensis*	8.8	10.3	7.9	9.3	9.1	9.08
10.	F_1 of *C. sinensis x C. japonica*	9.1	9.3	9.3	8.1	7.2	8.6
11.	*Gordonia excelsa*	12.7	12.1	8.5	13.0	9.7	11.2
12.	*Pyrenaria berringtoniaefolia*	12.4	10.9	12.3	10.8	11.1	11.5
13.	Tetraploid tea	10.7	11.0	11.7	9.9*	10.8	10.82

The foregoing results indicated that the incompatibility system operating in all the species of *Camellia* and related genera investigated was due to pre-fertilization barrier present in the female parent. The barrier was genetophytically controlled multiple alleles of 'S' genes and certain physiological factors also played important role in the mechanism.

4.6 FOLIAR MORPHOLOGICAL CHARACTERISTICS OF THE PARENTAL SPECIES

4.6.1 Foliar morphology

Various foliar morphological characters of nine species of *Camellia*, one interspecific F_1 hybrid of *C. sinensis* X *C. japonica*, two taxa of polyploid teas and three species of related genera *viz.*, *Eurya, Gordonia* and *Pyrenaria*, have been presented in Table 4.13. There was considerable variation among all the parameters studied, length of leaves varied from 6.05 cm in case of *C. rosaeflora* to 21.21 cm in *P. berringtoniaefolia*. Among the tea species, Assam tea, *C. assamica* had a length of 19.37 cm. While that of Cambod and China were 15.11 and 9.93 cm, respectively. Both the taxa of polyploid tea had comparatively longer leaves, only next to Assam tea. F_1 hybrid of *C. sinensis* X *C. japonica* had a length of 15.95 cm. Among the species of related genera, *Eurya japonica* had a length of only 7.31 cm whereas other two species *viz.*, *Gordonia* and *Pyrenaria* had 16.85 and 21.21 cm, respectively.

Table 4.13. Foliar morphological characters of parental species of *Camellia* and related genera[a]

Sl. No.	Name of the species	Length[b] (cm)	Width[c] (cm)	Length : width	No. of serration	Anthocyanin pigmentation[d]	Leaf angle, (degrees)
1.	*C. assamica*	19.37	6.14	3.10	98.8	0	88.0
2.	*C. cambodiensis*	15.11	6.15	2.51	98.4	3	62.0
3.	*C. drupifera*	6.30	3.31	1.96	56.0	4	48.2
4.	*C. irrawadiensis*	12.48	4.46	2.77	86.6	3	65.0
5.	*C. japonica*	9.70	4.61	2.18	78.6	3	42.8
6.	*C. kissi*	6.70	1.95	3.51	55.5	3	39.0
7.	*C. rosaeflora*	6.05	2.59	2.42	36.0	3	29.6
8.	*C. sasanqua*	6.53	4.00	1.66	42.4	3	44.0
9.	*C. sinensis*	9.93	3.56	2.91	103.4	2	92.5
10.	F_1 of *C. sinensis* X *C. joponica*	15.95	8.83	2.09	97.6	2	92.5
11.	*Eurya japonica*	7.31	2.75	2.76	65.6	2	48.4
12.	*Gordonia excelsa*	16.85	5.38	3.24	55.6	4	101.0
13.	*P. berringtoniaefolia*	21.21	8.34	2.59	90.8	3	37.3
14.	Tetraploid tea	16.99	8.51	2.08	103.0	0	72.8
15.	Triploid tea	16.59	6.64	2.55	122.2	0	70.6

a = average of 25 leaves
b = including petiole
c = maximum width
d = indicate number of leaf besides the terminal bud

Maximum width of 8.83 cm was observed in the case of F_1 hybrid of *C. sinensis* X *C. japonica*, followed by tetraploid tea (8.51 cm) and *P. berringtoniaefolia* (8.34 cm). Among the tea species, Assam tea and Cambod tea had almost similar width while that of China tea, *C. sinensis* was almost half (3.56 cm) as compared to the former two species. *C. kissi* had the minimum (1.95 cm) width followed by *C. rosaeflora* (2.59 cm) among all the species investigated. Non-tea diploid *Camellia* species had lesser width (less than 5 cm) as compared to tea species and other related genera.

Length to width ratio was, however, minimum (1.66) in the case of *C. sasanqua* while it was maximum (3.51) in *C. kissi*. In three tea species, Assam, Cambod and China, the length to width ratio was 3.10, 2.51 and 2.91, respectively. The ratio of leaf length to leaf width was found to be one of the most reliable criteria for identifying a particular species than either length or width alone.

4.6.2 Number of Serration

It was observed that in most of the hybrid seedlings, the number of serration per leaf increased from the lower portion towards the top portion of the stem, corresponding to the size of leaf. Generally, the older leaves were smaller and had lesser number of serration as compared to upper portion, which approached the mid-parental values.

4.6.3 Anthocyanin Pigmentation

There were slight variation in the brick red colouration of the young tender leaves along with the bud due to presence of anthocyanin in various species studied (Table 4.13). Anthocyanin pigmentation was observed in the terminal vegetative bud and up to a maximum of four leaves in case of *C. drupifera* and *G. excelsa*. In *C. assamica* and *C. sinensis*, however, only the bud and a leaf was pigmented while, two leaves were pigmented in the F_1 hybrid between *C. sinensis* X *C. japonica* and *E. japonica*, In all other species, except polyploid teas, exhibited the colouration up to third leaf from the terminal bud. In the later, two taxa, anthocyanin pigmentation was completely absent.

4.6.4 Leaf angle

Angle of the leaf lamina extended with the vertical axis of the stem was observed to be minimum (29.6°) in *C. rosaeflora* and maximum (101.0°) in *G. excelsa.* Among the tea species, China tea had an angle of 36.8°, while that of Assam and Cambod were 88.0° and 62.0°, respectively (Table 4.13). *C. irrawadiensis* had an angle of 65.0°. Among other non-tea species, there were not much variation in the leaf angle, ranging from 37.3° to 48.4°. Two taxa of polyploid tea also did not vary much in respect of leaf angle. In tetraploid tea, it was 72.8° while in the triploid tea it was 70.6°.

4.7 ANATOMICAL CHARACTERS OF THE PARENTAL SPECIES

4.7.1 Stomata

The frequency of stomata per 0.64 sq.mm. of leaf area, size and number of chloroplasts per guard cell have been presented in Table 4.14. In general, the frequency and size of stomata were inversely related. The size of the stomata was larger in the species in which the frequency was less and vice-versa. The maximum frequency of stomata (58.1) was observed in *P. berringtoniaefolia*, where they were of smallest size, 698.63 sq.µm. On the other hand, only14.9 stomata of size 1824.82 sq.µm were observed in tetraploid tea. In *C. japonica*, on an average 51.9 numbers of stomata of 708.68 sq.µm were observed. Among the three diploid tea species, Assam tea, *C. assamica* had the lowest frequency of 21.9 stomata per 0.64 sq.mm with a size of 1174.68 sq.µm; 25.3 stomata of size 1168.3 sq.µm in case of Cambod tea, *C. cambodiensis*. While in China tea, *C. sinensis*, the frequency and sizes were 28.5 and 1296.42 sq.µm, respectively. In two triploid taxa *viz.*, *C. rosaeflora* and triploid tea, there were 15.3 and 20.3 stomata with an average size of 1752.08 and 1656.08 sq.µm were observed. However, in another triploid F_1 hybrid between *C. sinensis* X *C. japonica* there were 39.6 number of stomata with a size of 1535.36 sq.µm. In *C. sasanqua*, which was a hexaploid species, the general relationship did not exist, the average frequency and size of stomata observed were 22.4 and 1089.08 sq.µm, respectively.

There was wide variation in the number of chloroplast observed in each guard cell in various species investigated. Maximum number of chloroplast (47.8) were observed in *C. sasanqua*, followed by tetraploid tea (42.6). It was generally observed that the number of chloroplast per guard cell increased with the increase in the ploidy level of the taxa. In diploid taxa, the number of chloroplast ranged from 14.9 in case of *E. japonica* to 22.4 in *P. berringtoniaefolia*. In triploid species *viz.*, *C. rosaeflora*, triploid tea and F_1 hybrid between *C. sinensis* X *C. japonica*, the number of chloroplast were 29.7, 33.8 and 30.8, respectively.

Table 4.14. Anatomical characters of the parents. I. Stomata

Sl. No.	Species	No. of stomata per 0.60 mm^2	Size, μm^2	No. of chloroplast per guard cell
1.	*C. assamica*	21.9	1174.68	21.4
2.	*C. cambodiensis*	25.3	1168.32	19.9
3.	*C. drupifera*	43.7	1011.37	23.1
4.	*C. irrawadiensis*	35.3	1102.63	20.7
5.	*C. japonica*	51.9	708.68	16.8
6.	*C. kissi*	38.9	1024.05	15.3
7.	*C. rosaeflora*	15.3	1752.08	29.7
8.	*C. sasanqua*	22.4	1089.08	47.8
9.	*C. sinensis*	28.5	1296.42	21.5
10.	F_1 of *C. sinensis* X *C. japonica*	39.6	1535.36	30.8
11.	*Eurya japonica*	19.8	1134.07	14.9
12.	*Gordonia excelsa*	17.1	1435.30	18.6
13.	*P. herringtoniaefolia*	58.1	698.63	22.4
14.	Tetraploid tea	14.9	1824.82	42.6
15.	Triploid tea	20.3	1656.08	33.8

4.7.2 Sclereid

The number of sclereids per 0.196 sq.cm area of leaf surface, their size (length and breadth) in micron and number of spicule per sclereid observed in various parental species have been tabulated in Table 4.15. There were wide variation in all the parameters or characteristics of sclereids studied. Maximum number of sclereid (148) was observed in *C. sinensis*, while in *C. sasanqua*, the number was minimum (only 48).

There was no regular pattern of distribution of sclereids in various ploidy levels. In diploid species, the frequency ranged from 52 in case of *C. kissi*, to 148 in China tea. Likewise, in triploid species also the frequency ranged widely - in triploid tea there were only 73 sclereids, while in *C. rosaeflora* and F_1 hybrid of *C. sinensis* X *C. japonica* the frequency observed were 102 and 132, respectively. In tetraploid tea, and hexaploid *C. sasanqua*, the frequencies were 81 and 48, respectively.

Table 4.15. Anatomical characters of the parents. II. Sclereid

Sl. No.	Species	No. of sclereid per 0.196 cm^2	Size, µm		No. of spicule per sclereid
			Length	Breadth	
1.	*C. assamica*	120	141.3	31.6	13.3
2.	*C. cambodiensis*	108	128.6	36.3	11.7
3.	*C. drupifera*	110	120.3	28.6	8.3
4.	*C. irrawadiensis*	96	92.6	35.3	13.7
5.	*C. japonica*	92	127.4	35.7	5.3
6.	*C. kissi*	52	90.8	31.6	5.6
7.	*C. rosaeflora*	102	113.7	43.2	7.7
8.	*C. sasanqua*	48	87.3	39.9	6.9
9.	*C. sinensis*	148	127.3	28.9	9.6
10.	F_1 of *C. sinensis* X *C. japonica*	78	117.9	30.6	3.9
11.	*Eurya japonica*	132	106.1	39.4	10.3
12.	*Gordonia excelsa*	72	158.7	58.9	8.9
13.	*P. berringtoniaefolia*	136	210.6	53.2	7.3
14.	Tetraploid tea	81	181.3	43.6	7.8
15.	Triploid tea	93	179.7	41.8	8.4

The size (length and breadth) of sclereid in various taxa studied was also variable. Longest sclereids (210.6 µm) were observed in *P. berringtoniaefolia*, while the shortest (87.3 µm) was observed in *C. sasanqua*. The breadth ranged from 28.6 µm in case of *C. drupifera*, to 58.9 µm in *G. excelsa*. Among the diploid tea species, Assam, Cambod and China, the length observed were 141.3, 128.6 and 127.3 µm, respectively, their corresponding breadth were 31.6, 36.3 and 28.9 µm. In tetraploid tea, the size was observed to be 181.3 µm X 43.6 µm, while in triploid taxa, it was observed to be 179.7 µm x 41.8 µm and the F_1 hybrid of *C. sinensis* X *C. japonica*, the size was 106.1 X 39.4 µm and in *C. rosaeflora* 113.7 µm X 43.2 µm.

Number of spicule ranged from 39 in *E. japonica* to 13.7 in *C. irrawadiensis*. In diploid tea species, Assam, Cambod and China tea had 13.3, 11.7 and 9.6 spiclues per sclereids respectively. In other non-tea *Camellia* species, there was slight variation - the numbers observed was approximately 5 to 9 per sclereid. In triploid taxa, the average number recorded were 7.7 in *C. rosaeflora*, 8.4 in triploid tea and 10.3 in the F_1 hybrid of *C. sinensis* X *C. japonica*. While in tetraploid tea and hexaploid *C. sasanqua*, there were slight variation in the average number 7.8 and 6.9 per sclereid, respectively.

4.8 GROWTH HABIT AND MORPHOLOGICAL CHARACTERISTICS OF INTERSPECIFIC AND INTERGENERIC HYBRIDS

Various growth characteristics like girth at collar, plant height, number and size (length and breadth) of leaf, and internodal length of the interspecific and intergeneric hybrids after six months of germination have been presented in Table 4.16. In total, there were 253 hybrid seedlings resulting from 58 compatible combinations, which produced viable hybrids. Number of seedlings in each hybrid combination ranged from a single plant to ten plants.

Table 4.16 indicated that the girth at a few centimeters above the ground level varied from 1.5 mm to 4.8 mm. In general, most of the seedlings were very thin, weak, with stunted growth and the thickness at collar was nearly 2.0 mm. Only the hybrids with tetraploid tea as seed parent exhibited thicker girth at collar. Height of the hybrids ranged from 6.2 cm in the hybrids of *C. rosaeflora* X *C. sasanqua*, to 23.5 cm in the hybrids between *C. cambodiensis* X *C. irrawadiensis*. However, most of the hybrids were around 15 cm height.

Internodal length of the hybrids and their ranges tabulated shows that in almost all hybrids the internodal lengths were very short and they were highly variable even within the progeny from the same parents. In some of the seedlings, the leaves were almost like a whorl, while in some others, they were like the seed parent. In the parental species also, there were distinct differences among the internodal lengths. In general, when the internodal length of the parental species were more, then the hybrids also inherited long internodes, dimensions nearly equal to the midparental value. However, when the parents with dissimilar lengths were used the hybrids approached the smaller internode indicating a partial dominance of the shorter internodes as compared to the longer ones.

Table 4.16. Growth habit and morphological characters of inter-specific and inter-generic hybrids (6 months old)

Sl. No	Hybrids	No. of plants observed	Girth (dia.) at collar (mm)	Plant height (cm)	No. of leaf	Internodal length (cm)	Size of leaf (cm)	
							Length	Width
1.	*C. assamica* X *C. cambodiensis*	10	2.9	21.8	14.8	2.1 (1.0-2.3)	6.30 (3.2-8.2)	3.10 (1.9-4.8)
2.	Reciprocal	10	2.7	18.4	13.3	1.93 (0.8-2.4)	5.1 (3.1-7.1)	2.98 (2.1-4.1)
3.	*C. assamica* X *C. drupifera*	5	1.8	13.5	8.6	0.98 (0.2-4.4)	5.42 (1.2-9.8)	2.44 (0.5-4.2)
4.	Reciprocal	5	2.5	10.3	6.6	0.87 (0.5-9.1)	3.06 (1.9-4.2)	1.98 (0.8-2.7)
5.	*C. assamica* X *C. irrawadiensis*	5	3.0	22.4	14.8	0.83 (0.1-4.1)	6.49 (1.5-12.9)	2.99 (0.9-5.4)
6.	Reciprocal	4	1.7	20.6	11.3	1.73 (0.91-2.3)	6.23 (3.7-8.1)	3.15 (1.9-4.8)
7.	*C. assamica* X *C. japonica*	5	1.8	16.5	9.8	0.92 (0.3-2.8)	6.66 (1.1-13.3)	2.91 (0.2-4.7)
8.	*C. assamica* X *C. kissi*	10	2.5	13.8	10.3	1.58 (0.3-3.8)	8.39 (1.0-15.8)	3.02 (0.5-5.2)
9.	Reciprocal	3	1.8	10.7	11.0	1.23 (0.3-2.3)	5.88 (2.7-7.1)	2.03 (0.4-3.4)
10.	*C. assamica* X *C. sinensis*	10	2.3	19.3	12.7	2.83 (1.8-3.9)	7.9 (4.8-9.9)	3.56 (2.2-4.3)
11.	Reciprocal	10	2.1	15.7	13.1	2.41 (1.7-3.3)	6.3 (3.8-8.7)	2.98 (1.2-3.8)
12.	*C. assamica* X *P. berringtoniaefolia*	1	1.7	10.9	7.0	0.93 (0.6-1.8)	4.3 (3.3-6.0)	2.1 (0.8-2.2)
13.	Reciprocal	8	2.3	12.9	8.1	0.43 (0.2-0.9)	6.6 (2.8-7.8)	3.3 (2.0-4.8)
14.	*C. cambodiensis* X *C. drupifera*	7	1.7	18.3	12.4	1.03 (0.2-2.8)	5.19 (1.0-10.1)	2.4 (0.4-4.5)
15.	Reciprocal	4	2.3	11.1	11.7	0.45 (0.1-1.6)	3.71 (2.0-6.0)	1.67 (1.0-2.4)
16.	*C. cambodiensis* X *C. irrawadiensis*	2	2.1	23.5	10.5	2.07 (1.3-3.3)	7.43 (1.0-7.9)	2.69 (0.7-4.3)
17.	Reciprocal	1	1.9	18.3	11.0	1.33 (0.80-3.9)	5.62 (3.1-7.4)	2.2 (1.1-3.9)
18.	*C. cambodiensis* X *C. kissi*	4	1.5	13.5	12.3	0.89 (0.5-2.1)	4.78 (2.1-7.3)	2.4 (1.0-3.9)

Table 4.16. (Continued) ....

Sl. No	Hybrids	No. of plants observed	Girth (dia.) at collar (mm)	Plant height (cm)	No. of leaf	Internodal length (cm)	Size of leaf (cm)	
							Length	Width
19.	*C. cambodiensis* X *C. sinensis*	10	2.1	19.7	15.6	1.92 (1.3-3.6)	6.8 (3.4-8.9)	3.7 (2.2-3.9)
20.	Reciprocal	10	1.7	18.6	16.3	1.77 (0.9-3.3)	6.1 (2.7-8.1)	3.5 (2.1-4.3)
21.	*C. cambodiensis* X *G. excelsa*	3	1.3	12.7	8.2	1.03 (0.6-2.6)	4.7 (2.3-7.1)	2.8 (1.8-3.3)
22.	Reciprocal	2	2.4	17.3	8.5	0.3 (0.1-0.7)	4.1 (3.3-6.2)	1.8 (0.9-3.1)
23.	*C. cambodiensis* X *P. berringtoniaefolia*	4	2.1	15.7	9.5	1.83 (0.5-2.7)	6.83 (2.5-11.4)	3.44 (0.8-5.2)
24.	Reciprocal	9	2.7	17.7	9.6	0.3 (0.1-0.8)	6.3 (3.1-8.2)	3.9 (1.9-3.4)
25.	*C. drupifera* X *C. irrawadiensis*	3	1.6	12.3	10.7	0.59 (0.1-2.0)	2.77 (0.9-6.0)	1.43 (0.3-2.9)
26.	*C. drupifera* X *C. kissi*	6	1.6	11.7	10.3	1.91 (0.1-2.9)	3.12 (1.8-4.7)	0.93 (0.7-1.8)
27.	Reciprocal	3	1.5	10.9	7.7	0.42 (0.1-0.8)	2.54 (1.3-4.0)	1.11 (1.0-1.5)
28.	*C. drupifera* X *C. sinensis*	5	1.7	13.7	10.8	1.59 (0.1-5.6)	5.56 (0.7-8.0)	2.30 (0.7-4.5)
29.	Reciprocal	6	1.9	19.9	13.5	1.1 (0.8-2.3)	4.1 (0.8-26.3)	2.2 (0.3-3.3)
30.	*C. drupifera* X *G. excelsa*	1	2.3	13.2	7.0	0.9 (0.4-1.7)	3.3 (0.8-4.4)	1.6 (0.4-2.3)
31.	*C. irrawadiensis* X *C. sinensis*	1	2.1	16.3	10.0	1.0 (0.7-2.2)	4.2 (2.1-6.8)	2.0 (1.2-2.3)
32.	Reciprocal	4	2.9	20.3	13.7	0.93 (0.7-1.9)	4.2 (1.9-6.3)	1.9 (0.4-2.8)
33.	*C. japonica* X *C. drupifera*	2	2.5	13.4	9.5	0.76 (0.2-1.9)	3.56 (1.1-6.3)	1.73 (0.6-2.8)
34.	*C. japonica* X *C. kissi*	1	2.1	10.3	7.0	1.2 (0.6-1.9)	3.4 (2.1-5.3)	1.6 (0.8-3.4)
35.	*C. japonica* X *C. sinensis*	2	2.3	11.9	6.5	1.1 (0.5-1.8)	3.3 (1.9-4.2)	1.3 (0.7-1.7)
36.	Reciprocal	1	1.8	10.5	9.0	0.79 (0.2-1.4)	4.8 (0.7-8.3)	1.97 (0.3-3.2)
37.	*C. kissi* X *C. sinensis*	2	1.8	15.7	9.5	0.97 (0.3-1.7)	3.6 (1.8-4.2)	1.2 (0.9-2.0)
38.	Reciprocal	3	1.9	16.2	12.3	1.67 (0.2-4.8)	7.16 (2.4-13.9)	3.01 (1.3-5.4)

Table 4.16. (Continued) ....

Sl. No	Hybrids	No. of plants observed	Girth (dia.) at collar (mm)	Plant height (cm)	No. of leaf	Internodal length (cm)	Size of leaf (cm)	
							Length	Width
39.	*C. rosaeflora* X *C. sasanqua*	1	1.7	6.2	4.0	1.2 (0.9-1.6)	1.9 (1.3-2.4)	1.1 (0.9-1.3)
40.	*C. rosaeflora* X *C. sinensis*	2	1.6	7.7	3.5	1.4 (1.1-1.9)	2.4 (1.5-3.5)	1.4 (0.9-1.7)
41.	*C. sasanqua* X *C. assamica*	1	2.1	11.3	7.0	0.68 (0.3-1.6)	2.02 (1.2-3.1)	1.59 (0.9-2.3)
42.	*C. sasanqua* X *C. cambodiensis*	3	1.9	12.6	6.7	0.32 (0.1-0.5)	2.45 (1.2-3.1)	1.16 (0.6-1.4)
43.	*C. sasanqua* X *C. drupifera*	1	1.8	11.6	8.0	0.95 (0.1-3.7)	3.82 (2.3-6.3)	2.22 (1.1-3.7)
44.	*C. sasanqua* X *C. kissi*	1	1.7	12.3	9.0	0.33 (0.2-0.7)	2.30 (1.2-2.8)	1.49 (0.82-0.9)
45.	*C. sasanqua* X *C. sinensis*	4	2.3	11.7	7.5	0.84 (0.2-2.1)	3.00 (0.6-4.7)	1.85 (0.6-3.2)
46.	Reciprocal	1	1.8	15.3	9.0	0.37 (0.1-0.8)	3.17 (1.2-5.2)	1.78 (1.0-2.3)
47.	*C. sasanqua* X Tetraploid tea	2	2.6	13.3	8.0	1.5 (0.8-2.4)	6.8 (3.2-8.2)	3.3 (1.7-3.9)
48.	*C. sinensis* X *P. berringtoniaefolia*	2	1.9	13.5	6.5	1.56 (0.4-3.5)	4.72 (2.0-8.4)	2.53 (1.4-3.8)
49.	Reciprocal	5	2.2	17.1	8.4	0.82 (0.4-1.4)	4.64 (2.9-9.7)	1.88 (1.4-3.6)
50.	*G. excelsa* X *C. assamica*	6	2.4	16.7	6.5	0.32 (0.1-0.8)	5.25 (3.6-7.96)	1.90 (0.9-2.3)
51.	*G. excelsa* X *C. sinensis*	5	2.1	13.8	6.2	0.30 (0.1-0.7)	5.3 (3.3-7.2)	1.9 (0.9-2.6)
52.	Tetraploid tea X *C. assamica*	10	4.7	22.8	14.9	0.92 (0.6-2.3)	6.4 (3.1-8.3)	3.1 (1.1-3.8)
53.	Tetraploid tea X *C. cambodiensis*	10	4.7	20.5	14.3	0.93 (3.5-2.2)	5.8 (3.8-7.8)	3.4 (2.1-4.3)
54.	Tetraploid tea X *C. drupifera*	8	4.3	14.5	7.5	0.83 (0.6-2.9)	4.1 (2.8-5.8)	2.3 (0.8-3.8)
55.	Tetraploid tea X *C. irrawadiensis*	3	4.1	16.7	8.3	1.1 (0.8-2.7)	6.3 (2.0-8.3)	2.5 (0.9-3.3)
56.	Tetraploid tea X *C. japonica*	1	3.6	13.2	8.0	0.80 (0.2-1.2)	3.83 (3.5-4.0)	1.60 (1.3-1.8)
57.	Tetraploid tea X *C. kissi*	5	3.1	16.7	11.2	0.73 (0.4-1.7)	4.32 (1.8-6.9)	2.8 (1.9-3.8)
58.	Tetraploid tea X *C. sinensis*	10	4.8	22.6	14.6	0.37 (0.2-0.6)	2.73 (1.8-3.8)	1.78 (1.2-2.8)

Various growth characteristics and foliar morphological characteristics observed on six months old interspecific and intergeneric hybrids (Table 4.16) were very small and stunted. As the seedlings were too young and weak, so they generally, did not reflect the characteristics of either parents. However, the hybrids survived for more than one year and the comparative performance of the morphological characteristics were observed. Observations made on the hybrid seedlings when they grew for one and half year have been presented in Table 4.17. Besides the tabulated parameters, some non-parametric observations were also made, which have not been included in the table. Details of the parametric characters could be seen from Table 4.17.

In total, there were 31 combinations of interspecific and intergeneric hybrids survived for 18 months. Out of which, there were 20 successful crosses and 11 reciprocal crosses. In general, the morphological characters of most of the hybrids were intermediate between the parental species, when morphologically similar parents were crossed. However, when the parents with morphologically dissimilar characters were crossed, the characters of the seed parent partially dominated over the pollen parent.

Table 4.17. Growth habit and morphological characters of interspecific and intergeneric hybrids (18 months old)

Sl. No	Hybrids	Girth at collar (mm)	Size of leaf			No. of serration	Leaf angle (°)	Anthocyanin pigmentation
			Length (cm)	Width (cm)	Length : width			
1.	*C. assamica* X *C. cambodiensis*	5.91	17.86	6.13	2.78	64.6	77	3
2.	Reciprocal	5.45	16.26	6.27	2.59	58.8	73	3
3.	*C. assamica* X *C. drupifera*	3.74	14.73	5.07	2.90	60.4	65	3
4.	Reciprocal	3.57	12.24	4.93	2.48	52.2	70	4
5.	*C. assamica* X *C. irrawadiensis*	4.83	21.04	6.91	3.04	68.0	77	4
6.	Reciprocal	5.97	20.63	8.09	2.59	70.4	72	4
7.	*C. assamica* X *C. kissi*	5.03	14.33	6.93	2.07	50.8	69	3
8.	*C. assamica* X *C. sinensis*	5.37	15.07	5.01	3.01	88.2	67	2
9.	Reciprocal	4.72	13.67	4.83	2.83	76.6	52	2
10.	*C. cambodiensis* X *C. drupifera*	4.03	12.73	5.08	2.1	57.0	60	5
11.	Reciprocal	3.91	10.54	4.72	2.23	58.8	53	4
12.	*C. cambodiensis* X *C. irrawadiensis*	3.08	14.08	5.33	2.4	78.2	64	4
13.	Reciprocal	4.17	16.89	6.49	2.58	74.0	70	4
14.	*C. cambodiensis* X *C. sinensis*	5.39	13.06	5.11	2.56	87.8	52	4
15.	Reciprocal	5.04	11.58	4.8	2.47	84.6	48	4
16.	*C. drupifera* X *C. kissi*	3.87	5.9	2.9	2.27	33.6	45	4
17.	Reciprocal	3.42	5.37	2.36	2.27	32.4	44	4
18.	*C. drupifera* X *C. sinensis*	4.09	6.96	2.40	2.90	30.8	43	3
19.	Reciprocal	4.89	8.20	3.41	2.40	42.4	45	3
20.	*C. irrawadiensis* X *C. sinensis*	5.03	13.51	5.28	2.58	82.0	54	3
21.	Reciprocal	4.09	10.33	3.98	2.59	88.8	42	3
22.	*C. kissi* X *C. sinensis*	2.83	7.77	3.01	2.58	60.8	39	3
23.	Reciprocal	3.61	9.01	2.71	3.32	58.2	37	3
24.	*C. sasanqua* X *C. drupifera*	3.23	6.40	3.70	1.73	42.8	46	4
25.	*C. sasanqua* X *C. sinensis*	3.41	8.39	4.98	1.68	50.2	45	4
26.	*P. berringtoniaefolia* X *C. assamica*	3.89	19.39	8.07	2.40	64.4	38	4
27.	*P. berringtoniaefolia* X *C. cambodiensis*	4.07	19.20	8.23	2.33	83.2	40	5
28.	Tetraploid tea X *C. assamica*	5.87	17.83	6.98	2.55	92.8	75	2
29.	Tetraploid tea X *C. cambodiensis*	5.39	16.11	7.23	2.23	90.4	70	3
30.	Tetraploid tea X *C. drupifera*	4.23	10.83	6.01	1.80	62.4	63	2
31.	Tetraploid tea X *C. sinensis*	5.93	14.07	6.13	2.29	86.0	58	2

4.9 ANATOMICAL CHARACTERISTICS OF INTERSPECIFIC AND INTERGENERIC HYBRIDS

Anatomical characters like the frequency and size of stomata, number of chloroplast in each guard cell and frequency, length and breadth of sclereid and number of spicule per sclereid have been presented in Table 4.18 and 4.19. In most of the hybrids, the stomatal characteristics were intermediate between the parental species. When *C. irrawadiensis* was used as seed parent with *C. assamica, C. cambodiensis* and *C. sinensis* as pollen parent, the anatomical characteristics of the seed parent were dominant. In *C. sinensis* X *C. assamica, C. cambodiensis* X *C. drupifera, C. cambodiensis* X *C. sinensis* and reciprocal, *C. drupifera* X *C. sinensis, C. kissi* X *C. sinensis, C. sasanqua* X *C. drupifera, P. herringtoniaefolia* X *C. assamica* and *P. herringtoniaefolia* X *C. cambodiensis*, the maternal parent was dominant over the pollen parent. However, in triploids resulting from tetraploid tea X *C. assamica* and tetraploid tea X *C. cambodiensis* showed neither dominance of the parents. Like parental species, in the hybrids also, frequency and size of stomata were inversely related - the hybrids in which the frequency was more (e.g., *P. herringtoniaefolia* X *C. assamica, P. herringtoniaefolia* X *C. cambodiensis*, tetraploid tea X *C. assamica*), the sizes were smaller. On the other hand, the hybrids in which the stomata were less frequent, had larger size.

Table 4.18. Anatomical characters of the interspecific and intergeneric hybrids. I. Stomata*

Sl. No.	Hybrids	No. of stomata per 0.60 mm^2	Size, µm^2	No. of chloroplasts /guard cell
1.	*C. assamica* X *C. cambodiensis*	22.8	1130.6	20.7
2.	Reciprocal	24.4	1160.3	20.3
3.	*C. assamica* X *C. drupifera*	31.7	1110.5	22.7
4.	Reciprocal	36.3	1078.3	22.7
5.	*C. assamica* X *C. irrawadiensis*	30.7	1108.1	21.2
6.	Reciprocal	35.3	1110.3	20.3
7.	*C. assamica* X *C. kissi*	32.8	1100.4	19.5
8.	*C. assamica* X *C. sinensis*	26.7	1201.2	21.3
9.	Reciprocal	28.1	1261.2	20.8
10.	*C. cambodiensis* X *C. drupifera*	28.7	1113.7	21.7
11.	Reciprocal	35.3	1063.1	22.8
12.	*C. cambodiensis* X *C. irrawadiensis*	30.3	1124.0	20.2
13.	Reciprocal	33.1	1112.3	19.9
14.	*C. cambodiensis* X *C. sinensis*	27.4	1220.9	20.5
15.	Reciprocal	28.3	1268.3	21.0
16.	*C. drupifera* X *C. kissi*	40.2	1010.3	19.7
17.	Reciprocal	40.3	1020.5	21.6
18.	*C. drupifera* X *C. sinensis*	39.6	1118.0	22.8
19.	Reciprocal	36.1	1156.0	22.1
20.	*C. irrawadiensis* X *C. sinensis*	33.3	1089.3	21.0
21.	Reciprocal	31.4	1200.5	21.1
22.	*C. kissi* X *C. sinensis*	35.7	1098.1	16.3
23.	Reciprocal	32.5	1575.8	18.1
24.	*C. sasanqua* X *C. drupifera*	26.7	1076.3	36.6
25.	*C. sasanqua* X *C. sinensis*	24.6	1112.3	38.3
26.	*P. berringtoniaefolia* X *C. assamica*	49.3	813.3	22.2
27.	*P. berringtoniaefolia* X *C. cambodiensis*	50.8	801.7	20.3
28.	Tetraploid tea X *C. assamica*	42.7	923.3	21.8
29.	Tetraploid tea X *C. cambodiensis*	44.2	1376.2	33.1
30.	Tetraploid tea X *C. drupifera*	30.4	1484.8	34.3
31.	Tetraploid tea X *C. sinensis*	24.5	1538.3	33.7

* Mean of 20 representative observations.

Table 4.19. Anatomical characters of the interspecific and intergeneric hybrids. II. Sclereid*

Sl. No.	Hybrids	No. of sclereid per 0.196 cm^2	Size, µm		No. of spicule per sclereid
			Length	Width	
1.	*C. assamica* X *C. cambodiensis*	110	132.8	34.2	12.8
2.	Reciprocal	113	127.3	35.7	13.3
3.	*C. assamica* X *C. drupifera*	117	120.1	30.6	13.2
4.	Reciprocal	111	117.9	29.2	9.7
5.	*C. assamica* X *C. irrawadiensis*	100	98.0	34.3	13.1
6.	Reciprocal	107	95.2	33.8	13.5
7.	*C. assamica* X *C. kissi*	83	114.3	32.1	13.2
8.	*C. assamica* X *C. sinensis*	136	135.0	29.7	11.2
9.	Reciprocal	141	141.2	30.5	11.3
10.	*C. cambodiensis* X *C. drupifera*	100	126.2	31.3	9.6
11.	Reciprocal	103	122.3	30.2	9.5
12.	*C. cambodiensis* X *C. irrawadiensis*	102	122.7	35.3	12.2
13.	Reciprocal	108	94.4	35.8	13.4
14.	*C. cambodiensis* X *C. sinensis*	113	126.6	30.3	9.9
15.	Reciprocal	128	127.0	30.7	10.2
16.	*C. drupifera* X *C. kissi*	83	117.3	35.4	8.1
17.	Reciprocal	71	103.0	30.1	7.4
18.	*C. drupifera* X *C. sinensis*	120	121.7	28.3	9.0
19.	Reciprocal	126	124.4	28.6	8.8
20.	*C. irrawadiensis* X *C. sinensis*	106	103.3	33.8	11.3
21.	Reciprocal	146	122.8	30.3	12.0
22.	*C. kissi* X *C. sinensis*	63	100.7	30.2	6.9
23.	Reciprocal	117	118.3	30.4	8.3
24.	*C. sasanqua* X *C. drupifera*	68	91.3	36.7	7.1
25.	*C. sasanqua* X *C. sinensis*	88	93.7	36.3	7.2
26.	*P. herringtoniaefolia* X *C. assamica*	118	181.1	48.5	9.6
27.	*P. herringtoniaefolia* X *C. cambodiensis*	123	170.5	42.4	8.6
28.	Tetraploid tea X *C. assamica*	112	161.7	38.5	9.7
29.	Tetraploid tea X *C. cambodiensis*	86	156.3	40.1	9.3
30.	Tetraploid tea X *C. drupifera*	93	167.0	36.3	8.0
31.	Tetraploid tea X *C. sinensis*	95	170.3	35.7	8.4

* Mean of 20 representative observations.

Frequency, size and structure of sclereids of the interspecific and intergeneric hybrids have been presented in Table 4.19. Like stomata, in most of the hybrids, the frequency and sizes of sclereids were intermediate between the parental species. However, in a few hybrids e.g., *C. sinensis* X *C. cambodiensis, C. kissi* X *C. drupifera, C. irrawadiensis* X *C. sinensis, C. sinensis* X *C. irrawadiensis, C. kissi* X *C. sinensis, C sinensis* X *C. kissi, C. sasanqua* X *C. drupifera* and tetraploid tea X *C. sinensis*, slightly dominance of the maternal parent was observed. In general, the sizes of the sclereids were larger in the hybrids where the frequency was less and vice-versa. In a few interspecific hybrids e.g., *C. assamica* X *C. drupifera, C. drupifera* X *C. assamica, C. assamica* X *C. kissi* and *C. drupifera* X *C. sinensis*, however, the number of spicule per sclereid was more inclined towards the seed parent.

4.10 CYTOLOGICAL OBSERVATION OF INTERSPECIFIC AND INTERGENERIC HYBRIDS

Somatic chromosome numbers of interspecific and intergeneric hybrids involving *Camellia* and related genera have been recorded in Table 4.20. Cytological observation was made on 216 representative hybrids out of a total 529 hybrid seedlings obtained. Most of the hybrids had a chromosome number intermediate between the parents, that is, the somatic chromosome numbers was equal to the summation of the numbers of chromosome in the gametes of both the parents. However, in 36 hybrids representing 22 combinations, aneuploid number was recorded. Out of a total of 58 compatible combinations, which produced viable and germinated seeds, 42 combinations

Table 4.20. Chromosome numbers of interspecific and intergeneric hybrids of *Camellia* and related genera

Sl. No.	Hybrids	Total number of plants obtained	Number of plants observed	Chromo-some number
1.	*C. assamica* X *C. cambodiensis*	66	10	30
2.	Reciprocal	35	10	30
3.	*C. assamica* X *C. drupifere*	5	3	30
4.	Reciprocal	5	3	30
5.	*C. assamica* X *C. irrawadiensis*	5	2	30
6.	Reciprocal	4	3	30
7.	*C. assamica* X *C. japonica*	5	3	30, 29(1)* 31(1)
8.	*C. assamica* X *C.kissi*	14	10	30
9.	Reciprocal	1	1	32
10.	*C. assamica* X *C. sinensis*	43	10	30
11.	Reciprocal	54	10	30
12.	*C. assamica* X *P berringtoniaefolia*	1	1	30
13.	Reciprocal	8	5	30, 31(1), 33(2)
14.	*C. cambodiensis* X *C. drupifera*	7	4	30
15.	Reciprocal	4	4	30
16.	*C. cambodiensis* X *C. irrawadiensis*	2	2	30
17.	Reciprocal	1	1	30
18.	*C. cambodiensis* X *C. kissi*	4	3	30, 31(2)
19.	*C. cambodiensis* X *C. sinensis*	26	10	30
20.	Reciprocal	47	10	30
21.	*C. cambodiensis* X *G. excelsa*	1	1	33
22.	Reciprocal	2	2	30, 31
23.	*C. cambodiensis* X *P. berringtoniaefolia*	4	2	31, 33
24.	Reciprocal	9	4	30
25.	*C. drupifera* X *C. irrawadiensis*	3	3	30, 31(1), 33(1)
26.	*C. drupifera* X *C. kissi*	6	5	30
27.	Reciprocal	3	3	30
28.	*C. drupifera* X *C. sinensis*	5	5	30
29.	Reciprocal	6	3	30

Table 4.20. (Continued) ....

Sl. No.	Hybrids	Total number of plants obtained	Number of plants observed	Chromo-some number
30.	*C. drupifera* X *G. excelsa*	1	1	28
31.	*C. irrawadiensis* X *C. sinensis*	1	1	30
32.	Reciprocal	4	4	30
33.	*C. japonica* X *C. drupifera*	2	2	29, 31
34.	*C. joponica* X *C. kissi*	1	1	31
35.	*C. joponica* X *C. sinensis*	2	2	29, 33
36.	Reciprocal	1	1	30
37.	*C. kissi* X *C. sinensis*	2	2	30
38.	Reciprocal	3	3	30
39.	*C. rosaeflora* X *C. sasanqua*	1	1	75
40.	*C. rosaeflora* X *C. sinensis*	2	1	45
41.	*C. sasanqua* X *C. assamica*	1	1	59
42.	*C. sasanqua* X *C. cambodiensis*	1	1	61
43.	*C. sasanqua* X *C. drupifera*	1	1	60
44.	*C. sasanqua* X *C. kissi*	2	1	58
45.	*C. sasanqua* X *C. sinensis*	4	4	60
46.	Reciprocal	2	2	59, 61
47.	*C. sasanqua* X Tetraploid tea	6	2	75
48.	*C. sinensis* X *P. berringtoniaefolia*	2	1	31
49.	Reciprocal	5	3	28, 31, 33
50.	*G. excelsa* X *C. assamica*	6	3	30, 32(2)
51.	*G. excelsa* X *C. sinensis*	5	3	31, 33(2)
52.	Tetraploid tea X *C. assamica*	27	10	45
53.	Tetraploid tea X *C. cambodiensis*	15	10	45
54.	Tetraploid tea X *C. drupifera*	8	5	45
55.	Tetraploid tea X *C. irrawadiensis*	3	3	45
56.	Tetraploid tea X *C. japonica*	1	1	43
57.	Tetraploid tea X *C. kissi*	5	3	45, 41, 42
58.	Tetraploid tea X *C. sinensis*	39	10	45

had diploid X diploid and 16 crosses involved one or both polyploid parental species. Parental combinations involving *C. assamica* with *C. japonica, C. kissi* and *P. berringtoniaefolia; C. cambodiensis* with *C. kissi, G. excelsa, P. berringtoniaefolia; C. drupifera* with *C. irrawadiensis, G. excelsa; C. japonica* with *C. drupifera, C. kissi, C. sinensis*, in the diploid x diploid crosses produced one or more aneuploids. All other combinations produced diploid seedlings. In the crosses involving polyploid species, *C. sasanqua* with *C. assamica, C. cambodiensis, C. kissi* and *C. sinensis* in reciprocal cross; *C. sinensis* with *P. berringtoniaefolia*, reciprocal cross, *G. excelsa* with *C. assamica, C. sinensis* and *C. japonica* and *C. kissi* as pollen parent on to tetraploid tea resulted in aneuploids. All other combinations including diploid and polyploid species, resulted into euploids ranging from diploid, triploid, tetraploid and pentaploid. Hybrids resulting from 27 combinations did not survive more than a few weeks after germination. Rest of the combinations produced hybrids which survived for more than eighteen months. All the lethal hybrids and a few others, were initially very weak stunted growth habit with aneuploid number of chromosomes. In tetraploid x diploid crosses, all the 35 viable hybrids were triploid with 45 chromosomes, while in the hexaploid x diploid crosses, e.g., *C. sasanqua* with *C. drupifera* and *C.* **assamica** produced five plants with 60 chromosomes. Only 17 hybrid plants survived in the intergeneric crosses between *Pyrenaria* and *Camellia*, out of which only 9 plants could be observed cytologically, which showed 30 chromosomes. Disjunction of chromatids during the anaphase stage was normal without any bridge formation or lagging chromosomes.

CHAPTER - V

Discussion

CHAPTER - V

DISCUSSION

5.1 SCANNING ELECTRON MICROSCOPIC STUDIES ON MORPHOLOGY AND SURFACE TOPOGRAPHY OF POLLEN GRAINS OF VARIOUS SPECIES OF *Camellia* AND RELATED GENERA

Sometimes it become difficult to distinguish and identify cultivars by using visible morphological characters such as leaf, growth habit or fruit appearance (Matsuta *et al.*, 1982). The problem became more prominent in cross-pollinated crops grown for their vegetative parts. It is thus necessary to seek new character(s) for distinguishing and identifying cultivars. They can be used independently or in combination with other vegetative or reproductive characters to avoid confusion. Micromorphology of pollen (Nowicke and Skvarla, 1979; Arguc, 1980; Walker and Walker, 1980), trichome (Hardin, 1979) and seed-coat (Hill, 1976; Crow, 1976) have been utilized for taxonomic purposes in divergent as well as closely related taxa (Matsuta *et al.*, 1982). Morphology and surface topography or ultrastructure of pollen grains have been utilized in blueberry, *Vaccinium; Vitis*; raspberry, *Rubus*; blackberry; strawberry, *Fragaria* and their hybrids (Maas, 1977; Goldy *et al.*, 1984), peach, nectarine, *Prunus*; plum *Prunus* spp.; apricot, *P. armeniaca*; cherry, apple, *Malus*; pear, *Pyrus* (Fogle, 1977a, 1977b; Westwood, 1978; Matsuta *et al.*, 1982), *Cotyledon grandiflora*, *Echeveria* spp. and their hybrids (Mamatha

Rao, 1979), crabapple, *Malus* spp. (Martens and Fretz, 1980), olive, *Olea europaea* (Pacini and Vosa, 1979).

However, reports on the morphology of the pollen grains of *Camellia* species are scanty. Erdtman (1952), Ikuse (1956), Keng (1962) studied the morphology of tea and a few other species. Shape and size of the pollen grains of three main varieties of tea by Bezbaruah (1971a), various clones of tea in South India by Mohanan and Sharma (1981) and that of a triploid tea byBezbaruah (1975d). While Ackerman and Kondo (1980) studied and correlated the size, variability and chromosome number of various species of *Camellia* under light microscope. Shu and Chen (1996) investigated the morphology and surface ornamentation of tea under scanning electron microscopy for studying the origin and evaluation of tea plants in China. Therefore, the present investigation was carried out to study the morphology and ultrastructure of the pollen grains of nine species of *Camellia*, one interspecific hybrid, two polyploid tea and two species of related genera *via.*, *Gordonia* and *Pyrenaria*.

In the present investigation, the shape of the pollen grains from equatorial view was observed to range from elliptical with tapering to flattened end to depressed oval. From polar view, they were either circular or semi-angular with the furrows intruding or open from the angles at a distance of 120°. The length to width ratio among the species varied within a narrow range of 1.14 (sub-prolate) in case of tetraploid tea to 1.96 (prolate) in all other species studied.Bezbaruah (1971a, 1975c) and Mohanan and Sharma (1981) reported the pollen grains of tea to be triangular.

The length and width of the pollen grains of three tea species, *C. assamica*, *C. cambodiensis* and *C. sinensis* ranged from 34.88 µm to 50.50 µm and 21.42 µm to 32.04 µm, respectively (Table 4.1). Though the size of pollen grains was smaller in case of *C. sinensis* than those of *C. assamica* and *C. cambodiensis* in between the two. Interspecific differences in size of the pollen grains of three tea species were not well marked. Bezbaruah (1971a) also reported slight variation in the diameter of the pollen grains of three main varieties. However, Mohanan and Sharma (1981) reported marginal differences in size in 22 clones, while in two others, there were wide variation. In all the three varieties of tea studied (Bezbaruah, 1971a), the size of the pollen grains was larger than the present investigation, which might be due to varietal difference in the plant material and the method followed. For light microscopy, Bezbaruah (1971a) followed wet method of preparation - by staining with 0.5 per cent acetocarmine solution which might result in equatorial swelling. Dry pollen grains just after dehiscence were used in the present study for the elimination of chance swelling. In SEM preparation also Lombardo *et al.* (1976) reported such swelling of the pollen grains, which were prepared by critical point drying method.

In other diploid species of *Camellia*, there was a wide range of variation in length, 20.00 µm in case of *C. rosaeflora*, to 56.95 µm in case of *C. irrawadiensis*. Similar inter-specific differences were also mentioned by Ackerman and Kondo (1980) in *Camellia*, Maas (1977) in strawberry and other small fruit crops, Fogle (1977a, 1977b) in tree fruit species - peach, plum, sweet cherry, nectarine and apple.

In the present investigation, three triploids, including one interspecific hybrids were observed for the gross morphology of the pollen grain and the ultrastructure of the exine. In all the cases, the pollen grains were shrivelled, poorly filled and had irregular or inconsistence in shape. The germinal furrows were also irregular and comparatively smaller, in the intercolporate regions there were concave areas. The surface sculpture of the exine was variable, with very less or without distinct perforations. In *C. rosaeflora* the surface was smooth without ridges or well-developed muri, in *C. japonica* X *C. sinensis* hybrid both ridged and papillate surface were present, however, in triploid tea, comparatively regular but rough surface was observed.

In the present investigation, in most of the species the length and width of the pollen grains were consistent. However, in a few species *viz.*, *C. irrawadiensis*, *C. japonica* and triploid tea had almost similar length and the ranges were also overlapping. In these species for identification any two measurements and exine sculpture would be very useful. Sizes recorded in the present study are in confirmation with the dimensions reported by Ackerman and Kondo (1980). In triploid tea, average length and width were observed to be 54.68 µm and 37.45 µm, respectively. **Bezbaruah (1975a)** and Ackerman and Kondo (1980) also reported the diameter of the pollen grains of triploid tea to be well within the range. Considerable variation in pollen size was recorded in the genus *Camellia*. Variation in size, shape and exine pattern were found to be more in widely cultivated species than the wild species. In general, the size of the pollen grains were observed to be larger in triploid and tetraploid tea species. **Bezbruah (1975a)** also observed large sized pollen grains in triploid tea and Ackerman and Kondo (1980) observed a positive correlation between size of the pollen grains and levels of ploidy. Variation in size of pollen grains of cultivated species might occur due to difference in mineral nutrition (Bell, 1959; Schoch-Bodmer, 1940), effects of moisture and other environmental factors (Fogle, 1977a), position of the flower on the plant (Kurtz and Liverman, 1958). Ackerman and Kondo (1980) also reported variation in size of pollen grains to be more in widely cultivated species than the wild species of *Camellia*. In the present investigation, the variation in size of the pollen grains was the greatest in *C. sasanqua*, which was almost three times as compared to others. Similarly, in triploid tea, the extent of variation was about double in comparison to other diploid species. Pollen size was shown (Stanley and Linskens, 1974) to be positively correlated with various degrees with chromosome numbers and ploidy levels. Erdtman (1969) reported that the differences encountered among ploidy levels tended to be obvious and were considered genetically and morphologically significant. The size and shape of the pollen grains were uniform for a particular species of *Pyrus* (Westwood and Challice, 1978). Length to width ratio did not indicate the shape actually observed under SEM. Similar conclusion was made by Westwood and Challice (1978) after observing 18 species of *Pyrus*. Pollen size was also influenced by the number of germination pores. Funke (1956) reported that the number of germination pores increased in autopolyploid and allopolyploid plants with the increase in the ploidy level except in grasses. The size of the pollen grains was larger in polyploid *Vaccinium* as compared to the diploid (Maas, 1977). Mean pollen diameter of tetraploid *Vaccinium* species was 11 per cent larger than in the diploid and 11 per cent larger in hexaploid than in the tetraploid (Cockerham and Galleta, 1976). They suggested that the variation in the size of the pollen grains in various ploidy levels could be used as a taxonomic tool.

Pollen grains of two species of related genera *viz.*, *Gordonia* and *Pyrenaria* also showed significant interspecific differences in length and width (Table 4.1). In *Pyrenaria*, dimorphism in the exine sculpturing was observed (Plate 4E). Mohanan and

Sharma (1981) reported polymorphism in the pollen grain of tea. Renen (1919) also observed dimorphism, in the production of two or more classes of pollen grains. Finger like prominent ridges were present on the exine of *Pyrenaria* which was completely different from all other species. In *Fragaria nipponica,* pollen grains were completely different from other strawberry pollen samples examined (Maas, 1977). In the closest allies to *Fragaria* in the Rose family, *Duchesnea* and *Potentilla* pollens were having ridges frequently anastomose with adjacent ridges. Pores in pollen exine were not found in *Fragaria* pollen, although they were reported from taxonomically related plants *viz., Malus, Pyrus, Prunus* (Fogle, 1977a) and *Rubus* (Maas, 1977).

The exine sculpture varied from psilate to rough reticulate through verrucate or papillate. In *Pyrenaria,* pollen surface was longitudinally ridged but without perforations. The shape, sizes and frequency of perforations in other species varied considerably but were almost uniform for a particular species. Exine sculpturing could be used in classification and identification of various species in the family *Theaceae.* Details of exine sculpturing was used as a valuable taxonomic tool in crabapple (Martens and Fretz, 1980) *Vitis, Rubus* and *Fragaria* (Maas, 1977), *Malus, Pyrus* and *Prunas* (Fogle, 1977a, 1977b; Matsuta *et al.*, 1982) in olive (Pacini and Vosa, 1979). The most useful characteristics for catagorizing crabapple pollen were exine topography, perforation type and size and frequency of perforations (Martens and Fretz, 1980). Based on progeny tests (Quiros, 1975; Rao *et al.*, 1979) and exine ontogeny studies (Heslop-Harrison, 1968; Rogers and Harris, 1969; Pandey and Troughton, 1974), it was presumed that the exine pattern was controlled sporophytically by the pollen mother cell. To estimate the morphological differences quantitatively, Matsuta *et al.* (1982) developed a numerical model using twelve characters. Using the model they correctly assigned 76 per cent of the total number of pollen grains examined to a particular group or cultivar.

In the present investigation, besides normal, well filled pollen grains representative of a species, shrivelled and poorly filled grains were also observed in almost all the species. Similar observation of shrivelled or incompletely developed pollen grains in cherry was reported by Fogle (1977a). Pear also had small and abortive pollen grains (Westwood and Challice, 1978; Matsuta *et al.*, 1982).

In *Pyrus* species, Westwood (1978) studied nine characters of the pollen grains and concluded that none of the characters showed consistency among the geographic groups of species. However, when all the nine features considered, each species had unique pattern. When within species variability was minimal those measurements could be used along with those of leaf, stem, flower and fruit (Challice and Westwood, 1973; Lee, 1948; Rubstov, 1944; Westwood and Bjornstad, 1971; Westwood, 1978). Use of pollen characters was best suited to distinguish between a few clones that are not separable by major fruit or vegetative characters, in pollen studies to identify the distribution of pollen under open pollination, to determine the collection patterns and distances covered by bees, determining ploidy in progenies from interspecific crosses (Fogle, 1977b).

5.2 STORAGE AND VIABILITY OF POLLEN GRAINS

Various investigators emphasized the effect of humidity and temperature of storage on the longevity of pollen grains. Nebel and Ruttle (1937) concluded that for the pollen grains of various species and varieties of *Pyrus, Prunus* and *Vitis*, a humidity of 50 per cent was optimum for storage. Gollmick (1942) reported that the longevity was best at a relative humidity between 6 and 60 per cent and for most species the optimum was below 60 per cent. In general, the longevity of pollen grains was negatively correlated with the relative humidity required for optimal storage (Visser, 1955). However, in *Gramineae*, the pollen lost viability within one day under low humidity. The loss of viability was slightly slower at low temperature. It was only at a high humidity (80-100%) that the pollen of *Gramineaceous* plants retained viability and that too only for a very short period (Pahler and Linskens, 1972; King, 1961; Hoekstra and Bruinsma, 1975; Shivanna and Heslop-Harrison, 1981). Pollen grains of *Arachis hypogaea, Brassica nigra, Pennisetum typhoideum, Solanum melongana* and *S. tuberosum* showed maximum viability at 0 or 31-40 per cent relative humidity. Sometimes, specially at low temperatures, 50 to 60 per cent relative humidity was also favourable (Vasil, 1962).

Stanley and Linskens (1974) listed 36 species, the viability of which could be substantially extended at a temperature of about 0°C. Viability could be extended beyond one year and up to three years or longer for some pollens by storage at 0°C or -15°C with relative humidity between 10 and 50 per cent (Neble and Ruttle, 1937). King (1965) after investigating for a long time, over a diversified taxa also listed the details of pollen storage under controlled temperature and humidity. Low temperature storage of pollen has been reported in a number of species - *Citrus* sp. (Kobayashi *et al.*, 1998), *Artocarpus* sp. (Sinha, 1972), tree pollen (Ichikawa and Shidei, 1971), sunflower, *Helianthus* sp. (Frank *et al.*, 1982) avocado (Sedgley, 1981).

The primary reason for decreased pollen viability in storage is probably related to enzyme activities which decrease respiratory substrates. The mechanism by which pollen retain its viability during storage is related to the intracellular rates of respiration, e.g., the conversion of sugars to organic acids (Neble and Ruttle, 1937; Stanley and Poostchi, 1962). This was confirmed by the observation that, stored pollen required higher concentrations of sugar for normal germination than fresh pollen (Vasil, 1962). The rate of respiration decreased with time in storage while, sensitivity of the stored pollen to boron increased (Visser, 1955). There is no detail report on the genetic behaviour of the pollen in storage, however, it has been suggested that old pollen gave rise to a higher number of mutations (Stanley and Linskens, 1974). Pfeiffer (1955) reported that the germination capacity was related to changes in oil deposits in the pollen exine. Mineral nutrition of the plant during pollen development also influenced the longevity of the pollen grains. Contamination of the pollen grains either at the time of collection or during storage might also reduce the viability or longevity of pollen in storage.

In the present investigation, it was observed that except in triploids and interspecific hybrids, the viability of pollen grains of diploids and polyploids species was about 90 per cent. Pollen fertility in *C. sinensis* and *C. japonica* was reported to be more than 85 per cent (Kato and Simura, 1970) and more than 90 per cent (Kato and Simura, 1978). Viability of pollen grains of 23 tea clones in South India has also been found to be more than 75 per cent (Mohanan and Sharma, 1981). While Rashid *et al.* (1985) reported more than 83 per cent viability of the fresh pollen of diploid and tetraploid tea. Ackerman and Kondo (1980) observed more than 80 per cent viability on the basis of first 500 pollen grains. However, the viability of the pollen grains of triploid tea and interspecific hybrid was observed to be about 78 per cent and about 80 per cent, respectively. Previous workers reported the viability of triploid or hybrid pollens to be 41 per cent (Ackerman, 1980), 38 per cent (Mohanan and Sharma, 1981) and about 78 per cent (Rashid *et al.*, 1985). Bezbaruah (1975c) observed a very high pollen sterility in triploid tea, while in the interspecific hybrids between tea and Wilson's *Camellia, C. irrawadiensis* pollen viability was above 95 per cent (Bezbaruah, 1975b).

The longevity of the pollen grains of almost all the species of *Camellia* and related genera was maximum, up to 203 days at 0°C and 35 per cent relative humidity (Table 4.2). Pollen grains stored in desiccators at 40 per cent relative humidity and 0 to –2°C maintained viability at least for five months in Sri Lanka (Anon., 1956). Amma and Watanabe (1983) reported that the pollen grains of tea packed in nitrogen gas, wrapped with parafilm paper, sealed and stored at 1°C remained viable for four years. However, at ultra-low temperatures (–40°C and –80°C) the grains retained viability for more than five years. Bezbaruah (1971a)could store the freshly collected pollen with a viability of 89.6 to 98.8 per cent up to about 8 to 10 days at ambient conditions of temperature and humidity during the flowering time in North East India. Tea pollen remained viable up to 105 days when stored at 0°C (Bezbaruah, 1971a). Ahmed *et al.* (1966) in Bangladesh reported that about 20 per cent of the tea pollen remained viable for up to 50 days at 5 to 7°C.

The results of present study are in agreement with the observation of Kato and Simura (1978), who were also successful in storing pollen grains of tea, *C. sinensis* for 6 months at -2°C with a viability of 52.8 per cent, and Visser and Tillekeratne (1958) who observed more than 60 per cent germination of the pollens stored for 120 days at 0°C and 40 per cent relative humidity. The pollen grains soaked in acetone, n-amyl alcohol, butyl alcohol, ether, petroleum ether and pentane and stored for three days at 5 to 6°C showed better pollen tube growth than fresh pollen of *C. japonica, Lilium* spp. and *Impatiens balsamina* (Iwanami and Nakamura, 1972). The pollen grains lost viability either due to dehydration resulting in shrinkage in pollens or due to bacterial, fungal and/or algal infection, particularly at higher levels of temperature and humidity. Bezbaruah (1971) also encountered similar problem of infection by fungi and bacteria in storing the pollen grains of tea which resulted in loss of viability.

5.3 INTERSPECIFIC AND INTERGENERIC HYBRIDIZATION

In the present investigation out of a total of 13,195 flower buds artificially pollinated, representing 154 interspecific and intergeneric combinations, 465 capsules (4.88%) were harvested resulting from 58 compatible combinations. Ackerman (1971) reported that out of 8741 controlled pollination among ornamental *Camellia* and related genera representing 219 combinations only 459 hybrid plants from 106 combinations were obtained. Ackerman and Williams (1982) also reported 6574 crosses during 1968 to 1976 between genera within *Theaceae* including *Camellia, Franklinia, Stewartia* and *Tutcheria* which resulted in 179 seed capsule (2.72%), out of which, only 16 seeds germinated. The higher percentage of success in fruit-set obtained in the present study was due to inclusion of four taxa of tea which combined better among themselves as compared with non-tea species. Among the tea species, the success percentage was as high as 50.79 per cent (*C. sinensis* X *C. assamica*). Bezbaruah and Saikia (1977) reported up to 50.6 per cent fruit set among tea species. Wight (1939) reported 40 per cent fruit set and Satyanarayana and Sharma (1981) obtained 32 per cent capsules in the interspecific crosses involving Assam X Cambod teas.

The present successful report of hybridization between *C. sasanqua* and all the tea species were similar to the reports of Nagata and Sakai (1981), Nagata *et al.* (1986), Fuchinone (1975), Kondo (1977), Kato and Simura (1970) and Ackerman (1971). However, in the reciprocal cross with tea species, fruit could be harvested only with *C. sinensis* as seed bearer. Similar observation was reported by Nagata and Sakai (1985), Kato and Simura (1970). Other two tea species, *viz.*, *C. assamica* and *C. cambodiensis* failed to produce seeds. Bezbaruah and Saikia (1977) also failed to get a single fruit set out of 144 flower pollinated using Assam tea as female parent and *C. sasanqua* as pollen parent.

All the three tea species, Assam, Cambod and China produced fruits 4.96, 2.78 and 3.87 per cent, respectively, when used as female parent with *C. irrawadiensis* as pollen parent. Wight and Barua (1957) first reported successful hybridization between Assam tea, *C. sinensis* var. *assamica* X *C. irrawadiensis*. Bezbaruah (1975) obtained hybrid seeds from Assam tea X *C. irrawadiensis* and Takeda (1990) reported successful hybridization between *C. sinensis* and *C. irrawadiensis*. In the reciprocal crosses using *C. irrawadiensis* as seed parent and all the three tea species, Assam, Cambod and China as pollen parent, *C. assamica* was found to be more closely related (with a fruit set of 4.61%) as compared to *C. cambodiensis* (4.00%) and *C. sinensis* (3.33%). This was the first report of successful hybridization using *C. irrawadiensis* as seed bearer. Bezbaruah

(1975) reported satisfactory fruit-set in *C. irrawadiensis* crossed with *C. assamica*, however, all the developing fruits dropped off before attaining maturity.

Out of 43 flower buds pollinated on *C. assamica* X *C. japonica*, only 5 fruits (11.63%) matured and harvested. *C. sinensis* was also observed to be compatible with *C. japonica* (4.69% fruit set). However, *C. japonica*, as female parent was only compatible with *C. sinensis*. Assam tea, *C. assamica* and Cambod tea, *C. cambodiensis* when used as female parent, did not set fruit. Hybrids between *C. japonica* X *C. sinensis* were reported by Kitamura (1970), Nagata *et al.* (1986), Kondo (1977) in Japan and Ackerman (1971) in the U.S.A. Reciprocal cross was only reported by Nagata *et al.* (1986). Kato and Simura (1978) obtained only 11 fruits from controlled pollination of 226 flowers. However, they also failed to get any fruit from 1579 flowers pollinated in the reciprocal cross involving *C. sinensis* X *C. japonica*. Takeda *et al.* (1987) obtained 9 hybrid plants from 16 seeds produced from 13 fruits resulted from 300 pollination in *C. sinensis* X *C. japonica* and named them as 'Chatsubaki'. The present report of successful hybridization between tetraploid tea and *C. japonica* was in accordance with the earlier report by Bezbaruah and Gogoi (1972). Kitamura (1970) speculated that the Wabisuke group might have originated in the hybridization between *C. japonica* X *C. sinensis*. Bezbaruah (1977) reported successful hybridization between Assam tea X *C. japonica* with a fruit set of 30.0 per cent.

All the three tea species and other non-tea species *viz.*, *C. drupifera*, *C. irrawadiensis*, *C. japonica* and *C. kissi* were found to produce viable seeds with tetraploid species of tea. Among the tea species, China tea, *C. sinensis* observed to be the best pollen parent with a fruit set of 37.24 per cent, followed by Assam tea and Cambod tea species with 26.75 and 22.27 per cent fruit set. Among the non-tea species, *C. drupifera* had close relationship, which was reflected by a fruit set of 10.16 per cent. However, all the reciprocal crosses failed. Toyao (1960) reported 35.0 per cent fruit-set in tetraploid X diploid tea and only a few fruits with abortive seeds in the reciprocal crosses. Singh *et al.*, (1981) also reported tetraploid X diploid tea species. They (Singh *et al.*, 1981) obtained 29 sinker seeds from 757 flowers artificially pollinated.

It has been observed that with a few exception like *C. sasanqua* and *C. drupifera* inter-specific hybrid within a sub-genus having similar chromosome number or ploidy level, usually show greater fertility than hybrids between sub-genera with different chromosome numbers. Hilsman (1968) and Savige (1967) reported that the most successful crosses in *Camellia* breeding were found within the same section particularly the species with the same ploidy. Hagiya (1982, 1986) also found that the *Camellia* species belonging to the same section were more compatible than the species of different sections and in the cases of intersectional crosses, those with the same ploidy were more compatible than those with different chromosome numbers. Ackerman (1973) showed that the species of certain section were more closely related to each other than to species of other section based on the cross compatibility between their representative species.

C. japonica was the most cold resistant and hardiest species among various species tested in the genus *Camellia*, followed by the plants belonging to the section *Paracamellia* as a species of *C. sasanqua* (Toyao *et al*, 1974). In the present investigation, both the species, *C. japonica* and *C. sasanqua* produced viable seeds when hybridized with *C. sinensis* and *C. assamica*. So, there is a good potentiality to transfer winter hardiness to the hybrid progeny and thereby it may offer great opportunity to produce tea plants with lesser winter dormancy. These hybrids would be valuable material for the breeders to increase the plucking rounds and in turn production during winter or dormant months. Takeda *et al.* (1987) investigated inter-specific hybridization to introduce favourable characters of *C. japonica* like cold hardiness and disease resistance to tea plants. These hybrid progeny would certainly increase the genetic variability in tea and thereby broaden the genetic base of tea population. Barua (1965) after detailed study of various tea taxa, concluded that the complex hybrids among the three kinds (species) of tea and one or more of the wild species of *Camellia* occur in the cultivated tea population, particularly in the Darjeeling district of North East India, which produce distinctive quality and flavour.

Bezbaruah and Saikia (1977) and Ackerman (1971, 1973) also reported that a number of related species of tea was found to be compatible with tea which provide ample scope for incorporation of desirable genome from their sources for breeding and improvement of the tea plant.

Two fruits were obtained from 50 flowers pollinated in the cross *C. japonica* X *C. kissi*. However, all the reciprocal crosses failed. Nagata and Sakai (1981, 1985) reported natural interspecific hybridization between *C. japonica* X *C. kissi* in Japan, where the seed parent is endemic. Ackerman (1971) also reported successful controlled pollination between *C. japonica* X *C. kissi* and reciprocal cross.

In the present study of interspecific and intergeneric crosses involving tea species as one of the parents, it was observed that China tea, *C. sinensis* combined best with other species followed by Assam tea, *C. assamica* and Cambod tea. *C. cambodiensis* was the poorest as both pollen and seed parent. Similar observation was also recorded by Bezbaruah and Saikia (1977) in the crosses involving various species of tea that the China type plant was the best as a female parent while Assam type was the best as pollen parent. Satyanarayana and Sharma (1981) reported that the percentage of fruitset was consistently high in the predominantly "China" type of clones while it was low in the clones of Cambod origin. Assam type of clones as female parents gave moderate to high fruit-set. On the basis of fruit-set, Assam type plants were the best followed by 'China type'; Cambod type as the females registered the lowest fruit set. In the study of Satyanarayana and Sharma (1981) observation, one of the two Cambod parents pollinated did not set fruit when crossed with various types of male parents while another clone did set fruit.

Among the intergeneric hybridization, all the three species of tea were found to be compatible with *P. berringtoniaefolia*. All the crosses produced viable seeds. This was the first report of successful intergeneric hybridization between tea species and *P. berringtoniaefolia*. However, only Assam tea, *C. assamica* and China tea, *C. sinensis* were found to be compatible with *G. excelsa*, when the later was used as female parent. Reciprocal crosses and crosses involving Cambod tea, *C. cambodiensis* failed. Wight and Barua (1939) attempted hybridization between *Pyrenaria* and tea but were unsuccessful. Other intergeneric hybrids reported in *Theaceae* include *C. japonica* X *Franklinia alatamaha*, *C. sasanqua* X *F. alatamaha* (Ackerman and Williams, 1982). They attempted 6574 controlled pollination which resulted in only three intergeneric hybrids, which survived for one year.

5.4 RECEPTIVITY OF STIGMA

The current findings of receptivity of stigma for a short period were in support of the observation of Maheshwari (1971) in a number of taxa. Wight (1938) also was of the opinion after observing the pollen germination and pollen tube growth of various crosses including reciprocals and self, that for hybridization, the flower buds should be emasculated 24 hours prior to anthesis. Low percentage of germination of pollen grains on the stigmatic surface and successive slow rate of growth of elongation of pollen-tube observed in most of the species after 1.00 p.m. might be due to comparatively dry stigmatic surface which resulted in the failure of pollen adhesion, hydration and germination. Shivanna and Johri (1985) mentioned that the lipoidal substances of the stigmatic surface help in tapping the pollen and in protecting the stigma from desiccation or wetting. The phenolic compounds play important roles in promotion and inhibition of pollen grains on stigma (Martin, 1972; Tara and Namboodiri, 1976). The first pollen tube penetrate the proximal end of the style in about 18 hours, under conditions morbid changes could be seen in the style of the tea flower, 24 to 48 hours after pollination (Wight, 1938). The period of receptivity varied from species to species and was influenced by temperature and humidity. In *Lilium* (Ascher and Peloquin, 1966) and *Petunia* (Shivanna and Rangaswamy, 1969) observed that the unpollinated stigma remained receptive for more than eight days after anthesis.

Maximum percentage of dropping off of flower buds within 15 to 30 days when pollinated three days prior to anthesis was in agreement with the observation of Sharma *et al.* (1981), Wight (1938) and Bezbruah (1971a) on tea. Sharma *et al.* (1981) reported that all the flower buds dropped when young flower buds of about 6 mm to 8 mm diameter in tea were pollinated. However, when pollinated in the pre-balloon stage and balloon stage (with 11 mm and 13-18 mm diameter) 68 per cent of the ovaries enlarged in size. While only 5 per cent of the ovaries remained on the bush when young buds were pollinated. Shivanna and Johri (1985) also reported that the receptivity of stigma was generally maximum soon after anthesis and the period of receptivity varied from species to species.

5.5 SELF-INCOMPATIBILITY AND ITS MECHANISM

In the present investigation the percentage of fruit-set in various species of *Camellia* and related genera, as a result of selfing ranged from 0 to 10.0 per cent, with an average of 2.78 per cent (Table 4.11). Various authors also observed similar range of fruit-set percentage from selfing in various tea species - 0 to 6.2 per cent (Bezbaruah, 1971a),0.98-9.88 per cent (Bezbaruah and Saikia, 1977), 2 to 3 per cent in South India (Satyanarayana and Sharma, 1981), up to 1.00 per cent with Japanese varieties (Toya, 1966), 0 to 4.0 per cent (Sebastiampillai, 1961) in Sri Lanka and Bakhtadze (1940) in Russia. Wight (1938) obtained only 9 fruit from a total of 246 flower buds artificially selfed with a success of 3.66 per cent, the range of success varied from 0 to 8.0 per cent among various types of clones of tea. All of them recorded variation among various types of clones and even individual plants within a particular clone or *jat* of tea. Kondo (1979) after carrying out detailed compatibility studies concluded that tea, *C. sinensis* was self incompatible and the degree of compatibility decreases when individual clones were inbred. The tea plant showed an appreciable degree of self-sterility and invariably set a better crop of seed with pollen from another bush. In particular cases, both greater and lesser successes might be from self-pollination (Wight and Barua, 1939). The complete self-incompatibility or low success in fruit-set observed in various taxa of *Camellia* and related genera might be due to the differences in the genomic constitution of the individual plants.

There was considerable variation in the degree of self-incompatibility among various taxa studied. *C. irrawadiensis*, *C. rosaeflora* and F_1 hybrids of *C. sinensis* x *C. japonica* were completely self-sterile. The highest percentage of fruit-set (10.0%) was recorded in *C. drupifera*. Among the tea species, self-incompatibility was lowest in China tea and more prominent in Cambod tea, *C. cambodiensis*. Variation in the degree of self-incompatibility in various types, clones and even individual plants of tea has been reported by various authors. Wight (1948) after conducting hybridization experiments for many years concluded that the more heterozygous the plant, the greater its self-fertility. China tea was more self-fertile as compared to extreme Assam tea. Bezbaruah and Saikia (1977) observed that the degree of self-incompatibility was generally more prominent in China and less prominent in Cambod, while Assam type was intermediate between the two extremes. However, Satyanarayana and Sharma (1981) observed that Assam type of clones were highly compatible and Cambod was least compatible, which was in agreement with the present findings.

A total of 242 seeds were obtained from 125 fruits, out of which, only 101 seeds were sinkers. Rest of the seeds were either empty, partially developed or deformed. Among the sinker seeds, 58 seeds germinated and only 30 seedlings survived for more than three months. Wight (1938) also obtained only 10 seedlings out of 12 seeds obtained from only 9 fruits resulting from artificially selfing 246 flower buds. Bakhtadze (1940) artificially selfed 20 tea plants in Russia, from which, only 53 seeds resulted, 10 seeds germinated and only five seedlings survived for more than 18 months. Most of the seeds resulting from self-pollination were empty, without embryo, which might have degenerated at a very early stage of development. Partially developed or shrivelled seeds resulting from self-pollination having reduced germinability was also reported by Wight and Barua (1938), Wight (1948) and Fuchinoue (1979). Shrivelled seeds with reduced germination was also reported in grasses - *Festuca* and *Lollium* (Matzk, 1976). Hwang *et al.* (1990) recorded only 13 embryo from a total of 132 seeds resulted from hybridization among the species which were cross-incompatible. Except a few, most of the seedlings were very weak, stunted and segregation of morphological characters reflected the highly heterozygous condition of the genotypes of the parents, which might be due to inbreeding depression. Similar observation was reported by Wight (1938), Wight and Barua (1939), Bakhtadze (1932) in tea.

In the present investigation, detailed study has been made to ascertain the mechanism of self-incompatibility in various species *Camellia* and related genera. It was observed that the pollen grains germinated almost similarly in selfed as well as crossed stigma. Rate of elongation of pollen tube was comparatively slower in the selfed style as compared to crossed. So the nature of incompatibility system was not caused by any factor inhibiting pollen germination and tube growth in the stigmatic surface or because of sporophytic reaction. Similar observation was also recorded by Bezbaruah (1971) Bezbaruah and Saikia (1977). However, Tomo *et al* (1956) and Fuchinoue and Fuchinoue (1966,1979) reported inhibition of pollen tube growth at the base of the style in the case of self-pollination.

Self-incompatibility is very common in flowering plants, more than 300 species belonging to 20 families of angiosperms exhibited self-incompatibility Darwin carried out first systematic studies on many self-incompatible taxa during 1876 and 1877 and emphasized its significance as an out-breeding mechanism. The most commonly accepted definition of self-incompatibility is the inability of the plant producing functional gametes to set seeds upon self-pollination (Brewbaker, 1957). de Nettancourt (1977) modified the definition as the inability of a fertile hermaphrodite seed plant to produce zygote after self-pollination.

Lewis (1954) classified the self-incompatibility into two heteromorphic system and homomorphic system. Later may be either gametophytically controlled or sporophytically controlled. Genetic interpretation of the gametophytic incompatibility in *Nicotiana sanderae* was put forwarded by East and Mangelsdorf (1925). According to which, the incompatibility reaction is determined by a single gene having multiple alleles as in *Nicotiana*, *Lycopersicon*, *Solanum* etc. However, the mechanism of self-incompatibility is quite complex and is poorly understood. The location of incompatibility factors in gametophytic system is not clear (Shivanna and Johri, 1985). Different forms of interactions between genetically specified proteins, haploid pollen grains and the diploid stigma have been grouped into three broad catagories–pollen stigma interaction, pollen tube-style interaction and pollen tube–ovule interaction.

In the present investigation, it was observed that after germinating normally on the selfed and crossed stigmatic surface, the pollen tube extended almost equally to a certain extent in the styles of both compatible and incompatible pollination, which indicated that the mechanism of self-incompatibility in tea was not sporophytically controlled. However, after traversing a few millimeters in the style canal, the selfed pollen tubes produced a bulb like swelling and in certain cases, the growth was inhibited and dicposition of callose was observed, which plugged the pollen tube. In certain other instances, the growth of the pollen tube at the proximal end of the tube was irregular and the growth was inhibited and they did not reach the micropyler end to the tube nucleus for fertilization, which indicated that the self-incompatibility system was gametophytically controlled. This was in support of Brewbaker (1957) findings that the species in which pollen granis were shed at the two-celled stage showed gametophytic type of incompatibility and the zone of inhibition was in the style.

It could also be concluded from the findings of the present investigation that the self-incompatibility operating in *Camellia* might be due to multiple allele system. The incompatibility was caused by the relationship between the genotypes of pollen and that of style. When both of them were of the same genotype, fertilization could not take place. However, the genetics of self-incompatibility has been poorly understood. Fuchinoue and Fuchinoue (1979), Heslop-Harrison *et al* (1975a) and Heslop-Harrison and Shivanna (1977) devised a correlation between the nature of the stigmatic surface and the type of incompatibility. The taxa characterised by gametophytic system was associated with wet stigma. The stigma of all the species studied were also wet and helped the germination of the pollen grains. This is the confirmatory observation in support of the present investigation. Tupy (1959), Schlosser (1961), de Nettancourt *et al.* (1974) observed that in gametophytic system, the pollen tube inhibition was associated with extensive deposition of callose in the tube. Often, the entire tip of the tube got plugged with callose (Heslop-Harrison *et al.*, 1974; Sastri and Shivanna, 1979). The tips of incompatible tubes might swell and/or burst, de Nettancourt (1972,1973a, 1973b, 1974) findings was that the compatible pollen tubes had a wall of two layers – an outer layer of loose fibrils and an inner homogeneous layer. Incompatible tubes displayed a similar wall structure during initial growth. As it grows one third of the style, the inner layer became thinner and burst open in the styler tissue and numerous particles accumulated in the tube cytoplasm. They also hypothesized that the tube wall was the site of action for the incompatibility proteins and suggested that self-incompatibility was not a passive process but an active phenomenon, which lead to the dismetelling of the tube-wall into large number of particles. All the diploid species of the genus *Coffee* were self-sterile and when selfed, the pollen tubes after entering the stigmatic or styler tissue produced bulb-like swelling at their tips and usually burst without reaching the ovule proper (Mendes, 1949, de Vreaux *et al.*, 1959; Santa Ram *et al.*, 1981) which closely pointed out that certain other developmental factors interferred with the reproduction of the hybrid and resulted in hybrid sterility.

It was observed that the seedlings resulting from selfing or incompatible crosses were inferior to compatible hybridization, many of the selfed progenies died soon after germination or after growing for a few weeks. While, some others produced empty seeds without embryo and cotyledon. On the outer side, they were normal, in those cases the hard seed coat developed from the outer integuments. However, due to lack of fertilization, syngamy and triple fusion development of embryo, endosperms and cotyledon was either blocked or they degenerated soon after formation. Stebbins (1950) divided hybridization barriers into two broad categories – pre-fertilization and post-fertilization barriers. The formar included those mechanisms which prevented fertilization, such as geopraphical location, apomixis and pollen-pistil incompatiblities. Post-fertilization barriers which on the other hand, imposed greater hindrance to hybridization and could be resulted from ploidy differences, chromosome elimination, incompatible cytoplasm, seed dormancy and hybrid lethality or mortality (Collins *et al.*, 1987). Gill *et al.* (1981) however, postulated that the non - viability or weaknesses of the F_1 hybrids could be due to lack of harmony between genomes of the parental species, between genomes of one species and cytoplasm of the other, between genotypes of the F_1 zygotes of the genotypes of endosperms or maternal tissue.

In *Prunus* (Raff and Knox, 1977) and tea (Masuda, 1955; Bezbaruah, 1971), removal of the stigma and pollination of the cut end of the style was effective in overcoming the self-incompatibility, which indicated that the incompatibility factors were confined to the style. In certain crosses, e.g., *C. irrawadiensis* X tea species, *C. japonica* X tea species, *G. excelsa* X tea species, low percentage of fruit - set might be due to longer length of the style of the species used as female parent as compared to the length to which the pollen tube would normally grow. In those cases, pollen tube failed to reach the embryo-sac. Similar mechanism was reported in intergeneric crosses involving *Zea mays* X *Tripsacum* species (Reeves and Bockholt, 1964). In some other cases, involving polyploid species like - tetraploid tea, hexaploid *C. sasanqua* or triploid *C. rosaeflora* as male parent, the failure of zygote formation might be resulted from comparatively thicker and slower rate of growth of pollen tube in the styles of diploid species. Similar mechanism was also reported in *Datura* (Singh, 1983). In *Oenothera* (Makinen and Lewis, 1962) and some grasses (Matzk, 1976) where inhibition took place in the stigma itself, the pistillate factors were located on the surface of the stigma and / or intercellular spaces in the transmitting tract of the stigma. This was true even in a few systems in which inhibition was in the style. The recognition of the incompatible pollen took place in the stigma, soon after pollination, whereas, inhibition of pollen tubes was completed in the style about 24 hours later.

Recently, Mulcahy and Mulcahy (1983) put forward the heterosis model to explain gametophytic incompatibility as a passive mechanism. According to which, there were many S-genes, number of which depended on the genetic composition of the plant. Following cross - pollination, most of the S-genes would be heterozygous in the pollen and style, this resulted in interaction between them leading to an increase in the rate of pollen tube growth. However, when self-pollination was followed, a high proportion of S-genes would be homozygous resulting in a reduction in the rate of pollen tube growth, the extent of reduction dependent on the number of homozygous S-genes. Incompatibility, therefore, is due not to the specific inhibitory molecules but rather to pollen-tube growth being too slow to allow fertilization before abscission of flower.

5.6 FOLIAR MORPHOLOGY OF THE PARENTAL SPECIES

Though the morphological characters are considered more often than others to distinguish the species or varieties, in tea, but most of the morphological studies have been done mainly to classify tea into three forms, types, subspecies or to the specific rank. In recent years, various morphological characters of a particular clone or stock was studied only when they were included in the hybridization or breeding programmes to compare the performance of the hybrids with that of the parents. Therefore, in the present study morphological characters of all the species of *Camellia* and related genera available in Tocklai Experimental Station, Jorhat were included. The results of the present investigation on tea species were in agreement with earlier findings, though very scanty. Assam (Naga) tea, *C. assamica* leaf was observed to be of 19.37 cm long, 6.14 cm width and with a leaf angle of 88°. Watt and Mann (1903) also reported that the Naga tea possessed 15 to 22.5 cm long and 5 to 8.75 cm wide leaf. Hashimoto (1973) found wide variability in size and shape of Assam type of tea. Length, width and length to width ratio were observed to be 19.2 ± 1.78 cm, 9.8 ± 0.1 cm and 2.1 ± 0.13, respectively. Similarly Cambod and China teas were also within the range observed by him (Hashimoto, 1973). Vegetative characters give reliable criteria for identification of a species (Wight, 1939), there were considerable variation in floral characters (Barua, 1963). Morphological characters are important as they exhibit positive correlationship between size and yield in tea population in USSR (Memedov, 1961), Japan (Toya, 1966). Hadfield (1968) after studying the morphological characters of various forms of tea concluded that large leaved Assam type of plants having more leaf angle or droppy nature of leaf were unsuitable for higher crop production under North East Indian climatic condition. Satyanarayana and

Sharma (1981) studied various biometric parameters and showed a positive association with yield. Wickremaratne (1981) also studied various morphological characters to identify and distinguish between clones and concluded that it was not usually possible to recognize clones on the basis of a single character but rather a combination of characters was required. Depending on the leaf size, ratio of leaf length to width, leaf angle and internodal length, twelve tea clones could be distinguished in Sri Lanka. However, Bezbaruah (1968) could find correlationship between leaf size and yield only in China and China hybrid plants but failed to observe any correlation among other teas. In tea, morphological characters are important for selection by visual observation of the bushes. The present results of length, breadth, length to breadth ratio, leaf angle of Assam, Cambod and China teas were also similar to the observation of Sharma and Venkataramani (1974). Leaf angle of tetraploid and triploid tea was observed to be 72.8° and 70.6°, respectively. Sarmah and Bezbaruah (1984) observed a leaf angle of 66.8° in a tetra ploid stock and in the triploid F_1 hybrid ranged from 59.34 to 63.54°, respectively. Foliar morphological characters observed in the present investigation on other species *viz., C. drupifera, C. irrawadiensis, C. japonica, C. kissi, C. rosaeflora, C. sasanqua* and *C. sinensis* were within the range as recorded by Sealy (1958). Bezbaruah (1975c) also observed leaf length of 8.5 to 15.7 cm, width 3.2 to 5.9 cm in the case of *C. irrawadiensis.* While Kondo *et al.*, (1986) studied the length, breadth and length to breadth ratio of *C. sasanqua* which were also within the range as observed by the present investigation.

Leaf area plays an important role influencing the carbohydrate metabolism of a plant, the determination of which has profound effect in predicting the productivity of a cultivar, particularly in a leaf crop like tea. Comparatively more bigger leaf, observed in tetraploid and triploid plants was in support of the observation by Sarmah and Bezbaruah (1984) and Bezbaruah (1975b).

Anthocyanin pigmentation was observed in most of the species investigated, particularly in *C. cambodiensis, C. irrawadiensis, G. exclsa, C. drupifera* etc. (Table 4.13). Roberts *et al.* (1958) also observed that the leaves of many plants of Cambod variety developed leaf colouration ranging from coppery yellow to ox-blood red, particularly to the end of the season. Autumnal colour never developed on young leaves of indigenous Assam tea while in Chinery plants, garnet brown through ox-blood to purple colouration developed on the young leaves. The colouration of tender leaves was closely related to the quality of made tea manufactured from the shoots with green or yellow green colouration was of good quality in most cases. The violet shoots do not produce good quality of made tea, especially to green tea (Wang *et al.*, 1987). Visser (1969) also pointed out that the occurrence of slight red anthocyanin pigment in the leaf petiole with pubescence resulted in better quality made tea.

5.7 ANATOMICAL STUDIES ON PARENTAL SPECIES

5.7.1 Stomata

The number of stomata per 0.64 sq.mm ranged from 14.9 to 58.1 among all the species of *Camellia* and related genera (Table 4.14). The sizes ranged from 698.63 sq.µm to 1824.82 sq.µm. In general, the frequency and sizes of stomata were inversely related in soya bean (Guha and Braun. 1975) and Coffee (Mishra *et al.*, 1991). The three tea species *viz.*, Assam, Cambod and China, the frequencies were 21.9, 25.3 and 28.5, respectively, while their sizes were 1168.32, 1011.37 and 1296.42 sq.µm. Chaudhuri and Bezbaruah (1985) also observed 42.0 to 103.7 stomata per 0.64 sq.mm among tea plants and the size varied from 1034.16 sq.µm to 1893.39 sq.µm. They could not find any correlationship with the ploidy level. In tetraploid teas, the sizes were 1824.82 sq.µm. In the present investigation, the sizes of stomata in triploid and tetraploid were comparatively larger. In *C. sasanqua* which was a hexaploid species, had 22.4 stomata with a size of 1089.08 sq.µm. In other species, the number and sizes of stomata observed were similar to the observation of Ackerman and Chang (1983), Mazumdar and Bezbaruah (1978) in 20 TV clones of tea and Bezbaruah (1976) in aneuploids and polyploid teas. Rashid *et al.* (1985) also reported only fifteen stomata in diploid and only six in case of tetraploid taxa.

The variation in stomatal density observed in the present investigation among the species were in agreement with the findings in other crop plants. The frequency of stomata may vary on different parts of the leaf lamina and on different leaves of the same plant (Esau, 1962). The present findings of lesser frequency of the larger stomata and vice-versa was in agreement with the observation by Mayer (1973). Eames and Mac Daniels (1947) also indicated that the number of stomata per unit area generally increases towards the margin and the apex of leaf. Rao and Vishveshwara (1960) and Mayer (1973) observed that in a species with smaller stomata were more densely present, as compared to the frequency of large stomata which were more dispersed. Grice (1972) and Barua (1975) could not correlate drought tolerance of clones with either size or density of the stomata. Raman and Chang (1981) also investigated the stomatal density of four different clones of tea, the frequency varied from 310 ± 10 to 550 ± 25. Karasawa (1932) also observed that the sizes of stomata of polyploid species were significantly larger than diploid species.

5.7.2 Chloroplast

The number of chloroplast per guard cell of stomata ranged from 14.9 in *E. japonica* to 42.8 in *C. sasanqua*. It was generally observed that the number of chloroplasts increased with the increase in ploidy level in various species studied. In diploid species, the number was around twenty with slight variation except in *E. japonica*, which had around 15 chloroplasts per guard cell. In triploid taxa, there were around 30 chloroplasts, while in tetraploid and hexaploid taxa, the number was observed to be around 40. Chen and Ye (1992) observed slightly less than 16, 24 and 32 chloroplasts in each guard cell of diploid, triploid and tetraploid tea, respectively. Ahmed and Singh (1993), however, recorded 21.9 ± 2.07 chloroplasts in diploid, 32.5 ± 3.15 for triploids and 41.4 ± 4.26 for tetraploid taxa of tea in North-East India. Nuesch (1966) reported that the guard cells of lower leaf epidermis of tetraploid red clover contained 80 per cent more chloroplasts than the diploids. Significantly, more chloroplast number in tetraploids as compared to the diploids have also been recorded in *Solanum* (Hermsen and De Boer, 1971), *Brassica* (Speckman *et al.*, 1967). Saikia and Dey (1987) observed a significant difference in the frequency and nature of distribution of stomata in two tea clones in North East India.

5.7.3 Sclereid

In the present investigation, the number of sclereids per 0.196 sq.cm of leaf lamina in three diploid tea species was observed to be 120, 108 and 148 in case of *C.*

assamica, *C. cambodiensis* and *C. sinensis*, respectively. Chaudhuri and Bezbaruah (1985) observed 742 sclereids per sq.cm of leaf area of diploid tea. In *C. kissi* and *C. sasanqua* the sclereids were restricted to near the margin of the leaf lamina, which ranged from 52 and 48, respectively. These observations were in agreement with the observation of Barua and Wight (1956). The tetraploid tea leaf had a frequency of 81 sclereids per 0.196 sq.cm. Chaudhuri and Bezbaruah (1985) recorded wide variation in the frequency of sclereid, which ranged from 29 and 403 per sq.cm of leaf area. In the non-tea species of *Camellia* related genera *Eurya* had 78, *Gordonia*, 72 and *Pyrenaria* had a frequency of 136 sclereids per 0.196 sq.cm.

Except in *C. sinensis*, *C. kissi* and sporadically in a few other species, most of the sclereids were observed to be of irregular shape so accurate measurements of the length and width was difficult. The mean length of the sclereids ranged from 87.3 µm to 210.6 µm, while the width ranged from 28.6 to 58.9 µm. Number of spicule also varied considerably, with the mean number of 3.9 to 13.7 per sclereid in the cases of *Eurya japonica* and *C. irrawadiensis*, respectively. All other species had intermediate frequency between these two extremes. Bera *et al.* (1994) observed sclereid without spicule in TV29, up to 35.0 spicule per sclereid in TV19, a Cambod type of clone of tea.

5.8 GROWTH HABIT AND MORPHOLOGICAL CHARACTERISTICS OF INTERSPECIFIC AND INTERGENERIC HYBRIDS

Growth habit and various morphological characters of interspecific and intergeneric hybrids at 6 months and 18 months after germination revealed that initially there were 58 compatible combinations, the seeds of which germinated. However, at 18 months there were hybrids representing only 31 combinations. The lesser number of survivals might be due to poor vigour, stunted growth habit of the distant hybrids. The measurement of dimensions of plant height, girth at collar, internodal length, number and size of leaf at six months old hybrids did not reflect the hybrid nature and inheritance pattern. So, these dimensions were measured at 18 months old plants to study the comparative morphological characters of the hybrids with their parents. Shanmugarajah and Kulasegaram (1991) observed that the interspecific and intergeneric hybrids were of poor vigour and at four months there were no significant differences between the plant height of various clones of tea. They also reported that the plants at 8 months reflected the yield potential and was better indicator of yield of resulting tea. Harler (1964) mentioned that nursery selection on vigour of seedlings was a general practice till recent time. Watanabe and Harada (1955), Harada *et al* (1961) and Green (1969) showed that initially growth habit of the hybrids may be slow but produced higher crop on maturity. Kulasegaram (1980) observed low vigour in the F_1 hybrids of the crosses between the clones of TRI-2000 series in Sri Lanka, might be due to inbreeding effect. Wide morphological variation observed with respect to plant and leaf might be due to heterogeneous origin of the parents which was in support of the prediction made by Satyanarayana and Sharma (1981).

In the present investigation, hybrid validity was based on comparison of morphological characters of parents and hybrids and subsequently anatomical and cytological observation. Though, there were considerable variation in all the characters studied, still in most of the cases, the ranges were in between the extents of the parents. In a few cases, only there were dominance of one of the parents. Rashid *et al.* (1985)

observed that all the F_1 hybrids between TV1 and tetraploid TRI clones of tea showed variability in their morphological attributes in comparison to their parental types. They ranged from tiny narrow to large broadly elliptical leaves. A few plants showed stunted abnormal growth, while leaves were narrow and very small, while certain others had vigorous growth habit and close morphological resemblance to the tetraploid plants. From these observation, they concluded that progenies showed variation and provided a fair degree of hybridity which usually implied polygenic determination. Wickremaratne (1981) identified and distinguished twelve tea clones depending on the leaf shape (length to width ratio) and concluded that it could be regarded as a reliable marker, most useful in grouping clones. Ackerman (1971) reported that the first evidence of validity of a young hybrid plant was the comparison of its vegetative morphological characters with those of its parents. He (Ackerman, 1971) identified 211 interspecific and intergeneric hybrids based on foliar and floral characters, particularly in the hybrids whose ploidy status was similar to that of its parents (e.g., diploid). The variation observed in the present investigation in respect of the vegetative characters presumably owing to the heterozygous nature of the parents. Considerable variation in growth habit, the shape, size and texture of leaves in the hybrids of tea was also observed by Bezbaruah (1975). However, none of the parental characters was dominant in the hybrids and they were not easily distinguishable from the cultivated tea. He described the variation observed in the hybrids due to the heterozygous nature of the parents and polygenic pattern of inheritance of the characters in the tea plant. However, Kato and Simura (1970) reported that in the gross morphology, the F_1 hybrids between *C. japonica* X *C. sinensis* was more resembled to *C. japonica*. While, in the foliar morphological characters like form, size and thickness were intermediate between the parents. From these observations they concluded that the F_1 plant was a true hybrid between *C. japonica* and *C. sinensis*.

Intermediate forms of most of the hybrids observed in the present study was in support of the observation made by various authors – Kondo (1978) in tea, Bezbaruah (1975) in the morphological and anatomical characters in the hybrids between tea and *C. irrawadiensis*. Interspecific hybrids between mung bean, Adzuki bean and their hybrids were intermetidate with respect to foliar characters and plant height was reported by Patel *et al.* (1986) in *Coffea* (Santa Ram *et al.*, 1981). *Nicotiana* (Ramanujan and Joshi, 1942). Wight and Barua (1957), Wood and Barua (1958) showed that species hybrids involving tea and *C. irrawadiensis* existed in the wild state in some parts of Upper Burma and some might have found their way into cultivated tea undetected because of their superficial similarity to the tea plants. Though the hybrids were more or less intermediate in characters between the parents, still they showed considerable variation in their growth habit, shape, size and texture of the leaves. Bezbaruah (1975) also observed that the hybrids between tea, *C. sinensis* X *C. irrawadiensis* were intermediate in morphological characters between the parents.

Morphological resemblance of the hybrids between the three tea species Assam, Cambod and China with *C. irrawadiensis* with the maternal parent supports the earlier findings at Tocklai (Anon., 1970-71). Bezbaruah and Gogoi (1972) observed the dominance of maternal parent in a few characters, while in certain others, the pollen parent was dominant. A few hybrids exhibited very weak, stunted and abnormal growth with very small, narrow leaves, which was in support of the observation made by Rashid *et al.* (1985), Hwang *et al.* (1990) in interspecific hybrids in the genus *Camellia*, might be due to distant hybridization. Parks and Kondo (1974) scored five recognizable segregation patterns on the hybrids between *C. japonica* and other species of *Camellia*, including intensity of anthocyanin pigmentation of flower. Ramanujan and Joshi (1942) also recorded the interspecific hybrids of *Nicotiana* were more or less intermediate in respect of many characters of the parents such as growth habit, leaf form and structure of flower etc. Extreme vigour recorded in some of the hybrids was in support of the findings of Bezbaruah (1974) and Bezbaruah (1987) in the interspecific hybrids between *Camellia* species. While, Toyao (1960) and Patel *et al.* (1988) observed poor growth of the F_1 hybrids in tea and beans, respectively.

Intensity of anthocyanin pigmentation in the interspecific and intergeneric hybrids in *Camellia* and related genera generally inherited the dominant parent. Stalker (1980) stated that anthocyanin pigmentation has attracted the attention of geneticists and breeders in all crop plants as this could be used in various species as a morphological marker in identification of true hybrids. Wight and Barua (1957), however, reported that the characteristic red pigment of young leaves of *C. irrawadiensis* disappeared in the hybrids between *C. sinensis* var. *assamica* X *C. irrawadiensis* and was visible as a seasonal phase in others, which was at variance with the present findings. Wang *et al.* (1987), however, found that the seedling colour under the natural hybridization had a tendency towards the maternal parent. They analysed the results of pattern of inheritance of colouration of leaves in the hybrids and concluded that if the leaves of the parents were violet or violet-red the proportion of violet or red coloured plants were more (68.5%) as compared to the parents. If the leaf colour of maternal parent was green or yellowish green, the proportion of green coloured seedlings was larger (60%). The heredity of leaf colour between the maternal parents and their progenies under artificial hybridization showed that if the colour of young tender leaves of the parents was yellow or yellowish green, the proportion of plants with yellowish-green colour generally reached 80 to 100 per cent. However, if the parents were with medium or small leaf and where the parents had slight violet shoots, the proportion of green shoot plants in the F_1 progeny were relatively low.

As observed in the present study, the hybrids between diploid *C. sinensis* X hexaploid, *C. sasanqua* were also almost maternal. All plants were of hybrid type, a few of them were vigourless and lethal from one to three years after germination (Fuchinoue, 1975). However, Ikeda (1993) observed the morphological character of the seven hybrid seedlings between *C. sinensis, C. oleifera* and *C. brevistyla,* which were very similar to those of the maternal parent. The interspecific and intergeneric hybrids obtained exhibited segregation of characters of the parents (TES, Ann. Rep., 1995) while in a few cases, they could not be distinguished from tea (Bezbaruah, 1996). Bezbaruah (1975b) mentioned that easy crossability between species, the genomes of these species might have found their way into cultivated tea undetected because of their superficial similarity to the tea plant.

5.9 ANATOMICAL CHARACTERS OF INTERSPECIFIC AND INTERGENERIC HYBRIDS

5.9.1 Stomata

The results of the present investigation on the sizes of stomata were in agreement with the findings of Bezbaruah (1976), Mazumdar and Bezbaruah (1978), Chaudhuri and Bezbaruah (1985) on tea in North East India, Raman and Chang (1981) in South India and Rashid *et al.* (1985) in Bangladesh. Bezbaruah (1976) observed a size of 39.9 µm X 25.5 µm in the diploid tea species. Mazumdar and Bezbaruah (1978) recorded a size range of 1338.13 sq.µm to 1097.60 sq.µm in tea clones in North East India. While, Chaudhuri and Bezbaruah (1985) recorded the range of size of stomata to be between 1034.16 sq.µm to 1466.88 sq.µm in diploid clones and F_1 hybrids. The corresponding sizes in tetraploid tea ranged from 48.7 µm X 36.3 µm to 52.2 µm X 39.4 µm (Bezbaruah, 1976) and 1834.16 sq.µm to 1840.88 sq.µm (Chaudhuri and Bezbaruah, 1985). Sizes of stomata remained more or less constant irrespective of their position in the leaf. However, significant difference in sizes were observed between the species.

Larger sizes of stomata, as observed in the present investigation in the triploids as compared to the diploids was also reported by Karasawa (1932) in tea. Bigger stomata were observed in the species having larger leaf area. Mazumdar and Bezbaruah (1978) also observed that the clones with larger leaf possessed bigger stomata as compared to the normal sized leaf.

In the present investigation, it was observed that the frequency or the number of stomata per unit area of leaf surface was directly related to the ploidy levels of the species. Sax and Sax (1937) discovered that the number of stomata per sq. mm was almost double in diploid taxa of *Tradescantia canaliculata* as compared to their tetraploid counterparts. Differences in the frequency of stomata in various species of *Camellia* and related genera was observed. Mazumdar and Bezbaruah (1978) also found significant differences in the density of stomata not only between tea clones, but at different positions of the leaf as well. The stomatal frequency might vary on different parts of the same leaf and on different leaves of the same plant (Esau, 1962; Mayer *et al.*, 1973). In *C. sasanqua* and *C. kissi*, the frequency of stomata was observed to be more on the margins of the leaf as compared to the middle position. Similar observation was also noted by Eames and Mac Daniels (1947). Number of stomata in *Coffea* decreased from diploid to hexaploid taxa and whenever, the size of stomata were smaller in size, they were densely packed from the larger stomata (Mayer *et al.*, 1973; Rao and Vishveshwara, 1960).

In general, Grice (1972) and Basu (1975) classified the clones of tea into drought tolerant or susceptible depending on the characteristics of the stomata. Raman and Chang (1981) observed that most of the stomata remained closed on the drought resistant clone. While, in drought susceptible clone, majority of the stomata were fully open. Skoss (1955) in a detailed study, correlated the structure and composition of anatomical structure of plant with environmental factors and permeability as various physiological activities of a plant is dependent on occurrence and frequency of stomata. Mishra *et al.* (1991) observed a positive correlationship between stomatal length and ploidy level in *Coffea arabica* and *C. canephora*. Stomatal frequency decreased with increased ploidy level. Speckman *et al.* (1965) also observed similar tendency in diploid and tetraploid *Lolium*.

5.9.2 Chloroplasts in the guard cell

The mean number of chloroplast per guard cell in the interspecfic and intergeneric hybrids observed to be generally equal to or slightly more or less than 20, 30 and 40 in case of diploids, triploids and tetraploids, respectively. There were slight variation in the number of a particular hybrid progeny. There was a positive correlationship between the chloroplast number per guard cell and ploidy levels in various hybrids of *Camellia* and related genera. Similar correlationship was reported in mulberry (Hamada and Daba, 1930; Sikdar *et al.*, 1986; Dwivedi *et al.*, 1986), *Gossypium* spp. (Chaudhuri and Burrow, 1975; Krishnaswamy and Andal, 1978 and Mehetre, 1982). Chen and Ye (1989) observed the number of chloroplast per guard cell to be equal to or slightly less than 16, 24 and 32 in diploid, triploid and tetraploid strains of tea, respectively. Ahmed and Singh (1993) also reported a good positive correlation between stomatal chloroplast number and ploidy levels in tea and concluded that this technique could be conveniently used for rapid identification of ploidy levels in tea. The guard cells of stomata in the lower leaf epidermis of tetraploid red clover contained about 80 per cent more chloroplasts as compared to the diploid taxa (Nuesch, 1966). Similar increase in the number of chloroplasts has also been reported in *Solanum* (Hermsen and Deboer, 1971) and *Brassica* (Speckmann *et al.*, 1967).

5.9.3 Sclereid

There was considerable variation in the number, size and structure of the sclereids observed in the interspecific and intergeneric F_1 hybrids among various species of *Camellia* and related genera. The number of sclereid per 0.196 sq.cm area of leaf ranged from 63 to 146. Chaudhuri and Bezbaruah (1985) reported that the frequency of sclereid was variable, ranged from 0 to 742 per sq.cm of leaf area. The present investigation on the number and structure of sclereid to be generally intermediate between the parental species was in agreement with the observation made by Wight and Barua (1957) in the hybrids between Assam tea and *C. irrawadiensis*. However, Barua and Wight (1958) reported that the sclereids of hybrids of *C. irrawadiensis* with *C. sinensis* were more or less like those of *C. irrawadiensis*. Wood and Barua (1958) studied the sclereids of the probable hybrid plant collected by Kingdon-Ward from Upper Burma and observed that the sclereids were uniform and typical of *C. irrawadiensis* and in the experimental hybrids, they were of mixed character in the same plant. From the observation of Barua (1965) on the presence of sclereid with form types similar to those of *C. irrawadiensis* plant or punctuation in the leaf of tea plants were indicative of hybridity of tea with *C. irrawadiensis*. So, in the present investigation, the hybrids having intermediate forms of sclereid were also indicative of true hybrid. As breeding experiments suggested that sclereid forms to be valid as taxonomic types. Comparatively simple crosses between tea and *C. irrawadiensis* showed chromatographic spots of both the parents. However, in back crosses hybrids with tea, the chemical composition approximated to tea but the sclereids were like those of *C. irrawadiensis* (Barua, 1965). He (Barua, 1965) further concluded that the hybrid plants between Assam and China tea contained a mixture of sclereid types, the relative frequencies of which indicated that they might be regarded as complex hybrids derived at least from four taxa namely the Assam, Cambod and *C. irrawadiensis*. Bezbaruah (1975b) also reported that the sclereids of interspecific hybrids between Assam tea and *C. irrawadiensis* were intermediate in nature.

The structure of sclereids was also variable – in some of the hybrids, the spicules were not prominent, almost like rod shaped but in some others the structure was very complex, branched with numerous spicules. Bera *et al.* (1994) also observed two types of sclereids – macro-sclereids (rod cells) and astro-sclereids (stellate cells) in tea clones. The distribution and structural differences in shape and size of the sclereids have also been reported in *Olea europaea* (Arzee, 1953), Memecylon (Rao, 1957). Among the various structural parameters of sclereids studied, Bera *et al.* (1994) concluded that the length to breadth ratio was more suitable for identification and characterization of TV clones. Foster (1944) observed the structure of sclereids in *C. japonica, C. reticulata* and *C. sinensis* to be remarkably polymorphic, ranging in shape from fusiform types to elaborately ramified elements with all form-types, more or less numerous, short conical or irregular spicules. Roberts *et al.* (1958) used the presence or absence of sclereids to indicate the closeness of the different species in the genus *Camellia*. While Wu (1968) correlated the anatomical characters of tea leaves with the quality of made tea in Taiwan.

5.10 CYTOLOGY OF THE INTERSPECIFIC AND INTERGENERIC HYBRIDS

In the present investigation, on cytology of the interspecific and intergeneric hybrids, the chromosome number was observed to be of average value between the parents. Ackerman (1973) observed all the 402 interspecific hybrid seedlings except only 11, to be diploid in the genus *Camellia*. The karyotypes of five clones of *C. irrawadiensis* and 30 clones of *C. sinensis* had the chromosome number of 2n = 30. In all the karyotypes, only gradual decrease in size from the longest to the shortest chromosome was observed. Satellite chromosomes were not found in the five clones of *C. irrawadiensis* studied. 28 out of 30 clones of *C. sinensis* var. *sinensis* had four sat-chromosome which was more in number as previously documented by Kato and Simura (1971), while one had sat-chromosome, which was the same in number as the previous report by Kondo (1978) and one had no sat-chromosome (Ackerman, 1971). Being similar to *C. sinensis* var. *sinensis*, a wider range of morphological variability in the cultivars of *C. japonica* might be directly correlated with their wider range of karyotypic variation (Kondo, 1978). Since cultivated *Camellia* is perennial and was artificially selected for economic purposes, all the genetical and cytological factors have been accumulated and incorporated into the genomes without regard to reproductive fitness (Kondo, 1979). Rashid *et al.* (1985) reported five growing hybrids between diploid and tetraploid tea. F_1 hybrids from *Nicotiana glauca* (2n=24) X *N. plumbaginifolia* (2n=20) were with 22 chromosomes.

Aneuploids observed in the present investigation were in agreement with the earlier findings of tetraploid x diploid *Camellia* reported by Ackerman (1973) and Ackerman and Kondo (1980). Ackerman (1973) recorded a hybrid with meiotic irregularities having 31 and 32 chromosomes from a cross between diploid *Camellia granthamiana* and *C. crapnelliana*, which might be due to interspecific hybridity resulting in the presence of partially non-homologous genomes. Ackerman (1971) mentioned that sterility was more pronounced among diploids than polyploids and the triploid produced both haploid and diploid pollen in the crosses involving *C. rosaeflora* X *C. sinensis* and *C. rosaeflora* X *C. sasanqua*. Other aneuploids resulted from hexaploid x diploid *Camellia* species had 58 (Ackerman, 1973), 52, 54 and 56 chromosome (Kondo, 1978). Bezbaruah (1976) also observed two hybrids with 32 chromosomes, one each with 63 and 38 chromosomes and three had 58 chromosome, resulting from open natural pollination of triploid parent. One plant out of 5 hybrids observed by Rashid *et al.* (1985) had 46 chromosomes.

Most of the parental species involved in the present observation were diploids, which also produced diploid hybrid with morphological similarity with tea. Takeda (1990) investigated the hybrids between various diploid species which resulted in diploid progeny. The parental species involved were *C. sinensis* with *C. japonica, C. irrawadiensis* and *C. caudata*. Only a single hybrid with 30 chromosomes was also reported by Kato and Simura (1978). Kondo (1977) observed 13 hybrid combinations between diploid species of *Camellia* and showed similar pattern in meiotic chromosome configuration and chiasma frequency at Metaphase I. These hybrid combinations showed a single chromosome configuration of 15 bivalents. The presence of sterility or partial sterility shown in many cultivated taxa of diploid *Camellia* was not correlated with chromosome configuration at Metaphase I in pollen mother cells (PMC) analysed to be due to either genetic differences or cryptic structural differences or chromosomal differences. Six hybrids developed between tea and *C. oliefera* and only one hybrid from tea with *C. brevistyla* had 30 chromosomes which implied that the seedlings might be developed through parthenogenesis and chromosome doubling of a reduced gamete.

Tetraploid (2n=60) and diploid (2n=30) crosses produced 98 seedlings, out of which, except three aneuploids, all showed triploid (2n=45) number of chromosomes. Karasawa (1932) identified a plant with larger morphological and anatomical structures to be a natural triploid tea. Bezbaruah and Gogoi (1972) reported that the interspecific hybrid resulted from tetraploid tea X *C. japonica*, to be a triploid (2n=45). Sarmah and Bezbaruah (1984) obtained 22 triploid hybrid from a combination of tetraploid and diploid tea. A range of diploid, triploid and tetraploids resulted from diploid x tetraploid teas by Rashid *et al.* (1985) in Bangladesh. Kondo (1978) and Nadamitsu (1986) also obtained viable tetraploid interspecific hybrids between *Camellia* species.

Seeds from 58 combinations germinated, however, hybrids from only 31 combinations survived for more than eighteen months. Seedlings resulting from other combinations were generally weak, with stunted growth habit, smaller internodes and leaves and died within a few weeks after germinations. Ackerman (1973) reported eleven aneuploids out of 402 interspecific and intergeneric hybrids which were also extremely weak, with short internode, small distorted leaves and considerable rosetting, one seedling died shortly and others died during their third year. Slow growth, small compact habit and lack of interstitial leaf lets was described by Vogt and Rowe (1968) as distinguishing morphological conditions of trisomic plants of *Solanum tuberosa* as compared with those of diploid sibs. Dobzhansky (1951) identified the phenomena of the inviability, weakness and sterility of interspecific hybrids connected with the development of barrier or reproductive isolation, which prevented or restricted gene exchange between populations – "the essence of the process of speculation." The causes of hybrid weakness or degeneration were grouped into three categories : the disharmony might reside entirely or chiefly in the chromosomes and genes of the two parental species, as they are combined in the hybrid nuclei, there might be a disharmonious interaction between the chromosomes or genes of one species and the cytoplasm of the other as it has been contributed by the egg of the maternal parent and the hybrid embryo might be perfectly capable of developing so far as its own constitution was concerned, but might be inhibited by the action of the maternal tissue which surrounded it or in higher plants by the endosperm which nourished it. Brieger (1928) studying the hybrids of *Nicotiana* found that breakdown of the embryo occurred most often at the stage when the vegetative growing point was differentiated. Inviability due to the action of specific genes and chromosomes was described by Hollingshed (1930) in *Crepis tectorum* X *C. capillaris*, *Nicotiana longiflora* X *N. sanderae* (Brieger, 1929), in *Gossypium* by Hutchinson (1932), Silow (1941) and Gerstel (1954) in wheat affecting crossability with rye (Lein, 1943), in *Triticum monococcum* affecting crossability with *Aegilops* and *Haynaldia* (Sears, 1944) and in *Vigna* (Sanders, 1952). In *Datura*, Cole (1956) found that the ability of *D. stramonum* to form hybrids with other species, particularly *D. ceralocaula* was greatly influenced by the trisomic condition for various chromosomes and chromosome segments. However, the exact causes of inviability or weakness of the hybrids in the present hybrids could not be studied, so more in depth study of the genetical, biochemical and physiological causes are needed.

In the present study, successful intergeneric hybrids were obtained involving *Camellia*, *Gordonia* and *Pyrenaria*, which have not been reported earlier. A total of 44 hybrids resulted from the crosses, out of which cytological observation on 25 seedlings could be made (Table 4.20). All of which showed the chromosome numbers around

2n=30. Meristematic cells of the root tips or shoot tips of the hybrids showed a chromosome number of around 30, including normal diploids and aneuploids. Ackerman (1971, 1973) reported production of eleven intergeneric hybrids among three intergeneric combinations *viz., C. pitardii* X *Tutcteria spectabilis, T. virgata X C. granthamiana* and *T. virgata* X *C. miyagii*. The cytological investigation revealed that the chromosome number ranged from diploid (2n=30) to heptaploid (2n=105) level.

Intergeneric crosses within *Theaceae* and the successful hybridization of *Camellia japonica* and *C. sasanqua* with *Franklinia alatamaha* were also reported by Ackerman and Williams (1982). Cytological observation on the *C. japonica* (2n=30) X *F. alatamaha* (2n=36) hybrid had 33 chromosomes, while, *C. sasanqua* (2n=90) X *F. alatamaha* (2n=36) hybrid had 63 chromosomes.

CHAPTER - VI

Summary

CHAPTER - VI

SUMMARY

The present investigation was undertaken for studying the morphological, cytological and genetical studies on various species of *Camellia* and related genera *viz.*, *Eurya*, *Gordonia* and *Pyrenaria* and their hybrids. The results have been summerised below :

1. Scanning Electron Microscopic study on the pollen grains of various species revealed that the size (length and width) was smallest in *C. rosaeflora*, while it was largest in tetraploid tea. In *Camellia* species the length ranged from 20.00 µm to 65.42 µm while the width ranged from 14.44 µm to 41.50 µm. The length to width ratio ranged from 1.11 to 1.96 though the average values were species specific. However, in a few species they were very close or the ranges overlapped with one another. The length of the germinal furrows were related to the length of the pollen grains in all the species except *P. berringtoniaefolia.* The surface exine pattern was also variable with distinct perforation, ridges and muri. Depending upon the size, shape, length : width ratio and surface topography of the pollen grains, various species of *Camellia* and two species of related genera could be identified.

2. Viability of the pollen grains of all the species were observed at various combinations of storage temperatures (0, 5 and 20°C) and relative humidities (0, 35 and 65%). Generally, most of the species remained viable for maximum number of

days when stored at 0°C and 35 per cent relative humidity. Both the triploid species, *C. rosaeflora* and F_1 hybrid between *C. sinensis* X *C. japonica* pollen grains remained viable for shorter period than other diploid and tetraploid species. Diploid China tea, *C. sinensis* remained viable for a maximum period of 203 days when stored at 0°C and 35 per cent relative humidity. The pollen growing of all the diploid and tetraploid species of *Camellia* remained viable over three months when stored at 0°C and 35 per cent relative humidity. The viability of the pollen grains of two *Camellia* related species, *G. excelsa* and *P. berringtoniaefolia* were retained for lesser period than the *Camellia* species when stored at similar condition.

3. A total of 13,195 flower buds were artificially (hand) pollinated during 1993-94 and 1994-95 involving 154 combinations including reciprocals among the species of *Camellia* and related genera. Out of which only 58 combinations yielded seeds which germinated to give 529 interspecific and intergeneric hybrids. However, only 209 hybrids representing 31 combinations survived for more than 18 months while others died at various times - from a few days to about six months after germination. In general, most of the interspecific and intergeneric hybrids were weak and slow in growth habit, stunted with very small leaf and internodes. Among the compatible combinations, the average per cent fruit-set varied from 1.77 in *P. berringtoniaefolia* X *C. sinensis*, to 46.65 in *C. sinensis* X *C. assamica*. Generally, higher percentage of fruit-set was observed among the tea species having similar ploidy levels (21.29 to 46.65%) followed by the combinations involving tea and non-tea species. However, the fruit-set was minimum when two non-tea *Camellia* species with different ploidy levels or distantly related combinations involving

species of related genera, as one of the parents were crossed. In a substantial number of crosses, seed capsules developed to apparent maturity but were fallen off at later stages of development, which contained partially developed or aborted embryo. Morphological characters of the interspecific and intergeneric hybrids revealed that except in a few combinations, where dominance and co-dominance of seed parent over the pollen parent was observed. Generally, the hybrids were intermediate between the parents.

The interspecific and intergeneric hybrids could be further utilized in the breeding programme for improvement of tea and other related species. The genetic base of tea in particular and *Camellia* in general, could be broadened by transferring desirable genes into popular clones by incorporating them into hybridization programme. The hybrids between *C. irrawadiensis* and *C. sasanqua* with tea species are of promise. However, they are to be assessed by analysing the agronomical, physiological and biochemical characters at maturity.

4. One of the most important factors responsible for successful seed production in artificial interspecific and intergeneric hybridization was the receptivity of stigma. In general, it was found that the *Camellia* species were protogynous and stigma remained viable from 24 hours prior to anthesis to about a few hours after anthesis. However, the observation on the fruit-set revealed that the stigmas were best receptive in the morning hours from 8.30 a.m. to 11.30 a.m. in all the species. Receptivity reduced to almost nil when pollinated after 1.00 p.m.

5. To determine the extent of self-incompatibility, all the species were artificially selfed and fruit-set was recorded. There was considerable variation among the species in fruit-set and maturity of fruits resulting from artificial selfing. Highest percentage (10.00%) of fruit-set was recorded in *Camellia drupifera* while *C. irrawadiensis, C. rosaeflora* and F_1 hybrids between *C. sinensis* X *C. japonica* were completely self-sterile. Among the tea species, *C. cambodiensis* had more prominent self-incompatibility as compared to *C. sinensis* and *C. assamica*. Among the non tea *Camellia* species, *C. japonica, C. kissi* and *C. sasanqua* produced a few fruits, so was the case with two related genera, *Gordonia* and *Pyrenaria*. Most of the seedlings resulted from selfing were very stunted, with deformed leaves and internodes were extremely small. There were considerable variation and segregation of characters in the selfed progeny.

   Anatomical observation of the selfed styles to ascertain the mechanism of self-incompatibility revealed that the rate of elongation of the pollen-tube was slower in the selfed styles than in the crossed styles. Deposition of callose tissue at the tip of the pollen tubes was observed, which inhibited further elongation, resulting in the failure fertilization. The results indicated that the incompatibility system operating in all the species of *Camellia* and related genera were gametophytically controlled. Multiple alleles of 'S' genes and certain physiological factors also played important role in the mechanism.

6. Morphological observation of the interspecific and intergeneric hybrids showed that there was wide variation in the hybrids and the characters segregated well in the hybrids and generally most of the hybrids were intermediate between the

parents. However, in a few hybrids, the dominance of maternal parent over the pollen parent was also observed.

In most of the cases, the frequency and sizes of stomata and sclereids were also observed to approach the mid-parental value. Though there was only slight differences in many species and their hybrids, so in many cases, overlapping of characters were observed.

7. Cytological observation of root tips or shoot tips to determine the chromosome number of interspecific and intergeneric hybrids disclosed that out of a total of 156 hybrids obtained from crosses between diploid X diploid, all were diploids except seven aneuploids. The number of chromosome in aneuploids were 28, 29 and 31. Aneuploids were obtained in *C. assamica* X *C. japonica, P. berringtoniaefolia* X *C. assamica, G. excelsa* X *C. cambodiensis* and reciprocal, *C. drupifera* X *C. irrawadiensis, C. drupifera* X *G. excelsa* and *P. berringtoniaefolia* X *C. sinensis*. In the tetraploid X diploid crosses, all but two hybrids had 45 chromosomes. Both the aneuploid hybrids were hypo-triploid with 43 (tetraploid tea X *C. japonica*) and 41 (tetraploid tea X *C. kissi*) chromosomes. Other aneuploids were recorded in *C. rosaeflora* with *C. sasanqua* and *C. sinensis* having a chromosome number of 63 and 44, respectively. Hybrids between hexaploid *C. sasanqua* X diploid *Camellia* species were tetraploid with 60 chromosomes. Only one hybrid involving *C. sasanqua* X *C. sinensis* was aneuploids with 59 chromosomes. In one combination between *C. sasanqua* X tetraploid tea produced two hybrids with pentaploid (2n=75) chromosomes.

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Appendix

APPENDIX

Table 4.9.1 Interspecific and intergeneric crosses in various species of *Camellia* and related genera failed to produce hybrid plants during 1993-94(I) and 1994-95(II)

Sl. No.	Crosses	Number of									
		Flowers pollinated		Capsule harvested		Seeds obtained		Sinker seeds		Seeds germinated	
		I	II	I	II	I	II	I	II	I	II
1.	*Camellia assamica* X *C. japonica*	39	+*	4	0	5	0	3	0	1	0
2.	Reciprocal	53	17	2	0	3	0	0	0	0	0
3.	*C. assamica* X *C. rosaeflora*	59	30	0	0	0	0	0	0	0	0
4.	Reciprocal	62	32	0	0	0	0	0	0	0	0
5.	*C. assamica* X *C. sasanqua*	76	76	0	0	0	0	0	0	0	0
6.	Reciprocal	73	+	6	0	7	0	3	0	1	0
7.	*C. assamica* X (F$_1$ of *C. sinensis* X *C. japonica*)	25	55	0	0	0	0	0	0	0	0
8.	Reciprocal	30	20	0	0	0	0	0	0	0	0
9.	*C. assamica* X *G. excelsa*	16	65	5	0	5	0	0	0	0	0
10.	*C. assamica* X *P. berringtoniaefolia*	103	+	7	0	9	0	4	0	2	0
11.	*C. assamica* X Tetraploid tea	–**	67	0	0	0	0	0	0	0	0
12.	*C. cambodiensis* X *C. irrawadiensis*	+	53	0	0	0	0	0	0	0	0
13.	Reciprocal	+	38	0	0	0	0	0	0	0	0
14.	*C. cambodiensis* X *C. japonica*	86	54	0	0	0	0	0	0	0	0
15.	Reciprocal	63	13	2	0	3	0	2	0	0	0
16.	*C. cambodiensis* X *C. rosaeflora*	95	64	0	0	0	0	0	0	0	0
17.	Reciprocal	59	34	0	0	0	0	0	0	0	0
18.	*C. cambodiensis* X *C. sasanqua*	89	51	0	0	0	0	0	0	0	0
19.	Reciprocal	76	+	5	0	5	0	2	0	1	0
20.	*C. cambodiensis* X (F$_1$ of *C. sinensis* X *C. japonica*)	15	32	0	0	0	0	0	0	0	0
21.	Reciprocal	32	20	0	0	0	0	0	0	0	0
22.	*C. cambodiensis* X *G. excelsa*	10	+	0	0	0	0	0	0	0	0
23.	Reciprocal	+	51	0	0	0	0	0	0	0	0
24.	*C. cambodiensis* X Tetraploid tea	–	63	0	0	0	0	0	0	0	0
25.	*C. drupifera* X *C. irrawadiensis*	88	+	3	0	5	0	3	0	1	0
26.	*C. drupifera* X *C. japonica*	60	43	2	0	4	0	2	0	1	0
27.	*C. drupifera* X *C. rosaeflora*	69	68	3	0	3	0	0	0	0	0
28.	Reciprocal	52	47	0	0	0	0	0	0	0	0
29.	*C. drupifera* X *C. sasanqua*	81	51	0	0	0	0	0	0	0	0
30.	Reciprocal	93	+	3	0	5	0	3	0	1	0
31.	*C. drupifera* X (F$_1$ of *C. sinensis* X *C. japonica*)	41	52	0	0	0	0	0	0	0	0
32.	Reciprocal	47	20	0	0	0	0	0	0	0	0
33.	*C. drupifera* X *G. excelsa*	23	+	0	0	0	0	0	0	0	0
34.	Reciprocal	26	57	2	0	10	0	0	0	0	0
35.	*C. drupifera* X *P. berringtoniaefolia*	7	11	0	1	0	3	0	1	0	0
36.	Reciprocal	–	17	0	0	0	0	0	0	0	0
37.	*C. drupifera* X Tetraploid tea	–	36	0	0	0	0	0	0	0	0
38.	*C. irrawadiensis* X *C. drupifera*	61	38	0	0	0	0	0	0	0	0
39.	*C. irrawadiensis* X *C. japonica*	35	42	0	0	0	0	0	0	0	0
40.	Reciprocal	47	10	0	0	0	0	0	0	0	0
41	*C. irrawadiensis* X *C. kissi*	52	44	1	0	3	0	2	0	1	0
42.	Reciprocal	43	83	0	0	0	0	0	0	0	0
43.	*C. irrawadiensis* X *C. rosaeflora*	51	58	0	0	0	0	0	0	0	0
44.	Reciprocal	60	20	0	0	0	0	0	0	0	0
45.	*C. irrawadiensis* X *C. sasanqua*	65	62	0	0	0	0	0	0	0	0

Table 4.9.1 Continue........

Sl. No.	Crosses	Number of									
		Flowers pollinated		Capsule harvested		Seeds obtained		Sinker seeds		Seeds germinated	
		I	II	I	II	I	II	I	II	I	II
46.	Reciprocal	52	54	7	0	8	0	2	0	0	0
47.	*C. irrawadiensis* X *C. sinensis*	+	40	0	0	0	0	0	0	0	0
48.	*C. irrawadiensis* X (F$_1$ of *C. sinensis* X *C. japonica*)	10	32	0	0	0	0	0	0	0	0
49.	Reciprocal	27	20	0	0	0	0	0	0	0	0
50.	*C. irrawadiensis* X *G. excelsa*	17	26	0	0	0	0	0	0	0	0
51.	Reciprocal	24	52	0	0	0	0	0	0	0	0
52.	*C. irrawadiensis* X *P. berringtoniaefolia*	25	23	0	0	0	0	0	0	0	0
53.	Reciprocal	78	48	0	0	0	0	0	0	0	0
54.	*C. irrawadiensis* X Tetraploid tea	–	34	0	0	0	0	0	0	0	0
55.	*C. japonica* X *C. kissi*	+	13	0	0	0	0	0	0	0	0
56.	Reciprocal	36	49	2	0	2	0	2	0	1	0
57.	*C. japonica* X *C. rosaeflora*	54	–	0	0	0	0	0	0	0	0
58.	Reciprocal	51	38	0	0	0	0	0	0	0	0
59.	*C. japonica* X *C. sasanqua*	54	9	0	0	0	0	0	0	0	0
60.	Reciprocal	77	47	5	0	5	0	2	0	0	0
61.	*C. japonica* X (F$_1$ of *C. sinensis* X *C. japonica*)	35	20	1	0	1	0	0	0	0	0
62.	Reciprocal	29	20	0	0	0	0	0	0	0	0
63.	*C. japonica* X *G. excelsa*	10	4	1	0	1	0	0	0	0	0
64.	Reciprocal	32	23	0	0	0	0	0	0	0	0
65.	*C. japonica* X *P. berringtoniaefolia*	–	16	0	0	0	0	0	0	0	0
66.	Reciprocal	–	13	0	0	0	0	0	0	0	0
67.	*C. japonica* X Tetraploid tea	–	23	0	1	0	1	0	0	0	0
68.	Reciprocal	32	+	7	0	11	0	7	0	5	0
69.	*C. kissi* X *C. assamica*	51	+	3	0	3	0	1	0	1	0
70.	*C. kissi* X *C. cambodiensis*	46	52	4	0	4	0	3	0	1	0
71.	*C. kissi* X *C. rosaeflora*	29	–	0	0	0	0	0	0	0	0
72.	Reciprocal	52	20	0	0	0	0	0	0	0	0
73.	*C. kissi* X *C. sasanqua*	43	89	2	0	2	0	1	0	0	0
74.	Reciprocal	90	+	7	0	8	0	5	0	2	0
75.	*C. kissi* X (F$_1$ of *C. sinensis* X *C. japonica*)	17	33	0	0	0	0	0	0	0	0
76.	Reciprocal	37	20	0	0	0	0	0	0	0	0
77.	*C. kissi* X *G. excelsa*	13	65	0	0	0	0	0	0	0	0
78.	Reciprocal	41	34	0	0	0	0	0	0	0	0
79.	*C. kissi* X *P. berringtoniaefolia*	8	5	0	0	0	0	0	0	0	0
80.	Reciprocal	3	10	0	0	0	0	0	0	0	0
81.	*C. kissi* X Tetraploid tea	–	43	0	1	0	1	0	0	0	0
82.	*C. rosaeflora* X *C. sasanqua*	42	+	0	0	0	0	0	0	0	0
83.	Reciprocal	43	33	0	0	0	0	0	0	0	0
84.	*C. rosaeflora* X *C. sinensis*	+	39	0	0	0	0	0	0	0	0
85.	*C. rosaeflora* X (F$_1$ of *C. sinensis* X *C. japonica*)	13	41	0	0	0	0	0	0	0	0
86.	Reciprocal	41	15	0	0	0	0	0	0	0	0
87.	*C. rosaeflora* X *G. excelsa*	14	29	0	0	0	0	0	0	0	0
88.	Reciprocal	19	20	0	0	0	0	0	0	0	0
89.	*C. rosaeflora* X *P. berringtoniaefolia*	–	5	0	0	0	0	0	0	0	0
90.	*C. rosaeflora* X Tetraploid tea	–	17	0	0	0	0	0	0	0	0
91.	Reciprocal	77	37	3	0	6	0	5	0	3	0

Table 4.9.1 Continue……..

Sl. No.	Crosses	Number of									
		Flowers pollinated		Capsule harvested		Seeds obtained		Sinker seeds		Seeds germinated	
		I	II	I	II	I	II	I	II	I	II
92.	*C. sasanqua* X *(*F$_1$ of *C. sinensis* X *C. japonica)*	17	44	0	0	0	0	0	0	0	0
93.	Reciprocal	29	20	0	0	0	0	0	0	0	0
94.	*C. sasanqua* X *G. excelsa*	16	32	2	0	2	0	0	0	0	0
95.	Reciprocal	30	29	0	0	0	0	0	0	0	0
96.	*C. sasanqua* X *P. berringtoniaefolia*	12	–	0	0	0	0	0	0	0	0
97.	Reciprocal	–	6	0	0	0	0	0	0	0	0
98.	*C. sinensis* X *C. japonica*	71	+	6	0	9	0	5	0	3	0
99.	*C. sinensis* X *C rosaeflora*	71	26	0	0	0	0	0	0	0	0
100.	*C. sinensis* X *C. sasanqua*	104	+	8	0	13	0	9	0	7	0
101.	*C. sinensis* X *(*F$_1$ of *C. sinensis* X *C. japonica)*	26	32	0	0	0	0	0	0	0	0
102.	Reciprocal	57	20	0	0	0	0	0	0	0	0
103.	*C. sinensis* X *G. excelsa*	18	52	0	0	0	0	0	0	0	0
104.	*C. sinensis* X *P. berringtoniaefolia*	107	+	0	0	0	0	0	0	0	0
105.	*C. sinensis* X Tetraploid tea	–	61	0	0	0	0	0	0	0	0
106.	*(*F$_1$ of *C. sinensis* X *C. japonica)* X *G. excelsa*	13	20	0	0	0	0	0	0	0	0
107.	Reciprocal	20	30	0	0	0	0	0	0	0	0
108.	*(*F$_1$ of *C. sinensis* X *C. japonica)* X *P. berringtoniaefolia*	–	5	0	0	0	0	0	0	0	0
109.	*(*F$_1$ of *C. sinensis* X *C. japonica)* X Tetraploid tea	–	54	0	0	0	0	0	0	0	0
110.	Reciprocal	47	51	0	0	0	0	0	0	0	0
111.	*G. excelsa* X *P. berringtoniaefolia*	5	–	0	0	0	0	0	0	0	0
112.	Reciprocal	–	7	0	0	0	0	0	0	0	0
113.	*G. excelsa* X Tetraploid tea	–	63	0	0	0	0	0	0	0	0
114.	Reciprocal	20	65	0	0	0	0	0	0	0	0
115.	*P. berringtoniaefolia* X Tetraploid tea	–	28	0	0	0	0	0	0	0	0
116.	Reciprocal	25	21	3	0	7	0	5	0	2	0
117.	*P. berringtoniaefolia* X *C. sasanqua*	64	36	0	0	0	0	0	0	0	0

* = resulted seed
** = failed to produce seed