आम (*मैंजीफेरा इंडिका* एल.) में असंगतता एवं भण्डारित पराग की जीवनक्षमता का अध्ययन

STUDIES ON INCOMPATIBILITY AND VIABILITY OF STORED POLLEN IN MANGO (*Mangifera indica* L.)

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NEW DELHI - 110 012

2011

Studies on incompatibility and viability of stored pollen in mango (*Mangifera indica* L.)

A Thesis

By

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Submitted to the Faculty of Post-Graduate School,
Indian Agricultural Research Institute, New Delhi
in partial fulfillment of the requirements
for the award of the degree of

DOCTOR OF PHILOSOPHY
IN
HORTICULTURE
2011

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C E R T I F I C A T E

This is to certify that the thesis entitled, **"Studies on incompatibility and viability of stored pollen in mango (*Mangifera indica* L.)"** submitted to the Faculty of the Post-Graduate School, Indian Agricultural Research Institute, New Delhi, in partial fulfilment of the requirements for the award of the degree of **Doctor of Philosophy** in **Horticulture** is a record of *bona fide* research work carried out by **Mr. Sudip Kumar Dutta, Roll No. 9550** under my guidance and supervision, and that no part of this thesis has been submitted for any other degree or diploma. It is further certified that all the assistance and help availed during the course of investigation as well as all sources of information have been duly acknowledged by him.

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ACKNOWLEDGEMENT

I bow my head to the Almighty, without whose blessing I could never have overcome the hurdles and complete this endeavour successfully.

I wish to record my sincere innate respect, appreciation and gratitude to Dr. Manish Srivastav, Senior Scientist, Division of Fruits & Horticulture Technology, IARI, New Delhi and the Chairperson of my advisory committee, for his undivided dedication, guidance, constructive criticism and wholehearted support not only during the entire course of investigation but preparation of manuscript as well. It is pleasure to acknowledge the affection and constant encouragement given by him. I consider it to be my proud privilege having been his student during my PhD. programme at I.A.R.I., New Delhi.

I express my sincere thanks to Dr. A. K. Dubey, Senior Scientist, Division of Fruits & Horticulture Technology, IARI, New Delhi, for providing his valuable suggestions during my course of research as Co-Chairman of my advisory committee. I would like to extend my heartfelt gratitude to member of my advisory committee, Dr. A.K. Singh, Head, Division of Fruits & Horticulture Technology, IARI, New Delhi, Dr. Rekha Chaudhury, Principal Scientist Division of Tissue culture and Cryopreservation unit, NBPGR, New Delhi, Dr. Vinod, Senior Scientist, Division of Genetics, IARI, New Delhi and Dr. Krishan Lal, Principal Scientist, IASRI, New Delhi for their valuable help rendered in relation to planning and execution of this work.

It is great privilege for me to express my esteem and profound sense of gratitude to Dr. A. K. Singh, Head of the Division of Fruits & Horticulture Technology, Dr. Subodh Joshi, Professor, Discipline of Horticulture, for their cooperation and providing the necessary facilities to carry out this study.

I owe a debt of gratitude to Dr. S. K. Singh, Senior Scientist, Dr. M. K. Verma, Senior Scientist, Dr. S.K. Bhagat, Dr. Nagaraja and Dr. V. B. Patel, Division of Fruits & Horticulture Technology, IARI, New Delhi, for their valuable help and constant inspiration to me during my research programme. I express my special thanks to Dr. Ramamurthy, Division of Entomology and Dr. K. V. Prasad, Division of Floriculture and Landscaping for providing the microscopic facilities.

I express my hearty gratitude to Rampyari mam for her encouragement and kind concern throughout the study.

I express my gratefulness to MR. S.P. Singh, Technical Officer, Division of Fruits and Horticultural Technology, IARI, New Delhi and Pravin, Jyotsna, Digvendra and Ravish SRF, NBPGR, for their unconditional help during my research

work. I shall always remain thankful to them for their unconditional and timely help during research work.

I convey my heartiest thanks to Dipak da, Nipendra sir, Virendra Sir, Kiran Sir, Arun and Sharifa mam for their help during the entire span of study. My heartfelt thank to my affectionate junior Mohan, Arvind, Ankit, Rymbai, Prativa, Flemine, Arindam and Manoj for their kind support throughout my research programme.

No words can describe the unending love and moral support by my friends, Arnab, Manoj, Dibyendu, Jayanta, Kali, Rakesh, Ajay, Sadanand and Bhaskar who deserve a special mention for being always there during my difficult time and all my class mates during my course of study. The unceasing affection and support of seniors Totan da, Biraj da, Pradip da, Tanmay da, Tarak da and Asit da would be in my memory for all the time. I extend my heartfelt thanks to my juniors like Nintu, Sujit, Tanumoy, Kalyan, Dhruva, Niladri and Nilimesh; I feel really proud of them.

The endless love, affection, sacrifice and constant inspiration from Maa and blessings from my Pappa have enabled me to reach the footsteps of my long cherished aspiration. My vocabulary utterly fails in expressing my love and respect to my affectionate didi, Jamai babu and Sontai for their unstinted support during all the years of study.

Finally, the financial assistance provided by the I.A.R.I. in the form of S.R.F. fellowship, ICAR- SRF fellowship and DST-INSPIRE fellowship during the tenure is gratefully acknowledged.

Place: New Delhi ***(Sudip Kumar Dutta)***
Dated:

CONTENTS

LIST OF TABLES

List of table contd…..

List of table contd…..

LIST OF FIGURES

LIST OF PLATES

LIST OF ANNEXURE

LIST OF ABREVIATIONS

DAP	Days after pollination
DMRT	Duncans multiple range test
g	Gram
MES	Morpholinoethanosulfonic acid
PEG	Polyethylene glycol
RH	Relative humidity
RBD	Randomised block design
CD	Critical difference
SD	Standard deviation
NS	Not significant
ANOVA	Analysis of variance
FDA	Fluorescein diacetate
Num DF	Numerator degrees of freedom
Den DF	Denominator degrees of freedom
SM	Storage method
^{o}C	Degree Celsius
V	Variety
D	Days

1. INTRODUCTION

The Mango (*Mangifera indica* L.), a member of family Anacardiaceae, is amongst the most important tropical fruits of the world. It is also called as 'king of the fruits' in India due to its historical and religious importance, attractive aroma and capitative taste. Mango originated in the South East Asian or Indo-Burma Region having 69 recognized species originating as forest trees with fibrous and resinous fruits (Kosterman and Bompard, 1993). In India, about 30 mango cultivars are being grown commercially. Majority of them posses narrow adaptability and show eco-geographical preferences for growth and yield (Yadav and Rajan, 1993). The ideal cultivar in mango should have characteristics like dwarf tree stature, precocity and regularity in bearing, attractive and good quality fruits, high productivity and resistance to major diseases and pests. Owing to the high heterozygosity and long juvenile period, conventional breeding in mango is intricate. Combining all the traits in a single cultivar is difficult and breeding objective may be defined for specific purpose. Mango hybridization work is in progress at various centres in India. Indian Agricultural Research Institute, New Delhi is notable among them credited with the development of some commercially important mango hybrids with desirable traits for domestic as well as overseas markets.

The problem of non-synchronised flowering in certain mango cultivars restricts their use as parent in mango hybridization programme. Mango blooming season in north India starts in February and lasts through April, whereas the regular harvesting season extends from May to August. In mango breeding programmes, circumstances usually make it necessary to keep the pollen of the male parent for later pollination of the female parent. Sensation, Tomy Atkins and Janardan Pasand are important polliniser cultivars which are extensively used at IARI in mango breeding programmes for imparting colour to the progeny. They are used for pollinating in both early and late flowering female parents that made it necessary to search for a suitable short term pollen storage method. Thus, in order to optimize the pollination for both early and late harvesting it could be necessary to conserve pollen collected during early season for pollination in late season. Mango pollen has a short viability and a high sensitivity to desiccation and, consequently, conservation is problematic (Issarakraisila and Considine, 1994). Therefore, need arised to determine the period of time over which pollen from important donor cultivars retains its viability under different storage conditions and to test the effect of genotype on pollen viability during storage.

Presence of self incompatibility in few cultivars can improve the breeding efficiency of mango. With the improved technique of mango hybridization and the report of self-

incompatibility in mango, it would be possible to evolve a larger number of hybrids having desirable horticultural traits. Embryological studies have shown that in mango pollen tubes grow down the style and effect fertilization but the development of zygote is blocked leading to a sporophytic type of self-incompatibility. From the earlier studies carried out at I.A.R.I. (Singh *et al.,* 1962), it has been shown that there is self-incompatibility in mango. In recent past number of improved mango hybrids has been developed at IARI, New Delhi by hybridizing parents which are known to have self incompatibility in their ancestry. Knowledge of variability in the degree of self incompatibility in these cultivars will help the breeders in selecting female parents for hybridisation programme without the need for emasculation. Moreover, at several occasions mango growers face the problem of poor fruiting in cultivars grown in isolation. A lot of research has been done regarding pollen-pistil interaction, fruit retention and fruit growth in many species *viz.*, apple, almond, citrus, pecan, olive, peach, plum, sour cherry and *ber*. With best polliniser selection with respect to pollen-pistil interaction and fruit growth, chances of fertilization can be increased which will result in increase of fruit yield. This signifies the indispensability for the pollen-pistil compatibility study in newly developed mango cultivars.

It has also been experienced at several occasions that mango growers faced the problem of poor fruiting due to lack of compatible pollinisers. The need of pollinisers in mango orchards was recognised by Popenoe as early as 1917. Further studies also supported his findings by showing increased fruit set through cross pollination (Sen *et al*., 1946) and self sterility was also suspected by Dijkman and soule (1951) and confirmed by Singh *et al*. (1962) in Dashehari. Later on some more cultivars were found to be self incompatible by Sharma and Singh, (1972). Cross incompatibility was also reported in Langra and Fazli (Saha and Chhonkar, 1972). Reddy and Ramayya (1976) observed that Himayuddin as polliniser resulted in 50 % more fruit set and increase in fruit size compared with that of open pollinated Rumani fruits. Ram *et al.* (1976) reported that Dashehari is cross incompatible with Chausa and Safeda Malihabad and it is cross compatible with Langra, Rataul and Bombay Green. Langra is cross incompatible with Alphonso, Bombay Green, Chausa and Fazli. Chausa is cross incompatible with Bombay Green and Rataul and it is cross compatible with Langra and Safeda Malihabad. They also reported that Bombay Green and Dashehari appear to be best polliniser for Dashehari and Chausa respectively. However, compatible pollinizers in case of majority of commercial varieties of mango are yet to be standardised. This emphasises the need for study on suitable pollinizers for mango cultivars. Amrapali, Pusa Arunima, Dashehari and Langra are commercial cultivars of mango which are gaining commercial status under North Indian conditions. Till now there has been very little work undertaken to search suitable pollinisers for them. Solid block planting of these cultivars may lead to a drastic reduction in fruit set due to the presence of self and cross

incompatibility in them. Therefore, suitable polliniser(s) have to be worked out and recommended to the farmers for good crop.

Keeping in view the above facts about mango, the present investigation was proposed to be carried out with following objectives:

1. To study the effect of different storage methods on pollen viability in pollen donor mango cultivars.
2. To determine self incompatibility in mango.
3. To determine cross incompatibility in mango.

2. BACKGROUND

Mango (*Mangifera indica* L.) belonging to family Anacardiaceae is one of the commercially important fruit crop of tropical and sub-tropical worlds. The problem of asynchronized flowering in certain mango cultivars restricts their use as parent in mango hybridization programme and circumstances usually make it necessary to store the pollen from male donor parent for later pollination of the female parents. Sensation, Tomy Atkins and Janardan Pasand are important polliniser cultivars which are extensively used at IARI in mango breeding programmes for imparting colour to the progeny. They are used for pollinating both early and late flowering female parents that made it necessary to ascertain the viability of pollens under different storage conditions. In recent past, number of improved mango hybrids has been developed at IARI, New Delhi by hybridizing parents which are known to have self incompatibility in their ancestry. Knowledge of variability in the degree of self incompatibility in these cultivars would help the breeders in selecting female parents for hybridisation programme without the need for emasculation. Also, it will address the problem of poor fruiting in cultivars grown in isolation. Compatible pollinizers in case of commercial cultivars of mango are yet to be worked out. Amrapali, Pusa Arunima, Dashehari and Langra are some of the commercial cultivars of mango which are grown widely under North Indian conditions and till now there has been very little work undertaken to search suitable pollinisers for them. Solid block planting of these cultivars may lead to a drastic reduction in fruit set due to the presence of self and cross incompatibility in them. Therefore, suitable polliniser have to be worked out and recommended to the farmers for good crop. The relevant literature corresponding to the objectives of the present investigation is reviewed here under.

2.1 Research Area I (Objective I): To study the effect of different storage methods on pollen viability in mango (*Mangifera indica* L.) varieties.

2.1.1 Pollen storage

Storage of pollen is an important component of plant improvement programs, when genotypes to be hybridized do not flower concurrently or are geographically separated. Conditions of storage which result in retention of viability vary; with freeze drying and liquid nitrogen are the most successful methods for long term pollen storage of a range of plant species (Sedgley and Griffin, 1989). In view of the effort invested in controlled hybridisation, it is important to ensure that the stored pollen retains viability to produce fertile seeds. Pollen staining and *in vitro* germination methods have long been used as indicators of viability prior to

use, including 2,3,5-triphenyltetrazolium chloride (TTC) for *Pinus* (Cook and Stanley, 1960), 5-bromo-4- chloro-3-indole-P-galactoside (X-Gal) for *Solanum* (Trognitz, 1991), fluorescein diacetate (FDA) for *Malus* (Bellani and Bell, 1986) and *in vitro* pollen germination for *Citrus* (Soost and Cameron, 1975). Staining methods rely on active enzymes in the pollen, which may persist after the ability to effect seed set has been lost, and *in vitro* pollen germination also does not always reflect the true viability of pollen (Sedgley and Griffin, 1989). Pollen performance, which includes pollen germination, pollen tube growth rate and pollen competition, is an important component of fertilization success in seed-producing plants. Pollen performance is clearly affected by the genotype of the pollen (Snow and Spira, 1991). Temperature is one of the most important environmental factors that could affect pollen performance during the progamic phase (Hedhly *et al*., 2005). It has been shown that temperature affects pollen germination (Shivanna *et al*., 1991), and pollen tube kinetics in the style (Elgersma *et al*., 1989). Temperature ranges and optimum temperature values for pollen germination and pollen tube growth were studied for different fruit species, including jojoba (Lee *et al*., 1985), pears (Vasilakakis and Porlingis, 1985), papaya (Cohen *et al*., 1989), cherimoya (Rosell *et al*., 1999), mango (Sukhvibul *et al*., 2000), *Prunus mume* (Wolukau *et al*., 2004), almond (Godini *et al*., 1987), apricot (Pirlak, 2002), sour cherry (Cerovic and Ruzic, 1992), and sweet cherry (Pirlak, 2002). The optimum temperature required for pollen germination was about 15 and 20 ºC for apricot, sour cherry and sweet cherry (Pirlak, 2002). Thus, temperature effects on pollen germination are inconsistent and seem to be cultivar or species dependent (Sukhvibul *et al*., 2000). In addition to collection methods, storage temperature and in *vitro* germination techniques, relative humidity (RH) of the storage environment, has been shown to have a significant influence on the subsequent germinability of walnut (Luza and Polito, 1985) and pistachio (Polito and I,uza, 1989) pollen.

2.1.2 Pollen viability

Longevity of partially hydrated pollen is generally determined by their rate of metabolic activity (Hoekstra and Bruinsma, 1975 and Shivanna *et al.*, 1991). Respiration of pollen is strongly restricted when pollen is in equilibrium with air of low relative humidity (RH) and longevity is considerably increased (Hoekstra and Bruinsma, 1975). But even in the dry state, large differences in longevity between pollen of different species exist (Pfundt, 1910). Many applications related to plant breeding, such as cross-pollination of plants that flower at different times or locations, conservation of germplasm in a minimum volume and/or the production of haploids may require long-term storage of viable pollen (Hanna and Towill, 1995). Storage under cryogenic conditions (*i.e*., temperatures below –70°C) seems to be the most effective means to maintain pollen viability for long periods, while temperatures of approx. –20°C enable the maintenance of pollen viability for 1 year (*i.e*., season to season) or even longer (Towill and

Walters, 2000). However, the optimum temperature for long-term storage of pollen varies between species or even between different cultivars of the same species. For example, a temperature of –20°C was more favourable for the storage of kiwi (*Actinidia delisiosa*) and almond (*Prunus dulcis*) pollen than –80°C (Abreu and Oliveira, 2004 and Martinez-Gómez *et al*., 2002). A crucial factor for successful storage of pollen under cryogenic conditions is its moisture content (Towill and Walters, 2000). In most cases, partial desiccation is required before storage, to ensure maintenance of viability (Parton *et al*., 2002) because this reduces the risk of intracellular ice formation (Ching and Slabaugh, 1966). However, critical moisture levels for cryogenic storage of pollen vary among species (Connor and Towill, 1993). In some cases, pollen may be exposed to cryogenic temperatures immediately after collection, indicating that some desiccation has already occurred after release from the anthers (Rajasekharan *et al*., 1994); but in most cases, desiccation to a certain moisture content is needed (Parton *et al*., 2002). On the other hand, excessive desiccation may be harmful to pollen viability during storage at cryogenic temperatures (Marchant *et al*., 1993).

2.1.3 *In vitro* pollen germination

The evaluation of the germination capacity (viability) during the storage of pollen grains from the male parent is crucial in the process of artificial hybridization. During the plant maturation, the pollen viability can be affected by several endogenous and exogenous factors, such as the stage of flower development (Lacerda *et al.,* 1994), high temperatures (40 °C) (Giordano *et al.,* 2003) and low temperatures (15°C) (Chira, 1963), nutritional status of the plant (Howlett, 1936), luminosity (Goss, 1971), agricultural pesticides and other chemicals (MacDaniels and Hildebrand, 1939). A successful system of *in vitro* pollen germination is a prerequisite for pollen research (Williams *et al.,* 1982) and is important for testing the capacity and viability of pollen for controlled pollinations (Griffin, 1982; Heslop-Harrison, 1979). The preservation of viable pollen for future study and for plant breeding is of considerable theoretical and practical value (Shivanna and Rangaswamy, 1992). Germination capacity of stored pollen can be maintained in hybridization and crops improvement programs. Fruit tree pollen are generally required to be stored for controlled crossing, either to achieve a desired breeding objective or to overcome a constraint involved in commercial fruit production (Ganeshan and Alexander, 1991). The pollen grains of different plant species required varying range of growth media like water, sugar solution, inorganic salts and vitamins for their successful germination (Amma and Kulkarni, 1979). The pollen grain viability, a measure of male fertility may be determined by different techniques (Dafni, 1992). These can be grouped into direct methods such as the induction of *in vitro* (Dutra *et al*., 2000) and *in vivo* (Oliveira *et al*., 2001) germination or indirect methods based on cytological parameters such as colour (Kearns and Inouye, 1993). The *in vitro* germination test is a methodology to germinate a small

sample of pollen grains in an appropriated culture medium. Through this technique, it is possible to observe in a microscope the percentage of pollen grains that developed pollen tubes after a certain period. The composition and pH of the medium are among the factors that affect pollen germination. The angiosperms pollen requires a carbon source, boron, and often other nutrients to promote their germination (Galleta, 1983). According to Pfahler (1967), the addition of boron is important and their responses vary according to species. Its mechanism of action occurs through its interaction with the sugar, forming a complex sugar-borate which reacts more rapidly with the cell membranes. Thompson and Batjes (1950) found that the addition of boron to the medium markedly increased the percentage of germination and pollen tube length of various fruit species of temperate climates. Calcium added in a culture medium for germination provides physiological characteristics to the pollen tube and pollen grain with less sensitivity to changes in the basic medium, lower permeability and growth in a linear and rigid appearance of pollen tube (Bhojwani and Bhatnagar, 1974). In the absence of calcium there is increased permeability of the pollen tube membrane, causing the release of internal metabolites to the external environment (Stanley and Linskens, 1974). Beyoung (1965) observed in 46 vegetable species that the addition of calcium promoted the germination of pollen grain and pollen tube growth in all species. Brewbaker and Kwack (1963), working with 86 species of 39 families showed that the addition of calcium and boron acts as a primary factor controlling the germination of the pollen tube *in vitro*. Sugar is used in the culture medium to provide the osmotic balance between the solution and pollen germination and provides energy to assist the development process of the pollen tube (Stanley and Linskens, 1974 and Miranda and Clement, 1990). Among sugars, sucrose is the most suitable for the ovule culture of *Lycopersicum peruvianum* because it is crucial for *in vitro* ovary growth and seed formation (Torres and Murashige, 1985). For the tomato culture (Torres and Murashige, 1985), tobacco (Loguercio, 2002) and *Acacia mearnsii* (Stiehl-Alves and Martins-Corder, 2007), the culture medium of Brewbaker and Kwack (1963) should be calibrated with 10 g L^{-1} of sucrose. The *in vitro* germination test tries through the culture medium, to simulate the plant ovary providing the balanced environment for the development of the pollen tube. The *in vivo* germination consists in placing the pollen grains in the receptive stigma of the flower and evaluating the developing pollen tube under the microscope (Stanley and Linskens, 1974). An indirect way of evaluating the percentage of pollen germination is by fruit (Galleta, 1983) or the seed production (Stanley and Linskens, 1974 and Akihama *et al*., 1978). Galleta (1983) considered that the stain method overestimates the percentage of pollen grain germination while the *in vitro* test underestimates it. However, samples of pollen that don't seem viable when tested *in vitro* germination tests can produce a high percentage of seeds *in vivo* (Einhard *et al*., 2006). The staining methods although simple and low cost, don't provide information about the germination capacity of pollen which can be obtained by testing the *in vitro* (Techio *et al*., 2006) or *in vivo* germination (Einhard *et*

al., 2006). In mango 10% sugar solution has been found to give maximum pollen germination but highest pollen germination (28.2%) in *var*. Chausa has been recorded with 25% sucrose solution at an incubation temperature of 30ºC (Randhawa and Damodaran, 1961). Popenoe, (1917) reported 10-15% pollen germination with 25% sugar solution and 0.5% agar at 75-80°F, in the variety Mulgoa. He considered it unlikely that mango pollen could germinate below 60°F. The guava pollen required comparatively lower sugar concentration for optimum germination. The pollen germination capacity of almond was studied by Martinez-Gomez *et al*., (2001) and that of strawberry by Aslantus and Pirlak, (2002). Vasil, (1960) concluded that boron promotes absorption and metabolism of sugars by forming sugar borate complexes, increase oxygen uptake and is involved in the synthesis of pectic materials for the wall of the actively elongated pollen tubes. This was the first systematic study carried out on 3-mango cultivars *viz*., Chausa, Dashehari and Langra. Lora *et al.* (2006) in order to optimize the process of hand-pollination, studied the conservation of cherimoya pollen at -20, -80 and -196 ^{0}C for up to 3 months. *In vitro* pollen germination of fresh pollen was 57.1% and it was progressively reduced with conservation time at the three temperatures studied reaching a minimum after 3 months of storage of 10.4, 14.2 and 13.6% at -20, -80 and -196 ^{0}C, respectively. Differences in germination among temperatures were only significant during the first 2 weeks of storage. Field pollinations with pollen stored for up to 3 months at the three temperatures show no yield differences compared to pollinations performed with fresh pollen also pollen collected and stored at sub-zero temperatures at the beginning of the cherimoya blooming season can be used along the whole blooming season avoiding the need of collecting fresh pollen daily.

2.1.4 FDA (Fluorescein diacetate reaction) test

FDA tests the plasmalemma and the intactness of the plasma membrane of the cell. Heslop-Harrison, (1992) reported that FDA test correlates with germination tests, tests two aspects of viability enzymatic activity and an intact cell membrane and is easier and quicker than other viability testing methods. It correlates with germination of pollen for a wide range of species (Heslop-Harrison *et al*., 1984). Pinney and Polito (1990) reported that the FDA test correlates well with *in vitro* germination which indicates that this assay is suitable for determinations of olive pollen viability. Because pollen can be assayed more quickly and easily by the fluorescein diacetate test, this would be the method of choice for evaluation of olive pollen viability. Cerovic *et al.* (1998) investigated that *in vivo* test as the most exact method for pollen viability evaluation, a degree of positive correlation was assessed in relation to the other tests which indicates a certain validity of the *in vitro* germination test and the fluorescein diacetate test in estimating pollen viability when determining varietal composition with sweet cherry cultivar Carna as well as in choosing a potential male parent cultivars in future breeding work. Pacini *et al.* (1997) investigated pollen longevity under field greenhouse conditions in

relation to pollen packaging, and mode of pollination using FDA in species viz. *Cucurbita pepo* (Cucurbitaceae), *Festuca arundinaceae* (Poaceae), *Mercurialis annua* (Euphorbiaceae), *Acanthus mollis* (Acanthaceae), *Charnaerops humilis* (Arecaceae) and *Spartiurn junceum* (Fabaceae).

2.2 Research Area II (Objective II): To determine self incompatibility in mango (*Mangifera indica* L.).

2.2.1 Self Incompatibility

Self-incompatibility (SI) is one of the most important systems used by many flowering plants to prevent self-fertilization and thereby generate and maintain genetic diversity within a species (de Nettancour, 2001)). The SI response is comprised of a self and nonself recognition process between pollen and pistil that is followed by selective inhibition of the self-pollen tube development. Some self-incompatible flowering plants produce morphologically distinct flowers, in which the relative positions of the reproductive organs within a flower pose an additional topological barrier to the already existing intra-specific barrier of self-incompatibility (Kao and McCubbin, 1996). In other self-incompatible species, the flowers possess the same morphological character but the phenotype of the pollen can be either sporophytically or gametophytically derived (Nettancourt, 1977). In the gametophytic systems, the self-incompatibility phenotype of the pollen is determined by its own haploid genotype, whereas in sporophytic self-incompatibility systems, the self-incompatible behaviour of the pollen is determined by the genotype of the pollen parent. Gametophytic self-incompatibility is the most common system and has been described in more than 60 families of flowering plants (Kao and Mc Cubbin, 1996). Despite its widespread prevalence, gametophytic self-incompatibility has only been studied in detail at the molecular level in the *Papaveraceae* (poppy) and in members of the *Solanaceae* family including *Nicotiana* (tobacco), *Petunia*, *Solanum* (potato), and *Lycopersicon* (tomato). Sporophytic self-incompatibility, which is less common, has been studied in detail in the *Brassicaceae* (mustard) family. The existence of self-incompatibility systems as strategies to promote genetic variability has been documented since Darwin's classical genetic studies dating back to the end of the 18th century. Only within the last two decades have scientists been able to complement these genetic observations with molecular and biochemical analyses which have significantly contributed to elucidating the complex series of interactions occurring at the pollen-stigma interface. From an evolutionary perspective, it is interesting to note that many families of flowering plants have evolved different mechanisms of self-incompatibility even though they share the function of preventing self-fertilization.

2.2.2 Self incompatibility in mango

In fruit crops, gametophytic incompatibility caused by stylar inhibition has been

reported in apple (Crane and Lawrence, 1931), citrus (Soost, 1969), plum (Crane and Lawrence, 1929), sweet cherry (Crane and Lawrence, 1929), pear (Crane and Lewis, 1942), guava (Seth, 1962), loquat (Singh and Rajput, 1964) and pineaaapple (Majumdar *et al*. 1964). However, post fertilization breakdown following selfing is reported in black currants (Arasu, 1970) and in mango (Singh *et al*., 1962). The existence of pollination barrier in the form of self incompatibility remained unknown until Dijkman and Soule (1951) suspected self sterility in mango and its prevalence was established in cv. Dashehari by Singh *et al*. (1962). The cultivars of mango like Dashehari, Langra and Chausa were found to be self-incompatible (Sharma and Singh, 1970).

2.2.3 Mango floral biology

Mango inflorescence is terminal with frequent emergence of the multiple axillary panicles. Both perfect (2-70%) and hermaphrodite flowers occur on the same panicle (Fraser, 1927). Total number of flowers per panicles is 1000-6000 depending upon the variety (Mukherjee, 1953). Anthesis starts early in the morning and completes at noon. Stigma receptivity remains for 72 h but most receptive period is for the first 6 h. Minimum pollen germination time is 1.5 h (Spencer and Kennard, 1955). Initial fruit set depends upon the ratio of the perfect to male flowers (Iyer *et al*., 1989). Proportion of perfect flowers required for optimum fruit set must not be less than 1%. Detailed floral morphology in mango was described by Juliano and Cuevas, (1933) for the variety 'Pico'. Scholefield (1982) investigated that hermaphrodite flowers have a 10-part perianth consisting of 5 sepals and 5 petals. The round carpel is supported on 5-lobed nectar; the short style has a small stigmatic surface without the prominent papillae of the other stigmas. The groove in the style appears to be a factor in common with the avocado, litchi and macadamia. One fertile stamen and 4 short infertile staminodes are present. Male flowers are similar to the hermaphrodite flowers except that the carpel has aborted. The single, 4-lobed anther dehisces longitudinally releasing pollen. The staminodes consist of a lobed mass of tissue on a short filament. Mango is considered to be a cross-pollinated plant (Allard, 1960; Mukherjee, 1953) with flies as the pollinating agent. The flowers exhibit some form of separation of the sexes, although the species are all monoecious. Although some mango flowers are perfect, evidence has been presented (Urata, 1954; Ito and Hamilton, 1980 and Sharma and Singh, 1970) that some degree of self incompatibility exists in these species. Therefore, it is essential that pollen from one flower is transmitted to the receptive stigma of another for successful pollination, fertilization and fruit production. All the flowers possess nectaries and this indicates that insects are the vector for cross pollination (McGregor, 1976). The small stigmatic area of the mango reduces the possibility of wind pollination. A better understanding of the peculiarities of the floral structure and biology will allow us better to manipulate the flowering-period, either by the use of pollinating insects for increasing yield or

by increasing the efficiency of hybridising in controlled-pollination breeding-programs (Scholefield, 1982).

2.2.4 Pollination

Mango is self-fertile (Sturrock, 1944) but cross pollination increases fruit set (Popenoe, 1917). Some self unfruitful cultivars may get benefit from cross-pollination. There is almost no air-borne pollen since it is heavy and adherent. The eye irritation (dermatitis) may result from volatile oils from flowers, mangiferol (sesquiterpene alcohol) and mangiferone (ketone). Young (1942) studied pollination of 'Haden' mango in Florida and found no significant difference between percentages of set in selfed and cross-pollinated flowers. Naturally more than 50% flowers don't receive any pollen. Self-pollination may also occur in some cultivars (Dijkman and Soule, 1951). Though the ratio of hermaphrodite to male flowers is cultivar related, cool temperatures may also influence sex expression to favour majority of male flowers. There are several hundred flowers in a panicle and less than 1% only develops fruits because of pollination failure and premature fruit drop. Singh *et al.* (1962) reported that crossed flowers set fruit whereas, selfed ones did not indicating the presence of self-sterility. The actual degree of self-fertility and sterility in individual cultivars had not been determined but there is some variation. Though self-sterility is not a major problem in fruit set but within cultivar, there is a definite need for a pollinating agent. Popenoe (1917) stated that some of the embryos are capable of development without fertilization, however, Naik and Rao (1943) obtained no parthenocarpic fruit set of more than 100,000 flowers studied. The effect of cool weather adversely affects pollen tube growth but this was not considered to be of major importance (Young, 1955). Wolfe (1962) concluded that getting flowers to set fruit was more of a problem than getting trees to bloom. The studies indicate that the need for cross-pollination between mango cultivars is not critical at least for most cultivars but pollinating insects are needed to pollinate within cultivar to get satisfactory crop.

2.2.5 Pollinisers

Several agents have been credited as pollinators of mango. Wagle (1929) showed that there was some selfing and wind pollination but insects (bees, ants and flies) played an important role. Popenoe (1920) disagreed with him and stated that there is no wind pollination observed in mango rather it is strictly an insect-pollinated plant. Galang and Lazo (1937) and Singh and Sturrock (1969) supported him. Studies showed that plants caged to exclude all insects set no fruit but a plant caged with honeybees set a heavy crop (Sharma, 1987). Popenoe (1917) reported that honeybees were the most important hymenopterous insect visitors to the mango flowers with variability in number. Young (1942) recommended placing colonies of honeybees in mango groves. Simao and Maranhao (1959) reported population of honeybees in

mango orchards. Singh (1954) listed mango as a nectar source for bees. While, Singh (1960) stated that honeybees do not visit mango flowers. Singh (1961) reported that over 65% of the perfect flowers were never pollinated showing that wind is not an effective pollinating agent. Complaints about lack of adequate fruit set in large plantings particularly of monoclonal cultivars are frequent (Singh and Sturrock, 1969). The mango flowers do not appear to be attractive to honey bees as they tend to open when many other flowers are also available leading to poor visitation in commercial groves. Pollination occurs by mainly wild insects while the uses of the honeybees are unnecessary.

2.2.6 Pollen tube growth

Pollen tube elongates by tip growth and vegetative cells provide many of the activities needed for this process. In the pistils pollen tube remain anchored to the stigmatic surface via a trail of spent pollen tube materials. In addition to polysaccharides, proteins are also secreted to the pollen cell surface- extra cellular matrix continuum and provide for both the chemical and physical factors necessary for pollen tube growth. Pollen tube inhibition in the transmitting tissue of the style and such morphological abnormalities as swelling and callose deposition at the tips have been described as expression of gametophytic self incompatibility in almond (Pimienta *et al*., 1983). Continuous deposition of callose plugs behind the extending pollen tube tips restricts the pollen tube cytosol to the most proximal segment of the pollen tube (Cheung *et al*., 2000). Jayaprahasam *et al.* (2001) reported that amongst the different cross combinations the highest number of pollen tubes noted up to the base of the style was recorded in the cross Dashehari x Chausa followed by Amrapali x Sensation while the lowest pollen retention was registered in the cross Dashehari x Langra after 72 hours of pollination. In the case of selfing, the highest number of pollen tubes reaching upto in the base of the style was reported in the Bangalora selfed while, the lowest pollen tubes number was recorded in Langra followed by Chausa and Dashehari, respectively. Kahan and Demason (1988) observed in citrus between 1 and 3 days after pollination generative cell division occurred in pollen tubes in stigmas of Dancy tangerine. Although generative cell division was observed between 1 and 3 days in a few pollen tubes growing on Orlando stigmas. Hampson *et al.* (1993) found that in Hazelnut compatible crosses, penetrable of the style was visible sometimes after 12 hours or more readily at 18 hours or later. No evidence for penetration of the style was found in the incompatible pollinations. This difference was almost entirely due to delayed pollen germination in self pollinated pistils rather than slower rate of growth in style. Similar report is also available in tobacco (Cruzan, 1986), pistachio (Shuraki and Sedgley, 1994) and litchi (McConchie *et al*., 1992). Sedgley and Annels (1981) reported low temperature inhibition of pollen tube growth in avocado. In almond, the early reception of the a tree‘s own pollen by the stigma may limit the physical space available for the reception of compatible pollen but more importantly perhaps,

given the heterotrophic characteristic of pollen tube growth (Herrero and Dickinson, 1981), it may drain the reserves of the style (Herrero and Dickinson, 1979) and prevent the tubes of compatible pollen from developing and fecundating the ovule. Heslop-Harrison, (1975) reported that in the gametophytic system which is the one present in apricot, the stigma bears a secretion fluid and the pollen germinates in this. The style is solid, and the tubes grow in a matrix material between the widely separated and elongated stylar cells. Incompatible tubes are inhibited at some point during their passage usually penetrate through the first third of the length of the style and then commonly burst at the tip. In apricot the most common situation is that pollen tubes are arrested about the third fourth of the style.

2.2.7 Fertilization and post fertilization developments

Singh *et al.* (1962) while analyzing causes of self incompatibility in the varieties Dashehari, Langra, Chausa and Bombay Green reported that the pollen germination on the stigma to be normal, the pollen tubes started entering the embryo sac within 12 hours of pollination, irrespective of the type of mating. Eighteen hours after pollination, the pollen tubes had entered the embryo sac of 40 per cent and 70 per cent of selfed and crossed flowers, respectively also by 24 hours the percentage was as high as 90 in the selfed and 100 in crossed flowers respectively. In the same study one of the selfed fruitlets, fixed 25 days after pollination, the embryo sac together with the proembryo and the endosperm were completely degenerated, whereas the ovules of comparable crosses had well developed endosperm and embryo. The types of degeneration encountered were similar to the ones reported in *Secale cereal* (Landes, 1939), *Medicago sativa* (Brink and Cooper, 1939), *Beta sp*. (Savitsky, 1950) and *Vicia faba* (Rowlands, 1963). Singh *et al*. (1962) reported that unlike the typical cases of self incompatibility where the incompatibility reaction occurs at the stigma or style, the S allele action in his study was delayed and inhibition occurred in the embryo sac after fertilization. As yet *Theobroma cacao* (Cope, 1958), *Gasteria verrucosa* (Sears, 1937), black currants (Ledeboer and Rietsema, 1937), hence it is indicated that the self-incompatibility system in mango may be of sporophytic type.

In most examples of homomorphic, gametophytic SI the growth of incompatible pollen tubes is arrested in the stylar transmitting tissue, sometimes with swelling or bursting of the tip. However, an increasing number of examples have accumulated in the literature which report that the incompatible pollen tubes are not inhibited before they reach the ovary where they may actually penetrate the ovules. *Theobroma cacao*-Sterculiaceae (Knight and Rogers, 1955; Cope, 1962) has been traditionally cited as the sole example of this phenomenon but there are several other early studies from diverse families, of *Gasteria verrucosa* Liliaceae (Sears, 1937), *Medicago sativa* - Leguminosae (Brink and Cooper, 1939), *Lotus corniculatus*- Leguminosae (Bubar, 1959), and an increasing number of more recently described cases, eg. *Sterculia chichi*

Sterculiaceae (Taroda and Gibbs, 1982), *Acacia retinodes* - Leguminosae (Kenrick *et al.*, 1984. In the classic example of *Theobroma Cacao,* Knight and Rogers (1955) and Cope (1962) reported discharge of the male gametes into the embryo sac but failure of syngamy. Ovaries with a critical number of non-fusion ovules failed to develop fruits. In *Acacia retinodes* Kenrick *et al*. (1984) suggest that self pollen tube inhibition occurs in the nucellus and syngamy does not occur. However, in *Asclepias syriaca* both syngamy and endosperm nucleus formation are reported but only the endosperm nucleus begins to divide and early abortion follows (Sparrow and Pearson, 1948). Clearly, some of these cases of ovarian self-incompatibility intergrate with other situations where self-incompatibility has not been established but in which differential seed-set occurs following self- versus cross-pollinations eg. *Fagus sylvatica* Fagaceae (Blinkenberg *et al*., 1958), *Lotus jacobeus* and *L. tenuis* Leguminosae (Bubar, 1958) in which a polygenic `feed-back' type of mechanism causes increasing self-sterility in the progeny of initially self-fertile plants.

Unfortunately, in most species which appear to have late-acting self incompatibility we simply do not know what happens after self-pollen tubes reach the ovary and detailed histological studies, either with sectioned material or cleared ovules, are urgently required for such taxa. It seems certain that late-acting SI' covers a range of situations including some pre-zygotic mechanisms *i.e*., SI in a strict sense and others involving postzygotic events. Some of the former may simply represent examples of species with essentially gametophytic SI (e.g. *Acacia retinodes)* or heteromorphic SI (e.g. *Anchusa officinal*) but with a delayed incompatibility response. Other cases may represent a novel gamete-gamete level of SI reaction. The post-zygotic situations involving embryo abortion may likewise represent a novel form of SI mechanism or, perhaps more likely, comprise a whole range of self-sterility phenomena with rather generalised genetic control. At present we simply do not know enough about the nature or control of the threshold or `trigger' events which lead to selfed ovule abortion. These examples of late-acting SI' also seem to overlap with current ideas on 'sexual selection' (Stephenson and Bertin, 1983), although this is a general area where theory tends to outstrip empirical evidence. Certainly a few studies, eg. Stephenson and Winsor (1986) have produced good evidence for the occurrence of sexual selection in *Lotus corniculatus.* These authors were able to show that seed of surviving fruits in plants which had naturally self-thinned fruit set were fitter (as assessed by comparison of vegetative growth and reproductive output of progeny) than such seed of plants subjected to random hand-thinning. However, other studies (Wiens *et al.*, 1987) indicate that embryo abortion can have a simple mechanistic cause due to competition, *e.g*. for hormonal stimuli between young fruits with few versus many developing seeds.

2.2.8 Fruit set

Singh *et al.* (1962) found that fruit set obtained after selfing in Dashehari and Langra

was much less than that obtained from crossing. The adverse effect of selfing was more pronounced in varieties Chausa and Bombay Green, where none of the selfed fruitlets attained even marble size and majority of the selfed fruitlets in varieties Dashehari, Langra, Chausa and Bombay Green dropped within about four weeks of pollination. Mukherjee, *et al.* (1968) reported that out of 966 flowers self-pollinated in Dashehari only two fruits developed beyond pea stage, which also dropped subsequently. On examination, such dropped fruit revealed a tiny and shrivelled ovule, creamy white or black in colour. Of the 657 flowers sib-mated, the result was the same as that of selfing, whereas in crossing, a large number of mature fruits were obtained in Dashehari. In Chausa although the fruit set beyond pea stage was very high in crossed flowers, fruits dropped before these could reach maturity. The evidence of inbreeding, shown by a consistent low bud density, a high flower sterility and a lower fruit set after self- than after cross-pollination, recommends that a first step to increase productivity is the diversification of the parents in the breeding programmes (Alonso and Socias i Company, 2005), at present mostly limited to 'Tuono' for self-compatibility transmission (Socias i Company, 2002). Thus, in addition to bud density, flower sterility, pollination success and environmental conditions, other traits must be taken into account when evaluating yield in selfcompatible almond cultivars, such as the inbreeding effect and the effective autonomous self-pollination.

2.2.9 Fruit growth

Singh *et al.* (1962) investigated that in the varieties Dashehari, Langra and Chausa there were practically no differences in ovary and ovule size of fruitlets obtained from self and cross pollination up to the tenth day after pollination but in Bombay Green, however, the deleterious effect of selfing was more evident even at the same age. From fifteen days onwards the ovaries and ovules of selfed fruitlets of all the four varieties were invariably smaller than those of crossed fruitlets of the same age.

2.3 Research Area III (Objective III): To determine cross incompatibility in mango (*Mangifera indica* L.).

2.3.1 Cross incompatibility

Ram *et al.* (1976) investigated that pollination studies revealed self incompatibility in Dashehari, Chausa and Langra cultivars of mango. Dashehari was cross incompatible with Chausa and Safeda Malihabad and Chausa with Bombay green and Rataul. Langra appeared to be cross incompatible with Dashahari, Safeda Malihabad, Bombay Green, Chausa, Fazri, Surkha Verma, Rataul, Safeda Lucknow and Totapuri. Langra, Rataul and Bombay Green were compatible pollinizers for Dashehari, whereas Langra and Safeda Malihabad for Chausa also

Bombay Green and Dashehari proved to be the best pollinizer for Dashehari and Chausa respectively.

Ruehle and Lynch (1936) found that self incompatibility has not been reported from Floridan cultivars but in Florida Haden has been suggested to be more productive when located in close proximity to other cultivars. In Israel, caged Turpentine trees produce a good yield. However, a significant advantage was found in hand pollination study for pollination with foreign pollen over self pollination (Roizman, 1984). In South Africa, Zill pollen was found to be significantly more effective than self pollen in Haden hand pollination (de Wet *et al*., 1989). The development of isozyme as genetic marker in mango (Degani *et al*., 1990 and Truscott *et al*, 1994) has made it possible to distinguish hybrid offspring from selfed ones and to determine outcrossing rates between cultivars in orchards (Yukoto, 1995).

2.3.2 Inter-varietal hybridization

Mukherjee *et al*. (1961) was the first to describe inter-varietal hybridization with 1.45% success. Pinto and Byrne (1993) suggested an improvement to the technique and increased fruit set to 6 %, which promotes the enlargement of the hybrid population and shortening of cultivar release. Although the caging of top-worked cultivars is time consuming and expensive, the number of pollinated flowers is much higher than that obtained by hand pollination. The caging technique and floral induction are essential for the poly-cross method, since there is synchronous flowering of the top-worked cultivars. Tagging and bagging fruits give 100% of crossing assurance, allowing the breeder to know the generation obtained. Recently, Pinto (1999) suggested a new strategy to improve open pollination efficiency by using a Latin square experimental design method.

2.3.3 Fruit retention

Under north Indian conditions low, temperature condition consistently induce flowering with inflorescence carrying large numbers of flowers that is often followed by low fruit set and high fruit drop during the early stages of fruit development. Whiley *et al.* (1988) found that exposure of flower to low temperature below a minimum 12^0C during flowering interfered with pollination and/or fertilization of polyembryonic mango cultivars. In mango, Issarakraisila *et al.* (1992) found that Kensington trees produced abnormal flowers with short styled, small sized ovaries and black anthers when the daily mean temperature of the orchard was below 15^0C. They suggested that the cause of low fruit set in mango in sub tropical climate was related to abnormal flower morphology. There was a direct relationship between temperature and pollen viability. There were significant differences in pollen viability between cultivars grown at day/night temperature of 20/10 ^{0}C and 30/20 ^{0}C but no significant differences occurred between cultivars at 25/15 ^{0}C. At 20/10 ^{0}C Sensation pollen viability (7.4 %) was significantly lower

than Kensington (85 5%). The much reduced viability of sensation pollen indicates that pollen development for this cultivar is highly sensitive to low temperature. Issarakraisila *et al.* (1992) reported that 60 % of styles in Kensington flower developed under field condition were shortened when daily mean temperature were 7 and 15 ^{0}C. They suggested that short styled mango flower had reduced capacity to set fruit (6 %) when compared to long styled flower (90 %). They observed low rate of effective pollination may result from concurrent environmental factors, particularly inadequate pollinator activity and suboptimal temperatures. Sukhvibul *et al.* (2000) reported that the effect of temperature on duration of anthesis had important implication for potential fruit set and yield in mango. A prolonged anthesis period at low temperature increases the opportunity for successful pollination and ovule fertilization while shorter anthesis period and reduced flower number at high temperature contributed to the lower yield recorded for mango in tropical climate (5 ton ha^{-1}) compared with production in subtropics (15-20 ton ha^{-1}). In contradiction to these reports Sharma and Singh (1970) and Singh *et al.* (1962) had reported low fruit set in mango as a genotypic character (*i.e.*, self and cross incompatibility).

3. MATERIALS AND METHODS

The present investigation on **"Studies on incompatibility and viability of stored pollen in mango (*Mangifera indica* L.)"** was carried out at the Division of Fruits and Horticultural Technology, IARI, New Delhi, Division of Entomology, IARI, New Delhi, Division of Floriculture and Landscaping, IARI, New Delhi and National Phytotron Facility, IARI, New Delhi during the year 2010-11. Fluorescent microscopic studies for compatibility were undertaken at NRC on DNA Fingerprinting, NBPGR, New Delhi. Cryopreservation and other storage studies were done with the help of facilities available from the Tissue Culture and Cryopreservation Unit (TCCPU) NBPGR, New Delhi. The materials and methodologies adopted for the experimentations are described in this chapter.

3.1 Experiment No. 1: To study the effect of different storage methods on pollen viability in mango cultivars.

3.1.1 Experimental material

In the present study, three popular mango pollen parents *viz*., Sensation, Tommy Atkins and Janardan Pasand were selected from the main orchards of Division of Fruits and Horticulture Technology, IARI, New Delhi. Trees of these pollen parents were fairly old (20-25 years), healthy and free from diseases and pests. Description of pollen parents are as follows.

Sensation

An exotic collection from Florida (USA), used for imparting red peel colour in regular bearer cultivars for export purposes. The original tree grew from a seed planted in North Miami, Florida in 1935. For some decades, the parents of 'Sensation' were unknown but a 2005 pedigree analysis estimated that 'Sensation' was likely a cross between 'Haden' and 'Brooks. An important red colour donor parent in mango breeding programme at IARI, New Delhi. The fruit is of oval shape and typically weighs less than a pound. It has a small beak above the apex, sometimes no beak at all. The distinctive feature is the colour of the skin, which is a dark plum red. The skin often contains numerous pale yellow lenticel dots. The fruit are often born in clusters. Fruit size small (250 g) to sometimes just over 450 g, oval; skin beautifully coloured. The flesh is light yellow in colour with very fine fiber and flavor is mildly sweet with a light aroma. The fruit contains a monoembryonic seed. It ripens in August making it a late-season cultivar.

Tommy Atkins

The 'Tommy Atkins' mango developed from a seed planted in the early 1920's near Ft. Lauderdale, Florida. The heavy fruit production and outstanding red colour of the fruit at tracted favorable attention of local people. It is an important red colour donor parent in mango breeding programme at IARI, New Delhi. 'Tommy Atkins' trees are vigorous in growth, with a dense, rounded canopy. The fruit is medium to large in size, weighing 450 to 700 g. It is oval to oblong in shape with a broadly rounded tip and an inconspicuous nak. The fruit surface is smooth and the skin is thick and resistant to mechanical injury. The fruit is resistant to infection by the fungus which causes anthracnose disease. The external colour of the fruit is excellent. Ground colour is orange-yellow and the blush is bright to dark red. The flesh is medium to dark yellow in color. Fruit quality is very good. Flavor is fair to good. The firm flesh renders the fruit quite resistant to handling damage and gives it a long storage and shelf life.

Janardhan Pasand

Red colour donor male parent in mango breeding programme at IARI, New Delhi. Under Delhi conditions it flowers during mid of 15th March to 25th of March. Maturity is mid to late season. Fruits medium, oblong; shoulder equal and level, sinus shallow; skin medium thick, cadmium with a blush of red feather on most parts of the fruit; flesh firm, empire yellow and fibreless. Fruit quality is good. Flavour pleasant, taste sweet, juice scanty to moderately abundant. Keeping quality is medium. Moderately resistant to hopper and wind.

3.1.2 Pollen collection

Pollens were collected between 8.00 and 10.00 hours in the morning. Freshly and fully opened male flowers with red or purple anthers were collected from selected pollen parents. These pollens were subjected to four storage conditions and six storage periods with three replications in each treatment. For each replication, a minimum number of 50 flowers were placed in petridishes lined with moist paper and brought immediately to the laboratory. The flowers were then placed under sun to induce dehiscence and pollens were collected in small cryo vials and kept into desiccators for 3-4 hours for reducing the moisture content. After desiccation, the cryo vials were sealed and transferred to respective storage conditions. The weekly weather data is given in Annexure 4.1.1.

3.1.3 Storage condition

Pollens collected in cryo vials were subjected to four different storage conditions (room temperature, -4 ^{0}C, -20 ^{0}C and -196 ^{0}C). For room temperature storage, the sealed vials were kept at clean and dry place free from any light exposure (which served as control treatment). For storage at -4 ^{0}C, refrigerator was used with constant temperature maintenance.

Storage at -20 ^{0}C was also achieved through refrigeration. Storage of pollens at -196 ^{0}C (cryostorage) was achieved through dipping the cryo vials in sealed liquid nitrogen cylinders. Pollens collected in cryo vials were stored for six different time intervals *viz*. 4, 8, 12, 16, 20 and 24 weeks under different storage conditions. Pollen samples were taken out from the lot and were used for the viability testing using *in vitro* germination (Stanley and Linskens, 1974), fluorescein diacetate (FDA) (Helpson–Harrison and Helpson–Harrison, 1970) and acetocarmine tests (Nassar *et al*., 2000).

3.1.4 Observation recorded

Pollen viability of fresh and stored pollen from pollen donor parents was confirmed using *in vitro* germination, Fluorescein diacetate (FDA) and acetocarmine tests at different intervals i.e., 4, 8, 12, 16, 20 and 24 weeks.

3.1.4.1 *In vitro* germination test

In vitro pollen germination was assessed by using hanging drop technique suggested by Stanley and Linskens (1974). The liquid germination media contained 150 g l^{-1} polyethylene glycol (PEG 4000), 4.88 g l^{-1} N- morpholinoethanosulfonic acid in potassium hydroxide buffer (MES- KOH, pH 6.4), 200 mg l^{-1} $MgSO_4$, 100 mg l^{-1} KNO_3, 100 mg l^{-1} H_3BO_3, 700 mg l^{-1} $Ca(NO_3)_2$ and 200 g l^{-1} sucrose at pH 5.5 (modified Vivian-Smith *et al*., 1992). To obtain uniform pollen samples, pollens were thoroughly mixed in 1 ml of liquid germination medium. A 15-µl aliquot of this pollen mixture was placed on a glass slide inside an 8 mm diameter ring. Slides were inverted and placed on a rack in a polycarbonate sealed container lined with moistened blotting paper and incubated in darkness for 24 h at room temperature. At the end of incubation period, a minimum of 100 pollen grains per slide were randomly counted under a light microscope (Leica DM 1000) at X 20 magnification (Plate 4.1.1.). Pollen was adjudged as having germinated when the length of the pollen tube was equal to or exceeded pollen diameter (Stanley and Linskens, 1974).

3.1.4.2 Fluorescein diacetate (FDA) test

To asses pollen viability FDA test was used as suggested by Helpson–Harrison and Helpson–Harrison (1970). Stock solution of FDA was prepared in acetone (2 mg/ml). Sucrose solution of 10 % concentration was used to prevent bursting of pollen grains. Addition of 300 mg/ml of calcium nitrate into sucrose solution improved the response of pollen. To 2-5 ml of sucrose solution in a small glass vial drops of stock solution of FDA was added until the resulting mixture showed persistent turbidity. A drop of sucrose FDA mixture was taken on a micro slide. Sufficient amount of pollen grains were suspended in the drop and uniform distribution of the pollen in the preparation was ensured. The preparation was incubated in a

humidity chamber (>90% RH) for 5-10 min. At the end of incubation period, a cover glass was lowered and the preparation was observed under the fluorescent microscope (Leica DM 5000B) with fluorescence filters. Pollen grains fluoresce brightly were taken as viable and scored (Plate 4.1.2.).

3.1.4.3 Acetocarmine test

The pollen grains were distributed with the assistance of a brush and after, were dyed with the 1% acetocarmine (Nassar *et al*., 2000). Pollen grains were observed through an optical microscope (light microscope 20x magnification) and were subsequently classified as fertile or not fertile. Pollens grains that presented visibly abnormal sizes, light colouring and reduced and/or absent protoplasm were considered nonviable (does not have potential to germinate), while those that presented intact exines and strongly coloured protoplasm with homogeneous distribution were classified as viable (having potential to germinate) (Plate 4.1.2.).

3.1.5 Statistical analysis

The data has been analysed by using Repeated Measure Technique. For the analysis of data, mixed procedure in SAS 9.2 has been used. Mixed procedure takes care of correlated structure of the error variance-covariance matrix. In mixed procedure, data is analysed using the iteration procedure and ANOVA has been obtained by this procedure.

3.2 Experiment No. 2: Determination of self incompatibility in mango.

3.2.1 Botanical description of *Mangifera indica* L.: Inflorescence, flower and pollen

Mango flowers are borne on terminal pyramidal and are glabrous or pubescent; the inflorescence is rigid and erect, up to 30 cm long and is widely branched, usually tertiary, although the final branch is always cymose. The inflorescence is densely flowered with hundreds of small flowers, which are 2-10 mm in diameter. The flowers are either monoecious or polygamous and both monoecious and polygamous flowers are borne within a single inflorescence. The pistils abort in male flower. The ratio of monoecious to polygamous flower is strongly influenced by environmental and cultural factors. The flowers have four or five sepals and petals that are ovate to ovoid to lanceolate and also thinly pubescent. The floral disc also is four or five lobed, fleshy and large and located above the base of petals. The ovary is sessile, one-celled, oblique and slightly compressed in its lateral aspect. It is placed on the disc. The ovule is anatropous and pendulous and shows one-sided growth. The style arises from the edge of the ovary and ends in a simple stigma. Sometimes three carpels may develop in a flower (Singh, 1960). There are five large, fleshy stamens, only one or two of them being fertile; the remaining stamens are sterile stamenoides that are surmounted by a small gland. In

addition, two or three smaller filaments arises from the lobes of the nectaries. The stamens are central. The pollen grains are of variable shapes, with the size varying from 20 to 35 micron (Singh, 1954)**.** Small amount of pollen is produced in *M. indica*; the grains are sphaeroidal to prolate sphaeroidal, radially symmetrical, subangular in polar view, isopolar, with a few giant triploid ones of up to 50 micron, they are 3-monocolporate, goniotreme, sides convex-subprolate; apertures equidistant and zonal; ecto-aperture (colpus) extends slit-like from pole to pole.

3.2.2 Experimental material

Four IARI released mango cultivars *viz.,* Amrapali (Dashehari x Neelum), Mallika (Neelum x Dashehari), Pusa Arunima (Amrapali x Sensation) and Pusa Surya (Selection) were chosen for the present study. Fully grown healthy grafted plants free from diseases and pests of four mango cultivars were selected from the orchards of Division of Fruits and Horticulture Technology, IARI, New Delhi. During the course of study, all the trees received uniform cultural management practices. Description of mango cultivars are as follows.

Amrapali

It has been evolved at IARI as a result of a cross between Dashehari and Neelum. This was released in 1978. It is precocious, distinctly dwarf, highly regular and prolific bearing. It is suitable for high density orcharding (Majumdar *et al*., 1982). Fruit size is relatively smaller (143.0 g) than Dashehari but are compensated by other good points viz., regularity and prolific bearing, high pulp percentage (74.8) and total soluble solids (22.8 ^{0}Brix). The flesh is deep orange red and has about 2.5-3.0 times more β-carotene content, which indicates high vitamin A than its parents. This cultivar appears to better suited for preparing a good quality, highly colourful mango nectar for which there is considerable demand in foreign markets (Singh 1990).

Mallika

Among the promising hybrids Mallika a cross between Neelum and Dashehari has been released in 1971. Its tree is semi vigorous. It is medium to heavy cropper and has a tendency to bear regularly. The fruits have an attractive appearance and the average fruit weight is 307 g, pulp percentage is 74. The pulp is fibreless and firm and the stone is very thin. The percentage of total soluble solids are higher (25) than that of Dashehari (21). It has a better keeping quality and also mature latter than Dashehari.

Pusa Arunima

It has been evolved as a result of crossing between Amrapali X Sensation and released in the year 2002. The plants are of semi-vigorous nature and are suitable for closer planting (6m x 6m). It is a regular bearer cultivar and starts bearing after 4th year of planting. Late in maturity

and ready for harvest in 1st week of August. Fruits are of medium size (250g/fruit) with red peel colour, TSS 19.5^0 Brix, excellent sugar : acid blend pulp is firm, fibreless, flavoured aroma and excellent in taste, rich in Vit.C (43.6mg/100g pulp) and carotene content, good shelf-life (10-12 days at room temperature after ripening). This cultivar is suitable for entire mango growing areas of India

Pusa Surya

This cultivar is a selection from exotic cultivar 'Eldon' released in the year 2002. It is Semi-vigorous, suitable for closer planting (6m x 6m), less susceptible to malformation. It is a regular bearer cultivar and starts bearing after 4th year of planting. Late in maturity and ready for harvest in 3rd week of July. Fruits are of medium to large in size (270g/fruit) with apricot yellow peel colour, TSS 18.5^0 Brix, excellent sugar : acid blend pulp is firm, fibreless, flavoured aroma and excellent in taste, rich in Vit.C (42.6mg/100g pulp) and carotene content, good shelf-life (8-10 days at room temperature after ripening). This cultivar is suitable for entire mango growing areas of India

3.2.3 Number of male, hermaphrodite and unusual flowers

The male flowers are similar to the hermaphrodite flowers but are without the pistil, which has been aborted. Hermaphrodite flowers are small (5–10 mm) with four to five ovate, pubescent sepals and four to five oblong, lanceolate, thinly pubescent petals. Only one or two of the four to five stamens that arise from the inner margin of the disc are fertile. The single ovary is born centrally on the disc with the style arising from one side. There are five large, fleshy stamens, only one or two of them being fertile; the remaining stamens are sterile stamenoides. In general, mango ovaries are green, spherical and easy to make out, its slender style pointing skyward and ending with a tiny stigmatic area where pollen grains germinate. Mango ovaries are unusual in that their styles do not emerge from their centres but rather a bit to one side or there is more than one ovary in a single flower. For counting male, hermaphrodite and unusual flowers, 10 panicles were tagged in each tree on four directions. Observation on male, hermaphrodite and unusual flowers were recorded during middle of flowering season when specific cultivar attained > 50% bloom. Total numbers of flowers of above discussed kinds were counted in fully opened panicles with naked eyes and with the help of magnifying lens.

3.2.4 Selfing and open pollination of mango cultivars

For self pollination, total 30 panicles (10 panicles/replication) were taken on three different trees of each mango cultivars. Hand pollination was attempted using the technique described by Mukherjee *et al*. (1961). Panicles directly arising from secondary or tertiary branches were selected because of their more retention capacity. These panicles were bagged in

finely perforated alkathane bags ($8^{//}$ x $5^{//}$) of 100 gauge thickness preceding evening, after removing all opened flowers. The next morning 10-12 freshly opened perfect flowers/panicle were kept for pollination and all other unopened buds were removed. Hermaphrodite flowers on middle of panicle were selected. As the experiment was on self incompatibility therefore emasculation was not necessary and not attempted. The panicles were then rebagged. A stock of freshly opened flowers for the pollen of same parents was collected from the panicles bagged earlier and kept in separate petridish in shade. Prior to pollination, anthers were dehisced in semi shade conditions by transferring them to different petridishes. Bags from the panicles were removed and hand pollination was done on each panicle one by one. Each panicle was properly labelled and rebagged immediately after pollination. The bags were removed after 72 hrs and the fruit set was recorded at different intervals. A set of 30 panicles (10 panicles/replication) were allowed for open pollination and observations on fruit set, fruit growth and pollen-pistil interaction were recorded at different intervals. For this purpose panicles of similar size and age to that of selfed panicles were tagged and observations were taken on intervals.

3.2.5 Fruit setting

Fruit setting percentage was calculated by observing the number of fruitlets that developed in response to self and open pollination at intervals viz., 6, 10, 14, 18, 22 and 26 days after pollination. Fruitlet retention up to marble size (26 days after pollination) was taken as final fruitlet set.

3.2.6 Pollen tube growth measurement under fluorescence microscopy

Pollen tube growth measurement at different intervals under fluorescence microscopy was studied using the procedure suggested by Martin (1959). Collection and fixation of pollinated flowers were done after 6, 24, 48 and 72 hours after pollination in FAA solution (Formalin: Acetic acid: Ethyl alcohol; 20:10:70). Later, the flowers were rinsed with distilled water and preserved in 70% ethanol under constant refrigeration (-4 ^{0}C) until further processing of the samples for observation. Fixed flowers were carefully excised and softened with 4N NaOH for 45 minute in a hot air oven (60 ^{0}C). Softened pistils were stained for observing pollen tubes and callose growth using freshly prepared Aniline Blue (0.005 %) stain in 0.05M Na_2HPO_4 (pH 8.2) for 3-4 hours. After staining pistils were mounted in 1:1 glycerol: aniline blue dye solution covered with coverslips and moderately squeezed and counts immediately made under fluorescent microscope (Leica DM 5000 B) for pollen tube growth. The observations were made at different sampling time of 6, 24, 48 and 72 hours and pollen tube penetrance *i.e.*, less than 10% of style, 1/3rd of the style, 2/3rd of the style and up to micropyle were graded symbolically (0, +, ++ and +++, respectively). Fertilization was considered to

complete when the pollen tube entered the micropyle and fertilized ovule (Jayaprakasham, 2001).

3.2.7 Ovule and fruit growth of selfed and open pollinated fruitlets

Size of fruitlets (length and breadth) and ovules (ovule length and breadth) were measured using stereo microscope (Leica M 205 FA) and Vernier's Calliper and were expressed in mm. Fruitlets weight were measured with the help of precision balance (Adair Dutt 1620 C) and were expressed in gram (g).

3.2.8 Statistical analysis

The experiments were laid out in RBD with control design. Four cultivars were taken in three replications with ten panicles in each replication. ANOVA was calculated to separate the means. The data on number of male, hermaphrodite, unusual flowers per panicle were analysed through post-hoc test (DMRT) using software SPSS 17. The data on percentage of fruit set were angular transformed before analysis and means were back transformed for analysis. In the ovule and fruit growth studies data were analysed using software Agristat. The C.D. at 5% was used to compare treatment means.

3.3 Experiment No. 3: Determination of cross incompatibility in mango.

3.3.1 Experimental material

Healthy grafted trees of mango cultivars namely, Amrapali (Dashehari x Neelum), Pusa Arunima (Amrapali x Sensation), Dashehari and Langra free from diseases and pest were selected from orchards of the Division of Fruits and Horticulture Technology, IARI, New Delhi. During the course of study, all the trees received uniform cultural management practices. Description of the cultivars are as follows.

Amrapali

Please refer section 3.2.2 (Description of the cultivars) in Experiment No. 2

Pusa Arunima

Please refer section 3.2.2 (Description of the cultivars) in Experiment No. 2

Dashehari

It is one of the most important and popular cultivar of north India, also known as Dasehri and Aman Dasehri**.** The tree is of medium height and moderate vigour, spreading with a rounded, medium dense canopy; the fruit is primrose to canary yellow with abundant light yellow dots, oblong to oblique with base rounded to obliquely rounded, medium sized, skin

smooth, medium thick, tough and non adhering, the flesh is yellow, firm with almost no fibre, scanty juice and a delightful aroma, very sweet taste and of excellent quality (Singh, 1960)

Langra

Originated as a chance seedling tree. It is an important commercial cultivar of north India. Tree tall and spreading. It is binneal bearer and a mid season cultivar, with good quality fruits. Flesh is firm, lemon yellow in colour and scarcely fibrous. It has a characteristic turpentine flavor. Keeping quality is medium. Stone almost oblong with a narrow and empty basal end and rounded apical end; small and fine fibre all over the surface, long and coarse tuft of fibre on the entire ventral edge; veins slightly depressed to almost an the surface level. It has a wide range of adaptability.

3.3.2 Crossing of selected cultivars

Crossings were attempted following the technique of Mukherjee *et al*. (1961), where panicles of female parents directly arising from secondary or tertiary branches were selected because of their more retention capacity. These panicles were bagged in finely perforated alkathane bags ($8^{//}$ x $5^{//}$) of 100 gauge thickness preceding evening, after removing all opened flower. The next morning 8-12 freshly opened perfect flowers were retained for pollination per panicle and all other opened and unopened buds were removed. Retained flowers were emasculated by removing the anther with the help of forceps. The panicles were then rebagged immediately. A stock of freshly opened flowers of the desired male parents was collected from the panicles bagged earlier and kept in separate petridish in shade. Prior to pollination, anthers were dehisced in semi shade conditions by transferring them to different petridishes. Bags from the panicles were removed and hand pollination was done on each panicle one by one. Each panicle was properly labeled and rebagged immediately after pollination. The bags were removed 48 hours after pollination and the fruit set was recorded at different intervals.

3.3.3 Fruit set

Fruits were counted on each panicles at different intervals *viz.*, 6, 10, 14, 18, 22 and 26 days after pollination. Fruitlet retention up to marble size (26 days after pollination) was taken as final fruitlet set. Per cent fruit set was then calculated based on intial and final counts.

3.3.4 Pollen tube growth

Pollen tube growth measurement at different intervals under fluorescence microscopy was studied by the procedure as suggested by Martin (1959). Collection and fixation of pollinated flowers were done after 6, 24, 48 and 72 hours after pollination in FAA solution (Formalin: Acetic acid: Ethyl alcohol; 20:10:70). Later, the flowers were rinsed with distilled water and preserved in 70% ethanol under constant refrigeration (-4 ^{0}C) until further processing

of the samples for observation. Fixed flowers were carefully excised and softened with 4N NaOH for 45 min in a hot air oven (60 ^{0}C). Softened pistils were stained for observing pollen tubes and callose growth using freshly prepared Aniline Blue (0.005 %) stain in 0.05M Na_2HPO_4 (pH 8.2) for 3-4 hours. After staining pistils were mounted in 1:1 glycerol: aniline blue dye solution covered with coverslips and moderately squeezed and counts immediately made under fluorescent microscope (Leica DM 5000 B) for pollen tube growth. The observations were made at different sampling time of 6, 24, 48 and 72 hours for their penetrance in stylar region. Fertilization was assumed to happen when the pollen tube entered the micropyle and fertilized the ovule (Jayaprakasham, 2001).

3.3.5 Ovule and fruit growth

Size of fruitlets and ovules and weight of fruitlet were measured at 7, 14, 21 and 28 days intervals after crossing. Size of the ovules (length and breadth) and fruitlets (length and breadth) were measured using stereo microscope (Leica M 205 FA) and Vernier's Calliper and were expressed in mm. Fruitlets weight were measured with the help of high precision electronic balance (Adair Dutt 1620 C) and were expressed in gram (g).

3.3.6 Statistical analysis

The experiments were laid out in factorial randomised block design (RBD). Four cultivars were crossed in all possible combinations (4*3) and a total of 12 cross combinations were attempted. Three plants of each mango cultivar were taken as replication and 10 panicles on each plant (replication) making 30 panicles per cross combination were attempted for crossing. ANOVA was calculated to separate the means. The data on percentage of fruit set were angular transformed before analysis and means were back transformed for analysis. In the ovule and fruit growth studies data were analysed using software Agristat. The C. D. at 5% was used to compare the treatment means.

4.1. RESEARCH PAPER I

Effect of storage methods on pollen viability in mango (*Mangifera indica* L.) cultivars

4.1.1 Abstract

The problem of asynchronised flowering in certain mango cultivars restricts their use as parent in mango hybridization programme and circumstances usually make it necessary to store the pollen from male donor parent for later pollination of the female parents. In the present investigation, pollen viability of three mango pollen parents *viz*., Sensation, Tommy Atkins and Janardan Pasand were investigated up to 24 weeks under four different storage conditions (room, -4^{o}C, -20^{o}C and -196^{o}C). Three different methods of viability estimations were utilized *viz*., *in vitro* germination, FDA and acetocarmine tests. Fresh pollens from all the three pollinisers gave higher pollen viability in terms of *in vitro* germination, FDA and acetocarmine tests as compared to stored pollens. ANOVA clearly indicated that storage methods and interaction between storage methods and days of storage had highly significant effect on pollen viability as confirmed by *in vitro* germination test ($p < 0.0001$). In case of room temperature storage, Sensation, Tommy Atkins and Janardan Pasand showed only 2.11%, 1.80% and 1.03% pollen viability up to 4 weeks of storage as confirmed by *in vitro* pollen germination test, after which pollens were not able to germinate in any of the pollen parents. Pollen storage of mango cultivars at -20 ^{0}C showed almost similar trend as it was observed in case of -4 ^{0}C storage. The only difference noticed was the percentage of viability was higher in all three pollen parents at all dates of observations compared to pollen storage at -4 ^{0}C. Cryostored pollens showed significantly higher viability percentage as compared to all the other storage conditions as confirmed by *in vitro* germination, FDA and acetocarmine tests of pollen viability in all pollen donor parents. Moreover, the differential results obtained by using different pollen viability assay confirmed that *in vitro* germination test was more reliable compared to FDA and acetocarmine tests, in which germination is often overestimated. From the present study we suggest storage of pollen at -20^{o}C for pollination among cultivars having non synchronized flowering in the same season. However for long term pollen storage cryo storage proved to be the best method.

Key words: Mango, pollen viability, *in vitro* germination, FDA, acetocarmine.

4.1.2 Introduction

Mango (*Mangifera indica* L.) belonging to family Anacardiaceae is one of the commercially important fruit crop of India having centre of origin in Indo-Burma region. Mango hybridization programme is underway at several research institutes of India and several important mango hybrids have been developed over the last few decades. Indian Agricultural Research Institute, New Delhi is notable among them credited with the development of some commercially important mango hybrids with desirable traits for domestic as well as overseas markets. Existing diversity of mango with respect to important traits provides huge scope for breeding new cultivars with desirable character (Ram and Rajan, 2003). Flowering period in mango ranged from December to April depending on agroclimatic zones and mango hybridization programmes are on several occasions hampered by spatial and temporal isolation of the parents (Chaudhury *et al.,* 2010). Red peel colour is important trait from export point of view and attention have been paid to develop red peeled mango cultivars at Indian Agricultural Research Institute, New Delhi over last 2-3 decades. For imparting red colour in the peel, mango cultivars *viz*., Sensation, Tommy Atkins and Janardan Pasand are preferentially used as male donor in breeding programmes. Mango blooming season in north India starts in February and lasts through March, whereas the regular harvesting season extends from June to August. Problem of asynchronised flowering in male and female mango cultivars restricts their use as parents in mango hybridization programme. Therefore, circumstances usually make it necessary to keep the pollen of the male parent for later pollination of the female parent. Thus, in order to optimize the pollination for both early and late blooming cultivars, it would be necessary to conserve pollen from early flowering cultivars for pollination in late season in late blooming cultivars. Mango pollen has a short viability and a high sensitivity to desiccation and consequently, conservation is problematic (Issarakraisila and Considine, 1994). Pollen germination capacity depends on the conditions of storage; critical external factors include relative humidity, temperature surrounding the pollen (Luza and Polito, 1985 and Polito and Luza, 1989). Therefore, an attempt was made to determine the period of time over which pollens from male donor parents retain viability under different storage conditions.

4.1.3 Material and methods

4.1.3.1 Experimental material

In the present study, three popular mango pollen parents *viz*., Sensation, Tommy Atkins and Janardan Pasand were selected from the main orchards of Division of Fruits and Horticulture Technology, IARI, New Delhi. Trees of these pollen parents were fairly old (20-25 years), healthy and free from diseases and pests.

4.1.3.2 Pollen collection

Pollens were collected between 8.00 and 10.00 hours in the morning. Freshly and fully opened male flowers with red or purple anthers were collected from selected pollen parents. These pollens were subjected to four storage conditions and six storage periods with three replications in each treatment. For each replication, a minimum number of 50 flowers were placed in petridishes lined with moist paper and brought immediately to the laboratory. The flowers were then placed under sun to induce dehiscence and pollens were collected in small cryo vials and kept into desiccators for 3-4 hours for reducing the moisture content. After desiccation, the cryo vials were sealed and transferred to respective storage conditions. The weekly weather data is given in Annexure 4.1.1.

4.1.3.3 Storage condition

Pollens collected in cryo vials were subjected to four different storage conditions (room temperature, -4 ^{0}C, -20 ^{0}C and -196 ^{0}C). For room temperature storage, the sealed vials were kept at clean and dry place free from any light exposure (which served as control treatment). For storage at -4 ^{0}C, refrigerator was used with constant temperature maintenance. Storage at -20 ^{0}C was also achieved through refrigeration. Storage of pollens at -196 ^{0}C (cryostorage) was achieved through dipping the cryo vials in sealed liquid nitrogen cylinders. Pollens collected in cryo vials were stored for six different time intervals *viz*., 4, 8, 12, 16, 20 and 24 weeks under different storage conditions. Pollen samples were taken out from the lot and were used for the viability testing using *in vitro* germination (Stanley and Linskens, 1974), fluorescein diacetate (FDA) (Helpson–Harrison and Helpson–Harrison, 1970) and acetocarmine tests (Nassar *et al*., 2000).

4.1.3.4 *In vitro* germination test

In vitro pollen germination was assessed by using hanging drop technique suggested by Stanley and Linskens (1974). The liquid germination media contained 150 g l^{-1} polyethylene glycol (PEG 4000), 4.88 g l^{-1} N- morpholinoethanosulfonic acid in potassium hydroxide buffer (MES- KOH, pH 6.4), 200 mg l^{-1} $MgSO_4$, 100 mg l^{-1} KNO_3, 100 mg l^{-1} H_3BO_3, 700 mg l^{-1} $Ca(NO_3)_2$ and 200 g l^{-1} sucrose at pH 5.5 (modified Vivian-Smith *et al*., 1992). To obtain uniform pollen samples, pollens were thoroughly mixed in 1 ml of liquid germination medium. A 15-µl aliquot of this pollen mixture was placed on a glass slide inside an 8 mm diameter ring. Slides were inverted and placed on a rack in a polycarbonate sealed container lined with moistened blotting paper and incubated in darkness for 24 h at room temperature. At the end of incubation period, a minimum of 100 pollen grains per slide were randomly counted under a light microscope (Leica DM 1000) at X 20 magnification (Plate 4.1.1.). Pollen was adjudged as

having germinated when the length of the pollen tube was equal to, or exceeded pollen diameter (Stanley and Linskens, 1974).

4.1.3.5 Fluorescein diacetate (FDA) test

To asses pollen viability FDA test was used as suggested by Helpson–Harrison and Helpson–Harrison (1970). Stock solution of FDA was prepared in acetone (2 mg/ml). Sucrose solution of 10 % concentration was used to prevent bursting of pollen grains. Addition of 300 mg/ml of calcium nitrate into sucrose solution improved the response of pollen. To 2-5 ml of sucrose solution in a small glass vial drops of stock solution of FDA was added until the resulting mixture showed persistent turbidity. A drop of sucrose FDA mixture was taken on a micro slide. Sufficient amount of pollen grains were suspended in the drop and uniform distribution of the pollen in the preparation was ensured. The preparation was incubated in a humidity chamber (>90% RH) for 5-10 min. At the end of incubation period, a cover glass was lowered and the preparation was observed under the fluorescent microscope (Leica DM 5000B) with fluorescence filters. Pollen grains fluoresce brightly were taken as viable and scored (Plate 4.1.2.).

4.1.3.6 Acetocarmine test

The pollen grains were distributed with the assistance of a brush and after, were dyed with the 1% acetocarmine (Nassar *et al*., 2000). Pollen grains were observed through an optical microscope (light microscope 20x magnification) and were subsequently classified as fertile or not fertile. Pollen grains that presented visibly abnormal sizes, light colouring and reduced and/or absent protoplasm were considered nonviable (does not have potential to germinate), while those that presented intact exines and strongly coloured protoplasm with homogeneous distribution were classified as viable (having potential to germinate) (Plate 4.1.2.).

4.1.4 Statistical analysis

Data has been analysed by using Repeated Measure Technique**.** For the analysis of data, mixed procedure in SAS 9.2 has been used. Mixed procedure takes care of correlated structure of the error variance-covariance matrix. In mixed procedure, data is analysed using the iteration procedure and ANOVA has been obtained by this procedure.

4.1.5 Results

Germination of fresh pollen of three mango pollen parents was examined using *in vitro* germination, FDA and acetocarmine test. It was interesting to note that regardless of cultivars different viability tests showed differential results. Comparatively higher pollen viability was depicted by acetocarmine test, however, *in vitro* germination test depicted less pollen viability.

In vitro pollen germination of fresh pollens was 50.14 % for Sensation, 48.78 % for Tommy Atkins and 47.30 % for Janardan Pasand, which was statistically *at par* and did not differ significantly from each other (Table 4.1.4). It was evident from the data presented in Table 4.1.4 that fresh pollen viability confirmed by *in vitro* germination and acetocarmine tests in all three pollen parents was non-significant ($P \geq 0.05$). However, fresh pollen viability tested using FDA test showed significant differences among mango cultivars ($P \leq 0.05$) and Sensation had highest pollen viability (88.27%) followed by Janardan Pasand (85.48%) and Tommy Atkins (83.242%) (Table 4.1.4). All three pollen viability tests proved that fresh pollen in all the three pollen parents had higher pollen viability during the peak flowering season compared to stored ones.

Table 4.1.1 to 4.1.3 shows the analysis of variance (ANOVA) for *in vitro* germination, FDA and acetocarmine tests for three mango cultivars, four storage methods and six storage periods. ANOVA clearly indicated that storage methods and interaction between storage methods and days of storage had highly significant effect on pollen viability as confirmed by *in vitro* germination test ($p < 0.0001$). Similarly, the effect of mango cultivar and interaction between mango cultivars and storage methods was significant at $p < 0.01$ level. Result pertaining to pollen viability as confirmed using FDA test showed highly significant effect of storage methods, days of storage and interaction between storage methods and days of storage on pollen viability ($P < 0.0001$) (Table 4.1.2). Results pertaining to pollen viability obtained from acetocarmine tests also showed a similar trend with highly significant effect of storage methods, days of storage and interaction of storage method and days of storage on pollen viability ($p < .0001$) (Table 4.1.3).

Results obtained on pollen viability of three mango cultivars under different storage methods are depicted in Fig 4.1.1 to 4.1.4. At room temperature storage, Sensation, Tommy Atkins and Janardan Pasand showed only 2.11%, 1.80% and 1.03% pollen viability up to 4 weeks of storage as confirmed by *in vitro* pollen germination test, after which the germination percentage declined to zero at later stages of storage (Fig 4.1.1. A). In case of room temperature storage, similar trend was observed when pollen viability was tested using FDA and acetocarmine assay methods as it was observed in *in vitro* germination test. In all pollen viability tests in all three pollen parents pollen viability was found to be 0.0 per cent at 8, 12, 16, 20 and 24 weeks after storage (Fig 4.1.1B and 4.1.1C).

Pollen storage at -4 ^{0}C showed non-significant pollen viability differences among three mango cultivars on all dates of observation as confirmed by FDA and acetocarmine viability tests. However, pollen viability determined by *in vitro* germination test showed significant variation among pollen donor parents (LSD=2.88, $P \leq 0.05$). In all mango male donor parents, the pollen viability was found to be decreasing significantly ($P \leq 0.05$) with the increase in

storage period as confirmed by all three pollen viability tests (Fig 4.1.2 A to C). In Sensation, the fresh pollen viability was found to be 50.14% (*in vitro* germination test), 88.27% (FDA test) and 87.71% (acetocarmine test), which reduced to 6.75%, 14.77% and 21.01%, respectively after 24 weeks of storage at -4 ^{0}C. Similarly in Tommy Atkins the fresh pollen viability was found to be 48.78% (*in vitro* germination), 83.42% (FDA test) and 88.28% (acetocarmine test), which further reduced to 6.52%, 12.00% and 20.32% , respectively after 24 weeks of storage at -4 ^{0}C. Janardan Pasand showed almost similar trend and fresh pollen viability was reduced to 5.49% in *in vitro* germination, 15.73% in FDA test and 16.43% in acetocarmine test after 24 weeks of storage at -4 ^{0}C as compared to the viability of fresh pollen (47.30%, 85.48% and 90.25%, respectively).

Pollen storage at -20 ^{0}C showed almost similar trend as it was observed in case of -4 ^{0}C storage (Fig 4.1.3 A to C). The only difference noticed was the percentage of viability was high in all three pollen parents at all dates of observations compared to pollen storage at -4 ^{0}C. In Sensation, pollen viability showed decrease of 79.23% tested by *in vitro* germination test, 79.04% by FDA test and 62.15% by acetocarmine test compared to fresh pollen viability after 24 weeks of storage at -20^{0}C. Similarly, in Tommy Atkins, the decrease in pollen viability after 24 weeks of storage at -20^{0}C was 74.94% (*in vitro* germination test), 75.90% (FDA test) and 61.62% (acetocarmine test) and for Janardan Pasand the decrease was 76.57% (*in vitro* germination test), 78.07% (FDA test) and 60.46% (acetocarmine test) as compared to fresh pollen viability.

Storage of pollens at -196^{0}C showed a significantly higher viability percentage as compared to all the other storage conditions as confirmed by *in vitro* germination, FDA and acetocarmine tests of pollen viability (Plate 1 and 2). Pollens of Sensation showed a significantly higher *in vitro* germination percentage than Janardan Pasand and Tommy Atkins at all the stages of viability test (Fig 4.1.4 A). Similarly, FDA pollen viability test also confirmed highest pollen viability in case of Sensation (66%) after 24 weeks of storage (Fig 4.1.4 B). Contrastingly, results obtained from acetocarmine test showed highest pollen viability in Janardan Pasand (78.15%) compared to pollen viability of other two cultivars stored at -196 ^{0}C on all dates of observation (Fig 4.1.4 C). Rate of reduction in pollen viability in all three pollen parents was minimal in case of -196 ^{0}C storage compared to other methods of storage which makes it most suitable technique for conserving pollen for both short as well as long term period. Pollen viability under -196 ^{0}C storage condition as confirmed by *in vitro* germination test in all three pollen donor mango cultivars showed non-significant differences between 4 and 24 weeks of storage. Similarly, pollen viability depicted by FDA and acetocarmine tests almost showed similar trend in all three pollen parents with few exceptions. For example, in case of FDA test, the pollen viability of Sensation cultivar decreased between 4 to 8 weeks and after 8

weeks, non-significant decrease in pollen viability were noticed. Likewise, in case of Tommy Atkins, the pollen viability tested by using acetocarmine test showed significant decrease in pollen viability between 4-8 weeks; however, after 8 weeks pollen viability did not differ significantly up to 24 weeks.

4.1.6 Discussion

Our results showed that freshly collected pollens of all the three cultivars had high pollen viability compared to stored pollen. Results were obvious and had strong conformity with findings in other species like litchi (Chaudhury *et al.* 2010), cherimoya (Lora *et al*., 2006), *Zea mays* (Inagaki, 2000), and *Brassica campestris* (Mulcahy and Mulcahy, 1988).

Pollens stored at room temperature were viable up to 4 weeks. However, per cent viability significantly reduced during this period (Fig. 4.1.1) compared to fresh pollen viability. The decrease in pollen viability at room temperature during first 4 weeks might be attributed to high sensitivity of mango pollen to high temperature and low humidity. The differential rate of reduction in pollen viability stored at different temperature may be because of rate of metabolic activities in pollen is temperature dependent. Mango pollen is known to be highly susceptible to desiccation and loose water from pollen when kept at a temperature above 25-27 ^{0}C. The metrological data observed during April month suggest that the average mean minimum temperature ranged between 17.6 to 22.3 ^{0}C and mean maximum temperature ranged between 37.7 to 42.2 ^{0}C further support present results. Our results are in strong agreement with the earlier report of Chaudhury *et al.* (2010) in mango and litchi.

All pollen viability tests such as *in vitro* germination, FDA and acetocarmine test revealed that significantly higher pollen viability was maintained at -196 ^{0}C followed by -20^{0}C and -4^{0}C at all dates of observation in all three pollen parents and the trend was same up to 24 weeks of storage. Pollen longevity has been reported to be extended by using lower temperatures such as 5^{0}C, -20^{0}C, -80 ^{0}C and -196 ^{0}C and low moisture content (Towill and Walters, 2000). The pollen once cryostored at temperature below -160 ^{0}C would theoretically have infinite period of longevity (Stanwood, 1985). In the present study high pollen viability was maintained by storage at -196 ^{0}C followed by -20^{0}C and -4 ^{0}C in all the three mango pollen parents as evidenced by pollen viability observed using *in vitro* germination test, FDA test and acetocarmine tests. The differential results obtained by using different pollen viability assay confirmed that in vitro germination test was more reliable and in FDA and acetocarmine tests germination is often overestimated. A similar overestimation was observed with mango pollen for fresh pollens and after storage at various storage conditions. Chaudhury *et al.* (2010) and Shivanna and Helpson-Harrison (1981). Thus, we have been able to devise a short-term pollen storage method with the help of simple refrigeration and through cryostorage. Trend

revealed by cryostorage showed the possibility of long-term storage of mango pollen. The studies undertaken by Indian Institute of Horticultural Research (Ganeshan, 2003) on pollen storage also confirmed our results. Results revealed that at -196 ºC pollen could be stored for long-term, for efficient conservation of genetic resources, for hand pollination both for commercial fruit production and for breeding. However, pollen can be efficiently stored for short periods at -20ºC and -4 ºC temperatures for few weeks. In mango, blooming season start from February and lasts up to April, consequently, pollen collected and stored at different storage conditions in February can be used for pollinations throughout mango blooming season. These make availability of pollen of differentially blooming parents in circumstances of non-synchronised flowering and to avoid the need of daily pollen collection to perform manual pollination the following day.

4.1.7 Conclusion

Pollen viability of three mango polliniser cultivars *viz*., Sensation, Tommy Atkins and Janardan Pasand were investigated up to 24 weeks of storage under four different storage conditions (room, -4 ^{o}C, -20 ^{o}C and -196 ^{o}C). Three different methods of viability estimations were utilized *viz*., *in vitro* germination, FDA and acetocarmine tests, respectively. Fresh pollens from all the three pollen parents gave higher pollen viability in terms of *in vitro* germination, FDA and acetocarmine tests, respectively. Storage at -196 ^{o}C resulted in significantly higher viability percentage compared to other methods, it gave the highest viability percentage after 24 weeks of storage followed by -20 ^{o}C and -4 ^{o}C storage conditions. Storage at room temperature resulted in non-viable pollen after 4 weeks of storage. Based on the findings of the present investigation, we suggest that pollen of these pollen parents could be stored for long period only under -196 ^{o}C. However, for short duration storage in refrigerators at -4 ^{o}C and -20 ^{o}C seems to be more convenient methods. Furthermore, it was worth mentioning that genotypic differences were not pronounced and all three pollen donor parents behaved in similar way. Among different viability tests, *in vitro* germination test was found to be most reliable followed by FDA test.

Table 4.1.1. ANOVA for pollen viability as confirmed by *in vitro* germination test for three pollen parents, four different storage methods and six storage periods.

Effect	Num DF	Den DF	F Value	Pr > F
Cultivars (C)	2	4	20.16	0.0081
Storage methods (SM)	3	6	1348.22	<.0001
Days (D)	5	10	63.95	<.0001
C*SM	6	12	4.41	0.0139
C*D	10	20	0.43	0.9141
SM*D	15	30	20.98	<.0001

Table 4.1.2. ANOVA for pollen viability as confirmed by FDA test for three pollen parents, four different storage methods and six storage periods.

Effect	Num DF	Den DF	F Value	Pr > F
Cultivars (C)	2	4	0.25	0.7872
Storage methods (SM)	3	6	2640.09	<.0001
Days (D)	5	10	181.54	<.0001
C*SM	6	12	1.22	0.3602
C*D	10	20	0.99	0.4804
SM*D	15	30	48.75	<.0001

Table 4.1.3. ANOVA for pollen viability as assayed by acetocarmine test for three pollen parents, four different storage methods and six storage periods.

Effect	Num DF	Den DF	F Value	Pr > F
Cultivars (C)	2	4	1.61	0.3069
Storage methods (SM)	3	6	2416.84	<.0001
Days (D)	5	10	100.10	<.0001
C*SM	6	12	2.02	0.1413
C*D	10	20	0.64	0.7663
SM*D	15	30	26.85	<.0001

Table 4.1.4. Viability of fresh pollen of mango cultivars.

Cultivars	Pollen viability test		
	In Vitro	**FDA**	**Acetocarmine**
Sensation	50.14±4.06 (45.06)	88.27± 0.93 (69.95)	87.71± 4.01 (69.62)
Tommy Atkins	48.78± 3.64 (44.28)	83.42± 1.59 (65.96)	88.28± 4.47 (70.21)
Janardan Pasand	47.30±7.75 (43.43)	85.48 ±0.57 (67.58)	90.25± 3.58 (71.96)
CD at 5%			
Method of viability test	3.94		
Cultivar	N.S.		
Method of viability test x Cultivar	N.S.		

Values in the table shows mean ± SD. N.S. not significant ($p \leq 0.05$).

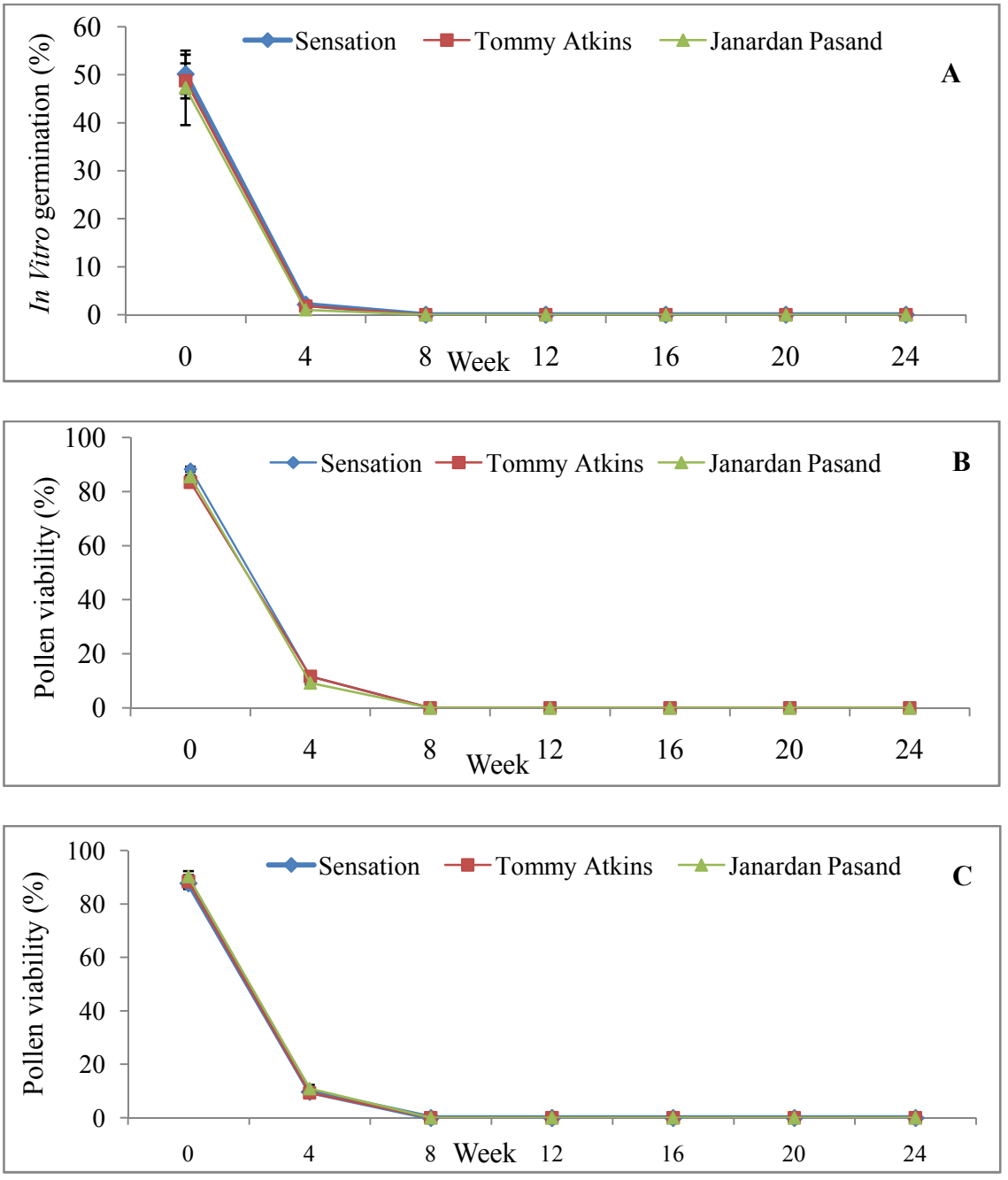

Fig. 4.1.1. Pollen viability of three mango cultivars stored at room temperature as evidenced by *in vitro* germination (A), FDA (B) and acetocarmine (C) tests. Vertical bars are standard deviation ± mean value. LSD for pollen viability as confirmed by *in vitro* germination test, cultivar=NS, days of storage= 1.99 and interaction of cultivar and storage period= NS at $P \leq 0.05$. LSD for pollen viability as evidenced by FDA test, cultivar= 0.34, days of storage= 0.52 and interaction of cultivar and storage period= 0.91 at $P \leq 0.05$. LSD for pollen viability as evidenced by acetocarmine test, cultivar= NS, days of storage= 1.61 and interaction of cultivar and storage period= NS at $P \leq 0.05$.

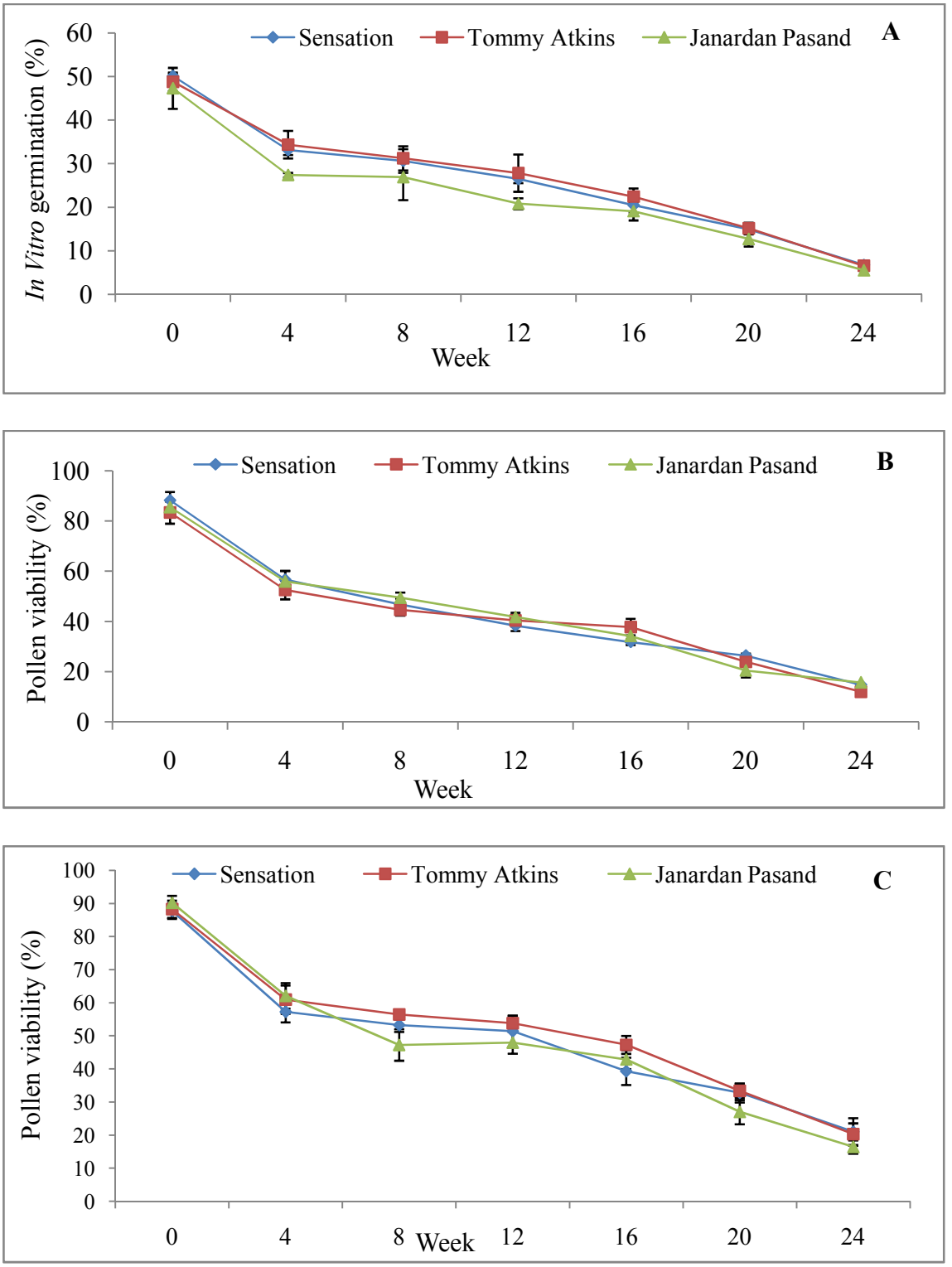

Fig. 4.1.2. Pollen viability of three mango cultivars stored at -4 ºC as evidenced by *in vitro* germination (A), FDA (B) and acetocarmine (C) tests. Vertical bars are standard deviation ± mean value. LSD for pollen viability as confirmed by *in vitro* germination test, cultivar=2.88, days of storage= 4.39 and interaction of cultivar and storage period= NS at $P \leq 0.05$. LSD for pollen viability as evidenced by FDA test, cultivar= NS, days of storage= 4.18 and interaction of cultivar and storage period= NS at $P \leq 0.05$. LSD for pollen viability as evidenced by acetocarmine test, cultivar= NS, days of storage= 5.30 and interaction of cultivar and storage period= NS at $P \leq 0.05$.

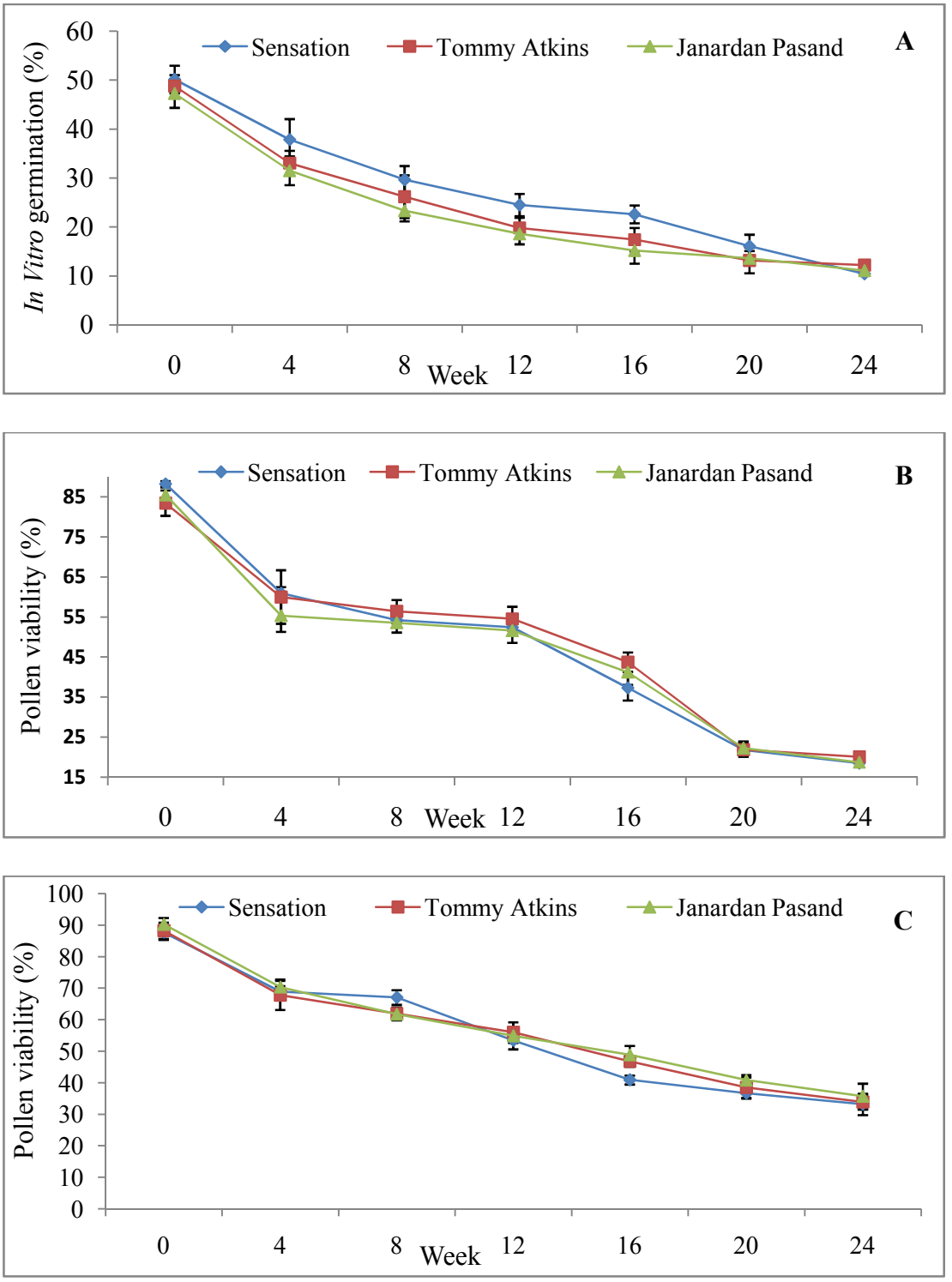

Fig. 4.1.3. Pollen viability of three mango cultivars stored at -20 ºC as evidenced by *in vitro* germination (A), FDA (B) and acetocarmine (C) tests. Vertical bars are standard deviation ± mean value. LSD for pollen viability as confirmed by *in vitro* germination test, cultivar=2.98, days of storage= 4.56 and interaction of cultivar and storage period= NS at $P \leq 0.05$. LSD for pollen viability as evidenced by FDA test, cultivar= NS, days of storage= 4.38 and interaction of cultivar and storage period= NS at $P \leq 0.05$. LSD for pollen viability as evidenced by acetocarmine test, cultivar= NS, days of storage= 4.33 and interaction of cultivar and storage period= NS at $P \leq 0.05$.

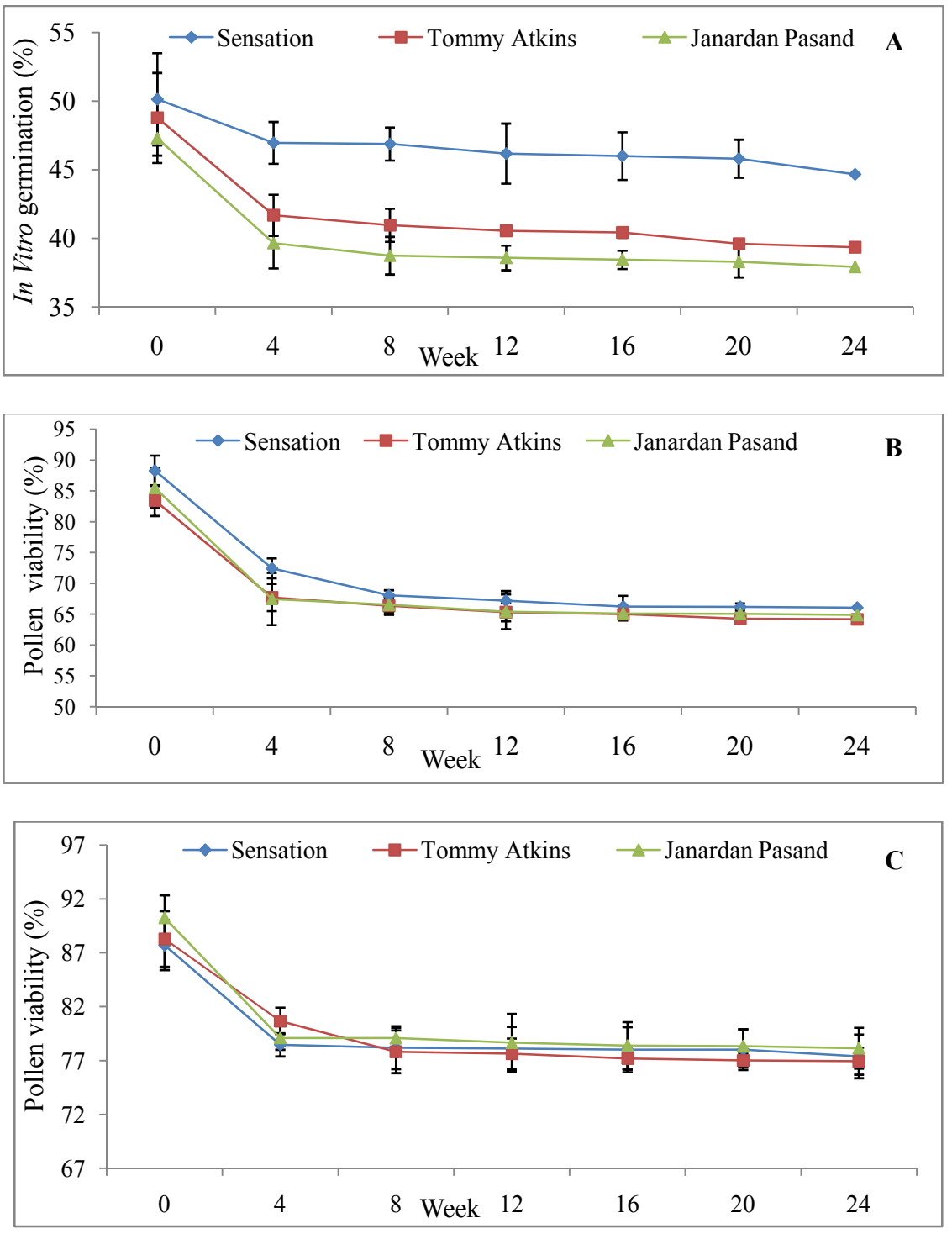

Fig. 4.1.4. Pollen viability of three mango cultivars stored at -196 ºC as evidenced by *in vitro* germination (A), FDA (B) and acetocarmine (C) tests. Vertical bars are standard deviation ± mean value. LSD for pollen viability as confirmed by *in vitro* germination test, cultivar=2.09, days of storage= 3.19 and interaction of cultivar and storage period= NS at $P \leq 0.05$. LSD for pollen viability as evidenced by FDA test, cultivar= 2.00, days of storage= 3.05 and interaction of cultivar and storage period= NS at $P \leq 0.05$. LSD for pollen viability as evidenced by acetocarmine test, cultivar= NS, days of storage= 3.91 and interaction of cultivar and storage period= NS at $P \leq 0.05$.

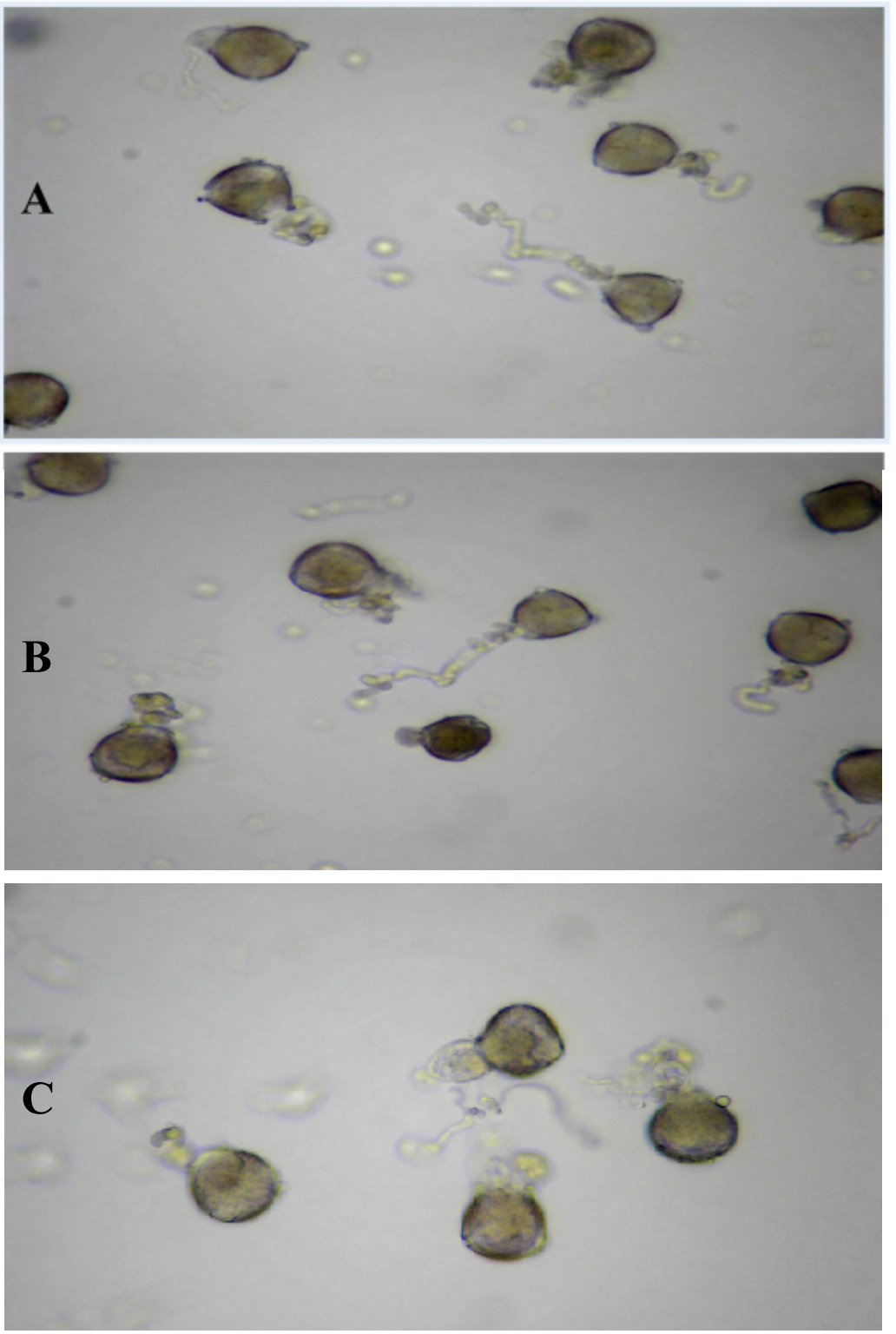

Plate 4.1.1. *In vitro* pollen germination of Sensation (A), Tommy Atkins (B) and Janardan Pasand (C) after 24 weeks of cryo storage, magnification 20x.

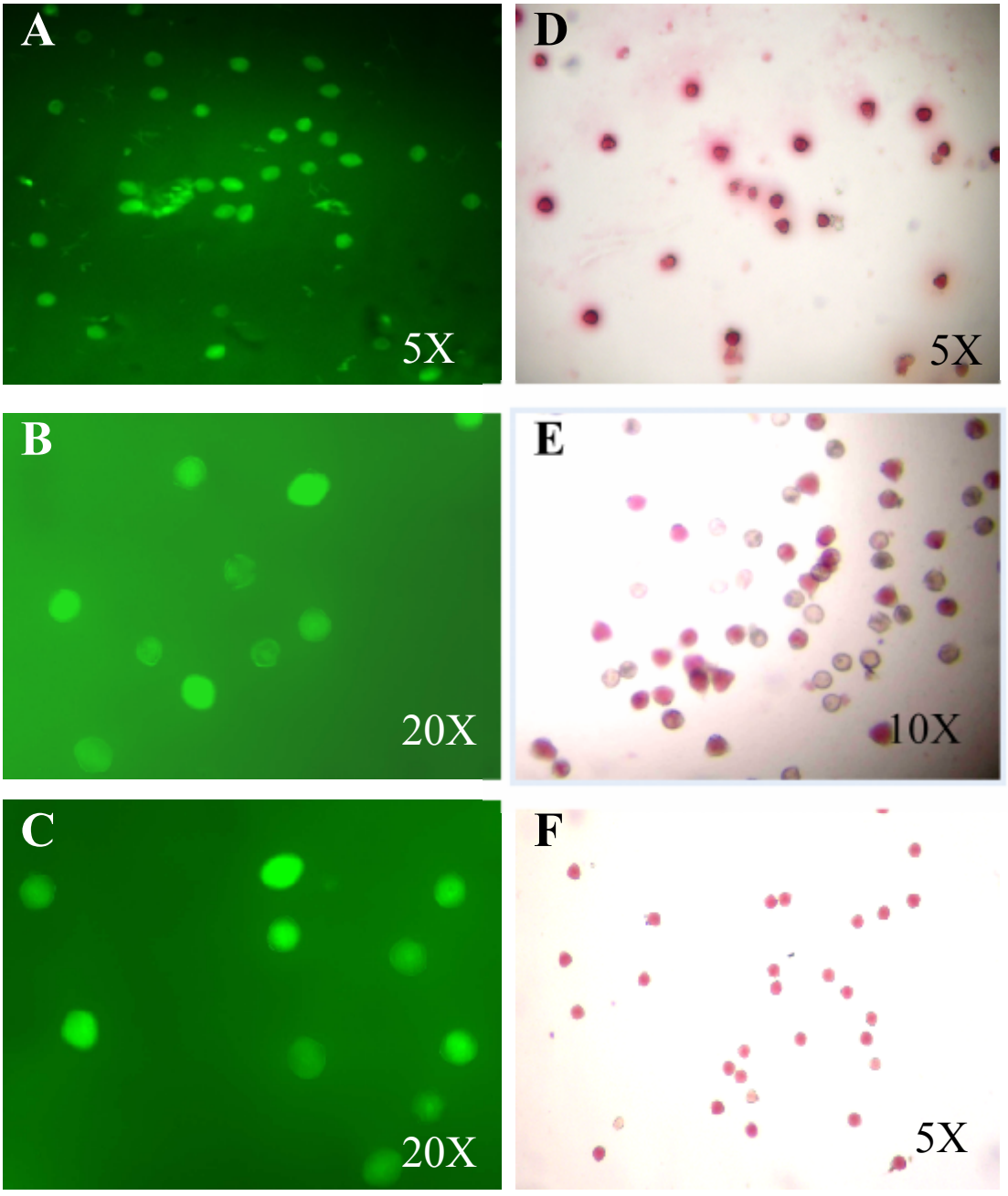

Plate 4.1.2. Pollen viability testing of Sensation (A), Tommy Atkins (B) and Janardan Pasand (C) by FDA test and Acetocarmine pollen test Sensation (D), Tommy Atkins (E) and Janardan Pasand (F) after 24 weeks of cryo storage.

4.2. RESEARCH PAPER II

Determinination of self incompatibility in mango (*Mangifera indica* L.)

4.2.1 Abstract

The present investigation was carried out in mango cultivars developed at Indian Agricultural Research Institute such as Amrapali, Mallika, Pusa Arunima and Pusa Surya. The effects of self and open pollination on the fruit set, ovule degeneration and growth rate of selfed and open pollinated fruitlets were compared. Self pollination resulted in very rapid decline in fruit setting percentage as compared to open pollination and which was much more pronounced in Amrapali and Mallika compared to Pusa Arunima and Pusa Surya which showed comparable fruit set upon selfing. During 48 hours after pollination, pollen tube growth in stylar region of selfed Amrapali and Mallika were considerably slower compared to selfed Pusa Arunima and Pusa Surya. After 48 hours of self pollination, pollen tube reached up to 2/3 of stylar region in Amrapali and Mallika but reached up to lower end of style in case of Pusa Arunima and Pusa Surya. Degeneration of ovule as a result of self and open pollination was much more apparent after 14 days after pollination. Amrapali and Mallika showed more than 75 degenerated ovules as a result of self pollination after 21 days and majority of selfed fruitlets dropped thereafter. However, open pollination resulted only 20% degenerated ovules in these mango varieties. Contrastingly, Pusa Surya (10%) and Pusa Arunima (20%) resulted less percentage of degenerated ovules after 21 days of self pollination. The growth of fruitlets and ovule obtained from self and open pollination of all the four cultivars revealed that the fruitlets weight and dimensions of fruitlets and ovule were significantly less after selfing as compared to open pollinated fruits in case of Amrapali, Mallika and Pusa Arunima, whereas there was no significant difference in fruitlet weight and dimension of fruitets and ovule between self and open pollinated Pusa Surya. The findings of present investigation bring out clearly that self incompatibility in mango unlike typical sporophytic self incompatibility is post zygotic and mango cultivar Mallika is self-incompatible, whereas, cultivar Amrapali is partially so. However, Pusa Arunima and Pusa Surya are self compatible.

Key words: Mango, self incompatibility, pollen tube growth, fluorescence microscopy, fruit set, fruit growth.

4.2.2 Introduction

Mango (*Mangifera indica* L.), one of the 73 genera of the family Anacardiaceae in order Sapindales, is amongst the most important tropical fruits of the world. It is also called as "King of the fruits" in India due to its historical and religious importance and attractive aroma and capitative taste. It originated in the South East Asian or Indo-Burma region having 69 recognized species of mango originating as forest trees with fibrous and resinous fruits (Kesterman and Bompard, 1993). In India, about 30 mango cultivars are being grown commercially. Majority of them posses narrow adaptability and show eco-geographical preferences for growth and yield (Yadav and Rajan, 1993). The ideal cultivar in mango should have characteristics like dwarf tree stature, precocity and regularity in bearing, attractive and good quality fruits, high productivity and resistance to major diseases and pests. Owing to the high heterozygosity and long juvenile period, conventional breeding in mango is intricate. Combining all the traits in a single cultivar is difficult and breeding objective may be defined for specific purpose.

Mango hybridization work is in progress at various centres in India. Indian Agricultural Research Institute, New Delhi is notable among them credited with the development of some commercially important mango hybrids with desirable traits for domestic as well as overseas markets. With the improved technique of mango hybridization and the report of self-incompatibility in mango, it would be possible to evolve a larger number of hybrids having desirable horticultural traits. The existence of self incompatibility in mango remained unknown until Singh *et al*. (1962) reported it in the cultivar Dashehari. Embryological studies have shown that in mango pollen tubes grow down the style and effect fertilization but the development of zygote is blocked leading to a sporophytic type of self-incompatibility (Mukherjee *et al*., 1968).

In recent past, number of improved mango hybrids have been developed at IARI, New Delhi by hybridizing parents, which are known to have self-incompatibility in their ancestry. Moreover, at several occasions mango growers faced the problem of poor fruiting in some mango cultivars grown in isolation. A very high fruit drop in different phases of fruit growth has been reported in many of the commercial cultivars and self-sterility had also been suspected but very few attempts have been made to study the varietal situation in this regard. There are not many reports with thorough investigation of the self incompatibility phenomena in most of the commercially cultivated mango cultivars. The present studies were, therefore, undertaken to investigate into this phenomenon in three mango hybrid varieties *viz*., Amrapali, Mallika and Pusa Arunima and one selection cultivar Pusa Surya developed at IARI. Attempts have been made to investigate the fruit set, fruit growth and pollen-pistil interaction under self and open pollinated condition in four mango cultivars.

4.2.3 Material and methods

4.2.3.1 Experimental plants

Four IARI released mango cultivars *viz.,* Amrapali (Dashehari x Neelum), Mallika (Neelum x Dashehari), Pusa Arunima (Amrapali x Sensation) and Pusa Surya (Selection) were chosen for the present study. Fully grown healthy grafted plants free from diseases and pests of four mango cultivars were selected from the orchards of Division of Fruits and Horticulture Technology, IARI, New Delhi. During the course of study, all the trees received uniform cultural management practices.

4.2.3.2 Number of male, hermaphrodite and unusual flowers

The male flowers are similar to the hermaphrodite flowers but are without the pistil, which has been aborted. Hermaphrodite flowers are small (5–10 mm) with four to five ovate, pubescent sepals and four to five oblong, lanceolate, thinly pubescent petals. Only one or two of the four to five stamens that arise from the inner margin of the disc are fertile. The single ovary is born centrally on the disc with the style arising from one side. There are five large, fleshy stamens, only one or two of them being fertile; the remaining stamens are sterile stamenoides. In general, mango ovaries are green, spherical and easy to make out, its slender style pointing skyward and ending with a tiny stigmatic area, where pollen grains germinate. Mango ovaries are unusual in that their styles do not emerge from their centres but rather a bit to one side or there is more than one ovary in a single flower. For counting male, hermaphrodite and unusual flowers, 10 panicles were tagged in each tree on four directions. Observation on male, hermaphrodite and unusual flowers were recorded during middle of flowering season when specific cultivar attained > 50% bloom. Total numbers of flowers of above discussed kinds were counted in fully opened panicles with naked eyes and with the help of magnifying lens.

4.2.3.3 Selfing and open pollination of mango cultivars

For self pollination, total 30 panicles (10 panicles/replication) were taken on three different trees of each mango cultivars. Hand pollination was attempted using the technique described by Mukherjee *et al*. (1961). Panicles directly arising from secondary or tertiary branches were selected because of their more retention capacity. These panicles were bagged in finely perforated alkathane bags ($8^{//}$ x $5^{//}$) of 100 gauge thickness preceding evening, after removing all opened flower. The next morning 10-12 freshly opened perfect flowers/panicle were kept for pollination and all other unopened buds were removed. Hermaphrodite flowers on middle of panicle were selected. As the experiment was on self incompatibility, therefore, emasculation was not necessary and not attempted. The panicles were then rebagged. A stock of freshly opened flowers for the pollen of same parents was collected from the panicles bagged

earlier and kept in separate petridish in shade. Prior to pollination, anthers were dehisced in semi shade conditions by transferring them to different petridishes. Bags from the panicles were removed and hand pollination was done on each panicle one by one. Each panicle was properly labelled and rebagged immediately after pollination. The bags were removed after 72 hrs and the fruit set was recorded at different intervals. A set of 30 panicles (10 panicles/replication) were allowed for open pollination and observations on fruit set, fruit growth and pollen-pistil interaction were recorded at different intervals. For this purpose panicles of similar size and age to that of selfed panicles were tagged and observations were taken on intervals.

4.2.3.4 Fruit setting

Fruit setting percentage was calculated by observing the number of fruitlets that developed in response to self and open pollination at intervals viz., 6, 10, 14, 18, 22 and 26 days after pollination. Fruitlet retention up to marble size (26 days after pollination) was taken as final fruitlet set.

4.2.3.5 Pollen tube growth measurement under fluorescence microscopy

Pollen tube growth measurement at different intervals under fluorescence microscopy was studied using the procedure suggested by Martin (1959). Collection and fixation of pollinated flowers were done after 6, 24, 48 and 72 hours after pollination in FAA solution (Formalin: Acetic acid: Ethyl alcohol; 20:10:70). Later, the flowers were rinsed with distilled water and preserved in 70% ethanol under constant refrigeration (-4 ^{0}C) until further processing of the samples for observation. Fixed flowers were carefully excised and softened with 4N NaOH for 45 minute in a hot air oven (60 ^{0}C). Softened pistils were stained for observing pollen tubes and callose growth using freshly prepared Aniline Blue (0.005 %) stain in 0.05M Na_2HPO_4 (pH 8.2) for 3-4 hours. After staining pistils were mounted in 1:1 glycerol: aniline blue dye solution covered with coverslips and moderately squeezed and counts immediately made under fluorescent microscope (Leica DM 5000 B) for pollen tube growth. The observations were made at different sampling time of 6, 24, 48 and 72 hours and pollen tube penetrance i.e. less than 10% of style, 1/3rd of the style, 2/3rd of the style and up to micropyle were graded symbolically (0, +, ++ and +++, respectively). Fertilization was considered to complete when the pollen tube entered the micropyle and fertilized ovule (Jayaprakasham, 2001).

4.2.3.6 Ovule and fruit growth of selfed and open pollinated fruitlets

Size of fruitlets (length and breadth) and ovules (ovule length and breadth) were measured using stereo microscope (Leica M 205 FA) and Vernier Callipers and were expressed in mm. Fruitlets weight were measured with the help of precision balance (Adair Dutt 1620 C) and were expressed in gram (g).

4.2.4 Statistical analysis

The experiments were laid out in RBD with control design. Four cultivars were taken in three replications with ten panicles in each replication. ANOVA was calculated to separate the means. The data on number of male, hermaphrodite, unusual flowers per panicle were analysed through post-hoc test (DMRT) using software SPSS 17. The data on percentage of fruit set were angular transformed before analysis and means were back transformed for analysis. In the ovule and fruit growth studies data were analysed using software Agristat. The C.D. at 5% was used to compare treatment means.

4.2.5 Results

4.2.5 .1 Male, hermaphrodite and unusual flower percentage

Mango cultivars were found to be significantly varying ($P \leq 0.05$) with respect to the percentage of male flowers per panicle (Table 4.2.1). Male flower percentage was found to be highest in case of Amrapali (50.21%) which was statistically *at par* with Mallika (48.01%) and Pusa Surya (40.08%). However, the minimum male flowers were recorded in Pusa Arunima (10.37%). Pusa Arunima showed significantly lower percentage of male flowers as compared to these three mango cultivars.

Similarly, variation in terms of number of hermaphrodite flower per panicle was also significant ($p \leq 0.05$) among mango cultivars (Table 4.2.1). Hermaphrodite flower percentage was highest in case of Pusa Arunima (89.62%) followed by Pusa Surya (59.91%) which did not differ significantly with Mallika (51.98%) and Amrapali (49.78%).

In present investigation, the maximum percentage of flowers having unusual stamens was noted in Amrapali (5.68%) followed by Pusa Arunima (2.42%), Mallika (1.08%) and Pusa Surya (0.68%). Amrapali had a significantly higher percentage ($p \leq 0.05$) of unusual stamened flowers as compared to the rest of the three mango cultivars (Table 4.2.1). However, percentage of unusual stamened flowers did not differ significantly among Pusa Arunima, Mallika and Pusa Surya.

Mango ovaries are considered unusual in which style does not emerge from their centres but rather a bit to one side or there is more than one ovary in a single flower. The extent of deformation varied significantly in mango varieties ($p \leq 0.05$). The percentage of hermaphrodite flowers showing unusual ovary was found highest in Pusa Arunima (5.38%) followed by Amrapali (1.43%) which did not have significant edge over Mallika (1.31%) and Pusa Surya (0.45%) (Table 4.2.1).

4.2.5 .2 Fruit set

The data presented in Table 4.2.2 to Table 4.2.5 showed fruit set after self and open pollination in four mango cultivars at an interval of 4 days. It was interesting to note that in all mango cultivars, self pollination resulted less fruit set as compared to open pollination on all stages of fruit growth (Plate 4.2.1 and 4.2.2).

In Amrapali, fruit set on 6th day after self pollination (DAP) was 47.97% which reduced to 0.37% on 26th DAP. It was also evident that in self pollinated Amrapali, fruit drop was remarkably high during first 18 DAP and around 99% selfed flowers dropped during this period. However, in case of open pollinated Amrapali, on 6th day fruit set was 80.0% which reduced to 11.08% on 26th DAP. In event of open pollination the reduction in fruit set during first 26 DAP was 85.0% as compared to 99% fruit drop in self pollination. This clearly suggests preferential response of mango cultivar Amrapali for open pollination as compared to self-pollination.

Similarly in case of Mallika, fruit set on 6th DAP was 65.20% which reduced to 3.30% after 14th DAP after which all the fruits dropped and there was no final fruit retention. Maximum fruit drop was reported up to 10th DAP where 89.32% of the fruitlets dropped. Whereas, in case of open pollination, fruit set after 6th DAP was 86.32% which reduced to 7.29% on 26th DAP. In case of self pollination reduction in fruit set was 100% but it was 91.55% in open pollination at 26th DAP. Fruit set data clearly shows that Mallika is completely self incompatible with no final fruit retention.

With self pollination in Pusa Arunima, fruit set on 6th DAP was 49.04% which reduced to 1.92% on 26th DAP. It was clear that in self pollinated Pusa Arunima fruit drop was remarkably high during initial 10 days after pollination, where 78.11% selfed flowers dropped. However, in case of open pollination, on 6th DAP fruit set was 88.32% which reduced to 16.06% on 26th DAP. Reduction in fruit set during first 26 DAP was 96.08% in self pollination, whereas it was 81.81% in open pollination.

Contrastingly, Pusa Surya selfed flowers showed a completely different trend, where fruit set on 6th DAP was 85.56% which reduced to 9.63% on 26thDAP, however, in open pollination fruit set on 6th DAP was 87.85% which reduced to 10.50% on 26th day after pollination. Reduction in fruit set during first 26 DAP was 88.74% in self pollination, whereas, it was 88.04% in open pollination. Fruit set on 26th day after pollination in both self and open pollination were statistically *at par*. Results showed that there was no prevalence of self incompatibility in case of mango cultivar Pusa Surya. The summary of initial (6 DAP) and fruit retention 26 days after self and open are depcited in Table 4.2.8.

4.2.5 .3 Pollen retention on stigmatic surface as a result of self pollination

The microscopic observation of pollen retention on stigmatic surface, post pollination clearly showed variation in the cultivars (Table 4.2.7). The pollen adhesion was highest (20.01 Pollens) in case of the Pusa Surya selfed stigmas, followed by the Amrapali selfed (18.67 pollens) after 6 hours of self pollination. The minimum pollen retention (13.02 pollens) was observed in case of Malika selfed (13.0 pollens) followed by Pusa Arunima (15.67 Pollens) after 6 hours of self pollination.

At 6 hours after self pollination pollen retention on stigmatic surface was higher and number declined with the passage of time. Observations taken 24 hours after self pollination indicated reduction in number of pollen retained on stigmatic surface in all four mango cultivars and in Pusa Surya selfed flowers a maximum of 16.33 pollens were observed while in Mallika selfed flowers had the lowest value (10.33 pollens).

4.2.5 .4 Pollen tube growth under fluorescence microscopy

Genotypes of pollens and pistils have a profound influence on pollen tube growth, which varied with self and open pollination. The pollen adherence on stigmatic surface and the extent of pollen tube growth in the pistil was observed under fluorescent microscope. Observations made under fluroscence microscope are given it Table 4.2.6 and Plate 4.2.5 and 4.2.6. In self pollinated Amrapali and Mallika, there was pollen adherence at stigmatic surface, pollen germination and subsequent growth inside the pistil.

After 6 hours of self pollination the pollen tube could travel only 1/3 of stylar region in Pusa Arunima and Pusa Surya. However, in self pollinated Amrapali and Mallika pollen tube travelled less than 10% of stylar region during this period. Interestingly, after 24 hours of self pollination, pollen tube travelled 2/3 stylar region in Pusa Arunima and Pusa Surya whereas in selfed Amrapali and Mallika pollen tube reached upto only 1/3 of stylar region. Simlarly, after 48 hours of self pollination, pollen tube reached upto 2/3 of stylar region in Amrapali and Mallika but reached up to micropyle end in case of Pusa Arunima and Pusa Surya. It was also evident that after 72 hours of self pollination, pollen tube reaching to micropyle was seen in all mango cultivars .

4.2.5.5 Fruit and ovule growth of selfed and open pollinated fruitlets

Data pertaining to weight of self and open pollinated fruitlets at 7 days interval from the event of pollination is depicted in Table 4.2.9. Irrespective of mango cultivars, the weight of fruitlets obtained from self pollination was significantly less (45%) (C.D. =0.011; $p \leq 0.05$) as compared to open pollinated fruitlets on 7^{th} day of observation. Similarly, weight of self pollinated fruitlets was 31% less as compared to open pollinated fruitlets on 14^{th} day after the

event of pollination (C.D.=0.042; $p \leq 0.05$). In case of Mallika, on 14th day onward all selfed fruilets dropped and in Amrapali only one fruitlet out of 271 initially set fruitlets retained but their open pollinated counterparts developed normally. However, in Pusa Arunima and Pusa Surya fruit set was apparent even on 21 and 26 day after self as well as open pollination. Regardless of self and open pollination attempts, the genotypic effect on weight of fruitlet of mango cultivars was also found significant ($p \leq 0.05$) on 7, 14 and 28 day after self and open pollination. On 7th day after pollination higher fruitlet weight was noticed in Pusa Surya (0.120 g). However, on 14th day Amrapali showed more fruitlet weight (0.211 g). The interaction between pollination method and mango cultivars was found significant on 7th and 28th days after pollination. On 28th day the maximum fruit weight was noted in Mallika open pollinated fruitlets, followed by open pollinated Amrapali fruitlets.

Table 4.2.10 and Plate 4.2.3 show the data on fruit length at 7, 14, 21 and 28 days after self and open pollination. At 7th and 14th DAP there was significant difference between selfed and open pollinated fruits in the fruit length. At 7th DAP fruit length was found 22.75% (C.D. =0.277; $p \leq 0.05$) higher in open pollination than self pollinated flowers while it was 22.27% higher (C.D.=0.467; $p \leq 0.05$) at 14 DAP. Regardless of self and open pollination, the genotypic effect on fruit length of mango cultivars was also found significant ($p \leq 0.05$) on 14, 21 and 28 day after self and open pollination. On 7th day after pollination higher fruit length was noticed in Amrapali (3.667 mm). However, also on 14th day, Amrapali showed more fruit length (8.603 mm). The interaction between pollination method and mango cultivars was found significant on 14th, 21th and 28th DAP. On 28th day, the maximum fruit length was noted in Mallika (16.423 mm) open pollinated fruitlets followed by open pollinated Amrapali (15.260 mm) fruitlets.

Irrespective of mango cultivars, the fruit breadth obtained from self pollination was significantly less with 19.15% (C.D. =0.160; $p \leq 0.05$) on 7th day, 15.55% (C.D. =0.566; $p \leq 0.05$) on 14th day, 21.86% (C.D. =0.626; $p \leq 0.05$) on 21th day and 24.87% (C.D.=0.731; $p \leq 0.05$) on 28th day less as compared to open pollinated fruits. Genotypic effect was also found significant at 14, 21 and 28 days after pollination. Open pollinated Amrapali (6.167 mm) gave highest fruit breadth on 14 DAP, while Pusa Surya showed highest fruit breadth on 21 DAP (9.573 mm) and open pollinated Mallika at 28 DAP (13.153 mm), respectively. The interaction between pollination method and mango cultivars was found significant on 7th, 21th and 28th days after pollination (Table 4.2.11).

There was significant differences in ovule length between selfed and open pollinated fruits at 7th DAP (C.D. =0.158; $p \leq 0.05$) and 14th DAP (C.D. =0.383; $p \leq 0.05$). At 7 DAP ovule length was found to be 26.61% higher in open pollination than self pollinated flowers, while it was 38.16% higher at 14 DAP. Open pollinated Amrapali (1.560 mm) showed highest ovule

length at 7th DAP while also at 14th DAP open pollinated Amrapali (4.203 mm) showed maximum ovule length. Genotypic effect of cultivar on ovule length was only significant on 14th DAP (C.D. =0.542; $p \leq 0.05$). Similarly, the interaction between pollination method and mango cultivars was also significant on 14th DAP (C.D. =0.767; $p \leq 0.05$) (Table 4.2.12).

Differences in ovule breadth between self and open pollinated fruits were significant irrespective of varieties at 7th (C.D. =0.088; $p \leq 0.05$) and 14th DAP (C.D. =0.316; $p \leq 0.05$). Increase in ovule breadth of 19.99% at 7th DAP and 31.36% at 14th DAP was found in open pollination as compared to self pollination. Genotypic effect of cultivars on ovule breadth was also evident significantly ($p \leq 0.05$) on 7, 14 and 21 days after self and open pollination. Open pollinated Mallika (1.130 mm) recorded higher ovule breadth at 7th DAP while at 14th DAP open pollinated Pusa Arunima (3.197 mm) revealed highest ovule breadth. The interaction between pollination method and mango cultivars was found significant on 14th DAP. After 28th DAP open pollinated Pusa Arunima recorded maximum ovule breadth followed by open pollinated Amrapali (Table 4.2.13).

The data recorded on ovule degeneration at various fruitlet developmental stages are depicted in Table 4.2.14. Degeneration of ovule as a result of self and open pollination was much more apparent after 14 days after pollination (Plate 4.2.4). In selfed Amrapali and Mallika showed more than 75 degenerated ovules as a result of self pollination after 21 days of self pollination and majority of selfed fruitlets dropped thereafter. However, open pollination resulted only 20% degenerated ovules in these mango varieties. Contrastingly, in Pusa Surya (10%) and Pusa Arunima (20%) resulted less percentage of degenerated ovules after 21 days of self pollination.

4.2.6 Discussion

Our results showed that there was a considerable differences in the male and hermaphrodite flower percentage among different cultivars. However, the differences in the numbers of male and hermaphrodite flowers and sex ratios among the four cultivars implies the important role of genetic background and prevailing environmental conditions. A similar result was also recorded by Shu (2009) who found differences in sex distribution and sex ratio of Haden, Irwin, Keitt and Tsai Suan under field conditions. Unusual or abnormal stamen flower and hermaphrodite flowers showing deformed ovary were both genetic and epigenetic character whose number varied in different cultivars. Amrapali showed a significantly higher percentage of abnormal stamen flowers and Pusa Arunima showed a significantly higher percentage of hermaphrodite flowers showing deformed ovary.

Self pollination resulted in very rapid decline in fruit setting as compared to open pollination and which was much more pronounced in Amrapali and Mallika compared to Pusa

Arunima and Pusa Surya which showed relatively higher fruit set upon selfing. The complete failure of fruit set in self pollination indicates that Mallika was completely self incompatible while Amrapali was partially so. Similar observations have been reported by Singh *et al.* (1962), Mukherjee *et al*. (1968), and Sharma and Singh (1970) in some other popular cultivars of mango which were reported to be self incompatible. Rapid decline in fruit setting upon selfing may be attributed due to degeneration of the ovule that leads the arrest of fruit growth and subsequent abscission.

The adhesion of pollen on stigma of flowering plants is a critical step for the success of reproduction in angiosperm (Lush *et al.,* 1997). The observations made with regard to pollination and fertilization therafter indicated that it is not only pollination that is effective towards the overall fertilization behaviour of a genotype but also the pollen number retained on the stigma after pollination. Pollen retention on the sigmatic surface depends upon the genotype and the biochemical reactions initiated after pollen transfer (Lush *et al.,* 1997). After 6 and 24 hours of self pollination interestingly maximum number of pollen retention was recorded in case of Pusa Surya. This might be due to the higher number of pollen production in Pusa Surya.

There was a considerable variation in the extent of pollen tube growth among different cultivars in the event of self pollination up to 48 hours. During 48 hours after pollination pollen tube growth in stylar region of selfed Amrapali and Mallika was considerably slower compared to selfed Pusa Arunima and Pusa Surya. After 48 hours of self pollination, pollen tube reached upto 2/3 of stylar region in Amrapali and Mallika but reached up to lower end of style in case of Pusa Arunima and Pusa Surya. It was also noted that after 72 hours in all mango cultiars pollen tube reached upto micropyle. Similar study by Pimienta *et al.* (1983) showed that the time required for compatible and incompatible pollen tubes to reach the base of the style in almond pistil was different and this was almost entirely due to delayed pollen germination in self pollinated pistils rather than slow rate of growth in the style. Similar reports are also available in Pistachio (Shuraki and Sedgley, 1996) and Litchi (Mc Conchie *et al*., 1992).

The data recorded on ovule degeneration at various fruitlet developmental stages clearly indicated degeneration of ovule as a result of self and open pollination was much more apparent after 14th DAP. In Mallika and Amrapali cultivars, self pollination resulted more number of ovule showing sign of degeneration than their open pollinated counterpart. This clearly indicated that these mango varieties does not favour self pollination. However, in Pusa Arunima and Pusa Surya showed less difference in terms of ovule degeneration as a result of self and open pollination and suggesting that they may not be having self incompatibility.

The growth of fruitlets and ovule obtained from self and open pollination of all the four cultivars revealed that the fruitlets weight and dimensions of fruitlets and ovule were

significantly less after selfing as compared to open pollinated fruits in case of Amrapali, Mallika and Pusa Arunima, whereas there was no significant differences in fruitlet weight and dimension of fruitets and ovule between self and open pollinated Pusa Surya. Previously Mukherjee *et al*. (1968) and Sharma and Singh (1970) have reported that the selfed fruitlets were invariably smaller than the ones obtained from cross pollination. It may be possibly due to that compatible open pollination leads to greater upsurge of auxin like substances resulting in increased growth rate as compared to that after selfing (Pandey *et al*., 1973). A faster growth rate of fruitlets of open pollinated fruits as compared to selfed fruits upto 28 DAP also suggests the possibility of a similar condition operating in the open pollinated fruits as has been reported by Pandey *et al*., 1973.

4.2.7 Conclusion

Based on the results it can be concluded that the number of male and hermaphrodite flowers varied with the genotype of mango, also there was a contrasting difference in the number of unusual stamen and deformed ovary flower with respect to different cultivars. Amrapali had higher proportion of abnormal male flowers. However, Pusa Arunima had more flowers with unusual ovary. Comparing the effects of self and open pollination on the initial fruit set and final fruit retention, Amrapali and Mallika showed significantly low fruit set in comparison to their open pollinated counterparts with no fruit retention after 28 days after pollination. Pusa Arunima and Pusa Surya gave normal initial and final fruit retention in both selfing and open pollination. Growth measurement of fruitlets of comparable age resulting from self and open pollination revealed that the selfed fruitlets of Mallika and Amrapali were invariably smaller than the ones obtained from open pollination. Mallika and Amrapali showed very shriveled ovule growth as a result of after self pollination and fruitlets of which dropped afterwards, whereas, it was normal in the case of other two cultivars. Pollen tube growth showed that there was no problem in pollen adherence to stigma surface, pollen germination and growth. Up to 48-72 hours pollen tube reached the micropyle and might resulted in fertilization.The findings of present investigation bring out clearly that self incompatibility in mango unlike typical sporophytic self incompatibility is post zygotic and mango cultivar Mallika is self-incompatible, whereas, cultivar Amrapali is partially so. However, Pusa Arunima and Pusa Surya are self compatible.

Table 4.2.1. Male, hermaphrodite and unusual flowers in mango cultivars.

Cultivars	Male flowers (%)	Hermaphrodite flowers (%)	Flowers with unusual stamens (%)	Hermaphrodite flowers showing unusual ovary (%)
Amrapali	50.21[b]	49.78[a]	5.68[b]	1.43[a]
Mallika	48.01[b]	51.98[a]	1.08[a]	1.31[a]
Pusa Arunima	10.37[a]	89.62[b]	2.42[a]	5.38[b]
Pusa Surya	40.08[b]	59.91[a]	0.68[a]	0.45[a]

Same letter within column indicates no significant differences among cultivars ($P \leq 0.05$) according to DMRT.

Table 4.2.2. Fruit setting percentage of selfed and open pollinated Amrapali.

Pollination	Fruit set (%) at various days after pollination					
	6	**10**	**14**	**18**	**22**	**26**
Self	47.97(43.92)	15.50(23.14)	6.27(14.39)	0.37(1.85)	0.37(1.85)	0.37(1.85)
Open	80.00(63.94)	64.62(53.47)	57.23(49.28)	32.92(34.81)	22.46(28.21)	11.08(19.35)

	SEm ±	C. D. at 5%
Pollination Method (P)	0.70	2.08
Day (D)	1.22	3.60
P x D	1.73	5.96

Table 4.2.3. Fruit setting percentage of selfed and open pollinated Mallika.

Pollination	Fruit set (%) at various days after pollination					
	6	**10**	**14**	**18**	**22**	**26**
Self	65.20(54.05)	6.96(15.23)	3.30(10.32)	0.00(0.00)	0.00(0.00)	0.00(0.00)
Open	86.32(68.78)	48.02(43.17)	24.92(29.47)	13.37(21.49)	9.73(18.12)	7.29(15.48)

	SEm ±	C. D. at 5%
Pollination method (P)	0.78	2.31
Day (D)	1.36	4.01
P x D	1.93	5.67

Table 4.2.4. Fruit setting percentage of selfed and open pollinated Pusa Arunima.

Pollination	Fruit set (%) at various days after pollination					
	6	**10**	**14**	**18**	**22**	**26**
Self	49.04(43.86)	10.73(18.83)	7.28(15.44)	4.21(11.48)	3.45(10.56)	1.92(7.62)
Open	88.32(70.38)	53.28(47.55)	29.93(33.28)	21.90(27.88)	18.25(25.15)	16.06(23.49)

	SEm ±	C. D. at 5%
Pollination method (P)	1.05	3.10
Day (D)	1.83	5.37
P x D	2.59	7.60

Table 4.2.5. Fruit setting percentage of selfed and open pollinated Pusa Surya.

Pollination	Fruit set (%) at various days after pollination					
	6	**10**	**14**	**18**	**22**	**26**
Self	85.56(68.28)	47.59(43.34)	31.02(33.21)	19.79(26.12)	16.04(23.23)	9.63(17.86)
Open	87.85(71.59)	72.93(60.00)	40.61(39.31)	27.35(31.43)	18.51(25.49)	10.50(18.75)

	SEm ±	C. D. at 5%
Pollination method (P)	1.17	3.45
Day (D)	2.04	5.98
P x D	2.88	N.S.

Table 4.2.6. Pollen tube growth in style under self and open pollinated conditions in mango cultivars.

Cultivar	Pollen tube growth at various hours after self-pollination			
	6	24	48	72
Amrapali (self)	0	+	++	+++
Pusa Arunima (self)	+	++	+++	+++
Mallika (self)	0	+	++	+++
Pusa Surya (self)	+	++	+++	+++

0 = < 10% of style; += 1/3 of style; ++ = Up to 2/3 of style; +++ = Up to micropyle.

Table 4.2.7. Pollen retention on stigmatic surface as a result of self pollination in mango cultivars.

Cultivar	Pollen retention after self-pollination at various hours	
	6	24
Amrapali (self)	18.67^{bc}	12.62^{a}
Pusa Arunima (self)	15.67^{ab}	13.20^{ab}
Mallika (self)	13.02^{a}	10.33^{a}
Pusa Surya (self)	20.01^{c}	16.33^{b}

Same letter within column indicates no significant differences among cultivars ($P \leq 0.05$) according to Duncans multiple range test.

Table 4.2.8. Initial and final fruit set following self and open pollination in mango cultivars.

Cultivars/ Pollination type	Panicle	Flower pollinated	Fruit set (%) 6 DAP	Fruitlets / panicle 6 DAP	Fruit set (%) 26 DAP	Fruitlet/ panicle 26 DAP
Amrapali (self)	30	271	47.97	4.33	0.37	0.03
Amrapali (open)	30	1,050	80.00	28.00	11.08	3.88
Mallika (self)	30	273	65.20	5.93	0.00	0.00
Mallika (open)	30	967	86.32	27.82	7.29	2.34
Pusa Arunima (self)	30	261	49.04	4.27	1.92	0.17
Pusa Arunima (open)	30	1,523	88.32	44.83	16.06	8.15
Pusa Surya (self)	30	187	85.56	5.33	9.63	0.60
Pusa Surya (open)	30	1,336	87.85	39.12	10.50	4.68

Table 4.2.9 Fruit weight of self and open pollinated mango cultivars at different intervals.

Cultivars	Fruit weight (g)											
	7 Days			14 Days			21 Days			28 Days		
Pollination	Self	Open	Mean	Self	Open	Mean	Self	Open	Mean	Self	Open	Mean
Amrapali	0.045	0.102	0.074	0.107	0.211	0.159	--	0.508	--	--	2.229	--
Pusa Arunima	0.028	0.047	0.038	0.088	0.094	0.091	0.407	0.529	0.468	0.472	1.536	1.004
Mallika	0.034	0.049	0.041	0.062	0.132	0.097	--	0.642	--	--	2.293	--
Pusa Surya	0.084	0.157	0.120	0.164	0.180	0.172	0.374	0.418	0.396	1.187	1.680	1.433
Mean	0.048	0.089		0.105	0.154		0.391	0.473		0.830	1.608	

	SEm ±	C. D. at 5%	SEm ±	C. D. at 5%	SEm ±	C. D. at 5%	SEm ±	C. D. at 5%
Pollination method (PM)	0.003	0.011	0.014	0.042	0.025	N.S.	0.083	0.287
Cultivar (C)	0.005	0.016	0.020	0.060	0.025	N.S.	0.083	0.287
PM x C	0.007	0.023	0.028	N.S.	0.035	N.S.	0.117	N.S.

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Table 4.2.10 Fruit length of self and open pollinated mango cultivars at different intervals.

Cultivars	Fruit length (mm)											
	7 Days			14 Days			21 Days			28 Days		
Pollination	Self	Open	Mean	Self	Open	Mean	Self	Open	Mean	Self	Open	Mean
Amrapali	2.627	3.667	3.147	5.723	8.603	7.163	--	10.863	--	--	15.260	--
Pusa Arunima	2.640	3.540	3.090	4.937	5.563	5.250	7.757	9.963	8.860	8.810	11.923	10.36
Mallika	2.470	3.513	2.992	3.893	4.443	4.168	--	11.777	--	--	16.423	--
Pusa Surya	3.233	3.483	3.358	4.067	5.347	4.707	10.100	10.283	10.192	13.890	14.027	13.95
Mean	2.743	3.551		4.655	5.989		8.928	10.123		11.350	12.975	
	SEm ±	C. D. at 5%		SEm ±	C. D. at 5%		SEm ±	C. D. at 5%		SEm ±	C. D. at 5%	
Pollination method (PM)	0.091	0.277		0.154	0.467		0.209	0.722		0.232	0.802	
Cultivar (C)	0.129	N.S.		0.218	0.661		0.209	0.722		0.232	0.802	
PM x C	0.183	N.S.		0.308	0.936		0.295	1.021		0.328	1.134	

Table 4.2.11 Fruit breadth of self and open pollinated mango cultivars at different intervals.

Cultivars	Fruit breadth (mm)											
	7 Days			14 Days			21 Days			28 Days		
Pollination	Self	Open	Mean	Self	Open	Mean	Self	Open	Mean	Self	Open	Mean
Amrapali	2.193	3.147	2.670	4.763	6.167	5.465	--	8.310	--	--	10.560	--
Pusa Arunima	2.497	3.303	2.900	4.533	5.160	4.847	5.377	9.043	7.210	6.213	11.467	8.840
Mallika	2.377	3.033	2.705	3.717	4.380	4.048	--	9.350	--	--	13.153	--
Pusa Surya	2.723	2.627	2.675	3.800	4.200	4.000	9.170	9.573	9.372	11.647	12.307	11.97
Mean	2.448	3.028		4.203	4.977		7.273	9.308		8.930	11.887	
	SEm ±	C. D. at 5%		SEm ±	C. D. at 5%		SEm ±	C. D. at 5%		SEm ±	C. D. at 5%	
Pollination method (PM)	0.052	0.160		0.186	0.566		0.181	0.626		0.211	0.731	
Cultivar (C)	0.074	N.S.		0.263	0.800		0.181	0.626		0.211	0.731	
PM x C	0.105	0.321		0.373	N.S.		0.256	0.886		0.299	1.034	

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Table 4.2.12 Ovule length of self and open pollinated mango cultivars at different intervals.

Cultivars / Pollination	Ovule length (mm)											
	7 Days			14 Days			21 Days			28 Days		
	Self	Open	Mean	Self	Open	Mean	Self	Open	Mean	Self	Open	Mean
Amrapali	0.947	1.560	1.253	1.050	4.203	2.627	--	4.847	--	--	6.807	--
Pusa Arunima	1.047	1.287	1.167	2.863	3.350	3.107	4.690	4.697	4.693	5.097	6.490	5.793
Mallika	0.887	1.093	0.990	1.507	2.160	1.833	--	5.863	--	--	7.100	--
Pusa Surya	0.947	1.277	1.112	1.717	1.827	1.772	4.080	4.163	4.122	4.780	5.080	4.930
Mean	0.957	1.304		1.784	2.885		4.385	4.430		4.938	5.785	
	SEm ±	C. D. at 5%		SEm ±	C. D. at 5%		SEm ±	C. D. at 5%		SEm ±	C. D. at 5%	
Pollination method (PM)	0.052	0.158.		0.126	0.383		0.247	N.S.		0.264	N.S.	
Cultivar (C)	0.074	N.S.		0.178	0.542		0.247	N.S.		0.264	N.S.	
PM x C	0.104	N.S.		0.252	0.767		0.349	N.S.		0.374	N.S.	

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Table 4.2.13 Ovule breadth of self and open pollinated mango cultivars at different intervals.

Cultivars	Ovule breadth (mm)											
	7 Days			14 Days			21 Days			28 Days		
Pollination	Self	Open	Mean	Self	Open	Mean	Self	Open	Mean	Self	Open	Mean
Amrapali	0.627	0.823	0.725	0.773	2.147	1.468	--	2.643	--	--	4.607	--
Pusa Arunima	0.670	0.900	0.785	2.663	3.197	2.930	2.943	3.517	3.230	4.240	4.777	4.508
Mallika	0.900	1.130	1.015	0.990	1.560	1.275	--	3.590	--	--	4.340	--
Pusa Surya	0.823	0.920	0.872	1.097	1.143	1.120	2.460	2.547	2.503	4.303	4.383	4.343
Mean	0.755	0.943		1.381	2.012		2.702	3.032		4.272	4.580	
	SEm ±	C. D. at 5%		SEm ±	C. D. at 5%		SEm ±	C. D. at 5%		SEm ±	C. D. at 5%	
Pollination method (PM)	0.029	0.088		0.104	0.316		0.147	N.S.		0.151	N.S.	
Cultivar (C)	0.041	0.124		0.147	0.447		0.147	0.508		0.151	N.S.	
PM x C	0.058	N.S.		0.208	0.633		0.208	N.S.		0.213	N.S.	

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Table 4.2.14 Fruitlet degeneration as a result of self and cross pollination in four mango cultivars.

Age (days)	Pollination	Amrapali		Mallika		Pusa Arunima		Pusa Surya	
		Fruitlet sectioned	Fruitlet degenerated	Fruitlet sectioned	Fruitlet degenerated	Fruitlet sectioned	Fruitlet degenerated	Fruitlet sectioned	Fruitlet degenerated
7	Self	10	5	10	4	10	3	10	2
	OP	10	4	10	5	10	4	10	0
14	Self	9	7	8	5	8	4	10	2
	OP	10	2	9	2	9	3	8	2
21	Self	8	6	8	6	10	3	10	1
	OP	10	1	10	0	10	0	10	0
28	Self	-	-	-	-	8	2	9	1
	OP	10	2	8	1	7	1	7	0

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Amrapali

Mallika

Pusa Arunima

Pusa Surya

Plate 4.2.1. Panicles of mango cultivars after seven days of self pollination

Amrapali

Mallika

Pusa Arunima

Pusa Surya

Plate 4.2.2. Panicles of mango cultivars after 14 days of self pollination

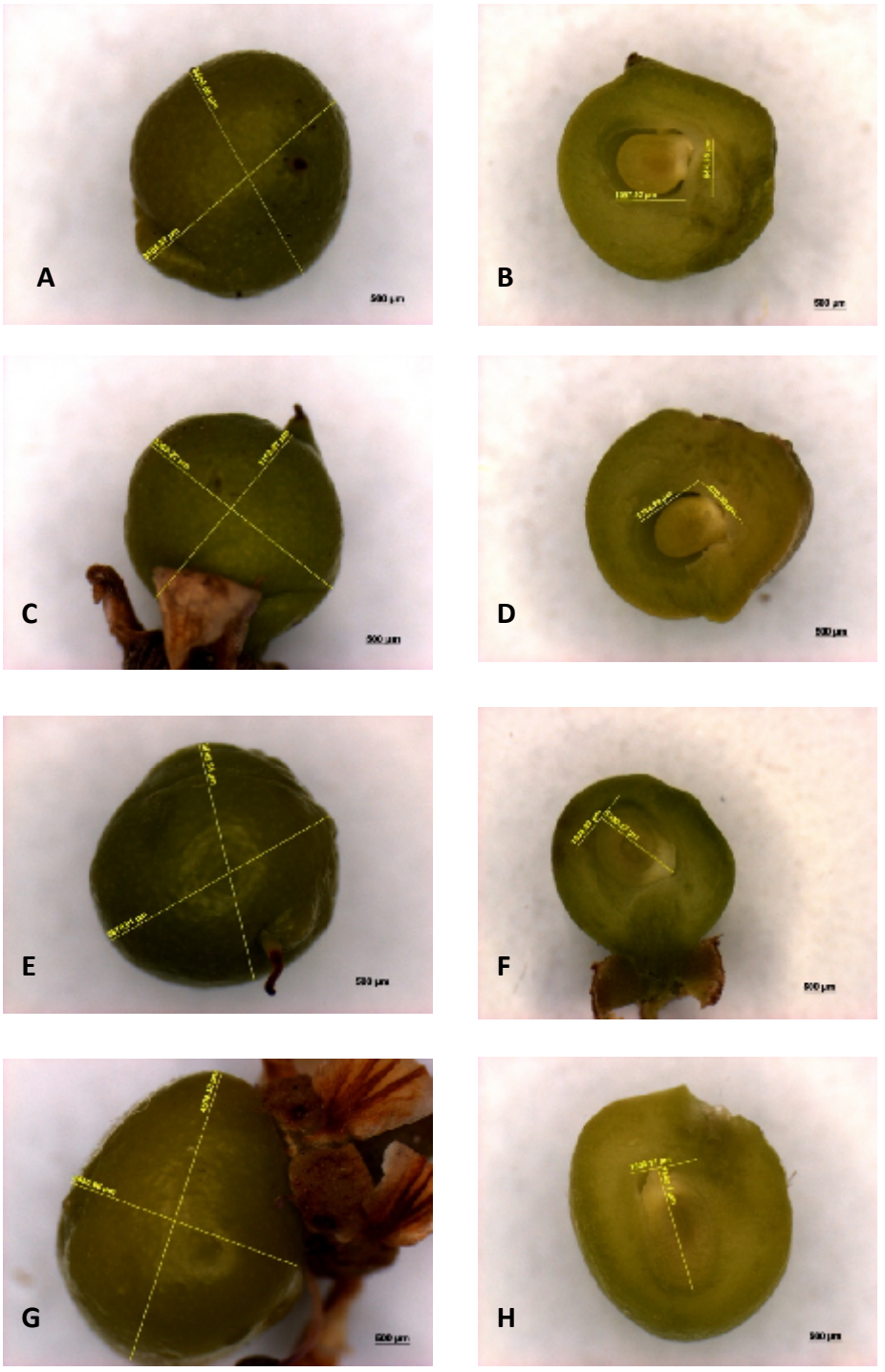
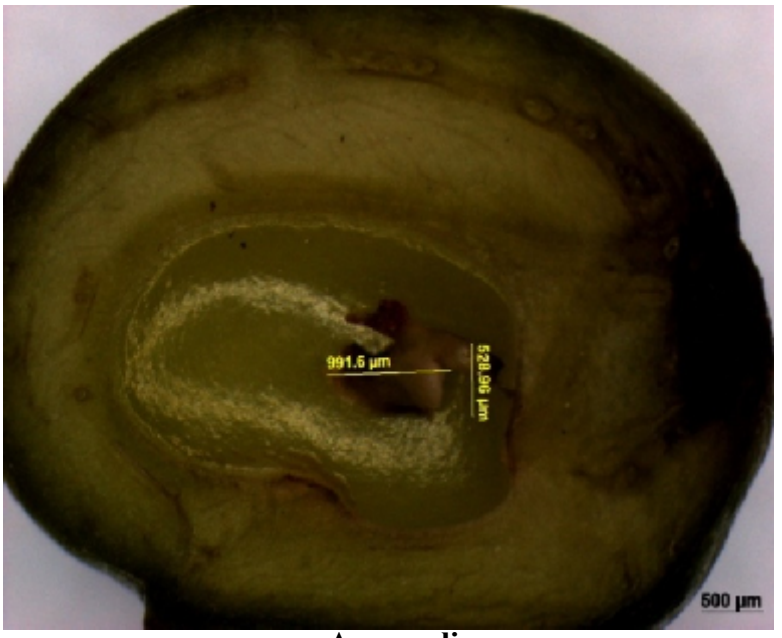
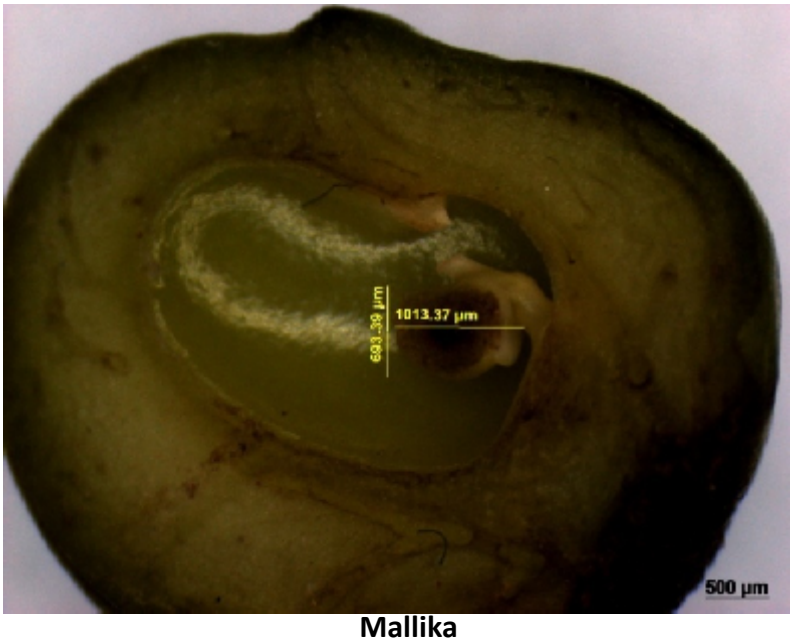


Plate 4.2.3. Fruitlets and ovule dimensions of Amrapali (A, B), Mallika (C,D), Pusa Arunima (E,F) and Pusa Surya (G, H) after seven days of self pollination



Amrapali



Mallika

Plate 4.2.4. Amrapali and Mallika fruitlets showing *in vivo* degeneration of ovules after 14 days of self pollination.

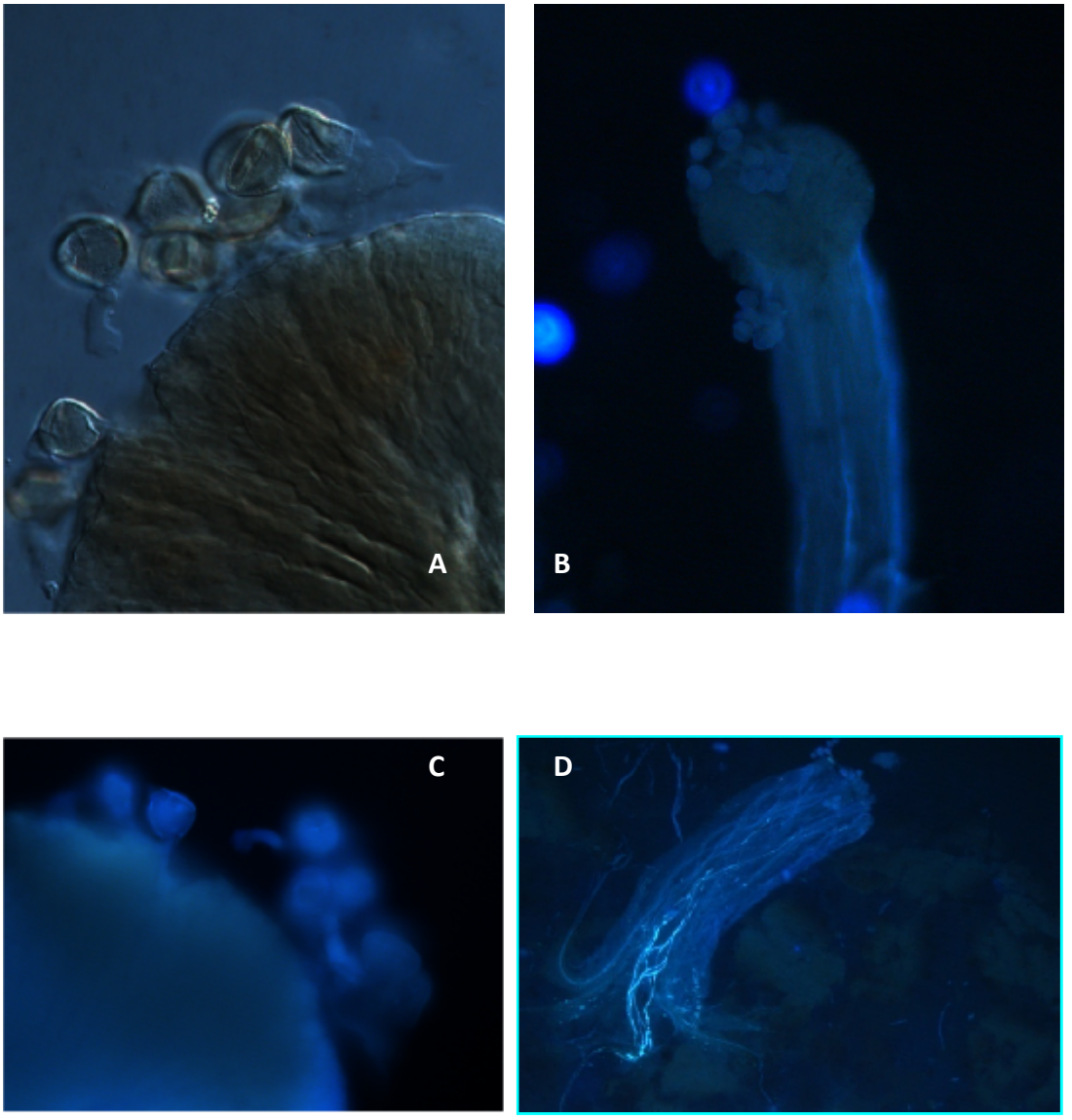


Plate 4.2.5. Pollen germination in self pollinated Amrapali. Pollen retention on stigmatic surface (A and B), germination of pollen in stigmatic fluid (C), pollen tube entering in ovular region (D).

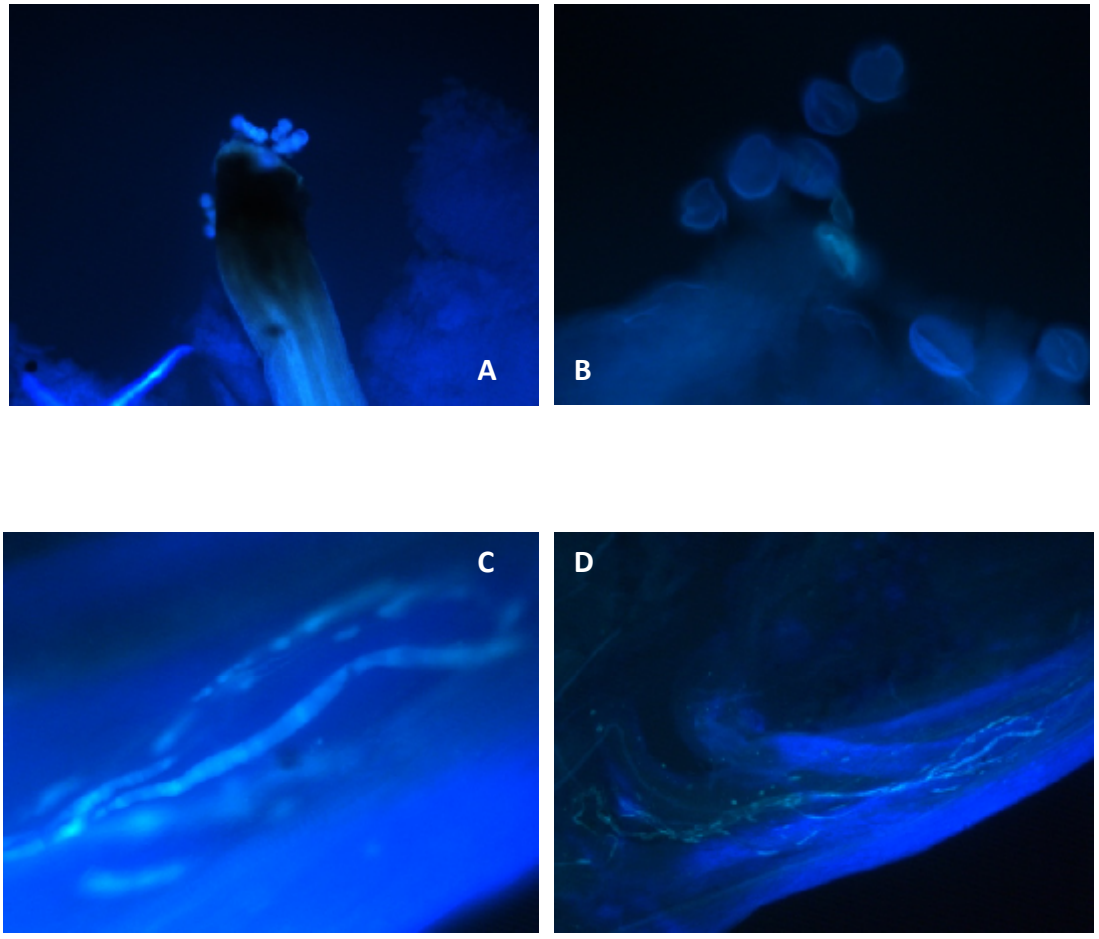


Plate 4.2.6. Pollen germination in self pollinated Mallika. Pollen retention on stigmatic surface (A), germination of pollen in stigmatic fluid (B), pollen tube in style (C) and pollen tube entering in ovular region (D).

4.3. RESEARCH PAPER III

Determination of cross incompatibility in mango (*Mangifera indica* L.)

4.3.1 Abstract

An investigation was carried out during 2009-10 to discover the cross compatibility behaviour some mango cultivars. Present investigation evidenced significant effects of pollen donor parent on pollen tube growth, fruitlet development and fruit set. In crosses where Amrapali was used as a female parent, the differences in fruit set throughout different stages of fruitlets development were non-significant. However, when Pusa Arunima was used as female parent resulted in significant differences. Pusa Arunima crossed with Dashehari and Amrapali retained fruitlets only up to 14 days after cross pollination and found to be incompatible crosses. Results pertaining to Pusa Arunima clearly showed that Langra is better polliniser cultivar for Pusa Arunima. Pollen tube growth was faster in those cross combinations which showed higher fruit set compared to those cross combination which had poor fruit set. The findings of pollen tube growth in different cross combinations further confirmed Langra as a better pollen donor parent for Pusa Arunima and Dashehari. The results obtained on fruit set and pollen tube growth were also confirmed by growth rate of fruitlets and ovule in the present investigation. In general, the increase in fruit weight and dimension of fruitlets and ovule were much more in those cross combinations which had higher fruit set and fast pollen tube growth. On the basis of results we suggest that Langra was compatible and proved to be better polliniser cultivar for Pusa Arunima and Dashehari. However, Dashehari and Amrapali showed incompatibility with Pusa Arunima.

Key words: Mango, cross incompatibility, fruit setting, pollen tube growth, ovule growth

4.3.2 Introduction

Mango (*Mangifera indica* L.) is one of the most important fruit crop of India. It is believed to have originated in the Indo Burma region and has been in cultivation in India for a long time. It is known as “King of fruits” because of its wide adaptability, nutritive richness, wide diversity, delicious taste, excellent flavour, attractive appearance and is liked by young and old alike (Purseglove, 1972). Understanding the dependence of mango cultivar on cross-pollination is crucial when planting a new orchard. If the cultivar is self-fruitful, a solid block can be planted but if it depends on cross-pollination, at least two compatible cultivars must be interplanted. Postzygotic self-incompatibility has been reported in several commercial Indian

cultivars, and this prompted us to study this phenomenon in mango cultivars developed at Indian Agricultural Research Institute, New Delhi, India and gaining popularity among mango growers. Mango cultivars developed at IARI predominate in commercial orchards throughout India and other neighbouring mango growing countries. Little information has been available regarding the need for cross-pollination in these mango cultivars. It has been found that at several occasions mango growers faced the problem of poor fruiting in mango orchards due to lack of compatible and suitable pollinizing mango cultivars.

The need of pollinisers in mango orchards was recognised by Popenoe as early as 1917. Subsequent investigators further supported his view point by showing increased fruit set through cross pollination (Sen *et al*., 1946) and self sterility was also suspected by Dijkman and Soule (1951) and confirmed by Singh *et al*. (1962) in Dashehari. Later on some more cultivars were found to be self incompatible by Sharma and Singh (1972). Cross incompatibility was also reported in Langra and Fazli (Saha and Chhonkar, 1972). Reddy and Ramayya (1976) observed that Himayuddin as polliniser resulted in 50 % more fruit set and increase in fruit size compared with that of open pollinated Rumani fruits. Ram *et al*. (1976) reported that Dashehari is cross incompatible with Chausa and Safeda Malihabad and it is cross compatible with Langra, Rataul and Bombay Green. Langra is cross incompatible with Alphonso, Bombay Green, Chausa and Fazli. Chausa is cross incompatible with Bombay Green and Rataul and it is cross compatible with Langra and Safeda Malihabad. They also reported that Bombay Green and Dashehari appear to be best polliniser for Dashehari and Chausa, respectively. This emphasises the need for study on suitable pollinizers for mango cultivars. However, compatible pollinizers in case of majority of commercial cultivars of mango are yet to be worked out.

Amrapali released by the institute in the year 1979 gained commercial status in last few years due to dedicated efforts of the institute and as per one estimate, Amrapali is the most preferred mango cultivar in the eastern mango growing belts of India for replacement of old senile orchards. Pusa Arunima is another mango hybrid cultivar released in the year 2002 and gained popularity very quickly due to its regularity, medium sized fruits, better quality and more importantly red coloured peel which makes it more suitable for export. The hypothesis behind taking Dashehari and Langra in the study was their wider cultivation. Dashehari and Langra are some of the commercial cultivars of mango which are grown widely under North Indian conditions and till now there has been very little work undertaken to search suitable pollinisers for them. Therefore, present study was undertaken to find out the most effective pollinizer(s) for commercial mango cultivars of North India such as Amrapali, Pusa Arunima, Dashehari and Langra.

4.3.3 Material and methods

4.3.3.1 Experimental plants

Present study was conducted on the grafted plants of Amrapali, Pusa Arunima, Dashehari and Langra selected from the orchards of Division of Fruits and Horticulture Technology, IARI, New Delhi. Healthy plants free from diseases and pests and at their full blooming stages were selected for crossing purpose. During the course of study, all the trees received uniform cultural treatment.

4.3.3.2 Crossing of selected cultivars

Crossings were attempted following the technique of Mukherjee *et al*. (1961), where panicles of female parents directly arising from secondary or tertiary branches were selected because of their more retention capacity. These panicles were bagged in finely perforated alkathane bags ($8^{//}$ x $5^{//}$) of 100 gauge thickness preceding evening, after removing all opened flower. The next morning 8-12 freshly opened perfect flowers were retained for pollination per panicle and all other opened and unopened buds were removed. Retained flowers were emasculated by removing the anther with the help of forcep's. The panicles were then rebagged immediately. A stock of freshly opened flowers of the desired male parents were collected from the panicles bagged earlier and kept in separate petridish in shade. Prior to pollination, anthers were dehisced in semi shade conditions by transferring them to different petridishes. Bags from the panicles were removed and hand pollination was done on each panicle one by one. Each panicle was properly labeled and rebagged immediately after pollination. The bags were removed 48 hours after pollination and the fruit set was recorded at different intervals.

4.3.3.3 Fruit set

Fruits were counted on each panicles at different intervals *viz.*, 6, 10, 14, 18, 22 and 26 days after pollination. Fruitlet retention up to marble size (26 days after pollination) was taken as final fruitlet set. Per cent fruit set was then calculated based on initial and final counts.

4.3.3.4 Pollen tube growth

Pollen tube growth measurement at different intervals under fluorescence microscopy was studied by the procedure as suggested by Martin (1959). Collection and fixation of pollinated flowers were done after 6, 24, 48 and 72 hours after pollination in FAA solution (Formalin: Acetic acid: Ethyl alcohol; 20:10:70). Later, the flowers were rinsed with distilled water and preserved in 70% ethanol under constant refrigeration (-4 ^{0}C) until further processing of the samples for observation. Fixed flowers were carefully excised and softened with 4N NaOH for 45 min in a hot air oven (60 ^{0}C). Softened pistils were stained for observing pollen tubes and callose growth using freshly prepared Aniline Blue (0.005 %) stain in 0.05M

Na_2HPO_4 (pH 8.2) for 3-4 hours. After staining pistils were mounted in 1:1 glycerol: aniline blue dye solution covered with coverslips and moderately squeezed and counts immediately made under fluorescent microscope (Leica DM 5000 B) for pollen tube growth. The observations were made at different sampling time of 6, 24, 48 and 72 hours for their penetrance in stylar region. Fertilization was assumed to happen when the pollen tube entered the micropyle and fertilized the ovule (Jayaprakasham, 2001).

4.3.3.5 Ovule and fruit growth

Size of fruitlets and ovules and weight of fruitlet were measured at 7, 14, 21 and 28 days intervals after crossing. Size of the ovules (length and breadth) and fruitlets (length and breadth) were measured using stereo microscope (Leica M 205 FA) and Vernier's Calliper and were expressed in mm. Fruitlets weight were measured with the help of high precision electronic balance (Adair Dutt 1620 C) and were expressed in gram (g).

4.3.4 Statistical analysis

The experiments were laid out in factorial randomised block design (RBD). Four cultivars were crossed in all possible combinations (4*3) and a total of 12 cross combinations were attempted. Three plants of each mango cultivar were taken as replication and 10 panicles on each plant (replication) making 30 panicles per cross combination were attempted for crossing. ANOVA was calculated to separate the means. The data on percentage of fruit set were angular transformed before analysis and means were back transformed for analysis. In the ovule and fruit growth studies data were analysed using software Agristat. The C. D. at 5% was used to compare the treatment means.

4.3.5 Results

4.3.5.1 Fruit set

The results pertaining to the cross compatibility as ascertained by fruit set from different cross combination among four parents are presented in Table 4.3.1 to 4.3.4. In crosses, where Amrapali is used as a female parent and Pusa Arunima, Dashehari and Langra as male donor parents, the differences in fruit set throughout different stages of fruitlets development were non-significant ($P \geq 0.05$). The fruit retention after 26 days after pollination was found to be the highest in cross Amrapali x Langra (14.68%) followed by the cross Amrapali x Dashehari (9.09%) and Amrapali x Pusa Arunima (8.27%). It was also evident from the Table 4.3.1 that in all cross combinations where Amrapali was used as female parent showed significant reduction in fruitlet retention with increasing in fruitlets age (C.D.= 5.67, $P \leq 0.05$). However, the interaction effect of crosses and days were found to be non-significant.

In contrast to this, crosses where Pusa Arunima was used as female parent and Amrapali, Dashehari and Langra as male donor parents resulted in significant differences (C.D.= 4.30, P ≤0.05) in fruitlets retention among different cross combinations on all stages of fruitlets development. It was interesting to note that in crosses where Dashehari was used as male donor parent with Pusa Arunima as female parent, there was no fruitlet retention after 18 days of pollination. Similarly, in crosses where Amrapali was used as male parent, fruit retention was only up to 18 days after pollination and after that all crossed fruitlets were dropped. However, in crosses where Langra was used as male donor parent with Pusa Arunima as female parent, 6.02% fruitlet retained after 26 days after pollination as compared to 0.0% fruitlets retention in crosses Pusa Arunima x Amrapali and Pusa Arunima x Dashehari.

Langra as a female parent along with Amrapali, Dashhari and Pusa Arunima as male donor parent showed similar trend as it was observed in crosses where Amrapali was used as female parent (Table 4.3.4). Langra as female parent gave non-significant differences in fruitlets retention with Amrapali, Dashhari and Pusa Arunima as male donor parent at all the stages of observation. The fruit retention after 26 days of pollination was 1.12% in cross Langra x Pusa Arunima, 4.08% in Langra x Amrapali and 2.91% in Langra x Dashehari. The effect of days on fruit retention and interaction effect of cross and days were found to be significant (C.D.= 3.49 and 6.04, P ≤0.05, respectively) and reduction in fruitlet retention was 77.8% in cross Langra x Amrapali than 85.0% in Langra x Dashehari and 95.7% in Langra x Pusa Arunima.

In crosses where Dashehari was used as a female parent gave significant differences in fruit set percentage with all the pollen parents (Amrapali, Pusa Arunima and Langra) at all the stages of observation (C.D.= 2.07, P ≤ 0.05). After 26 days of pollination, in cross Dashehari x Langra, the fruit retention was 6.36-fold more compared to the cross Dashehari x Amrapali and 1.81-fold more than Dashehari x Pusa Arunima. The effect of days on fruit retention was also found to be significant (C.D.=3.91, P ≤ 0.05) and reduction in fruit retention was 89.7% in cross Dashehari x Pusa Arunima, 97.0% in Dashehari x Amrapali. The interaction effect of cross and days was significant ((C.D.= 5.11, P ≤ 0.05) and the fruit retention after 26 days of pollination was 5.61% in Dashehari x Langra, 3.09% in Dashehari x Pusa Arunima and 0.88% in Dashehari x Amrapali (Table 4.3.3).

4.3.5.2 Pollen tube growth

The extent of pollen tube growth in stylar region of mango cultivars as observed under fluorescent microscope has been presented in the Table 4.3.5. There was a marked genetic effect of male donor parent and female parent on the extent of pollen tube growth. In the crosses where Amrapali was used as female parent with Pusa Arunima, Dashehari and Langra, pollen tube travelled to 1/3 of stylar region in 6 hours after pollination which reached to 2/3 of stylar

region in 24 hours. Interestingly, after 48 hours after pollination in all cross combinations, where Amrapali was used as female parent, pollen tube reached to the micropyle end.

In crosses where Pusa Arunima was taken as female parent, only in cross Pusa Arunima x Langra, pollen tube travelled to 1/3 of stylar region in first 6 hours. However, in crosses Pusa Arunima x Dashehari and Pusa Arunima x Amrapali, pollen tube travelled less than 10% of the stylar region in first 6 hours after pollination. It was also evident that in crosses such as Pusa Arunima x Dashehari and Pusa Arunima x Amrapali, pollen tube travelled upto 2/3 of stylar region, whereas, in cross Pusa Arunima x Langra it reached to micropyle in 24 hours after pollination. Observations made after 48 hours showed that in other cross combinations where Pusa Arunima was taken as female, pollen tube reached to micropyle end.

In the cross combinations viz., Dashehari x Pusa Arunima and Dahshehari x Langra, pollen tube penetration was observed upto 1/3 of stylar region 6 hours after pollination which extended up to micropyle end after 48 hours of pollination. However, in cross Dashehari x Amrapali pollen tube growth was slow and it travelled less than 10% of stylar region in 6 hours and reached 2/3 of stylar region in 24 hours which further extended up to micropyle end in 48 hours after pollination.

The pollen tube of Dashehari and Amrapali in the stylar region of Langra travelled 1/3 of style in first 6 hours. However, pollen tube of Pusa Arunima travelled less than 10% region of Langra style in first 6 hours after pollination. After 24 hours, pollen tube reached up to 2/3 of stylar region in all cross combinations. Observations made after 48 hours showed that in all cross combinations, where Langra was taken as female, pollen tube reached to micropyle.

It was clearly evident from the results presented in Table 4.3.5 that 48 and 72 hours after pollination, pollen tube reached up to micropyle in all twelve cross combinations and assumed to effected fertilization.

4.3.5.3 Fruitlet weight

Observations made with regard to fruitlets growth at different interval as a result of different cross combinations are presented in Table 4.3.6. Among the crosses involving Amrapali as female parent with Pusa Arunima, Dashehari and Langra as male donor parent, fruitlet weight was found to be significantly (C.D.= 0.17, $P \leq 0.05$) higher in cross Amrapali x Langra (0.76 g) which was statistically at par with fruitlet weight obtained in cross Amrapali x Dashehari (0.65g). Irrespective of cross combinations, the fruitlets weight significantly increased with the passage of time. Moreover, in general the rate of growth was higher during the 7^{th} to the 14^{th} day in all combinations, where Amrapali was used as female parent. In cross Amrapali x Langra, the fruitlets weight was higher on all dates of observations suggesting Langra as better pollen donor parent for Amrapali than Pusa Arunima and Dashehari.

The results obtained in terms of fruitlet weight observed from crosses, where Pusa Arunima as female parent with Amrapali, Dashehari and Langra as male parents showed interesting trend. Fruitlet retention was only in cross Pusa Arunima x Langra up to 28 days after pollination, however, fruitlets from cross Pusa Arunima x Dashehari and Pusa Arunima x Amrapali dropped during 14 to 21 days after pollination. The findings clearly suggest that out of three pollen parents used to cross Pusa Arunima, only Langra resulted in fruit set up to 28 days and may be a better pollinizer cultivar for Pusa Arunima.

Crosses employing Dashehari as female along with Amrapali, Langra and Pusa Arunima as male donor parents showed significant effect of cross, days and interaction of cross x days on fruitlets weight ($P \leq 0.05$). Among the crosses, Dashehari x Langra resulted in the maximum fruitlets weight (0.56 g) followed by Dashehari x Pusa Arunima (0.50 g). Interestingly the rate of growth was the maximum in cross Dashehari x Langra than other crosses from 7^{th} days to the 28^{th} days suggesting Langra as a better pollen donor parent for Dashehari followed by Pusa Arunima.

In crosses where Langra was used as female parent along with Dashehari, Pusa Arunima and Amrapali as male donor parents, the fruitlets weight significantly varied among different crosses (C.D.= 0.06, $P \leq 0.05$). Similarly, the mean effect of days and interaction effects of cross x days on fruitlets weight were also significant. The maximum fruitlet weight was observed in Langra x Amrapali cross after 28 days, however, the minimum fruit weight (0.04 g) was noted after 7 days in all cross combinations.

4.3.5.4 Fruit dimension

Data pertaining to fruitlets and ovule dimensions with regards to different cross combinations are presented in Table 4.3.7 to 4.3.10. The increase in fruitlets and ovule size belonging to the crosses, where Amrapali taken as female with Dashehari, Pusa Arunima and Langra as male parents showed similar trend as it was noted in case of fruitlets length. The increase in fruitlet as well as ovule dimensions were significantly ($P= \leq 0.05$) higher in cross Amrapali x Langra. The fruit length in cross Amrapali x Langra was 31.83% and 5.42 % more than fruit length observed in crosses Amrapali x Pusa Arunima and Amrapali x Dashehari, respectively. Similar trend was also noted in case of fruitlet breadth and ovule weight. However, the ovule breadth did not differ among different crosses, where Amrapali was used as female parent.

Among crosses, where Pusa Arunima was used as female parent, the only cross Pusa Arunima x Langra retained fruilets up to 28 days after pollination. However, in crosses Pusa Arunima x Dashehari and Pusa Arunima x Amrapali could retain fruitlets only up to 14 days after pollinations which further dropped between 14 to 21 days. In cross Pusa Arunima x

Langra, the fruitlet length (12.51 mm), fruit breadth (10.93 mm), ovule length (8.07 mm) and ovule breadth (5.09 mm) were observed after 28 days after cross pollination.

Data pertaining to fruitlet and ovule dimension in crosses employing Dashehari as female parent with Amrapali, Pusa Arunima and Langra as male parent are presented in Table 4.3.10. Among the crosses, Dashehari x Langra resulted significantly (P= ≤0.05) more fruit length, ovule length and ovule breadth than other two crosses. The fruit length was 48.43% more than the fruit length observed in cross Dashehari x Pusa Arunima, however, had non-significant difference with fruit length observed in cross Dashehari x Pusa Arunima. Similarly, ovule length in cross Dashehari x Langra was 20.71% more compared to cross Dashehari x Amrapali and had non-signifcant difference with ovule length in cross Dashehari x Pusa Arunima.

Among crosses, where Langra was used as female parent, the cross Langra x Dashehari resulted in significantly (P ≤0.05) higher fruit and ovule length compared to other crosses. However, higher fruit breadth was noted in cross Langra x Amrapali. The fruit length in cross Langra x Dashehari was 28.72% more than fruit length observed in cross Langra x Pusa Arunima. Simlarly, ovule length was 19.73% more in cross Langra x Dashehari than cross Langra x Pusa Arunima.

4.3.6 Discussion

Results from the present investigation clearly indicated significant effects of pollen donor parent on fruit set and fruitlet development. On the basis of data observed on fruit set it was evidenced that in crosses, where Amrapali and Langra have been used as female parents, the differences in fruit set throughout different stages of fruitlets development were non-significant suggest that tested pollen parents behaved equally well for these two female parents. However, in crosses where Pusa Arunima was used as female parent with Amrapali, Dashehari and Langra as male donor parents resulted in significant differences in fruit set up to 14 days after cross pollination. Interestingly, Pusa Arunima crossed with Dashehari and Amrapali retained fruitlets only up to 14 days after cross pollination and found to be incompatible crosses, whereas Pusa Arunima x Langra retained fruits even up to 28 days after cross pollination. Results pertaining to Pusa Arunima clearly showed that Langra is better polliniser cultivar for Pusa Arunima. Similarly for Dashehari, the better polliniser was found to be Langra. Our results pertaining to Dashehari had strong conformity with the findings of Ram *et al*. (1976) who reported that Langra, Rataul and Bombay Green were compatible pollinizers for Dashehari. The differential response of pollen donor parents in all four mango cultivars may be primarily attributed to their genotypic interaction between male and female parents. Earlier Hang (1999)

and Desai and Bhandwalker (1995) obtained similar results while attempting cross pollination among different cultivars.

Pollen tube growth is governed genetically which results from the interaction of pollen-pistil of parent genotypes. Pollen adhesion, retention, germination and growth inside the style vary considerably with respect to compatibility reactions. Fluorescence microscopic study of pollen pistil interaction in the present study clearly revealed that there was a marked genetic effect of male donor parent and female parent on the extent of pollen tube growth. In the crosses where Amrapali was used as female parent with Pusa Arunima, Dashehari and Langra, pollen tube travelled to the micropyle end in 48 hours after pollination. However, in crosses, where Pusa Arunima was taken as female parent with Langra as male parent pollen tube reached to micropyle in 24 hours after pollination. Similarly, in the cross combinations viz., Dashehari x Pusa Arunima and Dahshehari x Langra, pollen tube penetration was observed up to 1/3 of stylar region 6 hours after pollination which extended up to micropyle end after 48 of pollination. It was interesting to note that pollen tube growth was faster in those cross combinations which showed higher fruit set compare to those cross combination had poor fruit set. The findings of pollen tube growth in different cross combinations further confirmed Langra as a better pollen donor parent for Pusa Arunima, Amrapali and Dashehari.

The results obtained on fruit set and pollen tube growth were also confirmed by growth rate of fruitlets and ovule in the present investigation. In general the increase in fruit weight and dimension of fruitlets and ovule were much more in those cross combinations, which had higher fruit set and fast pollen tube growth.

Sharma and Singh (1970) had earlier reported highest numbers of styles with pollen tubes when Dashehari was crossed with Totapuri Red Small. Similarly, Dhaliwal and Dhaliwal (1992) noted better pollen tube growth in cross of Dashehari x Langra. The findings of present investigation had strong agreement with the reports in apple (Modlibowska, 1945), prunes (Roy, 1938) and almond (Pimienta *et al*. 1983). They found that pollen tube growth in compatible crosses was better than incompatible crosses. Jayprakasham (2001) suggested that in mango delay in pollen tube growth to reach the micropyle was not due to inhibition of pollen tube growth but due to delayed pollen germination and pollen in compatible crosses germinate efficiently than incompatible crosses.

4.3.7 Conclusion

Understanding the dependence of mango cultivar on cross-pollination is crucial factor in commercial mango growing. If the cultivar is self-fruitful, a solid block can be planted but if it depends on cross-pollination, at least two compatible cultivars must be interplanted. Present investigation evidenced significant effects of pollen donor parent on fruit set, pollen tube

growth and fruitlet development. Fluorescence microscopic study of pollen pistil interaction in the present study showed marked genetic effect of male donor parent and female parent on the extent of pollen tube growth. On the basis of results, it can be concluded that Langra was compatible and proved to be better polliniser cultivar for Pusa Arunima and Dashehari. However, Dashehari and Amrapali showed incompatibility with Pusa Arunima. The use of compatible pollinizers in commercial plantations of mango seems to be quite congenial because a considerable overlapping in the periods of panicle emergence and flowering occurs between the cultivars. Therefore, for proper pollination, fertilisation, ovule and fruit growth and development the most effective pollinizers like Langra should be either interplanted or top-worked in commercial orchards of Amrapali, Pusa Arunima and Dashehari respectively.

Table 4.3.1. Fruit set in crosses having Amrapali as female parent.

Cross	Fruit set (%)					
	6 DAP	**10 DAP**	**14 DAP**	**18 DAP**	**22 DAP**	**26 DAP**
A X PA	73.68(59.36)	45.86(43.83)	38.35(39.02)	27.82(32.42)	12.03(20.50)	8.27(16.94)
A X D	85.23(69.52)	37.50(38.00)	28.41(32.13)	21.59(27.38)	13.64(21.20)	9.09(17.24)
A X L	78.90(63.05)	42.20(39.79)	36.70(36.75)	23.85(27.81)	16.51(23.12)	14.68(22.02)

		SEm ±	C.D. at 5%
	Cross	1.39	NS
	Day	1.97	5.67
	Cross X Day	3.41	NS

Table 4.3.2. Fruit set in crosses having Pusa Arunima as female parent.

Cross	Fruit set (%)					
	6 DAP	**10 DAP**	**14 DAP**	**18 DAP**	**22 DAP**	**26 DAP**
PA X A	27.93(31.64)	16.22(23.30)	10.81(18.63)	3.60(10.73)	0.00(0.00)	0.00(0.00)
PA X D	35.58(36.83)	9.62(17.34)	5.77(11.56)	0.00(0.00)	0.00(0.00)	0.00(0.00)
PA X L	37.35(36.37)	33.73(33.41)	31.33(30.84)	18.07(19.92)	7.23(12.43)	6.02(10.50)

		SEm ±	C.D. at 5%
	Cross	1.49	4.30
	Day	2.11	6.89
	Cross X Day	3.07	9.13

Table 4.3.3. Fruit set in crosses having Dashehari as female parent.

Cross	Fruit set (%)					
	6 DAP	**10 DAP**	**14 DAP**	**18 DAP**	**22 DAP**	**26 DAP**
D X PA	29.90(32.79)	26.80(31.03)	12.37(20.47)	7.22(15.27)	4.12(11.62)	3.09(8.51)
D X A	29.82(33.69)	14.04(22.21)	14.04(22.21)	4.39(9.95)	0.88(3.50)	0.88(3.50)
D X L	25.23(30.20)	21.50(27.74)	14.95(22.80)	11.21(19.53)	7.48(15.49)	5.61(13.64)

	SEm ±	C.D. at 5%
Cross	0.71	2.07
Day	1.34	3.91
Cross X Day	1.76	5.11

Table 4.3.4. Fruit set in crosses having Langra as female parent.

Cross	Fruit set (%)					
	6 DAP	**10 DAP**	**14 DAP**	**18 DAP**	**22 DAP**	**26 DAP**
L X PA	25.84(30.57)	17.98(25.19)	12.36(20.74)	6.74(14.47)	3.37(8.31)	1.12(3.62)
L X A	18.37(23.80)	15.31(22.19)	8.16(14.79)	5.10(10.07)	4.08(9.24)	4.08(9.24)
L X D	19.42(26.37)	7.77(15.14)	5.83(13.45)	5.83(13.45)	3.88(11.34)	2.91(7.89)

	SEm ±	C.D. at 5%
Cross	0.85	NS
Day	1.21	3.49
Cross X Day	2.10	6.04

Table 4.3.5. Pollen tube growth measurement at different intervals.

Cross	Pollen tube growth at various hours after pollination			
	6	**24**	**48**	**72**
A X PA	+	+ +	+ + +	+ + +
A X D	+	+ +	+ + +	+ + +
A X L	+	+ +	+ + +	+ + +
PA X A	0	+ +	+ + +	+ + +
PA X D	0	+ +	+ + +	+ + +
PA X L	+	+ ++	+ + +	+ + +
D X PA	+	+ + +	+ + +	+ + +
D X L	+	+ + +	+ + +	+ + +
D X A	0	+ +	+ + +	+ + +
L X PA	0	+ +	+ + +	+ + +
L X D	+	+ +	+ + +	+ + +
L X A	+	+ +	+ + +	+ + +

0 = < 10% of style; += 1/3 of style; ++ = Upto 2/3 of style; +++ = Upto micropyle.

Table 4.3.6. Fruit weight (g) of various crosses at different intervals.

Days	Cross combinations															
	A X PA	**A X D**	**A X L**	**Mean**	**PA X A**	**PA X D**	**PA X L**	**Mean**	**L X PA**	**L X D**	**L X A**	**Mean**	**D X PA**	**D X L**	**D XA**	**Mean**
7	0.06	0.06	0.10	0.07	0.04	0.04	0.04	0.04	0.03	0.04	0.04	0.04	0.05	0.04	0.05	0.04
14	0.18	0.20	0.24	0.21	0.23	0.22	0.23	0.23	0.22	0.24	0.24	0.23	0.19	0.24	0.23	0.22
21	0.53	0.68	0.78	0.66	0.00	0.00	0.47	0.47	0.63	0.71	0.60	0.65	0.53	0.63	0.58	0.58
28	0.86	1.66	1.92	1.48	0.00	0.00	1.44	1.44	0.75	1.33	1.40	1.16	1.24	1.34	0.71	1.10
Mean	0.41	0.65	0.76		--	--	0.55		0.41	0.58	0.57		0.50	0.56	0.39	

	SEm ±	C.D. at 5%	SEm ±	C.D. at 5%	SEm ±	C.D. at 5%	SEm ±	C.D. at 5%
Cross (C)	0.06	0.17	0.01	0.05	0.02	0.06	0.01	0.04
Day (D)	0.07	0.20	0.02	0.06	0.02	0.07	0.01	0.05
C x D	0.12	0.36	0.03	0.10	0.04	0.13	0.02	0.08

--,dropped

Table 4.3.7. Fruit lets and ovule dimensions of Amrapali crosses at different intervals.

Cross	Fruit length (mm)					Fruit breadth (mm)					Ovule length (mm)					Ovule breadth (mm)				
Day	**7**	**14**	**21**	**28**	**Mean**	**7**	**14**	**21**	**28**	**Mean**	**7**	**14**	**21**	**28**	**Mean**	**7**	**14**	**21**	**28**	**Mean**
A X PA	3.20	7.19	9.23	11.50	7.78	3.06	5.66	7.38	9.32	6.35	1.52	5.37	5.44	6.25	4.64	0.81	1.63	3.52	4.90	2.71
A X D	3.44	7.08	13.05	14.38	9.49	2.70	5.68	9.43	10.28	7.02	1.27	5.34	6.08	7.59	5.07	0.81	2.54	3.99	5.03	3.09
A X L	3.40	7.27	14.69	15.16	10.13	3.17	5.69	9.90	11.62	7.59	1.48	4.57	6.70	7.75	5.12	0.92	2.54	4.60	5.33	3.35
Mean	3.35	7.18	12.32	13.68		2.97	5.68	8.90	10.40		1.42	5.09	6.07	7.20		0.85	2.24	4.03	5.09	

	SEm ±	C.D. at 5%	SEm ±	C.D. at 5%	SEm ±	C.D. at 5%	SEm ±	C.D. at 5%
Cross (C)	0.16	0.48	0.17	0.50	0.10	0.30	0.19	NS
Day (D)	0.18	0.55	0.19	0.58	0.12	0.35	0.22	0.65
C x D	0.32	0.96	0.34	1.01	0.21	0.61	0.38	NS

Table 4.3.8. Fruit lets and ovule dimensions of Pusa Arunima crosses at different intervals.

Cross	Fruit length (mm)					Fruit breadth (mm)					Ovule length (mm)					Ovule breadth (mm)				
Day	**7**	**14**	**21**	**28**	**Mean**	**7**	**14**	**21**	**28**	**Mean**	**7**	**14**	**21**	**28**	**Mean**	**7**	**14**	**21**	**28**	**Mean**
PA X A	3.09	5.04	0.00	0.00	--	3.08	4.48	0.00	0.00	--	1.23	3.27	0.00	0.00	--	0.79	2.05	0.00	0.00	--
PA X D	3.39	5.11	0.00	0.00	--	2.49	4.09	0.00	0.00	--	1.11	3.44	0.00	0.00	--	0.89	1.40	0.00	0.00	--
PA X L	3.91	5.25	10.43	12.51	8.02	2.90	4.73	8.63	10.93	6.79	1.48	3.89	5.74	8.07	4.80	0.96	1.25	4.42	5.09	2.93
Mean	3.46	5.13	10.43	12.51		2.87	4.43	8.63	10.93		1.27	3.53	5.74	8.07		0.88	1.57	4.42	5.09	

	SEm ±	C.D. at 5%	SEm ±	C.D. at 5%	SEm ±	C.D. at 5%	SEm ±	C.D. at 5%
Cross (C)	0.06	0.19	0.12	0.35	0.10	0.29	0.04	0.14
Day (D)	0.07	0.22	0.13	0.40	0.11	0.34	0.05	0.16
C x D	0.13	0.39	0.24	0.70	0.20	0.59	0.09	0.28

--,dropped

Table 4.3.9. Fruit lets and ovule dimensions of Langra crosses at different intervals.

Cross	Fruit length (mm)					Fruit breadth (mm)					Ovule length (mm)					Ovule breadth (mm)				
Day	**7**	**14**	**21**	**28**	**Mean**	**7**	**14**	**21**	**28**	**Mean**	**7**	**14**	**21**	**28**	**Mean**	**7**	**14**	**21**	**28**	**Mean**
L X PA	3.65	5.12	8.17	12.28	7.31	3.08	3.90	7.41	9.533	5.98	1.13	3.50	5.67	8.13	4.61	0.76	2.34	2.94	4.34	2.59
L X D	3.58	6.35	11.48	16.22	9.41	2.72	5.03	8.31	11.89	6.99	1.52	4.14	7.00	9.44	5.52	0.74	2.51	3.81	5.21	3.06
L X A	3.96	5.14	11.66	16.67	9.36	3.17	4.09	8.79	12.68	7.18	1.36	3.28	6.48	9.61	5.18	1.01	1.68	3.43	5.44	2.89
Mean	3.73	5.54	10.44	15.06		2.99	4.34	8.17	11.37		1.34	3.64	6.38	9.06		0.84	2.17	3.39	4.99	

	SEm ±	C.D. at 5%	SEm ±	C.D. at 5%	SEm ±	C.D. at 5%	SEm ±	C.D. at 5%
Cross (C)	0.21	0.63	0.12	0.36	0.15	0.44	0.09	0.27
Day (D)	0.24	0.73	0.14	0.42	0.17	0.50	0.10	0.31
C x D	0.43	1.26	0.24	0.72	0.30	NS	0.18	0.55

Table 4.3.10. Fruit lets and ovule dimensions of Dashehari crosses at different intervals.

Cross	Fruit length (mm)					Fruit breadth (mm)					Ovule length (mm)					Ovule breadth (mm)				
Day	**7**	**14**	**21**	**28**	**Mean**	**7**	**14**	**21**	**28**	**Mean**	**7**	**14**	**21**	**28**	**Mean**	**7**	**14**	**21**	**28**	**Mean**
D X PA	4.58	6.72	12.36	16.17	9.96	3.21	5.58	9.10	10.69	7.31	1.25	4.54	6.31	9.26	5.34	0.75	1.35	3.33	5.64	2.77
D X L	3.82	6.83	13.30	17.66	10.42	3.88	5.06	9.31	10.97	7.13	1.60	4.47	6.51	9.12	5.42	1.04	1.47	3.42	5.46	2.85
D X A	3.53	5.36	8.94	10.24	7.02	2.98	4.47	7.41	8.55	5.85	1.81	4.54	5.10	6.53	4.49	1.00	1.50	2.84	5.02	2.59
Mean	3.98	6.30	11.56	14.69		3.36	5.03	8.61	10.07		1.55	4.52	5.97	8.30		0.93	1.44	3.19	5.37	

	SEm ±	C.D. at 5%	SEm ±	C.D. at 5%	SEm ±	C.D. at 5%	SEm ±	C.D. at 5%
Cross (C)	0.23	0.69	0.14	0.43	0.09	0.27	0.08	NS
Day (D)	0.27	0.80	0.17	0.50	0.10	0.31	0.10	0.29
C x D	0.47	1.39	0.29	0.86	0.18	0.54	0.17	NS

5. DISCUSSION

Mango (*Mangifera indica* L.) is one of the most important fruit crop of India. It is originated in the Indo Burma region and has been in cultivation in India for a long time. It is known as “king of fruits” because of its wide adaptability, nutritive richness, wide diversity, delicious taste, excellent flavour, attractive appearance and is liked by young and old alike (Purseglove, 1972). Mango has been considered to be a difficult plant species to improve in breeding programmes because of certain inherent characteristics.

Mango hybridisation work is in progress at various centres in India and at several occasions, it has been realised by the mango breeders that problem of asynchronized flowering in certain mango parental cultivars restricts their use in mango hybridization programme. Circumstances usually make it necessary to store the pollen from male donor parent for later pollination of the female parents. Pollen viability of three mango polliniser cultivars *viz*., Sensation, Tommy Atkins and Janardan Pasand were investigated up to 24 weeks under four different storage conditions (room, -4^{o}C, -20^{o}C and -196^{o}C). Three different methods of viability estimations were utilized *viz*., *in vitro* germination, FDA and acetocarmine tests. The results showed that freshly collected pollens of all the three cultivars had high pollen viability compared to stored pollen. Results were obvious and had strong conformity with findings in other species like litchi (Chaudhury *et al.,* 2010), cherimoya (Lora *et al*., 2006), *Zea mays* (Inagaki, 2000) and *Brassica campestris* (Mulcahy and Mulcahy, 1988). Pollen stored at room temperature was viable up to 4 weeks. However, the per cent viability significantly reduced during this period and only 2.11% (*in vitro* germination), 11.57% (FDA test) and 9.73% (acetocarmine test) pollen remain viable in Sensation. Similar trend was noted in case of Tommy Atkins and Janardan Pasand mango cultivars. In Tommy Atkins, the pollen viability was 1.80% (*in vitro* germination), 11.66% (FDA test) and 9.29% (acetocarmine test) and in Janaradan Pasand, it was only 1.03% (*in vitro* germination), 9.22% (FDA test) and 10.94% (acetocarmine test) compare to fresh pollen viability. The decrease in pollen viability at room temperature during first 4 weeks may be attributed to high sensitivity of mango pollen to high temperature and low humidity. Mango pollen is known to be highly susceptible to desiccation and loose water from pollen, when kept at a temperature above 25-27 ^{0}C. The results are in strong agreement with the earlier reports of Chaudhury *et al.* (2010). All pollen viability tests like *in vitro* germination, FDA and acetocarmine test revealed that significantly higher pollen viability was maintained at -196 ^{0}C followed by -20^{0}C and -4^{0}C at all dates of observation in all three cultivars and the trend was same up to 24 weeks of storage. Pollen longevity has been reported to be extended by using lower temperatures such as 5^{0}C, -20^{0}C, -80 ^{0}C and -196 ^{0}C and

low moisture content (Towill and Walters, 2000). Pollen viabilities at 5^0C, -20^0C and -80 ^{0}C differ considerably among species and genotypes (Mishra, 1984). The pollen once cryostored at temperature below -160 ^{0}C would theoretically have infinite period of longevity (Stanwood, 1985). In the present study high pollen viability was maintained by storage at -196 ^{0}C followed by -20^0C and -4 ^{0}C in all the three mango cultivars as evidenced by pollen viability observed using *in vitro* germination test, FDA test and acetocarmine tests. The differential results obtained by using different pollen viability assay confirmed that FDA tests seem to be less reliable than *in vitro* germination tests, since germination is often over estimated as demonstrated with pollen of various other species. A similar over estimation was observed with mango pollen for fresh pollens and after storage at various storage conditions (Chaudhury *et al.*,2010 and Shivanna and Helpson-Harrison, 1981). Thus, we have been able to devise a short-term pollen storage method with the help of simple refrigeration and also through cryostorage. Trend revealed by cryostorage showed the possibility of long-term storage of mango pollen. The studies undertaken by Indian Institute of Horticultural Research (Ganeshan, 2003) on pollen storage also confirmed our results. Results revealed that at -196 ºC pollen could be stored for long-term, for efficient conservation of genetic resources, for hand pollination both for commercial fruit production and breeding. However, pollen can be efficiently stored for short periods at -20ºC and -4 ºC temperatures for few weeks. In mango, blooming season start from February and lasts up to April, consequently, pollen collected and stored at different storage conditions in February can be used for pollinations throughout mango blooming season. These make availability of pollen of differentially blooming parents in circumstances of non-synchronised flowering and to avoid the need of daily pollen collection to perform manual pollination the following day.

Development of mango cultivars having combination of desirable traits should not be considered as the completion of mango breeding imporvement work. The flowering and fruiting behaviour of newly developed cultivars should be investigated further. The self and cross compatibility behaviour of mango cultivars has remarkable impact on commercialization of these new cultivars. Self incompatibility studies were carried out in mango cultivars developed at Indian Agricultural Research Institute such as Amrapali, Mallika, Pusa Arunima and Pusa Surya. The effects of self and open pollination on the fruit set, ovule degeneration and growth rate of selfed and open pollinated fruitlets were compared. The results showed that there was a considerable differences in the male and hermaphrodite flower percentage between different cultivars. However, the differences in the male and hermaphrodite flower numbers and sex ratios among the four cultivars implies the important role of genetic background and prevailing environmental conditions. A similar results were also recorded by Shu (2009) who found differences in sex distribution and sex ratio of Haden, Irwin, Keitt and Tsai Suan under field

conditions. Unusual or abnormal stamen flower and hermaphrodite flowers showing deformed ovary were both genetic and epigenetic character, whose number varied in different cultivars. Amrapali showed a significantly higher percentage of abnormal stamen flowers and Pusa Arunima showed a significantly higher percentage of hermaphrodite flowers showing deformed ovary. Self pollination resulted in very rapid decline in fruit setting percentage as compared to open pollination and which was much more pronounced in Amrapali, Mallika and PusaArunima compared to Pusa Surya which showed relatively higher fruit set upon selfing. Similar observations have been reported by Singh *et al.* (1962), Mukherjee *et al*. (1968), and Sharma and Singh (1972) in some other popular cultivars of mango, which were reported to be self incompatible. Rapid decline in fruit setting upon selfing may be attributed due to degeneration of the ovule that leads the arrest of fruit growth and subsequent abscission. The adhesion of pollen on stigma of flowering plants are a critical step for the success of reproduction in angiosperm (Lush *et al.,* 1997). The observations made with regard to pollination and fertilization, therafter indicated that it is not only pollination that is effective towards the overall fertilization behaviour of a genotype but also the pollen number retained on the stigma after pollination. Pollen retention on the sigmatic surface depends upon the genotype and the biochemical reactions initiated after pollen transfer (Lush *et al.*, 1997). After 6 and 24 hours of self pollination interestingly maximum number of pollen retention was recorded in case of Pusa Surya. This might be due to the higher number of pollen production in Pusa Surya. There was a considerable variation in the extent of pollen tube growth among selfed Amrapali, Mallika and Pusa Arunima up to 48 hours of pollination. During 48 hours after pollination pollen tube growth in stylar region of selfed Amrapali and Mallika were considerably slower compared to selfed Pusa Arunima and Pusa Surya. After 48 hours of self pollination, pollen tube reached up to 2/3 of stylar region in Amrapali and Mallika but reached up to lower end of style in case of Pusa Arunima and Pusa Surya. Similar study by Pimienta *et al.* (1983) showed that the time required for compatible and incompatible pollen tubes to reach the base of the style in almond pistil was different and this was almost entirely due to delayed pollen germination in self pollinated pistils rather than slow rate of growth in the style. Similar reports are also available in Pistachio (Shuraki and Sedgley, 1994) and Litchi (McConchie*et al*., 1992). The data recorded on ovule degeneration at various fruitlet developmental stages clearly indicated degeneration of ovule as a result of self and open pollination were much more apparent after 14 days after pollination. In Mallika and Amrapali cultivar self pollination resulted more number of ovule showing sign of degeneration than their open pollinated counterpart. This clearly indicated that these mango cultivars does not favour self pollination. However, in Pusa Arunima and Pusa Surya showed less difference in terms of ovule degeneration as a result of self and open pollination and suggesting that they may not be having self incompatibility.The growth of fruitlets and ovule obtained from self and open pollination of all the four cultivars revealed that

the fruitlets weight and dimensions of fruitlets and ovule were significantly less after selfing as compared to open pollinated fruits in case of Amrapali, Mallika and Pusa Arunima, whereas there was no significant differences in fruitlet weight and dimension of fruitets and ovule between self and open pollinated Pusa Surya. Previously, Mukherjee *et al*. (1968) and Sharma and Singh (1972) have reported that the selfed fruitlets were invariably smaller than the ones obtained from cross pollination. It may be possibly due to that compatible open pollination leads to greater upsurge of auxin like substances, resulting in increased growth rate as compared to that after selfing (Pandey *et al*., 1973). A faster growth rate of fruitlets of open pollinated fruits as compared to selfed fruits up to 28 DAP also suggests the possibility of a similar condition operating in the open pollinated fruits as has been reported by Pandey *et al*., 1973.

An investigation was also carried out to discover the cross compatibility behaviour of IARI released mango cultivars and other north Indian commercial cultivars. Results from the present investigation clearly indicated significant effects of pollen donor parent on fruit set and fruitlet development. On the basis of data observed on fruit set, it was evidenced that in crosses where Amrapali and Langra have been used as female parents, the differences in fruit set throughout different stages of fruitlets development were non-significant suggest that tested pollen parents behaved equally well for these two female parents. However, in crosses, where Pusa Arunima was used as female parent with Amrapali, Dashehari and Langra as male donor parents resulted in significant differences in fruit set up to 14 days after cross pollination. Interestingly, Pusa Arunima crossed with Dashehari and Amrapali retained fruitlets only up to 14 days after cross pollination and found to be incompatible crosses, whereas, Pusa Arunima x Langra retained fruits even up to 28 days after cross pollination. Results pertaining to Pusa Arunima clearly showed that Langra is better polliniser cultivar for Pusa Arunima. Similarly for Dashehari, the better polliniser was found to be Langra. These results pertaining to Dashehari had strong conformity with the findings of Ram *et al*. (1976) who reported that Langra, Rataul and Bombay Green were compatible pollinizers for Dashehari. The differential response of pollen donor parents in all four mango cultivars may be primarily attributed to their genotypic interaction between male and female parents. Earlier Hang (1999) and Desai and Bhandwalker (1995) obtained similar results while attempting cross pollination among different cultivars. Pollen tube growth is governed genetically, which results from the interaction of pollen-pistil of parent genotypes. Pollen adhesion, retention, germination and growth inside the style vary considerably with respect to compatibility reactions. Fluorescence microscopic study of pollen pistil interaction in the present study clearly revealed that there was a marked genetic effect of male donor parent and female parent on the extent of pollen tube growth. In the crosses, where Amrapali was used as female parent with Pusa Arunima, Dashehari and Langra, pollen tube travelled to the micropyle end in 48 hours after pollination. However, in crosses, where Pusa

Arunima was taken as female parent with Langra as male parent pollen tube reached to micropyle in 24 hours after pollination. Similarly, in the cross combinations viz., Dashehari x Pusa Arunima and Dahshehari x Langra, pollen tube penetration was observed up to 1/3 of stylar region 6 hours after pollination, which extended up to micropyle end after 48 hrs of pollination. It was interesting to note that pollen tube growth was faster in those cross combinations, which showed higher fruit set compare to those cross combination had poor fruit set. The findings of pollen tube growth in different cross combinations further confirmed Langra as a better pollen donor parent for Pusa Arunima, Amrapali and Dashehari. The results obtained on fruit set and pollen tube growth were also confirmed by growth rate of fruitlets and ovule in the present investigation. In general the increase in fruit weight and dimension of fruitlets and ovule were much more in those cross combinations, which had higher fruit set and fast pollen tube growth. Sharma and Singh (1970) had earlier reported highest numbers of styles with pollen tubes, when Dashehari was crossed with Totapuri Red Small. Similarly Dhaliwal and Dhaliwal (1992) noted better pollen tube growth in cross of Dashehari x Langra. The findings of present investigation had strong agreement with the reports in apple (Modlibowska, 1945), prunes (Roy, 1938) and almond (Pimienta*et al*. 1983). They found that pollen tube growth in compatible crosses were better than incompatible crosses. Jayprakasham (2001) suggested that in mango, delay in pollen tube growth to reach the micropyle was not due to inhibition of pollen tube growth but due to delayed pollen germination and pollen in compatible crosses germinate efficiently than incompatible crosses.

6. SUMMARY AND CONCLUSION

The problem of asynchronised flowering in certain mango cultivars restrict their use as parent in mango hybridization programme and circumstances usually make it necessary to store the pollen from male donor parent for later pollination of the female parents. In the present investigation, pollen viability of three mango polliniser cultivars *viz*., Sensation, Tommy Atkins and Janardan Pasand were investigated up to 24 weeks under four different storage conditions (room temperature, -4^{o}C, -20^{o}C and -196^{o}C). Three different methods of viability estimations were utilized *viz*., *in vitro* germination, Fluorescence diacetate (FDA) and acetocarmine tests. Fresh pollens from all the three pollinisers gave higher pollen viability in terms of *in vitro* germination, FDA and acetocarmine tests as compared to stored pollens. Storage methods and interaction between storage methods and days of storage had highly significant effect on pollen viability as confirmed by *in vitro* germination test. Pollen stored at room temperature was viable up to 4 weeks. However, the per cent viability significantly reduced during this period. Pollen storage of mango cultivars at -20 ^{0}C showed almost similar trend as it was observed in case of -4 ^{0}C storage. The only difference noticed was the percentage of viability was higher in all three mango cultivars at all dates of observations compared to pollen storage at -4 ^{0}C. Cryostored pollens showed significantly higher viability percentage as compared to all the other storage conditions as confirmed by *in vitro* germination, FDA and acetocarmine tests of pollen viability in all pollen donor mango cultivars. From the present study, we suggest storage of pollen at -20^{o}C for pollination among cultivars having non synchronized flowering in the same season. However, for long term pollen storage cryo storage proved to be the best method.

Self incompatibility studies were carried out in mango cultivars developed at Indian Agricultural Research Institute such as Amrapali, Mallika, Pusa Arunima and Pusa Surya. The effects of self and open pollination on the fruit set, ovule degeneration and growth rate of selfed and open pollinated fruitlets were compared. Self pollination resulted in very rapid decline in fruit setting percentage as compared to open pollination and which was much more pronounced in Mallika and Amrapali than Pusa Arunima and Pusa Surya, which showed fruit set upon selfing. During 48 hours after pollination, pollen tube growth in stylar region of selfed Mallika and Amrapali were considerably slower compared to selfed Pusa Arunima and Pusa Surya. After 48 hours of self pollination, pollen tube reached up to 2/3 of stylar region in Amrapali and Mallika but reached up to lower end of style in case of Pusa Arunima and Pusa Surya. Degeneration of ovule as a result of self and open pollination was much more apparent after 14 days after pollination. Selfed Mallika and Amrapali showed more than 75 degenerated ovules as

a result of self pollination and majority of selfed fruitlets dropped thereafter. However, open pollination resulted less degenerated ovules in these mango cultivars. The growth of fruitlets and ovule obtained from self and open pollination of all the four cultivars revealed that the fruitlets weight and dimensions of fruitlets and ovule were significantly less after selfing as compared to open pollination in Amrapali, Mallika and Pusa Arunima, whereas there was no significant difference in fruitlet weight and dimension of fruitets and ovule between self and open pollinated Pusa Surya. The findings of present investigation bring out clearly that self incompatibility in mango unlike typical sporophytic self incompatibility is post zygotic and mango cultivar Mallika is self-incompatible, whereas cultivar Amrapali is partially so. However, Pusa Surya is self compatible.

Cross compatibility investigation evidenced significant effects of pollen donor parent on fruit set, pollen tube growth and fruitlet development. In crosses, where Amrapali was used as a female parent, the differences in fruit set throughout different stages of fruitlets development were non-significant. In contrast to this, crosses where Pusa Arunima was used as female parent resulted in significant differences. Pusa Arunima crossed with Dashehari and Amrapali retained fruitlets only upto 14 days after cross pollination and found to be incompatible crosses. Results pertaining to Pusa Arunima clearly showed that Langra is better polliniser cultivar for Pusa Arunima. Pollen tube growth was faster in those cross combinations which showed higher fruit set compare to those cross combination, which had poor fruit set. The findings of pollen tube growth in different cross combinations further confirmed Langra as a better pollen donor parent for Pusa Arunima and Dashehari. The results obtained on fruit set and pollen tube growth were also confirmed by growth rate of fruitlets and ovule in the present investigation. In general the increase in fruit weight and dimension of fruitlets and ovule were much more in those cross combinations, which had higher fruit set and fast pollen tube growth. On the basis of results we suggest that Langra was compatible and proved to be better polliniser cultivar for Pusa Arunima and Dashehari. However, Dashehari and Amrapali showed incompatibility with Pusa Arunima.

Based on the results of the present investigation, following conclusions were drawn:

- Pollen viability of three mango pollen donor parents viz., Sensation, Tommy Atkins and Janardan Pasand were investigated up to 24 weeks of storage under four different storage conditions (room, -4^{o}C, -20^{o}C and -196^{o}C).
- Fresh pollens from all three pollen parents gave higher pollen viability in terms of *in vitro* germination, FDA and acetocarmine tests as compared to stored pollen.
- Storage at -196^{o}C resulted in significantly higher viability percentage compared to other storage methods, it gave the highest viability percentage after 24 weeks of storage followed by -20^{o}C and -4^{o}C storage conditions.

- Storage at room temperature resulted in non-viable pollen after 4 weeks of storage.
- From the present study we suggest storage of pollen at -20^{o}C for pollination among cultivars having non synchronized flowering in the same season. However, for long term pollen storage cryo storage proved to be the best method.
- Number of male and hermaphrodite flowers varied with the genotype of mango, also there was a contrasting difference in the number of unusual stamen and deformed ovary flower with respect to different cultivars.
- Comparing the effects of self and open pollination on the initial fruit set and final fruit retention, selfed Amrapali and Mallika showed significantly low fruit set in comparison to their open pollinated counterparts with no fruit retention after 28 days after pollination.
- Pusa Arunima and Pusa Surya gave higher initial and final fruit retention in both selfing and open pollination.
- Growth measurement of fruitlets of comparable age resulting from self and open pollination revealed that the selfed fruitlets of Mallika and Amrapali were invariably smaller than the ones obtained from open pollination.
- Mallika and Amrapali showed very shriveled ovule growth as a result of self pollination and fruitlets of which dropped afterwards, whereas, it was normal in the case of other two cultivars.
- Pollen tube growth showed that there was no problem in pollen adherence to stigma surface, pollen germination and pollen tube growth. Up to 48-72 hours, pollen tube reached the micropyle, and might resulted in fertilization.
- The findings revealed that self incompatibility in mango unlike typical sporophytic self incompatibility is post zygotic and mango cultivar Mallika is self-incompatible, whereas cultivar Amrapali is partially so. However, Pusa Arunima and Pusa Surya are self compatible.
- Cross compatibility studies revealed dependence of mango cultivar on cross-pollination for commercial mango growing. If the cultivar is self-fruitful, a solid block can be planted but if it depends on cross-pollination, at least two compatible cultivars must be interplanted.
- Present investigation evidenced significant effects of pollen donor parent on fruit set, pollen tube growth and fruitlet development.

- Fluorescence microscopic study of pollen pistil interaction in the present study showed marked genetic effect of male donor parent and female parent on the extent of pollen tube growth.
- Langra was compatible and proved to be better polliniser cultivar for Pusa Arunima and Dashehari.
- Dashehari and Amrapali showed cross incompatibility with Pusa Arunima. The use of compatible pollinizers in commercial plantations of mango seems to be quite congenial because a considerable overlapping in the periods of panicle emergence and flowering occurs between the cultivars.
- For proper pollination, fertilisation, ovule and fruit growth and development, the most effective pollinizers like Langra should be either interplanted or top-worked in commercial orchards of Pusa Arunima and Dashehari.

ABSTRACT

Pollen viability of three mango polliniser cultivars *viz*. Sensation, Tommy Atkins and Janardan Pasand were investigated up to 24 weeks under four different storage conditions (room temperature, -4^{o}C, -20^{o}C and -196^{o}C). Three different methods of viability estimations were utilized *viz*., *in vitro* germination, FDA and acetocarmine tests. Fresh pollens from all the three pollinisers gave higher pollen viability in terms of *in vitro* germination, FDA and acetocarmine tests as compared to stored pollens. Storage methods and interaction between storage methods and days of storage had highly significant effect on pollen viability as confirmed by *in vitro* germination test ($p < 0.0001$). In case of room temperature storage, Sensation, Tommy Atkins and Janardan Pasand showed only 2.11%, 1.80% and 1.03% pollen viability after 4 weeks of storage as confirmed by *in vitro* pollen germination test, after which they became non-viable. Pollen storage of mango cultivars at -20 ^{0}C showed almost similar trend as it was observed in case of -4 ^{0}C storage. The only difference noticed was the percentage of viability was high in all three mango cultivars at all dates of observations compared to pollen storage at -4 ^{0}C. Cryostored pollens showed significantly higher viability percentage as compared to all the other storage conditions as confirmed by *in vitro* germination, FDA and acetocarmine tests of pollen viability in all pollen donor mango cultivars.

Another study was carried out in mango cultivars developed at Indian Agricultural Research Institute such as Amrapali, Mallika, Pusa Arunima and Pusa Surya. The effects of self and open pollination on the fruit set, ovule degeneration and growth rate of selfed and open pollinated fruitlets were compared. Self pollination resulted in very rapid decline in fruit setting percentage as compared to open pollination and which was much more pronounced in Mallika and Amrapali than Pusa Arunima and Pusa Surya. During 48 hours after pollination pollen tube growth in stylar region of selfed Amrapali and Mallika was considerably slower compared to selfed Pusa Arunima and Pusa Surya. Degeneration of ovule as a result of self and open pollination was much more apparent after 14 days after pollination. In selfed Amrapali and Mallika showed more than 75 degenerated ovules as a result of self pollination after 21 days of self pollination and majority of selfed fruitlets dropped thereafter. Contrastingly, in Pusa Surya and Pusa Arunima resulted less percentage of degenerated ovules after 21 days of self pollination. The growth of fruitlets and ovule obtained from self and open pollination of all the four cultivars revealed that the fruitlets weight and dimensions of fruitlets and ovule was significantly less after selfing as compared to open pollinated fruits in case of Amrapali, Mallika and Pusa Arunima whereas there was no significant difference in fruitlet weight and dimension of fruitets and ovule between self and open pollinated Pusa Surya.

In cross compatibility studies where Amrapali was used as a female parent, the differences in fruit set throughout different stages of fruitlets development were non-significant. In contrast to this, crosses where Pusa Arunima was used as female parent resulted in significant differences. Pusa Arunima crossed with Dashehari and Amrapali retained fruitlets only up to 14 days after cross pollination and found to be incompatible crosses. Results pertaining to Pusa Arunima clearly showed that Langra is better polliniser cultivar for Pusa Arunima. The findings of pollen tube growth in different cross combinations confirmed Langra as a better pollen donor parent for Pusa Arunima and Dashehari. In general the increase in fruit weight and dimension of fruitlets and ovule were much more in those cross combinations which had higher fruit set and fast pollen tube growth.

From the pollen storage study we suggest storage of pollen at -20 ^{o}C for pollination among cultivars having non synchronized flowering in the same season. However, for long term pollen storage cryo storage proved to be the best method. The findings of self incompatibility study bring out clearly that self incompatibility in mango unlike typical sporophytic self incompatibility is post zygotic and mango cultivar Mallika is self-incompatible whereas cultivar Amrapali is partially so. However, Pusa Arunima and Pusa Surya are self compatible. On the basis of cross compatibility study we suggest Langra as better polliniser cultivar for Pusa Arunima and Dashehari. However, Pusa Arunima showed incompatibility with Dashehari and Amrapali pollens.

आम (*मैंजीफेरा इंडिका* एल.) में असंगतता एवं भण्डारित पराग की जीवनक्षमता का अध्ययन

सार

आम की तीन परागद कृषिजोपकिस्मों यथा, सेंसेशन, टॉमीएटकिन्स एवं जनार्दन पसन्द का चार भिन्न–भिन्न भण्डारण परिस्थितियों (सामान्य परिवेशी तापमान, -4^0 सें., -20^0 सें. एवं -196^0 सें.) के अन्तर्गत 24 हफ्तों तक अध्ययन किया गया। जीवन क्षमता निर्धारण की तीन अलग–अलग विधियों यथा, सजीव कोशिकाओं के बाहर प्रयोगशाला में अंकुरण, एफ.डी.ए. एवं एसिटोकार्माइन परीक्षण का उपयोग किया गया। सभी तीनों परागदों के ताजा परागकणों ने प्रयोगशाला में अंकुरण, एफ.डी.ए. एवं एसिटोकार्माइन परीक्षणों के अनुसार, भण्डारित परागकणों की तुलना में अधिक पराग–जीवनक्षमता दर्शायी। भण्डारण विधियों तथा भण्डारण विधियों एवं भण्डारण अवधि के बीच पारस्परिक क्रिया का पराग–जीवन क्षमता पर महत्वपूर्ण प्रभाव था जिसकी प्रयोगशाला में अंकुरण परीक्षण (पी <0.0001) द्वारा पुष्टि हुई। कमरे के तापमान पर भण्डारण करने पर भण्डारण के 4 हफ्ते बाद सेंसेशन, टॉमीएटकिन्स एवं जनार्दन पसन्द ने केवल 2.11 प्रतिशत, 1.80 प्रतिशत एवं 1.03 प्रतिशत पराग जीवनक्षमता दर्शायी जिसकी प्रयोगशाला में अंकुरण द्वारा पुष्टि हुई किन्तु उसके बाद उनकी जीवनक्षमता समाप्त हो गई। आम की कृषिजोपकिस्मों के -20^0 सेल्सियस पर पराग भण्डारण ने -4^0 सेल्सियस पर भण्डारण के लगभग समान ही व्यवहार दर्शाया। इसमें अन्तर केवल यही था कि अध्ययन की सभी तिथियों में आम की तीनों कृषिजोपकिस्मों में -4^0 सेल्सियस पर भण्डारण की तुलना में जीवनक्षमता का प्रतिशत अधिक देखा गया। सभी अन्य भण्डारण अवस्थाओं की तुलना में -196^0 सेल्सियस भण्डारित परागकणों ने महत्वपूर्ण रूप से अधिक जीवनक्षमता प्रतिशत दर्शाया जिसकी प्रयोगशाला में सभी परागदाता आम की कृषिजोपकिस्मों के पराग जीवनक्षमता संबंधी, प्रयोगशाला में अंकुरण, एफ.डी.ए. एवं एसिटाकार्माइन परीक्षणों द्वारा पुष्टि हुई।

एक अन्य अध्ययन, भारतीय कृषि अनुसंधान संस्थान द्वारा विकसित आम की कृषिजोपकिस्मों यथा, आम्रपाली, मल्लिका, पूसा अरूणिमा एवं पूसा सूर्या के साथ किया गया। स्वपरागण एवं मुक्त परागण के फल बनने, बीजाण्ड–ह्रास तथा स्वपरागित एवं मुक्त परागित नवविकसित फलों की वृद्धिदर पर प्रभावों की तुलना की गई। मुक्त परागण की अपेक्षा स्वपरागण के परिणामस्वरूप फलन निर्माण प्रतिशत में बहुत तेजी से कमी देखी गई तथा ऐसा पूसा अरूणिमा एवं पूसा सूर्या की अपेक्षा मल्लिका एवं आम्रपाली में अधिक था। परागण के 48 घण्टे के दौरान स्वपरागित आम्रपाली एवं मल्लिका के वर्त्तिका क्षेत्र में पराग नलिका की वृद्धि, स्वपरागित पूसा अरूणिमा एवं पूसा सूर्या की तुलना में महत्वपूर्ण परिणामस्वरूप धीमी थी। स्वपरागण एवं

मुक्त परागण के परिणामस्वरूप बीजाण्ड की ह्रास, परागण के 14 दिन पश्चात अधिक देखा गया। स्वपरागण के परिणामस्वरूप स्वपरागित आम्रपाली एवं मल्लिका ने स्वपरागण के 21 दिन पश्चात 75 से भी अधिक नष्ट बीजाण्ड दर्शाए तथा अधिकांश स्वपरागित नवविकसित फल तत्पश्चात गिर गए। इसके विपरीत पूसा सूर्या एवं पूसा अरूणिमा में स्वपरागण के 21 दिन पश्चात नष्ट बीजाण्डों का प्रतिशत कम था। सभी चार कृषिजोपकिस्मों में स्वपरागण एवं मुक्त परागण द्वारा बने नवविकसित फलों की वृद्धि ने दर्शाया कि आम्रपाली, मल्लिका एवं पूसा अरूणिमा में मुक्त परागण की अपेक्षा स्वपरागण के पश्चात बने नवविकसित फलों का भार तथा फलों एवं बीजाण्ड का परिणाम महत्वपूर्ण रूप से कम था जबकि पूसा सूर्या में स्वपरागित एवं मुक्त परागित नवविकसित फलों के भार तथा फलों एवं बीजाण्ड परिणाम के बीच कोई महत्वपर्ण अन्तर नहीं था।

क्रॉस संगतता अध्ययनों में जहां आम्रपाली का मादा जनक के रूप में उपयोग किया गया, नवविकसित फलों के विकास की समस्त भिन्न–भिनन अवस्थाओं के दौरान फल निर्माण में कोई महत्वपूर्ण अन्तर नहीं थे। इसके विपरीत, उन क्रासों में जहां पूसा अरूणिमा का मादा जनक के रूप में उपयोग किया गया, महत्वपूर्ण अन्तर देखे गए। दशहरी एवं आम्रपाली के साथ क्रॉस किए गए पूसा अरूणिमा में पर परागण के पश्चात नवविकसित फल केवल 14 दिनों तक बने रहे तथा इस प्रकार से ये असंगत क्रॉस पाए गए। पूसा अरूणिमा के संबंध में परिणामों ने स्पष्ट रूप से दर्शाया कि पूसा अरूणिमा हेतु लंगड़ा बेहतर परागद है। विभिनन क्रॉस संयोजनों में पराग नलिका वृद्धि संबंधी परिणामों ने पुष्टि की कि पूसा अरूणिमा एवं दशहरी हेतु लंगड़ा एक बेहतर परागदाता जनक है। सामान्यतया फल भार तथा नवविकसित फलों एवं बीजाण्ड के परिणाम में वृद्धि उन क्रॉस संयोजनों में अधिक थी जिनमें फल निर्माण अधिक था तथा पराग नलिका वृद्धि तेज थी।

पराग भण्डारण संबंधी अध्ययन के आधार पर हमारा सुझाव है कि एक ही ऋतु में असमकालिक पुष्पन वाली कृषिजोपकिस्मों के बीच परागण हेतु पराग का -20^0 सेल्सियस पर भण्डारण किया जाना चाहिए। वैसे पराग को लम्बी अवधि हेतु भण्डारित करने के लिए -196^0 सेल्सियस भण्डारण सर्वश्रेष्ठ विधि सिद्ध हुई है। स्वअसंगतता संबंधी अध्ययन के परिणाम स्पष्ट रूप से दर्शाते हैं कि प्रारूपी बीजाणु उद्भिद संबंधी स्व असंगतता से भिन्न आम में स्वअसंगतता पश्च–युग्मनजी है तथा आम की कृषिजोपकिस्म, मल्लिका स्व–असंगत है जबकि कृषिजोपकिस्म आम्रपाली में ऐसा आंशिक रूप से है वैसे पूसा अरूणिमा एवं पूसा सूर्या स्वसंगत है। क्रॉस संगतता संबंधी अध्ययन के आधार पर हमारा सुझाव है कि पूसा अरूणिमा एवं दशहरी हेतु लंगड़ा एक बेहतर परागद है। वैसे दशहरी एवं आम्रपाली के परागों के साथ पूसा अरूणिमा ने असंगतता दर्शायी।

REFERENCES

Abreu, I. and Oliveira, M. (2004). Fruit production in kiwifruit (*Actinidia deliciosa*) using preserved pollen. *Australian Journal of Agricultural Research*, **55**: 565–569.

Akihama, T. Omura, M. and Kozaki, I. (1978). Further Investigation of freezer-drying for Deciduous Fruit Tree Pollen. In: Akihama, T. and Nakajima, K. (eds) Long term preservation of favorable germplasm in arboreal crops. *The fruit tree Research Statistics*, Fujimoto, p. 1-7.

Allard, R.W. (1960). Principles of Plant Breeding. John Wiley, New York, pp 485**.**

Alonso, J. M., Socias I. and Company, R. (2005). Self-compatibility expression in self-compatible almond genotypes may be due to inbreeding. *Journal of the American Society for Horticultural Science*, **130**: 868–869.

Amma, M. S. P. and Kulkarni. (1979). Pollen storage in organic solvents. *Journal of Palynology*, **15**: 100-104.

Arasu, N. T. (1970). Self incompatibility in Ribea. *Euphytica*, **19**: 373-378.

Aslantus, R. and Pirlak. (2002). Storage and germination of Strawberry pollen. IV International Symposium on Strawberry, pollen. (Eds.): Hietaranta, M.-M Linn., Palonen & Parikka, P. *Acta Horticulturae,* **2**: 567.

Bellani, L. M. and Bell, P. R. (1986). Cytoplasmic differences between the pollen grains of two cultivars of *Malus domestica* Borkh. correlated with viability and gemination. *Annals of Botany*, **58**: 563-568.

Beyoung, H. K. (1965). The effects of calcium on pollen germination. *Journal of the American Society for Horticultural Science*, **86**: 818-823.

Bhojwani, S. S. and Bhatnagar, S. P. (1974). The Embryology of Angiosperms. Vikas Publishing House, Delhi, 264p.

Blinkenberg, C., Brix, H., Schaffaltizzky de muckadell, M. and Vedel, H. (1958). Controlled pollinations in *Fagus*. *Silvae Genetica,* **7**: 116-122.

Brewbaker, J. L. and Kwack, B. H. (1963). The essential role of calcium ion in pollen germination and pollen tube growth. *American Journal of Botany*, **50**: 859-865.

Brink, R. A. and Cooper, D. C. (1939). Somatoplastic sterility in *Medicago sativa*. *Science, New York,* **90**: 545-546.

Bubar, J. S. (1958). An association between variablity in ovule development within ovaries and self-incompatibility in Lotus. *Canadian Journal of Botany,* **36**: 65-72.

Bubar, J. S. (1959). Differences between self-incompatibility and self-sterility. *Nature,* **183**: 411-412.

Cerovic, R. and Ruzic, D. (1992). Pollen tube growth in sour cherry (*Prunus cerasus* L.) at different temperatures. *Journal of Horticultural Science,* **67(3)**: 333–340.

Cerovic, R., Micid, N., Djuric, G. and Nikolic, M. (1998). Determination of pollen viability in sweet cherry. *Acta Horticulturae,* **468**: 559-565.

Chaudhury, R., Malik, S. K. and Rajan, S. (2010). An improved pollen collection and cryopreservation method for highly recalcitrant tropical fruit species of mango (*Mangifera indica* L.) and litchi (*Litchi chinensis* Sonn.). *Cryo Letters,* **31(3)**: 268-278.

Cheung, A. Y., Wu, H. M., Stilio, V. D., Glaven, R., Chen, C., Wong, E., Ogdahl, J. and Estavillo, A. (2000). Pollen pistil interaction in *Nicotiana tabacum. Annals of Botany*, **85** (Sup A): 29-37.

Ching, T. M. and Slabaugh,W. H. (1966). X-ray diffraction analysis of ice crystals in coniferous pollen. *Cryobiology*, **2**: 321–327.

Chira, E. (1963). The pollen sterility of scots and black pines (*Pinus silvestris* L., *P. nigra* Arnold.). *Lesn Aopis,* **9**: 821-826.

Cohen, E., Lavi, U. and Spiegel-Roy, P. (1989). Papaya pollen viability and storage. *Scientia Horticulturae,* **40**: 317-324.

Connor, K. F. and Towill, L. E. (1993). Pollen-handling protocol and hydration/dehydration characteristics of pollen for application to long-term storage. *Euphytica*, **68**: 77–84.

Cook, S. A. and Stanley, R. G. (1960). Tetrazolium chloride as an indicator of pine pollen germinability. *Silvae Genetica,* **9**: 134-136.

Cope, F. W. (1958). Incompatibility in *Theobroma cacao* L. *Nature, London,* 181-279p.

Cope, F. W. (1962). The mechanism of pollen incompatibility in *Theobroma cacao*. *Heredity,* **17**: 157-182.

Crane, M. B. and Lawrence, W. J. C. (1929). Genetical and cytological aspect of incompatibility and sterility in cultivated fruits. *Journal of Pomology and Horticulture Science*, **7**: 276-301.

Crane, M. B. and Lawrence, W. J. C. (1931). Sterility and incompatibility in diploid and polyploidy fruits. *Journal of Genetics,* **24**: 97-107.

Crane, M. B. and Lawrence, W. J. C. (1942). Genetical studies in peas, III. Incompatibility and sterility. *Journal of Genetics*, **43**: 31-43.

Cruzan, M. B. (1986). Pollen tube distribution in *Nicotiana glauca* : evidence for density dependent growth. *American Journal of Botany*, **73**: 902-907.

Dafni, A. (1992). Pollination ecology*:* a practical approach (the practical approach series). University press, New York, 250p.

De Nettancour, T. D. (2001). *Incompatibility and Incongruity in Wild and Cultivated Plants*. Berlin/Heidelberg/New York: Springer-Verlag. 322 p. 2nd ed.

De Wet, E., Robbertse, P. J. and Groeneveld, H. T. (1989). The influence of temperature and boron on pollen germination in *Mangifera indica* L. *South African Journal of Plant and Soil*, **6**: 230-234.

Degani, C., Elbatsri, R. and Gazit, S. (1990). Enzyme polymorphism in mango. *Journal of American Society for Horticultural Science*, **115**: 844-874.

Desai, U. T. and Bhandwalkar, S. M. (1995). Compatibility and fruit set studies in some mango cultivars with 'Kesar' as pollen parent. *South Indian Horticulture*, **43**: 115-116.

Dhaliwal, H. S. and Dhaliwal, G. S. (1992). Studies on incompatibility and fruit set in mango (*Mangifera indica* L.) II: *In vivo* pollen germination in various media in Dashehari and Langra cultivars. *Punjab Horticulture Journal*, **32** (**4**): 46-50.

Dijkman, M. J. and Soule, M. J. (1951). A tentative method of mango selection. *Proceeding of Florida State Horticulture Society*, **64**: 257-262.

Dutra, G. A. P., Sousa, M. M., Rodrigues, R., Sude, C. P. and Pereira, T. N. S (2000). Viabilidade em grãos de pólen fresco e armazenado em acessos de pimenta. *Horticultura Brasileira*, **18**: 729- 730.

Einhard, P. M., Correa, E. R. and Raseira, M. C. B. (2006). Comparação entre métodos para testar a viabilidade de pólen de pessegueiro. *Revista Brasileira de Fruticultura*, **28**: 5-7.

Elgersma, A., Stephenson, A. G. and Nijs, A. P. M. (1989). Effects of genotype and temperature on pollen tube growth in perennial ryegrass (*Lolium perenne L.*). *Sexual Plant Reproduction*, **2**: 225–230.

Fraser, S. (1927). *American Fruits, Their Propagation, Cultivation, Harvesting and Distribution*, 829 pp. Orange- Judd Publishing Co., Inc., New York.

Galang, F. G., and Lazo, F. D. (1937). The Setting of Carabo mango fruits as affected by certain sprays. *Phillipine Journal of Agriculture*, **8**: 187–210.

Galleta, G. J. (1983). Pollen and seed management. In: Moore, J.N. and Janick, J. (eds.) Methods in fruits breeding. Purdue University press, Indiana, 23-47p.

Ganeshan, S. (2003). In *In vitro Conservation and Cryopreservation of Tropical Fruit Species* (eds) Chaudhury, R., Malik, S.K. and Bhag, M. IPGRI, Office for south Asia, New Delhi, India/NBPGR, New Delhi, India, 215-227p.

Ganeshan, S. and Alexander, V. V. (1991). Cryogenic preservation of lemon (*Citrus limon* Burm.) pollen. *Gartenbauwissenschaft*, **56**: 228-230.

Giordano, L. B., Aragão, F. A. S. and Boiteux, L. S. (2003). Melhoramento genético do tomateiro. *Informe Agropecuário*, **24**: 43-57.

Godini, A., de Palma, L. and Petruzzella, A. (1987). Interrelationships of almond pollen germination at low temperatures, blooming time and biological behaviour of cultivars. *Advances of Horticulture Science*, **1**, 73–76.

Goss, J. A. (1971). Effect of salinity on pollen. *American Journal of Botany*, **58**: 721-725.

Griffin (1982). A preliminary examination of pollen germination in several *Eucalyptus* species. *Silvae Genetica*, **31**: 198-203.

Hampson, C. R., Azarenko, A. N. and Soelender, A. (1993). Pollen stigma interaction following compatible and incompatible pollinations in hazelnut. *Journal of American Society for Horticultural Science*, **118(6)**: 814-819.

Hang, N. T. N. (1999). Studies on embryo rescue in mango (*Mangifera indica* L.). A M.Sc. Thesis submitted to PG school IARI., New Delhi.

Hanna,W. W. and Towill, L. E. (1995). Long-term pollen storage. In: *Plant Breeding Reviews.* Volume 13. (Janick, J., Ed.).Wiley and Sons, New York, USA. 179–207p.

Hedhly, A., Hormaza, J. I. and Herrero, M. (2005). Influence of genotype–temperature interaction on pollen performance. *Journal of Evolutionary Biology*, **18**: 1494–1502.

Helpson–Harrison, J. and Helpson–Harrison, Y. (1970). Evaluation of pollen viability by enzymatically induced fluorescene; intracellular hydrolysis of fluorescien diacetate. *Stain Technology*, **45**: 115-120.

Herrero, M. and Dickinson, H. G. (1979). Pollen-pistil incompatibility in *Petunia hybrida*: Changes in the pistil following compatible and incompatible intraspecific crosses. *Journal of Cell Science*, **36**: 1-18.

Herrero, M. and Dickinson, H. G. (1981). Pollen tube development in *Petunia hybrida* following compatible and incompatible intraspecific matings. *Journal of Cell Science*, **47**: 365-383.

Heslop-Harrison J. and Heslop-Harrison Y. (1992). Germination of Monocolpate angiosperm pollen: effects of inhibitory factors and the Ca^{+} channel blocker, nifedipine. *Annals of Botany*, **69**: 395- 403.

Heslop-Harrison J. S., Heslop-Harrison Y. and Shivanna K. R. (1984). The evaluation of pollen quality and a further appraisal of the flouro-chromatic (FCR) test procedure. *Theoretical and Applied Genetics*, **67**: 367-375.

Heslop-Harrison, J. (1975). Incompatibility and the pollen-stigma interaction. *Annual Review of Plant Physiolog*, **26**: 403-425.

Heslop-Harrison, J. (1979). Aspects of structure, cytochemistry and germination of the pollen of rye (*Secale cereale* L.). *Annals of Botany*, **44**(Suppl.): 1-47.

Hoekstra, F. A. and Bruinsma, J. (1975). Respiration and vitality of binucleate and trinucleate pollen. *Physiologia Plantarum*, **34**: 221 225.

Howlett, F. S. (1936). The effect of carbohydrate and nitrogen deficiency upon microsporogenesis and the development of the male gamethophyte in the tomato (*Lycopersicom esculentum* Mill.). *Annals of Botany*, **50**: 767-804.

Inagaki, M. (2000). Use of stored pollen for wide crosses in wheat haploid production. In: Engelmann, F., Tagako, H. (Eds.), Cryopreservation of Tropical Plant Germplasm. Current Research Progress and Applications. International Plant Genetic Resources Institute, Rome, Italy, 130–135p.

Issarakraisila, M. and Considine, J. A. (1994). Effects of temperature on micro-sporogenesis and pollen viability in mango cv. `Kensington'. *Annals of Botany*, **72**: 231-40.

Issarakraisila, M., Considine, J. A. and Turner, D. W. (1992). Seasonal effects on floral biology and fruit set of mangoes in a warm temperature region of west Australia. *Acta Horticulturae*, **321**: 626 635.

Ito, P. J. and Hamilton, R. A. (1980). Quality and yield of Keauhou macadamia nuts in mixed and pure block plantings. *HortScience*, **15**: 307.

Iyer, C. P. A., Subbaiah, M. C., Subramanyam, M. D. and Prasada Rao, G. S. (1989). Screening of germplasm and correlation among certain characters in mango. *Acta Horticulturae*, **231**: 83–90.

Jayaprahasam, S., Sharma, H. C., Singh, R., Singh, S. K., Karihaloo, J. L., Bhat, S. R. and Srivastava, G. C. (2001). Thesis M.Sc. IARI, New Delhi. Pollen-Pistil compatibility studies in mango (*Mangifera indica* L.)

Juliano, J. B. and Cuevas, N. L. (1933). Floral morphology of the mango *(Mangifera indica* Linn.) with special reference to the Pico variety from the Philippines. *Philippines Agriculture*, **21**: 449-472.

Kahan, T. L. and Demason, D. A. (1988). Citrus pollen tube development in cross compatible gynoecia, self incompatible gynoecia and *in vitro*. *Canadian Journal of Botany*, **66**: 25-27.

Kao, T. H. and McCubbin, A. G. (1996). How flowering plants discriminate between self and non-self pollen to prevent inbreeding. *Proceeding of National Acadamy of Sciences, USA*. **93**: 12059–12065

Kearns, C. A. and Inouye, D. (1993). Techniques for pollinations biologists. University Press of Colorado, Colorado, 579 p.

Kenrick, J. & Knox, R. B. (1984). Self-incomopatibility in the nitrogen-fixing tree *Acacia retinodes*: quantitative cytology of pollen tube growth. *Theoretical and Applied Genetics*, **69**: 481-488.

Knight, R. and Rogers, H. H. (1955). Incompatibility in *Theobroma cacao*. *Heredity*, **9**: 69-77.

Kostermans, A. J. G. H. and Bompard, J. M. (1993). The Mangoes-Botany, Nomenclature, Horticulture and Utilization. Academic Press, London.

Lacerda, C. A., Almeida, E. C. and Lima, J. O. G. (1994). Estádio de desenvolvimento da flor de *Lycopersicom esculentum* Mill.Cv. Santa Cruz Kada ideal para coleta de pólen a ser germinado em meio de cultura. *Pesquisa Agropecuária Brasileira*, **29**: 169-175.

Landes, M. (1939). The cause of self sterility in rye. *American Journal of Botany*, **26**: 567-571.

Ledeboer, M. and Rietsema, I. (1937). A case of unfruitfulness in blackcurrants. *Journal of Pomo1ogy*, **15**:191 304.

Lee, C. W., Thomas, J. C. and Buchmann, S. L. (1985). Factors affecting germination of jojoba pollen. *Journal of American Society for Horticultural Science*, **110 (5)**: 671–676.

Loguercio, L. L. (2002). Pollen treatment in high osmotic potential a simple tool for in vitro preservation and manipulation of viability in gametophytic population. *Brasilian Journal of Plant Physiology*, **14**: 65-70.

Lora, J., Pe´rez de Oteyza, M. A., Fuentetaja, P. and Hormaza J. I. (2006). Low temperature storage and *in vitro* germination of cherimoya (*Annona cherimola* Mill.) pollen. *Scientia Horticulturae*, **108**: 91–94.

Lush, W. M., Opat, A. S., Nie, I. and Clarke, A. E. (1997). An *in vitro* assay for assessing the effect of growth factors on *Nicotiana alata* pollen tube. *Sexual Plant Reproduction*, **10**: 351-357.

Luza, J. G, and Polito, V. S. (1985). *In vitro* germination and storage of English walnut pollen. *Scientia Horticulturae*, **27**:303-316.

MacDaniels, L. H. and Hildebrand, E. M. A. (1939). Study of pollen germination upon the stigmas of apple flowers treated with fungicides. *Proceedings of the American Society for Horticultural Science*, **36**: 137.

Majumdar, S. K., Kerns, K. R., brewbaker, J. L. and Johannessen, G. A. (1964). Assesing self incompatibility in pineapple by a fluroscence technique. *Proceeding of American Society of Horticultural Sciences*, **84**: 217-223.

Marchant, R., Power, J. B., Davey, M. R., Chartier-houis, J. M. and Lynch, R. P. (1993). Cryopreservation of pollen from two rose cultivars. *Euphytica*, **66**: 235–241.

Martin, F. W. (1959). Staining and observing pollen tubes in the style by means of fluorescence. *Stain Techn*ology, **34**: 125-128.

Martínez-gómez, P., Gradziel, T. M., Ortega, E. and Dicenta, F. (2002). Low temperature storage of almond pollen. *Hortscience*, **37**: 691–692.

Martinez-Gomez., Dicenta, F. and Ortega, E. (2001). The germination of almond pollen. *Journal of Horticltural Sciences*, **58**: 229-255.

McConchie, C. A., Batten, D. J. and Vithanage, V. (1992). The effect of temperature on pollen tube growth in lychee (*Litchi chinensis* Sonn). In: Control of fruit set and fruit retention in lychee. Rural industries Research and Development Corporation Report Cs18A, CSIRO, Brisbane, Australia.

McGregor, S. E. (1976). Insect Pollination of Cultivated Crop Plants. U.S. Dep. Agric., Agric. Handb. No. 496,411 p.

Miranda, P. A. and Clement, C. R. (1990). Germination and storage of pejibaye (*Bactris gasipaes)* palmae pollen. *Revista de Biologia Tropical*, **38**: 29-33.

Modlibowska, I. (1945). Pollen tube growth and embryo sac development in apple and pear. *Journal of Pomology and Horticultural Science*, **21**: 57-89.

Mukherjee, S. K. (1953). The Mango- its botany, cultivation, uses and future improvement, especially as observed in India. *Economic Botany*, **7**: 130-162.

Mukherjee, S. K., Majumdar, P. K. and Chatterjee, S. S. (1961). An improved technique of mango hybridization. .). *Indian Jounal of Horticulture*, **18**: 302-304.

Mukherjee, S. K., Singh, R. N., Majumder, P. K. and Sharma, D. K. (1968). Present position regarding breeding of mango *(Mangifera indica* L.) in india. *Euphytica*, **17**: 462-467.

Mulcahy, G. B. and Mulcahy, D. L. (1988). The effect of supplemented media on the growth *in vitro* of bi- and trinucleate pollen. *Plant Science*, **55**, 213–216.

Naik, K. C. and Rao, M. M. (1943). Studies on the blossom biology and pollination in mangoes (*Mangifera indica* L.). *Indian Jounal of Horticulture*, **1**: 107–19.

Nassar, N. M. A., Santos E. D. and David, S. (2000). The transference of apomixis genes from *Manihot neusana* Nassar to cassava, *M. eculenta* Crantz. *Hereditas*, **132**: 167-170.

Nettancourt D. de (1977). Incompatibility in angiosperms. In: Monographs on Theoretical and Applied Genetics, No. 3, 1–96p, Frankel R., Gall G. A. E. and Linskens H. F. (eds), Springer, Berlin

Oliveira, M. S. P., Maués, M. M. and Kalume, M. A. A (2001) .Viabilidade de pólen *in vivo* e *in vitro* em genótipos de açaizeiro. *Acta Botânica Brasilica*, **15**: 63-67.

Pacini, E., Franchi, G. G., Lisci, M. and Nepi, M. (1997). Pollen viability related to type of pollination in six angiosperm species. *Annals of Botany*, **80**: 83-87.

Pandey, R. M., Singh, R.N. and Rathore, D. S. (1973). A study of self incompatibility and its physiological cause in mango (*Mangifera indica* L.) cv. Dashehari. News. Indian Society for Nuclear Techniques in Agriculture and Biology. **2**: 21-23.

Parton, E., Vervaeke, I., Delen, R., Vandenbussche, B., Deroose, R. and De Proft, M. (2002). Viability and storage of bromeliad pollen. *Euphytica*, **125**: 155–161.

Pfahler, P. L. (1967). In vitro germination and pollen tube growth of maize (*Zea mays* L.) pollen: calcium and boron effects. *Canadian Journal of Botany*, **45**: 839-845.

Pfundt, M. (1910). Der Einfluss der Luftfeuchtigkeit auf die Lebensdauer des B1/itenstaubes. *Jahrb Wiss Bo*tany, **47**:1-40.

Pimienta, F., Polito, V. S. and Kester, D. E. (1983). Pollen tube growth in cross and self pollinated Nonpareil almonds. *Journal of American Society for Horticultural Science*, **108(4)**: 643-647.

Pinney, K**.** and Polito, V. S. (1990). Olive pollen storage and *in vitro* germination. *Acta Horticulturae*, **286**: 207-210.

Pinto, A. C. de Q. (1999). Hibridação em mangueira. 357-378p. In: A. Borém (ed.), Hibridação Artificial de Plantas, UFV, Viçosa.

Pinto, A. C. de Q. and Byrne, D. H. (1993). Mango hybridization studies in tropical savannah (Cerrados) of Brazil Central Region. *Acta Horticulturae*, **341**: 98-106.

Pirlak, L. (2002). The effects of temperature on pollen germination and pollen tube growth of apricot and sweet cherry. *Gartenbauwissenschaft*, **67(2)**: 61–64.

Polito, V. S. and Luza, G. (1989). Low temperature storage of pistachio pollen. *Euphytica*, **39**:265-269.

Popenoe, W. (1917). The pollination of the mango. Bur. P1. Ind., U.S. Department of Agriculture, 542p.

Popenoe, W. (1920). *Manual of Tropical and Subtropical Fruits*, 474p. The Macmillan Co., New York.

Purseglove, J. W. (1972). Mangoes of west India. *Acta Horticulturae*, **24**: 107–74.

Rajasekharan, P. E., Rao, T. M., Janakiram,T. and Ganeshan, S. (1994). Freeze preservation of *Gladiolus* pollen. *Euphytica*, **80**: 105–109.

Ram, S. and Rajan, S. (ed) (2003). Status report on genetic resource of mango in Asia-pacific region, International Plant Genetic Resource Institute, Office for South Asia, Pusa campus, New Delhi, India, 196 p.

Ram, S., Bist, L. D., Lakhanpal S. C. and Jamwal, I. S. (1976). Search of suitable Pollinizers for mango cultivars. *Acta Horticulturae,* **57**: 253– 263.

Randhawa, G. S. and Damodaran, V. K. (1961). Studies on floral biology and sex ratio in mango var. Chausa, Dashehari & Krishanbhog. *Indian Journal of Horticulture*, **18**: 51-64.

Reddy, K. S. and Ramayya, B. (1976). Himmayudin variety of mango in Rumani groves may help in getting better sized fruits. *Curremt Research*, **5**: 60-61.

Roizman, Y. (1984). Involvement of various factors in the process of pollination, fruit set and embryo development of mono and polyembryonic mango varieties. M.Sc. thesis, The Hebrew University of Jerusalem, Faculty of Agriculture, Rehovot, Israel, 84 p.

Rosell, P., Herrero, M. and Sauco, V.G. (1999). Pollen germination of cherimoya (*Annona cherimmola* Mill.). *In vivo* characterization and optimization of *in vitro* germination. *Scientia Horticulturae*, **81**: 251–265.

Rowlands, D. G. (1963). Sterility in broad bean (*Vicia faba* L.) In: *Genetics Today*. **1**: 215-216. New York. The Macmillan Company.

Roy, B. (1938). Studies on pollen tube growth in prunus. *Journal of Pomology and Horticultural Science*, **16**: 320-328.

Ruehle, G. D., Lynch, S. J. (1936). Mango yields increased by cross pollination. *Florida grower*, 57.

Saha, B. P. and Chhonkar, V. S. (1972). Studies on floral biology pollen viability, and intervarietal compatibility in mango (*Mangifera indica* L**.).** Third International Symposium Sub-tropical and Tropical Horticulture (Abstract of papers), 5p.

Sant Ram, Bist, L. D., Lakhanpal, S. C. and Jamwal, I. S. (1976). Search of suitable polliniser for mango cultivars. *Acta Horticulturae,* **57**: 253-263.

Savitsky, H. (1950). A method of determining self sterility and self fertility in sugar beets based upon the stage of ovule development shortly after flowering. *Proceeding of American Society of Suger Beet Technology*, 198-201.

Scholefield, P. B. (1982). A scanning electron microscope study of flowers of avocado, litchi, macadamia and mango. *Scientia Horticulturae,* **16**: 263-272.

Sears, E. R. (1937). Cytological phenomena connected with self-sterility in the flowering plants. *Genetics,* **22**: 130-181.

Sedgley, M. and Annels, C. M. (1981). Flowering and fruit set response to temperature in the avocado cultivar Hass. *Scientia Horticulturae,* **14**: 27-33.

Sedgley, M. and Griffin, A. R. (1989). 'Sexual Reproduction of Tree Crops.' Academic Press: London.

Sen, P. K., Mallik, P. C. and Ganguly, B. D. (1946). Hybridization of the mango. *Indian Journal of Horticulture*, **4**: 4-15.

Seth, J. N. (1962). Varietal cross incompatibility in guava. *Horticulture Advances,* **4**: 161-164.

Sharma, D. K. (1987). Mango Breeding. *Acta Horticulturae,* **196**: 61–67.

Sharma, D. K. and Singh, R. N. (1970). Self-incompatibility in mango *(Mangifera indica L.). Horticultural Research,* **10**: 108-118.

Sharma, D. K. and Singh, R. N. (1972). Investigation of self-incompatibility in *Mangifera indica L. Acta Horticulturae*, **24**: 126-130.

Shivanna, K. R. and Helpson-Harrison, J. (1981). *Annals of Botany*, **47**: 759-770

Shivanna, K. R. and Rangaswamy, N. S. (1992). *Pollen biology*. A laboratory manual. New Delhi. India.

Shivanna, K. R., Linskens, H. F. and Cresti, M. (1991). Response of tobacco pollen to high humidity and heat stress: viability and germinability *in vitro* and *in vivo*. *Sexual Plant Reproduction*, **4**:104–109.

Shu, Z. H. (2009). Sex distribution, sex ratio and natural pollination percentage of mango (*Mangifera indica* L.). Proc.VIIIth Int. Mango Symposium. *Acta Horticulturae*, 820.

Shuraki, Y. D. and Sedgley, M. (1996). Fruit development of *Pistacia vera* (Anacardiaceae) in relation to embryo abortion and abnormalities at maturity. *Australian Journal of Botamy,* **44**: 35-45.

Simao, S. and Maranhao, Z. C. (1959). Insects pollinating mangoes. Anais Esc. sup. *Agr. 'Luiz Queiroz',* **16**: 299-304.

Singh, J. P. and Rajput, B. S. (1964). Pollination and fruit set studies in loquat. *Indian Journal of Horticulture,* **21**: 143-147.

Singh, L. B. (1960). *The Mango-Botany, Cultivation and Utilization.* Leonard Hill, London

Singh, L. B. and Sturrock, D. (1969). Mango. *In:* Ferwerda, F. P. and F. Wit (Eds.), *Outlines Of Perennial Crop Breeding In The Tropics*,309–327p. H. Veenman and Zonen, N.V. Wageningen, The Netherlands.

Singh, R. N. (1954). Studies on floral biology and subsequent development of fruits in mango (*Mangifera indica* L.). *Indian Journal of Horticulture,* **31**: 16-22.

Singh, R. N., Majumdar, P. K. and Sharma, D. K. (1962). Self incompatibility in mango (*Mangifera indica* L.) Var. Dashehari. *Current Science*, **31**: 209

Singh, S. N. (1961). Studies on the morphology and viability of the pollen grains of mango. *Horticulture Advances, **5***: 121–144.

Snow, A. A. and Spira, T. (1991). Pollen vigor and the potential for sexual selection in plants. *Nature*, **352**: 796–797.

Socias i Company, R., (2002). Latest advances in almond self-compatibility. *Acta Horticulturae,* **591**: 205-211.

Soost, R. K. (1969). The incompatibility gene system in citrus. In H. D. Chapman (ed) Proc. 1st International Citrus Symposium Vol.1: riverside. University of California189-190p.

Soost, R. K. and Cameron, J. W. (1975). Citrus. In 'Advances in Fruit Breeding'. (Eds J. Janick and R. N. Moore.) 507-40p. (Purdue University Press: West Lafayette, IN.)

Sparrow, F. K. and Pearson, N. L. (1948). Pollen compatibility in Asclepias syriaca. *Journal of Agriculture Research,* **77**: 187-199.

Spencer, J. L. and Kennard, W. C. (1955). Studies on Mango fruit set in Puerto Rico. *Tropical Agriculture,* **32**: 323–330.

Stanley, R. G. and Linskens, H. F. (1974). Pollen: biology, biochemistry and management. Springer-Verlag, Berlin, Germany.

Stanwood, P. C. (1985). Cryopreservation of seed germplasm for genetic conservation. In: Kartha, K. K., ed. Cryopreservation of plant cells and tissues. Boca Raton, FL: CRC Press:199-226p.

Stephenson, A. G. and Bertin, K. E. (1983). Male competition, female choice, and sexual selection in plants. In REAL, L. (ed.) *Pollination Biology,* 109-149. New York.

Stephenson, A. G. and Winsor, J. A. (1986). *Lotus corniculatus* regulates offspring quality through selective abortion. *Evolution,* **40**: 453-458.

Stiehl-Alves, E. M. and Martins-Corder, M. P. (2007). *Acacia mearnsii* (Fabacea) reproductive biology: pollen tube viability and growth. *Crop Breeding and Applied Biotechnology*, **7**: 29-35.

Sturrock, T. T. (1944). *Notes On The Mango.* Sturart Daily News, Inc., Sturart, Fla., 122p.

Sukhvibul, N., Hetherington, S. E., Whilley, A. W., Smith, M. K., Vithanage, V., Subhadarabandhu, S. and Pichakum, A. (2000). Effect of temperature on pollen germination,pollen tube growth and seed development in mango (*Mangifera indica* L.). *Acta Horticulturae*, **509** : 609-616.

Sukhvibul, N., Whiley, A. W., Vithanage, V., Smith, M. K., Doogan, V. J. and Hetherington, S. E. (2000). Effect of temperature on pollen germination and pollen tube growth of four cultivars of mango (*Mangifera indica* L.). *Journal of Horticultural Science and Biotechnology*, **75 (2)**, 64–68.

Taroda, N. and Gibbs, P. E. (1982). Floral biology and breeding system of *Sterculia chicha* St. Hiz. (Sterculiaceae). *New Phytology,* **90**: 735-743.

Techio, V. H., Davide, L. C., Pedrozo, C. A. and Pereira, A.V. (2006). Viabilidade do grão de pólen de acessos de capim-elefante, milheto e híbridos interespecíficos (capim-elefante x milheto). *Acta Scientiarum*, **28**: 7-12.

Thompson, A. H. and Batjer, L. P. (1950). The effect of boron in the germination medium on pollen germination and pollen tube growth of several deciduous tree fruits. *Journal of the American Society for Horticultural Science*, **56**: 227- 230.

Torres, A. C. and Murashige, T. (1985). *In vitro* culture of ovularies of *Lycopersicon esculentum* Mill. and *L. peruvianum* (L.) Mill. *Horticultura Brasileira*, **3**: 95.

Towill, L. E. and Walters, C. (2000). Cryopreservation of pollen. In: *Cryopreservation of Tropical Plant Germplasm: Current Research Progress and Application* (Engelmann, F. and Tagaki, H., Eds.). International Plant Genetic Resource Institute, Tsukuba, Japan. 115–129p.

Trognitz, B. R. (1991). Comparison of different pollen viability assays to evaluate pollen fertility of potato dihaploids. *Euphytica*, ***56***: 143-148.

Truscott, M., Visser, G. J. and Anderson, S. H. (1994). Isozyme description of mango genotypes. *African Society Horticulture Sciences*, **4(2)** : 16-18.

Urata, U. (1954). Pollination requirements of macadamia. Hawaii *Agric. Exp. Stn. Tech. Bull*., **22**; 40.

Vasil, I. K. (1960). Effect of boron on pollen germination and pollen tube growth. In: *pollen physiology and fertilization*. (ED.): H. F. Linskens. 107-119p. North Holland Amsterdam.

Vasilakakis, M. and Porlingis, I. C. (1985). Effect of temperature on pollen germination, pollen tube growth, effective pollination period, and fruit set of pear. *HortScience*, **20 (4)**: 733–735.

Vivian-Smith, A., McConchie, C. A. and Batten, D. J. (1992). *In vitro* germination and growth of lychee (*Litchi chinensis* Sonn.). In: Control of fruit set and fruit retention in lychee. Rural Industries Research and Development Corporation Report, No. CS18A, CSIRO, Brisbane, Australia.

Wagle, P. V. (1929). A preliminary study of the pollination of the Alphonso mango. *Agriculture Journal of India*, **24**: 259–263.

Whiley, A. W., Saranah, J. B., Rasmussen, T. S., Winston, E. C. and Wolstenholme, B. N. (1988). Effect of temperature on growth of 10 mango cultivars with relevance to production in Australia. Proceedings of the 4th Australasian Conference on Tree and Nut Crops (Batten, D., Ed.). Acotanc, Lismore, Australia, 176-185p.

Wiens, D., Calvin, C. L., Wilson, C. A., Davern, C. 1., Frank, D. R. and Seavey, S. R. (1987). Reproductive success, spontaneous embryo abortion and genetic load in flowering plants. *Oecologia*, **71**: 501-509.

Williams. D., Ganeshan, S., Rajasekharan, P. E. and Shashikumar, S. (1982). Cryopreservation of pollen. *A Practical Guide*, 443-464.

Wolfe, H. S. (1962). The mango in Florida 1887-1962. *Foridla State Horticulture Society Proceeding*, ***75***: 387–91.

Wolukau, J. N., Zhang, S. L., Xu, G. H. and Chen, D. (2004). The effect of temperature, polyamines and polyamine synthesis inhibitor on *in vitro* pollen germination and pollen tube growth of *Prunus mume*. *Scientia Horticulturae*, **99**: 289–299.

Yadav, I. S. and Rajan, S. (1993). Genetic resources of Mangifera in *Advances in Horticulture*. (Chadha, K. L. and Pareek, O.P. eds) Malhotra publishing House, New Delhi, **1**: 77-82.

Young, T. W. (1942). Investigations of the unfruitfulness of the Haden mango in Florida. *Florida State Horticulture Society Proceeding*, ***55***: 106–110.

Young, T. W. (1955). Influence of temperature on growth of mango pollen. *Florida State Horticulture Society Proceeding*, **68**: 308–13.

Yutko, O. (1995). Self and cross pollination in open and caged mango trees and its effect on fruit set and yield. M.Sc. thesis, Faculty of Agriculture, The Hebrew university of Jerusalam, Israel.

ANNEXURES

*, ** Significant at 5% and 1% probability level and NS, non significant.

Annexure 4.1.1 Mean weekly weather data during 2010

Week nos	Max. T. (°C)	Min. T. (°C)	Rain fall (mm)	RH %
1	19.3	4.2	0	92
2	15.2	6.9	0	92
3	15.0	6.0	0	93
4	17.6	5.8	0	93
5	23.8	8.4	0	94
6	23.4	7.0	0	91
7	23.6	11.1	11.8	91
8	22.7	7.4	0	90
9	27.7	12.8	1.2	89
10	31.2	15.4	0	82
11	28.9	12.5	0	85
12	33.4	14.7	0	88
13	37.9	17.6	0	78
14	37.7	18.1	0	71
15	39.2	19.6	0	44
16	42.2	22.3	0	49
17	40.9	24.4	0	57

Annexure 4.1.2(Table 4.1.4) ANOVA for viability of fresh pollen of mango varieties.

Source of variation	df	SS	MS	F
Method of viability test	2	8933.23	4466.61	281.55**
Variety	2	15.91	7.96	0.50 NS
Method of viability test x variety	4	42.42	15.86	0.67 NS
Error	18	285.56		

Annexure 4.2.1 (Table 4.2.1) ANOVA of variance for variable male flower.

Source of variation	df	SS	MS	F
Variety	3	3042.44	1014.14	9.65*
Replication	2	141.28	70.64	0 .67 NS
Error	6	629.97	104.99	

Annexure 4.2.2 (Table 4.2.1) ANOVA for variable hermaphrodite flower.

Source of variation	df	SS	MS	F
Variety	3	3042.23	1014.07	9.65*
Replication	2	141.36	70.68	0.67 NS
Error	6	630.04	105.00	

Annexure 4.2.3 (Table 4.2.1) ANOVA for variable unusual stamen flower.

Source of variation	df	SS	MS	F
Variety	3	46.41	15.47	12.36*
Replication	2	2.05	1.02	.82 NS
Error	6	7.50	1.25	

Annexure 4.2.4 (Table 4.2.1) ANOVA for variable deformed ovary flower.

Source of variation	df	SS	MS	F
Variety	3	43.77	14.59	13.74*
Replication	2	1.42	.71	0.67NS
Error	6	6.37	1.06	

Annexure 4.2.5 (Table 4.2.2) ANOVA for variable fruit set in Amrapali.

Source of variation	df	SS	MS	F
Replication	2	52.73	26.37	
Pol.Met	1	6566.94	6566.94	725.49*
Day	5	8147.06	1629.41	725.49*
Pol. Met X Day	5	372.36	74.47	8.23*
Error	22	199.14	9.05	

Annexure 4.2.6 (Table 4.2.3) ANOVA for variable fruit set in Mallika.

Source of variation	df	SS	MS	F
Replication	2	27.56	13.78	
Pol.Met	1	3417.58	3417.58	304.73*
Day	5	12606.99	2521.40	224.82*
Pol. Met X Day	5	173.82	34.76	3.10**
Error	22	246.73	11.22	

Annexure 4.2.7 (Table 4.2.4) ANOVA for variable fruit set in Pusa Arunima.

Source of variation	df	SS	MS	F
Replication	2	118.23	59.12	
Pol.Met	1	3596.97	3596.97	178.64*
Day	5	7289.22	1457.84	72.40*
Pol. Met X Day	5	274.08	54.82	2.72**
Error	22	442.98	20.14	

Annexure 4.2.8 (Table 4.2.5) ANOVA for variable fruit set in Pusa Surya.

Source of variation	df	SS	MS	F
Replication	2	190.90	95.45	
Pol.Met	1	298.10	298.10	11.93*
Day	5	11210.47	2242.09	89.72*
Pol. Met X Day	5	241.89	48.38	1.94 NS
Error	22	549.81	24.99	

Annexure 4.2.9 (Table 4.2.7) ANOVA for pollen retention after self pollination at 6 hours of mango varieties.

Source of variation	df	SS	MS	F
Variety	3	88.07	29.35	8.87*
Replication	2	3.31	1.65	0.50 NS
Error	6	19.85		

Annexure 4.2.10 (Table 4.2.7) ANOVA for pollen retention after self pollination at 24 hours of mango varieties.

Source of variation	df	SS	MS	F
Variety	3	55.03	18.35	7.18*
Replication	2	0.34	0.17	0.06 NS
Error	6	15.32	2.55	

Annexure 4.2.11 (Table 4.2.9) ANOVA for variable fruit weight 7 days.

Source of variation	df	SS	MS	F
Replication	2	0.00	0.00	
Pol.Met	1	0.01	0.01	59.14**
Var.	3	0.03	0.01	52.11**
Pol. Met X Var.	3	0.00	0.00	7.37*
Error	14	0.00	0.00	

Annexure 4.2.12 (Table 4.2.9) ANOVA for variable fruit weight 14 days.

Source of variation	df	SS	MS	F
Replication	2	0.00	0.00	
Pol.Met	1	0.01	0.01	6.06*
Var.	3	0.03	0.01	4.35*
Pol. Met X Var.	3	0.01	0.00	1.37 NS
Error	14	0.03	0.00	

Annexure 4.2.13 (Table 4.2.9) ANOVA for variable fruit weight 21 days.

Source of variation	df	SS	MS	F
Replication	2	0.02	0.01	
Pol.Met	1	0.02	0.02	5.36 NS
Var.	1	0.02	0.02	4.07 NS
Pol. Met X Var.	1	0.00	0.00	1.16 NS
Error	6	0.02	0.00	

Annexure 4.2.14 (Table 4.2.9) ANOVA for variable fruit weight 28 days.

Source of variation	df	SS	MS	F
Replication	2	0.09	0.05	
Pol.Met	1	1.82	1.82	43.73**
Var.	1	0.55	0.55	13.27*
Pol. Met X Var.	1	0.24	0.24	5.87 NS
Error	6	0.25	0.04	

Annexure 4.2.15 (Table 4.2.10) ANOVA for variable fruit length 7 days.

Source of variation	df	SS	MS	F
Replication	2	0.08	0.04	
Pol.Met	1	3.92	3.92	38.99**
Var.	3	0.43	0.14	1.43 NS
Pol. Met X Var.	3	0.64	0.21	2.13 NS
Error	14	1.41	0.10	

Annexure 4.2.16 (Table 4.2.10) ANOVA for variable fruit length 14 days.

Source of variation	df	SS	MS	F
Replication	2	1.97	0.99	
Pol.Met	1	10.68	10.68	37.42**
Var.	3	30.63	10.21	35.77**
Pol. Met X Var.	3	5.26	1.75	6.15**
Error	14	4.00	0.29	

Annexure 4.2.17 (Table 4.2.10) ANOVA for variable fruit length 21 days.

Source of variation	df	SS	MS	F
Replication	2	0.24	0.12	
Pol.Met	1	4.28	4.28	16.31**
Var.	1	5.32	5.32	20.26**
Pol. Met X Var.	1	3.07	3.07	11.69*
Error	6	1.58	0.26	

Annexure 4.2.18 (Table 4.2.10) ANOVA for variable fruit length 28 days.

Source of variation	df	SS	MS	F
Replication	2	0.62	0.31	
Pol.Met	1	7.92	7.92	24.45**
Var.	1	38.70	38.70	119.43**
Pol. Met X Var.	1	6.65	6.65	20.51**
Error	6	1.94	0.32	

Annexure 4.2.19 (Table 4.2.11) ANOVA for variable fruit breadth 7 days.

Source of variation	df	SS	MS	F
Replication	2	0.32	0.16	
Pol.Met	1	2.02	2.02	60.21**
Var.	3	0.22	0.07	2.14 NS
Pol. Met X Var.	3	0.98	0.33	9.76**
Error	14	0.47	0.03	

Annexure 4.2.20 (Table 4.2.11) ANOVA for variable fruit breadth 14 days.

Source of variation	df	SS	MS	F
Replication	2	0.17	0.09	
Pol.Met	1	3.59	3.59	8.59*
Var.	3	8.84	2.95	7.05**
Pol. Met X Var.	3	0.85	0.28	0.68 NS
Error	14	5.85	0.42	

Annexure 4.2.21 (Table 4.2.11) ANOVA for variable fruit breadth 21 days.

Source of variation	df	SS	MS	F
Replication	2	1.22	0.61	
Pol.Met	1	12.42	12.42	62.84**
Var.	1	14.02	14.02	70.91**
Pol. Met X Var.	1	7.99	7.99	40.40**
Error	6	1.19	0.20	

Annexure 4.2.22 (Table 4.2.11) ANOVA for variable fruit breadth 28 days.

Source of variation	df	SS	MS	F
Replication	2	1.37	0.68	
Pol.Met	1	26.23	26.23	97.37**
Var.	1	29.52	29.52	109.59**
Pol. Met X Var.	1	15.82	15.82	58.75**
Error	6	1.62	0.27	

Annexure 4.2.23 (Table 4.2.12) ANOVA for variable ovule length 7 days.

Source of variation	df	SS	MS	F
Replication	2	0.03	0.02	
Pol.Met	1	0.72	0.72	22.08**
Var.	3	0.22	0.07	2.22 NS
Pol. Met X Var.	3	0.15	0.05	1.56 NS
Error	14	0.46	0.03	

Annexure 4.2.24 (Table 4.2.12) ANOVA for variable ovule length 14 days.

Source of variation	df	SS	MS	F
Replication	2	0.17	0.08	
Pol.Met	1	7.27	7.27	37.94**
Var.	3	7.50	2.50	13.04**
Pol. Met X Var.	3	8.66	2.89	15.06**
Error	14	2.68	0.19	

Annexure 4.2.25 (Table 4.2.12) ANOVA for variable ovule length 21 days.

Source of variation	df	SS	MS	F
Replication	2	1.41	0.70	
Pol.Met	1	0.01	0.01	0.02 NS
Var.	1	0.98	0.98	2.67 NS
Pol. Met X Var.	1	0.00	0.00	0.01 NS
Error	6	2.20	0.37	

Annexure 4.2.26 (Table 4.2.12) ANOVA for variable ovule length 28 days.

Source of variation	df	SS	MS	F
Replication	2	0.10	0.05	
Pol.Met	1	2.15	2.15	5.11 NS
Var.	1	2.24	2.24	5.32 NS
Pol. Met X Var.	1	0.98	0.98	2.13 NS
Error	6	2.52	0.42	

Annexure 4.2.27 (Table 4.2.13) ANOVA for variable ovule breadth 7 days.

Source of variation	df	SS	MS	F
Replication	2	0.02	0.01	
Pol.Met	1	0.21	0.21	21.05**
Var.	3	0.29	0.19	9.40**
Pol. Met X Var.	3	0.02	0.01	0.59 NS
Error	14	0.14	0.01	

Annexure 4.2.28 (Table 4.2.13) ANOVA for variable ovule breadth 14 days.

Source of variation	df	SS	MS	F
Replication	2	0.35	0.18	
Pol.Met	1	2.39	2.39	18.26**
Var.	3	12.52	4.17	31.93**
Pol. Met X Var.	3	1.36	0.45	3.46*
Error	14	1.83	0.13	

Annexure 4.2.29 (Table 4.2.13) ANOVA for variable ovule breadth 21 days.

Source of variation	df	SS	MS	F
Replication	2	0.03	0.02	
Pol.Met	1	0.33	0.33	2.50 NS
Var.	1	1.58	1.58	12.14*
Pol. Met X Var.	1	0.18	0.18	1.36 NS
Error	6	0.78	0.13	

Annexure 4.2.30 (Table 4.2.13) ANOVA for variable ovule breadth 28 days.

Source of variation	df	SS	MS	F
Replication	2	0.35	0.17	
Pol.Met	1	0.29	0.29	2.08 NS
Var.	1	0.08	0.08	0.60 NS
Pol. Met X Var.	1	0.16	0.16	1.14 NS
Error	6	0.82	0.14	

Annexure 4.3.1 (Table 4.3.1) ANOVA of fruit set (%) using Amrapali as female parent

Source of variation	df	SS	MS	F
Replication	2	59.06	29.53	
Cross	2	15.66	7.83	0.22 NS
Day	5	12138.90	2427.78	69.34**
Cross X Day	10	377.24	37.72	1.08 NS
Error	34	1190.51	35.02	

Annexure 4.3.2 (Table 4.3.2) ANOVA of fruit set (%) using Pusa Arunima as female parent

Source of variation	df	SS	MS	F
Replication	2	1417.66	708.83	
Cross	2	1640.85	820.42	20.30**
Day	5	7064.03	1412.81	34.96**
Cross X Day	10	494.14	49.41	1.22 NS
Error	34	1374.10	40.41	

Annexure 4.3.3 (Table 4.3.3) ANOVA of fruit set (%) using Dashehari as female parent

Source of variation	df	SS	MS	F
Replication	2	178.00	89.00	
Cross	2	314.35	157.18	3.01 NS
Day	5	4056.57	811.31	15.56**
Cross X Day	10	349.23	34.92	0.67 NS
Error	34	1772.67	52.14	

Annexure 4.3.4 (Table 4.3.4) ANOVA of fruit set (%) using Langra as female parent

Source of variation	df	SS	MS	F
Replication	2	1438.27	719.13	
Cross	2	70.00	35.00	2.64 NS
Day	5	2479.44	495.89	37.38**
Cross X Day	10	347.97	34.80	2.62*
Error	34	451.10	13.27	

Annexure 4.3.5 (Table 4.3.6) ANOVA of fruit weight using Amrapali as female parent

Source of variation	df	SS	MS	F
Replication	2	0.16		
Cross	2	0.56	6.24	6.24**
Day	3	11.53	85.32	85.32**
Cross X Day	6	1.30	4.80	4.80**
Error	22	0.99		

Annexure 4.3.6 (Table 4.3.6) ANOVA of fruit weight using Pusa Arunima as female parent

Source of variation	df	SS	MS	F
Replication	2	0.00	0.00	
Cross	2	1.87	0.93	248.23**
Day	3	0.94	0.31	83.51**
Cross X Day	6	2.78	0.46	123.01**
Error	22	0.08	0.00	

Annexure 4.3.7 (Table 4.3.6) ANOVA of fruit weight using Langra as female parent

Source of variation	df	SS	MS	F
Replication	2	0.01	0.00	
Cross	2	0.23	0.11	18.86**
Day	3	0.66	2.22	368.30**
Cross X Day	6	0.56	0.09	15.54**
Error	22	0.13	0.01	

Annexure 4.3.8 (Table 4.3.6) ANOVA of fruit weight using Dashehari as female parent

Source of variation	df	SS	MS	F
Replication	2	0.01	0.00	
Cross	2	0.20	0.10	37.67**
Day	3	5.88	1.96	741.56**
Cross X Day	6	0.51	0.09	32.44**
Error	22	0.06	0.00	

Annexure 4.3.9 (Table 4.3.7) ANOVA of fruit length using Amrapali as female parent

Source of variation	df	SS	MS	F
Replication	2	0.86	0.43	
Cross	2	35.44	17.72	54.64**
Day	3	612.91	204.30	639.94**
Cross X Day	6	34.14	5.69	17.54**
Error	22	7.13	0.32	

Annexure 4.3.10 (Table 4.3.7) ANOVA of fruit breadth using Amrapali as female parent

Source of variation	df	SS	MS	F
Replication	2	0.27	0.14	
Cross	2	9.23	4.62	12.87**
Day	3	298.55	99.52	277.45**
Cross X Day	6	9.94	1.66	4.62**
Error	22	7.89	0.36	

Annexure 4.3.11 (Table 4.3.7) ANOVA of ovule length using Amrapali as female parent

Source of variation	df	SS	MS	F
Replication	2	0.09	0.04	
Cross	2	1.65	0.83	6.23**
Day	3	168.88	56.29	424.77**
Cross X Day	6	6.14	1.02	7.72**
Error	22	2.92	0.13	

Annexure 4.3.12 (Table 4.3.7) ANOVA of ovule breadth using Amrapali as female parent

Source of variation	df	SS	MS	F
Replication	2	1.12	0.56	
Cross	2	2.42	1.21	2.68 NS
Day	3	95.72	31.91	70.52**
Cross X Day	6	1.34	0.22	0.49 NS
Error	22	9.95	0.45	

Annexure 4.3.13 (Table 4.3.8) ANOVA of fruit length using Pusa Arunima as female parent

Source of variation	df	SS	MS	F
Replication	2	0.04	0.02	
Cross	2	282.92	141.46	2644.56**
Day	3	16.71	5.57	104.11**
Cross X Day	6	248.89	41.48	775.51**
Error	22	1.18	0.05	

Annexure 4.3.14 (Table 4.3.8) ANOVA of fruit breadth using Pusa Arunima as female parent

Source of variation	df	SS	MS	F
Replication	2	1.42	0.71	
Cross	2	202.80	101.40	578.57**
Day	3	15.50	5.17	29.48**
Cross X Day	6	186.36	31.06	177.22**
Error	22	3.86	0.18	

Annexure 4.3.15 (Table 4.3.8) ANOVA of ovule length using Pusa Arunima as female parent

Source of variation	df	SS	MS	F
Replication	2	0.30	0.15	
Cross	2	107.61	53.80	432.58**
Day	3	25.91	8.64	69.43**
Cross X Day	6	89.74	14.96	120.25**
Error	22	2.74	0.12	

Annexure 4.3.16 (Table 4.3.8) ANOVA of ovule breadth using Pusa Arunima as female parent

Source of variation	df	SS	MS	F
Replication	2	0.11	0.05	
Cross	2	42.05	21.03	734.00**
Day	3	3.51	1.17	40.79**
Cross X Day	6	49.95	8.32	290.60**
Error	22	0.63	0.03	

Annexure 4.3.17 (Table 4.3.9) ANOVA of fruit length using Langra as female parent

Source of variation	df	SS	MS	F
Replication	2	1.67	0.84	
Cross	2	34.49	17.24	30.77**
Day	3	703.01	234.34	418.16**
Cross X Day	6	26.82	4.47	7.98**
Error	22	12.33	0.56	

Annexure 4.3.18 (Table 4.3.9) ANOVA of fruit breadth using Langra as female parent

Source of variation	df	SS	MS	F
Replication	2	1.06	0.53	
Cross	2	9.98	4.99	27.01**
Day	3	389.43	129.81	702.51**
Cross X Day	6	11.66	1.94	10.52**
Error	22	4.07	0.18	

Annexure 4.3.19 (Table 4.3.9) ANOVA of ovule length using Langra as female parent

Source of variation	df	SS	MS	F
Replication	2	0.21	0.10	
Cross	2	5.18	2.59	9.56**
Day	3	302.43	100.81	372.08**
Cross X Day	6	2.92	0.49	1.80 NS
Error	22	5.96	0.27	

Annexure 4.3.20 (Table 4.3.9) ANOVA of ovule breadth using Langra as female parent

Source of variation	df	SS	MS	F
Replication	2	0.34	0.17	
Cross	2	1.36	0.68	6.40**
Day	3	84.56	28.19	265.05**
Cross X Day	6	3.06	0.51	4.79**
Error	22	2.34	0.11	

Annexure 4.3.21 (Table 4.3.10) ANOVA of fruit length using Dashehari as female parent

Source of variation	df	SS	MS	F
Replication	2	3.53	1.76	
Cross	2	82.02	41.01	60.15**
Day	3	642.62	214.21	314.20**
Cross X Day	6	48.89	8.15	11.95**
Error	22	15.00	0.68	

Annexure 4.3.22 (Table 4.3.10) ANOVA of fruit breadth using Dashehari as female parent

Source of variation	df	SS	MS	F
Replication	2	1.71	0.86	
Cross	2	15.21	7.60	28.99**
Day	3	260.24	86.75	330.65**
Cross X Day	6	4.95	0.83	3.15*
Error	22	5.77	0.26	

Annexure 4.3.23 (Table 4.3.10) ANOVA of ovule length using Dashehari as female parent

Source of variation	df	SS	MS	F
Replication	2	0.04	0.02	
Cross	2	6.37	3.18	30.27**
Day	3	215.69	71.90	683.57**
Cross X Day	6	11.88	1.98	18.83**
Error	22	2.31	0.11	

Annexure 4.3.24 (Table 4.3.10) ANOVA of ovule breadth using Dashehari as female parent

Source of variation	df	SS	MS	F
Replication	2	0.19	0.10	
Cross	2	0.42	0.21	2.31 NS
Day	3	109.01	36.34	402.94**
Cross X Day	6	0.96	0.16	1.77 NS
Error	22	1.98	0.09	