HETEROMORPHY AND INCOMPATIBILITY IN SOME COLOUR VARIANTS OF *PENTAS LANCEOLATA* (Forssk.) Deflers

THESIS SUBMITTED
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IN BOTANY

By

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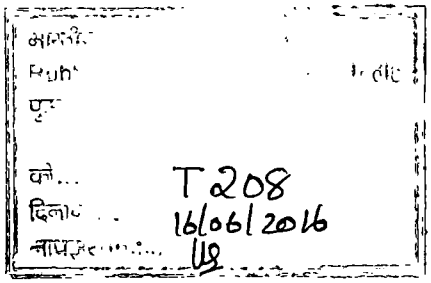
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Dedicated to the cherished memory of my beloved husband, the late Dr. K. K. Mohandas....

A sweet companion.....

" From giant Oaks that

Wave their branches dark

To the dark Moss that clings upon their bark

What Beaux and Beauties

Crowd the gaudy groves

And woo and win their vegetable loves"

Erasmus Darwin, 1789

DECLARATION

I do hereby declare that the thesis entitled ***"Heterostyly and incompatibility in some colour variants of Pentas lanceolata (Forssk.) Deflers"*** *is a record of independent research work carried out by me in the Department of Botany, Mahatma Gandhi College, Thiruvananthapuram and no part of this thesis has previously formed the basis for the award to me, of any degree, diploma, associateship, fellowship or other similar titles of this or any other university or society.*

Thiruvananthapuram,
May 2002.

Jayasree 22-05-02
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Reader and Head

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CERTIFICATE

This is to certify that the thesis entitled ***"Heterostyly and incompatibility in some colour variants of Pentas lanceolata (Forssk.) Deflers"****, submitted to the University of Kerala, by* ***Smt. Jayasree M.*** *for the award of the degree of Doctor of Philosophy, is a record of bonafide research carried out by her under my supervision in the Department of Botany, Mahatma Gandhi College, Thiruvananthapuram. No part of this work has been submitted by the candidate for the award of any degree, diploma or other similar titles from this or any other university or society.*

Thiruvananthapuram,
May 2002.

22/5/2002

Dr. P. Sreedevi

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Jayasree, M.

CONTENTS

ABBREVIATIONS

CD	-	Critical difference
f. wt.	-	Fresh weight
g	-	gram
h	-	hour
ISI	-	Index of self incompatibility
L	-	Litre
L_P	-	Lilac pin
L_T	-	Lilac thrum
µl	-	Micro litre
mg	-	milli gram
min	-	minute
ml	-	milli litre
M_P	-	Magenta pin
M_T	-	Magenta thrum
N	-	Normality
OD	-	Optical density
PAGE	-	Polyacrylamide gel electrophoresis
SDS	-	Sodium dodecyl sulphate
SEM	-	Scanning electron microscopy
UV	-	Ultra violet
v/v	-	Volume by volume
var.	-	variant
w/v	-	Weight by volume
W_P	-	White pin
W_T	-	White thrum

Introduction

Heterostyly was recognized as a morphological feature of certain groups of flowering plants as early as 16th century when it was noted in *Primula* by Clusius (vanDijk, 1943). Not many attempts were made to interpret the adaptive significance of this floral heteromorphism until Charles Darwin and Hildebrand studied the phenomenon just after the middle of the 19th century (Darwin, 1862; Hildebrand, 1863). After discussing the floral morphology of several species of *Primula*, Darwin concluded that the existence of two forms is very general in the genus, and these represent a physiological and morphological adaptation to promote outcrossing between the two morphs. An American botanist, Asa Gray, a contemporary of Darwin noted distyly in the eastern American *Hedyotis purpurea* as early as 1842. Another contemporary of Darwin who played a key role in the early development of the knowledge of the significance and functioning of heterostyly was Hildebrand. Hildebrand published the results of his studies with *Primula sinensis*, *Linum perennale*, *Pulmonaria officinalis*, *Lythrum salicaria*, Fsyth*ia* and several species of *Oxalis*. Burck was an important 19th century contributor to our

knowledge on heterostyly in woody plants of Rubiaceae, Connaraceae and Oxalidaceae (Burck, 1883, 1884). An important study on heterostyly in Pontederiaceae was made by Fritz Muller (1871).

After his first paper on heterostyly in 1862, Darwin published papers on heterostyly in 1864, 1865 and 1869. In 1877, he published a general review of the subject of heterostyly in the book 'The Different Forms of Flowers on Plants of the Same Species'. A version with the same text but corrections of errors in the first version, was published in 1880 as 'Forms of Flowers'. Edward Bell, an Englishman published a lengthy remodel to Darwin's primrose work, which was published (anonymously). Despite Darwin's observations on *Primula* as well as those of other 19th century naturalists, Bell maintained that self fertilization of heterostylous plants "is the natural fertilization", which seems quaint in the modern context (Ornduff, 1992). Darwin's work inspired others to conduct a number of field studies of heterostylous plants. A particularly interesting and generally overlooked example of such a study was published in 1884 by Christy, 'On the species of the genus *Primula* in Essex'. Unlike Bell, Christy accepted the notion that the floral structure of the distylous *Primula* is a mechanism designed to promote inter-morph pollinations. The latter portion of the 19th century

saw a few dozen papers published on heterostyly, in groups in which it was previously unrecorded. Thus the 19th century can be viewed largely as a time when the morphological nature of heterostyly was described, its functional significance suggested, its occurrence documented, and some field studies conducted, but no progress made in understanding inheritance patterns until the advent of Mendelian genetics in the first year of the 20th century.

The inheritance of distyly in *Primula sinensis* was studied by Bateson and Gregory in 1905. Short styled *Primula sinensis* was shown to be dominant and long styled, recessive. Since *Primula sinensis* has a high degree of intramorph compatibility, Bateson and Gregory were able to obtain 'Ss' shorts via illegitimate pollinations. Longs were found to be 'ss'. Important contributions to an understanding of the genetics of heterostyly include those of Gregory (1911), Ubisch (1926), Bodmer (1927), Mather and De Winton (1941), Ernst (1943), Mather (1950) and Dowrick (1956).

According to Darwin's interpretation, the morphological features of heterostyly and sterility in self and intra-morph cross pollinations are two distinct outbreeding mechanisms. This interpretation has received wide acceptance among students of heterostyly (Baker, 1966; Vuilleumier,

1967 ; Ganders, 1979a,b ; Charlesworth and Charlesworth, 1979 ; Lewis, 1982 ; Barrett, 1988a).

It has been shown that heterostyly may also be accompanied by multiallelic incompatibility, as appears to be the case in *Narcissus tazetta* (Dulburger, 1964), *Anchusa hybrida* (Dulberger, 1970a) and *A. officinalis* (Philipp and Schou, 1981; Schou and Philipp, 1984), or it may be accompanied by self compatability, as in *Amsinckia species* (Ray and Chisaki, 1957; Ganders, 1975), *Eichhornia crassipes* (Barrett, 1977a, 1979), *E. paniculata* (Barrett, 1985a) and in *Cryptantha flava* (Casper, 1985).

In the course of the last two decades, there has been resurgence of interest in heterostyly and studies of this phenomenon have been extended to many taxa. The pertinent literature of the 1980s, includes articles on members of Boraginaceae (Philipp and Schou, 1981, Schou and Philipp, 1983, 1984 ; Casper, 1983, 1985 ; Casper *et al.*, 1988; Olesen, 1979; Weller, 1980 ; Weller and Ornduff, 1989), Menyanthaceae (Barrett, 1980; Ornduff, 1982, 1986, 1987a, 1988a,b; Olesen, 1986), Oxalidaceae (Weller, 1979, 1981a,b ; Ornduff, 1987b), Pontederiaceae (Barrett, 1985a, 1988b; Anderson and Barrett, 1986) and Turneraceae (Shore and Barrett, 1984, 1985a,b ; Barrett and Shore, 1987). For same taxa, new observations related to heterostyly have been reported (Lewis, 1982;

Riveros *et al.*, 1987; Ornduff, 1987a, 1988b). The efficiency of pollen transfer has been examined in natural populations of numerous species (Barrett and Glover, 1985 ; Piper and Charlesworth, 1986 ; Piper *et al.*, 1986 ; Barrett, 1990). The role of heterostyly in enhancing pollen carryover has been explored (Nicholls, 1985a; Feinsinger and Busby, 1987; Wolfe and Barrett, 1989). Development of heterostyly was traced in *Eichhornia* and *Pontederia* species (Richards and Barrett, 1984, 1987) and in *Linum* and *Fagopyrum* (Dulburger, 1984). Theoretical models have been proposed for the evolution and breakdown of distyly and tristyly (Charlesworth and Charlesworth, 1979). Ecological genetics and evolutionary modification of the heteromorphic mating system have been studied (Barrett, 1985b ; Shore and Barrett, 1986).

Since the pioneering work of Darwin and Hildebrand in the last century, evolutionary biologists have been intrigued by the complex sexual arrangements of reproductive organs in heterostylous plants. "In their manner of fertilization, these plants offer a more remarkable case, than can be found in any other plant or animal" is what Darwin wrote about *Lythrum species* (Darwin, 1865). How heterostyly originated, what selective forces maintain the polymorphism, and why it often becomes

evolutionarily modified into other breeding systems are questions often posed by workers investigating heterostylous groups.

Heterostyly is a simply inherited polymorphism, in which floral morphs are easily identified under field conditions. Population studies using ecological genetic approaches (Ford, 1964) offer attractive opportunities for investigations of natural selection, maintenance and breakdown of heterostyly (Bodmer, 1960 ; Weller, 1976a ; Barrett, 1985a). Experimental field studies of the pollination biology of heterostylous plants have enabled analysis of the function and adaptive significance of the polymorphism (Ganders, 1979a ; Barrett, 1990).

Although majority of the plants possess reciprocal herkogamy, diallelic incompatibility and various ancillary floral polymorphisms, research over the last few decades has revealed a significant number of cases, where plants with style length polymorphisms exhibit various combinations of heterostylous and non-heterostylous characters (Dulberger, 1964 ; Arroyo and Dafni, 1995 ; Barrett *et al.*, 1996; Sage, 1999 ; Baker *et al.*, 2000a,b). The latter include strong self compatibility, multiallellic incompatibility, monomorphic stamen heights and an absence of ancillary polymorphism.

The majority of the studies have focussed on the functioning of heterostyly, strictly on the basis of reciprocal herkogamy (Webb and Lloyd, 1986) as a floral mechanism for promoting pollinations between morphs and avoiding those within each morph. This approach has continued to eclipse an elucidation of the possibility that heterostyly functions to prevent inbreeding through incompatibility.

Pollen and stigma polymorphisms have been the least investigated component of heterostyly and their functional significance and evolution have remained obscure. Apart from the style and stamen lengths, the only polymorphism to which adaptive significance has been ascribed is the disparate pollen production by the morphs. The detection of structural and cytochemical dimorphism of the stigma in species of *Linum* and various Plumbaginaceae, coupled with dissimilar behavior of the male gametophyte in the two types of incompatible pollinations have led to the suggestion that dimorphism of stigmas and of the pollen exine function in the physiology of incompatibility mechanism (Dulberger, 1987a,b,c, 1991). This conclusion has also been extended to polymorphism of pollen size and of style and stamen lengths, thus leading to a hypothesis on the developmental relationships between

incompatibility and morphological and structural traits of heterostyly (Dulberger, 1975b).

Pollen–pistil relationships have been investigated in many heterostylous plants like species of *Linum* (Dulberger, 1989, 1990, 1991 ; Ghosh and Shivanna, 1980a,b, 1983 ; Murray, 1986) *Primula species* (Heslop-Harrison *et al.*, 1981 ; Shivanna *et al.*, 1981, 1983 ; Richards and Ibrahim, 1982 ; Stevens and Murray, 1982 ; Schou, 1984 ; Richards, 1986), Rubiaceae (Bawa and Beach, 1983), *Pontederia species* (Glover and Barrett, 1983 ; Barrett and Anderson, 1985 ; Scribailo and Barrett, 1986, 1989 ; Scribailo, 1989) and in *Anchusa officinalis* (Schou and Philipp, 1983). These investigations have yielded much information on stigmas and pollen grains and on the behaviour of the male gametophytes, thereby considerably enhancing our understanding of the relationships between the male and female partners after compatible and incompatible pollinations. Most of the above studies are based on the idea that the morphological characters of heterostyly and the physiology of incompatibility are independent phenomena.

The number of families containing heterostylous species have grown with increased botanical exploration, particularly of tropical regions. On the other hand, many species originally separated as

heterostylous have on closer examination proven to be otherwise (Barrett and Richards, 1990).

Distyly occurs in more than 28 flowering plant families and it is clear from its taxonomic distribution that it has arisen independently, a number of times (Ganders, 1979a; Charlesworth, 1982; Barrett, 1992; Lloyd and Webb, 1992b). The short-styled morph is commonly determined by the dominant 'S' allele and long-styled morph is homozygous recessive 'ss' (Ornduff, 1979; Lewis and Jones, 1992), but the dominance relationships are reversed in *Hypericum aegypticum* (Ornduff, 1979) and possibly in the genus *Limonium* (Baker, 1966). All characters distinguishing the morphs, including style length and its incompatibility response, stamen length, pollen size, production and pollen incompatibility (Ganders, 1979b; Dulberger, 1992; Richards and Barrett, 1992) appear to be determined by two alternative alleles at a single locus (Ganders, 1979b; Ornduff, 1979; Lewis and Jones, 1992).

Studies of *Primula* (Mather and De Winton, 1941; Mather, 1950; Lewis, 1954; Ernst, 1955; Dowrick, 1956; Lewis and Jones, 1992) and to a lesser extent, *Armeria* (Baker, 1966) and *Turnera* (Shore and Barrett, 1985a; Barrett and Shore, 1987), however indicate that distyly may in fact be determined by a supergene, a number of tightly linked genes held in

complete disequilibrium with the dominant alleles of at least three loci linked in coupling and inherited *en masse* (Althanasiou and Shore, 1997). Rare recombination within the supergene (Lewis and Jones, 1992) can lead to the formation and spread of unusual and potentially high self fertilizing genotypes (Crosby, 1949; Dowrick, 1956; Bodmer, 1960; Charlesworth and Charlesworth, 1979 ; Piper *et al.*, 1984, Barrett and Shore, 1987; Belaoussof and Shore, 1995).

Despite considerable success using distyly as a model system in ecological genetics (Crosby, 1949 ; Mather, 1950 ; Bodmer, 1960 ; Ornduff, 1975; Ganders, 1974, 1975, 1979a,b ; Charlesworth and Charlesworth, 1979 ; Piper *et al.*, 1984 ; Cahalan and Glidon, 1985) details of its molecular genetics and mechanisms of incompatibility remain largely obscure.

Theoretical models differ in both the selective mechanisms invoked and in the sequence in which the morphological and physiological components of the syndrome are assembled (Charlesworth and Charlesworth, 1979; Lloyd and Webb, 1992a; Richards, 1998). Unfortunately, very little comparative information is available to evaluate the different pathways by which heterostyly has evolved and there have been few experimental studies that have examined the contrasting

predictions of theoretical models (Kohn and Barrett, 1992; Stone and Thompson, 1994).

Heteromorphy is found in all habitats from desert to aquatic and from tropics to the arctic (Richards, 1997). Heteromorphy has a scattered distribution in angiosperms. Some major families have only single heteromorphic representatives (*Jepsonia* in the Saxifragaceae, *Sebaea* in the Gentianaceae). Other families which contribute many heteromorphic genera and species include Boraginaceae, Plumbaginaceae and Primulaceae. *Primula* has 426 species, 91% of which are heterostylous (Wedderburn and Richards, 1992).

Barrett *et al.* (1996) have reported that five Spanish *Narcissus* species are distylous, and that one of these, *N. triandrous* has tristylous populations as well. All of these are to a greater or lesser extent self compatible (Barrett and Cruzan, 1994) but, act as if possessing multiallelic self incompatibility for most inter-morph crosses are compatible. Possibly, some have a late-acting self incompatibility.

In recent years, many more examples of self compatible heterostyles have become available, including members of the genera *Cryptantha, Decodon, Melochia, Nivenia, Oplonia* and *Quinchamaluim* (Barrett and Cruzan, 1994).

In the commonest departure from the 'primula syndrome' of heteromorphy, there is no heteromorphy for anther position, so that reciprocal herkogamy does not occur. This condition is best known in *Linum*, but also occurs in *Villarsia*, *Quinchamalium*, *Anchusa*, *Epacris* and *Chlorogalum* (Barrett, 1992). It is also found in one species of *Primula*, *P. boveana*. Al Wadi and Richards (1993) argue that in this primitive self compatible species, anther position monomorphy may represent an intermediate condition in the evolution of full distyly. Lloyd and Webb (1992a,b) argued that the first stage is the evolution of distyly from an approach herkogamous ancestor, involving the establishment of a polymorphism for stigma height, but not anther height. Charlesworth and Charlesworth (1979) investigated the stability of stigma height dimorphism and found that it was difficult to maintain in populations. One source of evidence in favour of the Lloyd and Webb (1992a,b) model would be the co-occurrence of stigma–height dimorphism and distyly among closely related species. Because of the absence of reciprocal herkogamy, dimorphic species of *Narcissus* are not considered to be distylous, but instead are best described as possessing stigma–height dimorphism (Barrett *et al.*, 2000). In contrast to most heterostylous groups, heterostyly is rare in *Narcissus*, whereas stigma-height dimorphism is common, implying that there are strong constraints on the evolution of

reciprocal herkogamy and also there is no evidence for the occurrence of diallelic incompatibility or ancillary polymorphisms of pollen and stigmas in any *Narcissus* species (Dulberger, 1964 ; Arroyo and Dafni, 1995 ; Barrett *et al.*, 1996 ; Sage *et al.*, 1999 ; Baker *et al.*, 2000a,b).

Some of the earlier investigated tropical heterostylous species are *Oldenlandia umbellata* (Bahadur, 1968, 1970), *Pentas lanceolata* (Bahadur, 1968), *Morinda tomentosa* (Reddy and Bahadur, 1977)and *Guettarda species* (Bahadur, 1968 ; Zapota and Arroyo, 1978 ; Richards and Koptur, 1993), all belonging to Rubiaceae.

The present investigation was carried out to study the heterostyly and incompatibility shown by *Pentas lanceolata* (Forssk.) Deflers ; a member of Rubiaceae. Heirn (1878) was the first to notice heterostyly in three species of *Pentas*, while studying the peculiarities and distributions of Rubiaceae in tropical Africa. Extensive study of heterostyly in this genus was carried out by Verdcourt (1958). Attributing taxonomic significance to the character of heterostyly, he recognised thirty four species from Africa alone, out of which fifteen species exhibit dimorphic heterostyly. *Pentas lanceolata* was one among them which showed heterostyly (Baker, 1958).

The objectives of the present study in *Pentas lanceolata* were the following :

- Screening the heterostylous and homostylous plants of *P. lanceolata* colour variants to select a suitable material for the study.
- Morphological studies to determine the degree to which the plant shows the typical heterostylous syndrome.
- SEM investigations to get a detailed information on the morphology of stigma and pollen.
- Anatomical studies to determine the nature of pistil.
- Studies on pollination biology of *Pentas lanceolata.*
- Physiological studies to understand the pre- and post-pollination changes of the pistil.
- *In vitro* and *in vivo* pollen germination studies to confirm the viability of pollen.
- Aniline blue fluorescence studies to determine the nature of pollen tube growth in the pistil and to locate sites of incompatibility, if any.
- *In vitro* bioassay to understand the nature of inhibitors/promoters of pollination on the stigma.

Materials and Methods

- Biochemical analysis to find out the changes on the stigma following self and cross pollinations.
- Fruit and seed set studies to find out the degree of incompatibility.
- SDS PAGE studies to identify the nature of proteins involved in inducing incompatibility.
- Genetic studies to understand the nature of inheritance of heterostylous morphs.
- Population studies from different localities to understand the morph frequencies.
- To understand the overall significance of the reproductory systems of these plants, which might aid in distinguishing among various evolutionary models for the origin of heterostyly.

MATERIALS

Heterostyly and its influence in controlling pollination pattern and fruit set was a curious topic for biologists from Darwin's time onwards. Rubiaceae is one of the advanced dicot families, exhibiting heterostyly. On the basis of an elaborate screening, *Pentas lanceolata* (Forssk.) Deflers., a species of the tribe *Oldenlandeae* of Rubiaceae was found to be the best suited material for heterostyly studies. This genus is interesting, due to the occurrence of both homomorphic and heteromorphic (heterostylous) varieties, in attractive colour variants.

The heterostylous plants are of the floral morphs, the pin form, which bears flowers with long styles and short stamens. The other morph, thrum bears flowers with short styles and long stamens.

The homomorphic varieties include only the pin forms with long styles and short stamens.

Pentas lanceolata plants are woody erect shrubs, growing to a height of four to ten meters. The plants produce flower clusters in beautiful colours, throughout the year.

The following colour variants grown in the botanic garden of Mahatma Gandhi College, Thiruvananthapuram were used for the present investigation.

Pentas lanceolata **- white**

This variety is characterised by white flowers. It is heterostylous, producing both pin and thrum flowered plants. Seed set is generous.

Pentas lanceolata **- lilac**

This variety produces light pink or lilac flowers. It is heterostylous, having both pin and thrum morphs. Setsseeds profusely.

Pentas lanceolata **- magenta**

This plant produces purple coloured flowers. It is heterostylous, producing both pin and thrum morphs. Setsseeds sparsely.

Pentas lanceolata - red

This variety is characterised by red coloured flowers. It is monomorphic producing only pin morphs. Sets no seeds.

Pentas lanceolata - crimson

This variety is characterised by bright red coloured flowers of smaller size. Only pin flowered plants are seen. No seed set was observed.

METHODS

Floral Morphology

To study the size relationship and position of the male and female floral parts, the flowers were observed closely with a hand lens and a needle. Fresh flowers collected early in the morning were used for the study. Measurements of flower length, length of petals, width of petal lobes, length of pistil, style and stigma, length of anthers and filaments were taken using a metric scale. Mean values of fifty flowers were taken.

Cytological studies

For cytological studies flower buds of suitable stages were fixed in Carnoy's fixative (absolute ethanol : acetic acid, 3 : 1) between 8.30am and 10.30am. Anthers were squashed in 1% acetocarmine and observed under light microscope. Relevant photomicrographs were made.

Anatomical Studies

In order to compare the anatomical features of the pistils of the two morphs, the pistils were fixed in acetic alcohol (absolute ethanol : glacial acetic acid, 3 : 1) for 24 h. It was then cleared in NaOH at laboratory temperature for 24 h. The pistils which became soft after clearing were rinsed well in tap water and then mounted in glycerine to prevent drying. The pistils were longitudinally split up before lowering the cover glass.

Physiological Studies

Estimation of pollen sterility

Estimation of pollen sterility was made from fresh flower buds collected early in the morning, shortly before anthesis, which is between 6.30 am and 7.30 am. The pollen grains were dusted on to micro slides

and stained with 1:1 acetocarmine - glycerine mixture. The micro slides were kept for 1 h and the pollen then examined under light microscope. Uniformly stained, full pollen grains were taken as fertile and unstained and shrunken pollen grains were taken as sterile. The micro slide was scored for the fertility of the pollen and also the size of the pollen was measured using micrometer. Pollen grains from ten arbitrarily selected microscopic fields were taken. Relevant photomicrographs were also made.

Estimation of pollen viability

The most commonly used technique in pollen physiology is *in vitro* germination. (Heslop-Harrison, 1981). It is rapid and reasonably simple, in many species the test shows correlation with fruit set and seed set (Visser, 1955). Freshly collected pollen grains were dusted on to clean slides to which is added a drop of the culture medium and the preparation was incubated in a humidity chamber for 4 h. The humidity chamber consisted of a pair of large Petri plates, lined with moist filter paper, on the lower side. Two glass rods placed parallel and apart, on the moist filter paper, facilitated the easy handling of the culture. At the end of the incubation period, the cultures were fixed by adding 10% ethanol and glycerine in 1:1 ratio. The

cultures were then scored for pollen germination and average pollen tube length measurements were made after sliding a cover slip over the micro slides and examining under light microscope. The data was tabulated and relevant photographs were made.

Different culture media like Robert's medium (Robert *et al.*, 1983), Hodzkin and Lyon's medium (Hodzkin and Lyon, 1986) and Brewbaker and Kwack's medium (Brewbaker and Kwack, 1963) were tried. Of these Brewbaker and Kwack's medium with 20% sucrose was found to give the best results. The composition of this medium is the following :

Sucrose	10 %
Boric acid	100 mg/l
Calcium nitrate	300 mg/l
Magnesium sulfate	200 mg/l
Potassium nitrate	100 mg/l

Acetolysis of pollen grains

Pollen preparations were made according to the acetolysis method (Erdtman, 1952).

The pollen grain samples were put in glacial acetic acid for 10 min. centrifuged and decanted; the supernatant was discarded. The pellet

collected was dissolved in 6 ml of freshly made mixture of acetic-anhydride and conc. sulphuric acid (9:1 v/v). It was heated gently to boiling point in a water bath for 3 min stirring continuously with a glass rod. The supernatant obtained by centrifugation was decanted carefully into running water. It was resuspended in glacial acetic acid, centrifuged, decanted and again resuspended in distilled water, centrifuged and again decanted the supernatant. The sample was then embedded on a micro slide with polyvinyl lactophenol and observations were made. Relevant microphotographs were also taken.

In vivo germination studies

For *in vivo* germination studies, flowers of three stages were used. Fresh flower buds just before anthesis, on the day of anthesis and one day after anthesis were collected. Stigmas from these were excised and observed under the light microscope. The stigmatic papillae were measured using micrometer. Hundred measurements from each sample were taken for calculating the mean. Relevant microphotographs were also made.

Scanning Electron Microscopic Studies

For studying the surface view of the stigma before and after pollination with self pollen and cross pollen, SEM studies were conducted. The stigmas collected from fresh flowers were of three types. Unpollinated virgin stigmas of both pin and thrum morphs tested earlier under light microscope, self pollinated pin stigma and thrum stigma and cross pollinated stigmas of both pin and thrum morphs. The stigmas were first kept in 10 ml of glutaraldehyde fixative, prepared by making a 4% solution of glutaraldehyde in phosphate buffer (pH 7.0), taken in a centrifuge tube and kept for 2-15 h. The specimen was centrifuged and later was taken out and washed three times in phosphate buffer. Then, to the specimen, 2% osmium tetroxide in phosphate buffer (pH 7.0) was added and incubated at 0-5°C for 5 h. Then the specimen was taken out, washed again with buffer and dehydrated with alcohol series. The dehydrated specimen was mounted on a stub and coated with gold-palladium coating and then SEM photographs were made.

SEM studies were conducted in the following cases :

- Fresh unpollinated stigmas on the day of anthesis of both pin and thrum.
- Stigmas of pin and thrum after 24 h of self pollination.

- Stigmas of pin and thrum after 24 h of cross pollination.
- Acetolysed and unacetolysed pollen grains.

Self and Cross pollination studies under laboratory conditions

Self and cross pollination studies were carried out in excised stigmas from fresh unpollinated and bagged flowers collected afresh.

Selfing

Intra morph - intra varietal crosses of the following combinations were carried out.

White pin	×	White pin
Lilac pin	×	Lilac pin
Magenta pin	×	Magenta pin
White thrum	×	White thrum
Lilac thrum	×	Lilac thrum
Magenta thrum	×	Magenta thrum

The stigmas were mounted on to a clean glass slide and dusted with self pollen, collected from the same plant. The slides were then kept in incubation chamber, undisturbed for some time. The data of percentage of adherence, percentage of pollen germination and length of

pollen tube growth after 8 h and 24 h were collected under light microscope and fluorescent microscope. The data was tabulated and relevant photographs were made.

Crossing

The following inter morph–intra varietal crosses were carried out.

White pin	×	White thrum
Lilac pin	×	Lilac thrum
Magenta pin	×	Magenta thrum
White thrum	×	White pin
Lilac thrum	×	Lilac pin
Magenta thrum	×	Magenta pin

The stigmas kept on a glass slide were dusted with cross pollen from the same variety and kept inside the incubation chamber undisturbed for a few hours. The percentage of adherence, percentage of germination of pollen and also the length of pollen tube after 8h and 24h of pollination were studied under the light microscope and the data was tabulated and the relevant stages photographed.

Inter morph - inter varietal crosses of all possible combinations between the three colour variants of pin and thrum were carried out in incubation chambers, and the data as in the above cases were tabulated and relevant photographs made.

In all the laboratory studies of *in vivo* germination the self and crossed pistils were collected after 8 h and 24 h. These were boiled in lactophenol to soften the tissues and then stained with cotton blue for 15 min. The stained pistils were mounted in glycerine and data tabulated.

Field Studies

For confirmation of the data from laboratory studies, field studies under controlled conditions were conducted in the two different morphs of the three colour variants of *Pentas lanceolata.* For selfing and crossing flowers from inflorescences, bagged the previous day of anthesis, were used.

Selfing

Intra morph- Intra varietal

The stigmas were dusted with fresh pollen collected from flowers of the same morph, from the same colour variant, in the following combinations.

White pin	×	White pin
Pink pin	×	Pink pin
Magenta pin	×	Magenta pin
White thrum	×	White thrum
Pink thrum	×	Pink thrum
Magenta thrum	×	Magenta thrum

For pin and thrum selfing, young buds were bagged, the day before anthesis. Fresh uncontaminated pollen from the same plant were used for pollination, the following day in the morning between 6 am and 7 am. In thrum plants, the corolla tube was split open with a needle and then the stigma was pollinated. The pollinated styles were marked and the rest pinched off and the seeds collected at the appropriate time.

Crossing

For crossing also, the flowers were bagged the day before anthesis. In crosses pin × thrum, emasculation was carried out, the previous day, to prevent any accidental selfing. In the cross, thrum × pin, removal of corolla upto the throat including stamens, was done, the previous day of pollination. The bagged flowers were then pollinated using pollen from another plant of the same colour variant. Here also, the

pollinated stigmas were marked and the rest pinched off. Seeds were collected at the appropriate time before the capsule dehisced.

Crosses of the following combinations were conducted:

Inter morph - Intra varietal crosses

White pin	×	White thrum
Lilac pin	×	Lilac thrum
Magenta pin	×	Magenta thrum
White thrum	×	White pin
Lilac thrum	×	Lilac pin
Magenta thrum	×	Magenta pin

The data on fruit and seed set was tabulated for these crosses by collecting seeds at the appropriate time, before the capsule dehisced.

Fluorescent studies

Details of pollen germination and pollen tube growth in pollinated pistils were studied using aniline blue fluorescent method (Shivanna and Rangaswamy, 1992). The pistils after 24 h of pollination were fixed in acetic alcohol (absolute ethanol : glacial acetic acid, 3 : 1) for 24 h and then cleared in NaOH at laboratory temperature for 24 h. The pistils which became soft after clearing were carefully rinsed, twice with tap water and mounted in 0.005 % aniline blue, prepared in 0.05 M Na_2HPO_4

(pH > 8.2). A drop of 50 % glycerine was added to the stain to prevent drying of pistils. The pistils were given a longitudinal slit with a scalpel before lowering the cover glass. The tissue was spread by applying gentle pressure on the coverglass. The preparations were observed under fluorescence microscope (Nikon), using UV-2A filter combination (excitation filter 330 - 380 nm, dichromatic mirror, 400 nm and barrier filter 420 nm).

Index of self incompatibility

To find out the measure of self incompatibility, both selfing and crossing were carried out and data up to fruit set and seed set was collected. Based on the number of seeds realized by selfing and crossing, the ISI was calculated using the formula of Zapota and Arroyo (1978).

$$ISI = \frac{\text{No. of seeds obtained per self pollination}}{\text{No. of seeds obtained per cross pollination}}$$

And can be categorized as :

Self incompatible	=	ISI < 0.02
Partially self compatible	=	ISI 0.2–0.9
Fully self compatible	=	ISI 1.0
Preferentially selfed	=	ISI > 1.0

In vitro bioassay

In vitro pollen germination studies of pollen from both pin and thrum were carried out in stigmatic washes and stigmatic extracts prepared from the stigmas of both the morphs.

In stigmatic leachates (washes)

For taking stigmatic leachates, fresh unpollinated stigmas were excised from flowers on the day of anthesis and fifty stigmas were tied together with a piece of thread and kept with the stigmas completely immersed under 1 ml of water taken in small screw cap bottles for 1 h. After that, the stigmas were removed and the stigmatic wash was mixed with 1 ml of germination medium. Pollen grains collected from fresh flowers of both pin and thrum were taken on clean micro slides and a drop of the stigmatic wash + medium (1:1) was added to it and kept inside the incubation chamber for 6 h. After that, the cultures were terminated using the fixative ethanol (10%) and then placing a cover glass on to the micro slide, the pollen cultures were scored.

In stigmatic extracts

For preparing stigmatic extracts, fifty stigmas excised from fresh flowers on the day of anthesis were collected. These stigmas were ground

BIOCHEMICAL STUDIES

Estimation of soluble proteins

The stigmas used for the estimation of soluble proteins were of three types ; unpollinated stigmas from bagged flowers, to serve as control, cross pollinated and self pollinated stigmas collected after 24 h of pollination. The stigmas from fifty flowers were homogenised with phosphate buffer (pH 7.4) in a tissue homogeniser, centrifuged at 3000 rpm for 15 min and the collected supernatant was again centrifuged for 3 min. The supernatant collected was made upto 10 ml. The solution was kept in an ice bath for 15 min. The precipitated protein was separated by centrifugation. The pellet containing protein was dissolved in 1 ml of extraction buffer. The extracted protein was estimated using Lowry's method (Lowry *et al.*, 1951).

Estimation of soluble sugars

All the three types of unpollinated stigmas from bagged flowers, cross pollinated and self pollinated stigmas collected after 24 h of pollination were used for the estimation.

Soluble sugars were estimated using anthrone method (Packer, 1967). The sample (0.5mg) was homogenized in 10 ml of cold acid

alcohol mixture, prepared by mixing 80% ethyl alcohol in 0.1 N percholoric acid. The homogenate was centrifuged, supernatant collected and volume made upto 10 ml. 0.5 ml of aliquot made upto 1 ml was used for the estimation by adding 4 ml of anthrone reagent. The absorbance was read at 620nm.

Estimation of total phenols

For the estimation of total phenols also the three types of stigmas were used. Unpollinated stigmas with styles were collected from bagged flowers, cross pollinated and self pollinated stigmas with styles after 24 h. of pollination were also collected. 50 mg of the sample was boiled in 80% methanol at 70°C for 10 min. This was homogenised with a tissue homogeniser and centrifuged for 2 min. The supernatant was made upto 10 ml, using distilled water. To 0.5 ml of the extract now made upto 3 ml with distilled water, 0.5 ml of Folin reagent and 2 ml of 20% Na_2CO_3 solution were added. After keeping for 30 min, the absorbance was read at 650 nm against the proper blank. The amount of phenol was estimated using the standard graph of chlorogenic acid (Swain and Hellis, 1955).

Estimation of soluble peroxidase

Stigmas with styles collected from unpollinated, selfed and crossed flowers were used for the estimation .The soluble peroxidase content was estimated using the method of Putter (1974).

The sample (0.5 g) was homogenized in 1.5 ml of 0.1 M phosphate buffer (pH 7.0) by grinding in a precooled mortar and pestle. The homogenate was centrifuged and the supernatant collected was used as the enzyme source.

Pippetted out 3.0 ml of buffer solution, 0.05 ml of guaiacol (20mM) and 0.03 ml of H_2O_2 (12.3mM) and mixed well. The assay mixture was incubated at room temperature for 20 min and the reaction was terminated by keeping it in a boiling water bath for 2 min. The absorbancy was measured at 436 nm.

Estimation of proline

The estimation of proline was done with the three different types of stigmas with styles collected from unpollinated, selfed and crossed flowers.

The sample (0.5 g) was homogenized in 10 ml of 30% aqueous sulphosalycilic acid. The homogenate was filtered through Whatman No.2 filter paper. Two ml of glacial acetic acid and 2 ml of acid ninhydrin were added to 2 ml of the filtrate. It was heated in a boiling water bath for 1h. The reaction was terminated by placing the tube in an ice bath. Four ml of toluene was added to the reaction mixture and stirred well for 20-30 sec. The toluene layer was separated and warmed to room temperature. Measured the absorbancy at 520 nm (Bates *et al.*, 1973).

SDS PAGE analysis

The Protean II Electrophoresis system (Biorad, USA) was used to study the protein profiles of thrum self, pin self, thrum cross and pin cross combinations of *Pentas lanceolata* on polyacrylamide gels.

Extraction of protein

The samples were collected from the respective plants and homogenized in pre-cooled mortar and pestle with 2 ml of extraction buffer. The homogenate was centrifuged at 12000 rpm for 20 min and the clear supernatant was used for the electrophoresis.

Stock solution preparation

A. Acrylamide/Bis (30% T, 2.67% C)

Acrylamide	29.2 g
N,N_1-Methylene Bisacrylamide	0.8 g
Water to	100 ml

B. Separating Gel Buffer (1.5 M Tris-HCl, pH 8.8)

Tris base	18.15 g
Water to	50 ml

Adjust to pH 6.8 with HCl and make up to 50 ml with distilled water

C. Stacking Gel Buffer (0.5 M Tris HCl, pH 6.8)

Tris base	3.0 g
Water to	25 ml

Adjust to pH 6.8 with HCl and makeup to 50 ml with distilled water.

D. Polymerising Agents

Ammonium persulphate 10%

(Dissolved 100 mg ammonium persulphate in 1ml distilled water)

TEMED (N,N,N^1,N^1, tetra methyl ethylene diamine)

E. Sodium dodecyl sulphate 10%(w/v)

Dissolved 5 g SDS in 30 ml distilled water and made up to 50 ml.

F. Electrode (Running) Buffer (5 X)

(1 X = 25 mM Tris, 192 mM Glycine, 0.1% SDS, pH 8.2 – 8.4)

Tris base	45.0 g
Glycine	216.0 g
SDS	15.0 g
Distilled water to	3 L

300 ml of stock solution was diluted with 1.2 L distilled water for one electrophoretic run

G. Sample Buffer (5X concentration)

Tris-HCl Buffer (pH 6.8)	5 ml
SDS	0.5 g
Sucrose	5.0 g
Mercaptoethanol	0.25 ml
Bromophenol blue	1 ml
(0.5 % w/v solution in water)	
Water to	10 ml

H. Protein stain solution

Coomassie brilliant blue R 250	0.1 g
Methanol	40 ml
Acetic acid	10 ml
Water	50 ml

I. Destaining solution

Methanol	40 ml
Acetic acid	10 ml
Water	50 ml

Gel Preparation

	Separating gel (12%)	Stacking gel (4%)
Acrylamide/Bis stock	40.0 ml	1.3 ml
Distilled water	33.5 ml	6.1 ml
1.5 M Tris HCl, pH 8.8	25.0 ml	--
0.5 M Tris HCl, pH 6.8	--	2.5 ml
10% SDS	1.0 ml	100 µl
10% Ammonium persulphate	500 µl	50 µl
TEMED	50 µl	10 µl
Total volume	100 ml	10 ml

Thoroughly clean and dry glass plates and spacers were assembled properly in the gel-casting unit. White petroleum jelly was then applied around the edges of the spacers to hold them in place and sealed the chamber between the glass plates. The separating gel mixture was prepared carefully and poured in the chamber between the glass plates. A

small amount of butanol was layered on top of the gel and allowed to set for 20-30 min.

The butanol was removed from the top of the gel and washed with little stacking gel solution. Then the stacking gel solution was poured and the comb was placed in it and allowed to set.

After the stacking gel had polymerized, the comb was removed without distorting the shapes of the well. Then the gel was carefully installed in the electrophoresis apparatus and the electrode buffer was filled in the tanks.

The protein concentration of each sample was adjusted using 5X sample buffer in such a way that the same amount of protein was present per unit volume. The sample solutions were heated in boiling water for 2-3 min to ensure the complete interaction between proteins and SDS. With the help of a microsyringe 50 µl samples were injected into each sample well through the electrode buffer. Then the electrophoretic apparatus was connected with the power pack unit and 15 mA current was applied for 30 min (until the samples had run through the stacking gel). After that the run was continued at 30 mA until the bromophenol blue reached the bottom of the gel (approximately 4 h).

When the run was completed the gel was carefully removed from the plates and immersed in the staining solution overnight (at least 6 h) with uniform shaking. Then the gel was transferred to a suitable container containing 300 ml of destaining solution. The destainer solution was changed until the background of the gel became colourless. The proteins fractionated into bands appeared blue and it was scanned and photographed with the Gel documentation unit (Biorad, USA).

Results

Heterostyly is always correlated with incompatibility. The plant *Pentas lanceolata*, though conventionally propagated by stem cuttings, is also propagated through seeds. The occurrence of a broad spectrum of colour variants and the existence of both pin and thrum forms associated with varying degrees of incompatibility and sterility, makes the plant interesting and unique.

Pentas lanceolata was found to be the best suited material for the type of investigations carried out in the present study. This genus includes both monomorphic and heteromorphic forms, in attractive colours (Fig. 1). The heteromorphic plants of *Pentas lanceolata* exhibit distyly, with pin and thrum flowers, of which, the pin form bears long style and short stamens, whereas the thrum form, bears short style and long stamens.

The homomorphic forms were all pin types. Their corresponding thrum forms or monomorphic thrum plants were not observed, throughout the study.

Fig. 1. ***Pentas lanceolata*** **colour variants from Mahatma Gandhi College Botanic Garden, Thiruvananthapuram.**

Pollen grains of all the heterostylous colour variants were fertile resulting in good percentage of fruit set (24.78 to 76.06) while monomorphic members did not produce seeds. Among them, some were pollen fertile and others were completely pollen sterile. Propagation through seed was possible only in heterostylous forms.

General features of *Pentas lanceolata* (Forssk.)Deflers

Leaves

Leaves are opposite, petiolate, ovate, lanceolate with inter petiolar stipules, covered with trichomes. The leaves are two to seven inches in length with prominent veins. The leaves are also covered with trichomes.

Flowers

The flowers are produced in corymbose cymes. Each inflorescence contains twenty to twenty-five flowers with slight variation in their numbers per inflorescence, between the different colour variants. The time of anthesis is between 6.30 and 7.30 in the morning.

The floral characters recorded were taken on the day of anthesis.

Calyx

The calyx is made up of four to six sepals which are unequal, gamosepalous and persistent.

Corolla

The corolla is gamopetalous with five red petal lobes. The corolla tube is 17 ± 1 mm long, with a narrow basal portion and a comparatively broader neck region. The inner surface of the corolla tube is pubescent, with upwardly directed hairs. The corolla is valvate in aestivation.

Androecium

Stamens are five in number, epipetalous with introrse and dorsifixed anthers. Each stamen is 2.5 to 3.0 mm long.

Gynoecium

Gynoecium is bicarpellary, syncarpous. Ovary is inferior, two-chambered, with numerous ovules on axile placenta. Style is 10-20 mm long with a bifid papillate stigma, 3.0 to 4.0 mm long.

Although the characters mentioned above are more or less common in all the colour variants, each colour variant showed one or more features, characteristic to that variety, of which variation in flower colour was the most conspicious one. Hence they were referred on the basis of flower colour as colour variants (var.).

Pentas lanceolata - Monomorphic (Homostylous) forms

Pentas lanceolata var. red

(*Ophiorrhiza lanceolata* Forssk., *P. carnea* Benth.)

This plant is a bright red flowered erect shrub growing to a height of 8 to 10 ft. It is monomorphic producing only pin flowers (Fig. 2 a, b). The pollen grain fertility was 70.56%, but produced no fruits. The floral characters are typical of the species (Table 1).

Pentas lanceolata var. crimson

This is a bushy beautiful garden ornamental plant with crimson red flowers. The black stigmatic lobes enhance the beauty of the bloom (Fig. 3a, 3b). The flowers are smaller than those of other varieties (Table 2). This homomorphic form showed high percentage of pollen sterility and hence sets no fruits.

Pentas lanceolata - Dimorphic (Heterostylous) forms

In contrast to the varieties mentioned above the following three colour variants exhibited distyly where the stigmas are placed at two different levels in the morphs, pin and thrum (Fig. 34).

Homostylous colour variants of *Pentas lanceolata*

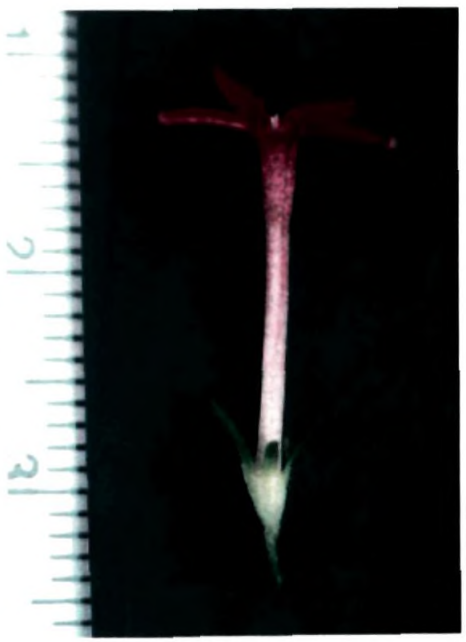
Fig. 2. *Pentas lanceolata* var. red

a. An inflorescence

b. L.S. of pin flower

Fig. 3. *Pentas lanceolata* var. crimson

a. An inflorescence



b. A pin flower

Table. 1. Floral characters of *Pentas lanceolata* var. red

Characters	Measurement (mm)	Frequency*			Mean
		I	II	III	
Flower Length	23.5	11	8	10	24.5 ± 0.62
	24.0	13	12	17	
	25.0	26	30	23	
Length of Style	20.0	11	10	15	20.5 ± 0.41
	20.5	29	26	24	
	21.0	10	14	11	
Length of Stigma	3.0	13	16	11	3.5 ± 0.41
	3.5	22	26	30	
	4.0	5	8	9	
Length of Anther	2.5	45	42	36	2.75 ± 0.25
	3.0	5	8	14	
Level of Stigma	24	32	38	33	24.25 ± 0.25
	24.5	18	12	17	
Level of Anther	15	22	18	15	15.5 ± 0.5
	16	28	32	35	
Length of Petal	7.5	18	15	10	7.75 ± 0.25
	8.0	32	35	40	
Width of Petal	2.9	10	10	8	3.0 ± 0.08
	3.1	12	14	12	
	3.0	28	26	30	

*All values represent a mean of 50 flowers

Table. 2. Floral characters of *Pentas lanceolata* var. crimson

Characters	Measurement (mm)	Frequency*			Average
		I	II	III	
Flower Length	21.0	20	18	22	20.5 ± 0.41
	20.5	18	19	15	
	20.0	12	13	13	
Length of Style	16	28	30	26	15.5 ± 0.5
	15	22	20	24	
Length of Stigma	2.0	32	40	37	1.75 ± .25
	1.5	18	10	13	
Length of Anther	2.0	30	28	26	2.25 ± 0.25
	2.5	20	22	24	
Level of Stigma	18.5	32	36	28	18.75 ± 2.25
	19.0	18	14	22	
Level of Anther	10.0	4	7	9	11.0 ± 0.82
	11.0	29	30	26	
	12.0	17	13	15	
Length of Petal	5.0	7	9	10	5.5 ± 0.4
	5.5	15	16	13	
	6.0	28	25	27	
Width of Petal	3.0	50	50	50	3.0

*All values represent a mean of 50 flowers

***Pentas lanceolata* var. white**

This heterostylous variety is characterised by white flowers. A very good percentage of pollen fertility (71.84 to 73.6%) and fruit set were observed in both the floral morphs. The pin and thrum morphs showed characteristic features (Fig. 4 a-f ; Table 3).

***Pentas lanceolata* var. lilac**

Both pin and thrum morphs of this distylous variety are lilac flowered, with a high percentage of pollen fertility (82.3 to 83.8%) and it sets fruits in abundance. The floral characteristics of pin and thrum morphs are shown in Table 4 (Fig. 5 a-f).

***Pentas lanceolata* var. magenta**

This distylous plant produces deep magenta coloured flowers with slight variation in floral size (Fig. 6a-f ; Table 5). Both the morphs were pollen fertile (54.42 to 63.97%), but fruit set is sparse.

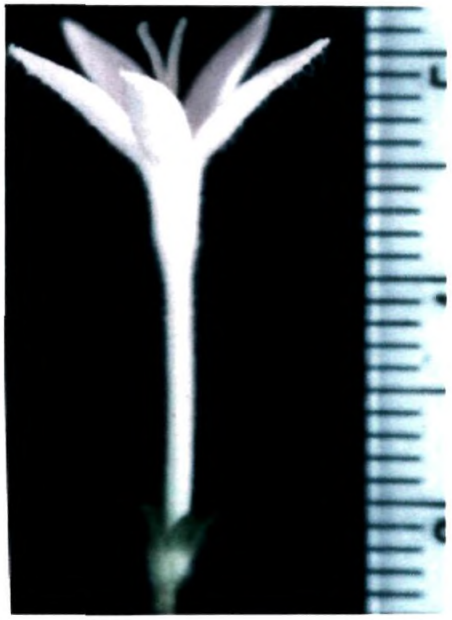
There are a series of common features by which pin and thrum differ, irrespective of the genera. Some such characters in which the heteromorphs of *Pentas* showed variation are given in Table 6.

In this investigation an attempt was made to find out the morphological, anatomical, physiological and biochemical differences

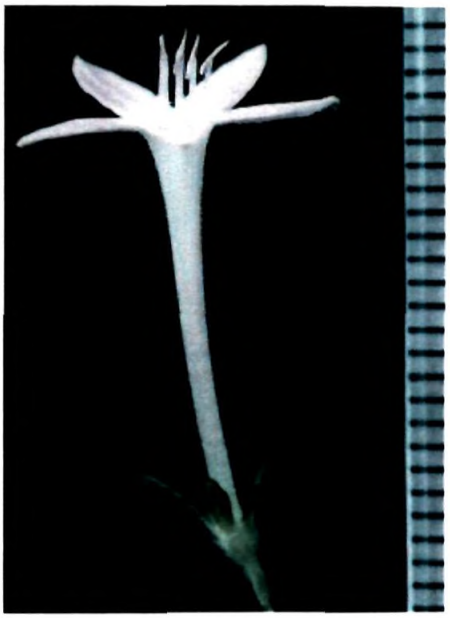
Fig. 4. *Pentas lanceolata* var. white

a. An inflorescence of pin flowers

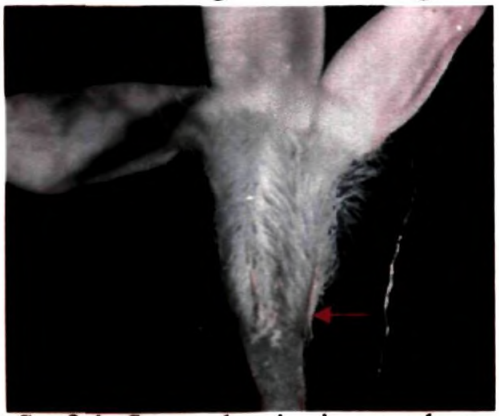
b. An inflorescence of thrum flowers



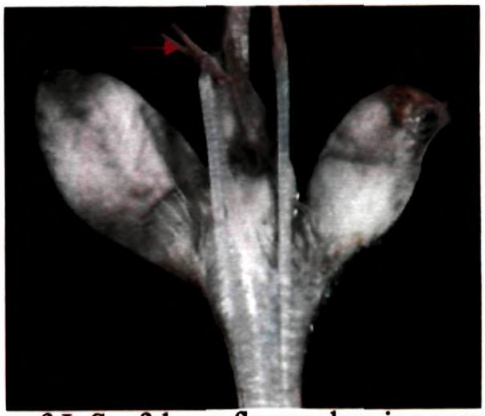
c. Pin flower showing exerted bifid stigma



d. Thrum flower showing exerted stamens



e. L.S. of pin flower showing inner pubescent corolla tube with the inserted stamens



f. L.S. of thrum flower showing scanty corolline hairs and exerted stamens

Table. 3. Floral characters of pin and thrum morphs of *Pentas lanceolata* var. white

Characters	PIN					THRUM				
	Measurement (mm)	Frequency*			Mean	Measurement (mm)	Frequency*			Mean
		I	II	III			I	II	III	
Flower Length	23.0	3	4	7	23.5 ± 0.41	25	10	10	12	25.5 ± 0.41
	23.5	19	21	20		25.5	25	27	28	
	24.0	28	25	23		26	15	13	10	
Length of Style	17	13	15	12	17.5 ± 0.41	9	15	18	15	9.5 ± 0.41
	17.5	11	6	13		9.5	13	12	10	
	18	26	29	25		10	22	20	25	
Length of Stigma	6	27	25	29	6.25 ± 0.25	4	28	30	26	4.25 ± 0.25
	6.5	23	25	21		4.5	22	20	24	
Length of Anther	3.0	38	33	40	2.5 ± 0.41	1.5	24	22	21	2.0 ± 0.41
	2.5	12	17	10		2.0	6	3	7	
						2.5	20	25	22	
Level of Stigma	23	21	22	20	23.5 ± 0.5	15	29	26	28	15.5 ± 0.5
	24	29	28	30		16	21	24	22	
Level of Anther	20	38	35	33	20.5 ± 0.5	23	22	21	24	23.25 ± 0.25
	21	12	15	17		23.5	28	29	26	
Length of Petal	9	23	21	24	9.5 ± 0.5	5	18	20	21	5.5 ± 0.5
	10	27	29	26		6	32	30	29	
Width of Petal	3	50	50	50	3.0	2.5	50	50	50	2.5

* Mean of 50 flowers

Fig. 5. *Pentas lanceolata* var. lilac

a. An inflorescence of pin flowers

b. An inflorescence of thrum flowers

c. Single pin flower

d. Single thrum flower

e. L.S. of pin flower with exerted stigma and inserted stamens

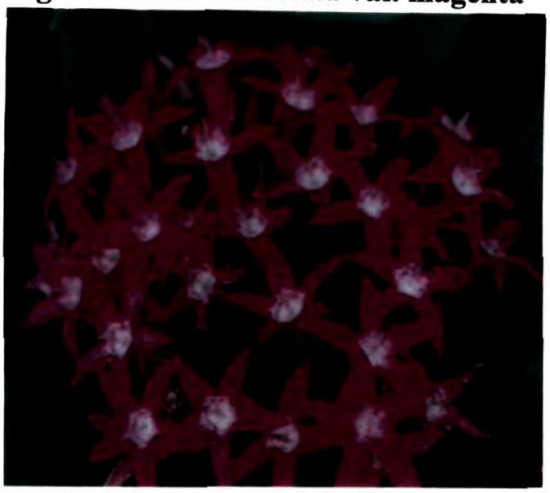
f. L.S. of thrum flower with inserted stigma and exerted stamens

Table. 4. Floral characters of pin and thrum morphs of *Pentas lanceolata* var. lilac

Characters	PIN					THRUM				
	Measurement (mm)	Frequency*			Mean	Measurement (mm)	Frequency*			Mean
		I	II	III			I	II	III	
Flower length	25	8	7	6	25.5 ± 0.41	27	10	10	12	27.5 ± 0.41
	25.5	29	33	30		27.5	12	14	14	
	26	13	10	14		28	28	26	24	
Length of Style	17	11	12	11	17.5 ± 0.41	14	26	28	27	14.5 ± 0.41
	17.5	10	13	16		14.5	21	20	20	
	18	28	25	23		15	3	2	3	
Length of Stigma	6.0	30	20	18	6.25 ± 0.2	6.0	29	30	32	6.25 ± 0.25
	6.25	10	16	21		6.5	21	20	18	
	6.5	10	14	11						
Length of Anther	2.5	50	50	50	2.5	2.5	50	50	50	2.5
Level of Stigma	23	12	9	14	23.5 ± 0.5	18	25	26	27	18.5 ± 0.41
	24	38	41	36		18.5	20	22	18	
						19.0	5	8	5	
Level of Anther	17	10	8	5	17.25 ± 0.25	27	12	10	13	27.5 ± 0.5
	17.5	40	42	45		28	38	40	37	
Length of Petal	8	16	18	16	9.0 ± 0.82	8	14	11	12	8.5 ± 0.41
	9	28	25	30		8.5	12	14	10	
	10	6	7	4		9	26	25	28	
Width of Petal	3.0	50	50	50	3.0	3.0	50	50	50	3.0

* Mean of 50 flowers

Fig. 6. ***Pentas lanceolata*** **var. magenta**



a. An inflorescence of pin flowers

b. An inflorescence of thrum flowers

c. Single pin flower

d. Single thrum flower

e. L.S. of pin flower

f. L.S. of thrum flower

Table.5. Floral characters of pin and thrum morphs of *Pentas lanceolata* var. magenta

Characters						THRUM				
	Measu-rement (mm)	Frequency*			Mean	Measu-rement (mm)	Frequency*			Mean
		I	II	III			I	II	III	
Flower Length	21 21.5 22	6 10 34	8 12 30	4 14 32	21.5 ± 0.41	22 23 23.5	9 38 3	10 35 5	10 33 7	22.8 ± 0.62
Length of Style	16.0 17.0 18.0	8 11 31	9 12 29	10 7 33	17.0 ± 0.82	8 9 9.5	12 28 10	8 30 12	10 27 13	8.8 ± 0.62
Length of Stigma	4.5 5.0	22 28	23 27	24 26	4.75 ± 0.25	4 5	22 28	23 27	25 25	4.5 ± 0.5
Length of Anther	3	50	50	50	3.0	3	50	50	50	3.0
Level of Stigma	21 22 23	1 20 29	2 23 25	2 22 26	22.0 ± 0.82	16 16.5 17	34 14 2	34 15 1	32 15 3	16.5 ± 0.41
Level of Anther	13 14 15	11 34 5	8 35 7	10 32 8	14.0 ± 0.82	21 22 23	2 38 10	3 35 12	5 30 15	22.0 ± 0.82
Length of Petal	8 9	39 11	36 14	35 15	8.5 ± 0.5	7.0 7.5 8.0	2 38 10	2 33 15	5 35 10	7.5 ± 0.41
Width of Petal	3	50	50	50	3.0	3	50	50	50	3.0

* Mean of 50 flowers

Table. 6. *Pentas lanceolata* - Characters which showed variation between pin and thrum morphs

Characters		Pin	Thrum
1	Corolla tube shape	Swollen at neck	Evenly broad
2	Corolla tube pubescence	Highly pubescent	Less pubescent
3	Style length	Longer	Shorter
4	Stylar cells	Long	Short
5	Style colour	Slightly coloured	White
6	Stigma size	Bigger	Smaller
7	Stigma shape	Lobes of equal size	Lobes unequal
8	Stigmatic papilla size	Longer	Shorter
9	Stamen length	Shorter	Longer
10	Anther size	Shorter	Longer
11	Pollen grain size	Small	Bigger
12	Pollen shape	Ovoid	Ovoid
13	Pollen colour	Yellowish	White

between the heteromorphs of *Pentas lanceolata* colour variants and their incompatibility reactions.

Propagation through seeds and stem cuttings was possible in heterostylous forms of *Pentas* but vegetative propagation through stem cuttings was the only mode of propagation in homostylous forms.

A preliminary screening of some of the available monomorphic and heteromorphic colour variants of *Pentas lanceolata* has shown that, they have many features in common, but differ in some morphological, anatomical, physiological and biochemical features.

Floral Morphology

Thrum flowers were one or two mm longer than pin flowers (Fig. 30). In both the morphs, the inner surface of the corolla was pubescent, but this was more prominent around the stamens. In thrum flowers the throat of the corolla tube was more pubescent, where the stamens were attached. But in pin flowers, the corolla tube was densely pubescent, starting from the throat of corolla tube upto about one third of its length, where the stamens were attached. The stamens are placed at two different levels in the morphs, pin and thrum (Fig. 35). The upwardly directed corolline hairs of pin were longer than those of thrum so that they remain interlocked at the throat.

In thrum flowers, the stamens were 6 to 7 mm in length with a long filament. But in pin flowers, the stamens were almost sessile, with the length of anther being more or less same, 3 mm in both the morphs (Fig. 33). Thrum stamens produce lesser number of dry and powdery pollen grains of larger size, whereas pin stamens produce more sticky pollen grains which were smaller in size when compared to that of thrum. Both the morphs exhibited pollen polymorphism with a diameter varying from 16.9 to 24.32 μm in thrum and 16.33 to 21.04 μm in pin (Table 7). Pollen grains were tri or tetra-colpate, according to their size.

The stigma of thrum morph was wet and short, 4.23 mm to 6.25 mm (Fig. 32) whereas that of pin was dry and comparatively long, 4.75 mm to 6.75 mm. The stigmatic papillae of thrum were shorter than those of pin. In thrum, the tips of stigmatic lobes were closely placed, while they were diverged in pin stigma.

Thrum flowers have a short style (8.8 mm to 14.5 mm), which was hidden inside the corolla tube, whereas the pin styles were long (17.55 mm) and remain protruded out of the corolla tube (Fig. 31). The level of anther and stigma was almost reciprocal in the two morphs (Fig. 36).

Table. 7. *Pentas lanceolata* -Pollen and papillae heteromorphism

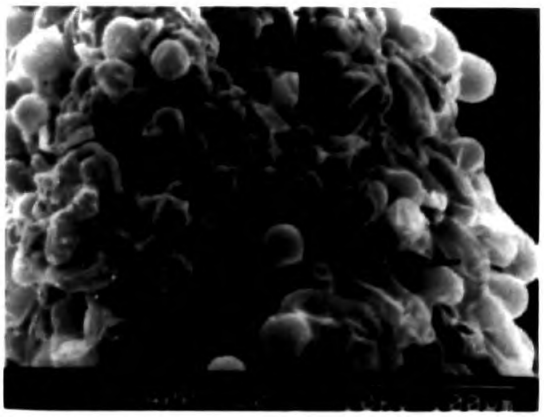
Colour variant	Diameter of pollen (μ)	Length of papillae (μ)
White pin	18.73 ± 1.28	100.72 ± 1.56
White thrum	20.13 ± 0.82	53.5 ± 1.36
Lilac pin	21.04 ± 1.68	151.33 ± 2.82
Lilac thrum	24.32 ± 1.39	62.81 ± 0.61
Magenta pin	16.33 ± 0.65	80.93 ± 1.74
Magenta thrum	16.9 ± 1.02	32.71 ± 0.8

All values represent a mean of 6 replicates

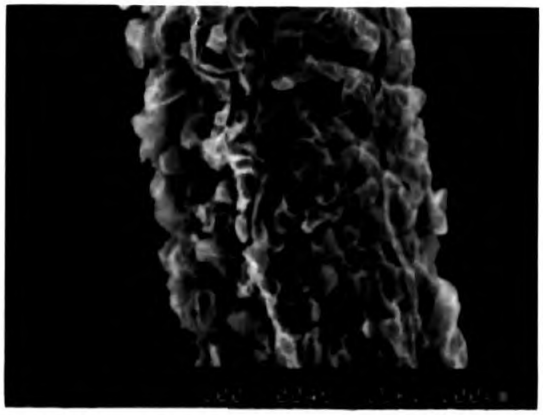
Stigmatic surface and papillae

The stigma was bifid in both the morphs. The receptive stigmatic surface was covered with club shaped papilla, having a length varying from 80.9 μm to 151.3 μm in pin morph. The papillae on thrum stigma were smaller, than those on pin stigma. The papillae showed a significant difference in length not only between the morphs, but also between colour variants (Table 7). The lilac pin stigmatic papillae were the longest with a mean length 151.33μm (Fig. 7c). The papillae on white pin stigma showed a mean length of 100.72μm (Fig. 7a), and those on magenta pin stigma were only 80.93μm long (Fig. 7e). The papillae on pin stigma showed a narrow base and swollen tip, while those on thrum stigma were broader at the base. The thrum papillae showed approximately one-third the length of the pin papillae. The papillae on lilac thrum were the longest among thrums, which showed a length of 62.81μm (Fig. 7d). The papillae on white thrum were 53.5μm long (Fig. 7b) and those on magenta were as short as 32.7μm (Fig. 7f). The papillae were single celled in both the morphs. The papillae stood erect in virgin unpollinated stigmas (Fig 7a-f), on the day of anthesis, whereas it collapsed one day after pollination, irrespective of self or cross, in both pin and thrum morphs (Fig. 8 a-b).

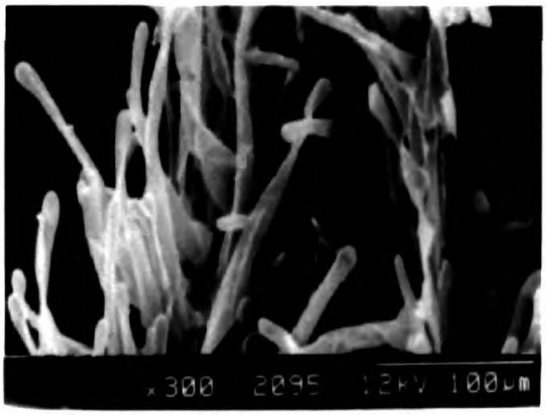
Fig. 7. SEM of *Pentas lanceolata* stigmatic papillae on the day of pollination



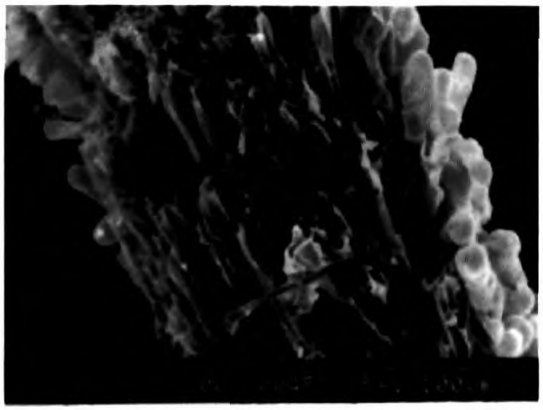
a. White pin - club shaped papillae holding germinating pollen grains



b. White thrum - with shorter papillae

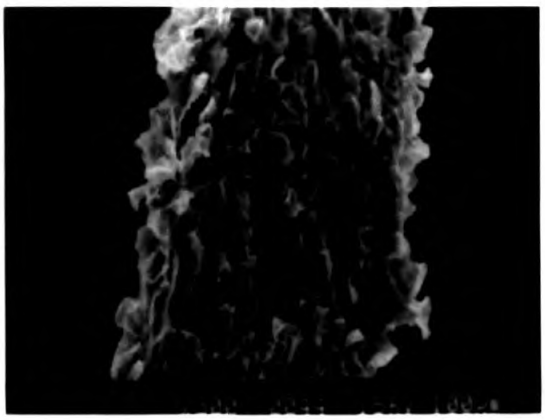


c. Lilac pin - with very long papillae



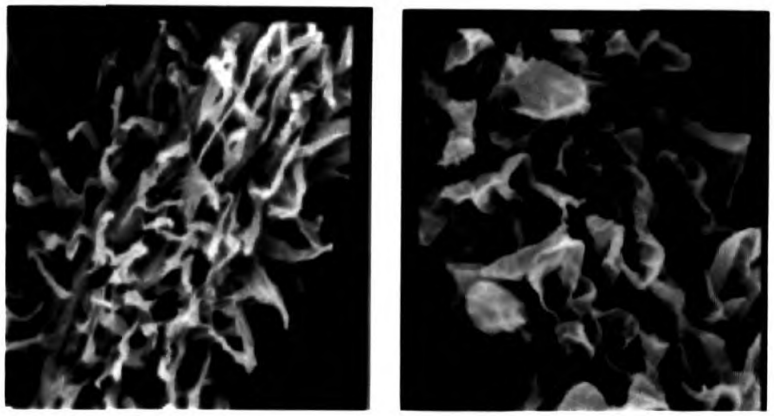
d. Lilac thrum-with medium sized papillae

e. Magenta pin-with medium sized papillae



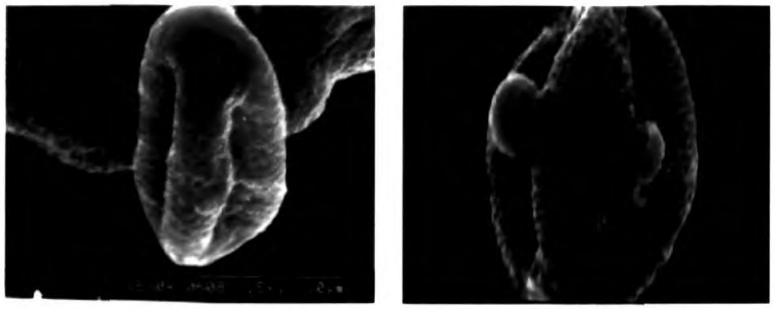
f. Magenta thrum-with short papillae

Fig. 8. SEM of *P. lanceolata* - collapsed stigmatic papillae after pollination



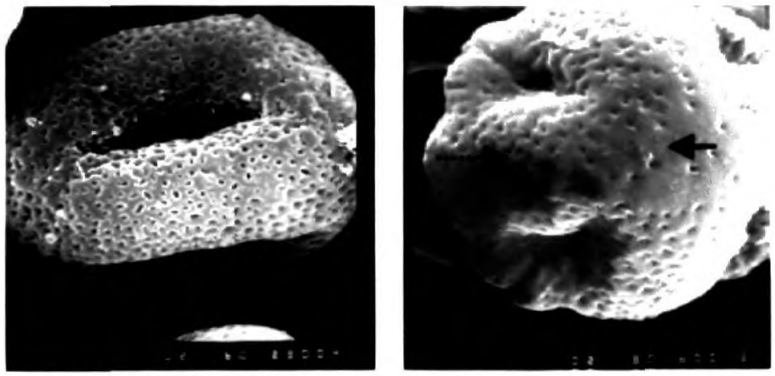
a. Pin (x 300) b. Thrum (x 300)

Fig. 9. SEM of *P. lanceolata* -acetolysed pollen grains



a. Pin (x 500) b. Thrum (x 500)

Fig.10. SEM of *P. lanceolata* pollengrains-exine pattern



a.Pin(x 600)-uniformly reticulate b. Thrum(x 800)-foveolate equatorial region(·····▶), scrobiculate polar region(→▶)

Pollen Morphology

Pollen grains of both the morphs of *Pentas lanceolata* colour variants showed pollen polymorphism. Pin pollen was found to be smaller than that of thrum in all the colour variants. The pollen grains were found to be 3-zonocolporate in pin morphs, whereas it was 4- zonocolporate in the thrum.

The exine of thrum pollen was found to be foveolate at the equatorial region with a scrobiculate area at the polar regions (Fig. 10b). The exine of pin pollen was uniformily reticulate (Fig. 10a). The presence of a centrally placed structure in the aperture is another distinguishing feature of thrum pollen (Fig. 9b), which was absent in pin pollen (Fig. 9a).

Cytology

All the fertile, heteromorphic varieties showed a normal cytological behaviour (Fig. 11) with a chromosome number 2n = 20. The sterile red colour variant was seen to show cytological aberrations (Fig. 12 a-d).

Anatomy

In both the morphs, the style was solid with uniform type of thin walled parenchymatous cells, which were more or less elongated. The

Fig. 11. ***P. lanceolata*** **- Normal meiosis**

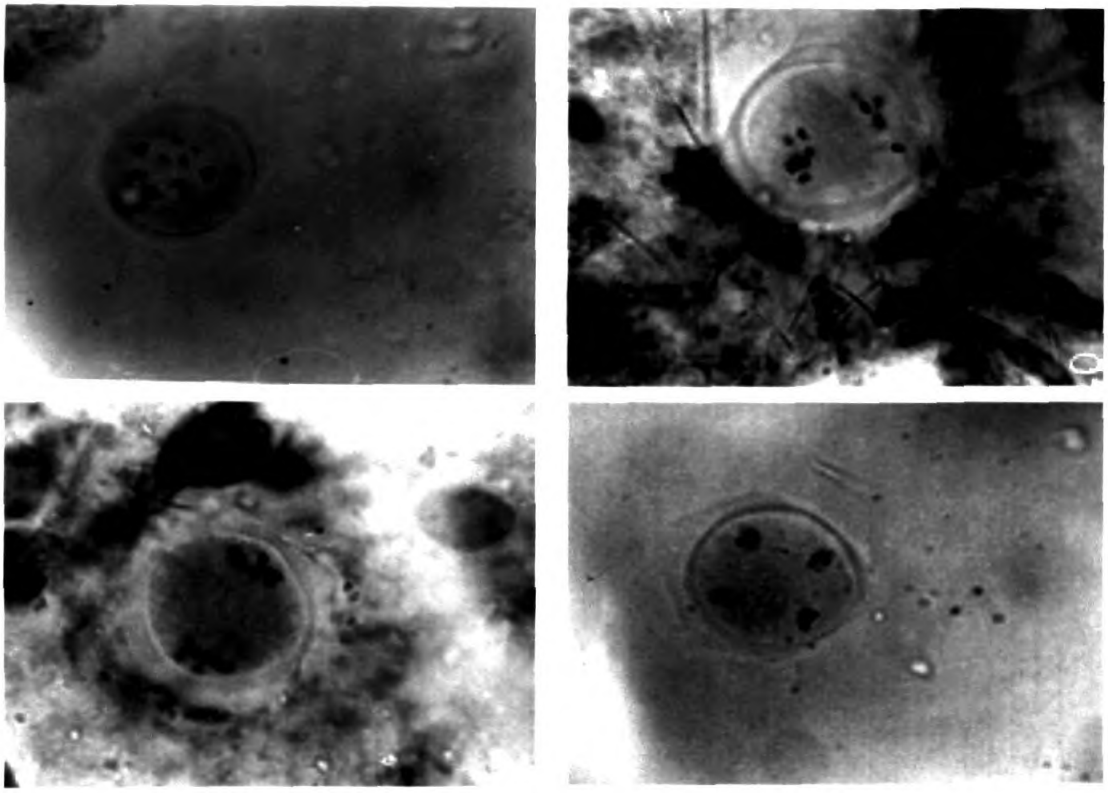
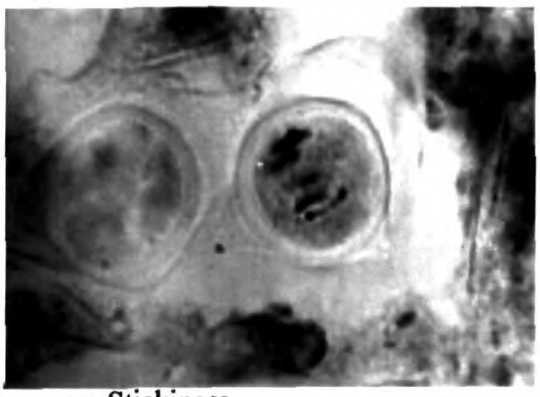
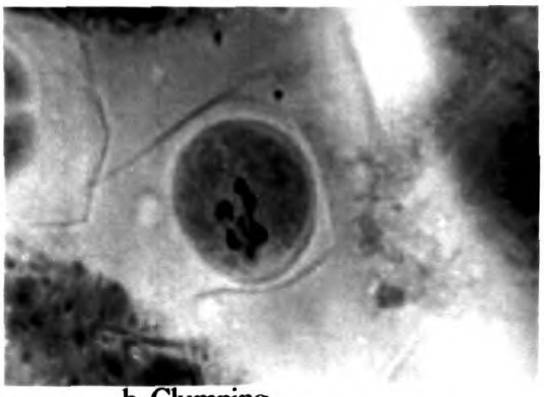


Fig. 12. ***P. lanceolata*** **- Meiotic aberrations**

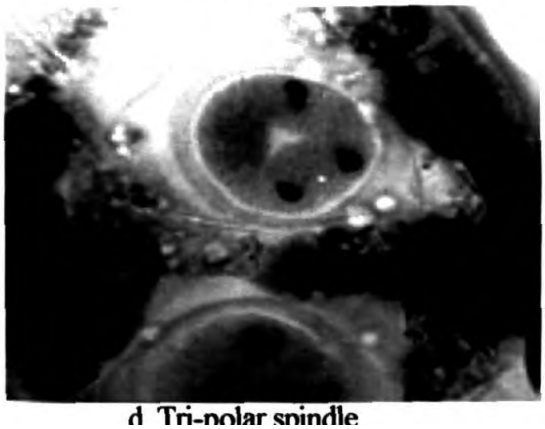


a. Stickiness



b. Clumping

c. Uneven separation



d. Tri-polar spindle

stylar cells of pin were longer than that of thrum. A vascular strand was seen extending upto the stigma.

Physiology

The different colour variants examined during this study showed a considerable degree of difference with regard to their physiological characters like the percentage of pollen viability, crossability and fruit set. Hence the different colour variants were considered separately with regard to the above mentioned characters.

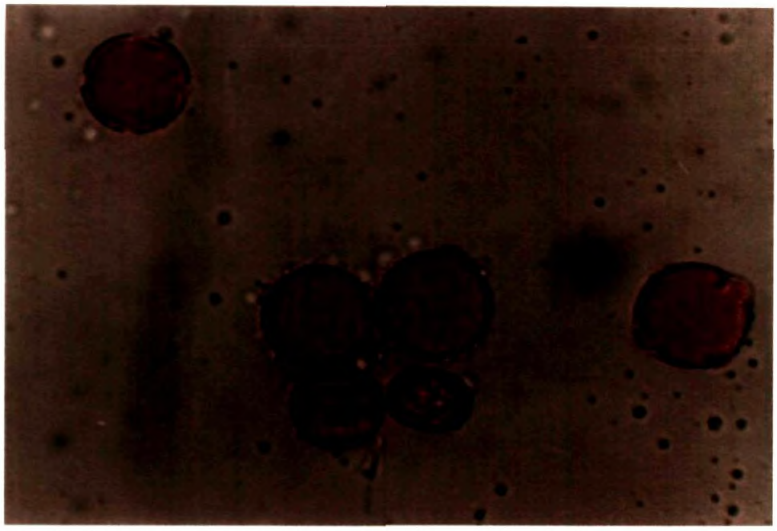
Pentas lanceolata var. white

In vitro germination studies

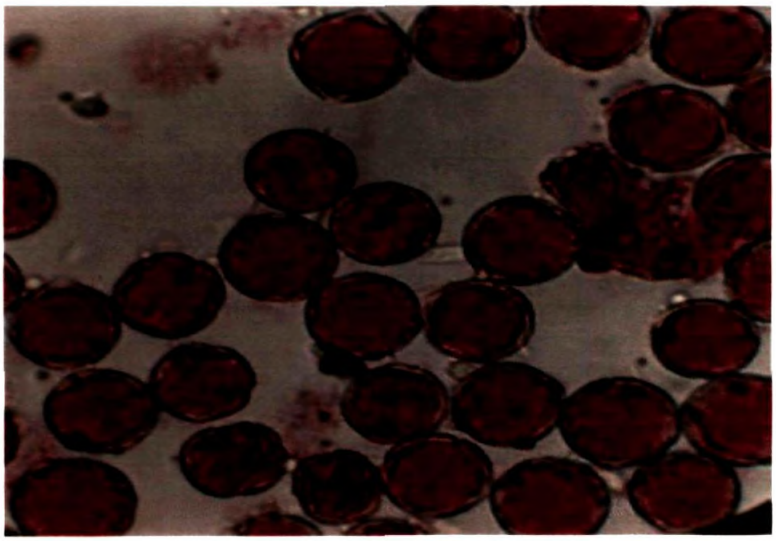
Pollen stainability with acetocarmine was 73.6% (Fig. 13a) in pin flowers of this variety. *In vitro* germination in Brewbaker's medium with 20% sucrose showed the maximum percentage of germination upto 62.87% (Fig. 15a).

In thrum plants, pollen stainability was almost equal to its pin morph, 71.84% (Fig. 14a) while *in vitro* germination was reduced to 53.88% (Fig. 15b).

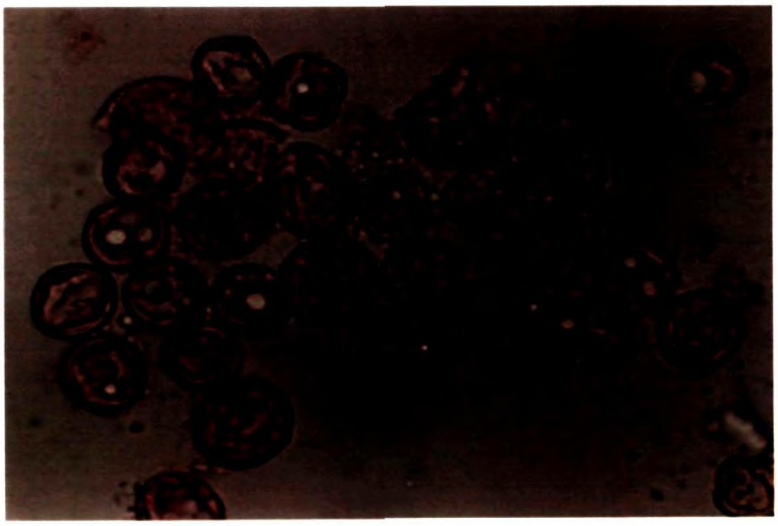
Fig. 13. Acetocarmine stained pin pollen grains of *P. lanceolata* (x 450)



a. *P. lanceolata* var. white-showing pollen polymorphism

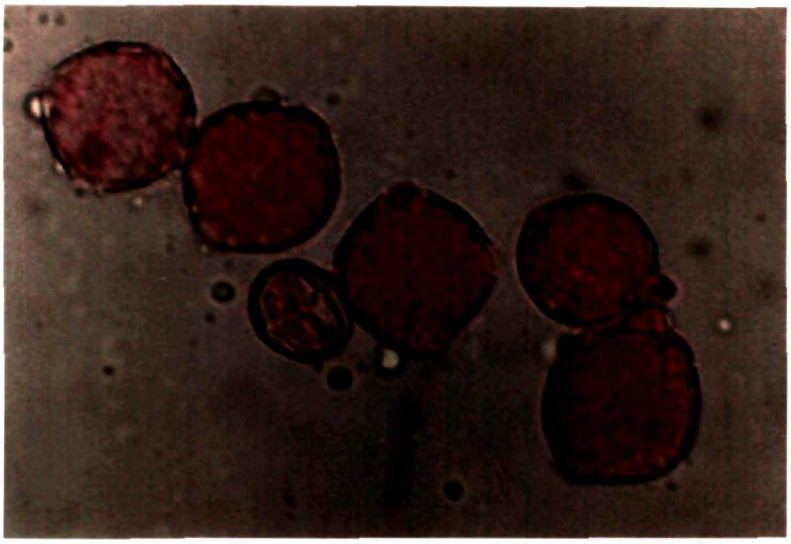


b. *P. lanceolata* var. lilac

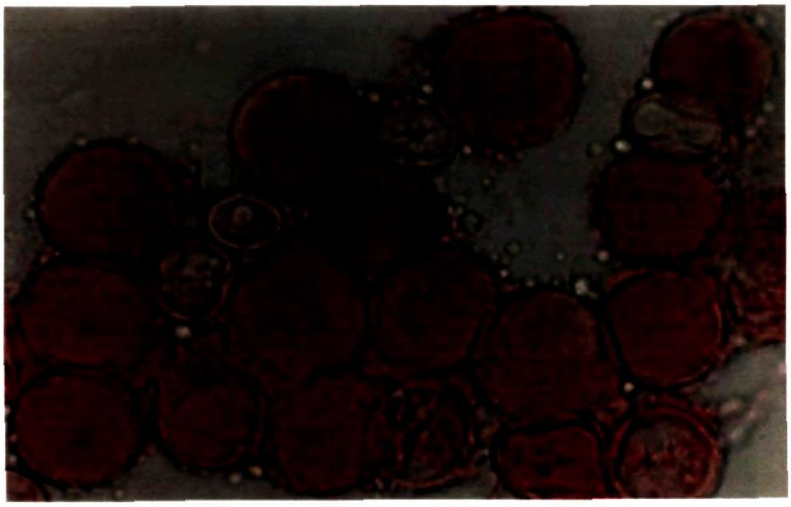


c. *P. lanceolata* var. magenta

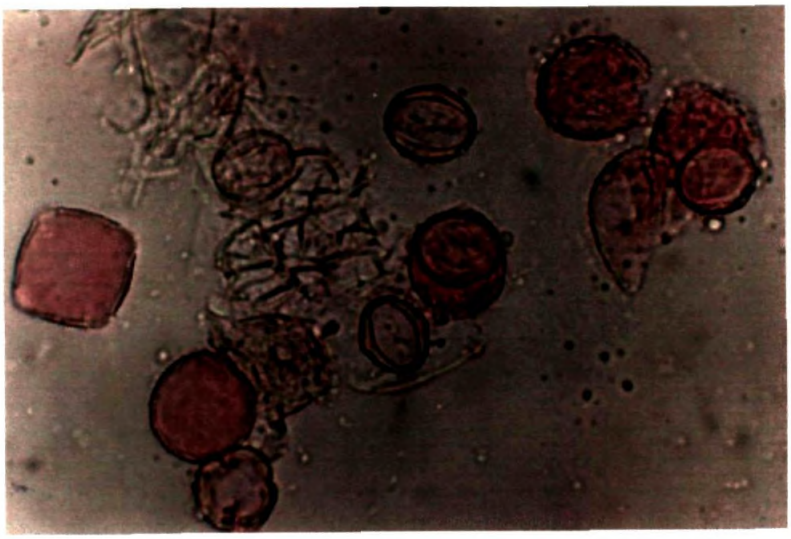
Fig.14. Acetocarmine stained thrum pollen grains of *P. lanceolata* showing polymorphism (x 450)



a. *P. lanceolata* var. white



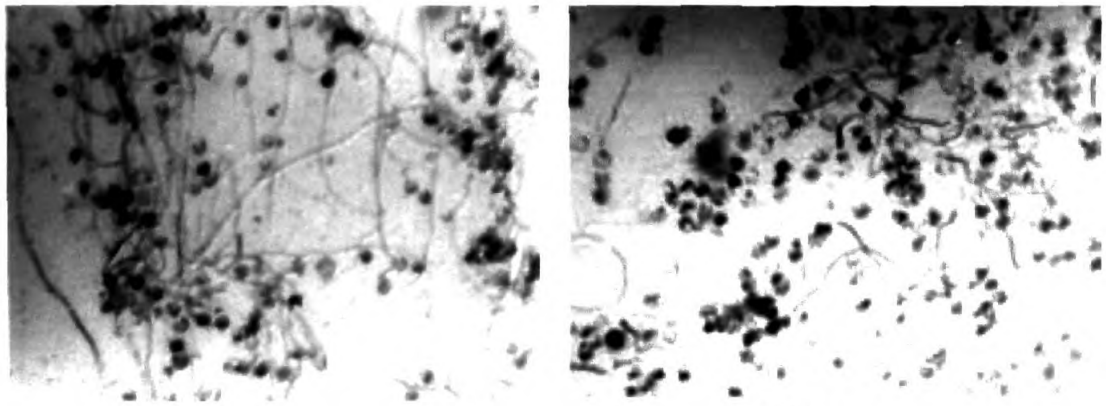
b. *P. lanceolata* var. lilac



c. *P. lanceolata* var. magenta

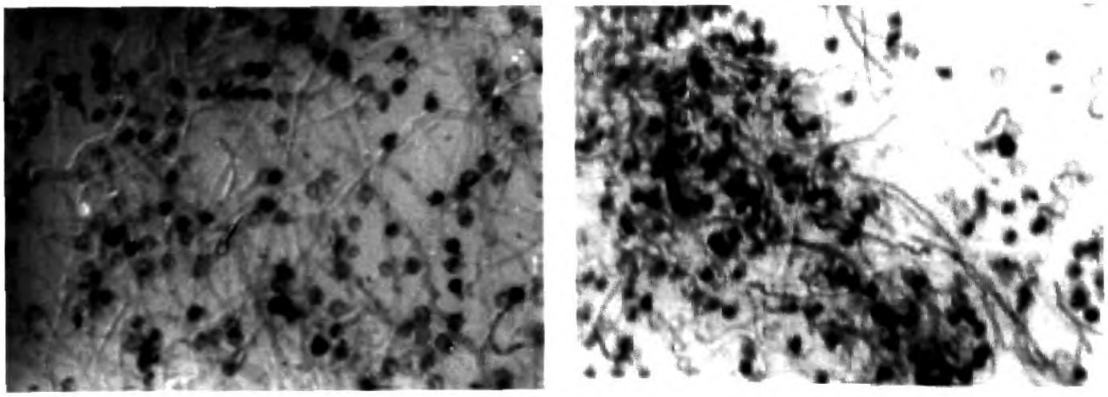
Fig. 15. *In vitro* germination of pollen grains in Brewbaker's medium (x 100)

***P. lanceolata* var. white**



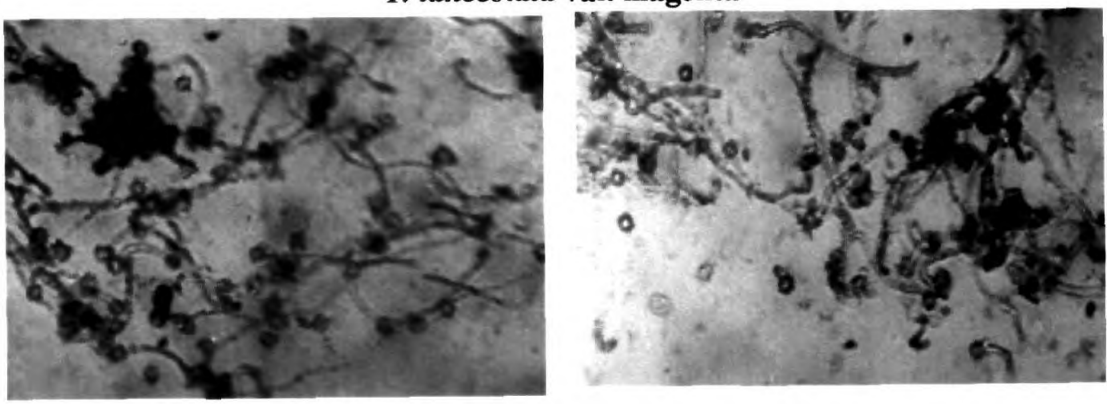
a. Pin b. Thrum

***P. lanceolata* var. lilac**



c. Pin d. Thrum

***P. lanceolata* var. magenta**



e. Pin f. Thrum

In vivo germination studies

The results of *in vivo* germination studies carried out both in the laboratory and in the field were found to be more or less similar in all the colour variants studied.

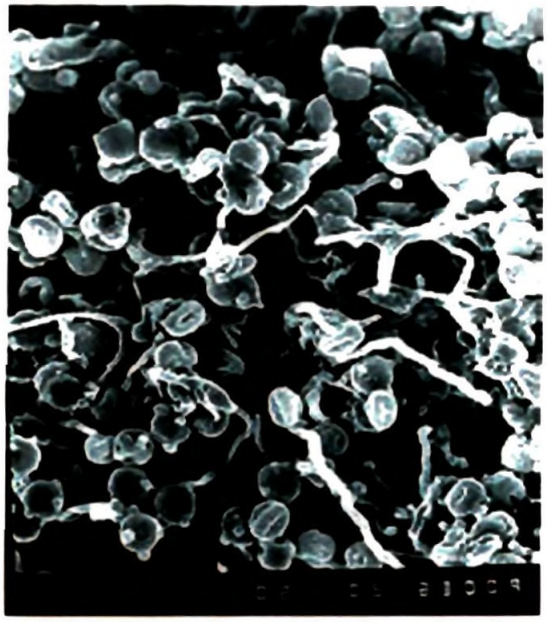
Laboratory Studies

Intra morph - intra varietal crosses

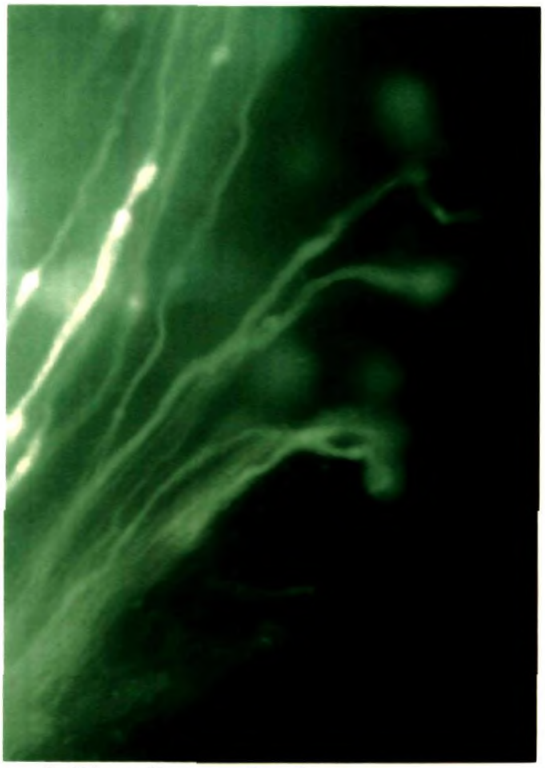
The stigma of *P. lanceolata* var. white, pin showed a very good percentage of self-pollen adherence. The percentage of pollen germination was 56.6 and the mean tube length after 8 h and 24 h were 11.4 mm and 30.3 mm respectively (Fig. 16 a-d, 38 ; Table 8). Numerous pollen tubes were seen to grow through the style and reaching beyond the base, which showed callose plugs interspersed at regular and large intervals (Fig. 16c-d).

P. lanceolata var. white, thrum stigma showed a poorer percentage of self pollen adherence, compared to pin stigma. The percentage of self pollen germination was 38.3 and the mean tube length after 8 h and 24 h were 9.8 mm and 17.8 mm respectively (Fig. 17a-b, 40 ; Table 8). The pollen tubes were not seen to traverse the whole length of the style.

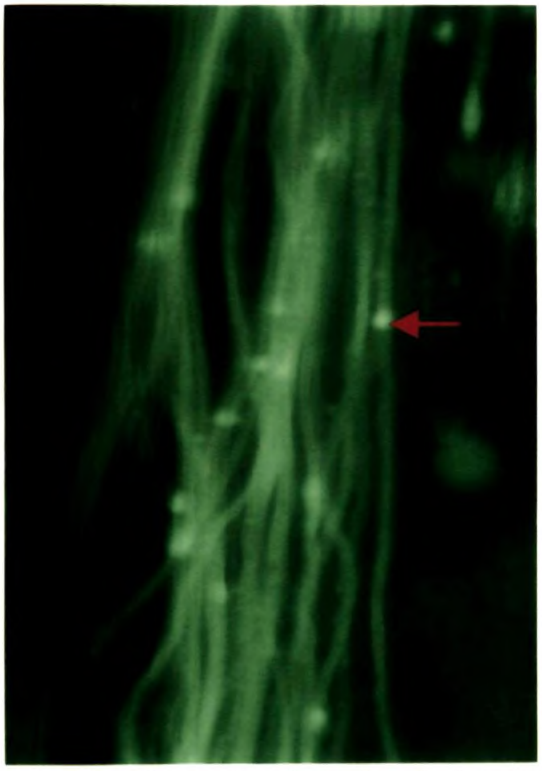
Fig. 16. *In vivo* pollen tube growth in *P. lanceolata* var. white
(b-d under UV illumination x 300)



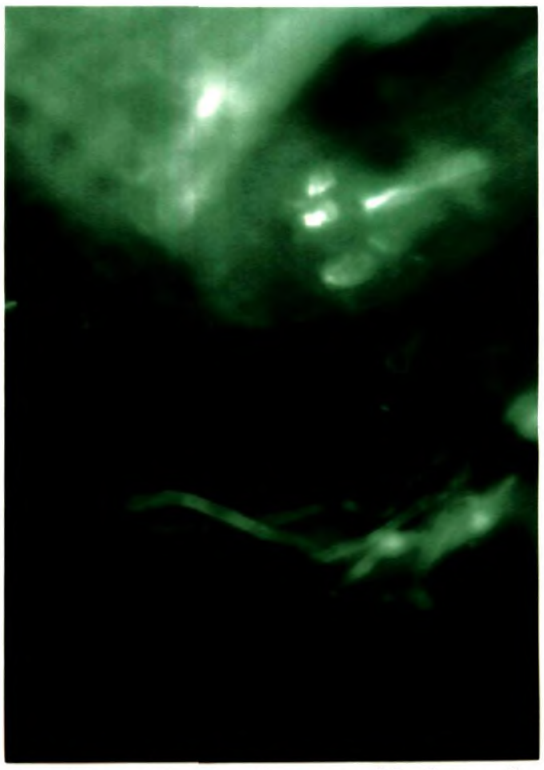
a. SEM of pin pollen on pin stigma showing a high percentage of pollen germination.



b. Pin pollen on pin stigma

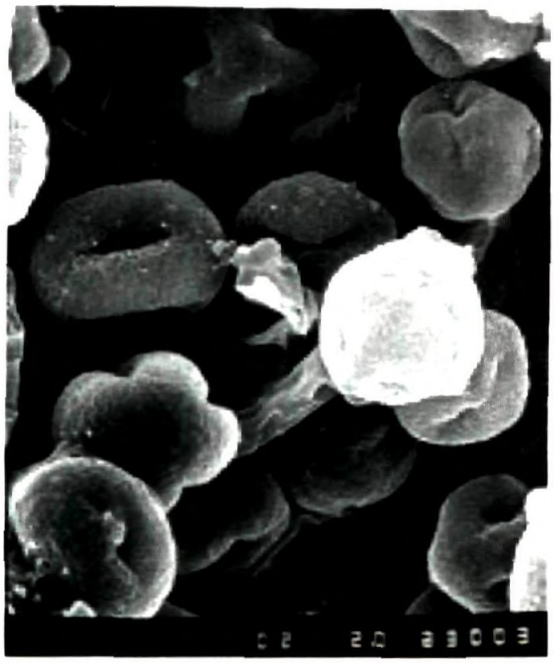


c. Pollen tube passing through style showing low callose deposition

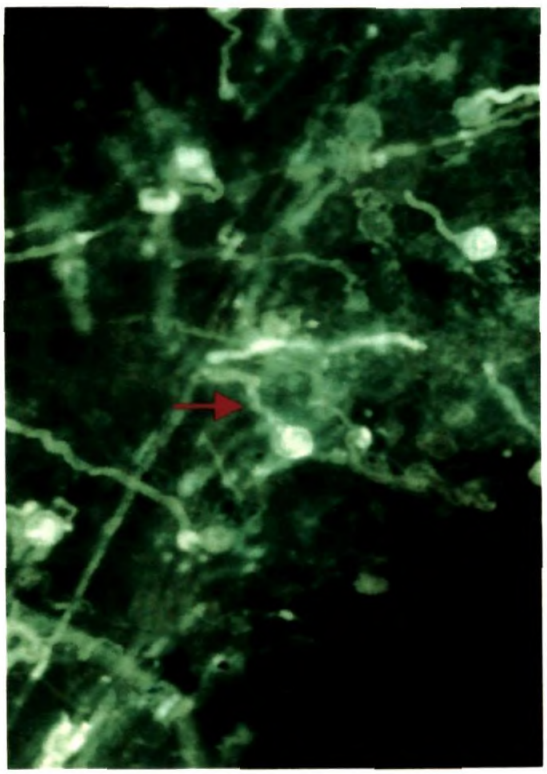


d. Pollen tube extending out of the base of style after compatible self pollination

Fig. 17. *In vivo* pollen tube growth in *P. lanceolata* var. white
(b. under UV illumination x 300)



a. SEM of thrum pollen on thrum stigma showing low pollen germination



b. Incompatible thrum pollen on thrum stigma showing short and curled pollen tubes

Table. 8. *Pentas lanceolata* - var. white intra-morph crosses

Combinations	% of pollen germination	Tube length (mm)	
		8 h	24 h
$W_P \times W_P$	56.6	11.4 ± 0.61	30.3 ± 1.06
$W_P \times L_P$	49.8	10.4 ± 0.59	25.8 ± 0.93
$W_P \times M_P$	18.5	7.5 ± 0.36	12.0 ± 0.63
$W_P \times R_P$	No germination	0.0	0.0
$W_T \times W_T$	38.3	9.8 ± 0.42	17.9 ± 0.89
$W_T \times L_T$	18.4	3.2 ± 0.18	11.8 ± 0.73
$W_T \times M_T$	9.5	2.5 ± 0.11	4.6 ± 0.25

All values represent a mean of 6 replicates

Table. 9. *Pentas lanceolata* - var. white inter-morph crosses

Combinations	% of pollen germination	Tube length (mm)	
		8 h	24 h
$W_P \times W_T$	34.10	56.2 ± 1.23	16.13 ± 0.65
$W_P \times L_T$	40.90	6.5 ± 0.23	18.9 ± 0.74
$W_P \times M_T$	18.50	5.0 ± 0.19	10.9 ± 0.59
$W_T \times W_P$	50.8	16.1 ± 0.56	25.5 ± 0.98
$W_T \times L_P$	50.1	15.0 ± 0.62	20.4 ± 0.87
$W_T \times M_P$	28.5	9.0 ± 0.31	17.1 ± 0.81
$W_T \times R_P$	17.0	2.1 ± 0.08	3.6 ± 0.12

All values represent a mean of 6 replicates

Intra morph - inter varietal crosses

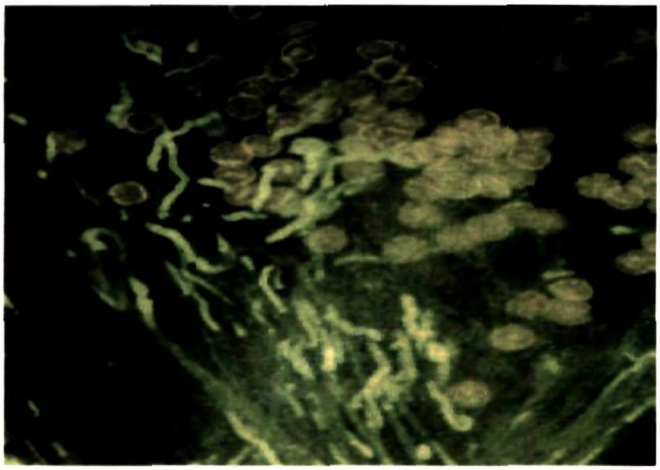
Pentas lanceolata var. white, pin stigma showed a good percentage of adherence of pin pollen from varieties lilac and magenta flowers. The germination percentage of lilac pin pollen was 49.8. The mean tube length after 8 h and 24 h were 10.4 mm and 25.8 mm respectively (Table 8). The percentage of magenta pin pollen germination was 18.5. The mean tube length after 8 h and 24 h were 7.5 mm and 12.0 mm respectively. The white pin variety when pollinated with pin pollen from var. red, showed poor adherence and no pollen germination (Table 8).

The thrum stigma of *P. lanceolata* var. white showed a lesser percentage of adherence of thrum pollen from lilac and magenta varieties. The percentage of germination of lilac thrum pollen was 18.4 and the mean tube length after 8 h and 24 h were 3.2 mm and 11.8 mm respectively (Fig. 18a ; Table 8). The germination percentage of magenta thrum pollen was only 9.5 and the mean tube length after 8 h and 24 h were 2.5 mm and 4.6 mm respectively (Fig. 18b ; Table 8).

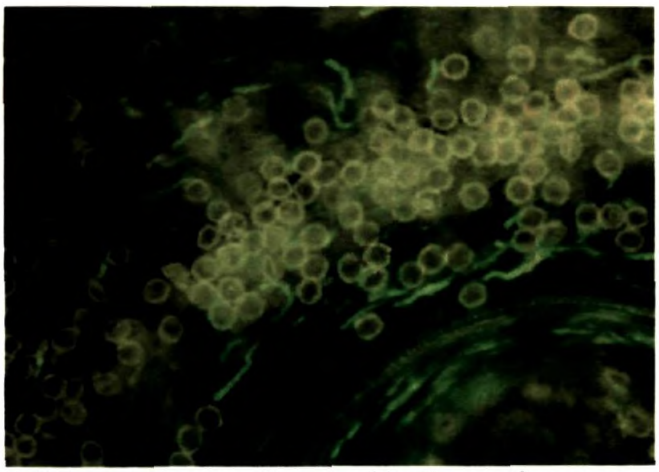
Inter morph- intra varietal crosses

P. lanceolata var. white, pin stigma showed lesser percentage of pollen adherence when pollinated with pollen from var. white thrum flowers. The percentage of pollen germination was 34.10. The mean

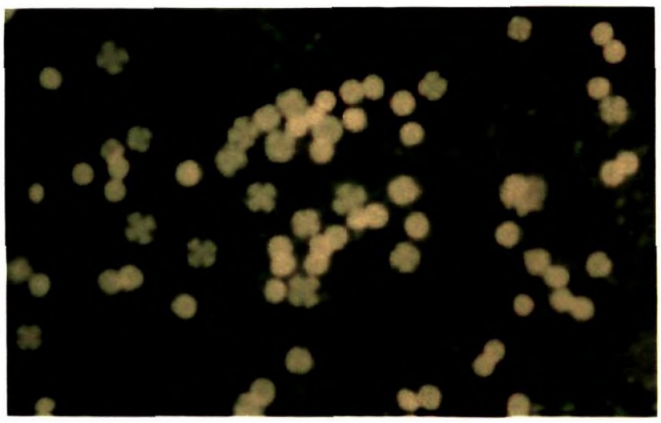
Fig. 18. *P. lanceolata*-Incompatible combinations
(a - c under UV illumination x 200)



a. Lilac thrum pollen on white thrum stigma showing more deposition of callose in pollen tubes



b. Magenta thrum pollen on white thrum stigma showing very short pollen tubes



c. Red pin pollen on magenta pin stigma showing no pollen germination

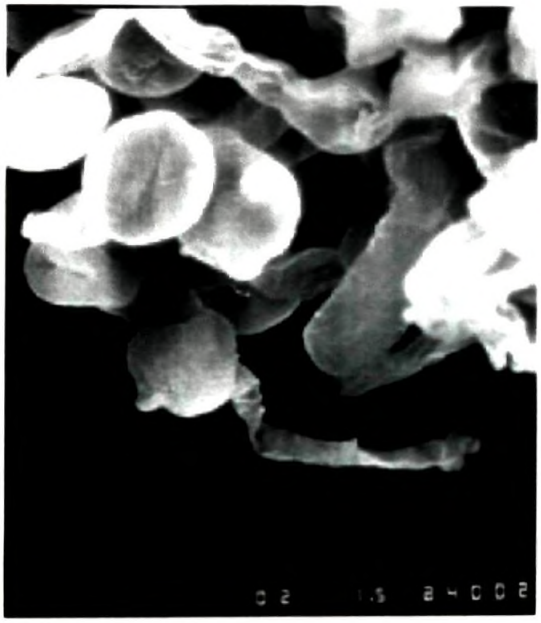
tube length after 8 h was 10.6 mm and after 24 h it was 26.1 mm (Fig. 19a-d, 39 ; Table 9). Many pollen tubes passed through the style and reached the base . The pollen tubes however showed larger and irregular callose plugs (Fig. 19c-d).

P. lanceolata var. white thrum stigma showed a higher percentage of pollen adherence and germination with pin pollen from var. white. The percentage of germination was 50.8 and the mean tube length after 8 h and 24 h were 16.1 mm and 25.4 mm respectively (Fig. 20a-d, 41;Table 9). More than 50% of pollen tubes passed through the style and extended beyond the base. The pollen tube showed very smaller and regularly arranged callose plugs, which were slightly bigger as it, reached the base (Fig. 20 c-d).

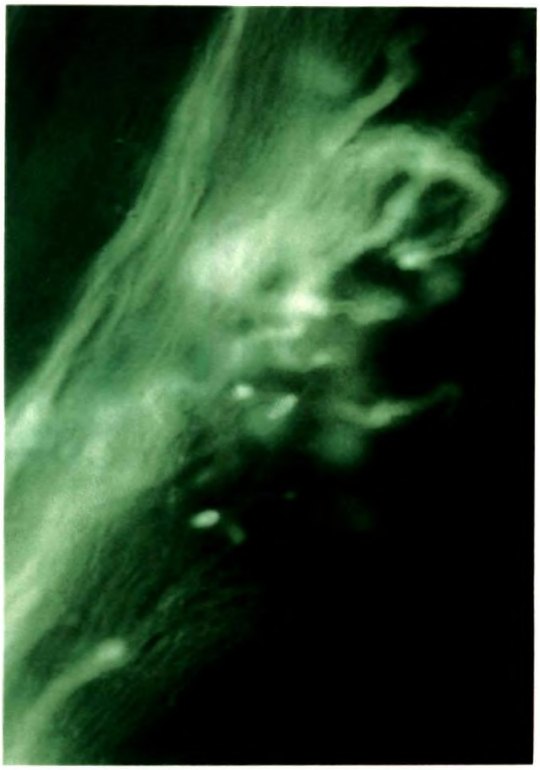
Inter morph – inter varietal crosses

When *P. lanceolata* var. white pin flowers were crossed with pollen from the thrum flowers of var. lilac, the percentage of adherence and pollen germination were found to be lesser than that of self pollen. The percentage of thrum pollen germination was 40.9 and the mean tube length after 8 h and 24 h of pollination were 6.5 mm and 18.9 mm respectively (Table 9).

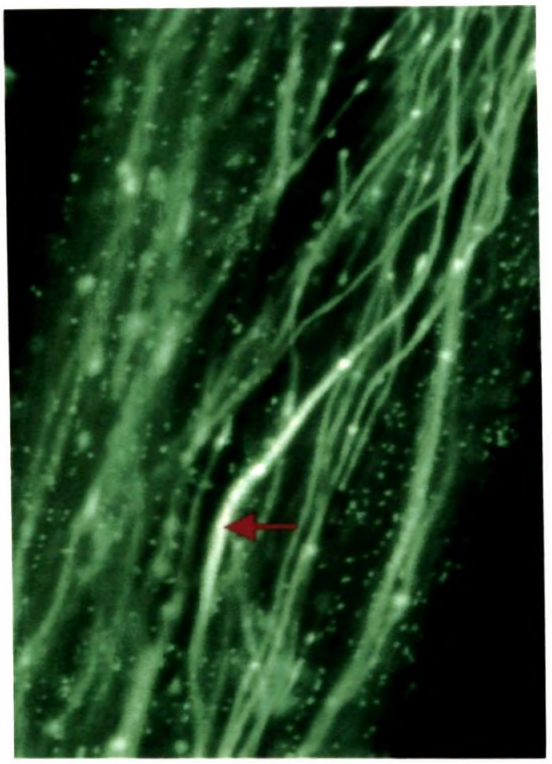
Fig. 19. *In vivo* pollen tube growth in *P. lanceolata* var. white
(b - d under UV illumination x 300)



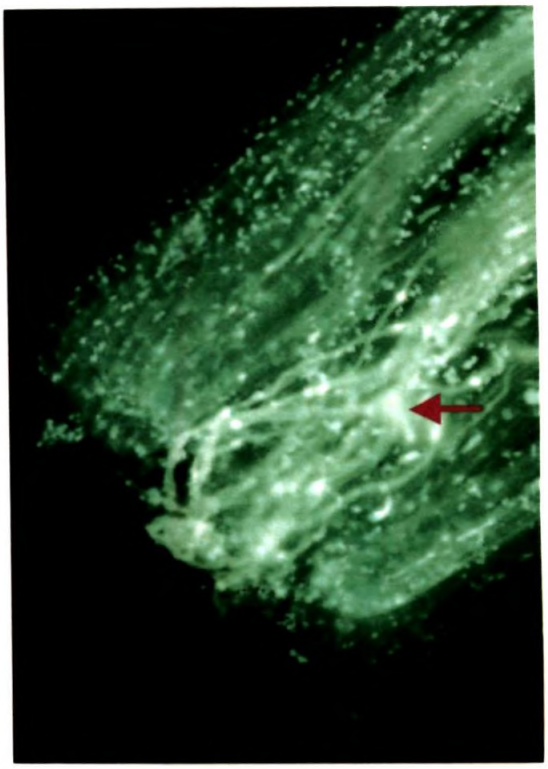
a. SEM of thrum pollen on pin stigma



b. Thrum pollen on pin stigma

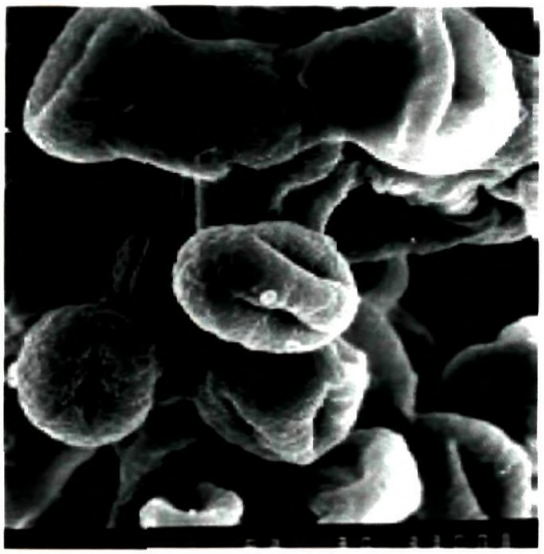


c. Pollen tube passing through style showing irregular deposition of callose

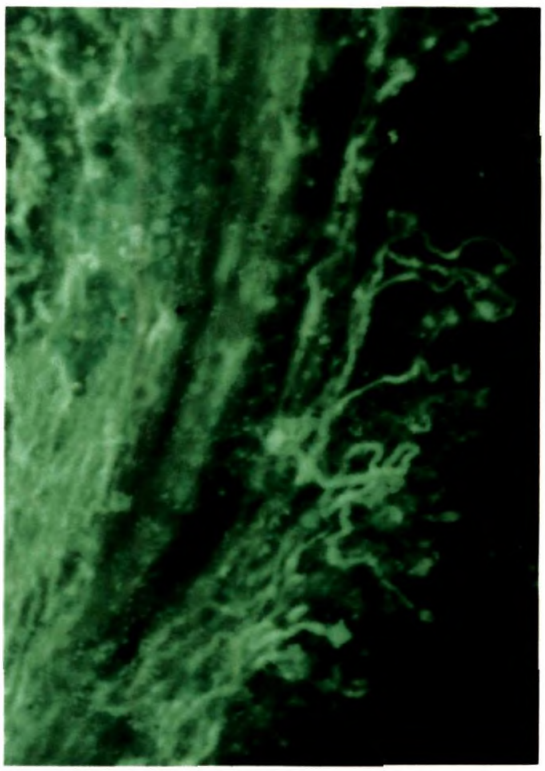


d. pollen tube at the base of style showing more callose depositions

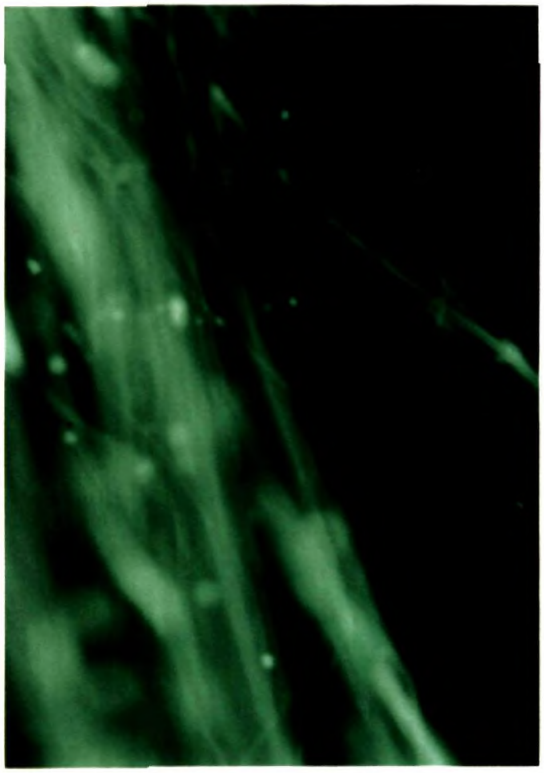
Fig. 20. *In vivo* pollen tube growth in *P. lanceolata* var. white
(b - d under UV illumination x 300)



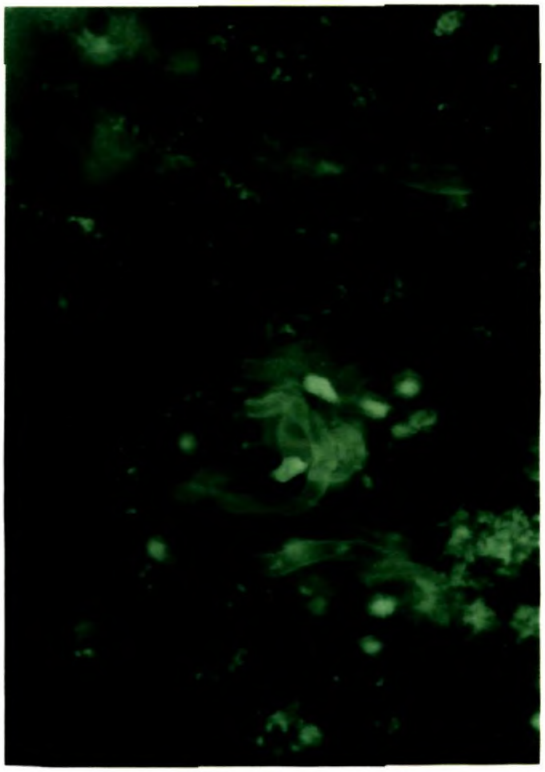
a. SEM of pin pollen on thrum stigma



b. Pin pollen on thrum stigma



c.Pollen tube passing through style showing low deposition of callose



d. Pollen tubes extending out of the base of style after compatible cross pollination

A cross of *P. lanceolata* var. white pin flowers with thrum flowers of var. magenta showed a very low percentage of pollen germination, 18.5, even though the pollen adherence was better. The mean tube length after 8 h was 5.0 mm and 24 h was 10.9 mm (Table 9).

Pentas lanceolata var. white thrum flowers when crossed with pollen from pin flowers of var. lilac, the percentage of pollen adherence and pollen germination was found to be fairly high. The percentage of pollen germination was 50.1 and the mean tube length after 8 h and 24 h were found to be 15.0 mm and 20.4 mm respectively (Table 9).

The thrum flowers of *P. lanceolata* var. white when crossed with pollen from the pin flowers of var. magenta, the percentage of pollen adherence and pollen germination was found to be low. The percentage of germination was found to be only 28.5 and the mean tube length after 8 h was 9.0 mm and after 24 h it was 17.1 mm (Table 9). The thrum flowers of var. white when crossed with pollen from red pin, the pollen adherence and germination were less. The percentage of pollen germination was 17 and the mean tube length after 8 h and 24 h were 2.1 mm and 3.6 mm respectively.

Field studies

Only intra morph-intra varietal and inter morph-intra varietal pollinations were carried out in the field. Of the 100 pollinated pistils, 50 were left on the plants until drying or fruit maturity and 50 were fixed after 8 h and 24 h after pollination for studies on *in vivo* pollen germination and pollen tube growth. The results were more or less the same in both field and laboratory studies.

The fruit set data was studied in the following crosses of *P. lanceolata* var. white pin. A set of fifty white pin flowers was selfed with pin pollen and another set of fifty flowers was crossed with thrum pollen var. white. The reciprocal crosses were also made using *P. lanceolata* var. white thrum as female parent, and pollinated with thrum pollen and pin pollen from var. white. *P. lanceolata* var. white pin on selfing showed 72.2 % fruit set and on crossing it was reduced to 64.3 % (Fig. 37). The result of selfing was better than that of crossing. But in *Pentas lanceolata* var. white thrum selfing showed only 64.7 % of fruit set, whereas it was enhanced to 70.6% after crossing. The percentage of fruit set was more in the case of crossing in thrum plants (Table 14).

Index of self incompatibility

The index of self incompatibility in *P. lanceolata* var. white, pin and thrum flowers were found out using the formula of Zapota and Arroyo. The index of self incompatibility was 1.125 in *P. lanceolata* var. white pin which proves that it is preferentially selfed. The index of self incompatibility in *P. lanceolata* var. white, thrum was 0.678, which has proved it to be only partially self compatible or preferentially cross compatible (Table 15).

Pentas lanceolata var. lilac

In vitro germination studies

Pollen stainability with acetocarmine was 83.8% in the pin flowers of this variety (Fig. 13b). *In vitro* germination in the Brewbaker's medium with 20% sucrose showed the maximum percentage of germination to be 65.8 (Fig. 15c).

Pollen stainability of thrum morph was more or less equal to that of pin morph *ie.* 82.3% (Fig. 14b); while *in vitro* germination percentage was 60.63 (Fig. 15d).

In vivo germination

The results on *in vivo* germination studies carried out both in the laboratory and in the field were found to be similar.

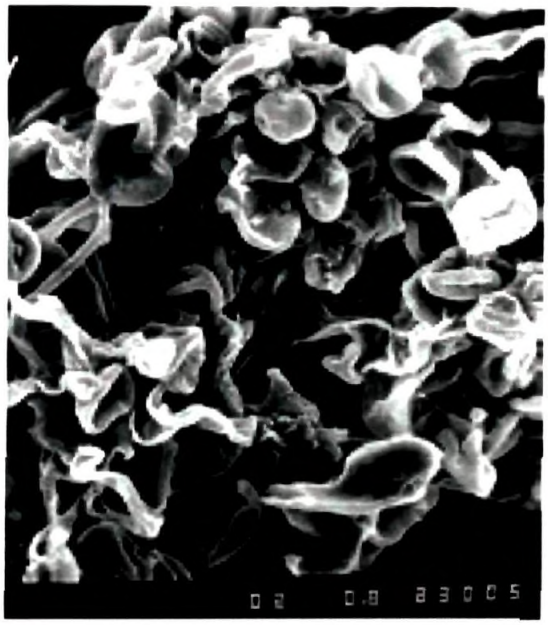
Laboratory studies

Intra morph - intra varietal crosses

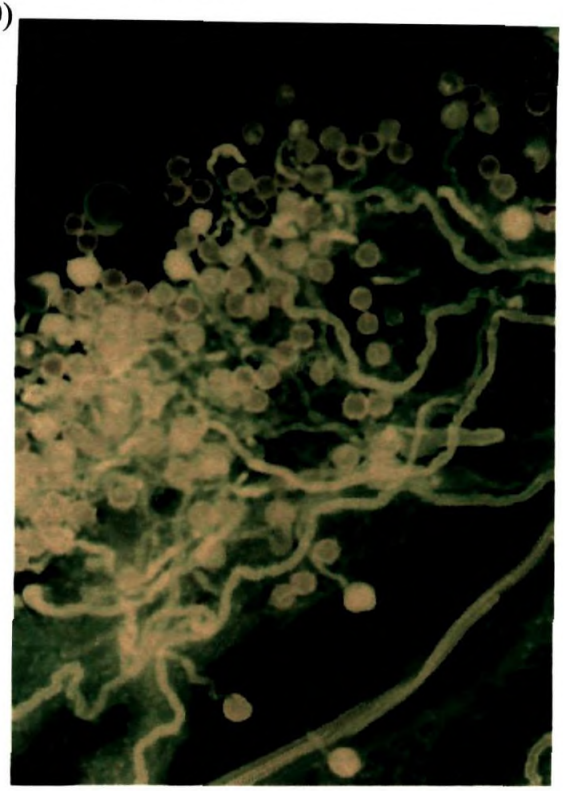
The pin stigmas showed very good percentage of self pollen adherence. The germination percentage was 68.4. The mean tube length after 8 h was 20.12 mm and after 24 h it was 37.04 mm (Fig. 21 a-d, 38 ; Table 10). Most of the germinated pollen produced tubes all of which passed through the style and reached beyond the base. The tubes showed only very little callose plug deposition (Fig. 21c-d).

In thrum flowers, the self pollen adherence was found to be poor, when compared to that of pin. The pollen germination percentage was reduced to 37.1 with a corresponding reduction in tube elongation which was 8.2 mm and 16.25 mm after 8 h and 24 h respectively (Fig. 22a-d ; Table 10). The pollen tubes passing through the style showed a greater deposition of the callose plugs which were arranged irregularly (Fig. 22c). The callose deposition increased as the tubes reached the base (Fig. 22d).

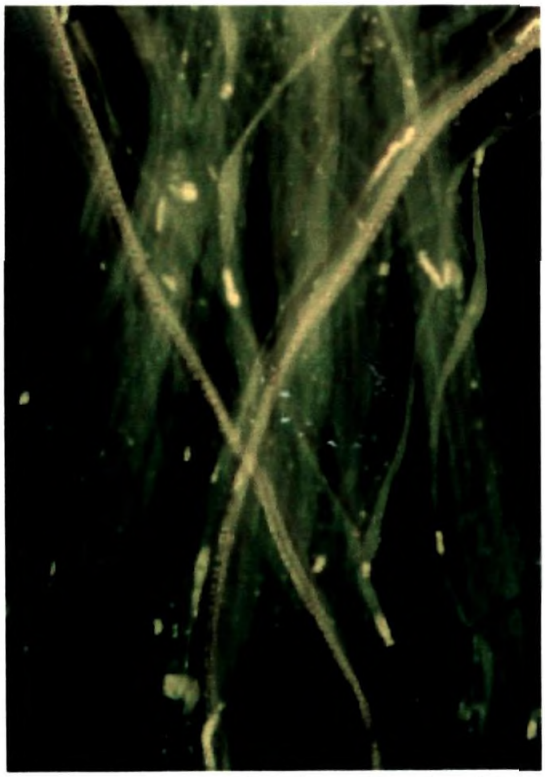
Fig. 21. *In vivo* pollen tube growth in *P. lanceolata* var. lilac
(b - d under UV illumination x 200)



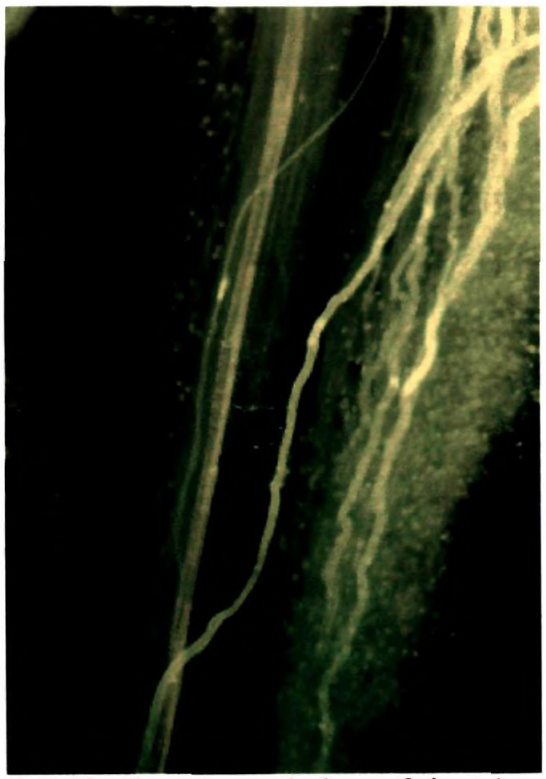
a. SEM of pin pollen on pin stigma



b. Pin pollen on pin stigma

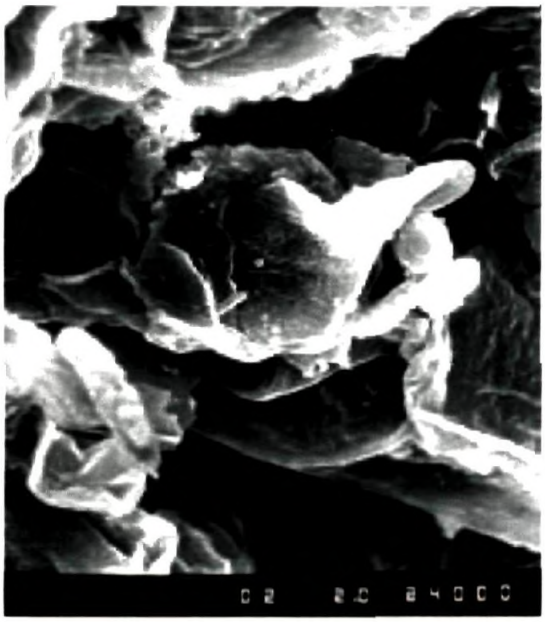


c. Pollen tubes passing through style with regular and low callose deposition

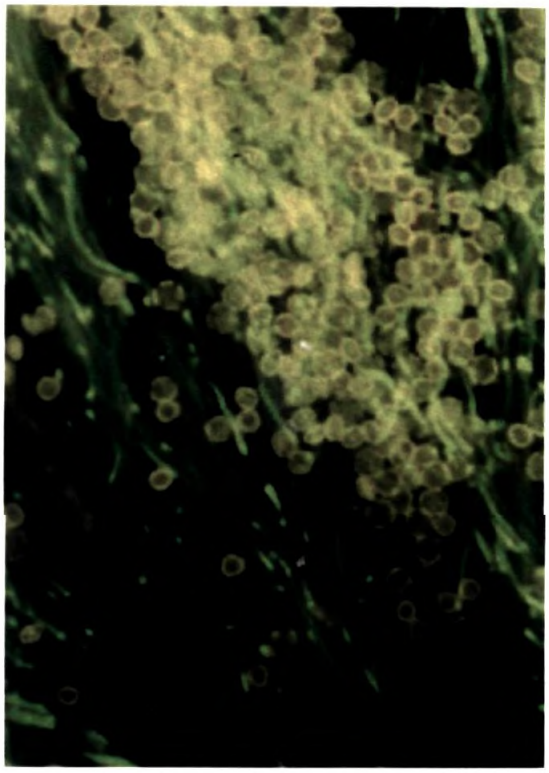


d. Self pollen tubes at the base of pin style after compatible pollination

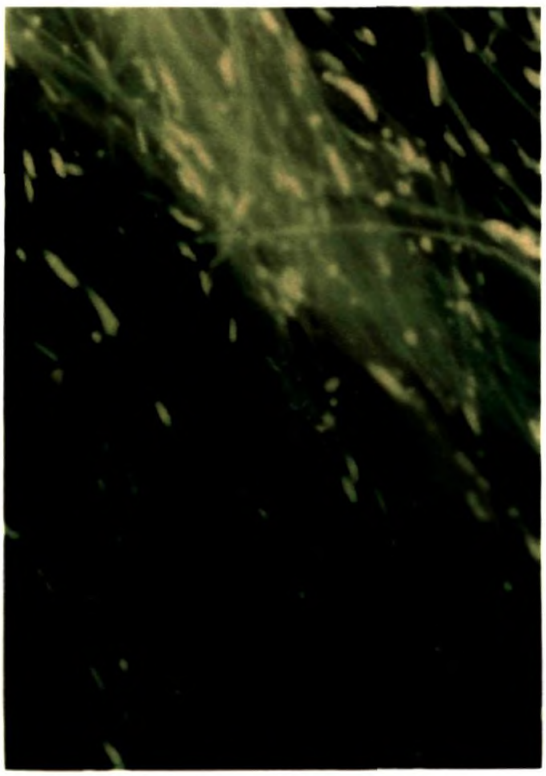
Fig. 22. *In vivo* pollen tube growth in *P. lanceolata* var. lilac
(b - d under UV illumination x 200)



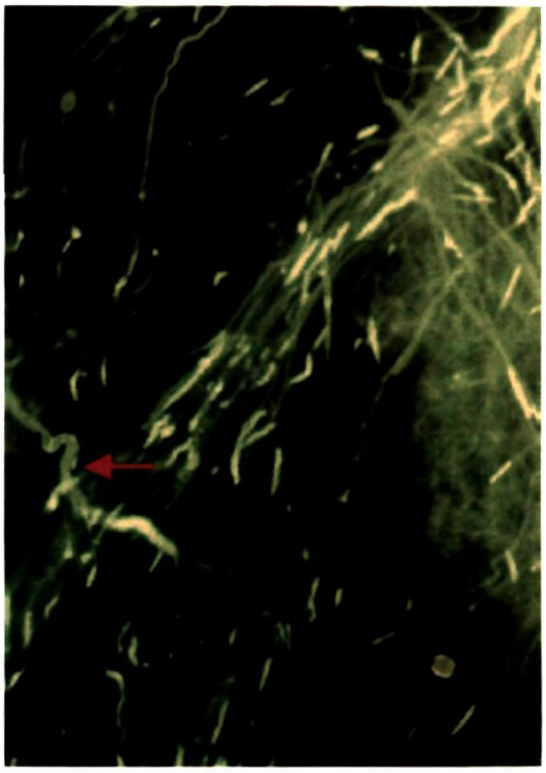
a. SEM of thrum pollen on thrum stigma



b. Thrum pollen on thrum stigma



c. Pollen tubes through style showing irregular and frequent callose plugs



d. Curled and irregular self pollen tubes at the base of style

Table. 10. *Pentas lanceolata* var. lilac intra-morph crosses

Combinations	% of pollen germination	Mean tube length (mm)	
		8 h	24 h
$L_P \times L_P$	68.4	20.12 ± 0.92	37.04 ± 0.95
$L_P \times W_P$	58.4	15.18 ± 0.76	25.99 ± 0.89
$L_P \times M_P$	19.7	7.85 ± 0.53	13.63 ± 0.69
$L_P \times R_P$	No germination	0.0	0.0
$L_T \times L_T$	37.1	8.2 ± 0.54	16.25 ± 0.71
$L_T \times W_T$	20.5	3.94 ± 0.21	9.69 ± 0.35
$L_T \times M_T$	18.4	3.31 ± 0.18	7.46 ± 0.45

All values represent a mean of 6 replicates

Table. 11. *Pentas lanceolata* var. lilac inter-morph crosses

Combinations	% of pollen germination	Mean tube length (mm)	
		8 h	24 h
$L_P \times L_T$	35.3	12.11 ± 0.69	24.25 ± 0.97
$L_P \times W_T$	21.2	9.67 ± 0.45	17.38 ± 0.89
$L_P \times M_T$	7.9	3.04 ± 0.26	5.86 ± 0.35
$L_T \times L_P$	53.8	18.14 ± 0.88	29.47 ± 0.98
$L_T \times W_P$	44.3	10.03 ± 0.51	20.51 ± 0.84
$L_T \times M_P$	31.2	8.56 ± 0.42	11.33 ± 0.61
$L_T \times R_P$	21.0	2.53 ± 0.22	4.07 ± 0.29

All values represent a mean of 6 replicates

Intra morph - inter varietal crosses

When *P. lanceolata* var. lilac pin flowers were pollinated with pollen from var. white pin, pollen adhered well on the stigma and the germination was 58.4%. The mean pollen tube length after 8 h was 15.18 mm and after 24 h was 25.99 mm (Table 10).

P. lanceolata var. lilac pin when crossed with pollen from var. magenta, the percentage of pollen germination was reduced to 19.7 with a low pollen adherence. The mean pollen tube length after 8 h and 24 h were 7.85 mm and 13.63 mm respectively (Table 10).

P. lanceolata var. lilac pin flowers when pollinated with pollen from var. red pin, germination was not observed even though a few pollen adhered on the stigma (Table 10).

P. lanceolata var. lilac, thrum flowers when pollinated with pollen from thrum flowers of var. white, the stigma showed poor pollen adherence and germination, 20.5%, with a mean tube length after 8 h as 3.98 mm and 24 h as 9.69 mm (Table 10).

When *P. lanceolata* var. lilac thrum flowers were crossed with pollen from var. magenta thrum, the pollen adherence and germination were very low. The percentage of germination was only 18.4 and the mean tube length after 8 h was 3.31 mm and 24 h was 7.46 mm (Table 10).

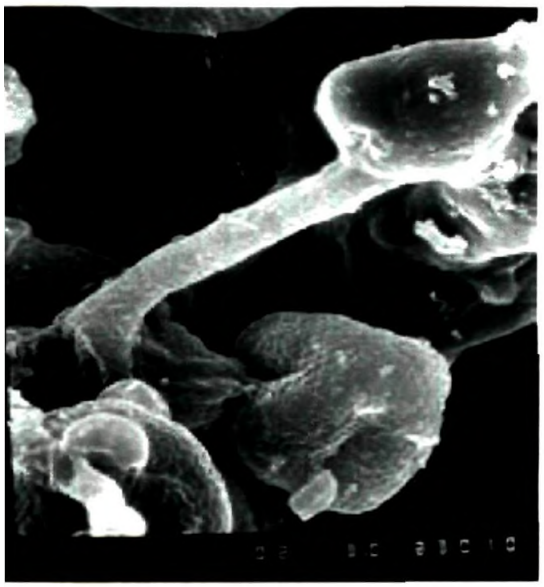
Inter morph - intra varietal crosses

Inter morph crosses of the different colour variants of *P. lanceolata* var. lilac produced the following results.

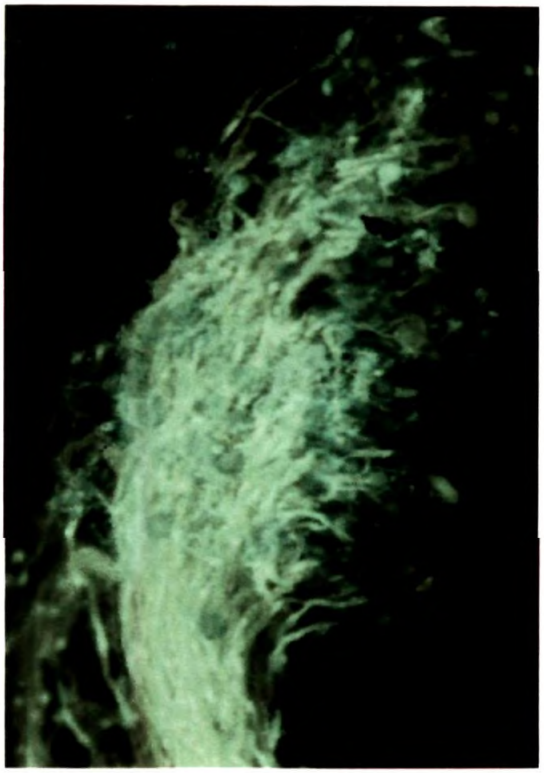
When *P. lanceolata* var. lilac coloured pin flowers were crossed with pollen from the thrum flowers of same colour variant, the percentage of pollen adherence was good. The percentage of pollen germination was 35.3 and the mean tube length after 8 h was 12.1 mm and after 24 h, it was 24.25 mm (Fig. 23a-d, 39 ; Table 11). Most of the pollen tubes passing through the style showed larger callose plugs, which were irregularly, placed (Fig. 23c). The callose deposition however was found to be less as the pollen tube reached the base (Fig. 23d).

When *P. lanceolata* var. lilac flowered thrum flowers were crossed with pollen from pin flowers of the same colour variant, the pollen adherence was better than the above cross. The percentage of pollen germination was 53.8 and the mean tube length after 8 h was 18.14 mm and that after 24 h was 29.47 mm (Fig. 24a-d, 41 ; Table 11). Larger number of pollen tubes passed through the style which showed smaller and regularly placed callose plugs (Fig. 24c). The callose plugs were larger as the tubes reached the base (Fig. 24d).

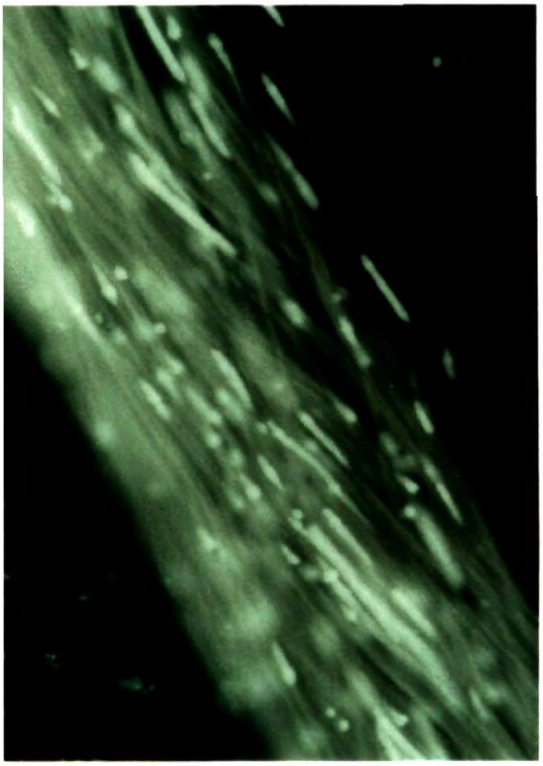
Fig. 23. *In vivo* pollen tube growth in *P. lanceolata* var. lilac
(b - d under UV illumination x 200)



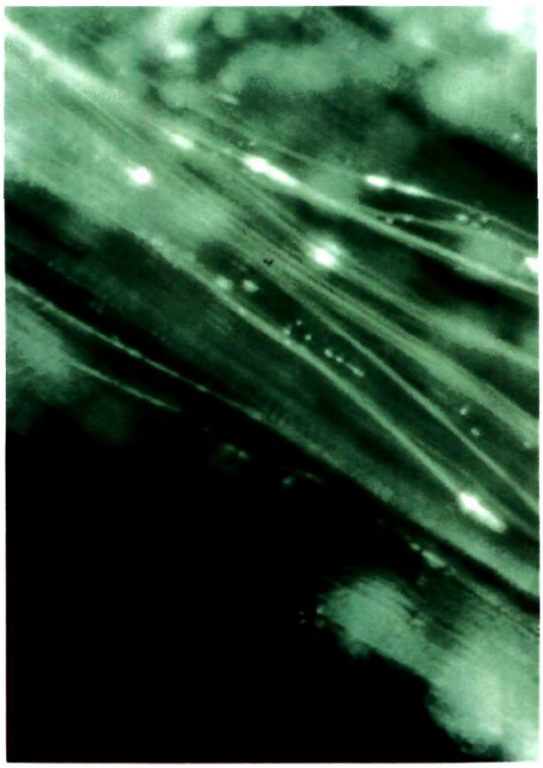
a. SEM of thrum pollen on pin stigma



b. Thrum pollen on pin stigma

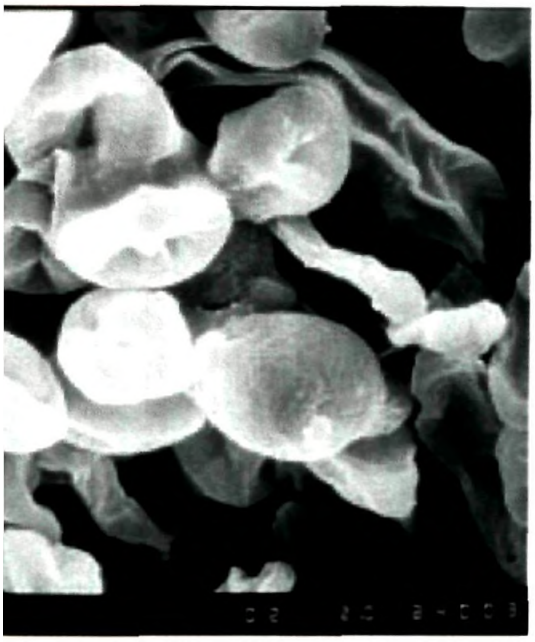


c. Pollen tubes through style showing frequent and irregular callose plugs

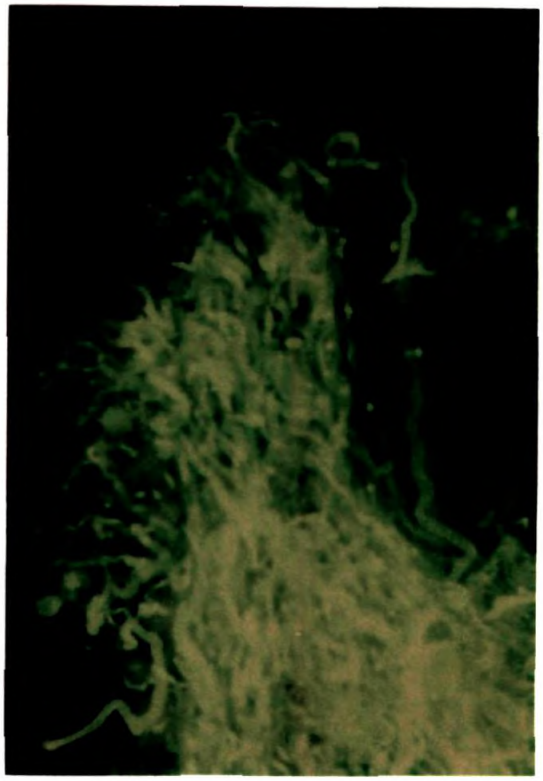


d. Pollen tubes at the base of style showing lesser callose deposition

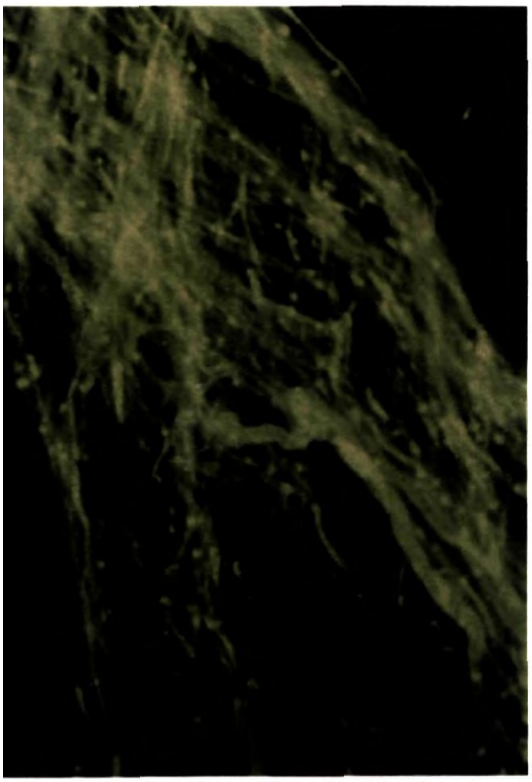
Fig. 24. *In vivo* pollen tube growth in *P. lanceolata* var. lilac
(b - d under UV illumination x 200)



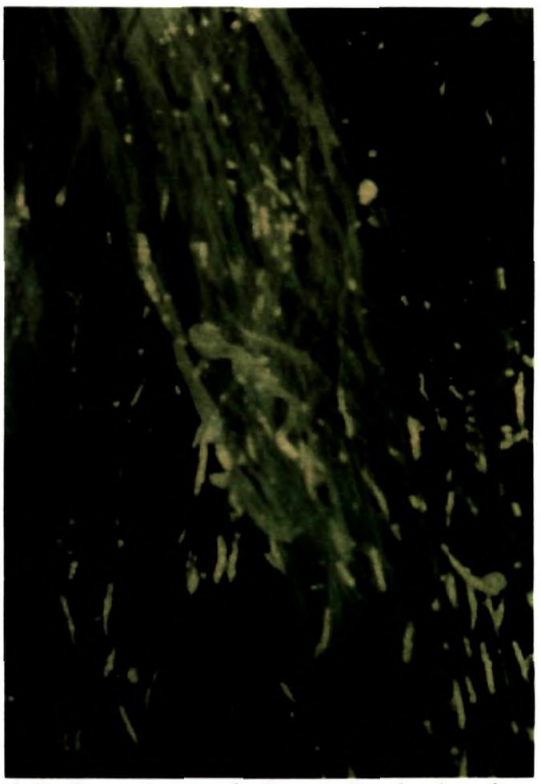
a. SEM of pin pollen on thrum stigma



b. Pin pollen on thrum stigma



c. Pollen tubes through style showing low callose deposition



d. Pollen tubes at the base of style showing more callose deposition

Inter morph - intervarietal crosses

Inter morph-intervarietal crosses between the different colour variants produced the following results.

When *P. lanceolata* var. lilac pin flowers were crossed with thrum pollen from var. white, the percentage of pollen adherence and pollen germination were less. The percentage of pollen germination was 21.2. The mean tube length after 8 h and 24 h were 9.67 mm and 17.38 mm respectively (Table 11).

The cross of lilac pin variety flowers with thrum pollen from var. magenta showed very low percentage of pollen adherence and germination. The percentage of germination was as low as 7.9 and the mean tube length after 8 h was 3.04 mm and after 12 h it was 5.86 mm (Table 11).

The cross of var. lilac thrum variety with pollen from var. white pin showed a good percentage of pollen adherence and pollen germination. The pollen germination percentage was 44.3 and the mean tube length after 8 h and 24 h were 100.3 mm and 20.51 mm respectively (Table 11).

When var. lilac thrum flowers were crossed using pollen from pin flowers of var. magenta, the pollen adherence and germination was

reduced to 31.2 with a corresponding reduction in mean tube length which was 8.56 mm and 11.33 mm after 8 h and 24 h respectively (Table 11). When lilac thrum stigmas were pollinated with red pin flowers, both pollen germination (21%) and tube elongation was reduced as 2.5 mm after 8 h and 4.07 mm after 24 h (Table 11).

Field studies

Only intra morph - intra varietal and inter morph - intra varietal pollinations were carried out in the field. Of the 100 flowers pollinated, 50 pistils were left on the plants until drying or fruit maturity and the rest were fixed after 8 h and 24 h after pollination, for studies on pollen germination and pollen tube growth. The results were in total agreement with that of laboratory studies.

The details on fruit development were closely observed and data collected in the following crosses. A set of fifty flowers of lilac pin plants was selfed with pin pollen and another set of fifty was crossed with thrum pollen from the same colour variant. The reciprocal crosses were also made in the above combinations. On selfing, pin flowers of lilac colour

variant produced 76.06% of fruits and on crossing the percentage of fruit set was reduced slightly as 72.61 (Fig. 37). But in the reciprocal cross, using lilac thrum as female parent, the fruit set was reduced to 36.62% on selfing, whereas on crossing it was increased to 70.49% (Fig. 37 ; Table 14). Hence in the case of pin morphs of lilac colour variant, fruit set was slightly increased on selfing, compared to crossing, whereas in thrum morphs crossing doubled the percentage of fruit set when compared to that of selfing.

Index of self incompatibility

The index of self incompatibility in P. lanceolata var. lilac, pin and thrum flowers were found out. The self incompatibility index was 1.138 in pin plants which proved it to be preferentially selfed. The index of self incompatibility in the thrum plants, was 0.688, which revealed that it is only partially self compatible and preferentially cross compatible (Table 15).

Pentas lanceolata var. magenta

In vitro germination studies

Pollen stainability with acetocarmine was 63.97% (Fig. 13c) but only 38.4% pollen germinated in Brewbaker's medium with 20% sucrose (Fig. 15e).

In the case of thrum pollen, stainability with acetocarmine was only 54.42% (Fig. 14c), which was less than that of pin pollen and the *in vitro* germination percentage was also reduced to 29.8 (Fig. 15f).

In vivo germination

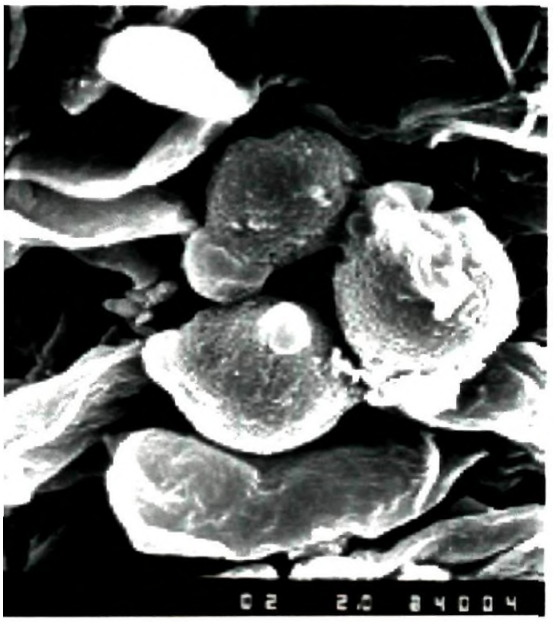
The results of *in vivo* germination carried out in the laboratory and field were found to be more or less the same.

Laboratory studies

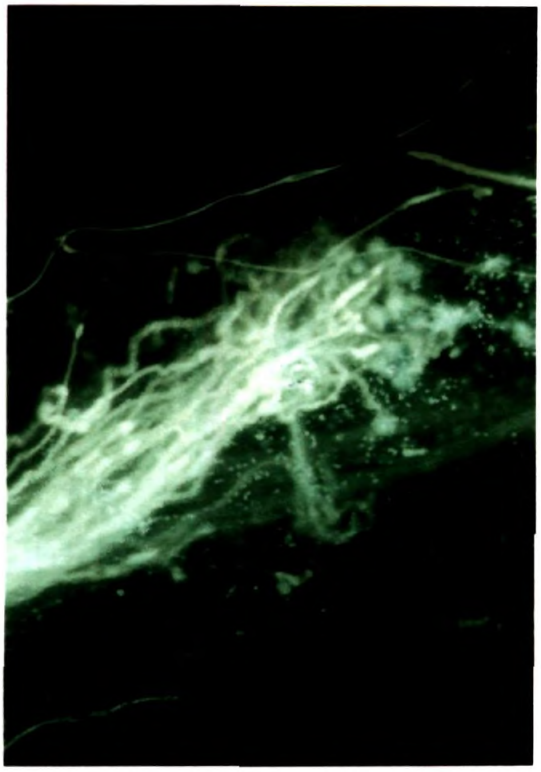
Intra morph - intra varietal crosses

The magenta pin stigmas showed a comparatively better percentage of self pollen adherence. The self pollen germination was 35.80 which is much less when compared to that of the other two colour variants. The mean tube length after 8 h was 10.29 mm and it was 15.83 mm after 24 h (Fig. 25a-d, 38 ; Table 12). The pollen tubes passing through the style showed low deposition of callose plugs (Fig. 25 c-d).

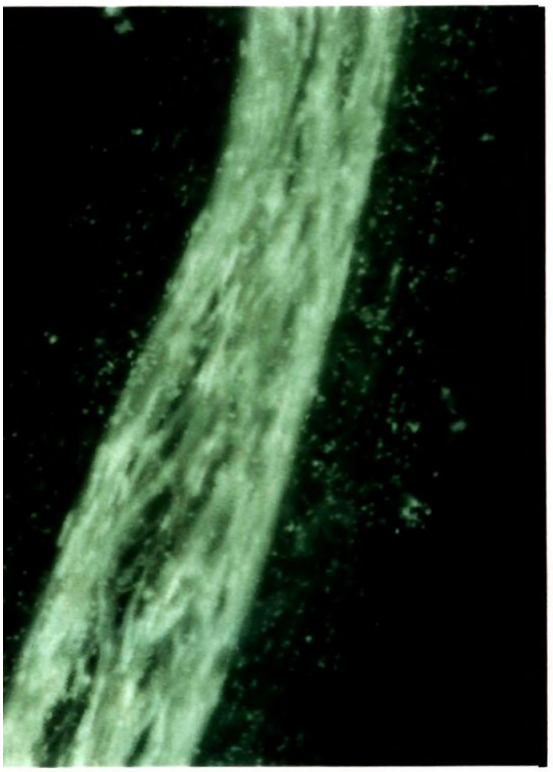
Fig. 25. *In vivo* pollen tube growth in *P. lanceolata* var. magenta
(b - d under UV illumination x 200)



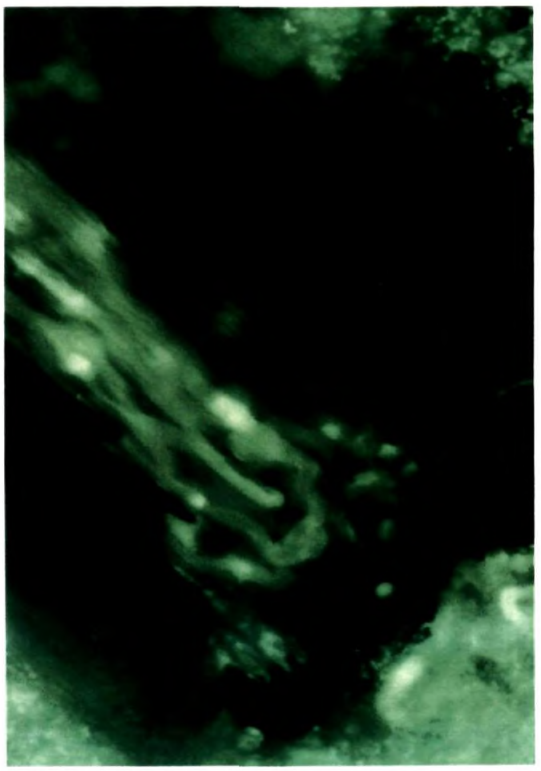
a. SEM of pin pollen on pin stigma



b. Pin pollen on pin stigma



c. Pollen tubes passing through style showing regular deposition of callose plugs



d. Pollen tubes at the base of style with less callose plugs

Table. 12. *Pentas lanceolata* var. magenta intra-morph crosses

Combinations	% of pollen germination	Mean tube length (mm)	
		8 h	24 h
$M_P \times M_P$	35.8	10.29 ± 0.61	15.83 ± 0.78
$M_P \times W_P$	12.3	5.91 ± 0.33	10.15 ± 0.59
$M_P \times L_P$	17.9	7.08 ± 0.41	12.51 ± 0.82
$M_P \times R_P$	No germination	0.0	0.0
$M_T \times M_T$	21.3	8.24 ± 0.52	17.52 ± 0.89
$M_T \times W_T$	16.3	2.93 ± 0.21	5.05 ± 0.32
$M_T \times L_T$	18.1	3.01 ± 0.28	6.53 ± 0.47

All values represent a mean of 6 replicates

Table. 13. *Pentas lanceolata* var. magenta inter-morph crosses

Combinations	% of pollen germination	Mean tube length (mm)	
		8 h	24 h
$M_P \times M_T$	26.2	3.41 ± 0.29	9.63 ± 0.57
$M_P \times W_T$	22.3	2.96 ± 0.18	7.85 ± 0.48
$M_P \times L_T$	25.8	4.01 ± 0.32	8.98 ± 0.62
$M_T \times M_P$	20.3	9.02 ± 0.58	19.86 ± 0.89
$M_T \times W_P$	58.9	1.68 ± 0.07	2.52 ± 0.21
$M_T \times L_P$	28.5	4.67 ± 0.29	8.13 ± 0.59
$M_T \times R_P$	5.5	0.91 ± 0.04	1.56 ± 0.17

All values represent a mean of 6 replicates

In thrum flowers, the self-pollen adherence was found to be poor, compared to that of the pin. The self-pollen germination was only 21.3% and the mean tube length was 8.24 mm after 8 h and 17.52 mm after 24 h (Fig. 26 a-d, 40 ; Table 12). The pollen tubes passing through the style were slightly curled and showed larger and irregular deposition of callose plugs (Fig.26 c-d).

Intra morph - intervarietal crosses

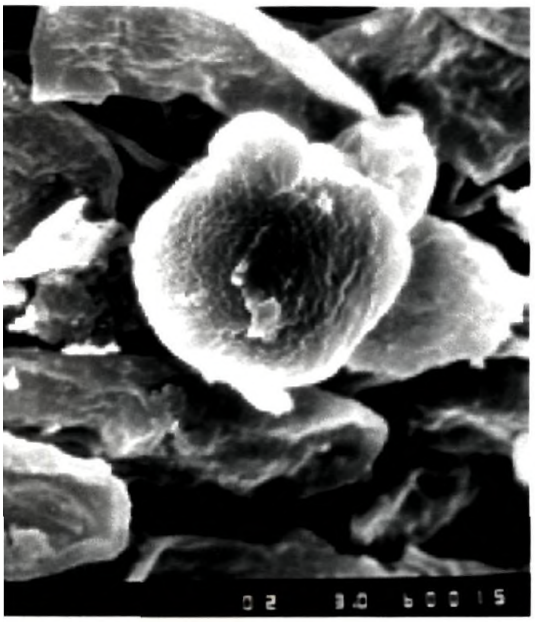
When *P. lanceolata* var. magenta pin flowers were pollinated with pollen from var. white, the combination exhibited low adherence and only 12.3% of pollen germination. The mean tube length after 8 h was 5.91 mm and after 24 h it was 10.15 mm (Table 12).

The same variety pin flowers on pollination with pollen from var. lilac, the percentage of adherence and pollen germination was slightly increased. The germination was only 17.9%. The mean tube length after 8 h was 7.80 mm and after 24 h it was 13.63 mm (Table 12).

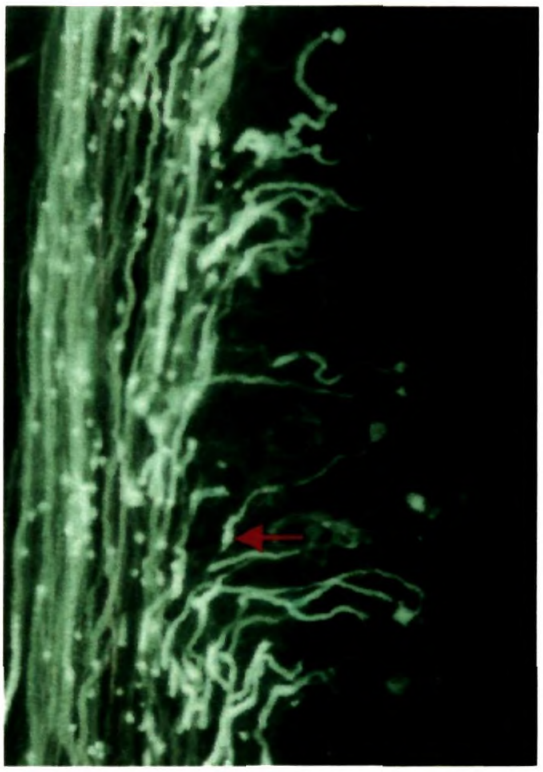
On pollinating with pollen from red pin variety, magenta pin flowers showed poor adherence and pollen failed to germinate on the stigma (Fig. 18c).

Pentas lanceolata var. magenta thrum flowers when pollinated with thrum pollen from var. white, the pollen adherence and germination was

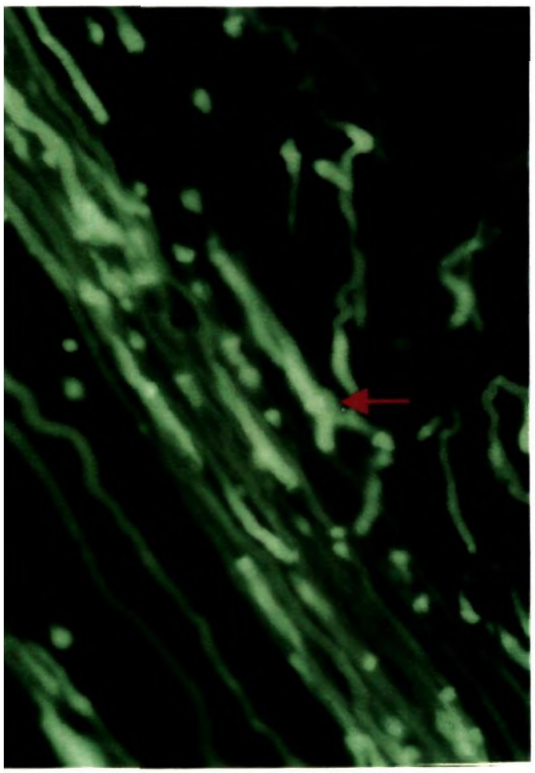
Fig. 26. *In vivo* pollen tube growth in *P. lanceolata* var. magenta
(b - d under UV illumination x 200)



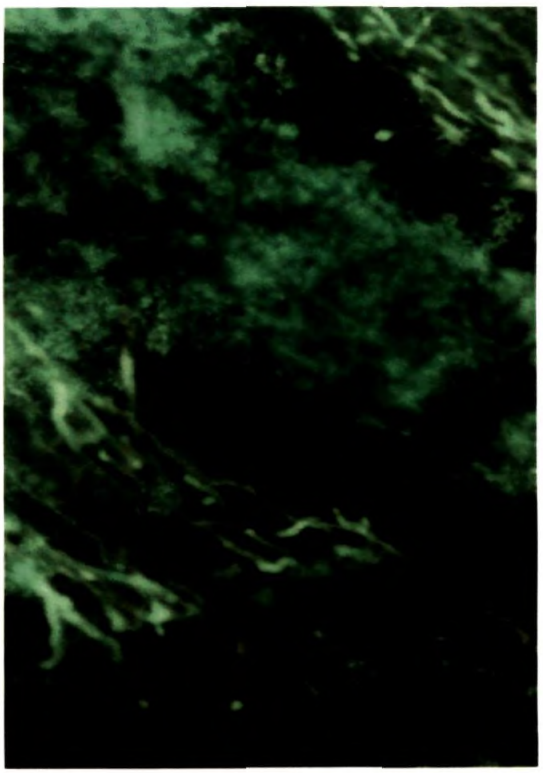
a. SEM of thrum pollen on thrum stigma



b. Thrum pollen on thrum stigma showing curled and bulged pollentubes



c. Pollen tubes through style showing irregular deposition of callose



d. Pollen tubes extending out of style after incompatible self pollination

less. The germination percentage was 16.3 and the mean tube length after 8 h and 24 h were 2.93 mm and 5.05 mm respectively (Table 12).

The same variety thrum, when crossed with pollen from variety lilac, the percentage of adherence and germination was better than that with white variety. The germination percentage was 18.1 and the mean pollen tube length after 8 h and 24 h were 3.01 mm and 6.53mm respectively (Table 12).

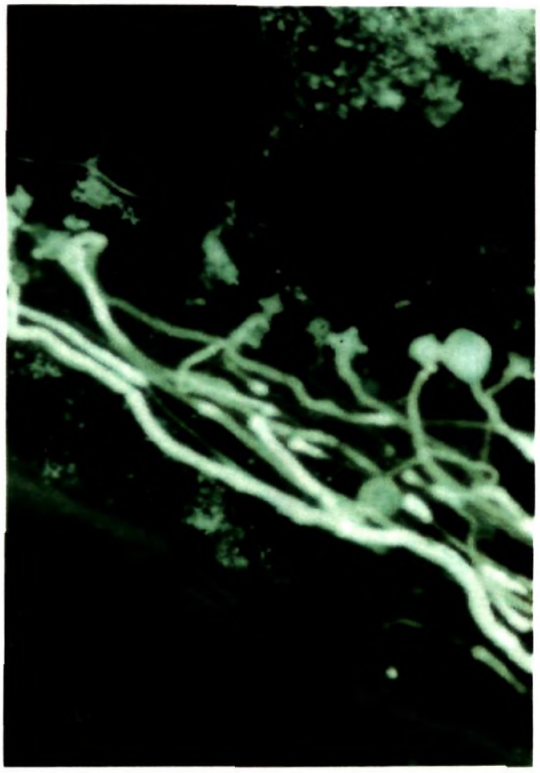
Inter morph - intra varietal crosses

When magenta pin flowers of *P. lanceolata* were crossed with pollen from thrum flowers of the same colour variant, the percentage of pollen adherence was found to be better. The percentage of pollen germination was 26.2 (Table 13). The mean tube length after 8 h was 3.41 mm which became 9.63 mm after 24 h of pollination (Fig. 27 a-d ; Table 13). Very few pollen tubes passed through the style and reached the base (Fig. 27c-d).

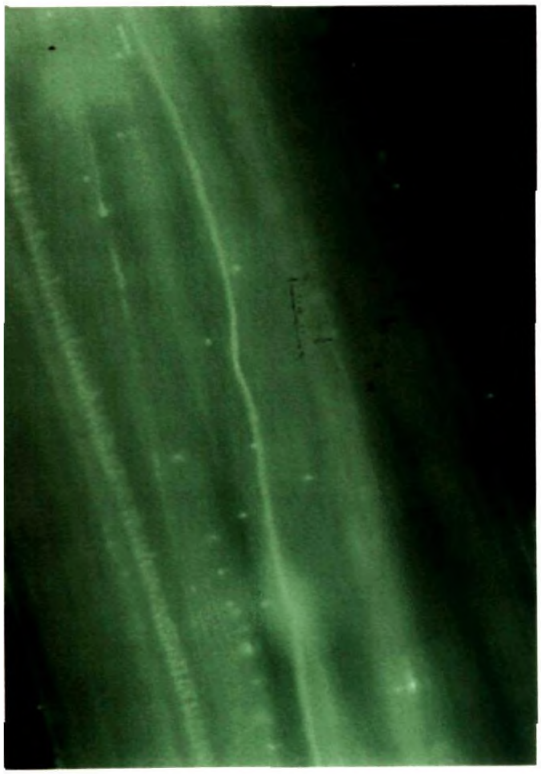
Magenta thrum flowers of *P. lanceolata* when crossed with pin pollen of same colour variant, the percentage of pollen germination was 26.3 (Table 13). The mean pollen tube length after 8 h was 9.02 mm and after 24 h it was 19.86 mm (Fig. 28a-d ; Table 13). Many pollen tubes

Fig. 27. *In vivo* pollen tube growth in *P. lanceolata* var. magenta
(b - d under UV illumination x 200)

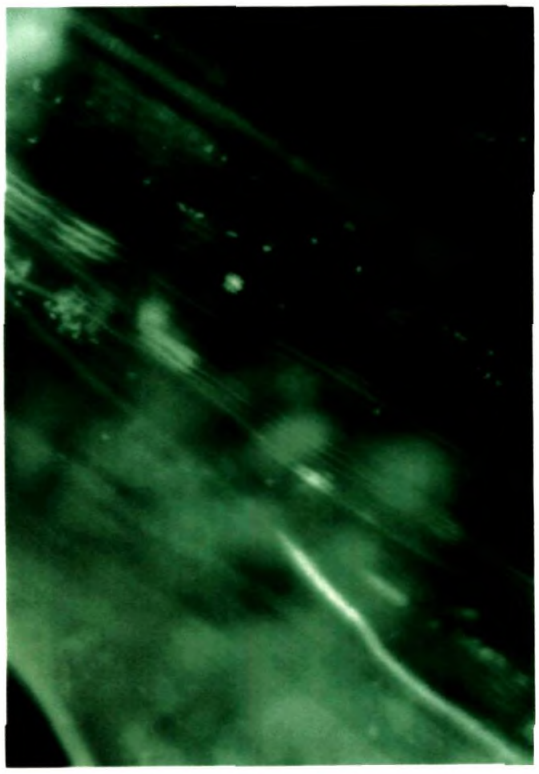
a. SEM of thrum pollen on pin stigma



b. Thrum pollen on pin stigma

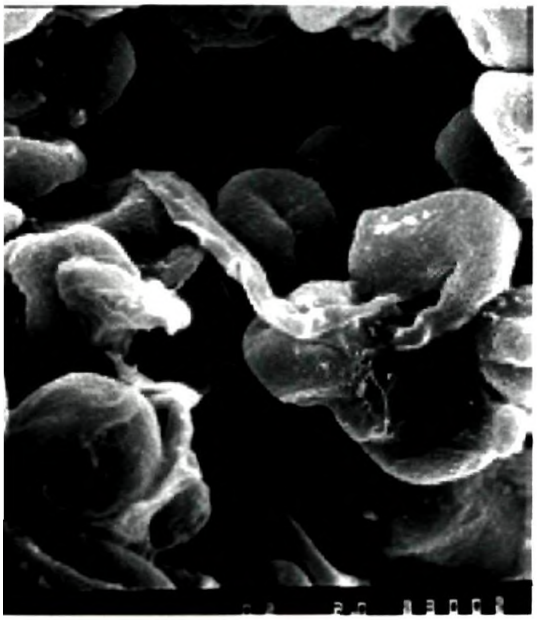


c. Pollen tubes passing through the style

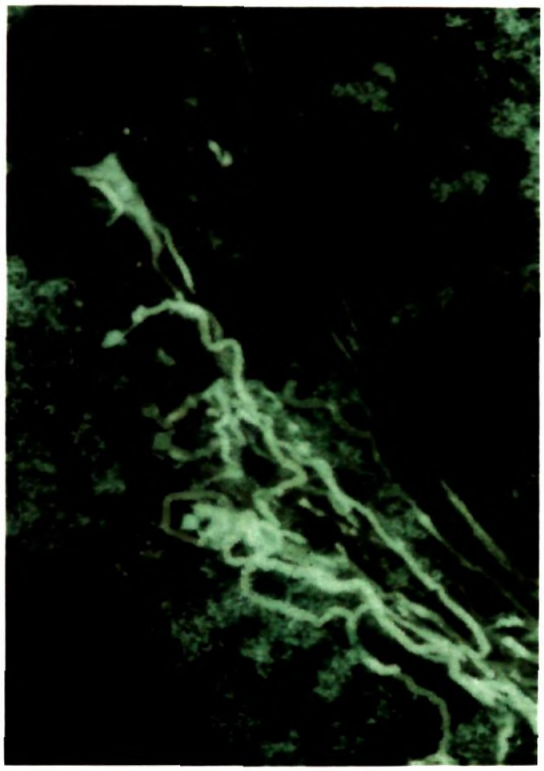


d. Pollen tubes at the base of style showing low callose deposition

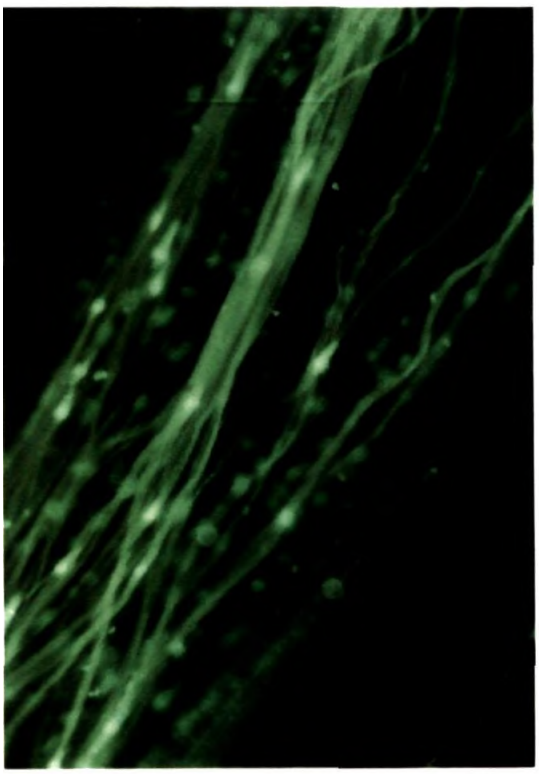
Fig. 28. *In vivo* pollen tube growth in *P. lanceolata* var. magenta
(b - d under UV illumination x 200)



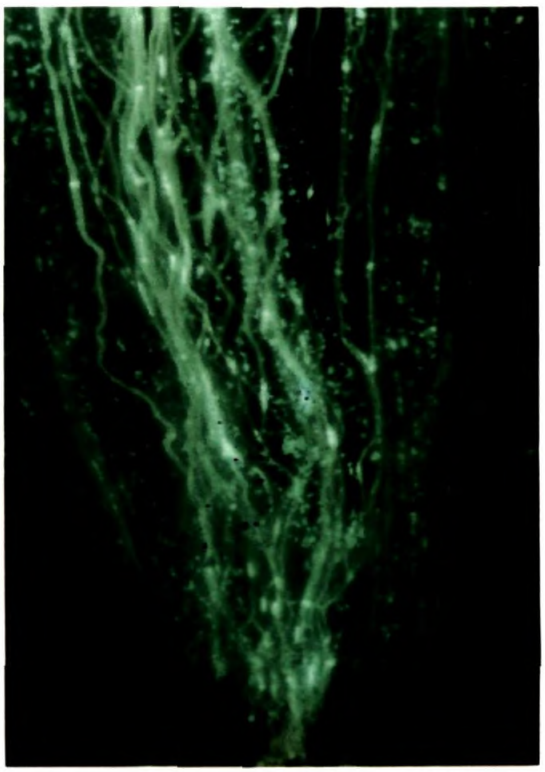
a. SEM of pin pollen on thrum stigma



b. Pin pollen on thrum stigma



c. Pollen tubes through style showing smaller and regular callose plugs



d. Pollen tubes extending out of the base of style after compatible pollination

passed through the style which showed smaller and regularly arranged callose plugs (Fig. 28c-d).

Inter-morph inter-varietal crosses

The pollen adherence was better when the same pin variety was pollinated with pollen from var. lilac thrum. The percentage of germination of pollen was 25.8 and the mean tube length after 8 h and 24 h were 4.01 and 8.98 mm respectively (Table 13).

When magenta variety pin flowers were pollinated with pollen from white thrum flowers, the pollen adherence and percentage of germination were low. The percentage of germination was only 22.3 and the mean tube length after 8 h and 24 h were 2.96 mm and 7.85 mm respectively (Table 13).

The same variety thrum flowers when pollinated with pin flowers of var. white, the pollen adherence and germination were very poor. The percentage of pollen germination was only 8.9 and the mean tube length after 8 h and 24 h were 1.68 mm and 2.52 mm respectively (Table 13). The cross with pollen from variety lilac showed a better percentage of pollen adherence and germination. The percentage of pollen germination was 28.50 and the mean tube length after 8 h and 24 h were 4.67 and 8.13 mm respectively (Table 13).

The thrum flowers of var. magenta on crossing with pollen from red pin variety, the adherence and germination were very poor. The percentage of germination was only 5.5%. The mean tube length after 8 h and 24 h were 0.91 mm and 1.56 mm respectively (Table 13).

Field studies

Only intra morph - intra varietal and inter morph - intra varietal pollinations were carried out in the field. Of the 100 pollinated pistils, 50 were taken for *in vivo* studies after 8 h and 24 h and the others were left on the plants for fruit set studies.

The fruit set data was taken from the following crosses. In *P. lanceolata* var. magenta, 50 pin flowers were selfed with magenta pin pollen and 50 flowers were crossed with thrum pollen from the same variety. The pin flowers on selfing produced 50.65% of fruits, whereas the crossed flowers produced only 25.84% of fruits (Fig. 37 ; Table 14).

The reciprocal crosses were also conducted with var. magenta thrum flowers taken as female parent. Fifty flowers were selfed with thrum pollen from same colour variant and 50 other flowers were crossed with pin pollen from var. magenta variety. In this case, the result of crossing was found to be better than that of selfing, in contrast to the pin flowers where selfing produced more fruits. The selfed flowers of thrum

Table. 14. *Pentas lanceolata* - Percentage of fruit set after selfing and crossing

Colour variants	% of fruit set*	
	Selfing	Crossing
White pin	72.2	64.3
White thrum	64.71	70.56
Lilac pin	76.06	72.61
Lilac thrum	36.62	70.49
Magenta pin	50.65	25.84
Magenta thrum	24.78	28.14

*All values represent a mean of 50 capsules

Table. 15. *Pentas lanceolata* -Index of incompatibility (ISI)

Colour variants	No. of seeds / capsule		Index of self incompatibilty
	Selfing	Crossing	
White pin	68.0 ± 5.56	60.4 ± 6.28	1.125
White thrum	70.2 ± 7.82	103.5 ± 8.56	0.678
Lilac pin	88.3 ± 6.45	77.6 ± 6.32	1.137
Lilac thrum	81.6 ± 5.78	118.5 ± 8.94	0.688
Magenta pin	15.5 ± 7.82	10.2 ±1.26	1.519
Magenta thrum	38.1 ± 6.45	51.4 ± 5.68	0.741

All values represent a mean of 50 capsules

produced only 24.78% of fruits whereas it was 28.14% after crossing (Fig. 37 ; Table 14).

Index of self incompatibility

The index of self incompatibility in *P. lanceolata* var. magenta variety pin plants was 1.49, which has proved it to be preferentially selfed. The index of self incompatibility in the thrum plants was 0.741, which has revealed that it is only partially self compatible (Table 15).

***In vitro* bioassay**

The stylar and stigmatic extracts when added to the germinating medium produced results, which were in agreement with *in vivo* studies. The pollen grains of var. lilac pin formed curled and deformed pollen tubes in thrum extract. The thrum pollen produced normal healthy pollen tubes in pin extract, even though the percentage of germination was not commendable.

The pin flowers of lilac colour variant was preferentially self-fertilized and their pollen grains produced normal pollen tubes in media containing low concentrations of self extracts while pollen grains of thrum flowers produced deformed tubes even in very low concentrations of self extract.

Population studies

The morph frequency studies conducted in six different localities of Thiruvananthapuram revealed the following data as given in Table 16 .

The results showed that in all the localities studied, the number of pin plants well exceeded the number of thrum plants. The ratio of pin to thrum was in excess of 2.5 : 1 in five localities whereas it was only 1.7 : 1 in one locality.

Genetic studies

In order to understand the nature of inheritance of the pin and thrum characters, an attempt was made in the present investigation. The pin and thrum plants were selfed and crossed and the seedlings were raised from the selfed and crossed seeds. The pin plants produced more fruits and seeds after selfing, whereas the thrum plants produced more fruits and seeds after crossing. The seeds produced were so very small in size that, many were lost during the process of germination itself and many at the early seedling stage. As a result, a very accurate count of seedlings could not be obtained. However, in all the six replications carried out, the number of thrum progeny obtained was very low. From the result of the genetic studies in *Pentas lanceolata*, it is tempting to assume

Table 16. *Pentas lanceolata* - Morph frequency ratio

Locality	Pin	Thrum	Ratio Pin : Thrum
M.G. College campus	73 ± 5.54	25 ± 2.75	2.9 : 1
Pattom	65 ± 5.35	38 ± 2.94	1.7 : 1
Perurkada	38 ± 4.08	14 ± 1.63	2.7 : 1
Balaramapuram	92 ± 7.79	33 ± 2.94	2.8 : 1
Neyyattinkara	87 ± 6.53	29 ± 2.45	3 : 1
Nedumangadu	68 ± 6.16	23 ± 3.27	3 : 1

All the values represent a mean of three replicates

Table. 17. *Pentas lanceolata* - Morph frequency of progeny raised

Type of seeds used	Morph	No. of progeny raised*		
		Total	Pin	Thrum
Self pollinated	Pin	19.0 ± 3.51	15.5 ± 2.81	3.5 ± 0.76
	Thrum	14.67 ± 2.98	7.33 ± 1.49	7.33 ± 1.49
Cross pollinated	Pin	10.67 ± 2.74	7.5 ± 1.71	3.17 ± 1.07
	Thrum	15.17 ± 3.39	9.5 ± 1.71	5.67 ± 1.7

*all values represent a mean of 6 replicates
in each replication 100 seeds were used

that the pin plants are heterostylous, producing both pin and thrum progeny even after selfing and the thrum plants are homozygous as they produced only thrum progeny, that too only in small numbers, may be because there is partial self incompatibility. The thrum plants produced pin progeny and a low number of thrum also following cross pollination. The results of genetic studies are given in Table 17.

BIOCHEMICAL STUDIES

In recent years, reproductive biologists have been giving much importance to the structural and biochemical aspects of the pistil for an overall understanding of the reproductive biology of flowering plants. Specifically, pollen-pistil interaction can also be explained from a different angle, using supporting evidences from the structure and biochemistry of stigma.

In the present investigation, the unpollinated stigmas of the three heterostylous and two homostylous colour variants were biochemically analysed to quantify soluble sugars, soluble proteins, phenols, peroxidase, proline. The three heterostylous colour variants produced fertile seeds, both from cross and self pollinations. Role of the above listed

compounds in self and cross combinations were analysed by quantifying them in selfed, crossed and unpollinated pistils. Pistils were processed for estimation after 24 h of pollination and unpollinated pistils were collected after 24 h of anthesis.

Soluble proteins

The soluble protein content was found to be comparatively very low in the stigmas of the three colour variants of *P. lanceolata* (Table 18).

In the unpollinated stigmas of the white pin variety, the protein content was found to be 3.135 mg/g which on selfing was increased slightly to 3.515 mg/g. However the protein content showed a significant reduction after crossing *ie.* 2.003 mg/g (Table 18).

The unpollinated stigmas of white thrum also showed a very low content of soluble protein, 2.222 mg/g. The protein content was lowered to 1.533 mg/g after selfing, but was increased to 2.882 mg/g after crossing (Table 18).

Table. 18. *P. lanceolata* - Soluble Proteins (mg/g fr. wt.)

PIN			
Colour variants	Unpollinated	Selfed	Crossed
White	3.135 ± 0.11	3.515 ± 0.17	2.003 ±0.14
Lilac	3.783 ± 0.15	5.185 ± 0.15	2.448 ± 0.12
Magenta	2.025 ± 0.09	2.432 ± 0.16	1.24 ± 0.09
THRUM			
Colour variants	Unpollinated	Selfed	Crossed
White	2.222 ± 0.13	1.533 ± 0.11	2.882 ± 0.12
Lilac	2.152 ± 0.17	1.845 ± 0.15	3.032 ± 0.14
Magenta	1.818 ± 0.11	1.553 ± 0.14	3.267 ± 0.17

All values represent a mean of 6 replicates

In the lilac pin unpollinated stigma the protein content was 3.783 mg/g, which on selfing was increased to 5.185 mg/gm and on crossing was reduced to a lower value, 2.448 mg/g (Table 18).

The unpollinated stigma of lilac thrum variety showed a protein content of 2.152 mg/gm, which on selfing was lowered to 1.845 mg/g. The protein content was increased after crossing, 3.032 mg/g (Table 18).

In the unpollinated stigma of magenta pin variety the protein content was only 2.025 mg/g, which was increased to a value of 2.432 mg/g, following selfing and to 1.240 mg/g following crossing (Table 18).

In magenta thrum variety, the unpollinated stigma showed a very low amount of soluble protein, 1.818 mg/g, which decreased to 1.553 mg/g after selfing and increased to 3.267 mg/g after crossing (Table 18).

The unpollinated stigmas showed generally, a very low content of soluble protein, which showed the highest value in the lilac pin, 3.783 mg/g and the lowest value in the magenta thrum variety, 1.818 mg/g. The protein content was found to be lowered after crossing

in the pin stigmas and after selfing in the thrum stigmas, whereas it showed a slight increase after compatible pollinations, *ie.* selfing in pin and crossing in thrum (Table 18a).

The soluble protein content was also analysed statistically at three levels, the result of which is shown in the (Table 18a-c).

The soluble protein content when analysed between morphs and varieties, the lilac pin variety showed the highest content of soluble protein and the magenta thrum contained the lowest amount. The protein content was significantly different between the two morphs in two of the colour variants, white and lilac, where as it was not significant in magenta (Table 18a).

The protein content when analysed between methods and varieties there was a more significant decrease in protein content after crossing in white pin than in white thrum (Table 18b).

The analysis of protein content between methods and morphs showed that the protein content was more significantly reduced after crossing in pin and selfing in thrum (Table 18c).

Table 18a. Variation in soluble proteins (mg/g fr. wt.) between morphs and varieties

Morphs	Varieties*			
	White	Lilac	Magenta	Mean
Pin	2.884	3.806	1.899	2.863
Thrum	2.212	2.343	1.846	2.134
Mean	2.548	3.074	1.872	2.498

*All values represent a mean of 18 observations
CD (0.05) = 0.247

Table 18b.Variation in soluble proteins (mg/g fr. wt.) between methods and varieties

Method	Varieties*			
	White	Lilac	Magenta	Mean
Unpollinated	2.678	2.967	1.922	2.522
Self	3.198	4.108	2.299	3.202
Cross	1.768	2.147	1.397	1.771
Mean	2.548	3.074	1.872	2.498

*All values represent a mean of 12 observations
CD (0.05) = 0.305

Table 18c. Variation in soluble proteins (mg/g fr. wt.) between method and morphs

Method	Morphs*		
	Pin	Thrum	Mean
Un-pollinated	2.981	2.064	2.522
Self	3.711	1.644	2.313
Cross	1.897	2.064	1.981
Mean	2.863	2.134	2.272

*All values represent a mean of 18 observations
CD (0.05) = 0.213

SDS PAGE Analysis

Protein profile of the colour variants of *P. lanceolata* revealed a picture, which was more or less in agreement with the quantification of soluble proteins. The soluble protein content was found to be increased after compatible pollinations *ie.* selfing in the pin morphs and crossing in the thrum morphs. Correspondingly two prominent new bands of protein were visible in the gel after selfing in the pin morphs, whereas in thrum morphs the appearance of new bands were seen after crossing (Fig. 29).

Protein profile of all the lanes showed four major bands which were more or less common to them having an *Rf.* which ranged from 0.945 to 0.659. Lanes 1 to 5 had two major bands unique to them having an *Rf.* which ranged from 0.400 to 0.332. The intensity of the bands increased after compatible combinations (Lanes 1-5). These combinations resulted in the appearance of new faint bands also.

Soluble sugars

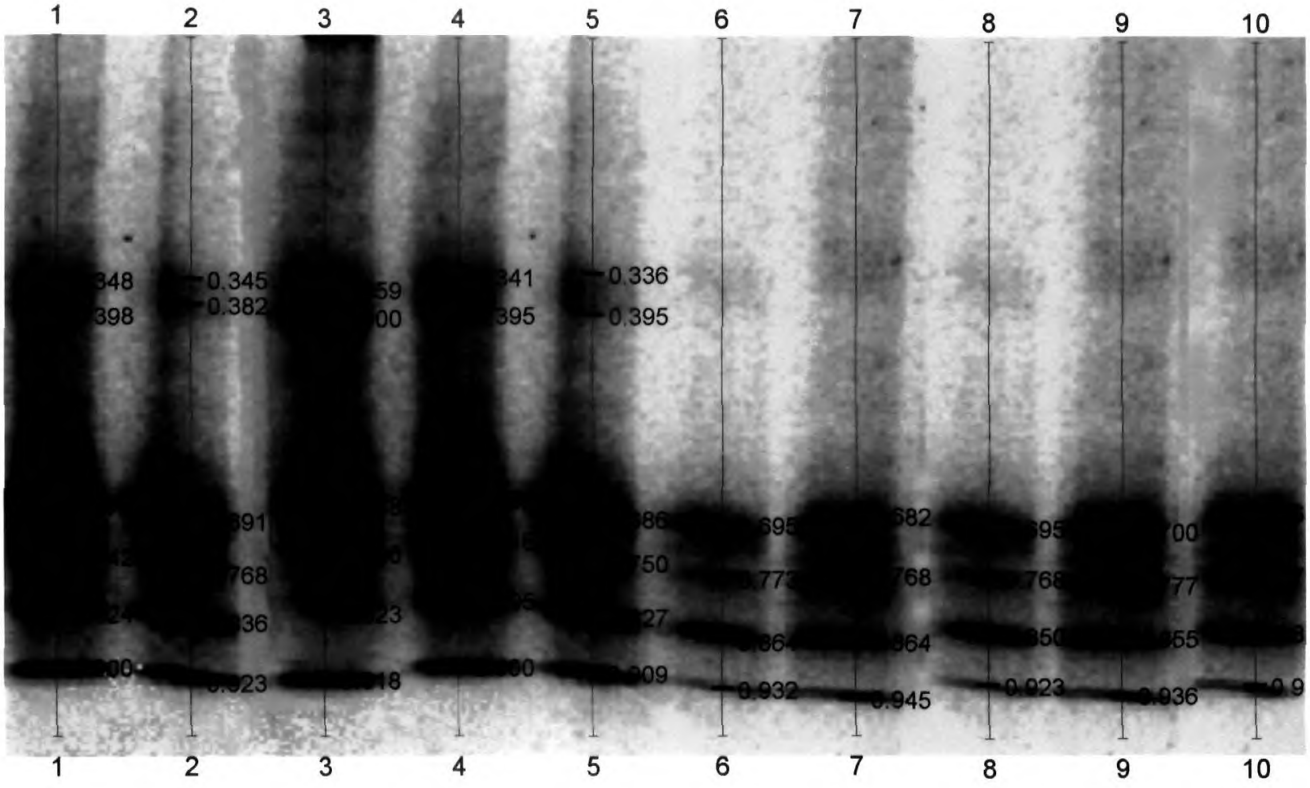
In general, the unpollinated stigmas contained a higher quantity of soluble sugars than the pollinated, whether it be selfed or crossed.

In the unpollinated stigma of white pin colour variant, the unpollinated stigma contained 56.211 mg/g of soluble sugar, but after self pollination, the value was reduced to 16.648 mg/g. The value was

Fig. 29. SDS PAGE profile of the stigmatic protein after selfing and crossing

Lane 1 - 5 Compatible combinations

Lane 6 - 10 Incompatible combinations



1. Lilac thrum cross
2. White thrum cross
3. Lilac pin self
4. White pin self
5. Magenta pin self
6. Lilac thrum self
7. White thrum self
8. Lilac pin cross
9. White pin cross
10. Magenta pin cross

Relative Front

reduced only to 22.317 mg/g when the pistils were cross-pollinated with thrum pollen (Table 19).

In the case of the stigma of white thrum the soluble sugar content was 115.978 mg/g before pollination, but after self pollination it was 89.457 mg/g, which was found to be still reduced to 74.064 mg/g after cross pollination (Table 19).

The lilac pin stigma before pollination showed a slightly higher content of soluble sugar, than the white pin unpollinated stigma. The value was found to be 60.794 mg/g. This value was reduced very much after self pollination, and was found to be only 32.31 mg/g. The soluble sugar content was reduced after cross pollination to a value of 44.210 mg/g (Table 19). The decrease in sugar content however was less in the cross pollinated, than the self pollinated.

In lilac thrum stigma the soluble sugar content was found to be 126.57 mg/g in the unpollinated, which was reduced to 90.98 mg/g after self pollination and was still reduced after cross pollination to a value of 60.359 mg/g (Table 19). The soluble sugar content was found to be still more reduced after cross-pollination in lilac thrum stigma.

In magenta pin variety, the unpollinated stigma contained about 50.703 mg/g of sugar which was reduced to 16.761 mg/g on selfing. The

Table. 19. *P. lanceolata* - Soluble Sugars (mg/g fr. wt.)

PIN			
Colour variants	Unpollinated	Selfed	Crossed
White	56.211 ± 2.87	16.648 ± 1.68	22.317 ± 1.72
Lilac	60.794 ± 3.25	32.31 ± 2.34	44.21 ± 3.04
Magenta	50.703 ± 2.65	16.761 ± 1.82	30.454 ± 2.54
THRUM			
Colour variants	Unpollinated	Selfed	Crossed
White	115.978 ± 6.24	89.457 ± 4.32	74.064 ± 3.78
Lilac	126.573 ± 5.88	90.98 ± 5.66	69.359 ± 4.22
Magenta	111.051 ± 5.24	79.615 ± 3.89	31.832 ± 3.42

All values represent a mean of 6 replicates

cross-pollinated stigmas however contained 30.454 mg/g of soluble sugar (Table 19). The soluble sugar content was very much reduced after self-pollination, whereas it was not much after cross-pollination.

In magenta thrum variety, the unpollinated stigma showed a high content of soluble sugar, 111.051 mg/g, which was lowered to 79.615 mg/g after self pollination and was still lowered to 31.832 mg/g in the cross pollinated stigmas (Table 19).

The soluble sugar content in the unpollinated stigmas was found to be significantly higher in the thrum than in the pin varieties. The sugar content was found to be much lowered after self-pollination in the pin stigma and cross-pollination in the thrum stigma. The lilac thrum variety was seen to show the highest content of soluble sugar and the white pin variety showed the lowest content of soluble sugar (Table 19a).

The soluble sugar content was analysed at different levels *ie.* between morphs and varieties, between varieties and methods and also between methods and morphs. The results are given in Table 19a-c.

Table 19a. Variation in soluble sugars (mg/g fr. wt.) between morphs and varieties

Morphs	Varieties*			
	White	Lilac	Magenta	Mean
Pin	31.725	45.771	32.640	36.712
Thrum	93.166	95.637	74.166	87.657
Mean	62.446	70.704	53.403	62.184

*All values represent mean of 18 observations

CD (0.05) = 4.387

Table 19b. Variation in soluble sugars (mg/g fr. wt.) between methods and varieties

Method	Varieties*			
	White	Lilac	Magenta	Mean
Un-pollinated	86.095	93.684	80.877	86.885
Self	53.052	61.645	48.188	54.295
Cross	48.191	56.784	31.143	45.373
Mean	62.446	70.704	53.403	62.184

*All values represent mean of 12 observations

CD (0.05) = 5.374

Table 19c. Variation in the amount of soluble sugars (mg/g fr. wt.) between methods and morphs

Method	Morphs*		
	Pin	Thrum	Mean
Unpollinated	55.903	117.867	86.885
Self	21.906	86.684	54.295
Cross	32.327	58.418	45.373
Mean	36.712	87.657	62.184

*All values represent mean of 18 observations

CD (0.05) = 4.387

When the soluble sugar content was analysed between methods and varieties, the sugar content was found to be significantly reduced after selfing and crossing. The sugar content reduction however was more significant in the magenta variety (Table 19b).

The soluble sugar content when analysed with methods and morphs, selfing more significantly reduced the sugar content in pin stigma than thrum, whereas crossing produced more significant sugar content reduction in thrum than pin (Table 19c).

Total Phenols

The unpollinated stigmas of *P. lanceolata* colour variants showed a low content of phenol, which was found to be significantly reduced following pollination, both self as well as cross. The highest content of phenol in the unpollinated stigma was found in white pin, 10.763 mg/g, and the lowest in magenta pin (Table 20). The phenol content reduction was more pronounced following self-pollination in pin stigmas and cross-pollination in thrum stigmas.

The unpollinated stigma of white pin variety showed 10.763 mg/g of phenol which was reduced to 2.657 mg/g after selfing and 3.218 mg/g after crossing (Table 20).

Table. 20. *P. lanceolata* - Total Phenol (mg/g fr. wt.)

PIN			
Colour variants	Unpollinated	Selfed	Crossed
White	10.763 ± 0.67	2.657 ± 0.21	3.218 ± 0.29
Lilac	6.61 ± 0.45	1.043 ± 0.12	1.473 ± 0.13
Magenta	1.705 ± 0.19	0.695 ± 0.08	1.082 ± 0.11
THRUM			
Colour variants	Unpollinated	Selfed	Crossed
White	8.128 ± 0.78	3.07 ± 0.37	1.082 ± 0.09
Lilac	4.498 ± 0.46	1.133 ± 0.08	0.742 ± 0.08
Magenta	7.927 ± 0.56	2.77 ± 0.13	0.732 ± 0.08

All values represent a mean of 6 replicates

In the white thrum unpollinated stigma, the phenol content was 8.128 mg/g, which decreased to 3.070 mg/g after selfing and 1.082 mg/g after crossing (Table 20).

The lilac pin unpollinated stigma contained 6.610 mg/g of phenol, which showed a significant reduction following selfing, 1.043 mg/g and a more or less equal content 1.473 mg/g after crossing (Table 20).

The phenol content in the unpollinated thrum stigma of lilac variety was only 4.498 mg/g, which was reduced to 1.133 mg/g after selfing and 0.742 mg/gm after crossing (Table 20).

The unpollinated stigma of magenta pin colour variant showed a phenol content of 1.705 mg/g, which was lowered though not significantly, following self pollination to 0.695 mg/g and still less significantly after crossing to 1.082 mg/g (Table 20).

The phenol content in the unpollinated magenta thrum stigma was 7.927 mg/g, which decreased significantly after selfing to 2.770 mg/g and to a much lower value 0.732 mg/g after cross pollination (Table 20).

The results of phenol content analysis at three different levels are given in Table 20a-c.

Table 20a. Variation in total phenol (mg/g fr. wt.) between morphs and varieties

Morphs	Varieties*			
	White	Lilac	Magenta	Mean
Pin	5.546	3.042	1.161	3.250
Thrum	4.093	2.124	3.809	3.342
Mean	4.820	2.583	2.485	3.296

*All values represent mean of 18 observations
CD (0.05) = 0.23

Table 20b.Variation in total phenol (mg/g fr. wt.) between methods and varieties

Method	Varieties*			
	White	Lilac	Magenta	Mean
Unpollinated	9.446	5.554	4.816	6.605
Self	2.863	1.088	1.733	1.895
Cross	2.150	1.108	0.907	1.388
Mean	4.820	2.583	2.485	3.296

*All values represent mean of 12 observations
CD (0.05) = 0.28

Table 20c. Variation in total phenol (mg/g fr. wt.) between methods and morphs

Method	Morphs*		
	Pin	Thrum	Mean
Unpollinated	6.359	6.851	6.605
Self	1.465	2.324	1.895
Cross	1.924	0.852	1.388
Mean	3.250	3.342	3.296

*All values represent mean of 18 observations
CD (0.05) = 0.23

Total phenol content when analysed between morphs and varieties the white and lilac pin contained significantly more quantity of phenol than its corresponding thrums whereas in magenta thrum the phenol content was significantly higher than its counterpart pin (Table 20a).

The phenol content between methods and varieties showed that there was a significant difference in phenol content following selfing and crossing. The phenol content was more significantly lowered after selfing in lilac than in the other two colour variants (Table 20b)

Between methods and morphs, selfing produced more significant lowering of phenol content in pins whereas crossing produced the same results in thrum varieties (Table 20c).

Soluble Peroxidase

The unpollinated stigmas of *P. lanceolata* colour variants showed a comparatively lower activity. The peroxidase activity increased after pollination, irrespective of the types of pollination. The peroxidase activity was however higher after cross-pollination in pin and self pollination in thrum.

The white pin unpollinated stigma showed an activity of 4.348 OD/h/g f.wt. which increased to 8.573 OD/h/g f.wt on selfing and to a still higher amount, 11.052 OD/h/g f.wt, on crossing (Table 21).

Table. 21. *P. lanceolata*-Soluble Peroxidase activity(OD/h/g fr. wt.)

PIN			
Colour variants	Unpollinated	Selfed	Crossed
White	4.348 ± 0.59	8.573 ± 0.78	11.052 ± 0.93
Lilac	3.175 ± 0.51	7.015 ± 0.65	7.057 ± 0.84
Magenta	4.162 ± 0.63	8.660 ± 0.97	15.128 ± 1.12
THRUM			
Colour variants	Unpollinated	Selfed	Crossed
White	8.988 ± 0.82	27.758 ± 1.94	17.713 ± 1.23
Lilac	3.262 ± 0.36	7.057 ± 0.91	0.703 ± 0.08
Magenta	6.015 ± 0.64	10.298 ± 0.95	7.227 ± 0.89

All values represent a mean of 6 replicates

In white thrum unpollinated stigma an activity of 8.988 mg/g of peroxidase was observed, which increased to 27.758 OD/h/g f.wt on selfing and 17.713 OD/h/g f.wt on crossing (Table 21).

The lilac pin unpollinated stigma showed an activity of 3.175 OD/h/g f.wt of peroxidase, which increased almost to an equal value of 7.015 OD/h/g f.wt on selfing and 7.057 OD/h/g f.wt on crossing (Table 21).

In lilac thrum stigma the enzyme activity was 3.262 OD/h/g/f.wt of peroxidase, which increased to 7.057 OD/h/g f.wt after selfing and decreased to the lowest value of 0.703 after crossing (Table 21).

The magenta pin unpollinated stigma showed an activity of 4.162 OD/h/g f.wt of peroxidase which increased to 8.660 OD/h/g f.wt on selfing and to a still higher value of 15.128 OD/h/g f.wt on crossing (Table 21).

The peroxidase activity of magenta thrum unpollinated stigma was found to be 6.015 OD/h/g f.wt which increased to 10.298 OD/h/g f.wt on selfing and 7.227 OD/h/g f.wt on crossing (Table 21).

The peroxidase activity was found to be maximum in the selfed stigmas of white thrum, 27.758 OD/h/g f.wt, and minimum in the crossed stigmas of lilac thrum, 0.703 OD/h/g f.wt ((Table 21).

The result of statistical analysis of peroxidase activity at three different levels is given in the Table 21a-c.

The soluble peroxidase activity when analysed between morphs and varieties, was highest in white thrum and lowest in lilac (Table 21a).

When peroxidase activity was measured between methods and varieties there was a significantly increased activity after selfing in two of the colour variants, white and lilac, whereas it was more so after crossing in the magenta colour variant (Table 21b).

Peroxidase activity between methods and morphs revealed that crossing produced a significant increase in peroxidase activity in pins, whereas selfing was found to do so in the thrum varieties (Table 21c).

Proline

The content of free proline was found to be very low in the unpollinated as well as pollinated stigmas of *P. lanceolata* colour variants.

The white pin unpollinated stigma contained only 0.245 mg/g of proline which showed a negligible rise to 0.285 mg/g after selfing and to 0.302 mg/g after crossing (Table 22).

The white thrum unpollinated stigma was seen to contain 0.330 mg/g of proline which increased to 0.523 mg/g after selfing and to 0.457 mg/g after crossing (Table 22).

Table 21a. Variation in Soluble Peroxidase activity (OD/h/g fr. wt.) between morphs and varieties

Morphs	Varieties*			
	White	Lilac	Magenta	Mean
Pin	7.991	5.749	9.317	7.686
Thrum	18.153	3.674	7.847	9.891
Mean	13.072	4.711	8.582	8.788

*All values represent mean of 18 observations
CD (0.05) = 0.374

Table 21b.Variation in Soluble Peroxidase activity (OD/h/g fr. wt.) between methods and varieties

Method	Varieties			
	White	Lilac	Magenta	Mean
Un-pollinated	6.668	3.218	5.088	4.992
Self	18.166	7.036	9.479	11.560
Cross	14.383	3.880	11.178	9.813
Mean	13.072	4.711	8.582	8.788

*All values represent mean of 12 observations
CD (0.05) = 0.46

Table 21c. Variation in Soluble Peroxidase activity (OD/h/g fr. wt.) between method and morphs

Method	Morphs*		
	Pin	Thrum	Mean
Unpollinated	3.895	6.088	4.992
Self	8.083	15.038	11.560
Cross	11.079	8.548	9.813
Mean	7.686	9.891	8.788

*All values represent mean of 18 observations
CD (0.05) = 0.374

Table. 22. *P. lanceolata* – Total Proline (mg/g fr. wt.)

PIN			
Colour variants	Unpollinated	Selfed	Crossed
White	0.245 ± 0.03	0.285 ± 0.04	0.362 ± 0.05
Lilac	0.226 ± 0.02	0.320 ± 0.04	0.377 ± 0.04
Magenta	0.207 ± 0.02	0.223 ± 0.02	0.405 ± 0.05
THRUM			
Colour variants	Unpollinated	Selfed	Crossed
White	0.330 ± 0.04	0.523 ± 0.04	0.457 ± 0.06
Lilac	0.529 ± 0.07	0.515 ± 0.04	0.254 ± 0.03
Magenta	0.623 ± 0.09	0.591 ± 0.07	0.431 ± 0.05

All values represent a mean of 6 replicates

The proline content of unpollinated lilac pin stigma was only 0.226 mg/g, which increased to 0.320 mg/g after selfing and 0.377 mg/g after crossing (Table 22).

The unpollinated stigma of lilac thrum contained 0.529 mg/gm of proline which changed to 0.515 mg/g after selfing and to 0.254 after crossing(Table 22).

The unpollinated stigma of magenta pin was seen to contain 0.207 mg/g of proline, which increased to 0.223 mg/g on selfing and to 0.405 mg/g on crossing (Table 22).

The unpollinated stigma of magenta thrum was seen to contain 0.623 mg/g of proline, which on selfing was found to decrease to 0.591 mg/g and to 0.431 mg/g on crossing (Table 22).

The proline content was found to be increased even though only slightly following selfing in pin stigmas and crossing in thrum stigmas (Table 22).

The data was analysed statistically at three different levels, the results of which are given in Table 22a-c.

When the proline content was analysed between morphs and varieties, the magenta thrum colour variant showed a significantly higher content of proline and pin of the same colour variant showed the lowest content (Table 22a).

Table 22a. Variation in Proline content (mg/g fr. wt.) between morphs and varieties

Morphs	Varieties*			
	White	Lilac	Magenta	Mean
Pin	0.297	0.308	0.278	0.294
Thrum	0.437	0.432	0.548	0.472
Mean	0.367	0.370	0.413	0.383

*All values represent mean of 18 observations
CD (0.05) = 0.003

Table 22b. Variation in Proline content (mg/g fr. wt.) between methods and varieties

Method	Varieties*			
	White	Lilac	Magenta	Mean
Unpollinated	0.288	0.377	0.415	0.360
Self	0.404	0.417	0.407	0.409
Cross	0.409	0.315	0.418	0.381
Mean	0.367	0.370	0.413	0.383

*All values represent mean of 12 observations
CD (0.05) = 0.006

Table 22c. Variation in Proline content (mg/g fr. wt.) between method and morphs

Method	Morphs*		
	Pin	Thrum	Mean
Unpollinated	0.226	0.484	0.360
Self	0.276	0.543	0.409
Cross	0.381	0.380	0.381
Mean	0.294	0.472	0.383

*All values represent mean of 18 observations
CD (0.05) = 0.003

The proline content when analysed between methods and varieties, crossing produced a significant increase in proline content in white and magenta varieties, whereas selfing produced an increase in the lilac colour variant (Table 22b).

Between methods and morphs, crossing produced a significant increase in the proline content in pins and selfing did so in the thrums (Table 22c).

Fig. 30. Flower length in the colour variants of *P. lanceolata*

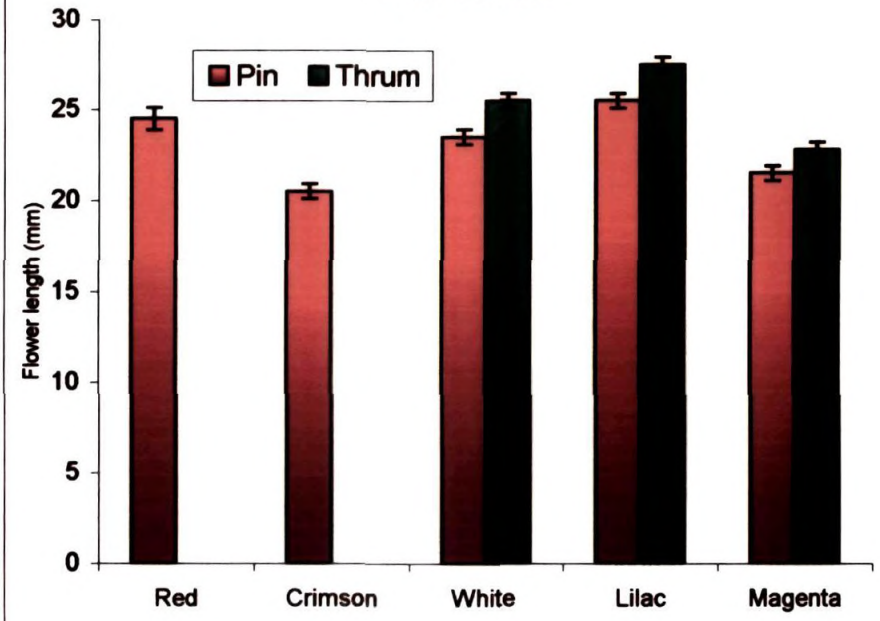


Fig. 31. Length of style in the colour variants of *P. lanceolata*

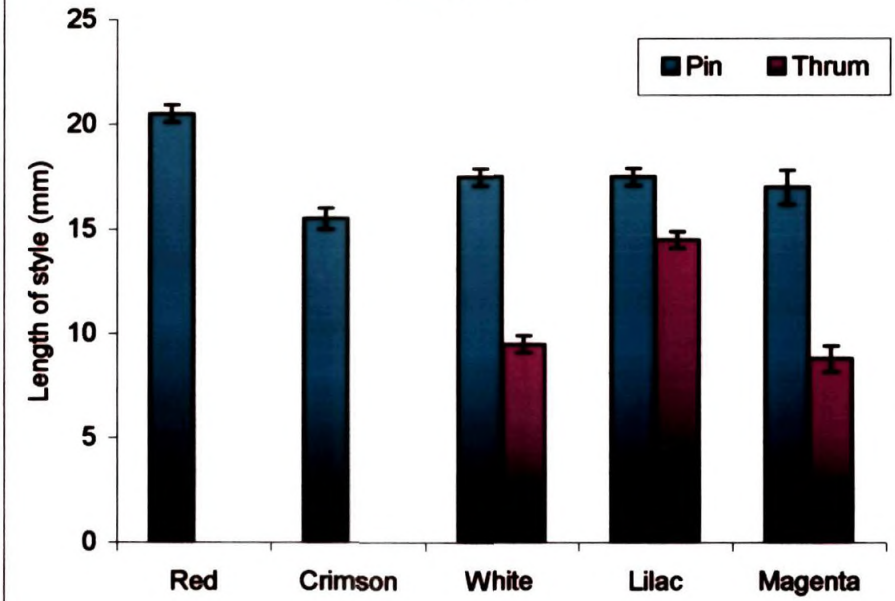


Fig. 32. Length of stigma in the colour variants of *P. lanceolata*

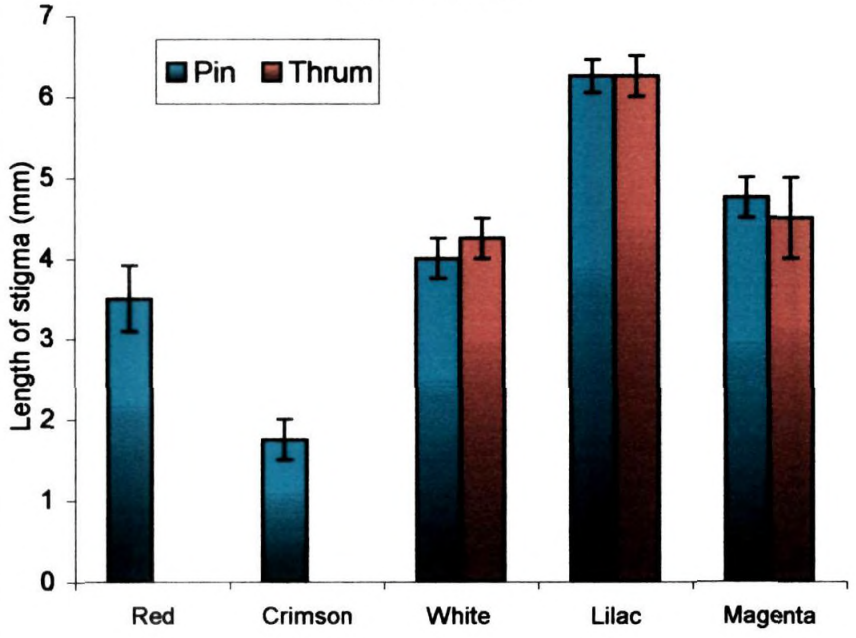


Fig. 33. Length of anther in the colour variants of *P. lanceolata*

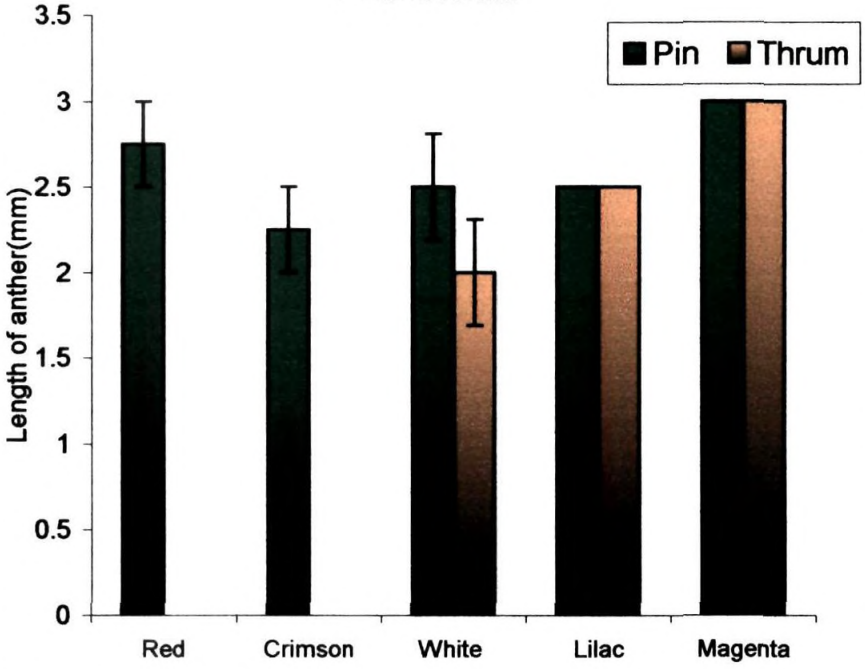


Fig. 34. Level of stigma in the colour variants of *P. lanceolata*

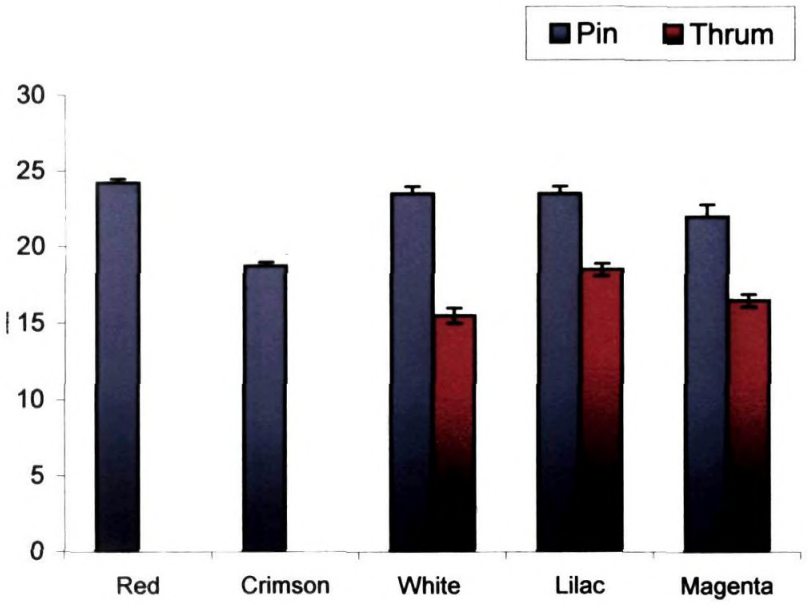


Fig.35. Level of anther in the colour variants of *P. lanceolata*

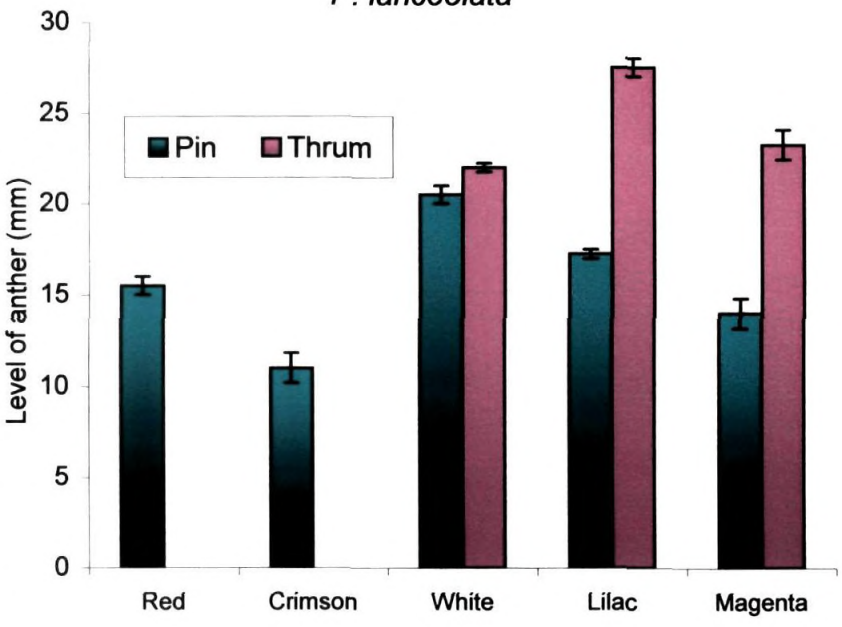


Fig. 36. Level of anther and stigma in the colour variants of *P. lanceolata*

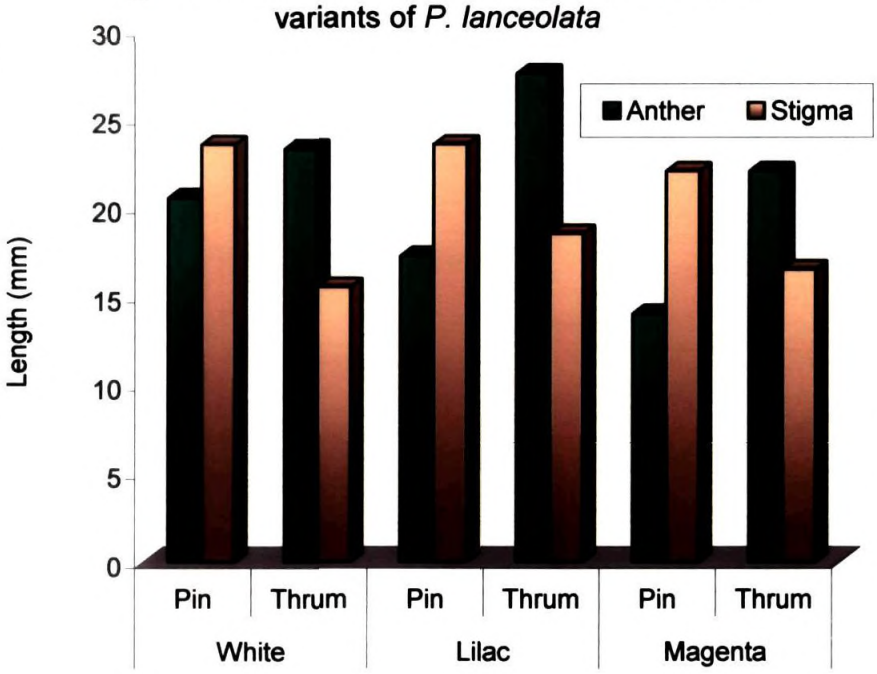


Fig. 37. Percentage of fruit set and seed set after selfing and crossing

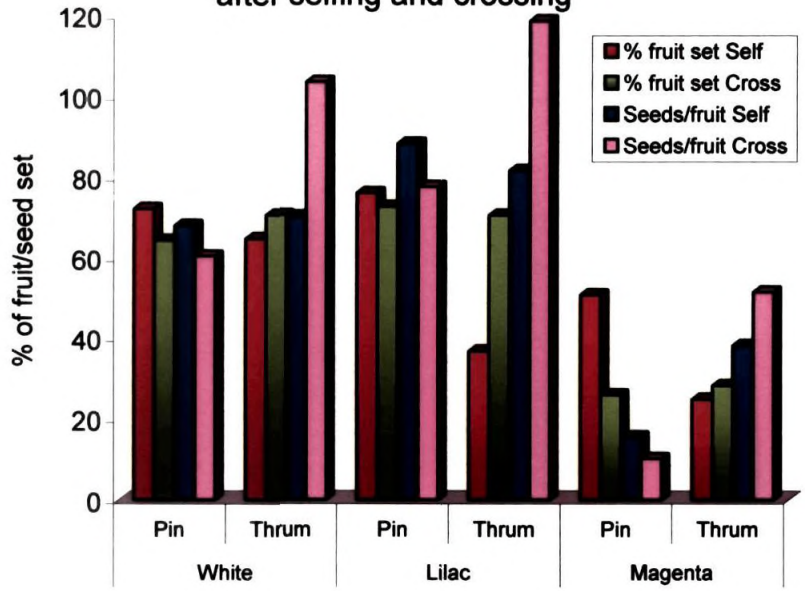


Fig. 38. Germination percentage and tube length after selfing in pin morphs

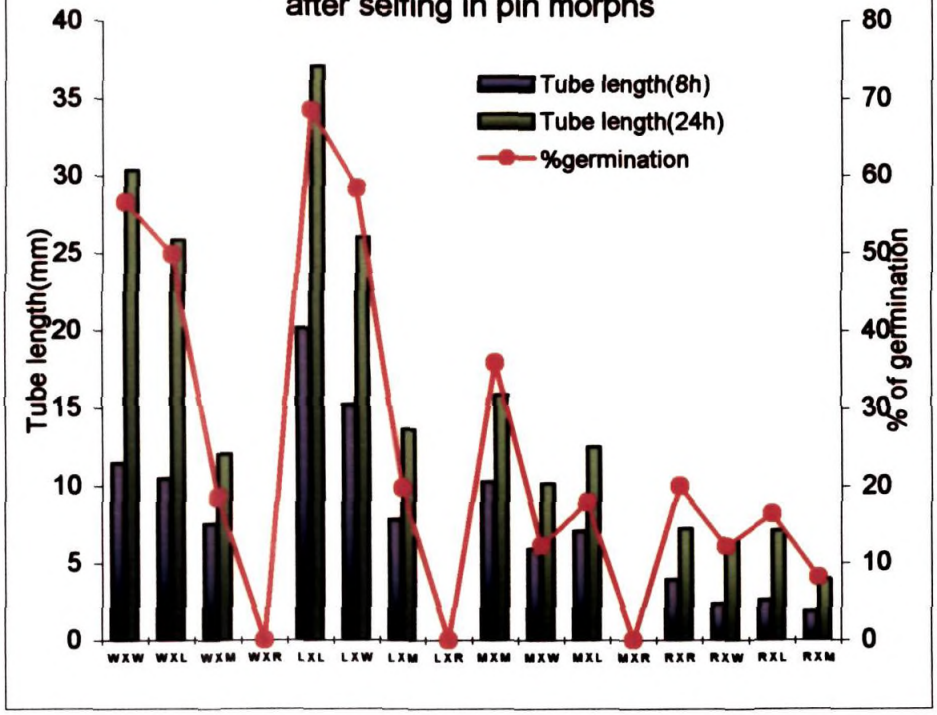


Fig. 39. Germination percentage and tube length after crossing in pin morphs

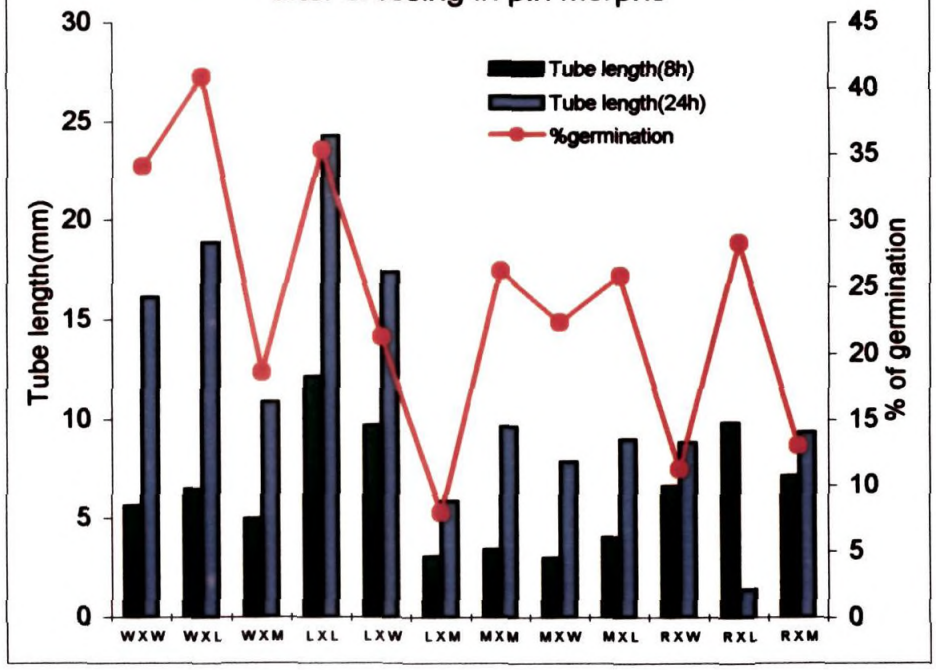


Fig. 40. Germination percentage and tube length after selfing in thrum morphs

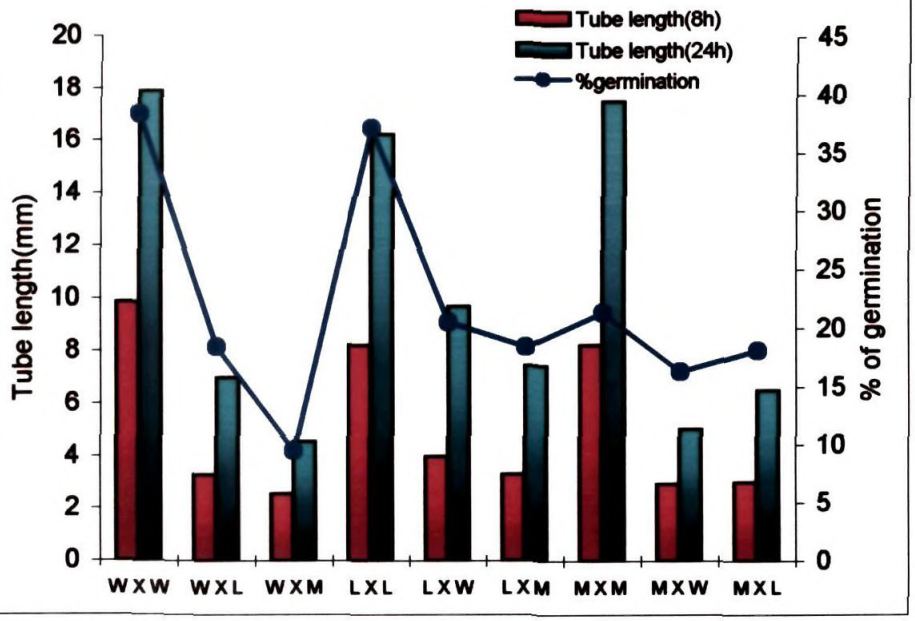
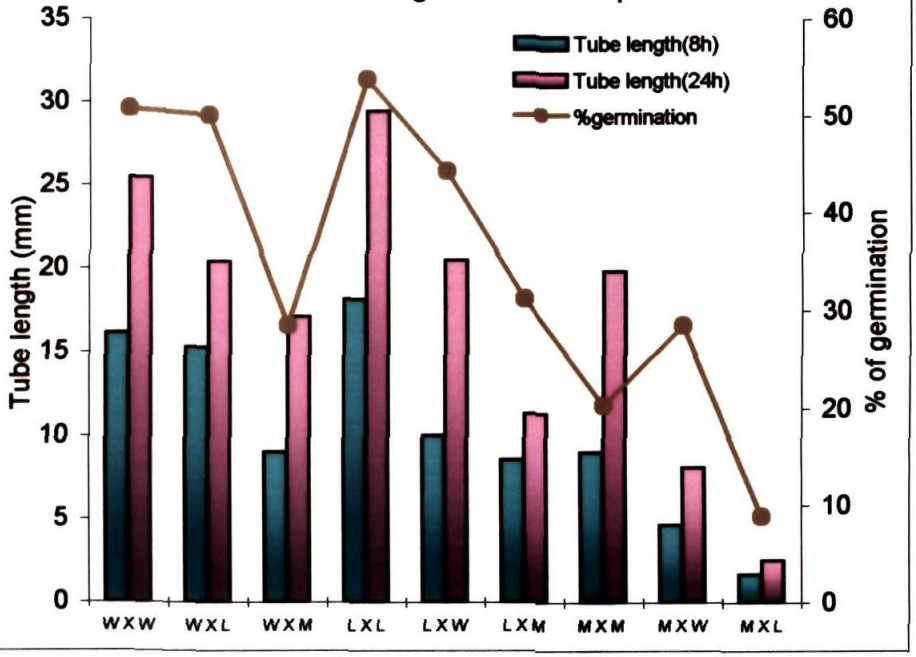


Fig. 41. Germination percentage and tube length after crossing in thrum morphs



Discussion

Heteromorphy in *Pentas lanceolata*

Heteromorphy is an accepted phenomenon in plants belonging to Rubiaceae (Bir Bahadur, 1968) and sizeable literature on the subject has accumulated (Ganders, 1979a ; Wyatt, 1984 ; Bawa and Beach, 1983 ; Barrett, 1992 ; Barrett and Cruzan, 1994 ; Barrett *et al.*, 1996).

Floral features - Pollination Biology

The three colour variants of *Pentas lanceolata viz*, white, lilac and magenta showed floral morphology, typical of distylous plants. The heterostylous species, nearly all have fused petals which form a bell, trumpet or tube (Richards, 1997). The colour variants of *Pentas lanceolata*, studied in this work, showed a corolla tube size ranging from two cm to three cm in length, which are frequently visited by bees, moths, butterflies and ants, which act as the pollinators. Ganders (1979) quote the four cm tubes of *Pentas*. Ants and butterflies are the most frequent pollinators, which carry both thrum and pin pollen on their body parts. According to Richards (1997) most pollinators of heteromorphic flowers are social

bees, moths, butterflies or birds, which can successfully visit the tubes. The corolla tubes of all the colour variants are pubescent, with their corolline hairs scanty, and confined only to the neck of the corolla tubes, in thrum flowers around the stamens in thrum flowers and abundantly distributed, below the neck in pin flowers, where stamens are placed. The corolla tube is swollen below the neck, due to the presence of upwardly directed profusion of corolline hairs around the stamens in pin morph. It seems as if the upwardly directed interlocking hairs block the entry of large insects upto the base of the corolla tube, whereas small visitors like ants, thrips or the proboscis of long tongued flies can easily effect pollination.

Ganders (1979b) stated that dimorphism in corolla size is rare, among heterostylous taxa and that, when examined, a short styled morph always has a larger corolla. Differences in corolla length between long styled and short styled flowers have been recorded for a number of other heterostylous Rubiaceae. Short styled flowers have larger corolla or corolla tubes in *Rudgea jasminoides* (Baker, 1958 ; *Palicourea fendleri* and *P. petiolaris* (Sobrevila *et al.*, 1983), *Luculia gratissima* (Murray, 1990), *Guettarda scabra* (Richards and Koptur, 1993), *Manettia luteorubra* (Passos and Sazima, 1995), *Hedyotis salzmannii* (Riveros *et al.*, 1995) and *Psychotria*

chiapensis and *Bouvardia ternifolia* (Faivre and Mc Dale, 2001). In *Pentas lanceolata* colour variants studied in the present investigation, all the thrum (short styled) flowers had large corolla tubes. Among the Rubiaceae studied, only in *Psychotria surrensis* (Stone, 1995) are long styled flowers larger than short styled flowers . The relationships between floral morph and corolla size suggest that heterostyly may be under phylogenetic or ontogenetic constraints in Rubiaceae (Faivre, 2000).

Stigma

The pin stigma is larger than the thrum stigma in two of the colour variants of *P. lanceolata viz*, white and magenta, whereas it is more or less equal in the lilac colour variant. The stigma in lilac is found to be larger than that of the other two, which may be one of the reasons for the highest percentage of seed set in this colour variant. Size polymorphism of stigmas is widespread among heterostylous species, with the receptive surface of long-styled morph typically larger than that of the short styled one (Dulberger, 1992). In *Jepsonia parryi*, the area of pin stigmas is 50% greater than that of thrum stigmas (Ornduff, 1970a). The length ratio of pin to thrum stigmas is 1.55 in *Plumbago carpensis* and about 2.0 in *Linum grandiflorum*, *L. pubescens* and *L. anueronalium* (Dulberger, 1992). In *P. lanceolata* var. white the ratio of pin to thrum stigma is 1.4.

Pronounced differences between the morphs in the shape and size of stigmas have also been described. In *Rudgea jasminoides*, thrum stigmas are long, narrow and considerably curled, whereas pin stigmas are short and broad (Baker, 1955). In *Villarsia capitata*, pin stigmas are bilobate and thrum stigmas are irregular, and lacerated and crateriform (Ornduff, 1982). Slight differences in stigma shape are also reported in *Anchusa hybrida* (Dulberger, 1970 a), *A. officinalis* (Philipp and Schou, 1981), *Primula vulgaris* (Heslop-Harrison *et al.*, 1981), *P. elatior* (Schou, 1983), *P. obeonica* and *Neandris montholoni* (Bir Bahadur, *et al.*, 1984 b). In *P. lanceolata*, the two lobes of the bilobed pin stigma remain much diverged after anthesis and after a day of anthesis, becomes elongated and slightly curled and the colour of the stigma changed from lighter to darker shades. But in thrum, the stigmatic lobes of the bilobed stigma showed a tendency to remain convergent, may be because of the limited space, inside the corolla tube. The thrum stigmas however did not show any colour change. Heteromorphism of stigma colour occurs in *Linum grandiflorum* where the short thrum stigmas are dark red with more crowded papillae, compared to the light red to pink pin stigmas. Similarly, in *L. pubescens* thrum stigmas are yellowish, whereas pin stigmas are white, probably as a result of cytochemical differences (Dulberger, 1974, 1987 a).

Stigmatic papillae

In *Pentas lanceolata*, the stigmatic papillae of pin flowers of all the three colour variants show a pronounced increase in length, compared to their counterpart, thrum morphs. The length of pin papillae range in size from 81µm to 151µm, whereas it was only 33µm to 63µm in the thrum flowers. The papillae are finger like with slightly swollen apex in pin flowers, whereas it is less swollen in the thrum flowers. The papillae in pin stigma are single celled with a homogenous outer film which turned yellow with Millon's reagent indicating the presence of proteins, whereas in thrum it is single celled and responded negatively to Millons reagent. The papillae in both the cases, collapse after pollination, indicating their positive role in pollination. In *Pentas*, the long papillae in the exposed, divergent dry stigmatic lobes facilitate, easy attachment of pollen. Since the stigmatic surface is exposed and the cross pollen grains are dry, the holding capacity increases the possibility of effective pollination. In thrum, the stigma is well protected inside the corolla tube and once it is pollinated, further protection is not essential. The exposed condition of pin stigma provides freedom for the papillae to elongate. In the case of thrum flower, space restriction occurs within corolla tube.

Perhaps the most frequently reported polymorphism of stigma is that of papillar size, with papillae in long styled flowers larger than in short styled flowers (Viulleumier, 1967; Dulberger, 1974). Thrum papillae are reported to be larger than pin papillae in *Anchusa officinalis* (Schou and Philipp, 1984). *Reinwardtia indica* (Bir Bahadur *et al.*, 1984b). The length of papilla tends to vary with the length of the style (Ganders, 1979a,b). Lewis (1949) was the first to conjecture that papillar length are merely developmental correlates of style length. Pin and thrum pistils have the same number of cells which become more elongate in pin flowers. Larger stigmatic papillae, in large thrum short styled flowers may be a consequence of differential style elongation in the morphs; style and stigma extension may have a common physiological basis (Dulberger, 1992). In *Pentas*, pin stigma is dry, while that of thrum is sticky.

It was observed that changes in elemental distribution took place in papillar cells and pollen grains in the process of cross and self pollination in a self incompatible species of *Brassica* (Iwano *et al.*, 1999). The elemental redistribution was correlated with the structural changes of the cell wall during the penetration of pollen tube which leads to callose formation.

Stamens

In the vast majority of heterostylous plants, the stamens of the morphs differ in anther level and reciprocal herkogamy is obvious, although it lacks precision (Dulberger, 1992). Exceptions are *Linum grandiflorum* (Darwin, 1877) and some populations of *L. suffruiticosum* (Rogers, 1979 ; Nicholls, 1985). As for *L. grandiflorum*, the measurements revealed a slight difference in anther level between the two floral morphs which was noted by Heitz (1980).

All the colour variants of *Pentas lanceolata* studied in this investigation, showed differences in anther level between the two morphs. In thrum flowers, the stamens are long with a prominent filament, but in the pin flowers the stamens are almost sessile. The length of anther however is the same in both the morphs. Generally anther size does not differ between pin and thrum (Richards, 1997), but a few exceptions are listed by Dulberger (1992). The dorsifixed anthers, remain well exerted above the corolla tube in thrum flowers, whereas it is placed inside the corolla tube, below the neck region in pin flowers. The pollen grains are usually seen on the corolline hairs surrounding the stamen in both the morphs, with more in pin than that in thrum. This may be due to the greater quantity of pollen produced or due to the sticky nature of

pollen grains produced from the well-inserted stamens, in the pin flowers. Differences between pollen production of the morphs have been found in most species examined, with stamens of long-styled flowers generally producing more pollen than those of short styled flowers (Ganders, 1979a ; Ornduff, 1980 ; Casper, 1983 ; Barrett and Shore, 1985).

The corolline hairs of thrum flowers show a lesser amount of pollen grains attached to it. This may be because of the extruded condition of the anthers and powdery nonsticky nature of the pollen grains which let them be carried away easily by wind currents.

Pollen size heteromorphism occurs in most heterostylous and tristylous species, belonging to diverse genera and families. Generally pollen grains from short styled flowers are larger than those from long styled ones. The ratios of thrum to pin pollen size may vary from 1.06 to 1.86. In *Pentas lanceolata*, thrum pollen grains are larger than those of pin, the ratio of which was about 1.1. Ganders (1979b) was the first to show an inverse correlation between pollen size and pollen production for a number of dimorphic species. Dulberger (1992) supported this by providing data from 19 species belonging to 12 genera. Piper and Charlesworth (1986) explained this to be due to the fact that reduction in the size of pin pollen allows pins to produce more pollen, without any

increased demand for male resource. It was seen that pin anthers with small pollen grains produced more than twice as many grains as do thrum anthers, in *Primula vulgaris* (Piper and Charlesworth, 1986). But there is no significant difference in the number of grains, that insects removed from the hidden pin anthers, even if the anthers are exposed as in thrum morph. This is true with the number of legitimate pollen grains in the stigmas of emasculated pin and thrum flowers of primrose (Piper and Charlesworth, 1986). This information suggests that the small size and large number of pollen grains have evolved in response to selection pressures to equalize male fitness between pins and thrums (Richards, 1997). Selection for variation in pollen grain size and number between pins and thrums should reflect differences in the availability of pollen for intermorph pollinations.

In *Pentas lanceolata*, pollen grains of pin flowers vary in their size from 16μm to 21μm, whereas those of thrum vary from 17μm to 24μm. Both pin and thrum pollen grains are seen to exhibit polymorphism, not only between morphs, but also among morphs. About 80% of thrum pollen are 4–zonocolporate, but in pin morphs 3,4-zonocolporate pollen grains are seen in equal proportion. Thrum pollen grains are lalongate and reticulate throughout their exine surface, but pin, pollen grains are

lalongate, but scrobiculate at the poles and foveolate towards the equatorial region.

Dissimilar pollen size in the morphs may be associated with slight differences in exine sculpture. Thus a coarse reticulation of exine in larger thrum grains are opposed to a more delicate one in the pin grains, as reported for *Nivenia binata* (Mulachy, 1965; Goldblatt and Bernhardt, 1990). *Oldenlandia umbellata*, *Pentas lanceolata*, *Hedyotis nigricans*, *Morinda tomentosa*, *Polygonum chinensis*, *Hedyotis procumbens* and *Turnera subulata* (Bir Bahadur, 1968, Bir Bahadur *et al.*, 1984 b), *Primula malacoides* (Pandey and Troughton, 1974) and *Primula vulgaris* (Heslop-Harrison, *et al.*, 1981). The differences in exine sculpturing may be a developmental outcome of size dimorphism (Dulberger, 1992).

Size polymorphism of pollen grains may also be accompanied by differing numbers or shape of apertures, with larger grains of high level anthers having more apertures (Lewis, 1965; Bir Bahadur, 1968; Kohler, 1976; Zavala, 1978; Bir Bahadur *et al.*, 1984b).

In *Pentas*, the pin morphs are characteristic with dry stigma having long papillae and sticky pollen while their corresponding thrum morphs are with wet stigma and dry pollen. These features facilitate both cross and self pollination in *Pentas*, which is reflected in their fruit set.

Cytology

All the heterostylous colour variants in this study revealed that they are diploid, having 2n = 20. Previous reports have shown the occurrence of both diploid with n = 10 (Fagerlind, 1937; Raghavan and Rangaswamy, 1941 ; Lewis, 1965 ; Selvaraj, 1982) and tetraploid with n = 2n (Lewis, 1965) in this species.

All the colour variants studied here showed varying degrees of pollen sterility. The heterostylous variants had high pollen fertility and viability, when compared to the homostylous forms. Of the homostylous forms, pollen grains of 'red' are highly fertile and viable whereas those of 'crimson' are sterile.

The sterility in the crimson colour variant may be due to the result of some genetic imbalance.

Anatomy

In *Pentas*, solid style is seen in both pin and thrum morphs. However, the stylar cells of pin are longer than those of thrum. Hence, the long styles in pin morph of *Pentas*, can be explained due to cell elongation, and not due to cell division. This is reflected in the longer papillae of pin morphs. A similar observation was reported in *Primula verticillata* by Al Wadi and Richards (1993).

In vitro pollen germination and tube growth

Pollen tube growth *in vitro* has been studied by a number of investigators to examine the mechanism of growth. In a comparative study of pollen tube growth *in vivo* and *in vitro*, Brewbaker and Majumder (1961) demonstrated that the growth rate of *Petunia* pollen tubes *in vitro* was only 10% of the rate *in vivo* (Kahn and De Mason, 1988). Pollen germination studies in *Sorghum* by Lansac *et al.* (1994) suggested that nutrients required for germination are contained within the grains themselves, and the medium was present to provide nutrients or to transport nutrients. Some species need only the required water imbibition as in the case of some Asclepiads (Sreedevi and Namboodiri, 1977), where pollinia can germinate even in distilled water. Maheswari and Mahadeva (1978) reported an extreme case of pollen germination in dilute HCl of *Datura innoxia* pollen.

The heterostylous as well as homostylous fertile color variants of *P. lanceolata* showed the maximum percentage of germination in Brewbaker's medium supplemented with 20% sucrose. Previous research has shown that sugar requirements of pollen vary with different plant species (Stanley and Linskens, 1974).

Studies of Dhawan and Malik (1981) on *Pinus roxburghii* pollen closely agreed with that of Calzoni *et al.*, (1979) on apple cultivars, where sucrose plays a vital role as a carbon source and as an osmotic agent. Boron has also been shown to enhance pollen tube growth of a large number of species (Stanley and Linskens, 1974).

In *P. lanceolata*, an increase in sucrose above 20% or a decrease below 20% might be causing an alteration in osmotic potential or deficiency in carbon source respectively, thus affecting the pollen germination negatively. The very shy nature shown by pollen tubes to come out in lower concentration of sucrose, showed its deficiency in carbon source and the bursting of pollen tubes in higher concentration of sugar proved that the osmotic potential was affected. The *in vitro* pollen tube growth rate was much less compared to the *in vivo* pollen tube growth, which agreed completely with that of *Petunia* pollen germination. The low growth rate of pollen tubes in *in vitro* medium and the increased growth of pollen tubes on stigma observed in *Pentas* can be explained by assuming that all the essential materials in required quantity is provided by the stigma, while *in vitro* medium can supplement only a few materials known to help pollen germination and tube growth. Acceleration of

germination in liquid medium possibly may relate to an increase in calcium, supplied from the medium (Lanssac *et al.*, 1994).

There is a positive correlation between the percentage of *in vitro* and *in vivo* germination and fruit set in *P. lanceolata* colour variants. The lilac colour variants are seen to show the maximum percentage of *in vitro* germination and fruit set, the magenta being the lowest in germination and fruit set, with white occupying a medium position. This observation suggests that even if *in vitro* and *in vivo* pollen germination efficiency of pollen is not identical, the fruit set is proportionate to *in vitro* and *in vivo* germination.

In vivo pollen germination and tube growth in *Pentas lanceolata*

The pattern of germination and growth of pollen tubes in the pistil has been of great interest to scientists since techniques were developed to observe them. Pollen tube growth is of great importance in assessing plant breeding systems using inhibition or abnormalities of growth, to indicate incompatibility (O' Brien, 1994).

Pollen–stigma interactions in angiosperms can be conveniently divided into a number of distinct phases (Heslop-Harrison, 1975a,b), namely, capture, adhesion, hydration, germination, tube penetration and tube growth. Each phase is characterized by the interaction of different

components of pollen and stigma (Roberts *et al.*, 1983). Adhesion between pollen and stigma is highly selective. In *Arabidopsis* the initial binding is independent of the extra cellular pollen coat, indicating that adhesion molecules reside elsewhere on the pollen surface, most likely within the exine walls (Zinki *et al.*, 1999). Differences in pollen grain adhesion have been detected between cross and self pollinations (Kroh, 1967 ; Roggen, 1972). Quantitative studies of these differences have shown that initially, self pollen is less firmly bound than cross pollen, but after 2 h on the stigma, self pollen becomes adhered to an extent comparable with cross pollen, whereas no further increase in adhesion of cross pollen over this period is detected (Stead *et al.*, 1979). Self and non-self pollen of *Grevillia banksii*, germinated on the stigma, both show normal protein mobilization and synthesis of wall proteins. In *Grevillia*, there is no barrier on the stigma, to foreign pollen tube penetrations (Herscovitch and Martin, 1990). In *Luculia gratissima* (Murray, 1990) there was a massive germination of self pollen in both pin and thrum stigmas. Clear cut differences in pollen tube growth behaviour of incompatible and in partially or completely incompatible crosses were detected in *Solanum commersonii* (Trognitz, 1995). Self pollen tubes grew slower than cross pollen tubes in some crosses and faster in others, in *Hibiscus moscheutos* (Snow and Spira, 1991). In *P. lanceolata*, a significant difference

between self and cross pollen adhesion is not noticed in any of the crosses conducted.

Investigations of stigmas in relation to self incompatibility have been carried out in many plants with heteromorphic systems (Ghosh and Shivanna, 1980a,b; 1983; Stevens and Murray, 1982; Schou, 1984; Richards and Ibrahim, 1982 ; Dulberger, 1987a; Lee *et al.*, 1992; Murfett *et al.*, 1996). From these studies, it is evident that in plants with dimorphic incompatibility systems, the final rejection response may be the outcome of diverse properties of the receptive surface, in the two floral morphs and of differing stages and sites at which the inhibition occur. This explanation is applicable to incompatibility reaction shown by *P. lanceolata* also.

In vivo germination behaviour of *Pentas lanceolata* seems to be close to that reported recently by Kenta *et al.* (2002), in *Dipterocarpus.* In *Dipterocarpus tempehes* the proportions of styles in which the longest pollen tubes reached the base of the style were almost the same in self and cross pollinations, indicating that self incompatibility was not caused by a defect in the elongation of pollen tube in the style. No incompatibility reaction was seen in pollen adhesion and germination on stigma or pollen tube elongation in the style. According to Kenta *et al.* (2002), the

incompatibility reaction in *Dipterocarpus tempehes* was not due to the absence of a component necessary for pollen germination or growth, nor to the existence of a substance interfering with the normal metabolism of the pollen grain or pollen tube as has been previously suggested (de Nettancourt, 1977), but only the guidance of pollen tubes to the style was inhibited. But in *Pentas*, compatible and incompatible pollen tubes exhibit variation in number and rate of tube growth. Similar guidance defects have been reported in previous mutant studies (Wilhelmi and Preuss, 1996; Smyth, 1997) and in a study of interspecific incompatibility (Shimizu and Okada, 2000). Wilhelmi and Preuss (1996) suggested that there was an inhibition of adhesion between pollen tubes and pistil tissues in a mutant of *Arabidopsis*. Shimizu and Okada (2000) studied the interspecific incompatibility in Brassicaceae and reported that there was a lack of directional guidance for pollen tubes to enter the ovules in ovaries. Either of these two models – a lack of adhesion substance or a lack of signal exchange for the directional guidance can also explain the reaction of self incompatibility in *D. tempehes*. Kenta *et al.* (2002) suggested that such a guidance defect as seen in *D. tempehes* contributed not only to interspecies incompatibility, but also to intraspecies incompatibility. For the non-inhibition of self pollen tube elongation in the style observed in *D. tempehes*, Kenta *et al.* (2002) has

suggested two possible explanations. The first, a threshold effect *i.*e. adhesion of a certain number of pollen grains is required for pollen germination in rare species (Hormaza and Herrero, 1994). In other plants, first pollen tubes "pave the way" for later arriving tubes, both mechanisms being controlled by maternal substance. It is possible that the number of pollen tubes entering the styles are below required threshold in most of the self-pollinated ovaries. In *P. lanceolata*, this is found to be the case with the thrum morphs. A possible explanation for low ovary survivorship in the self treatment may be a late-acting self incompatibility (Seavey and Bawa, 1986), which may work before formation of a multicellular embryo.

Despite the widespread occurrence of distyly in Rubiaceae, in most distylous taxa it is not known whether floral dimorphism is associated with a self incompatibility system (Ornduff, 1980). Intramorph cross pollinations and intermorph pollinations revealed the presence of a strong incompatibility system in many plants. In some distylous species, the efficiency of self incompatibility mechanism is greater in the thrum than that in pin morph and this is often correlated with an earlier or more superficial site of pollen tube inhibition (Stevens and Murray, 1982). In other taxa, like *Turnera* (Barrett, 1978), self incompatibility is equally

strong in both the morphs and in the case of *Linum*, the site of pollen tube inhibition is also the same (Murray, 1986, 1990).

Rare examples of heterostylous species in which intramorph crosses are compatible do exist, however, particularly in the Boraginaceae, *Cryptantha flava* (Casper, 1985), *Amsinckia spp.* (Ray and Chisaki, 1957; Ganders, 1979b), *Anchusa officinalis* (Philipp and Schou, 1981), *Anchusa hybrida* (Dulberger, 1970a) and also in *Narcissus tazetta* (Amaryllidaceae) (Dulberger, 1964), *Eichhornia paniculata* (Barrett *et al.*, 1985) and *Palicourea petiolaris* (Rubiaceae) (Sobrevila *et al.*, 1983). In *Cryptantha flava* and *Anchusa officinalis*, there is no apparent difference in germination and growth of pin and thrum pollen in intramorph or intermorph crosses. In *Anchusa officinalis*, pollen from both morphs reach the ovary within 18 h of pollination and can be traced into the ovules through the micropyle (Schou and Phillip, 1983). In *Cryptantha flava*, pollen from pin and thrum morphs reaches the base of the style within 2 days of pollination (Casper, 1985) and there is no difference between self, intramorph or intermorph except in pollen tube number or length at intervals ranging from 2 to 24 h following pollination.

In the present investigation in *P.lanceolata*, self and cross pollen show a massive germination, but self pollen tubes grow faster than cross

pollen in self as well as intramorph cross pollinations in the pin stigma. Both self as well as cross pollen could grow through the entire length of the style, without any inhibition at any region or abnormal growth pattern. The self pollen however show a more regular deposition of callose plugs, which is rather irregular in cross pollination. Self pollination could produce more fruits and seeds than cross pollination in pins. The self incompatibility index also indicate that the pin plants are preferentially self compatible. The result is found to be in agreement with the work of Snow and Spira (1991).

In the thrum flowers of *P. lanceolata*, the efficiency of self-incompatibility mechanism is greater. On thrum stigmas, the cross pollen tubes grow faster and show a regular deposition of callose plugs. The self pollen fail to germinate in the stigmas of magenta colour variant, whereas in the other two self pollen show a highly irregular deposition of callose plugs even though it could traverse the whole length of style. The fruit and seed set data is also more after cross pollination. The self incompatibility index revealed that the thrum plants are partially self incompatible or rather preferentially cross compatible. It may be assumed that in *P. lanceolata* thrum plants, there is a late acting

self incompatibility, comparable to that found in *Dipterocarpus tempehes* (Kenta *et al.*, 2002).

Analysis of cross among inbred lines of *Primula vulgaris* disclosed that the differences between morphs agree with those reported elsewhere, with intra thrum crosses being almost sterile and intra pin crosses relatively fertile (Vaerbak and Andersen, 1997). Inter morph crosses produced higher seed yield when thrum morph was used as the maternal parent, which is in total agreement with that of the thrum plants of *Pentas lanceolata* in the present investigation. Both the large reciprocal differences in inter morph crosses, where the thrum plants as maternal parents are more fertile than pin, as well as the higher sterility of intra thrum pollinations compared to intra pins have been reported previously (Ornduff, 1979 ; Piper and Charlesworth, 1986; Piper *et al.*, 1986 ; Wedderburn and Richards, 1990 ; Richards, 1993).

Ornduff (1980) reported a larger number of pin pollen in stigma of open pollinated *Hedyotis caerulea.* In *Pentas lanceolata* colour variants, the stigmas of pin as well as thrum flowers receive more pin pollen than thrum pollen after open pollination, perhaps due to increased number and dry nature of pin pollen grains. In this context, it is tempting to speculate that this may also be a contributing factor for the increased

effectiveness of pin pollen in pollinations than thrum pollen in self and cross pollinations.

Observations of pollen tube growth in *Nicotiana alata,* has shown that compatible tubes typically showed a uniform layer of callose deposition in the walls in small plugs spaced at regular intervals within the tube, whereas in incompatible tubes there was an irregularity of callose deposition in both walls and plugs (Mary Lush and Clarke, 1997). A similar observation was made in the pollen tubes of *Pentas lanceolata* also. Here self pollination is thus found to be more compatible, in pin flowers and cross pollination in thrum flowers, although both pollen could germinate on stigmas and produce fruits in all the colour variants of the pin morph and two of the colour variants of the thrum morph. A load of self pollen produced as many mature seeds as a load of outcross pollen in *Clarkia* (Jones, 1994) which may be compared to the observation in *P. lanceolata* colour variants.

In *P. lanceolata* colour variants, the percentage of fruit set is lowered following crossing in pin and selfing in thrum in the same colour variants, even though the pollen tubes from both the pollinations traverse the whole length of style. It appears thus that the timing and site of

incompatible pollen tube arrest varies in different species (Kahn and De Mason, 1988).

Thus in all colour variants of *Pentas lanceolata, in vivo* germination on self stigma of pin, suggest that there is no self incompatibility, which is atypical for a heterostylous plant, but the thrum stigmas show a reaction suggestive of incompatibility following self pollination.

Self compatibility / incompatibility in *Pentas lanceolata*

Heterostyly was recognized as a morphological feature of certain groups of flowering plants, as early as the 16th century, when it was noted in *Primula* by Clusius (vanDijk, 1943). Few attempts were made to interpret the adaptive significance of this floral heteromorphism until Charles Darwin and Friedrich Hildebrand studied the phenomenon just after the middle of the 19th century. Darwin, in his paper (1862) concluded that the floral dimorphism in *Primula* represents a physiological - morphological adaptation to promote outcrossing between the two morphs, long and short styled. Darwin (1877) suggested that reciprocal placement of stigmas and anthers serves as a mechanical device for promoting insect-mediated legitimate pollinations. According to Darwin's interpretations, the morphological features of heterostyly and sterility in self and intra morph cross-pollinations are two distinct

outbreeding mechanisms. This interpretation has received wide acceptance among students of heterostyly (Baker, 1966; Viulleumier, 1967; Ganders, 1979b; Charlesworth and Charlesworth, 1979; Lewis, 1982; Barrett, 1988a). Considering this, observations in *Pentas* are curious.

However, it has been shown that heterostyly may also be accompanied by multiallelic incompatibility, as appears in the case of *Narcissus tazetta* (Dulberger, 1970a) and *Anchusa officinalis* (Philipp and Schou, 1981; Schou and Philipp, 1984). It may be accompanied by self compatibility as in *Amsinckia species* (Ray and Chisaki, 1957; Ganders, 1975), *Eichhornia crassipes* (Barrett, 1977a,b, 1979), *E. paniculata* (Barrett, 1985a) and in *Cryptantha flava* (Casper, 1985).

A similar case of self compatibility is observed in the three heteromorphic colour variants of *P. lanceolata* studied here but in varying degrees. The self incompatibility index of all the three colour variants have shown that pin plants are preferentially selfed, but while their corresponding thrum morphs are partially self compatible. This gives a conclusive evidence that the heterostylous *P. lanceolata* plant is preferentially or partially self compatible. Moreover, pin plants are more self compatible, and the thrum plants are more cross compatible and partially self compatible. In recent years, many more examples of self

compatible heterostylous plants have become available, including members of the genera, *Cryptanthus*, *Decodon*, *Melochia*, *Oplonia* and *Quinchamalium* (Barrett and Cruzan, 1994).

In *Pentas lanceolata*, the two monomorphic colour variants studied show all the characteristics of a typical pin morph, but with not having their thrum morphs. Even though all the heterostylous pin morphs are preferentially self compatible, and set seeds in abundance, following self pollination, the monomorphic pin plants failed to set seeds. One of the monomorphic colour variants *viz.* crimson is pollen sterile also. Irrespective of their morphs, all the colour variants are conventionally propagated by stem cuttings.

There are two situations in which homomorphism occurs. The homomorphic types may be rare variants in wild populations or in cultivation or they may occur as a significant proportion of the individuals in a population (Lewis and Jones, 1992). The important point is that they are either of very recent origin or are of long standing and interbreeding with the distylous forms from which they have been derived. Lewis and Jones (1992) assumes that all the monomorphics are derived from heteromorphics, as being opposed to being primary *ie.* primitive (Ernst, 1955).

The long homostyles of the distylic *Amsinckia spectabilis* extremely variable and it has been suggested by Ganders (1975) that arose as a result of modifier genes, similar to those found in *Pr sinensis.* Shore and Barrett (1985a,b) conclude that the homostyle *Turnera ulmifolia* have arisen *via* the same genetic mechanism invol their distyly supergene.

Self incompatible long homostyles and self incompatible s homostyles were reported by Ernst (1943). These occur more rarely do self compatible homostyles. Homostyly in *Primula japonica* evolved from heterostylous ancestors by recombinations, retainin morphological and incompatibility features of the original heteros (Wedderburn and Richards, 1990). Experiments by Wedderburn Richards (1992) showed convincingly that the homostyle species invo were indeed secondary homostyles, which had evolved f heterostylous species by recombination. Here pin x pin selfs are sli self fertile, but thrum x thrum selfs usually set no seed whatsoever similar but less expressed condition is observed in *Pentas lanceolata* als which pin x pin is more successful than thrum x thrum. In *Primula*, al 6% of the species are secondary long homostyles and these were gene polyploid (Wedderburn and Richards, 1992). In *Pentas*, all the co

variants studied are diploid (2n = 20). Crosses between secondarily monomorphic and dimorphic individuals in Plumbaginaceae show that monomorphs, have lost the papillate stigma recognition (Baker, 1966). In *Pentas*, self and cross pollen grains are well recognized by stigma. Alwadi and Richards (1993) argue that some homostylous *Primula* are primary homostyles, representing the conditions in the genus before heterostyly evolved. Baker (1966) explained that the monomorphic species are derived from dimorphic ancestors and like most monomorphic species are self incompatible. The homostylous colour variants of *P. lanceolata* are however self and cross incompatible or sterile and hence set no seeds.

All the heterostylous colour variants were pollen fertile and show a variation in the percentage of pollen fertility and viability. The lilac colour variant had the highest percentage of pollen fertility and viability, the white colour variant comes next and the magenta colour variant showed the lowest percentage of pollen fertility and viability and this is reflected in seed set, also which is highest in lilac and lowest in magenta. The homostylous red variety however showed a very good percentage of pollen fertility and viability, but produced no seeds in any of the combinations. The pollen fertile heterostylous variants produced selfed as well as crossed seeds which help in recombination of characters,

common in the species, producing varying colour combinations. The seed propagated plants were quite often seen to show change in corolla colour. The heterostyles are maintained so, in order to carry out self and cross pollinations. The vegetatively propagated homomorphic plants are either pollen fertile or sterile, with no seed set because being monomorphic much variability is not expected in them.

Fruit set

The field studies conducted at two different levels, inter and intra varietal selfing and crossing produced very interesting results.

In pin plants, the highest percentage of pollen germination and fruit set were observed in selfed lilac colour variants. The percentage of pollen germination and fruit set were the second best in the white variety and the lowest in the magenta variety. The homostylous red pin plant show a low percentage of pollen germination and tube growth on stigma, but produced no fruits.

The pin plants on crossing produce the highest percentage of pollen germination and fruit set, when the lilac flowers were crossed with thrum flowers (inter morph, intra varietal). The second best result was seen in the white pin crosses and the lowest in the magenta pin colour variant.

The pin plants thus produced better results in terms of fruit set when selfed. However lower percentage of fruit set was observed when they were crossed. The lilac pin variety was the best fruit setter, in both self as well as cross pollinations.

Among thrum plants, the intra varietal as well as inter varietal selfing produced a lower percentage of germination, and fruit set compared to that in pin plants. The white thrum variety showed the highest percentage of fruit set, even though the percentage of pollen germination and tube elongation are lower than that of lilac variants. Lilac thrum variant showed a fairly good percentage of pollen germination, but a markedly lower percentage of fruit set, than that of the white thrum variety. Magenta variety showed very low percentage of pollen germination, tube elongation and fruit set.

The thrum plants however showed a higher percentage of pollen germination, tube elongation and fruit set following inter and intra varietal cross pollinations. The white as well as lilac colour variants showed a higher rate of pollen germination, and fruit set in intra varietal crossing with pin flowers. In lilac, cross pollination produced a remarkably higher percentage of fruit set compared to self pollination. In

magenta also the cross pollination produced a slightly higher rate of pollen germination and fruit set.

However the thrum plants showed a better performance following cross pollination than self pollination. The white thrum variety was found to be the best, with regard to fruit set, after selfing and crossing. The lilac variety showed a higher fruit set on crossing, but much lower on selfing, whereas the magenta variety was a poor performer with the lowest fruit set following selfing and crossing. This variation in their performance in seed set was reflected in populations as well, where magenta plants were lower in number compared to others.

In short, it may be concluded that the pin plants show higher percentage of fruit set than that in thrum plants, in all the three colour variants of *Pentas lanceolata* studied in the present work. Opler *et al.*, (1975) considered thrum as the morph that produces almost no seeds at all and the pin as the morph with aborted anthers or inviable pollen. Lack and Kevan (1987) observed in *Sarcotheca celebica* (Oxalidaceae) that pins produce very little pollen and yield more seeds per fruit following natural pollinations and are more likely to set fruit following inter morph crosses than thrums. Ornduff (1986) found variability in the seed set pattern of *Villarsia*, the pins producing more seeds than others. Inter

morph crosses produced a mean of 10.2 seeds per pollination in pins and 3.3 in thrums (Ornduff, 1982). Naturally pollinated pins also produced more seeds in *Linum perenne* (Hicks *et al.*, 1985).

Self pollinations of the short styled morphs in *Eichhornia paniculata* also result in lower mean seed set than self pollinations in others and some families are deficient in short styled progeny (Barrett *et al.*, 1989) which is in agreement with the observations in *Pentas lanceolata* thrum morphs also.

In vitro bioassay

In all the colour variants of *P. lanceolata*, the stigmatic extract added to the standard culture medium lowered the percentage of pollen germination, suggesting a possible inhibition both in compatible and incompatible pollen. Experiments with leachates however showed results, more or less comparable to the *in vivo* pollen germination and tube growth. The pin pollen grain showed an equally good percentage of germination in pin as well as thrum stigmatic leachate. The thrum pollen grains showed a lower percentage of germination in thrum stigmatic leachate and a better germination in pin stigmatic leachate.

Population studies

Population surveys of distylous species frequently report equality of the floral morphs, although exceptions are known (Crosby, 1949; Levin, 1974; Ornduff, 1979, 1980; Richards and Ibrahim, 1982). Morph frequencies in populations of self compatible species (Ganders, 1975; Riveros *et al.*, 1987) or those with extensive clonal growth (Mulachy, 1964; Barrett, 1980) frequently display unequal morph ratios, because of morph specific differences in selfing rate or founder effects. In *Anchusa officinalis*, the long styled morph predominated in all eleven populations sampled by Philipp and Schou (1981). A similar pattern was evident in 10 out of 11 dimorphic and trimorphic populations of *Narcissus spp*, surveyed in Southern Spain (Barrett *et al.*, 1993, 1996). Interestingly, the long-styled morph also predominates in the only two well documented non-heterostylous species with stigma height polymorphisms (Jernstedt, 1982; O'Brien and Calder, 1989).

In *Pentas lanceolata* also the survey conducted in six populations, all revealed a predomination of the long-styled plants. The ratio of pin to thrum is approximately 3:1, instead of the expected 1:1. This is in agreement with the finding of unequal ratio of the morphs in population of self compatible species.

These data suggest that the long-styled phenotype commonly has a selective advantage over the short-styled phenotype, a finding consistent with the overall prevalence of approach herkogamy and rarely of reverse herkogamy angiosperm families (Webb and Lloyd, 1986). According to Webb and Lloyd, this type of variation may represent an early stage in the evolution of heterostyly. Surveys of morph frequencies in heterostylous populations have provided valuable clues on the evolutionary processes responsible for the breakdown of heterostyly.

In some species of *Amsinckia* there seems to be a selection operating against the thrum allele. Intra morph crosses between heterozygous thrums result in an excess of pins (Weller and Ornduff, 1977, 1989 ; Ganders, 1979a,b). Selection may be operating against the thrum allele in thrum seed parent, during ovule formation and embryo development.

Inheritance of heterostyly in *P. lanceolata*

Early experimental studies on heterostyly were largely genetical in nature and concerned with determining the inheritance of polymorphism. Many leading geneticists, working in the early to mid-periods of twentieth century, were attracted to working on distyly and tristyly as a model system for studies on inheritance.

The inheritances of heterostyly has been determined for apparently 23 species, in 11 families by Lewis and Jones (1992) and suggest that the inheritance of distyly is due to a single factor with two allelomorphs, S and s. The dominance of short styled morph over long styled morph has been discovered in many species, but with three exceptions (Lewis and Jones, 1992). These cases where long style is dominant over short style are *Limonium* (Plumbaginaceae), *Hypericum* (Guttiferae) and *Armeria* (Plumbaginaceae).

The inheritance pattern of the colour variants of *P. lanceolata* in the present investigation suggests that the long-styled condition is dominant over short-styled. The long-styled or pin morphs are seen to produce both pin and thrum progeny, whereas the thrum plants invariably produce only thrum progeny. The frequency of thrum progeny produced is much lower when compared to pin progeny. Shore and Barrett (1984, 1985) also reported an excess of pin morphs obtained from selfing rare thrum plants of *Turnera ulmifolia* that exhibit a breakdown in self incompatibility. In this case, they suggest that the self compatibility gene imparts a selection advantage to male gametophytes carrying recessive alleles, a hypothesis which has not been tested experimentally. However, it seems that this hypothesis is not applicable to the colour variants of *P. lanceolata*, in the present investigation.

BIOCHEMICAL STUDIES

In recent years reproductive biologists have been giving increased attention to the structural and biochemical aspects of the pistil for an overall understanding of the reproductive biology of flowering plants. There have been a large number of studies on these aspects covering solid-styled systems (Heslop-Harrison and Shivanna, 1977; Heslop-Harison, 1981; Shivanna and Johri, 1985) and few on hollow styled systems such as *Gladiolus* (Clarke *et al.,* 1978), *Crocus* (Heslop-Harrison, 1977), *Ornithogalum* (Tilton and Horner, 1980) and *Citrus* (Ciampolini *et al.*, 1981, 1983 ; Cresti *et al.*, 1982). In the case of *P. lanceolata*, the style is solid in all the colour variants studied.

For understanding the nature of pollen-pistil interaction, it is important to know the structure and biochemistry of the stigma. In the last decade, considerable progress has been made in our understanding of the part played by the stigma surface in interactions with pollen grains and in the control of self incompatibility. (Mattson *et al.*, 1974; Heslop-Harrison and Barber, 1975 ; Heslop-Harrison, 1975a,b; Heslop-Harrison and Shivanna, 1977; Knox, 1984 ; Dulberger, 1987a,b,c).

The angiosperm stigma is generally considered as a glandular structure whose secretion is important in the pollen-stigma interaction.

(Williams *et al.*, 1982; Linskens, 1975) Pollen hydration, germination and penetration of the stigma by pollen tubes are influenced by the exudate on wet stigmas (Goldman *et al.*, 1994) and by the pollen coat in species with dry stigma. (Preuss *et al.*, 1993). The exudate allows pollen tubes to grow directly into the stigma, whereas the pollen coat establishes the contact with stigma. (Meike Wolters-Arts *et al.*, 1998). The components of the exudate or pollen coat that is responsible for pollen tube penetration are not yet clearly known.

In the present investigation, an attempt has been made to find out the role of the components in stigma in controlling self and cross fertilization. The stigmatic exudate is a complex mixture of different chemical compounds such as proteins, saccharides, fatty acids and phenols. (Cresti *et al.*, 1986). In *Sternbergia lutea*, the exudate is rich in pectins and other polysaccharides, but poor in proteins and lipids (Ciampolini *et al.*, 1990, 1995). The exudate consists of unsaturated lipids, reducing acids, proteins, phenols, insoluble polysaccharides and alkaloids in *Lablab purpureus* (Manoj Kumar and Bhatnagar, 1989).

There is an abundance of evidence that stigmatic secretions contain a wide variety of compounds. Some of them are involved in cell recognition reactions between pollen and stigma. (Linskens, 1975; Kanno

and Hinata, 1969; Martin, 1969, 1970 a,b; Martin and Ruberte, 1972 ; Hinata and Nishio, 1978; Nishio and Hinata, 1978; Knox *et al.*, 1976; Clarke and Knox, 1978; Shivanna *et al.*, 1978). Some enhance pollen germination and early pollen tube growth (Konar and Linskens, 1966 a; Martin, 1969, 1970 a, b; Martin and Ruberte, 1972) It is evident from this information that stigmatic transmitting tissue has several very crucial physiological and biochemical functions in addition to serving as a landing platform for pollen. (Tilton and Horner, 1980). The surface of the stigmatic papillar cells is covered by a proteinaceous pellicle (Mattson *et al.*, 1974) and by means of protease digesting experiments, proteins of this layer have been shown to be responsible for binding the pollen (Stead *et al.*, 1979). In *Pentas*, the dry pin stigmatic surface, has shown the presence of protein which was absent in the thrum.

In some plants, such as *Acacia* species (Kenrick and Knox, 1985) there is no exudate secretion before pollination, but pollination stimulates the secretion of the exudates. In *Acacia* and *Annona*, even foreign pollen may induce secretion of the exudate while there is no secretion in the unpollinated stigmas. In *Petunia hybrida*, pollination has no effect on the ongoing secretion of the exudates (Konar and Linskens, 1966a). Secretion in pistil appear to have a clear function in supporting pollen

tube growth (Gonzalez *et al.*, 1996) in kiwi fruit. The abundant stigmatic secretion ruptures the stigmatic cuticle, which occurs in other species also (Knox, 1984). This secretion favours pollen adherence, hydration and germination. The exudate is chiefly lipoidal in legumes (Ghosh and Shivanna, 1983), in some other plants like *Aptenia*, it consists of saccharides along with traces of proteins and pectic substances (Kristen *et al.*, 1979).

Soluble proteins

The protein component of the exudate has an important role in the pollen stigma interaction (Knox, 1984). In *Brassica*, the S-locus related glycoprotein 1 (SLR I), a stigma specific protein, belonging to the S-gene family of proteins, has been shown to be involved in the adhesion of pollen grains to the stigmatic surface (Takayama *et al.*, 2000). When the surface proteins are removed by enzyme treatment from the stigmas of some species of *Caryophyllaceae* and *Cruciferae*, the pollen tubes are unable to penetrate the cuticle, suggesting that, if the pollen contributes a cutinase precursor, activation depends on interaction with a factor held in the surface secretion of the stigma papilla.

In the colour variants of *Pentas lanceolota* studied, the virgin stigmas contain a comparatively low content of protein. The protein content

however is much lowered after incompatible pollinations *ie.*, cross pollination on pin stigmas and self pollination on thrum stigmas. In this context, it is tempting to speculate that the proteins essential for pollen tube penetration and growth are synthesized when compatible pollen lands on the stigma.

The electrophoretic profile also revealed a picture, identical to the above situation. The appearance of three new bands of proteins after compatible pollinations *i.e.* selfing in pin stigmas and crossing in thrum stigmas gave a supporting evidence. Vithanage and Heslop-Harrison (1979) reported the accumulation of pollen wall protein on stigma of *Secale cereale* after 24 h of pollination. The protein constituent of the stigmatic pellicle is critical for pollen capture. These proteins may not be involved in the initial stages of pollen adhesion on the stigma but they may be involved in the later stages. The patterns of pollen tube performance observed in *Erythronium* (Cruzan, 1990) indicate that compatibility substances may have been produced by the style after pollination and that their concentration and composition depend on the source of pollen present. Increased metabolic activity and post-pollination production of stylar substances are known. (de Nettancourt,

1977), but it is not clearly known whether these compounds play a role in the compatibility response.

The initial recognition event, which occurs within minutes of self pollination, appears to involve at least one glycoprotein from the stigmatic pellicle and one or more low molecular weight proteins of the sporophytically determined pollen grain coating (Roberts *et al.*, 1983). The S-allele associated ribonucleases in the pistil were found to be involved in self pollen rejection in *Petunia hybrida* (Entani *et al.*, 1999).

Pollen tubes have a trophic dependence on the pistil, since pollen tube growth in the style is supported at the expense of pistil reserves. (Herrero and Dickinson, 1975; Mulachy and Mulachy, 1983). The secretions of the pistil supply nutrition and provide a pathway for the pollen tubes towards the ovules. The trophic dependence plays a role in regulating pollen tube kinetics and in reducing the gametophytic population (Herrero and Arbeola, 1989). It has been suggested that through this interaction, mate choice can occur in plants and that particular pollen genotypes could be selected by the pistil (Hormaza and Herrero, 1994).

In plants with self incompatible pollination system, their pistil tissues are known to produce molecules important for pollen recognition

to reject incompatible pollen or their tubes (Newbegin *et al.*, 1993 ; Nasrallah *et al.*, 1994 ; Li *et al.*, 1994 ; Foote *et al.*, 1994). One of these molecules, the S-RNase from *Nicotiana alata*, has been shown to be taken up by pollen tubes (Gray *et al.*, 1991) and to hydrolyze pollen tube RNAs (Mc Clure *et al.*, 1990), indicating direct interactions between these pollen pistil proteins and pollen tubes.

Soluble sugars

In *Pentas lancelata* colour variants the exudates were found to consist chiefly of soluble sugars. Following pollination the content of soluble sugar decreased. In pin stigmas which were preferentially self compatible, the soluble sugar greatly decreased after selfing, but less so after crossing. But in the thrum stigmas, which is only partially self compatible, the sugar content is found to show a pronounced decrease following cross pollination and a comparatively low decrease after selfing.

The presence of chemically different saccharidic fractions has been revealed in numerous stigmatic exudates (Konar and Linskens, 1966a ; Kristen *et al.*, 1979 ; Sedgeley and Scholefield, 1980 ; Kadej *et al.*, 1985), even though there are plants that do not show carbohydrate secretions (Martin and Brewbaker, 1971). The large number of monosaccharides with different chemical structure in *Nicotiana* exudate did not help to

explain their role and function. (Cresti *et al.*, 1986). In some angiosperms, the sugar composition of the pollen tube wall (Nakamura and Suzuki, 1981) seems to be similar to the sugar present in the *Nicotiana* exudate. So the saccharidic fractions or the single monosaccharides in the exudate may be involved in the wall biosynthesis of growing pollen tubes and in the recognition reaction (Clarke and Geeson, 1981).

In *Pentas lanceolata*, also this may be true. The lowering of soluble sugars following more compatible pollinations may be due to the fact that the sugars may be involved in the wall biosynthesis of growing pollen tubes and in the recognition reaction. The longer pollen tubes of the pin pollen may be consuming more of the soluble sugars after selfing on the pin stigma, and after crossing on the thrum stigma. Hence a lowering of soluble sugars is observed after selfing in pin stigma and crossing in thrum stigma.

Phenols

The unpollinated stigmas of the three heterostylous colour variants of *P. lanceolata* showed a comparatively low content of phenols. The phenol content decreased much after compatible pollination rather than incompatible pollination. In the stigma of pin plants, the phenol content showed a significant decrease after compatible self pollination and in the

thrum stigma, the decrease was more pronounced after cross pollination which is also compatible.

Martin reported the presence of phenolic compounds on stigma surface as early as 1969. The stigmatic exudate in *Nicotiana* is principally composed of lipids, phenols, proteins and polysaccharides (Cresti *et al.*, 1986). Phenolic compounds are characteristic of the stigma exudate of many species (Knox, 1984; Manoj Kumar and Bhatnagar, 1989; Gonzalez *et al.*, 1996). In *Actinidia deliciosa* (kiwi fruit) the pollen germination depends on stigmatic papillar integrity (Gonzalez *et al.*, 1995). The papillae were associated with the liberation of some intracellular contents into the stigma, the most conspicuous of which were phenolic inclusions. They did not appear to be involved in the post pollen germination stages of fertilization and fruit development in kiwi fruit since they did not change with pollination. The phenolic compounds however may selectively stimulate or inhibit pollen germination (Vasil, 1974; Dhingra and Varghese, 1985) or conserve the sugar of the stigma by protecting it from insects. Martin and Ruberte (1972) reported that simple phenolic compounds could inhibit pollen germination and have a role in pollen rejection on stigma surface.

In *Pentas lanceolata,* it is tempting to assume that the phenols have a stimulatory effect on pollen tube germination. The stimulatory effect of flavonols on pollen tube growth was established in tobacco by Ylstra *et al.,* (1992), where kaempferol, quercetin and myricetin were found to increase pollen tube growth.

Flavonols, particularly kaempferol and quercetin (Vogt *et al.*, 1995), have been shown to be essential for pollen germination and tube growth in *Petunia* and maize (Mo *et al.*, 1992; Taylor and Jorgensen, 1992 ; Pollak *et al.*, 1995). According to Napoli *et al.* (1999), the biochemical complementation with nanomolar amounts of kaempferol, a flavonol aglycone has confirmed that the inability of white anther pollen in *Petunia* to germinatewas due to a lack of this essential flavonol.

Peroxidase

Numerous attempts have been made to relate the reaction of incompatibility to specific enzymes, which would act to prevent the growth of incompatible tubes. Among the enzymes, possibly involved in the manifestation of incompatibility, the most important ones are peroxidase isoenzymes, which regulate protein activity, destruct growth hormone IAA and as reviewed by Bredemeyer (1974) catalyze a number of essential reactions for regulation of pollen tube growth through the

style (de Nettancourt, 1977). Other possible roles of peroxidase are on ethylene formation (Mapson and Wardale, 1971), destruction of toxic hydrogen peroxidase (Cohen and Hochsetin, 1963). Pandey (1967a) suggested that peroxidase isozymes determine S-gene specificity. However Desborough and Peloquin (1968) working with *Lilium longiflorum*, and Nasarallah *et al.*, (1970), in *Brassica* could not detect a consistent relationship between peroxidase isozymes and S-gene specificity. Bredmeyer (1973, 1975b) analysed peroxidase activity and peroxidase isoenzyme patterns in the types of pollination performed (compatible or incompatible). His results showed that increase in peroxidase following pollination is more pronounced in cross than in self pollinated styles.

In *Pentas lanceolata*, Bredmeyer's observation was found to hold good in the pin morphs. But in the thrum morphs, a pronounced increase in peroxidase activity is observed after self pollination. These observations suggest that increased peroxidase activity is due to incompatible rather than compatible pollinations in both the morphs.

Bredmeyer proposed that peroxidase acted by inducing change in the structural glycoprotein, enzymes, pectin or cellulose present in pollen tube walls. Bredmeyer (1973) and Pandey (1967a) were of the opinion

that peroxidase plays a role in incompatibility, functioning as part of an oppositional mechanism of pollen tube-style interaction .

Although, a defined role is always referred to pollination process for each component of the pistil exudate, it may be concluded that the different components cannot be considered separately, but must be treated together in order to understand the regulation of the pollen-stigma interaction.

Proline

The accumulation of proline in a variety of species under various kinds of stress and its possible involvement in adaptive mechanisms have been reviewed (Palfi *et al.*, 1974; Aspinall and Paleg, 1981). Bhaskaran *et al.* (1985) found no correlation between proline level and stress tolerance in *Sorghum* and concluded proline increase in their system was an incidental consequence rather than an adaptive response to stress. Handa *et al.* (1986) however have reported a high relationship between proline level and stress tolerance in tomato. Hanson and Nelson (1978) suggested that proline accumulation was merely a symptom of injury.

Since pollen is a foreign living body, whether compatible or incompatible, it can create a stress on the stigma. So, pollination triggers the defence system of plants which leads to an increase in proline content

of the stigma. Palfi *et al.* (1988) reported that there is free proline in the pollen grains of many plants, which on pollination diffuse to the stigmatic surface.

The three heterostylous colour variants of *Pentas lanceolata* also showed a very low increase in proline content following pollination. The unpollinated stigmas were found to contain a very low amount of proline, which increased slightly after pollination. The increase though not pronounced was more after incompatible pollinations *i.e.* in pin stigma, the proline content increase was after cross pollination, whereas in thrum the increase was after self pollination. The increase however gave no indication as to whether it is having an inhibitory or stimulatory effect. It has been reported that proline upto an optimal concentration is capable of alleviating growth inhibition, but beyond a particular concentration it alters cellular physiology (Vinod Kumar, *et al.*, 1990).

Evolution of heterostyly

Heterostyly has originated on more than 20 separate occasions among angiosperm families, yet understanding its evolutionary development remains one of the most difficult problems in the evolution of reproductive systems. Perhaps, this is because the course of evolution in heterostylous groups, may have been complex and circuitous. Apart

from the Plumbaginaceae, where Baker's studies suggest the build up of distyly in several steps, beginning with diallelic incompatibility (Baker, 1966), in most families, the polymorphisms appear to arise *de novo*, without obvious clues as to the intermediate stages involved. In this respect, heterostyly differs from other polymorphic sexual systems, such as dioecy, where variation patterns among related taxa have enabled references on the evolutionary pathway involved in the separation of sexes. Many workers, except Anderson (1973) and Richards (1986) have favoured the view that diallelic incompatibility precedes the evolution of reciprocal herkogamy in heterostylous plants (Baker, 1966; Yeo, 1975; Charlesworth and Charlesworth, 1979; Ganders, 1979b; Lewis, 1982). However Lloyd and Webb (1992) has revived Darwin's original idea (Darwin, 1877) that reciprocal herkogamy developed first, followed by the evolution of an intramorph incompatibility system.

The phylogenetic status of self compatible heterostylous taxa as seen in *Pentas* is of particular interest in evaluating models for the evolution and function of heterostyly. In recent years, more cases have been reported in which the morphological components of the polymorphism are accompanied by high levels of self fertility. Several

genera most notably, *Amsinckia, Cryptantha, Eichhornia, Melochia, Nivenia, Quinchamalium* and monotypic *Decodon* contain species that are highly self compatible (Barrett, 1992). It has been assumed that this condition is derived through relaxation and eventual loss of diallelic incompatibility (Ganders, 1979b). Genes modifying the strength of incompatibility commonly occur in heterostylous species where related taxa possess normally functioning diallelic incompatibility systems. However, Lloyd and Webb's (1992) model of the evolution of heterostyly predicts the occurrence of self compatible heterostylous populations as an ancestral condition in heterostylous groups. This contrast with Charlesworth's model (Charlesworth and Charlesworth, 1979) where self compatible heterostyly is always likely to be a derived condition. Sound phylogenetic data on the relationships between self compatible and self incompatible heterostylous taxa would therefore be useful in evaluating the validity of these models.

A variety of evolutionary modification have also been documented in distylous groups. (Richards, 1986; Barrett, 1988 b). These include the shift from outcrossing to different degrees of selfing through the

evolution of homostyly (Barrett and Shore, 1987) and the evolution of various forms of gender specialization including gynodioecy (Baker, 1966) and dioecy primarily in Rubiaceae, Menyanthaceae and Boraginaceae (Baker, 1958; Opler, *et al.*, 1975; Charlesworth, 1982). According to Schoen *et al.* (1997) when distyly is treated as ancestral or when the loss of distyly is assumed to be more common than its gain, the results of the phylogenetic analysis of *Amsinckia* support the hypothesis that the self fertilizing taxa are of recent origin from out crossing relatives.

Lloyd and Webb (1992 a,b) emphasized functional criteria in their treatment of the evolution of heterostyly and proposed that distyly evolved from stigma-height dimorphism because of the influence of floral morphology on pollen transfer patterns. The occurrence of both stigma-height dimorphism and distyly in *Narcissus* provides evidence in support of their model (Arroyo and Barrett, 2000). This association is of particular significance because of the absence of diallelic incompatibility in *Narcissus* (Dulberger, 1964 ; Sage *et al.*, 1999; Baker *et al.*, 2000b). While diallelic incompatibility is generally a prerequisite for the evolution of

reciprocal herkogamy in some models, for the evolution of distyly (Charlesworth and Charlesworth, 1979), those of Lloyd and Webb (1992 a,b) are independent of the type of compatibility system present in ancestral populations. When Richards and Koptur (1993) and Eckert and Barrett (1994) found variation in stigma and anther heights, they suggested that evolutionary breakdown of heterostyly was underway. But the populations studied by Faivre and McDade (2001) were found to be in the process of gaining heterostyly. In most cases, in which the evolutionary status of heterostyly has been questioned, stigma and anther heights are not reciprocal and plants are also self compatible (Riveros *et al.*, 1987; Barrett, 1989). In *Pentas*, however the stigma and anther heights are reciprocal, but plants were self compatible. Evolutionary transition from heterostyly may be reflected not only by floral morphology and incompatibility system, but also by morph frequencies, within a population. (Barrett *et al.*, 2000). The study of Faivre and McDade (2001) and also those of Richards and Koptur (1993), Eckert and Barrett (1994), Pailler and Thompson (1997) all suggest that deviation from typical heterostyly as shown by the colour variants of *P. lanceolata* in the present investigation, is not uncommon. But it poses

the question of functionality of heterostyly. The evolutionary causes and consequences of heterostyly will not be clear until the functional aspects of heterostyly are fully understood.

As far as *Pentas lanceolata* is concerned, the present study has revealed that, both structural variants, pin and thrum, as well as functional variants, self compatible and incompatible are common in this species but the degree of incompatibility reaction varies. Hence, pollen sterile, pollen fertile but not fruit setting as well as self compatible and incompatible forms associated with heterostyly and homostyly are characteristic to this species.

Summary and Conclusions

Heterostyly is a genetic polymorphism in which two or three mating types in a population differ in floral morphology. The principal feature that distinguishes the floral morphs is that they differ in stigma and anther heights. The sex organs are reciprocally positioned with anthers in flowers of one morph at same level as stigmas in flowers of other morph. This structural difference is usually accompanied by a physiological self and intra morph incompatibility that limits mating to crosses between organs at the same level.

The evolution of heterostyly appears to occur within some general constraints on floral morphology. Heterostylous flowers are generally moderate sized and have a floral tube, a limited number of stamens, and a syncarpous ovary with few carpels. The reasons for these limitations are unclear but may depend on both developmental and functional constraints.

Present investigation on heterostyly and incompatibility in *Pentas lanceolata* colour variants was undertaken with the following objectives :

- Screening the heterostylous and homostylous plants of *P. lanceolata* colour variants to select, a suitable material for the study.
- Morphological studies to determine the degree to which the plant is showing the typical heterostylous syndrome.
- Studies on pollination biology of *Pentas lanceolata.*
- Fruit and seed set studies to find out the degree of incompatibility.
- SEM investigations to get a detailed information on the morphology of stigma and pollen.
- Anatomical studies to determine the nature of pistil.
- Physiological studies to evaluate the pre and post pollination changes of the pistil, on selfing and crossing.
- *In vitro* and *in vivo* pollen germination studies to confirm the viability of pollen.

- Aniline blue fluorescent studies to determine the nature of pollen tube growth in the pistil and to locate the sites of incompatibility.
- *In vitro* bioassay to understand the nature of inhibitors / promoters of pollination on the stigma.
- Biochemical analysis to find out the changes taking place in the stigma.
- SDS PAGE analysis to locate the nature of the proteins involved in inducing incompatibility.
- Genetic studies to understand the nature of inheritance of heterostylous morphs.
- Population studies from different localities to determine the morph frequencies.
- To understand the overall significance of the reproductory systems of these plants which might aid in distinguishing among various models for the origin of heterostyly.

Three heterostylous and homostylous colour variants of *Pentas lanceolata* were selected for the study after screening a number of colour variants. Morphological studies carried out revealed that with regard to morphology three of the colour variants showed the true

heterostylous syndrome with reciprocally placed anther and stigma, whereas two of them were homostylous or rather monomorphic with only the long styled morphs.

The heterostylous colour variants were all pollen fertile and set fruits and seeds. Of the two homostylous colour variants one of them was pollen fertile but set no fruits. The other one also set no fruits, but due to pollen sterility. The heterostylous colour variants showed varying degrees of pollen fertility and a corresponding percentage of fruit set under natural field conditions. The results of hand pollinations were interesting and contrary to what was expected in heterostylous plants. In *Pentas* heterostylous forms are self compatible, exhibiting different degrees of self/cross compatibility. Anatomical studies of the pistil revealed the style as solid with elongated cells in pin and of comparatively shorter cells in thrum.

Fluorescent studies on the pistil after 24 h of self and cross pollinations showed that both self and cross pollen germinated on the stigma in most of the intra as well as inter morph pollinations. On the pin stigma, self pollen showed a profuse growth of pollen

tubes passing through the style and reaching till the base with uniformly distributed callose plugs. The cross pollen also produced pollen tubes which were seen to traverse the whole length of style and reaching the base, with unevenly distributed callose plugs in them. But on the thrum stigma and style the cross pollen were found to show a profusion of pollen tube growth with evenly distributed callose plugs, reaching and extending out of the base, whereas the self pollen produced pollen tubes with uneven callose plugs which just reached the base of style.

But the intra morph inter varietal and inter morph inter varietal pollinations did not show an equally good pollen tube growth. In some of them the pollen tubes were arrested on the stigma itself, whereas in others it showed a slightly better tube growth.

The rate of pollen tube growth however was better after selfing in pin morphs, and crossing in thrum morphs. *In vitro* bioassay revealed that the stigmatic extracts in high concentrations inhibited the pollen germination. The pin pollen grain showed a

more or less good percentage of germination in pin as well as thrum stigmatic leachates. But the thrum pollen grains showed a lower percentage of germination in thrum stigmatic leachate and higher germination in that of pin.

Biochemical studies have shown that the stigma contained protein, phenol, peroxidase and proline in minor quantities and a fairly good amount of soluble sugars. The soluble sugars were lowered following compatible pollinations *i.e.* selfing in pin stigma and crossing in thrum stigma. The protein content on the other hand was increased after compatible pollination, which was proved to be true in the SDS PAGE studies also. The phenolic contents were lowered after compatible pollination than that in incompatible. The proline content though not worth mention, increased after incompatible pollination. The peroxidase activity was also increased after incompatible pollinations. From the biochemical studies it may be concluded that the soluble sugars, proteins and phenols have a positive role in germination whereas proline and peroxidase were seen to produce an inhibitory or negative effect on pollination.

The incompatibility index calculated from the seed set data showed that the pin plants were preferentially self compatible and the thrum plants were only partially self compatible or preferentially cross compatible. The plants could be very well graded with regard to their compatibility reactions.

The inheritance of the heterostylous morphs was found to be different from that in the typical. It looked as if the pin morphs were heterozygous and the thrum morphs homozygous recessive. The pin plants outnumbered the thrum plants in all the populations studied.

Based on the results and discussion the following conclusions have been drawn.

- *Pentas lanceolata* plants though heterostylous, did not show a true heterostylous syndrome with regard to its functioning.
- The pin of the heterostylous colour variants were self as well as cross compatible, having preferentially self compatibility.

- The thrum plants were also cross and self compatible, but they were more cross compatible.
- The incompatibility reactions typical to heterostylous plants were not met with either in stigma or style.
- All the heterostylous plants set fruits and fertile seeds in abundance both in controlled and open pollinated populations, whereas the homostylous colour variants red and crimson set no seeds.
- The heterostylous plants could be graded according to their incompatibility reaction. The lilac colour variant was found to be the best fruit and seed setter. The lilac pin being more so with selfing and the thrum with crossing. The white colour variant was found to be the second best with the pin producing more fruits and seeds by selfing and the thrum with crossing. The magenta colour variant was found to be the lowest in fruit and seed set.
- The heterostylous colour variants showed normal meiotic stages, but the homostylous forms showed slight

aberrations. Meiotic aberrations leading to pollen inviability may be the cause of sterility in crimson variety while in red variety the failure of seed set may be due to some genetic imbalance.

- The pin plants predominated all the populations studied, suggesting that the long styled phenotype has a selective advantage over the short-styled phenotype. It is tempting to assume that this type of variation might represent an early stage in the evolution of heterostyly.

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