Introduction

INTRODUCTION

The developmental stages in reproductive cycle from meiosis to seed formation play an important role in the life cycle of higher plants. For the many species propagated only by seed, effective functioning of these developmental pathways is essential for survival and performance. Even when asexual propagation is possible, the sexual reproduction creates new gene combinations which can result in novel and sometimes superior plant types. Significantly, the events of the sexual cycle frequency also influence the growth and development of vegetative plant parts. Inspite of years of efforts by plant physiologists, cytologists, embryologists and geneticists, our understanding of gametogenesis and fertilization is still quite limited and obscure. Use of modern biochemical, ultrastructural, immunological and cell culture techniques is increasing, nevertheless, a variety of difficulties still slow the progress.

Evolution of species and the induction of genetic variation solely depend on the interaction of male and female reproductive structures. Pollen-pistil interaction, the most important and vital process in the reproductive cycle, helps in the selection of right pollen for germination and effectively monitor its growth through the sporophytic tissue, till the embryo-sac which culminates in a successful fertilization. Although, the basic events

of pollen-pistil interaction leading to fertilization became known by the end of the nineteenth century, studies remained basically descriptive until 1950. During the past two-three decades, there has been a steady progress in our understanding of the biochemical, genetical and ultra-structural events that occur during pollen-pistil interaction which owe mainly to the advent of modern techniques. However, our knowledge of the sequential events in reproduction today is mainly due to the scientists of the past era who have toiled with limited facilities and profound imaginations to make a solid basis on which the modern embryology rests and has advanced. Hence, it is a tribute to these frontiers of embryology that I describe below, few historical events that occurred in angiosperm embryology and then describe the details of reproductive cycle in Angiosperms that are reported till today.

In tracing the history of a branch of natural science it is customary to go back to the days of Aristotle. Though, it seems fairly certain that he could not recognise the presence of sex in plants, he believed that the male and female principles were so blended that they generated of their own accord and the offspring arose from the superfluous food in the plant. Aristotle's pupil Theophrastus continued in the same line and in his book "Enquiry in to plants" written in the third century B.C. referred to the pollination of the date palm, probably for the first time. The problem of sexuality

in plants seems to have been laid aside and forgotten for almost hundred years. Indeed, many scientists of the fifteenth and sixteenth centuries totally denied the occurrence of sex in plants and regarded even the mention of it as inappropriate and obscene. Some thought the stamens to be excretory organs and the pollen to be a waste product (Maheswari, 1950).

It was Grew (1682) who in his 'Anatomy of plants' made the first explicit mention of the stamens as the male organs of the flower. After the role of pollen began to be understood, Giovanni Battista Amici (1824) made substantial observations. He found that the stigma of Portulaca oleracea was covered with hairs which contained some granules or particles inside them. Later he saw a pollen attached to the hair he had under observation and found that it grew along the side of the hair and entered the tissues of the stigma. This discovery stimulated the young French botanist Brongniart (1827) to examine a large number of pollinated pistils, with a view to understanding the interaction between the pollen and the stigma and the introduction of the fertilizing substance in to the ovule. Amici (1830) later came out with the opinion that the pollen tubes elongate bit by bit and finally come in contact with the ovules one tube for each ovule.

Discovery of sexual fusion in lower plants were made in the ninteenth century. The major contributions came from

Thuret (1854), Pringsheim (1855) and Oscar Hertwig (1875). In the phanerogams, where sex was supposed to be more apparent than in cryptogams, the actual demonstration did not come until a few years later. It was Strasburger (1877) and his pupil Elfving (1879) extended these observations to cover several families and demonstrated the widespread occurrence of the binucleate condition in pollen grains. However, the most notable advances of all was Strasburger's (1884) discovery of the actual process of syngamy or the fusion of the male and female gametes. The year 1900 marked the beginning of a new era in angiosperm embryology. By this time most of the facts on the development of the gametophytes and embryo had been discovered and an able summary of the literature was given by Coulter and Chamberlain (1903) in their book entitled " Morphology of Angiosperms". During the recent years, there has been considerable activity in the field of experimental embryology in India, Japan and Australia. The major aspects dealt being storage and viability of pollen, effect of environmental factors on pollen tube growth, control of fertilization, production of seedless fruits, pollen-pistil interaction, artificial induction of parthenogenesis and adventive embryony.

The Pistil

The pistil is the most vital organ of a flower since it contains the egg deep-seated within its basal ovary and

later protects the embryo that develops from it after fertilization. Basically it has three main parts, the upper stigma of varied nature and morphology, the middle style which is long and flexible and the basal ovary that contains the egg. Each of these parts, though morphologically different, serve the purpose of receiving the pollen grain, selecting them, aiding its growth and allowing the male gametes to make a successful fertilization.

Traditionally, two basic types of stigma have been recognised, the wet type in which the receptive surface becomes covered with a fluid secretion and the dry type in which the receptive surface has hydrated, extra-cuticular secretion which is not free flowing (Shivanna, 1982). Heslop-Harrison and Shivanna (1977) have classified the angiosperm stigmas based on their surface characteristics covering about 1000 species of 900 genera belonging to 250 families. They showed the taxonomic phylogenetic, and physiological significance of stigma-surfaces and broadly classified them according to the morphological characters. The variations occurring in the dry and wet categories of stigmas, have been further subdivided in to different groups.

Dry type

Group I - plumose with receptive cells dispersed in multiseriate branches,

Group II - receptive cells concentrated in distinct zones, ridges and heads,

Group IIA - surface non papillate,

Group IIB - surface papillate,

1) papillae unicellular,

ii) papillae multicellular.

Wet type

a) papillae uniseriate,

b) papillae multiseriate,

Group III - receptive surface with low to medium sized papillae,

Group IV - receptive surface non papillate, cells often necrotic at maturity.

While a considerable amount of information exists on the pollen (Stanley and Linskens, 1974) and the ovary (Maheswari, 1950) not much is known about the stigma. Studies carried out a few decades back do not give a detailed developmental account of the stigma (Jost, 1907; Lutz, 1911; Hanf,1935). However, from late 1960 onwards, more detailed information in the reproductive organs started coming up. Konar and Linskens (1966) in their studies on Petunia hybrida revealed the stigma as a bilobed structure with a central depression. The stigma surface shows a wavy outline with depressions and

raised portions, with a large number of papillae. A study of its developmental anatomy shows that the stigma can be separated in to two zones; the upper zone with the epidermis constituting the secretory zone and a lower 1-3 layers of laterally extended cells forming the storage zone. The cells of the secretory zone including the papillae and the epidermis are very rich in chloroplasts. The cells of the storage region also show considerable accumulation of starch. At maturity, the stigma surface shows large amount of oily exudate. More recent investigations like in Trifolium pratense, a highly impermeable cuticle investing the stigma receptive surface has been observed (Heslop-Harrison and Heslop-Harrison, 1982). Since Trifolium pratense is self-incompatible, the authors suggested that mechanical blocking of pollen at the stigma surface by the cuticle could be a significant determinant of breeding behaviour in self-fertile species. However, in the case of dry stigmas a layer of hydrated proteinaceous layer called pellicle of varying thickness above the cuticle has been reported in all the systems of this category (Mattsson et.al., 1974; Heslop-Harrison, 1977). The cuticular pores facilitate the extrusion of the pellicular layer synthesized and secreted by papillae cells at the receptive stage of the stigma. Hence, the dry stigma is not really dry as it was thought earlier, but covered with the pellicle which is physiologically comparable to the

exudate of the wet stigma (Shivanna, 1982). The pellicle can be easily localized at the receptive stage by the non-specific esterase activity and sensitivity to pronase digestion (Heslop-Harrison,1975 b). Apart from facilitating capture and subsequent hydration of pollen, the pellicle might also be concerned in pollen-stigma interaction.

Mattsson et.al, (1974) in a wider survey showed the presence of pellicle indicating that it may well be unusual in the dry group. The protein pellicle observed in the stigmas of Silene vulgaris (Caryophyllaceae) Brassica oleracea and Raphanus sativus (Cruciferae) showed distinct features which were not previously reported on the external surface of higher plant cells. Some of the important observations were that the pellicle ensheathing the cuticle did not have any close attachment. Electron microscopic studies further revealed stratification on the wall at the tip of the stigma papillae and the plasmalemma was found irregular. The pectocellulosic layer is bounded by a cuticle which according to them is discontinuous. The pellicle with its hydrophilic nature might increase wettability of the stigma surface. It is well documented that the pollen grains while on the pellicle becomes hydrated rapidly. In Silene vulgaris, the volume of the pollen grain increases by 50 % in the first 10 min after contact (Mattsson et.al.,1974).

In the wet stigma, the stigma surface at the receptive stage shows free flowing exudate. The composition of the exudate is highly variable and contains varying proportion of lipids, carbohydrates, phenolic compounds and proteins. The pistil of Lilium longiflorum secretes two forms of exudate, one from the stigma surface cells and the other from the canal epithelium. Electrophoretic studies of these exudates have revealed quantitative and qualitative differences in protein profiles. The exudate components which are transferred to the cell wall by endoplasmic reticulum and golgi vesicles are stored within the cell wall of the secretive tissues and secreted from the cell walls directly at the receptive stage (Miki Hirosige et.al., 1987). However, earlier works on Lilium longiflorum by Labarca et.al, (1970) showed 99% of water in the exudate. Apart from water, 95% is a high molecular weight protein containing polysaccharide, composed of galactose, arabinose, rhamnose, glucuronic acid, and galacturonic acid. Stigmatic exudate of L.longiflorum contains a small amount of low molecular weight carbohydrate also. Analysis of the stigmatic exudate on Zea mays (Martin, 1970) by fractionation of crude extracts by differential solubility and chromatography revealed anthocyanins, phenolic compounds (chiefly glycosides, and esters of hydroxycinnamic acids) esters of fatty acids and lipophilic phenolic compounds. The lipid compounds probably regulate availability of water to the pollen, and prevent dessication of the stigma and are

also nutritive. The phenolic compounds in addition to giving protection from microbes could also serve as a source of nutrients to the germinating pollen or may stimulate or inhibit pollen germination (Martin, 1970). Sedgley and Blesing (1985) in their studies on watermelon stigma showed the presence of galactose containing polysaccharide component in the exudate. They suggested that the polysaccharide component of the stigma exudate is produced in the golgi apparatus and secreted via the cell wall and wall thickenings. In Petunia the exudate is secreted by the cells of the stigmatic tissue and accumulates on the stigma surface by the rupture of the cuticle (Konar and Linskens,1966). According to them,the role of oily exudate is; (1) it serves the function of a liquid cuticle by checking excessive transpiration, (2) it helps to trap pollen. Stigma in Anona depicts an active secretory system and the cytochemical reactivity of the secretion indicates the presence of pectinaceous polysaccharides, proteins and lipids (Vithanage,1984).

Style in angiosperms is of two types, hollow and solid (Shivanna, 1982). In the former the style has secretory canals. The canal lumen usually is continuous with the stigma and is bordered by glandular cells. The canal cells in the case of open or hollow style are generally secretory and often multinucleate and polyploid. In Lilium, the canal cells have a characteristic thick dome shaped outer tangential wall (facing the stylar cavity) with a smooth outer surface and a highly

convoluted inner surface (Rosen and Thomas, 1970). The pistil of Anona has an open stylar canal lined by papillae, a character considered to be primitive (Hanf,1935). Monocots mostly have hollow styled pistils (Iwanami,1959; Vasil,1974; Clarke et.al., 1977). In the solid style, canals are absent but have elongated one or two zones of thin walled parenchymatous cells and the pollen tubes grow through the intercellular spaces of this tissue designated as the transmitting tissue (Shivanna, 1982). Electron microscopic studies carried out on the transmitting tissue cells have shown that these cells in general have thin transverse walls traversed by abundant plasmodesmata and thick longitudinal walls (Van der Pluijm and Linskens,1966; Sassen 1974; Cresti et.al.,1976; Bell and Hicks,1976). The growth of the transmitting tissue is through cell elongation (Sassen,1974).

Once a successful pollination is effected, it initiates structural and physiological changes in the pistil, namely degeneration of the adjoining cells of the stigma and transmitting tissue (Herrero and Dickinson, 1979; Heslop-Harrison, 1979 b; Sedgley, 1979; Heslop-Harrison and Heslop-Harrison,1980). In Lilium, the canal cells do not show any structural alteration following pollen tube growth but the underlying parenchymatous cells degenerate (Rosen and Thomas,1970).

Earlier it was thought that the pollen tube is guided from the stigma to the ovule by a gradient of chemotropic substance and Ca^{2+} ion was considered a universal chemotropic factor in pistils of angiosperms (Mascarenhas and Machlis,

1962 a, b; 1964). Rosen (1962) defined chemotropism as a movement by growth towards or away from an external substance. However, it may also be true that the cells of the transmitting tissue with their file-like arrangement provide a path of least mechanical resistance for the growth of pollen tubes, and there is no need for a chemotropic gradient in the style (Jensen and Fischer, 1969). In the hollow styled systems, the pollen tubes grow on the surface of the canal cells, and when the number of pollen tubes are large, they grew as a bundle filling the whole stylar canal (Malti and Shivanna, 1984). Secretions of the stigmatoid tissue found on the stigma surface and in the stylar canal have been implicated in chemotropism, nutrition and incompatibility (Gawlik, 1984).

Ultrastructure

Electron microscopic studies of the reproductive organs revealed many details on the mechanisms of secretion in the stigmatic as well as stylar cells. Observations on the stigma of Nicotiana sylvestris (Kandasamy and Kristen, 1987) have shown that the main secretion of the stigma is produced by the secretory zone comprising of the stigma papillae and few cells lying below them. The glandular cells of the stigma contain numerous plastids, mitochondria, ribosomes, ER, lipid droplets and dictyosomes. The plastids and the vacuoles in the secretory cells of the stigma have electron dense osmiophilic inclusions in the early and later stages of development. It

is suggested that the proteins are directly secreted by ER compartments, whereas SER is involved in the synthesis of lipidic materials. In Prunus avium (Uwate and Lin, 1981), the stigma tissue undergoes a specific pattern of development which is different from that of the papillae on the stigmatic surface. An elaborate system of intercellular spaces develop in the tissue having small lacunae and aerenchymatous tissue. Aerenchymatous tissue on the peripheral regions of the stigma is characterized by several cytological features which change during ontogeny, such as nuclear inclusions, amyloplast inclusions, dumbbell shaped mitochondria, cytoplasmic sequestration and isolated segments of ER.

In Nicotiana tabacum (Dumas et. al., 1978; Cresti et.al., 1982), two distinct zones of cells could be observed; a glandular zone formed by the papillae cells and 2-3 layers of basal cells immediately below them, and a non glandular region formed among the vacuolated cells which are in continuity with the transmitting tissue. Owens et.al,(1984) have described the ultrastructure of 37 species of 13 genera of Commelinaceae. In Aneilema and Commelina sp. (Owens and Horsefield, 1982), the stigma papillae cells have a bilayered cuticle. The outer layer is a thin electron dense lamella and the inner layer is composed primarily of radially oriented rodlets of cutin. The papillae cell cytoplasm showed much variation among themselves. Plastids associated with starch grains were prominent.

Vesicles of various sizes are either associated with ER cisternae or dictyosomes. Nuclei showed various forms of nuclear vacuoles. Intracellular protein transport follows a pathway suggested by Palade (1975) starting from ER → Golgi → Plasma membrane. However, according to Robins and Juniper (1980) in Dionaea, proteins are reported to be directly secreted by RER compartments in the digestive glands and similar observations have been made in the stigmatic papillae of Aptenia (Kristen et.al., 1979) and Crocus (Heslop-Harrison and Heslop-Harrison, 1975). Heslop Harrison and Heslop Harrison (1979) further report the involvement of paramural bodies in the granulocrine secretion of proteins in the stigmatic papillae of Secale and Hordeum. Lipid droplets in the exudate is a common feature observed in many taxa (Dumas et.al., 1978; Heslop-Harrison and Heslop-Harrison, 1983) and mostly they are found associated with ER. Involvement of plastids in the synthesis of osmiophilic material is presumed, since studies by Kandasamy and Kristen (1987) reveal the presence of osmiophilic material in the plastid as well as release from the plastid by a budding process.

Pollen grain

Pollen grains are surrounded by a two layered wall, the exine, comprising of sporopollenin, and the intine of pectocellulose. The exine is further differentiated into an outer sculptured sexine, and an inner non sculptured nexine.

The sculptured layer comprises the baculae or radially directe rods which may be roofed by a tectum, thus forming tectate grain. In the non-tectate grain, the baculae stand free or join together to form various patterning (Shivanna, 1977). During the earlier period, pollen morphology was studied mainly with the use of acetolyzed pollen; however, recent studies concentrate on unacetolyzed pollen. The wall layers exine and intine have now been demonstrated to contain large amounts of mobile proteins (Knox and Heslop-Harrison, 1969; 1970; 1971a; Heslop-Harrison et.al., 1973; Knox et.al., 1975). The intine proteins are concentrated mainly near the germ por Soon after the release of microspore from the tetrad, the deposition of the intine progresses. The plasmalemma of the pollen cytoplasm put forth radially oriented tubules in to th intine. Eventually, these tubules with their protein inclusi become cut off from the plasmalemma and sealed off from the cell surface by a layer of intine free from tubules (Heslop-Harrison et.al., 1973).

In exine, the proteins are located in the sculptured pa of the sexine. The exine proteins originate in the cells of the surrounding tapetum, a sporophytic tissue (Dickinson and Lewis, 1973b; Heslop-Harrison et.al., 1974). During meiosis of the microscope mother cell, proteins and lipids accumulate in the tapetal cells, the former being found in single membra bound vesicles derived from ER and the latter in spherosomes and plastids. When the tapetal cells break down towards

the end of pollen development, these proteins and lipids are released into the thecal cavity and eventually they become deposited in the surface depressions of the exine (Heslop-Harrison et al., 1973). Thus the intine proteins are the products of the pollen cytoplasm and exine proteins are the products of the tapetum which is a sporophytic tissue.

Pollen grains are released from the anther either in the 2-celled or 3-celled stage. In the latter, the generative cell undergoes one more division giving rise to 2 male gametes. Unlike in lower groups of plants, where the male gametes have direct access to the female gamete, in angiosperms a chain of sequential integrated processes in the pistil following recognition regulate the post-pollination behaviour of the pollen (Shivanna, 1982). Heslop-Harrison (1979a) remarked rightly that the gametophytic portion of the angiosperm life cycle as the forgotten generation. This neglect however came about, despite subtle but constant indication that pollen is the site of intense gene activity and selection. Pollen is often thought to be as serving a single function that of delivering the male gamates to egg. However, it should be noted that it serves to block the transmission of many defective alleles and gene combination into the next generation (Mulcahy, 1986).

Pollen tube growth

According to Shivanna (1982), the following sequential events take place when a pollen lands on the stigma.

Pollen lands on the stigma
↓
Pollen adhesion
↓
Pollen hydration
↓
Pollen germination
↓
Pollen tube entry in to the stigma,
↓
Pollen tube growth through the style.

Pollen tube development is a process of decisive importance in fertilization and determines the germination potential of a viable pollen. It is an expression of differentiation and represents one phase in a developmental process that controls seed and fruit formation (Malik, 1977). Upon pollination, the pollen tube starts emerging as an extension of the intine. The intine has to become less rigid before the tube can emerge (Johri and Shivanna, 1985). The pollen tubes are considered as the most rapidly growing cells in the plant world, since they are capable of attaining considerable length in a short duration under optimum conditions (Malik, 1977). In Crocus, the cuticle remains intact on the papillae, as well as the cells of the stylar canal, pollen tube bore through the cuticle of the stigma and grow down the stylar canal between cuticle and canal cells (Heslop-Harrison, 1975a). In many taxa, the stigmatic surface at the site of pollen tube entry shows an enhanced surface esterase activity (Shivanna and Sastri, 1981; Heslop-Harrison and Heslop-Harrison, 1981). In taxa such as Gladiolus and Crocus, a mucilaginous substance accumulates between the cuticle and cell wall and the pollen tube grow

through the mucilage and not through the pecto-cellulosic wall (Heslop-Harrison, 1977; Clarke et.al., 1977).

Pollination initiates many physiological changes in the pistil. It increases respiratory activity, changes pattern of RNA and protein synthesis (Linskens, 1975) and initiates marked increase in the activity of several enzymes. In Petunia, a wave of enzyme activity precedes the growing pollen tube (Roggen, 1967). Increased enzyme activity such as acid phosphatase, succinate dehydrogenase, cytochrome oxidase, peroxidase has been noticed on the stigma upon pollination (Malik, Mehan and Vermani, 1975), It is presumed that the pollen enzymes leach out and act on the substance present on the stigma and/or style (Malik and Gupta, 1976). The possibility that pistil provides an activator for pollen enzymes also may be involved in this phenomenon (Rosen, 1971).

It is now well known that RNA and protein synthesis take place during pollen germination and tube growth (Mascarenhas, 1975; Malik et,al., 1977; Shivanna et.al., 1979; Reynolds and Raghavan, 1982). However, much of the protein synthesis is reported to be initiated before tube growth (Mascarenhas and Bell, 1969). The experiments using density gradient analysis of the cytoplasmic extract of the germinated and ungerminated pollen in Tradescantia (Mascarenhas, 1975) revealed the following facts.

1) Activation of the pollen grain during germination is accompanied by the initiation of protein synthesis,
2) The initiation of the protein synthesis which occurs on imbibition of water is not tightly coupled to the morphogenetic events of pollen tube growth,
3) A sizeable fraction of ribosomes in ungerminated pollen grains are in the form of polysomes,
4) During germination there is a very rapid formation of additional polysomes,
5) A fraction of the ribosomes in the dehydrated metabolically inactive ungerminated pollen grain appears already to be prepackaged with stable messenger RNA,
6) Germination/tube growth are to some extent dependent on the translation of this mRNA. However, not much is known about the identity of this mRNA as to what specific proteins are synthesized on this stable mRNA.

Ultrastructural studies on pollen tube have shown clearly that tubes grow by the fusion of vesicles abundant at the tip and these vesicles originate from the activity of the dictyosomes (Vander Woude and Morre, 1968). It is also estimated that during the growth of the tube the vesicles are produced at the rate of 2150/min in Lilium longiflorum and 5,388/min in Tradescantia virginiana (Vander Woude and Morre, 1968; Picton and Steer, 1981). Calcium ion concentration at the tube tip is another factor which affects the rate of vesicle fusion (Picton and Steer, 1982, 1983).

Pollen viability and storage

The pollen grain after dehiscence of anther remains dormant till it reaches the stigma surface. The time period in which the viability of pollen is retained is crucial and varies from species to species. At normal conditions of temperature and humidity the viability of pollen is lost rather rapidly. Linskens and Pfahler (1973) indicate changes in 16 amino acids during storage in Zea mays pollen. They observed that some of the amino acids such as aspartic acid, isoleucine, leucine, phenyl alanine, ethanol amine and aminobutyric acid showed consistent increase and some others such as glutamic acid, proline, glycine and alanine, a consistent decrease. These studies indicate that the pollen grains, even after shedding undergo considerable metabolic change.

Viability should be considered as quite distinct from the germinability of pollen. In an artificial medium, viable pollen may fail to germinate due to lack of certain essential factors. It is however, necessary that viable pollen should give a high percentage germination and tube growth at optimum conditions (Johri et.al., 1977). Studies on this line indicate that pollen viability can be artificially prolonged by the following conditions (after Johri and Shivanna, 1985):

1) by controlling temperature by refrigeration,
2) by controlling relative humidity by dehydrating agents,

3) by controlling a correlated adjustment of temperature and humidity so as to create an optimal environment.

The pollen samples preserved by various techniques can be tested for viability and storage. King (1960) used peroxidase reaction as an indicator of pollen viability which is based on the oxidation of benzidine by peroxidase in the presence of hydrogen peroxide. Heslop-Harrison and Heslop-Harrison (1970) evaluated pollen viability by enzymatically induced fluorescence. The viable pollen grain immersed in fluorescein diacetate (FDA) solution rapidly accumulate fluorescein by fluorochromatic reaction which involves hydrolysis of certain nonfluorescent fatty acid esters of fluorescein by esterases. Fluorescein esters being non polar compounds, penetrate readily in to the cell (Rotman and Papermaster, 1966) where they are hydrolysed to produce fluorescein, a polar substance by the living cytoplasm which cannot pass through the plasma membrane and accumulates in the cell and can be detected by UV light. This vital fluorochromatization has been taken as an important and reliable technique to identify viable pollen grains by a number of investigators.

Pollen-pistil interaction

The first visible change, soon after the pollen lands on the stigma, is that the pollen gets hydrated and simultaneously with this, pollen wall proteins, first the exine proteins and

after a while, the intine proteins are released on to the stigma surface (Knox and Heslop-Harrison, 1971a; Knox, 1973, Heslop-Harrison et.al., 1975b). In dry stigma, the pellicle is the receptor site for the pollen wall proteins. Pollen wall proteins bind with the pellicle, establishing a close interaction and the pellicle looses its identity as a discrete layer. Recognition of the pollen appears to take place during this interaction, and results in the activation of male gametophyte and papilla (Heslop-Harrison, 1975a).

In the case of wet stigmas the exudate in some instances does not contain proteinaceous substance. In Petunia which possess a wet stigma, proteins were reported to be absent in the stigmatic exudate (Konar and Linskens 1966b). However, from the cytochemical investigation on the stigmatic exudate of Petunia hybrida, the presence of acid phosphatases were observed (Herrero and Dickinson, 1979). The recognition of the pollen therefore is as a result of the interaction between pollen wall proteins and stigma surface proteins.

Concanavalin A (Con A) was demonstrated to bind specifically to the pellicle. Stigmas of very young buds free from pellicle did not bind to Con A. However, Con A binding on the stigmatic surface did not inhibit pollen germination but prevented the entry of tube. Thus it was presumed that components involved in Con A binding were necessary for pollen tube entry, and not for pollen germination (Heslop-Harrison and Heslop-

Harrison, 1975; Sharma and Shivanna, 1983b; Sharma et.al., 1985). Studies carried out on bud receptivity also implicate stigma-surface proteins in pollen stigma interaction.

In Petunia, (Shivanna and Sastri, 1976) although younger buds are free from the exudate, they support pollen germination and pollen tube entry into the stigma. Thus following a compatible pollination, pollen will germinate and grow through the style. If incompatible, the pistil will initiate a rejection reaction mainly seen as callose plugging towards the tip of the pollen tube and also at the region of contact of the pollen tube with the papillae (Shivanna, 1982).

Incompatibility

Incompatibility basically falls in two groups; (1) Intraspecific and (2) Interspecific. In intraspecific incompatibility or self incompatibility, incompatibility occurs within the species, whereas in interspecific incompatibility it occurs between species. Intraspecific incompatibility has been grouped into two categories; (1) Heteromorphic, (2) Homomorphic. In heteromorphic incompatibility, different individuals of the species produce either two or three types of flowers differing in the length of stamens and style. Pollengrain, either from the same plant or from any other plant bearing same flower will be non-functional. In the homomorphic type, all individuals of the species produce only one type of flower and it is governed by multiple allelles called 'S' alleles. Pollen tubes having

a particular 'S' allele is inhibited in the style carrying the same 'S' allele. In the majority of taxa, incompatibility is determined by multiple alleles at one locus. In grasses, on the other hand, incompatibility is controlled by multiple alleles of two independent loci S and Z. In some members of Ranunculaceae and Chenopodiaceae (Lundquist, 1975; Larsen, 1977) and Cruciferae (Lewis, 1977), incompatibility is controlled by multiple alleles at three or four loci.

It was East and Mangelsdorf (1925) who first worked on the genetics of self incompatibility in Nicotiana (Solanaceae). Later investigations by East (1934) on the physiological response, have suggested that the incompatibility reaction in some sense is analogous with the vertebrate antigen-antibody reaction, and concluded that the factors concerned in the female side in Nicotiana were probably secreted in to the intercellular spaces of the pollen tube transmitting tract of the style where the interaction with the extending pollen tube takes place. Recent work on the pollen stigma interaction where cells of different genotype are found to influence each other have asserted that cells of vascular plants do communicate through the agency of large molecules, and it has long been supposed that the substances involved in self incompatibility were proteins (Lewis, 1952). It is also proposed that pollen wall proteins are concerned with compatibility reaction (Knox et. al., 1972a).

Brassica oleracea is one system where details in these line have been worked out. It has been shown that diffusates readily obtainable from the pellicle of intact stigmas contain antigens specific for the 'S' allele present on the stigmas (Heslop-Harrison; Heslop-Harrison and Barber, 1975) and since such diffusates can inhibit self pollen development, the presence of S-specific protein molecules in the papillae surface is presumed (Ferrari and Wallace,1975). In the morphological investigation of incompatibility studies, the deposition of heavy callose (1-3 β glucan) and its detection by fluorescent microscopy has been taken as one of the criteria for depicting a rejection reaction, since, it is found to be deposited in excess in incompatible pollen tube (Howlett et.al., 1975).

Depending on whether the system is sporophytic or gametophytic, the site of inhibition shows difference. In the case of sporophytic system, the pollen grain germ pore gets occluded with callose, and the site of inhibition is at the stigma tip, whereas in the case of gametophytic system, the pollen grain germinates on the stigma surface and inhibition take place mainly on the style, and the pollen tube normally gets loaded with heavy deposits of callose. (Heslop-Harrison, 1978). However,in grasses as an exception, the pollen tube inhibition is on the stigma though it belongs to the gametophytic system.(Shivanna et.al.,1982).

Though the incompatibility reactions occur as a natural phenomenon in angiosperms, many researchers have tried

different methods to overcome incompatibility reaction resulting in either successful pollen germination or the setting of seeds. Some of the few techniques used for overcoming incompatibility are described below.

a) By recognition pollen

By this technique, the incompatibility factors present on the stigma surface are masked by the proteins released from the wall of killed compatible pollen thus making it ineffective in inhibiting incompatible pollen tubes. Knox et.al, (1972-a,b) could obtain a number of hybrids by using this technique in crosses between Populus deltoides and Populus alba. In this method the killed compatible pollen is mixed with live incompatible pollen. This technique has been successfully used in Petunia (Sastri and Shivanna, 1976 a) and Nicotiana (Pandey, 1977) also.

b) Treatment of pollen/stigma with organic solvents

Treatments of stigmas with organic solvents such as anhydrous hexane and ethyl acetate has been effective in overcoming incompatibility in Populus (Willing and Pryor, 1976). By this technique also the pollen wall proteins as well as the stigma surface proteins involved in incompatibility reaction can be manipulated.

c) Intra-ovarian pollination

This technique enables one to eliminate the stigma /style totally. The pollen suspension is directly injected into the ovary thereby, achieving pollen germination, pollen tube entry into ovule and fertilization. Success has been obtained by this technique in the crosses between Argemone mexicana and Argemone ochroleuca (Kanta and Maheswari, 1963; Maheswari and Kanta, 1961). However, one limitation in this technique is that the ovary should have enough space so as to inject and accommodate the pollen.

d) In vitro fertilization

This technique comes in as of great advantage in cases where there is normal fertilization and initiation of embryo and endosperm, but there is later degeneration/abortion of embryo. To overcome this, young embryos are isolated and cultured on suitable nutrient medium. Zenktler et.al, (1975) used this technique successfully in raising interspecific and even intergeneric hybrids in Melandrium album (♀)× Melandrium rubrum (♂) as well as Melandrium album (♀)× Silene schafla (♂).

Other techniques used in overcoming incompatibility includes, stump pollination (Swaminathan,1955), where the pollination is carried out in the cut end of the style, irradiation of

flower buds (Nettancourt, 1977), and by somatic hybridization (Carlsson et.al.,1972).

It is clear from the descriptions, that though we have a sound consensus of the reproductive cycle in angiosperms, many aspects like pollen-pistil interaction, stigmatic secretion etc, show much variation in different taxa. It is very difficult to come to a definite conclusion unless a wide exploration of the cycle is being drawn in a wide representation of taxa. Most of the studies carried out give a clear account of the pistil at the mature stage of flower development. Studies pertaining to the development of pistil are relatively less. The present investigation on two dicots, namely Catharanthus roseus (L.)G Don (Apocynaceae) and Withania somnifera Dunal (Solanaceae) elucidates the developmental process of the stigma and style, and brings into light the organelle status of the stigma and style at early development, secretory and senescent stages. The studies look into the metabolic status of the stigma at different stages, and also lay emphasis on the in vitro and in vivo pollen germination and the role of polyamines in pollen germination.