# Discussion

# DISCUSSION

Catharanthus roseus L.G. Don belonging to the family Apocynaceae is a perennial herb, flowering allthroughout the year. The plant is highly prized in recent years for its importance as a medicinal plant, yielding two valuable alkaloids, vinblastine, and vincristine (Anderson et.al.,1986). The alkaloid content compared to other portions of the plant is more in roots and is highly influenced by environmental factors and varietal differences. It is essential to understand the reproductive cycle of this plant in order to take up breeding works, as well as increasing the alkaloid content through physiological, biochemical, and genetic manipulations and it is this need that has prompted me to undertake the present work in this plant which grows luxuriantly, unattended in waste lands.

Withania somnifera Dunal (Solanaceae) is also a perennial herb which grows equally well in semi-arid and wet lands. The plant is recognised for its valuble alkaloid withanin having narcotic properties (Bell and Charlwood 1980). To understand its reproductive cycle, giving due emphasis to the development of reproductive organs and their interaction, the present work on this plant was undertaken.

Though, I have separated my findings under different headings in the results, the discussion part has been compiled

together in such a way as to explain the similarities between the two unrelated taxa and discussing these data using the published reports on the reproductive cycle in angiosperms in general and dicots in particular for evolving the general trends.

Angiosperm stigmas, in general, have been classified by Heslop-Harrison and Shivanna (1977) covering about 900 genera belonging to 250 families. The classification is mainly based on the stigma morphology and the amount of secretion at the receptive period. Thus, angiosperm stigma, either falls into wet or dry type depending on the presence or absence of surface fluid. Again, the presence of low or medium sized papillae which are either unicellular or multicellular, in uniseriate or multiseriate arrangement or, the complete absence of papillae makes an angiosperm subject to further categorization. Stigma in C. roseus and W. somnifera are of the wet papillate type. Taking into account all the features described by Heslop-Harrison and Shivanna (1977) it seems fairly justified to categorize the stigmas in C. roseus and W. somnifera under group III of the wet type.

The stigma in C. roseus develops from a homogenous mass of cells outlined by a well defined epidermis. The epidermal cells which show slight protuberances in the early stages elongate further, the upper and lower ones attaining maximum elongation. Thus, a mature stigma in C. roseus shows

two morphologically distinct types of papillae cells arranged in uniseriate pattern.

In W. somnifera, stigma surface shows bifid nature even in the early developmental stage. The protodermal initials discernible at stage I later divide and elongate to become multicellular uniseriate thumb shaped papillae cells distributed uniformly on the upper surface of the stigma by stage III.

The papillae cells are considered as modified epidermal cells taking active part in secretion and are responsible for the majority of the secretion on the stigma surfaces (Considine and Knox, 1979; Owens and Horsefield, 1982). The secretory material bathes the stigma surface in C. roseus while in W. somnifera, the secretion is meagre and present only in between the interstices of the papillae cells. Most of the secretory material in C. roseus is confined to the lateral side of the stigma and is comparatively less on the upper side, clearly depicting the difference in the secretory status of the two types of papillae. Moreover, the stigma in C. roseus becomes receptive well before anthesis and along with the placement of anthers immediately above the stigma, makes easy transfer of pollen grain for self pollination. Thus, the flower morphology in C. roseus is conducive and favourable for self fertilization. However, Sedgley et al , (1985) in their investigation on Macadamia pistil, have noticed a complex

stigma surface having four morphologically different cell types. This complexity has been related by the authors to out-crossing mechanism, since the stigma attains maximum receptivity only two days post-anthesis.

The stigma structure in W. somnifera is similar to other solanaceous members like Petunia (Konar and Linskens, 1966), and Nicotiana (Kandasamy and Kristen,1987) in having a bifid stigma and multicellular uniseriate papillae. However, a distinct secretory zone, like the one present in Petunia is lacking in W. somnifera. In Zephyranthes, the stigma consists of three lobes having finger-like papillae (Ghosh and Shivanna, 1984) and no apparent secretion. Similarly, in the family Commelinaceae, investigations carried out in many species show a trifid or triangular stigma, each species showing considerable diversity in size, number, distribution and shape of the papillae (Owens and Kimmins, 1981).

In angiosperms, the receptive surface consisting of unicellular or multicellular papillae is invested by a cutinaceous covering. Presence of such a layer investing the stigma surface was first made by Jost (1907). The cuticle or the stigma surface membrane (Kambal et.al.,1976) is continuous and intact in the normal case. However, investigations carried out during the last decade on the stigma of several plant species indicate differences in the cuticular morphology. In Aneileima and Commelina (Owens and Horsefield, 1982), the

cuticle is ridged and shows a folded nature, while in Crocus (Heslop-Harrison and Heslop-Harrison, 1975), it exhibits many chambers containing stigmatic secretions. In Mangifera indica (Philip et.al.,1987) a thick ornamented cuticle invests the stigma papillae, which normally prevents dessication of the stigma surface, however, becomes a barrier for normal pollen tube penetration. Cuticle mainly functions in retaining the secretory material of the papillae as well as the underlying cells. The pollen grain germinating on the stigma surface, either has to enter through the torn-gaps or should release cutin splitting enzymes so that it can digest the cuticle at the point of contact and enter into the stigma papillae. In Trifolium pratense (Heslop-Harrison and Heslop-Harrison,1982b, 1984), the non-papillate stigma surface shows an inflated cuticle which retains the secretory material of the cells four to five layers below. The pollen grains germinate in the secretory material, and the pollen tubes enter through the torn-gaps in the cuticle to the intercellular spaces thereby coming in contact with the fluid held in the canal lumen of the upper style. However, self pollen tubes are retarded/ arrested in their later passage through the stylar canal, thus depitting a gametophytic self incompatibility. In Vicia faba (Lord and Heslop-Harrison, 1984), cuticle is thickened over the prominences left by the epidermal papillae. In all these wet stigmas, mechanical tripping of the cuticle is essential to allow the pollen to come in contact with the secretion for

its germination. In the dry stigma of Mango also, a mechanical tripping is needed to facilitate the entry of pollen tubes and the authors suggest this as the reason for relatively poor yield in many varieties of Mango (Philip et.al., 1987). According to Christ (1959), in a compatible pollination, an inactive precursor borne by the pollen is activated by a factor from the stigma to produce an effective cutin splitting enzyme. Though Dickinson and Lewis (1973b), from their ultrastructural studies on Raphanus showed that the barrier of the cuticle in both compatible and incompatible pollination may be breached, Heslop-Harrison and Heslop-Harrison (1975) support Christ's (1959) cutinase hypothesis in Caryophyllaceae, indicating that the penetration does depend on the activation of a pollen borne enzyme precursor by a factor held on the stigma surface.

In C.roseus and W.somnifera a distinct cuticle is present investing the papillae cells. The cuticle in C.roseus is intact in the early stages and in the receptive stage some discontinuities are formed which is responsible for the accumulation of exudates above the cuticle. W.somnifera shows distinct cuticular pores in stage IV. The pollen tubes in both C.roseus and W.somnifera do not penetrate the papillae cell, but enter the style through the intercellular spaces filled with the secretory material. Hence, in these two species it is certain that the cuticle does not become a barrier to pollen penetration as reported in other plants.

The stigma surface which is completely drenched in exudate at the receptive stage lacks a hydrated layer in C. roseus. The exudate is free flowing andgives a glistening appearance to the entire dumb-bell shaped stigma. W.somnifera has a distinct pellicle layer above the cuticle in addition to the meagre free exudate present only within the interstices of the papillae cells. Though the pellicle is invariably present in all the dry stigmas, its presence in wet stigmas is sporadic. Pellicle, a secretory product of the papillae functions as an activator for cutin splitting enzymes, and forms the binding site of proteins and glycoproteins released by pollen grain wall following pollen capture (Mattsson et.al., 1974). Moreover, it also enables to localize and expose the pollen recognition molecules which are proteins and glycoproteins (Clarke and Knox, 1978; Tilton et.al., 1984). Hence, the pellicle plays a major role in incompatibility reaction. This has been very well demonstrated by Heslop-Harrison and Heslop-Harrison (1975) in their experiments with Caryophyllaceous members. They observed that, in cases where pellicle was removed without killing the papillae, the entry of pollen tubes in a compatible pollination is prevented or delayed. In W.somnifera, a distinct pellicle, observed by stage IV at which the stigma becomes fully receptive, shows intense reaction for enzyme, non specific esterase, which is considered to be a pellicle marker (Mattsson et.al., 1974). This

may also be indicative of the sites of receptivity and may well serve as a guide to the onset of receptivity (Vithanage, 1984).

Style in angiosperms traditionally fall into two basic types, the hollow and solid (Shivanna, 1982). In the hollow style, it contains one or many stylar canals lined with glandular cells, while in the solid style, it has a rich core of transmitting tissue. In C.roseus, the style is much elongated, slender and flexible, while in W.somnifera it is short. Both species have solid style, which is considered to be an advanced character (Hanf, 1935). The transmitting tissue which makes a style solid is persumed to have formed from superficial carpellary derivatives (Satina, 1944). The transmitting tissue in addition to providing an easy passage for pollen tube growth supplies also the nutrients for its growth towards the ovary ( Maheswari, 1950).

Style in W.somnifera has two distinct vascular elements around a centrally placed transmitting tissue while, C.roseus has three. The presence of vascular elements around the transmitting tissue is also depicted in the style in Prunus avium (Uwate and Lin, 1981), and Petunia hybrida (Konar and Linskens, 1966).

In W.somnifera, the style develops from the middle of the ovary lobes. In the early stages, the style is very short with stigmatic end showing a furrow at the centre with two

zones of cells on either side. The protodermal cells undergo periclinal divisions and later elongate and the stigma surface expands. The style elongates and epidermis, cortex and central transmitting tissue become clearly demarcated. The cells of the transmitting tissue are loosely arranged, elongated parenchyma cells rich in cytoplasm. The intercellular spaces accumulate abundant secretory substances. Garg and Bhatnagar (1988) in their light microscopic study on the stigma in Withania are of the opinion that the young style has a narrow space in the centre which later disappears and the mature style is of solid type. The transmitting tissue according to them is continuous with the placental epidermis. However, my observation in this species show that the style develop from the region of fusion of the ovary lobes and not from the sides of the ovary as shown by the above authors. The structure described by Garg and Bhatnagar (1988) could be from a peripheral section of the young ovary lobes which depict the intervening space of the ovary lobes in the young bud. The style in W.somnifera does not have a space separating the two lobes and it is solid from the early stages of development.

The secretory product of the stigma contains carbohydrates, proteins, lipids and phenolic acids (Shivanna, 1982). In Lilium longiflorum (Labarca et.al., 1970), the stigma exudate analysis showed about 99% water, carbohydrate and proteins. Exclusive of water, 95% is a high molecular weight protein containing polysaccharide composed of galactose,

arabinose, rhamnose, glucuronic acid and galacturonic acids. In Trifolium pratense (Heslop-Harrison and Heslop-Harrison, 1982a), stigma and stylar fluid analysis shows similarities in protein. However, two major glycoproteins are present in the stigma elutes which is not found in the style. Sedgley and Blesing (1982) in their study on watermelon stigma demonstrated that pollen of all species can stimulate stigma secretion. Stimulation of stigma secretion by foreign pollen has also been reported earlier (Kenrick and Knox, 1981).

In C.roseus, the secretory materials contain abundant insoluble polysaccharides, lipids, and some proteins. The secretory substance confined to the interstices of the cells in W.somnifera also contains lipids and polysaccharides. In W.somnifera, the secretory substances come to the surface only when a slight pressure is applied on the stigma, which indicates that, the substances are contained deep in the stigma head and no free flowing exudate, as found in C.roseus is present in this species. However, its presence in addition to the pellicular layer, designate this stigma under the wet category, though it is very much different from C.roseus wherein, the exudate makes its appearance in the early stages and could be easily detected under a dissecting microscope. This clearly shows the degree of secretory activity among the species showing wet stigmas.

Lipids form a major constituent of the stigma exudate (Baker et.al., 1974; Vasil and Johri, 1964). It is also well

documented that lipid component of the exudate helps in trapping the pollen as well as prevent the stigma from dessication (Konar and Linskens, 1966b). Moreover, lipids also play a major role in the nutrition of pollen for its growth (Kuruvilla and Shah, 1988). The phenolic substances are helpful in protecting the stigma from microbes and pests (Martin, 1970; 1972; Tara and Namboodiri, 1976; Sedgley, 1975). The stigmatic exudate, apart from giving an ideal medium for pollen germination, also act as a food source to pollinating insects (Kenrick and Knox, 1981).

The stigma becomes receptive at an early stage in C.roseus and W.somnifera. While the onset of receptivity is 4-5 days pre-anthesis in C.roseus, in W.somnifera it becomes receptive 1-3 days pre-anthesis. This is evident following intense reaction for non-specific esterase. Though C.roseus lacks a distinct pellicle, esterase activity is localized in the exudate. In Nicotiana and Crinum having wet stigmas esters have been found even in early stages of pistil development (Shivanna and Sastri, 1981; Kandasamy and Kristen, 1987). The maximum activity of non-specific esterase at stages III and IV in W.somnifera could be due to the formation of a pellicle as a continuous layer above the cuticle.

Moderate to intense peroxidase activity at the I and II stages in both plants might be indicative of its involvement in the rapid turnover of peroxide in the cell during cell

differentiation. Peroxidase regulates the peroxide level in a cell by either decomposing it or using it in the oxidation of various substances (Brennen, Rychter and Frenkel, 1979). Peroxidase activity in stigma papillae is not worked out in the systems earlier studied. In Lily pollen, the peroxidase activity is distributed throughout the older regions of intact pollen tubes, not in the growing tip and the region immediately subjacent to it (Dashek et.al., 1979). Further, they also demonstrated the presence of two types of peroxidases; ionic and covalently bound forms in the purified wall fractions, thus supporting the report by Ridge and Osborne (1970) that plant cells contain two classes of wall peroxidases.

Succinate dehydrogenase (SDH) is a mitochondrial enzyme associated with cellular respiration leading to ATP synthesis. In C.roseus and W.somnifera, stages I and II of stigma papillae, depict moderate activity while stage III and IV showed intense activity. It has been well documented that during cell enlargement, extension and cell senscence, the respiratory activity increases (Brown 1972). Malik et.al. (1977) in their investigation on pollen grains detected particulate nature of SDH activity. Activity sites in the papillae of the two species of the present study also depicted a similar nature. Thus the high SDH activity in the papillae cells is accounted for the high metabolic rate to facilitate secretion as reported by various authors (Fahn and Benayoun, 1976; Setia et.al., 1977; Shah et.al., 1980).

Acid phosphatases are said to be associated with differentiation of cells during morphogenesis (Vanfleet, 1959) and as an enzyme enhancing cell senescence and death (Gahan, 1981). The wall bound nature of the enzyme observed in most of the reports is indicative of its involvement in cell wall formation and intercellular transport (McLean and Gahan, 1968). The intense acid phosphatase activity in stages IV and V in C.roseus and W.somnifera may be due to their presence in the lytic compartments to facilitate senescence and death of stigma papillae following a compatible pollination similar to the other reports (Matile, 1974; Moore and Walker, 1981). The lysosomal nature of the acid phosphatase has also been established by various authors (Novikoff, 1961; 1963; Malik et.al., 1969; Nagl, 1976; Singh et.al., 1980; Gahan, 1981). It may also be possible, as suggested by Matile and Winkenbach (1971) and Nagl (1976), that the release of the hydrolases due to the loss of compartmentation in the papillae cell is to effect the senescence and death of the papillae cell.

Wall bound ATP-ase activity which is meagre to moderate in all stages of stigma development in both the taxa could be indicative of the active transport of ions across the plasmalemma (Chaffey and Harris, 1985a). It is also reported in the secretory cells involved in active transport of metabolites (Lai and Thompson, 1972; Gahan, 1981; Didehvar and Baker 1986).

Biochemical analysis of total proteins in stigma and stylar extracts in C.roseus and W.somnifera also shows a

a similar pattern in the metabolic status when compared to the cytochemical tests. Though reports in this line are lacking, the results indicate a higher amount of protein in mature receptive stages in both the taxa. Proteins play a major role in the recognition rejection phenomena on the stigma surface (Nasrallah and Wallace, 1967) and they are present even at early stages of the stigma development (Shivanna and Sastri, 1976).

Pollen germination in vitro requires a medium containing the essential nutrients and carbon source. Brewbaker and Kwack (1963) had devised a medium containing macro and micro-nutrients, sucrose and boric acid for getting a comparable pollen germination as that of the stigma. I have used this medium with a slight modification in my in vitro experiments. The medium used contained 0.01% of boric acid and 10% sucrose for getting maximum pollen germination in C.roseus, while 0.02% boric acid 25% sucrose were needed in W. somnifera along with other nutrients. For C. roseus and W. somnifera the same elements calcium nitrate (0.06%), magnesium sulphate (0.04%) and potassium nitrate (0.02%) of Brewbaker and Kwack (1963) were needed for maximum germination.

Boron is essential for pollen germination and pollen grains of many species require only boron and a suitable sugar for its in vitro growth (Mc Leod, 1975; Vasil, 1960; Stanley and Linskens, 1974). Stimulation of pollen tube

growth by boron has been reported earlier (Schmucker, 1933). Moreover, it has been well documented that boron affects carbohydrate metabolism and in the synthesis of the wall polysaccharides (Stanley and Loewus, 1964; Young et.al.,1966; Chen and Loewus,1977). Sucrose has been found to be the best carbohydrate source for pollen germination and tube growth (Tupy, 1961; Hrabetova and Tupy, 1964). Sugars in the culture medium are found to serve two main purposes, maintenance of osmotic pressure of the medium and serving as a substrate for metabolism and carbon source essential for pollen germination (Johri and Shivanna, 1985).

Though the requirement of the nutrients were in traces, calcium play several major roles in pollen germination and tube growth. Calcium stimulates pollen tube growth, maintains membrane integrity and affects maintenance of permeability (Mascarenhas and Machlis, 1962b, Jones and Lunt,1967). Calcium also plays an active role in pollen tube tip growth, facilitating unidirectional movement of vesciles toward the tip and their fusion with the plasma membrane (Weissenseel and Jaffe, 1976, Picton and Steer, 1983). The reduced tube length in *in vitro* studies compared to *in vivo* very well indicate the lack of several other growth factors in germination medium.

Pollen grains once liberated from the anther are subjected to various factors in atmosphere before they land on the stigma surface. The pollen grain on its course to the

stigma encounters a variety of gases in the atmosphere, majority of which are harmful. Since pollen are supposed to be the most rapidly growing cells in the plant world (Malik, 1977), the effect of sulphur dioxide on pollen germination and tube growth seemed worth pursuing. Moreover, sulphur dioxide is one of the major pollutants affecting most of the plants in and around Baroda, which hosts a number of industries.

Air pollutants alter the size and chemistry of the pollen (Wolters and Martens, 1987). Absorption of sulphur dioxide and other compounds results in the acidification of the germinating medium (Karnovsky and Stairs, 1974) and acidification can affect pollen germination and tube elongation. The total or higher inhibition of pollen germination *in vitro* condition compared to *in vivo* could be due to the larger surface area of the agar blocks compared to the tiny stigma surface wherein, the former can absorb more sulphur dioxide gas which will be reflected on to a more pronounced impact. Fumigation with gaseous sulphur dioxide *in vitro* can bring pollen in contact with sulphur dioxide, sulphite, bisulphite and sulphuric acid, since the medium will accumulate them by conversions (Dubay, 1981, Dubay and Murdy 1983 b). Inhibition in pollen germination and tube growth *in vivo* could be mainly due to the chemical, physical and temporal differences between pollen germination and tube elongation which will be much less compared to *in vitro*. Comparatively less amount of sulphur

dioxide will be absorbed on to the surface of stigma due to the negligible build up of moisture on stigma surface. This could be the reason for pollen germination occuring even at 1.5 ppm sulphur dioxide in the *in vivo* condition.

The difference between the *in vivo* and *in vitro* results may reflect an extra buffering capacity of the stigma surface (Cox, 1984; Dubay, 1981; Dubay and Murdy 1983a,b). Plants utilize sulfate as their sulphur source for the synthesis of many sulphur containing components essential for life (Stumpf and Conn, 1981). Since gaseous sulphur dioxide can alleviate sulphur stress in plants grown on soils deficient in sulphur (Cowling and Lockyer, 1976), sulphur dioxide in the atmosphere enables the system to absorb and utilize sulphur for major pathways in the organism. Hence, at lower concentration, $SO_2$ can act as a growth promoter in many systems.

Polyamines are growth substances of higher plants (Bagni et.al., 1982; Galston and Kaur Sawhney, 1982), which are shown to interact with macromolecules and bio-membranes and regulate various cellular processes associated with growth and development (Tabor and Tabor, 1984; Feuerstein, Pattabiraman and Marton, 1986). Polyamines are also known to play a role in the regulation of plant senescence (Slocum, et.al., 1984). In apple pollen, it has been shown that biosynthesis of RNA and polyamines precedes tube emergence (Bagni et.al., 1981 ). However, it is not known whether polyamines are essential

for pollen germination and tube growth. To look into this aspect, spermidine a polyamine was used along with MGBG (methylglyoxalbis(guanylhydrazone)) which is an inhibitor of spermidine bio-synthesis to find out its role.

Pollen germination and tube growth in both the taxa were found to be stimulated following the incorporation of spermidine at $10^{-5}$M concentration into the nutrient medium. MGBG, when supplemented into the nutrient medium showed complete inhibition of pollen germination and tube growth at 1.5 M concentration in C.roseus and W.somnifera. However, the inhibitory effect of MGBG at 1.5 M concentration is reversed by the exogenous supply of spermidine at $10^{-5}$ M concentration which was found to be the optimum concentration. Thus a recovery of about 80% was observed in the case of C.roseus and about 20% in the case with W.somnifera. Spermidine at $10^{-4}$ M concentration however could not reverse the inhibitory role of MGBG. This could be due to the toxic effects of MGBG which makes irreversible changes in the pollen grain. A noticeable reversal in MGBG inhibition at lower concentration by spermidine suggests the requirement of polyamine for pollen germination which is in agreement with Bagni et al., (1982), who found similar results in apple pollen germination. The transition from dormancy to germination of pollen is characterized by a sudden rise in transcription and translation (Mascarenhas,1975) and polyamines are known to promote both these processes (Cohen, 1978; Tabor and Tabor, 1976). Another interesting

observation was that actinomycin-D inhibition of pollen germination could not be reversed by spermidine in C.roseus and W. somnifera. According to Čapkova et.al, (1983), apart from reducing the protein release, actinomycin-D interferes also with structures involved in pollen tube wall synthesis. This may be the reason for total inhibition of pollen germination by actinomycin-D and the subsequent failure of spermidine to reverse the impact. Bagni, Corsini and Serafini-Fracassini (1971) have demonstrated that spermidine reverses the inhibitory effect of actinomycin-D in Helianthus tuberosus. However, the present work could not lend support to these findings.

Pollen germination and tube growth were studied after the incorporation of self and cross pistil extracts into the nutrient medium in C. roseus and W.somnifera. In C.roseus, the supply of self pistil extracts into the nutrient medium showed an enhanced pollen tube growth compared to the control. In W. somnifera on the contrary, an enhancement in the tube length was observed in the media which contained cross pistil extracts.

In vitro analysis thus showed an indirect evidence to suggest whether the system favours self pollination or cross pollination. Though the in vitro and in vivo experiments together show that C. roseus as self compatible and W.somnifera as self incompatible, both self and cross pollen germinate

on the stigma surface in the bud stages. It may be mainly because either stigma factors which discriminates self or cross pollen and inhibiting the incompatible pollen are not yet present or are inactive in buds (Shivanna, Heslop-Harrison and Heslop-Harrison, 1978). In vivo experiments, moreover show that a distinct cuticle in the earlier stage does not become a barrier at all for pollen germination. However, once the stigma attains maximum receptivity at III and IV stages, the discrimination of self and cross pollen begins, indicating the presence of stigma receptors at these stages.

In C.roseus most of the pollen tubes enter the stigma through the lateral side of the bulky stigma head. This may be because of the availability of secretory material on the lateral sides than on the upper side and also because the lower region offers least resistance. The pollen grains germinate and the tube traverses through the space between the cells below the lateral papillae cells and enters the style through the base of the stigma head, thus avoiding the massive stigmatic tissue. In W. somnifera, the pollen tube does not penetrate the papillae but grows between the intercellular spaces of the papillae cells. The pollen tubes subsequently grow towards the ovary through the intercellular spaces of the transmitting tissue. Pollen tube entry in C.roseus is thus different from W.somnifera. Following pollination, the stigma withers off and shows a degenerated appearance in W.somnifera and C.roseus.

This has been a common feature in other species also (Heslop-Harrison, 1979; Sedgley, 1979; Considine and Knox, 1979).

In C.roseus, microsporogenesis follows in the normal pattern. The bithecous anther is tetrasporangiate, with a distinct secretory type of tapetum. Tapetum apart from playing a major role in nutrition, has been found to function in the synthesis of orbicules of sporopollenin also (Echlin,1971; Kapil and Tiwari,1976; Rudramuniappa and Panchaksharappa; 1980). The tapetum in C.roseus and W.somnifera remain intact, till the spores are formed, thereafterm, they loose contact with each other become highly vacuolate and later degenerate. In Psilotum (Parkinson 1987) individual tapetal chambers have been observed. In Taxus (Pennell and Bell; 1986), the tapetum invades into the loculus towards the end of its degeneration. There are several genera in which the walls of the tapetal cells break-down, but the protoplasts remain intact, and wander in the locules. This type of tapetum called as the 'amoeboid' type also serves for the nutrition of the spores (D. Maheswari, 1950).

Ovules in C.roseus and W.somnifera are of the anatropous type. While it is borne on parietal placenta in C.roseus, in W.somnifera, the ovules are borne on axile

placenta. The embroysac is of polygonum type in C.roseus and W.somnifera. Polygonum type of embryosac has been found in about seventy percent of angiosoperms investigated so far (: Maheswari, 1950).

Pollen viability tested at different conditions of storage showed that the response of pollen viability differed at different conditions. Pollen viability is lost rapidly in the normal conditions of temperature and humidity, and viability has been prolonged only through low temperature and low humidity (Johri, Sastri and Shivanna,1977). Low temperature storage technique has been used successfully in corn (Pfahler and Linskens,1973), pine, birch, tobacco and alfalfa pollen (Hanson and Campbell, 1972). In the studies carried out, -4°C has been found to be the condition where maximum viability was retained in both C.roseus and W.somnifera. King (1965) has reported that the optimum temperature for storage of pollen is -10°C to 10°C, since minimum pollen metabolism has been found at that temperature. Pollen grain viability can also be prolonged by keeping them in organic solvents (Iwanami and Nakamura, 1972).

## ULTRASTRUCTURE

### PISTIL

The angiosperm stigma is generally considered as a glandular stucture where secretion is important in the pollen-stigma interaction (Linskens,1981). The stigmas of many flowering plants have been classified morphologically (Heslop-Harrison and Shivanna, 1977; Heslop-Harrison,1981). In families having a dry stigma, a pellicle overlying the papillar surface is present (Heslop-Harrison and Heslop-Harrison,1980) and is the site of recognition and rejection reactions (Heslop-Harrison and Shivanna, 1977). In plants, with wet stigmas at the receptive stage, the surface is covered with a sticky secretion (Dumas et.al.,1978; Cresti et.al.,1982) consisting of many components.

The stigma in C.roseus and W.somnifera are of the wet category. The copious exudate bathes the entire dumb-bell shaped stigma at the receptive stage in C.roseus, in which the pollen grains are trapped and grow profusely. However, the stigma in W.somnifera, though categorised as wet, because of a scanty secretion present only between the interstices of the papillae, is much different from that of C.roseus. The only indication of a free flowing exudate in this species is when the stigma is squeezed to make the exudate come on to the surface. Garg and Bhatnagar (1988) in their light microscopic

study on Withania stigma, have also observed scanty exudate on the stigma surface. The presence of pellicle, in addition to the exudate present deep among the papillae cells is unique in wet categories of stigma. The stigma appears dry on the surface because of this thick pellicular layer. In the majority of the reports, like in Petunia and Nicotiana tabacum, in the young stigmas, the surface esterases (as markers of stigma receptivity) are present as a thin and continuous pellicle, comparable to that found on the dry stigmas (Shivanna and Sastri, 1981). The authors also have observed that in these species, the pellicle disrupts at random during the secretion of the exudate, and the flakes of disrupted pellicle drift on the surface of the exudate. However, in Crinum deflixum and Amaryllis vittata, having wet stigmas, the pellicle is not disrupted even though the exudate accumulates on the stigma surface (Shivanna and Sastri, 1981). The noteworthy difference between these reported plants and W.somnifera is that the copious exudate present over the pellicle in the above mentioned plants, is absent in this species. Stigmas in Liliaceae and Bromeliaceae are wet, but show dry appearance at anthesis (Heslop-Harrison and Shivanna,1977). According to the authors, though these plants could be easily misclassified as dry, because at a later stage, the stigmatic lobes open out further, and a copious secretion appears, and the papillae become partially inundated. Though, this phenomenon is not noticed in the stigma of W.somnifera, I have preferred to classify it under the

'wet' category though it has many characteristics of the 'dry' type. The ultrastructural evidence indicates that this species shows unique character of possesing hydrated and free-flowing exudates together, which makes it different from the other reported plants with wet stigmas. The pellicle keeps its identity even till the senescent stage of the papillae cells.

Stigma in C.roseus is a complex structure covered by two distinct types of papillae cells. The papillae over the surface and base of the dumb-bell shaped stigma are long with high vacuolation, while those on the sides are small and packed with little intercellular spaces. Since the exudate is more on the sides than at the tip and base, it is clear that the bulk of the secretion is contributed by the short papillae cells. Moreover, the pollen tubes prefer to grow through the intercellular spaces of these type of papillae cells which also indicate their secretory status. Compared to the complex stigma of C.roseus, the stigma in Withania is simple covered on the upper end of the stigma by multicellular thumb-shaped uniseriate papillae.

The papillae cells are thin walled and have highly dispersed microfibrillar texture in C.roseus, while in W. somnifera, the cell wall has compact microfibrils. The microfibrils merge with the secretory product which is suggestive of the mode of secretion of polysaccharide component of the stigmatic fluid in C.roseus. Though, it

is also possible that the polysaccharides are synthesized and secreted via dictyosomes as in many other secretory tissues (Fahn and Evert, 1974; Setia et.al.,1977, Nair et.al. 1983). Since the papillae cells are intact till the receptive stage, the incorporation of polysaccharide during their degeneration (holocrine mode) is not possible and hence the likely possibility is that there is constant addition of wall material through highly active dictyosomes, and subsequently the outer wall layers slough-off and get added to the secretion enriching it with polysaccharides. This may also be the possible mechanism in W.somnifera. However, in C.roseus in addition to this, the role of wall hydrolysing enzyme could not be ruled out, since the highly dispersed appearance of wall material is indicative of wall hydrolysis.

The role of dictyosomes in cell wall synthesis has been proved in many plants (Gunning and Pate,1969 ; Schnepf and Pross 1976; Fineran 1980). Barnabas et. al., (1982) in their study on wall ingrowth development in Zostera capensis have shown that paramural bodies, ER, dictyosomes and microtubules are associated with the initiation and subsequent development of ingrowth. Joel and Fahn (1980) have noted a close association of dictyosomes and ER in the protein polysaccharide mucilage secretion in mango fruits. In Commelinaceae the cuticle and wall are significantly disrupted in mid and basal region and the fibrils in the outer wall may contribute carbohydrate material to the secretion at the same

time increases the stigma permeability (Owens et.al.,1984). In Tradescantia ohiensis (Owens and McGrath, 1984), pollen grains adhere and germinate more rapidly when attached in the mid and basal regions of the papillae. It is expected that the receptive site on the papillae would provide the least resistance to transport of materials across the cell wall and through the cuticle (Heslop-Harrison and Heslop-Harrison,1982b). This is quite true in the case of C.roseus and W.somnifera and I strongly believe that apart from adding carbohydrate, the loose microfibrils of the papillae also would help in holding the pollen grains firmly on the stigma surface and increase the permeability of the wall.

The cell wall in the short papillae of C.roseus also shows ingrowth like structures bound by plasmamembrane. This is reminiscent of the wall membrane apparatus of transfer cells (Gunning and Pate, 1969). The development of wall membrane apparatus in some cells during certain physiological location is ascribed to a role in the transport of solutes either into or out of the cells. Labrynthine cell walls are also reported in the Lilium transmitting tissue (Gawlik,1984). The functional feature of plant cells possessing ingrowths of wall material is a high surface to volume ratio which facilitates an intensive trans-membrane flux of solutes. Rosen and Thomas (1970), also observed wall ingrowths in the secretory zone of stylar canal cells of Lilium longiflorum.

This specific type of cells, according to the authors resemble greatly to the transfer cells, however, they referred the canal cell as another type of transfer cell. However, in Lilium leucanthum (Gawlik, 1984), both the stigmatoid cells which line the canal and those found on the surface of the stigma possess cell wall ingrowths typical of transfer cells. The papillae cells in C.roseus showing wall ingrowth also might represent a different type of transfer cell, developed only at the time when the secretion is maximum, similar to those reported in Lilium longiflorum by Rosen and Thomas (1970).

The intercellular spaces between the papillae and sub-papillae cells and the cells below them in the stigmatic region in Withania somnifera accumulate lot of fibrillar and osmiophilic materials. The pollen grains which germinate along the sides of the papillae in this species have to pass through the inter-cellular spaces to reach the embryo sac. However, in C.roseus the dumb-bell shaped stigmatic head though made up of loosely arranged parenchyma cells, does not show enough deposition of secretory material in the intercellular spaces. Thus the pollen tubes in this species, from my critical study following structural and fluorescence microscope analysis, in no circumstances penetrate the massive stigmatic head, instead will grow along the papillae of the sides. The penetration occurs only through the basal junction between the stigma and style.

In the stigma of Nicotiana (Cresti et.al.,1986), a characteristic feature noticed was the presence of loosely

arranged thin cell walls and large intercellular spaces which is a general feature of secretory tissues (Fahn, 1979). Cresti et.al, (1986) also have demonstrated the occurrence of pollen tube growth inside these spaces which can easily enlarge by a slight mechanical pressure. According to them, cell wall dissolution is also necessary to enable pollen tube growth; the mainly pectic composition of the walls in this zone facilitates such a lytic process. This is very well true in the case of W.somnifera where many pollen tubes are found inside the intercellular spaces of the sub-papillae, cells below them and in the transmitting tissue. Since the pollen tubes found in the spaces are larger than the spaces observed, it is also certain that the pollen tube further exerts mechanical or enzymatic pressure on the space to widen so as to accommodate the tubes.

In C.roseus, the cytoplasm of the long papillae cell is comparatively less dense than that of the short papillae and contain many large vacuoles. The short ones have dense cytoplasm with a few small or no vacuolation in the early stages. The vacuolation in this type of papillae cell increases, depicted by a highly vesiculate cytoplasm towards late receptive stage. The vacuoles in the long papillae cells contain finely distributed fibrillar inclusions, while the short ones do not show similar inclusions in their vesicles. The fibrillar material which appears to occupy almost the total volume of many of the papillae cells and on the stigma surface may be the same material identified by Yamada (1965), as colloidal bodies and later

confirmed by Crang (1969) and Rosen and Thomas (1970) in Lily pistils.

The papillae cells in W.somnifera on the other hand, have large vacuoles containing osmiophilic droplets, while the sub papillae along with osmiophilic droplets, also show phenolic accumulation. Vacuoles are considered to be the most important ubiquitously distributed storage spaces of secondary products (Kuster, 1956; Blank, 1958). Matile (1982), opines that large vacuoles help to achieve a large surface contact area for the incoming pollen and secondly, that the vacuoles can act as a hydrostatic device and thus control pollen hydration. It seems that in C. roseus, the large vacuoles in the long papillae might serve a similar function. In the short ones, the increase in vesiculation is indictive of degeneration of the papillae cells similar to the views of Matile (1975), that cellular digestion requires the introduction of cytoplasmic material into the lytic compartment. The high vacuolation in the papillae and sub-papillae of W.somnifera act as storage spaces for the osmiophilic material, which is released only into the intercellular spaces since the secretion on the stigma surface is meagre.

The other secretory product, apart from the fibrillar materials (polysaccharides), is the osmiophilic droplets which could be easily detected following osmium fixation and uranyl acetate staining (Fahn,1979), in the stigma cells of both C. roseus and W.somnifera. The papillae cells in C.roseus

show osmiophilic materials associated with ER and mitochondria and are also distributed at the plasmalemma region and within the wall material suggesting that the osmiophilic material in this plant is synthesized within the ER cisternae and also in the mitochondria. However, in the case of W.somnifera, the plastids and chloroplasts are the only organelles which show association with the osmiophilic droplets.

The synthesis and secretion of osmiophilic material are attributed to different organelles in the various species secreting lipophilic materials studied (Fahn,1979). Wooding and Northcote (1965), who investigated the resin duct of Pinus pinea, have observed osmiophilic droplets in plastids and ER, while in P.halepensis (Fahn and Benayoun,1976), the occurrence of osmiophilic material is in the golgi apparatus and in the ground cytoplasm. In the gum-resin ducts of Anacardium occidentale, Nair et.al,(1983) have demonstrated the role of dictyosomes in the synthesis and transport of osmiophilic material.

In the stigma of Forsythia intermedia and Verbascum phlomoides Dumas (1973, 1974) has demonstrated the presence of osmiophilic globules in the plastids and such plastids are sheathed by endoplasmic reticulum. He suggested that the plastid - ER complex is the site of synthesis or accumulation and means of intracellular transport of a part of the stigmatic exudate. According to Kandasamy and Kristen (1987), the secretory pathway of osmiophilic materials, predominently

terpenes, is still a matter of speculation. However, in Niocotiana sylvestris, the authors have observed electron dense (osmiophilic) materials in and near the plastids, and sometimes, even appeared to be released from plastids by a process which resembles budding and they have assumed that plastids have a role in the synthesis of osmiophilic substances.

The secretion of lipophilic material has also been shown to be closely related to the occurrence of a tubular type of smooth endoplasmic reticulum (Schnepf, 1972; Schnepf and Klasova, 1972). Since the SER is well developed in the secretory tissue of stigma in N. sylvestris, Kandasamy and Kristen (1987) also believe that sER has a role in the secretion of lipophilic material. However, in C.roseus, the osmiophilic material is encountered only in the rER which is the predominant form of ER in the papillae cells. The association of ER with lipid droplets is also reported in Lycopersicum (Dumas et.al.,1978) and Trifolium (Heslop-Harrison and Heslop-Harrison, 1982b).

The plastids, in the secretory tissues are often encircled by endoplasmic reticulum (Dumas, 1973; Fahn and Evert, 1974; Fahn and Benayoun, 1976; Setia et.al., 1977; Fahn,1979, Joel and Fahn,1980; Heslop-Harrison and Heslop-Harrison, 1981; Nair, et.al., 1983). In the secretory tissues of Mangifera, the periplastidal ER transports the resin

synthesized in the plastids to the plasmalemma (Joel and Fahn, 1980 ). In the digestive glands of Pinguicula (Heslop-Harrison and Heslop-Harrison, 1981), the ER encircling the plastids have ribosomes only on the outer membrane. However, such a structural peculiarity is not observed either in Withania or Catharanthus. In Withania, where plastids are responsible for the synthesis of osmiophilic material, the ER-plastid association is significant for the transport of the resin to the plasmamembrane for its subsequent transfer to outside. In the pistil of Lilium, Miki-Hirosige et.al,(1987) attributed the function of transport of secretory substances to ER and golgi vesicles. Kristensen (1982), reported that the stigmatic exudate in Aptenia cordifolia is directly transferred by ER via ER vesicles to the plasmalemma. Since both the plants of the present investigations show many ER-plasmalemma connections which are direct evidences for the role of ER in transport. However, in C.roseus the plastid ER association is not significant in transport from plastids, at least not osmiophilic material, since such abundant accumulation of droplets, as found in the plastid of Withania, is not found in this plant. It is quite reasonable thus to assume that sheathing of plastids by ER is indicative of the co-ordination between these organelles in the synthesis of osmiophilic material on Withania. According to Bosabalidis and Tsekos (1982) and Galatis and Apstolakos (1977), the significance of plastid-ER association which becomes prominent in the secretory cells at the stage before secretion lies either in the involvement of ER in the

transfer of soluble carbohydrates or other products to and from plastids, or in that the ER cisternae are the site of synthesis of substances required for plastid differentiation, which may be very well applicable to the ER-plastid association in C.roseus.

The dictyosomes in C. roseus and W. somnifera lack osmiophilic contents and hence their role in lipid synthesis is doubtful. Dictyosomes, in C. roseus produce two distinct types of vesicles. In the early phase of secretion they produce small dense vesicles, while in the latter phase (i.e. during senescence) large translucent vesicles are formed from them. This distinct specific activity during the two demarcated stages of secretion indicate that the former may be active in polysaccharide synthesis and transport while the latter configuration may play a role in the senescence of the papillae cytoplasm. The role of dictyosomes in the synthesis of carbohydrates has enormous support in literature (Moore and McClelen 1983; Morrison and Polito, 1985; Gedalovich and Fahn, 1985). The secretion product of plant cells are frequently attributed to the activity of the dictyosome cisternae, which gives out vesicles that transport the secretion to the cell wall. This has been reported to be the case in the digestive glands of insectivorous plants (Schnepf, 1986; Scala, Schwab and Simmons, 1968), the secretory trichomes of Psychotria bacteriophila (Horner and Lersten, 1968), the root

cap cells of Zea mays (Moore, Jones and Mollenhauer,1967) gum-resin ducts of Anacardium occidentale (Nair et.al.,1983) and others (Schnepf, 1969; Fahn,1979). However, in contrast, the secretory papillae in W. somnifera lack signs of conspicuous dictyosome activity. The diversity in the association of different organelles with the osmiophilic materials and/or polysaccharides indicates that either all the organelles are capable of lipid/carbohydrate synthesis or that, different components of a secretory compound are synthesized by different organelles.

Transmitting tissue

In plants characterised by solid styles, the growing pollen tubes penetrate the central core of the style and grow intercellularly through the transmitting tissue which is cytologically different from the surrounding tissue. In C.roseus and W.somnifera, the styles fixed after pollination, revealed pollen tubes penetrating the intercellular substances of the transmitting tissue cells. These cells which are glandular show dense cytoplasm with profuse organelles. Osmiophilic droplets are found initially in the cytoplasm, while in later stages, the osmiophilic droplets are deposited in the widened intercellular spaces. The most remarkable feature of the transmitting tissue in C.roseus is that some of the cells are binucleate and the cytoplasm contains profuse dilated smooth ER. The presence of smooth tubular type of ER has been

depicted as its involvement in secretory products (Schnepf, 1972; Fahn,1979). However, osmiophilic material has not been encountered in its cisternae, hence its role may be in the synthesis of some other substance essential for pollen tube guidance and growth. Nevertheless, the occurrence of osmiophilic droplets, associated with the stacked rER in W.somnifera indicate its active role in lipid synthesis. The plastids and chloroplasts in the transmitting tissue cells of C.roseus are responsible for the lipid synthesis and secretion because, they are abundant in the cytoplasm and often have osmiophilic material in their matrix. This diverse occurrence of association of osmiophilic material with different organelles within the tissues of the same organ is remarkable and shows the complicated nature of functioning and division of labour of the tissues in general and the organelles in particular, of plants.

The most striking feature of the transmitting tissue at the receptive stage in both W.somnifera and C.roseus is the presence of large intercellular spaces filled with osmiophilic droplets and fibrillar materials. In soybean stigma (Tilton et.al., 1984), the cells in the stylar transmitting tissue are secretory, producing exudate detectable in the intercellular spaces. It is quite obvious that the pollentube while growing through the intercellular spaces, initially will have to exert pressure on the spaces to widen. The normal occurrence of longitudinal separation

of transmitting tissue cells ensures that there is an axial array of channels filled with exudate through which the pollen tube can grow, This feature in C.roseus and W.somnifera indicate that the intercellular spaces provide a kind of mechanical guidance and nutrition required for pollen tube growth in the lower part of the stigma.

One of the important roles of transmitting tissue is that it should control the direction of pollen tube growth by secreting a chemotropic exudate. Evidence for such possible control in C.roseus and W.somnifera is provided by the fact that the pollen tubes emerging from the stigma grow only towards the ovary. Though large amount of exudate is secreted and deposited in the intercellular spaces many pollen tubes in the stylar tract appear necrotic, similar to that found in other stigmas (Tilton et.al.,1984; Mulcahy, 1974). This may be due to a mechanism of natural selection viz. competition in terms of growth rate and the genetic superiority of gametophytes able to outgrow other individuals and effect fertilization.

Tapetum

C.roseus has a secretory type of tapetum which synthesizes predominantly lipids and protein. Plastids which are large, accumulate a number of osmiophilic droplets and are invariably encircled by rER. Pacini and Casadaro (1981)

also found that the tapetum plastids in Olea europea contain osmiophilic globules encircled by membranes. However, the encircling membrane, the ER, is peculiar in the species that the ribosomes are associated only with the outer membrane of the periplastidal ER and fine fibrils link the ER and the plastid envelope. In the digestive glands of Pinguicula, Heslop-Harrison and Heslop-Harrison (1981) also made a similar observation. However, the rER in C.roseus did not show such features. The plastid envelope breaks and the osmiophilic droplets are released into the cytoplasm and subsequently to the anther cavity during the degeneration of the tapetal cells.

The other striking feature of the tapetal cells in this species is the accumulation of fine crystallar proteins in the cisternae of the rough endoplasmic reticulum. This protein, which looses it crystallar nature in the later phases are released into the cavity of the anther for its subsequent incorporation into the pollen exine. This development is significant, because during pollen-pistil interaction, the first chemical substance that comes in contact with the stigma surface is the exine held proteins for a successful sporophytic incompatibility reaction (Shivanna,1979).

Synthesis and secretion of proteins usually require a well developed rough ER and golgi apparatus (Robinson and Kristen, 1982). However, a deviation from the classical

pathway of intracellular protein transport has been postulated for some gland types. Proteins are reported to be directly secreted by rER compartments in the digestive glands of Dionaea (Robins and Juniper, 1980), in the ligules of Isoetes (Kristen, Liebezeit and Biedermann,1982) and in the stigmatic papillae of Aptenia (Kristen et.al., 1979) and Crocus (Heslop-Harrison and Heslop-Harrison, 1975) and in the stigma of Nicotiana sylvestris (Kandasamy and Kristen, 1987). The tapetal cells of C.roseus also showed similar feature in which the protein is synthesized only by the rER and not by the dictyosomes.

The lipids and proteins are released into the thecal cavity of the anther by holocrine mode of secretion as postulated by Fahn (1979). The inner wall of the tapetal cell disintegrates and the lipids and proteins are released alongwith the other contents of the cell. Though Ubisch bodies are encountered along the inner tangential wall prior to the degeneration of the cell, their origin is not clear in the present investigation. However, there may be a possibility of the osmophilic droplets synthesized by the plastids becoming the precursor of the Ubisch bodies. The final emergence of large highly osmiophilic bodies coinciding with the plastid degeneration is suggestive of this. Moreover, the plastids contain bodies of different electron opacity and some of them would be pro-ubisch bodies. To prove

this more detailed analytical study in this line is required.

Pollen grains and pollen tubes

The pollen grain in both W.somnifera and C.roseus are characterised by electron dense intine, and highly dense ornamented exine. The cytoplasm of the pollen grain in both the species have abundant ribosomes, mitochondria, ER and plastids. While the pollen grains in C.roseus accumulate many starch grains possibly representing amyloplasts, those in W.somnifera lack such large grain accumulation. The notable feature in the starch grain is the development of cracks during hydration. These cracks, probably represent the hydrolysis of starch. Starch, normally a reserve material in the pollen grain, will have to be hydrolysed for the initial pollen tube growth. According to Chandorkar and Badenhuizen (1967), most of the starch grains in higher plants develop cracks through which the amylolytic enzymes penetrate. A similar morphology was also observed in the starch grains in wood parenchyma cells of Melia azedarach during transition from sapwood to heart wood (Pandalai et.al., 1985). They have speculated that the starch in these cells are mostly converted to fats, similar to the reports by Chandorkar and Badenhuizen (1967) and Badenhuizen (1966). However, this kind of conversion may not be the possibility in the pollen grain of C.roseus. The reserve food material is to be converted into compounds which can supply the required energy for the initial pollen tube emergence, till they can absorb the material contained in the

stigma fluid for their subsequent, sustained growth. Dickinson and Clare Wilson (1983), also have stated similarly that starch serves as a major energy reserve of pollen grain both during dispersal and subsequent development of pollen tube.

The growth of the pollen tube is accompanied by the localised fusion of secretory vesicles with the apical plasmamembrane. Normally, these vesicles arise from the numerous dictysomes present within the pollen tube cytoplasm and supply both the membrane and cell wall carbohydrates necessary for the growing tubes (Rosen, et.al.,1964). The growing pollen tubes of both C.roseus and W.somnifera show many hypertrophied dictysomes, little away from the tip which produce abundant vesicles which are concentrated at the tube tip and show fusion with the apical plasmalemma. According to Moore and Van Der Woude (1974), the rate of vesicle fusion determine the tube growth rate, but the mechanisms which control the vesicle fusion process and maintain a balance between the supply of vesicles and the requirements of the growing tubes are not known. However, in in vitro cultures $Ca^{+}$ ions have been known to be necessary for growth (Brewbaker and Kwack, 1963) and subsequently there have been suggestions that calcium may act as a chemotropic agent in the stigma and style (Mascarenhas and Machlis, 1962). Studies on the in vitro pollen tube growth in Lilium (Moore and Van der Wonde, 1974) and Tradescantia (Picton and Steer, 1983)

have clearly shown that during pollen tube extension the rate of production of vesicles, vesicle fusion, tip extension are all very closely linked and all these process appear to be sensitive to changes in $Ca^{+}$ ion concentration. The *in vivo* control of these process in the faster growth of pollen tubes in *C.roseus* and *W.somnifera* need to be examined to correlate the above mentioned findings.

Pollen tubes utilize the nutrients present in the pistil exudate or in the culture medium for their growth *in vivo* and *in vitro* respectively. The nutritional dependence of pollen tubes or stigma and stylar exudates has been clearly demonstrated by labelling experiments (Labarca and Loewus, 1972; Loewus and Labarca, 1973). The pollen tubes in *C.roseus* and *W.somnifera* are found growing in the stigmatic fluid and in the intercellular spaces of the sub-papillae cells below them and the transmitting tissue, which contain abundant secretion products, are indications to suggest this preference. Moreover, the pollen tubes show highly undulated plasmalemma which contain osmiophilic and fibrillar materials which are also suggestive of the active uptake of nutrients to facilitate their faster growth. Kandasamy, Kappler and Kristen (1988), in their study on *Nicotiana sylvestris* pollen tubes, have demonstrated the presence of plasmatubules formed by evaginations of the plasmamembrane. Plasma tubules, a specific type of plasmalemmasomes (Harris et.al., 1982) are suggested to facilitate high solute flux especially in the

area of uptake from apoplast to symplast (Harris and Chaffey, 1985; 1986). These tubular modifications of the plasma membrane in the pollen tubes would considerably increase the surface area similar to the wall in growth of transfer cells (Kristen, 1969; Pate and Gunning, 1972; Barnabas et.al., 1982). Though a comparable structure is not found in the pollen tube of C.roseus and W.somnifera, the undulation of the plasmamembrane is suggestive of an efficient mechanism to increase the surface area and thereby to an increased uptake of nutrients. The highly dispersed appearance of the pollen tube wall also would help an easy uptake of nutrients in these species.