DEVELOPMENTAL ULTRASTRUCTURAL CYTOCHEMICAL AND EXPERIMENTAL STUDIES OF THE PISTIL OF Catharanthus roseus L. G. Don (Apocynaceae) and Withania somnifera Dunal (Solanaceae)

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SUMMARY

Reproductive biology in angiosperms has been of interest to scientists during the past few decades. Though much is known about the biochemical, physiological and genetic aspects, detailed developmental study of the reproductive organs has been limited to a few taxa. Therefore, our understanding of gemetogenesis and fertilization has remained quite limited and obscure. There is need for extending the studies to many of the available species, so that a clear understanding of the complicated interaction, behaviour and the factors controlling them can emerge.

The present investigation elucidates the developmental study of the reproductive organs in two dicots, and brings into light the similarities and dissimilarities occurring in both the taxa. The important observations made and described in the thesis are as follows.

Catharanthus roseus L.G. Don (Apocynaceae) and Withania somnifera Dunal (Solanaceae) are perennial herbs prevalent in most parts of the country. Both are highly economically important and yield variety of alkaloids which have extensive use in medicine.

C.roseus and W.somnifera both have wet stigmas. In C.roseus, a free flowing exudate is discernible, while in

W.somnifera, the exudate is scanty and confined only to the interstices of the papillae cells. The stigma in W.somnifera shows a well defined pellicle above the papillae cell surface which persists till the senescent stages of the stigma. In C.roseus the stigma is dumb-bell shaped, while in W.somnifera it has a bifid appearance. The papillae cells in C.roseus are unicellular and arranged uniseriately, and are of two different types. The long papillae are found on the upper and lower regions of the dumb-bell shaped stigma, while the short papillae are arranged in a uniform pattern along the lateral side. In W.somnifera, the papillae cells are multicellular and thumb-shaped and arranged in uniseriate pattern.

In C.roseus, the papillae which are seen in the early stages as tiny protuberances, elongate in the later stages. However, those on the upper side of the stigma and lower region elongate maximum, thus producing two types of papillae of different lengths. The uniform sized papillae on the lateral side depict more secretory activity than the elongated upper and lower papillae cells. In W.somnifera, stigma in early stage I shows a homogenous mass of cells. The protodermal initials elongate and bulge forming dumb-bell shaped papillae initials. By stage V the papillae cells become highly elongated, multicellular densly stained and show degeneration.

In C.roseus, the long papillae show thin parietal

cytoplasm. The cytoplasm shows many rough ER, mitochondria, plastids and dityosomes. Osmiophilic droplets are associated with ER and mitochondria. The secretion product, osmiophilic as well as fibrillar, is found over the surface of the papillae cell, completely drenching them. The papillae on the lateral region are short and show dense cytoplasm with abundant well developed organelles. Dictyosomes produce two different types of vesicles at the early and late stages of secretion. The osmiophilic droplets are also found amidst the cellulosic microfibrils of the wall and the outer periphery of the papillae cells. The secretion is intense and maximum in stages III and IV, while stage V shows many papillae cells undergoing senescence.

In W. somnifera, active synthesis and secretion are observed in stages III and IV. The distinct pellicle observed above the cuticle in Withania, is thick and have osmiophilic droplets embedded in the dense layer. At stage V, the papillae show many degenerating organelles. The plastids which are electron dense and are the only organelle associated with the synthesis of osmiophilic material. They have few internal membrane system and some of them are well developed chloroplasts. ER is well represented and is mostly rough. Ribosomes at stage III are mostly in polysomal configuration.

Style in both the taxa are of the solid type with a central core of loosely arranged transmitting tissue.

Vascular bundles, observed in the stigma of C.roseus and W.somnifera are continuous in the style. Style in both species have elongated parenchymatous cell with little intercellular spaces surrounding the vascular bundles and the transmitting tissue.

The transmitting tissue cells in C.roseus are elongated, with little intercellular space in the early stages, while in the receptive stage, the cells show abundant intercellular spaces filled with secretion. The cytoplasm of the transmitting tissue cells is dense with few vacuoles. Plastids are abundant with poorly developed internal membrane and few of them are chloroplasts. Pollen tubes, growing through the intercellular spaces of the transmitting tissue cells are discernible in the style at stages IV and V.

In W.somnifera, the cells of the transmitting tissue depict blunt or tapering end walls. The cytoplasm shows many small vesicles without any contents. Osmiophilic droplets are seen in the cytoplasm in the early stages of the stigma, while, the later stages show intercellular spaces filled with the osmiophilic droplets.

Proteins, lipids, starch, insoluble polysaccharides, and enzymes, peroxidase, succinate dehyrogenase, acid phosphatase, adenosine triphosphatase and non-specific esterases were localized on the stigma at different developmental stages. Proteinaceous substances are present in the

exudate in both C.roseus and W.somnifera. A distinct pellicle is observed in Withania, though it belongs to a wet stigma type pellicle is localized as a distinct layer following staining with 8-Anilino naphthalene sulphonic acid Ca salts at stage III. Lipids are the major component in the exudate in both C.roseus and W.somnifera. The cuticle investing the papillae cell is readily observed following staining with Auromine O after washing off the stigma surface lipids using chloroform/methanol in the ratio 1:1. Insoluble polysaccharides are observed more in the stigma exudate, while starch deposits are more in the style in the stage III and IV.

In C.roseus, peroxidase activity was moderate at stage II and meagre in the later stages. Acid phosphatase activity was intense at stages IV and V, and the activity sites were confined to the tip of the papillae. SDH showed an intense reaction at III, IV and V stages of stigma development. ATPase activity was meagre to moderate in all stages. Non-specific esterase activity was intense at stage III and the stigma became receptive 3-4 days pre-anthesis.

In W.somnifera the papillae showed intense activity for peroxidase at stages II and III. However, the later stages revealed moderate reaction for peroxidase. Acid phosphatase showed intense reaction at IV and V stages. The base of the papillae cells at stages III and IV showed

an intense reaction for SDH. Non-specific esterase/enzyme activity depicted a distinct pellicle overlying the cuticle which was evident at stage III of the stigma development. The exudate in the intercellular spaces also showed esterase activity. ATPase activity was moderate at II, III and IV stages. The metabolic and enzymic levels in the papillae cells are well correlated with the secretory and senscent phases of the stigma.

In both C.roseus and W.somnifera, the anthers are bithecous and tetrasporangiate and dehisce by longitudinal dehiscence. Microsporongium showed typical structure as reported in other dicots with well defined epidermis, endothecium, wall layers and a distinct secretory tapetum, encircling a central mass of sporogenous cells.

The tapetum in C.roseus and W.somnifera are of the secretory type. In C.roseus the tapetal cells are rich in dilated rER, mitochondria, plastids, free ribosomes and dictyosomes. Rough ER and plastids show a accumulation of protein crystalloid and osmiophilic droplets respectively. The protein bodies in association with ER show a regular lattice net work which persist throughout the functioning stages of the tapetal cells. However, in later stages, this lattice net work appearance is lost and the protein is dispersed. The senescing stages reveal Ubisch bodies which are dark in appearance with prominent electron translucent middle regions.

The pollen grains in both the taxa have an electron dense exine and intine. In C. roseus the pollen grain cytoplasm has many starch grains which develop cracks during hydration. In W.somnifera, the exine shows osmiophilic material covering the surface. The pollen tubes in both the taxa show profuse dictyosomes and abundant vesicles, especially at the tip. The plasmalemma is undulated and accommodate many dark bodies depicting uptake of nutrients.

The ovule in C.roseus and W.somnifera are anatropous and unitegmic. In C.roseus, the ovule is borne on parietal placenta, while in W.somnifera, the ovule is borne on axile placenta. Both the species depict a polygonum type of embryosac.

In vitro pollen germination studies were carried out using Brewbaker and Kwack's (1963) medium. In C.roseus, maximum pollen germination and tube growth were observed in a medium which contained 0.02% boric acid 10% sucrose along with all the other nutrients of a standard Brewbaker and Kwack's medium. However, in W.somnifera, 0.03 % boric acid and 20% sucrose were required for maximum pollen germination and tube growth. By using this standard medium, effects of sulphur dioxide and polyamines in vitro were studied.

Pollen grains are found to be highly susceptible to sulphur dioxide in both the taxa. Exposure of pollen grains

dusted on nutrient medium to varying concentration of sulphur dioxide showed that pollen germination and tube growth are totally inhibited at 1.5 ppm and above concentration of SO_2 in both C.roseus and W.somnifera. In vivo experiments, run parallel, revealed that pollen germination and tube growth were less susceptible to sulphur dioxide compared to in vitro experiments. However, a gradual reduction in germination and tube growth was observed with increase in concentration of sulphur dioxide, and the maximum reduction was noted in the case were both the pollen and the pistil were exposed to sulphur dioxide prior to pollination.

Effects of polyamine spermidine and MGBG, an inhibitor of spermidine biosynthesis were studied on in vitro pollen germination. Incorporation of spermidine into the nutrient medium at 10^{-6} and 10^{-5}M concentration stimulated in vitro pollen tube growth in C.roseus and W.somnifera. MGBG at 0.5 x 10-^{3} and 1 x 10^{-3} M concentrations reduced the percentage of germination as well as tube growth and at a concentration of 1.5 x 10^{-3} M, germination was totally inhibited. However, pollen grains incubated in a medium containing 1.5 x 10^{-3} M MGBG, when transferred to a fresh medium with 10^{-5} M spermidine resulted in 80% and 20% germination recovery in C.roseus and W.somnifera respectively. Spermidine, however could not reverse the inhibition of pollen germination and tube growth caused by actinomycin-D

and tube growth in both C.roseus and W.somnifera.

In C.roseus, the pollen grains germinate in the exudate present on the stigma and grow through the interstices of the papillae cells on the lateral side of the dumb-bell shaped stigma. The pollen tube entry into the system is exclusively through the lateral basal side of the stigma, and reach the transmitting tissue of the style for the subsequent growth to reach the embryosac. In W.somnifera, the pollen grains germinate on the stigma and pollen tubes grow along the surface of the papillae cell towards the central depression and enter the stigma through the inter-cellular spaces of the papillae, sub-papillae and the transmitting tissue cells. The pollen tubes which grow in tufts reach the ovary in about 6 hours.

Pollination carried out using cross and self pollen indicated that C.roseus preferred self pollen while W.somnifera, cross pollen. The incompatibility barrier did not exist in the buds, since both self and cross pollen grew well, unaltered in both the species. Maximum pollen germination and unaltered growth were found following self pollination in C.roseus, while in W.somnifera the cross pollination showed maximum germination and growth.

Pollen viability tested at different stogage conditions showed that viability can be prolonged up to 10 days when

kept at -4°C in C.roseus and upto 15 days in W.sommifera Both C.roseus and W.smonifera, pollen lost the viability in 4 days and 10 days at room temperature respectively.