International Journal of Engineering Research & Management Technology
ISSN: 2348-4039
March-2017 Volume 4, Issue-2
Email: editor@ijermt.org www.ijermt.org

RECEPTIVITY OF STIGMA IN HIGHER PLANTS

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ABSTRACT
Reproduction in higher plants is topic that interests plant scientists' world over. The various structural and biochemical changes in stigma make it compatible for pollen to germinate and attain reproductive success. Various biomolecules include carbohydrates, protein, lipids, enzymes, elements like calcium and boron, flavonoids and reactive oxygen species. Various signaling events also occur in a receptive and mature stigma which aid in the identification of pollen. At receptivity, due to pollination, various pollen grains are bombarded on the stigmatic surface. It is only due to these biochemical components the stigma is able to recognize the correct pollen, allow its adhesion hydration and further penetration into the style.

Keywords: Carbohydrates, Glycoproteins, Lipids, Non specific esterase, Pollen, Stigma,

To understand reproduction in higher plants, it is imperative to know the process involved in floral evolution and seed setting. In sexual reproduction of plants, pistil is the female partner in flowering plants, whereas the anthers are the male components. A variation in the position, morphology, positioning and receptivity of stigma determines the reproductive success of plants. In addition, age of the flower, self incompatibility and pollinators may further affect the process of seed setting. Information on the biochemical components of the two sporophytes is still incomplete and scattered. An understanding of the biochemical components on the surface of stigma and pollen is essential in order to understand receptivity leading to pollen-stigma interactions.

Pistil is present in the central position of a flower, accepts the pollen and is composed of one or more fused carpel bearing the ovules. At the time of ovary closure the walls of carpel extend vertically to form the style. As the style elongates, the inner cells differentiate to form a specialized secretary zone, forming the stigma (Sanchez et al., 2004). At maturity a fully developed pistil consists of stigma, style and ovary. The cells of stigma encounter various cell to cell communications between the female sporophyte and the male sporophyte (pollen). Receptivity is defined as the ability of the stigma to capture pollen by adhesion, to let it hydrate and consequently germinate forming a pollen tube. (Sanchez et al., 2004). Stigma surface in angiosperms has been classified in dry and wet types based on the presence or absence of secretions at maturity. The surface cells of stigma are most commonly elongated as papillae. It is defined as a soft superficial gland or protuberance which may be unicellular or multicellular; uniseriate or multiseriate. (Heslop-Harrison, 1981). The surface of stigma contains extracellular components at maturity (Mattsson et al., 1974, Heslop-Harrison, 1977). The stigma surface contains heterogeneous extracelluar components which include, lipids, proteins, glycoproteins, carbohydrates, and phenols (Shivanna, 2003).

In dry stigma a pellicle is present, in which extracellular components are present in the form a a thin extracuticular hydrated layer. The pellicle originates from the epidermal cells of stigma and the components of pellicle are extruded onto the surface by the discontinuities present in the cuticle. The dry stigmas can be subdivided into two categories (Group I and II). In group I plumose form of stigma is present where the receptive cells are dispersed on multiseriate branches (*Dactylis glomerata*). In group II the receptive surface is concentrated in distinct ridges, zones or heads.Wet stigmas have a distinct surface secretion which may be

confined to the interstices of papillae or may flood the entire surface of stigma. The exudate may be lipoidal as in *Petunia* (Solanaceae) and *Oenothera* or aqueous carbohydrate rich as in Lilium (Liliaceae) are required for correct pollen hydration, germination and penetration of stigma by pollen tubes. (Hiscock et al., 2003) Two main categories (Group III and IV) of the wet sigma may be distinguished. In Group III are the stigmas that bear a receptive surface of medium to low length papillae or with marginal fringe of long papillae (eg. Orchids). Group IV where the receptive surface is non papillate and the secretory cells are flat or slightly bullate. In some cases the stigma may be dry at the time of anthesis but the secretions may follow later. In *Prunus dulcis*(Rosaceae) and *Malus* the surface may be wet at anthesis but the main secretions may follow later, and the base of papillae show internal ramifications of the transfer cell type,as found in the secretory tissue. The rupture of cuticle layer is associated with exudate production from the epidermal cells that accumulates in the intercellular spaces of stigmatic tissue below the cuticle of epidermal cells (Yi et al., 2006). In some tree crops like cherry and peach, surface cells of wet stigma may be disrupted with secretion and may degenerate. Recently, a semi-dry type of stigma has been characterized among the members of the family Asteraceae. They have characters, intermediate between wet and dry type of stigma. Like the dry stigma, they possess a surface cuticle which however, is not continuous at the base of the papillae and like the wet stigma at maturity, they possess a small amount of secretions containing lipid, carbohydrate and proteins (Hiscock et al., 2002, Sharma and Bhatla, 2013).

Stigma is the site of reception of pollen grains. The pollen grain adheres, hydrate and then germinate on the stigma. Dry stigmas are considered to be evolutionary more advanced than the wet stigmas. This evolution has also caused changes in pollen which enable them to adhere to the stigmas, extract water and penetrate into the surface of stigma. (Dickinson,1995). The extracellular components in a dry stigma are present in form of extracuticular hydrated layer pellicle (Mattsson, 1974). The pellicle components, (glyco) proteins, carbohydrates, enzymes (esterase, acid phosphatase, ATPase) and lipids (Graaf et al., 2001) originate from the stigmatic papillae, through discontinuities in cuticle. Stigma harbors pollen tube growth regulating substances (Ramasubbu et al., 2010). A number of studies have revealed an increase in sugar content in a mature stigma. An increase in the sugar content has also been noticed in apple and pear at anthesis (Pusey et al., 2008) In *Moringa oleifera*, the receptive stage of stigma showed a higher carbohydrate content of 11.8% than the non receptive stage 10.91% (Bhattacharya and Mandal, 2004). Carbohydrates have an important role in plant nutrition and metabolism. Glycoproteins present in the stigma surface act as signal directing growth of pollen tube towards the ovary. The transmitting tissue of stigma is rich in carbohydrates and serves as the nutrient media for the growth of the pollen tube. Pectins are structural hetropolysaccharides contained in the primary wall of cells. The characteristic structure of pectin is a linear chain of α 1-4 linked D galacturonic acid that forms pectin backbone, a homogalacturonan. Into this backbone there are regions where galacturonic acid is replaced by (1-2) L- rhamnose, forming rhamnogalacturonan. High and low methyl esterified homogalacturonans are common component of transmitting tissue. Homogalactronans are synthesized and secreted as highly methyl esterifed form by golgi bodies. High methyl esterification prevents calcium binding making the cell walls less rigid. The fluidity of the pectin gel also increases, allowing the cells to expand, while maintaining the intregretiy of their structure due to hydrophilic properties of pectin (Silverio and Mariath, 2010). High methyl estrified homogalacturonans are desterified within the cell wall by pectin methylesterase forming low ethyl esterifed homogalacturonans. Low methy esterifed homogalacturonans act as calcium reservoir to be released following pollination induced degradation of homogalaturonans (Hirstova et al., 2005). The stigmas also contain a number of proteins which play an important role in pollen germination, pollen tube entry into the stigma and incompatibility response. Papillae cells stain intensely for proteins, which correspond to the S locus proteins, related to self incompatibility. S locus glycoprotein (SLG) is secreted in the stigmatic wall (Kandasamy et al., 1989). S receptor kinase which phosphorylates serine/threonine residues is situated in plasma membrane of papillae showing an early self incompatible response of self pollen (Dickinson, 1995). In dry stigma like Arabidopsis and Brassica, a multivesicular body on stigmatic papillae where the compatible pollen adheres has been reported (Dantu et al., 2016). Other protein like Exo70A1 (Safavian and

Goring, 2013) and arabinogalactan proteins and extensions have been reported to play a role in pollen recognition and pollen tube elongation (Losada and Herrero, 2014). Cysteine rich peptides, have an N-terminal signal peptide and a divergent charged peptide with conserved cysteine residue (Higashiyama, 2010). Lipid transfer protein (LTP) having eight cysteine residues in conserved positions have been localized in the apoplast and proposed to be a group of multifunctional signaling molecule that bind receptors in the plasma membrane (Zhao et a., 2004). SCA (Stigma/stylar cystein rich adhesion) in lily, an LTP has three isoforms, and all show pollen tube adhesion activity. SCA increases the chemorophic activity of chemocyanin, a secreted peptide, is present in stigma and style of lily. SCA binds to pollen tube and may facilitate access of chemocyanin to pollen tubes (Higashiyama, 2010). There are few reports on the lipid composition of stigma. Most of the investigations have been carried out from stigmatic exudates (Cresti et al., 1986) or from wet stigmas (Dumas, 1977). Lipids present in the wet stigma are important for pollen hydration and pollen tube guidance by forming a water gradient between the hydrophobic and hydrophilic phases of extracellular matrix. In *Nicotiana*stigmaless pistils, lacking essential lipids were not able to support pollen germination, proving that lipids are essential for pollen tube to penetrate stigma. During the course of evolution of dry stigma from wet stigma, the role of lipids has been transferred to pollen grain extracellular matrix (Sage et al., 2009). Pollen grain contain a lipid and protein rich pollen coat with CER6 required for biosynthesis of long chain fatty acids, and CER1 required for conversion of long chain aldehydes to alkanes (Welter-Arts et al., 1998). GRP17 and EXL4 genes also help in hydration of pollen (Mayfield and Preuss, 2000). Pollen coat proteins include profilins, caleosin, Zea m2, β-expansin-10, exopolygalacturonase, Rho GDP-dissociation inhibitor-1, Ras-related protein Rab-2-A and putative subtilase (Dantu et al., 2016) Recent studies indicate that flavonoids are also important for pollen germination. Flavonoids provide colour, fragrance taste to flowers and seed thereby attracting insects to aid in pollen transmission (Mierziak et al., 2014)

Presence of esterase/peroxidases on the stigma surface has been often considered to indicate receptivity (Heslop Harrison 1977, McInnis et al., 2006). However activity of these enzymes does not confirm the receptivity of the stigma as in many stigmas, the enzyme may appear before the stigma is able to support pollen germination (Shivanna and Rangaswamy, 1992). Receptivity is defined as the ability of the stigma to capture pollen by adhesion, to let it hydrate and consequently germinate forming a pollen tube. (Sanchez et al.,2004). To enable successful germination of pollen a number of biomolecules and minerals (Onus, 2000; Sharma and Bhatla, 2014) are required which are involved in species recognition, pollen adhesion, pollen hydration, self incompatibility, pollen tube growth and pathogen defense (Allen et al., 2010). Non specific esterases (NE) (EC 3.1.1) include a group of enzymes that hydrolyze esteric bonds. It includes carboxylesterase, arylesterase and acetyl esterase. The profile of proteins isolated from Hibiscus, Gladiolus and Brassica show esterase isoforms as the major proteins (Heslop-Harrison, 1977) In *Brassica napus*, seven serine esterase (30-50kDa) have been identified which are required for pollen tube penetration in the stigma (Hiscock et al., 2002) Non specific esterase have the ability to hydrolyse cross-bonds of cell wall polysaccharides and are therefore important in the establishment and reorganization of cell walls. They play a role in host-pathogen interaction and in metabolism of fatty acids (Bikovà et al 2009). Secreted class III Perxoidase (EC 1.11.1.7) causes the breakdown hydrogen peroxide to form highly oxidizing intermediate which oxidize a variety of organic and inorganic reducing substrates in mature stigma. (McInnis et al., 2006).Peroxidases playrole in oxidative stress response, lignifications, cross linking of cell wall components and defense against pathogen attack. Class III peroxidases can also lead to the formation of highly reactive oxygen species (ROS), (Cosio and Dunand, 2009) which may play a role in signal transduction pathways leading to pollen germination/pollen tube growth (McInnis et al., 2006). Peroxidase has been detected in the stigma extract of many species. Receptivity of stigma is reflected by the presence of this enzyme (Galen et al., 1985). Recently, in stigmas of *Senecio squalidus* (Asteraceae) five peroxidase isoforms have been localized out which one is a stigma specific peroxidase (SSP). ATPase are also present in the stigmatic papillae which help in the recognition, hydration and germination of pollen. Recent studies suggest that

ACA13 (Auto-inhibited Ca^{2+} ATPase 13) helps in the export of Ca^{2+} to compatible pollen thereby aiding in successful fertilization (Dantu et al., 2016).

A receptive stigma undergoes various structural and biochemical changes. These alterations are essential for the stigma to attain reproductive success. As the stigma matures, it has to identify the correct pollen allow its adhesion, hydration and provide nutrition to the growing pollen tube. These events are associated with signaling processes in the form of self-incompatibility, calcium signaling and reactive oxygen species. An accumulation of complex biomolecules and signaling events indicate that pollen-stigma interaction is a highly complex association between the two sporophytes.

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