Light and Scanning Electron Microscopic Studies and Energy Dispersive X- Ray Analysis (EDAX) of Stigma in Sunflower

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Abstract

Stigma is an important component of female reproductive part in flowering plants. Stigma composition and elemental analysis of stigma was carried out using light microscopy, scanning electron microscopy and Energy Dispersive X- ray analysis (EDAX). Light microscopy and SEM studies reveal special characteristic features of stigma that are required for making it an effective platform for the landing of pollen. The chemical composition of stigma is enriched with potassium which makes the stigmatic cells unique and increases the volume for proper capturing of the pollen.

Keywords: Stigma, Pollen, SEM, EDAX, Flowering.

Introduction

Stigma is an important component of flowering plants and has a critical role in pollen stigma interaction. It is the site of reception of pollen grains and it causes cell to cell communication for successful pollination. The critical signaling events in the stigmatic cell and the pollen have a role in pollen-stigma adhesion, recognition, hydration and germination of the pollen grain. The stigma in angiosperms can be described as wet or dry depending on the presence of exudate in stigma (Heslop-Harrison and Shivanna, 1977; Hiscock and Allen, 2008). It has been revealed that the exudates present on the wet stigma is composed of various proteins, lipids, carbohydrates phenols, glycoprotein dissolved ion and various enzymes like esterase and peroxidase (McInnis et al., 2005; Shivanna, 2003). The exudate thus helps the pollen grain in adhesion, provide the pollen with nourishment allow the germination of the pollen. In dry stigmas, however the sigmatic exudates are absent and the epidermal cells are the key players in marking the adhesion and the hydration of pollen. They have a role in species specific adhesion coupled with species specific pollen stigma interaction (Ferrari et al., 1985). Epidermal cells in stigma are covered with a continuous layer of cuticle and proteinaceous pellicle (Mattsson et al., 1974). The pellicle is known to have various enzymes required for pollination. Investigations have revealed the presence of peroxidases, esterases, glycoproteins and flavonoids which have a role in pollen recognition, adhesion hydration and germination (Stead et al., 1980).

Pollen adhesion has been studied in Arabidopsis and Brasssica (Zinkl et al., 1999). It has been demonstrated that adhesion is a physical process which involves van-der-Waal forces between the two reproductive structures, pollen and the stigmatic papillae. In Brassica, the adhesion of pollen is dependent on PCP (pollen coat proteins) and S gene proteins on the stigmatic papillae, S gene proteins, SLG (S locus glycoprotein) and SLR-1 (S locus related-1) ; (Luu et al.,1999). Pollen hydration is dependent on constituents of stigmatic pellicle and pollen coat constituents which diffuse on the stigmatic surface and form a meniscus (Zinkl and Preuss, 2000). In members of Brassicaceae, pollen hydration is regulated by glycine-rich oleosin domain protein (GRP 17) and extracellular lipases, on pollen surface and aquaporins present on the stigmatic papillae (Losada and Herrero, 2012). Pollen hydration is faster in compatible pollen as compared to incompatible pollen. An interaction of serine esterase, lipids and signaling molecules present on the pollen and stigma cause the breakdown of cuticle, germination of the pollen grain and further growth of the pollen tube. Recent studies have revealed similarities in the pattern of self-incompatibility in Brassicaceae and Asteraceae (Wolters-Arts et al., 2002; Vithanage and Knox, 1977).

Mature stigma also contains a critical balance of all other biomolecules required for the development of the pollen. This includes carbohydrates, proteins, minerals and other signaling molecules. There has been evidence of nitric oxide (NO), reactive oxygen speices (ROS), cysteine-rich peptides and gamma-aminobutyric acid (GABA) (McInnis et al. 2006 a,b; Hiscock and Allen 2008; Sharma and Bhatla 2013a,b). Besides these, stigma also contains certain elements which act as signaling cues for the pollen germination and tube growth or are taken up by the pollen. Calcium potassium and boron have been known to play as key role in the germination and growth of pollen tube. Potassium acts as an osmotic regulator hence plays a role in maintaining the shape of stigmatic papillae, and has a role in the hydration of the pollen grains (Rehman and Yun 2006). Calcium acts as a signaling molecule and is present in bound to the pectins present in the cell wall of stigmatic cells. It is believed to be released, on pollination, from the different intercellular storage regions. Boron is also an essential micronutrient required for pollen germination and tube growth. It facilitates sugar uptake and helps in the production of pectins in pollen tube (Acar et al. 2010).

Material and Methods

Seeds of *Helianthus annuus* L. var KBSH were obtained from University of Agricultural Sciences, Bangluru, Karnataka, India. The seeds were washed and imbibed in water for 4 hours. The seeds were then grown in the botanical garden of Multanimal Modi College, Modinagar, Ghaziabad (U.P) India. The plants were raised till the bore flowers. When the capitulum reached anthesis and about 30% of the head was at flowering state, the flowers were excised ad used for experimental work

Stigmas of pollinated mature (pistillate) stigmas were fixed in a mixture of 2.5% glutaraldehyde and 2% paraformaldehyde prepared in 0.2M sodium cacodylate buffer (pH 7.4) for 4-5 h at room temperature, and then transferred to buffer solution. The material was then subjected to dehydration, according to Feder and O’Brien (1968). Briefly the fixed tissue samples were subjected to methoxy ethanol, ethanol and n-propanol for 24 h each, followed by n-butanol for 12 h. Glycol methacrylate (2-hydroxyethyl methacrylate) was used as embedding medium (Feder and O’Brien, 1968). Sections (2-3 µm) were obtained using a rotary microtome.

Cross sections (2-3 µm) of stigma were treated with 0.2% coomassie brilliant blue R-250 dissolved in a mixture of methanol: acetic acid: water (5:7:88) for five min. They were then treated with the same solvent mixture (minus stain) for five min to remove excess stain. The slides were rinsed with water, dried and mounted in DPX.

For scanning electron microscopic (SEM) imaging of stigmas, the samples were mounted on SEM stubs using double-sided tape and coated with gold layer using ion sputter (Emitech sputter coater) and observed using scanning electron microscope (Model: Zeiss EM EVO MA 15).EDAX analysis was carried out on Scanning electron microscope (SEM) fitted with an EDS 3D analysis with 3D software. (Make: JEOL Japan Mode: JSM 6610LV).

Results and Discussion

Development of capitulum in Sunflower

The inflorescence in the member of Asteraceae is a capitulum, which consists of disc florets and ray florets. The outer whorls of florets are the ray florets which are zygomorphic and neuter (Fig. 1A).

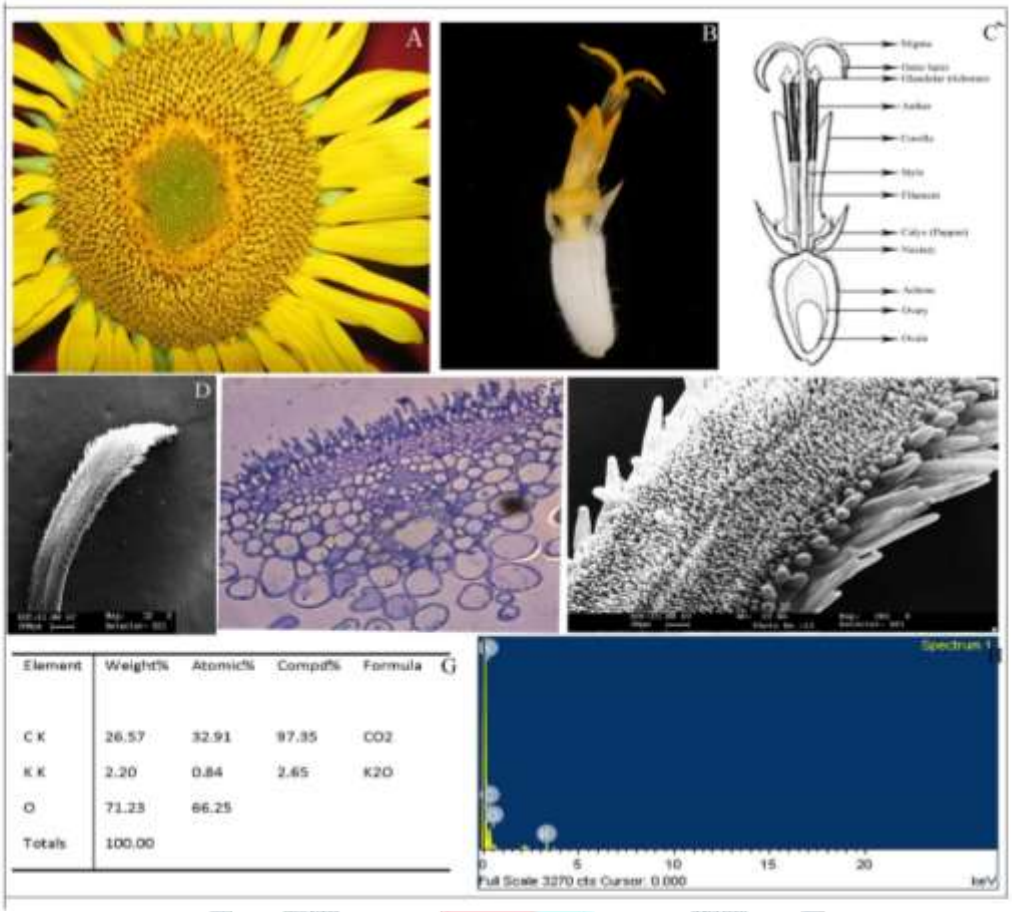

Fig. 1 A. Capitulum- Inflorescence of sunflower, B. Pistillate stage of disc floret in sunflower, C. Diagrammatic representation of the disc floret in sunflower. D. SEM image of the stigma of the mature disc floret (32X). E. Transverse section of stigma of sunflower, showing the papillae cells and the transmitting tissue (Magnification 100X). F. SEM image of the stigma (200X) showing the papillae cells enclosed by the pseudopapillae. G. Elements as detected by EDX in the stigma of sunflower. H. EDX spectra of the stigma of sunflower showing showing strong signals for K.

They have a long strap like fused petals on the outer side of the inflorescence. The ray florets serve to attract various pollinators, and also reflect the photosynthetically active radiations. The inner disc florets are arranged in a spiral manner. The disc florets are hermaphrodite and show mature in a centripetal manner. Thus, the duration of anthesis is elongated in the capitulum of sunflower, as anthesis is observed in successive whorls of capitulum. It has been reported that the anthesis is also in the influence of phytochromes and plant hormones, mainly auxins. Sunflower exhibits secondary pollen presentation, whereby the pollen grains are relocated from the anthers to other floral organs. The elongation of anther filament and the style is under the influence of phytochromes. The anther filaments elongate first and then the style which is in the middle elongates through the anther tube, pushing the pollen grains out. In the process, pollen grains become attached on the outer side of the stigma. The stigma then detaches medially, and the tips curl outwardly (Fig.1 B).

Scanning electron microscopy reveals the presence of papillae and pseudopapillae

Scanning electron microscope images reveal bifid stigma present on maturity. The stigma has two types of cells on its surface. In the middle region of the stigma, a median margin is observed which is broad towards the forked region. In the outer side are brush/bristle like pseduopapillae, and inner thin finger like projection called papillae. The finger like papillae is arranged in about forty rows, and aid in capturing pollen grains (Fig. 1D). The bristle like pseudopapillae act as secondary pollen presenter and the pollen grains that are left in the anther sac after the dehiscence are exposed to the pollinators. These pollen grains attach on the body of insects while they are visiting the stigma while collecting nectar (Fig. 1C). It has been reported that in Asteracae, pump and brush mechanism operate which aids the secondary pollen presentation in Sunflower (Erbar and Leins, 2015)

Light microscopic studies coincide with scanning electron microscopic images

Wet stigmas show the presence of stigmatic secretions at maturity, whereas dry types of stigmas are covered with a porteinaceous layer, called pellicle. Thin sections reveal the presence of receptive surface bearing finger like papillae (Fig. 1E). The papillae are coated with pellicle layer. Pellicle also contains proteins for S locus proteins related to self-incompatibility. S locus glycoprotein (SLG) is secreted in the cell walls of the stigmatic tissues (Dickinson, 2000). The protein also may correspond to arabinogalacton protein. Arabinogalactan proteins belong to a family of hydroxyproline-rich glycoproteins having diverse roles (Showalter, 2001).They are involved in gametophytic cell differentiation (Coimbra et al., 2007), cell signaling pathways (Ellis et al., 2010) and adhesion (Losada and Herrero, 2012).

EDAX (Energy Dispersive X-ray Analysis) studies on stigma

EDAX studies were carried out on the mature stigma of sunflower. It found out that the major elements that were present on the stigma were carbon, potassium and oxygen. Carbon and oxygen correspond to the organic compounds that are present of stigma. It was expected that potassium, calcium and boron would also be present on the stigmatic surface. However, only potassium was detected revealing that this element was present in majority. Calcium and boron are present in bound form. Potassium regulates several important events during anther dehiscence, pollen germination and papillae hydration, leading to successful pollination and pollen germination (Rehman and Yun, 2006). Stigma surface is the receptive area which receives the pollen grains. The pollen grains hydrate and germinate on contact with stigma. In grasses, potassium has been reported to cause stigma turgidity which aids in pollen capture and hydration (Heslop-Harrison and Reger, 1986). In the stigmatic papillae of *Hordeum vulgare*, the concentration of potassium increases with stigma maturity (Rehman and Yun, 2006). Present investigations on sunflower have revealed a similar trend in the accumulation of potassium in the stigmatic tissue. It has been reported that ROS signaling activates the uptake of potassium in plants (Ashley et al., 2006). Higher content of potassium at the pistillate stage of stigma (present work) suggests its role as osmoticum, thereby maintaining the turgidity of papillae, as has also been suggested in grasses and *Hordeum vulgare* (Rehman and Yun, 2006). A higher level of potassium in the mature papillae also suggests that hydration of pollen is caused by the hydration of the papillae (Rehman and Yun, 2006).

Conclusion

Microscopic studies are important to study the structure and the associated function of the plant component. The stigma is important as it functions as the landing platform for the pollen. The stigma recognizes the pollen (self/cross) and also provides it nutrients for the growth of the pollen tube. The stigma is associated with various molecules which act as signaling molecules which help in the germination of pollen. Further studies on pollen germination and signaling molecule cascade will aid in the studies of pollen-stigma interaction in sunflower.

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