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Stigma characteristics of *Abelmochus esculentus* (L.) Moench (Malvaceae)

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ABSTRACT

Angiosperm stigmata are unique in their structure are therefore very diverse in structurally. Okra, *Abelmoschus esculentus* (L.) Moench is self-pollinated crop with large, attractive hermaphrodite flowers. The morphology of stigma is described using scanning electron microscope (SEM) in order to extent current knowledge of stigmatic structure which may help to breeders working on genetic improvement program of okra.

Keywords: *Abelmoschus esculentus*, Malvaceae, Okra, papillae, stigma.

INTRODUCTION

On the account of reproductive parts of plant, flower and buds are composed of various reproductive organs. Among them pistil is the most vital organ of flower since it provides the fertilization ground by containing the egg deeply seated within its ovary. Also it protects the embryo which resulted by the successful fertilization. Pistil has three main parts, varied in nature and morphology- the uppermost stigma; long and flexible- the middle style and deeply seated egg within- the basal ovary. These parts, though morphologically different, might play the important role in receiving the pollen grain selectively and helping its growth and allowing male gametes to make successful fertilization. Among them, stigma is the most vital organ since it play crucial role in pollen attachment, following by pollen hydration, pollen germination, pollen tube elongation and its guidance to ovule.

The interesting floral structure which influence pollination and mating systems has always been intrigued evolutionary botanists. The Malvaceae contain herbs, shrubs or less often trees and usually have hermaphrodite and entomophilous flowers. The cultivated okra, *Abelmoschus esculentus* (L.) Moench., is one of the important multipurpose cultivated species of Malvaceae grown throughout tropical and sub-tropical low altitude regions of Asia and Africa. It is popularly known as '*bhindi*' in India and easy to cultivate and is adapted to varied ecological conditions. Being cultivated for its immature, edible fruits, are rich in valuable nutrients including calcium, potassium, carbohydrate and vitamin C (Patil *et al.,* 2015). Fruits are low in calories, are fat-free and provide a valuable supplementary food in the tropical diet. In Indian cooking, it is sauted or added to gravy-based preparations and is very popular in South India. The leaves are also occasionally eaten raw in salads.

In *A. esculentus*, pollen-pistil interaction includes the initial contacts between the male pollen and female stigma which is specific and next to the selective binding of appropriate pollen by the stigma, rapid pollen-recognition, pollen hydration, and pollen tube formation (Gregory and Daphne, 2000). In hybridization between diverse species of the same genus this pathway is often restricted by some barrier factors at stigma level. Therefore, knowledge on structural characteristics of stigma is very important to address the fertilization barriers in okra breeding program. Dissecting microscope or hand lens are often used to observe the stigma surface morphology with respect to its type and biochemical nature. Besides this, with the continuous development in microscopy, scanning electron microscopy (SEM) has been found to be extremely valuable for observing and recording stigmatic characteristics because of its potential to provide high resolution, large depth of field and ability to examine untreated and treated stigmata. Since there are no reports of this type of study involving SEM in *A. esculentus*, the objective of this work was to observe structural characteristics of stigma.

MATERIALS AND METHODS:

The stigmatic features of unpollinated flowers at different stages were assessed by SEM. Fresh stigmas were collected and fixed in 2.5% glutaraldehyde in 0.1M phosphate buffer (pH 7.3) followed by ascending series of alcohol and were transferred to 100% hexamethyldisilazane (HMDS) through a graded series of ethanol-HMDS mixtures then exchange with new 100% HMDS one final time and dried at room temperature (He-min *et al*., 1990). Samples were fixed on the brass specimen stubs with a double adhesive carbon tape and gold coated using ion sputter (JEOL JFC-1100). Each sample was uniformly coated with 20-30 nm thick gold film. Digital images were taken under Scanning Electron Microscope (JEOL JSM-840A) at accelerating voltage of 10Kv. Observations were also made on anther opening, pollen adhesion to stigma papillae and pollen tube penetration to stigma in various samples.

RESULTS AND DISCUSSION

At present, breeding programs of genetic improvement of Okra are primarily focused on obtaining new varieties that are resistant to biotic and abiotic factors which fulfill the demands of domestic and external markets (Patil *et al*., 2013). A number of wild relatives of okra such as *A. moschatus, A. manihot* subsp. *tetraphyllus, A. manihot* subsp. *tetraphyllus* var. *pungens, A. tuberculatus, A. ficulneus, A. angulosus* and *A. crinitus* have been identified as a potential source of resistance for *Fusarium* wilt, *Alternaria* blight, powdery mildew and YVMV as well as abiotic stresses (Sandhu *et al*., 1974; Arumugam *et al*., 1975). Though the procedure of emasculation and cross-pollination is less cumbersome in okra cultivated genepool, it is more difficult to cross *A. esculentus* with its wild species. Understanding the morphological characteristics of stigma is very important to overcome the pre-zygotic barriers in controlled pollinations. Besides this, studies of aspects further improve understanding of the compatibility between pollen grains and stigmas which influencing success rate of crossing program.

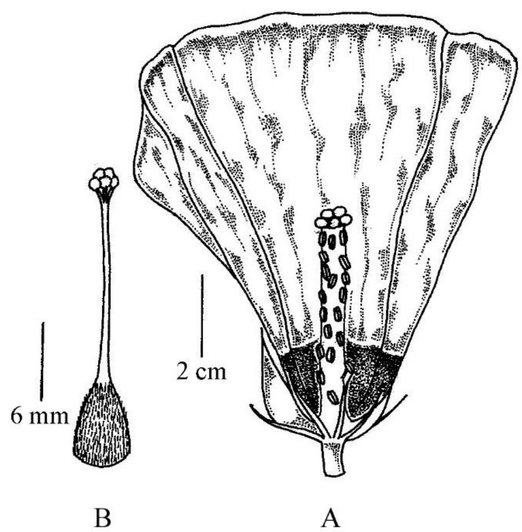

Figure 1. Schematic diagram of *Abelmoschus esculentus* flower showing pistil structure with respect to the stigma and style.

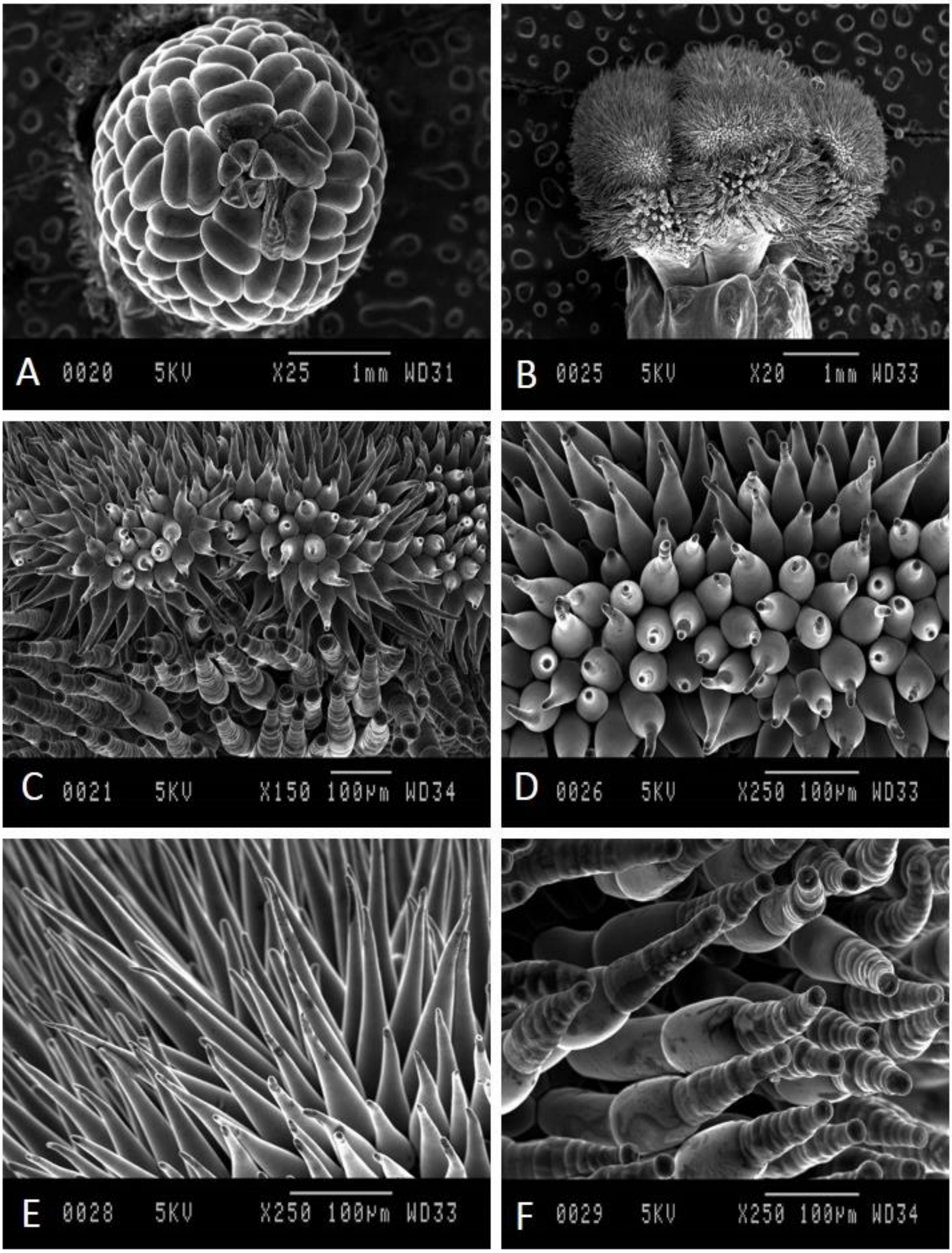

Figure 2. SEM micrographs of *A. esculentus* stigma showing: A) arrangement of anthers; B) stigma lobes; C to F) close view of stigmatic papillae.

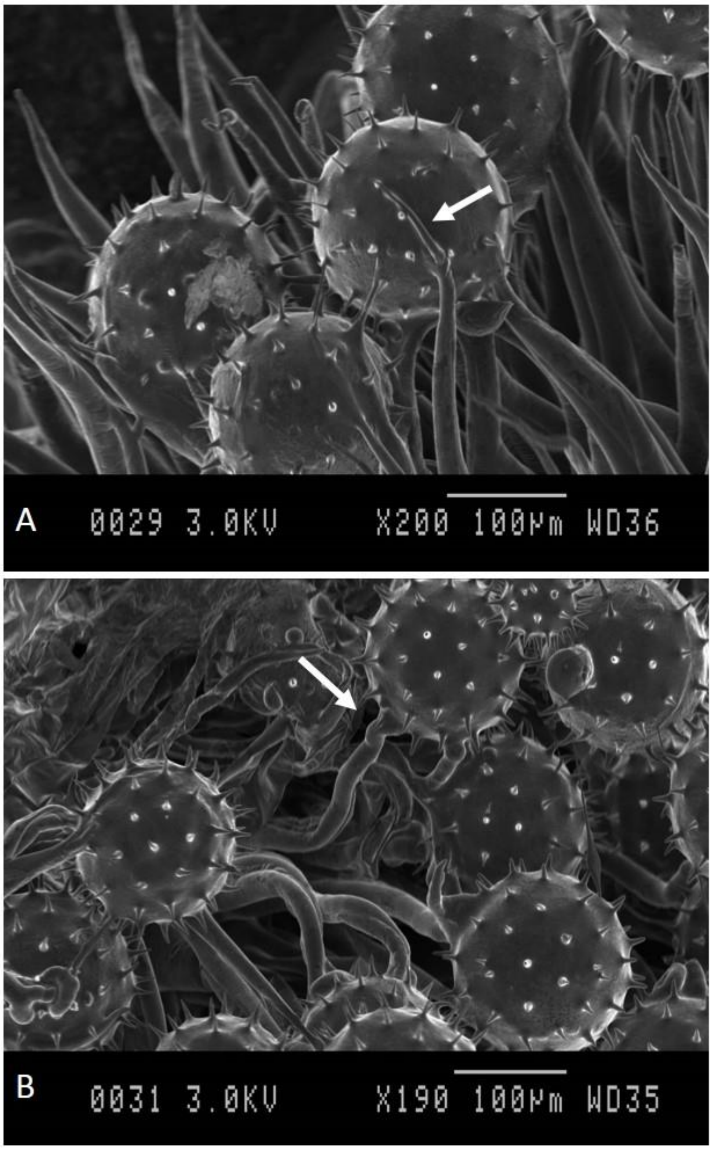

Figure 3. SEM micrograph showing pollen behavior on the stigma surface. A) Pollen attachment to the stigmatic papillae; B) germinated pollen grain.

Stigma morphology is variable and has been conveniently classified by Heslop – Harrison and Shivanna (1977) covering around 900 genera belonging to 250 families. In this paper we describe morphological characters of the stigma were studied using SEM. Style is terminal, elongated and one. Staminal tube is conical. Antheriferous filaments are sparsely arranged and are confined to upper half of the staminal tube (Figure 1). Stigma is sessile. Number of stigma is 5 and closely arranged (Figure 2A). On the basis of classification stigma of *A. esculentus* falls into wet type with densely arranged unicellular papillae (Figure 2C, 2D and 2E). This type stigma comes under Group III as classified by Hesslop- Harrison and Shivanna (1977) which has been reported in many plants (Cresti *et al*., 1982; Owens *et al*., 1982; Wetzstein and Law, 2012; Koshy *et al*., 2013). During the time of anthesis, the sticky nature of stigmatic surface makes papillae more visible. These papillae are considered to be responsible for the secretion on the stigma surface which taking active part in pollen adhesion (Figure 2F). These papillae shown to assist in the rehydration of pollen and promotes germination of pollen tube.

The results obtained demonstrate that primary knowledge of stigma provide useful information to study the stigma receptivity on pre-anthesis and postanthesis level by chemical assay (α-naphthyl-acetate, hydrogen peroxide, etc). Also, present study also helpful for planning and execution of breeding programs for genetic improvement of okra, by identifying incompatibility barriers. There is need to expand this study by investing stigma characteristics of other species of *Abelmoschus* as well as other aspects of reproductive biology with respect to the morpho-anatomy, biochemical studies of stigma. Additionally, and not less important this study can enhance efforts in taxonomic and phylogenetic studies.

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Conflicts of Interest: The authors declare no conflict of interest.

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