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Morphology, developmental ultrastructure and ultracytochemistry of staminal hairs in *Bulbine inflata* (Asphodelaceae) in relation to function

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ABSTRACT

Results of light and electron microscopy and preliminary ultracytochemical studies of the staminal hairs of *Bulbine inflata* at different stages of development are reported here. The staminal filaments are covered with yellow, unicellular, linear, erecto-patent hairs. These staminal hairs arise directly as single cell outgrowths from epidermal cells of the filament. The surface of each hair is patterned with helical wall thickenings in an anticlockwise direction. This wall is covered by a thick folded cuticle, and formed of a loosely fibrillar cellulose layer. The hair cell possesses a cytoplasm rich in organelles. Especially ribosomes are abundant. Plastids contain large starch grains and peripheral lipid droplets. The smooth endoplasmic reticulum cisternae (SER) encircle the plastids and mitochondria; it is extended in the cytoplasm along the hair length. These hairs have functions in flower pollination attracting pollinators visually, secreting specific substances, providing increased surface area, protecting the filaments and being involved in their movement and vibration.

Introduction

The family Asphodelaceae contains 15 genera and about 750 species distributed in the Old World especially in Southern Africa (Judd et al., 2006; Kubitzki and Huber, 1998). The genus *Bulbine* Wolf is one of the four major genera of the family Asphodelaceae including ca. 80 species, most of which are indigenous to Southern Africa, with about six species occurring in Australia. The flowers of this genus have yellow, rarely orange or white tepals, and are easily distinguished by their hairy filaments (Eggli, 2001; Judd et al., 2006; Kubitzki and Huber, 1998).

Dahlgren and Clifford (1982) and Dahlgren et al. (1985) conducted a broad survey of staminal hairs in the monocotyledons, and the taxonomic value of these structures is well established. Following earlier taxonomic studies of the genus *Bulbine* (Baijnath, 1977a,b; Ramdhani, 2002; Williamson and Baijnath, 1995, 1999), it was found that staminal hairs could provide characters for diagnostic purposes also in this genus. Although not used as key characters in *Bulbine* species, the following features of staminal hairs could be useful: length, shape, orientation of the apex and position of hairs on filaments. Most of the *Bulbine* species are characterized by clavate hairs, some display linear hairs, and rarely a mixture of both hair types are present. Hair orientation may be patent, erecto-patent or erect.

Pollination-related hairs may be present on all floral whorls, but they occur mainly on the androecium. Plant inflorescences and associated structures may attract pollinators visually by a showy corolla or calyx, while the androecium is attractive because it produces pollen (Faden, 1992). Faden (1992) reported also that yellow staminal hairs in Commelinaceae may deceptively attract insects and floral odors may also attract pollinators. Staminal hairs were also observed to play a role in pollination in *Osyris alba* (Santalaceae) where male flowers produce pollen, nectar and staminal hairs as a reward for pollinators (Aronne et al., 1993).

Recently, a study done by Vaughton et al. (2008) showed that the staminal hairs played a significant role in pollination of *Bulbine vagans* flowers. This was done by an emasculation and pollination experiment where the anthers and clavate hairs were left intact (control), the anthers were removed but the hairs left intact (emasculated), and both the anthers and the hairs were removed (hairless). Pollination of manipulated plants was shown to have been reduced by 50% clearly indicating a role in attracting pollinators.

Staminal hairs occur in different taxa and are known to occur in the genus *Bulbine* (Asphodelaceae). Very little information exists on details of the ultrastructure of staminal hairs in *Bulbine* species. Therefore, the present study is focused on the morphology, developmental ultrastructure and ultracytochemistry of the staminal

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hairs of *B. inflata*. Samples were taken from flowers at different stages of development to get information about their possible reproductive functions.

Materials and methods

Plant material

Bulbine inflata Oberm. was collected and identified from the Reservoir Hills area, Durban, South Africa (29°37′S, 30°23′E). A voucher specimen (Baijnath et al. sn.) is deposited in the Ward Herbarium of the University of KwaZulu-Natal, Westville Campus. This species is distributed in South Africa and Swaziland. It flowers during spring and summer. Numerous flowers are borne in the racemose inflorescence. Flower pedicels are up to 15 mm long and tepals are ca. 10 mm long and 3 mm wide. Flowers open only for a single day. The likely visitors are pollen collecting bees and pollen appears to be the only reward.

Stereomicroscopy (LM)

Opened buds of different sizes, corresponding to six stages of development, were measured and photographed using the Nikon AZ100 model AZ-LED Stereomicroscope with a Nikon Fiber Illuminator (Nikon, Japan).

Electron microscopy

For SEM microscopy, buds from flowers of *Bulbine inflata* were removed from the inflorescence just before midday. The smallest buds were processed in total, while larger buds were dissected and the stamens removed for processing. The stamens or whole buds were fixed for 2 h in 2.5% buffered glutaraldehyde in 0.05 M Na cacodylate buffer (pH 7.0) with a few drops of Triton X100 to facilitate wetting of the staminal hairs. The tissue was dehydrated in ethanol and critical-point dried. It was fixed onto brass stubs with double-sided adhesive tape and coated with gold for 3 min at 20 kV. Samples were viewed using a Philips SEM 500 at 12 kV.

For transmission electron microscopy (TEM), stamens were fixed for 2 h in 2.5% glutaraldehyde, post fixed in 1% osmium tetroxide in 0.05 M Na cacodylate buffer for 2 h, washed in water, stained in 2% aqueous uranyl acetate for 1 h, dehydrated in ethanol, briefly immersed in propylene oxide and embedded in epoxy resin (Spurr, 1969). Sections were stained in lead citrate (Reynolds, 1963) for 2 min and viewed using a Philips TEM 301 at 80 kV.

Ultracytochemistry

Ultrastructural cytochemistry was employed to elucidate specific and defined chemical reactions at the level of organelles and other cellular components. Four cytochemical tests were used namely ammoniacal silver hydroxylamine for ester linkages, detecting lipids, (Lawton, 1986) and silver proteinate for aldehyde localization, detecting polysaccharides such as cellulose (Thiéry, 1967). The other two stains for the enhancement of contrast in cytoplasmic membranes were zinc iodide osmium (Gilloteaux and Naud, 1979; Harris, 1978; Hawes, 1981; Marty, 1973, 1983) and potassium ferricyanide osmium (Hepler, 1980, 1981; White et al., 1979) as post-fixatives. The latter were used to determine the origin of those membranes in close association with secretory activity and to detect unsaturated lipids by osmium tetroxide (Lison, 1960).

Results

Morphology

The stamens of *B. inflata* are erect and the inner whorl slightly longer than the outer with 5–6 mm long slender filaments. A closer view of the flower showed that the staminal filaments were covered with yellow hairs which were observed to glisten and shine in the sunlight. These hairs were 2–3.5 mm long, unicellular, linear and erecto-patent (Fig. 1A–C). The long inner filaments (opposite the wide inner tepals) presented more hairs which were spread over a wider area than the outer ones (opposite the narrow outer tepals) (Fig. 1C). The short outer filaments have hairs massed medially; at the inner filaments hairs are densely spread from midway to the sub-apical part proximal to the anther (Fig. 1A). The hairs appear to arise directly as single cell outgrowths from the filament epidermal cells on the outermost surface (Fig. 1D). The surface of each hair is patterned with helical wall thickenings in an anticlockwise direction (Fig. 1E).

The developmental stages of the staminal hairs were visible with the eye or stereomicroscope and characterized numerically from 1 to 6 as seen in Table 1. Hairs appeared to be developed in most flower buds examined (stages 1–6), except the very youngest buds (stage 1) where hairs were not evident and appeared to be in the process of division. From stage 2 onwards staminal hairs developed rapidly and attained their full length by stage 3, before color developed in the bud. The hairs were crowded, closely and adpressed to both long and short filaments at stage 2, but were fully extended and expanding away from the filament at stages 3–5 (Fig. 1A and B). The yellow color of the staminal hairs was developed at stage 4 and the flower bud began to open at stage 5. At stage 6 the flower was fully open with collapsed staminal hairs and dehisced anthers (Fig. 1C). Once the anthers of the three long stamens had dehisced, the three anthers of the short stamens dehisced. When several flowers of *B. inflata* were dissected into tepals, stamens and gynoecium, only at the stamens a weak scent could be detected.

Developmental ultrastructure and cytochemistry

At stage 1 no visible signs of hairs were present on the smooth thick cell wall of the filament (Fig. 2A). The staminal hairs developed as tiny outgrowths of the filament epidermal cells. These epidermal cells of the filaments were rich in cytoplasm and a prominent nucleus with condensed chromatin, many mitochondria, profiles of endoplasmic reticulum (ER), plastids, and ribosomes were visible.

At stage 2, post-fixation with potassium ferricyanide osmium revealed the hair cell wall structure and the membrane system of plastids, ER, mitochondria and lipid droplets. The cell wall of a hair was covered by an irregular cuticle and structured into light outer and dark inner cellulose layers (Fig. 2B). There is a prominent nucleus, a central large vacuole, conspicuous plastids containing starch grains, few lipid droplets in the cytoplasm, many ribosomes, and fewer mitochondria than at stage 1 (Fig. 2B and C). Strands of tubular SER encircle plastids and mitochondria (Fig. 2D).

At stage 3 post-fixation of the hairs with zinc iodide osmium revealed the cell wall structure and lipid droplets. The cell wall cuticle of a hair began to become folded and the cell wall itself comprised the two light and dark cellulose layers as seen already at stage 2 (Fig. 2E). Ribosomes were abundant and the cytoplasm appeared as a dense granular background. There were large starch grains and numerous lipid droplets in plastids and cytoplasm (Fig. 2F).

At stage 4 the test for ester linkages rendered a strong reaction especially with lipid droplets. The cell wall development of the hairs was complete showing thick helical cuticular folds, and one dark fibrillar cellulose layer (Fig. 3A). This fibrillar cellulose