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Calcium distribution in the stigmas and styles of lettuce (*Lactuca sativa*)

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Antimonite precipitation was used to detect Ca^{2+} in the stigmas and styles of lettuce (*Lactuca sativa*) before and after pollination. Abundant calcium precipitates were detected in the papillae walls on the receptive surface of the stigma both before and after pollination. Meanwhile, few calcium granules were observed in the wall of epidermal cells on the non-receptive surface. Before pollination, calcium formed a gradient from the top to the base of the style in the transmitting tissue and parenchyma cells. After pollination, calcium levels increased in the transmitting tissue, and the gradient distribution became stronger. The calcium gradient was also observed in the tracheae of the vascular bundle of the style. These results indicate that a calcium gradient exists in the lettuce style, and that pollination induces an increase in stylar calcium levels.

Key words: antimonite precipitation, pollination, reproductive physiology, stigmatic surface, stylar calcium levels

Introduction

In the early 1960s, it was observed that the direction of growth of cultured pollen tubes could be determined by calcium. Ca^{2+} levels gradually increased from the stigma to the ovary in *Antirrhinum majus*, and that could explain why pollen tubes *in vivo* grow in the direction of this gradient (Mascarenhas & Machlis 1962). However, the phenomenon was not observed in several other plants (Glenk *et al*. 1971, Mascarenhas 1975). There were no subsequent reports in this area, but instead the research focused on Ca^{2+} function during pollen tube growth *in vitro*. From those studies, investigators concluded that cultured pollen tubes need to continuously absorb calcium to maintain a Ca^{2+} gradient at the tip of pollen tube for its polar growth (Holdaway-Clarke *et al*. 1997). Simultaneously, Ca^{2+} must be integrated into the pectic wall layer to strengthen the newly formed pollen tube wall (Li *et al*. 1994). The research on the relationship between Ca^{2+} and pollen tubes has led to the question as to how Ca^{2+} is distributed in pistil organization *in vivo*. Recently it was observed, using antimonite precipitation, that pollination in *Petunia hybrida* (Lenartowska *et al*. 1997) and lily (Zhao *et al*. 2004) induces an increase in exchangeable cellular calcium in the transmitting tissue.

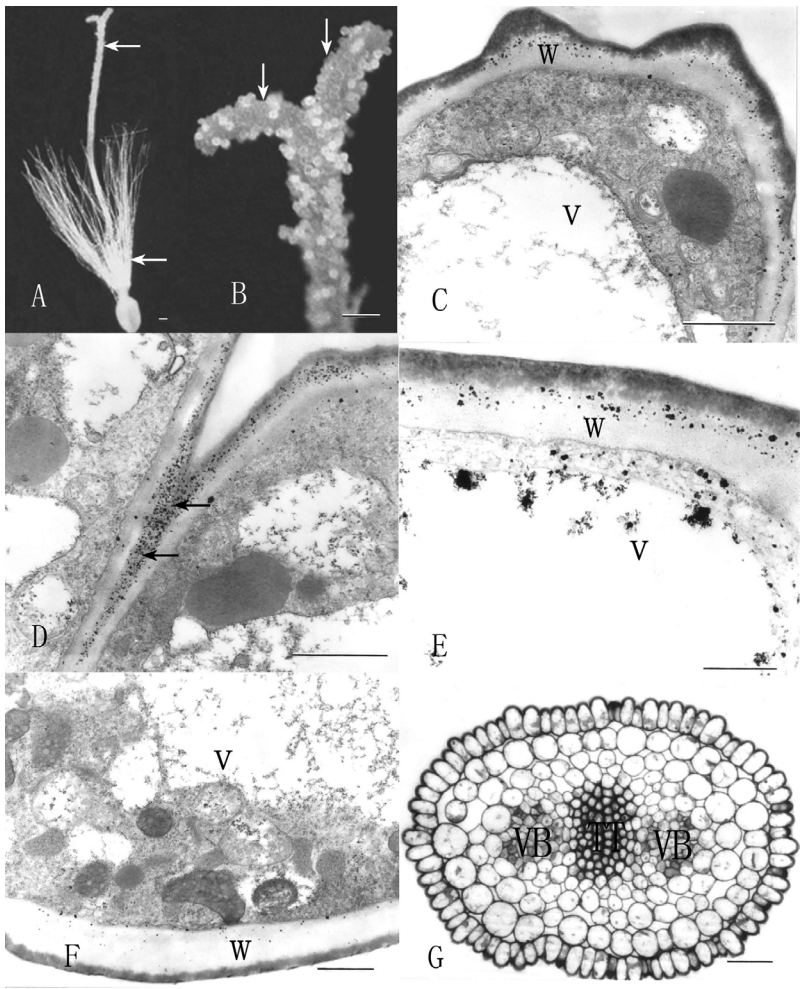

Fig. 1. — **A**: Lettuce gynoecium showing the positions (arrows) of stylar excision: the top of style and the base of the style. Bar = 300 μm. — **B**: Lettuce stigma with two slivers showing the receptive surface (arrows) and non-receptive surface. Bar = 10 μm. — **C**: Papillae cell of receptive surface of stigma nearly at anthesis displayed two layers of wall (W) with some calcium precipitates. Some flocculent calcium granules are present in vacuole (V). Bar = 1 μm. — **D**: Numerous calcium precipitates in the wall between two papillae cells (arrows). Bar = 1 μm. — **E**: Abundant calcium precipitates accumulated in papillae cell wall 1 h after pollination, evidently increased in cytoplasm and vacuole of the cell. Bar = 0.5 μm. — **F**: Calcium precipitates in the cell wall (W) of non-receptive surface of stigma, more precipitates in cytoplasm of the cell. Bar = 1 μm. — **G**: Cross section of style showing a transmitting tissue (TT) in center and two vascular bundles (VB) surrounded by parenchyma cells (P). Bar = 10 μm.

In the current study, we examined the distribution of the loosely-bound calcium in the stigma and style of lettuce to determine the relationship between loosely-bound calcium and pollen tube growth *in vivo*.

Material and methods

Lettuce (*Lactuca sativa*) was grown in a controlled growth chamber at 25 °C with a 15 h day length and illumination of 54 μmol m^{-2} s^{-1}, and at 20 °C for 9 h in the dark. The length of the lettuce style is about 1 cm. The style was cut into 1 mm long pieces between the stigma and the ovary (Fig. 1A). Stigmas and styles were sampled both before blooming and after pollination. The samples were fixed for 3 h at room temperature in 2% glutaraldehyde (v/v) in 0.1% M KH_2PO_4 buffer (pH 7.8), containing

1% potassium antimonite ($K_2H_2Sb_2O_7 \times 4H_2O$; from a fresh 4% (w/v) stock in double-distilled H_2O). The samples were washed (three 30 min washes in buffered 1% antimonite), postfixed in 1% (w/v) buffered OsO_4 containing 1% antimonite for 16 h at 4 °C, and washed in buffer (three 30 min washes). The samples were then dehydrated in a graded acetone series and finally embedded in Spurr's resin. For each experiment, at least 10 samples were sectioned and stained with 2% uranyl acetate (w/v) in 50% methanol (v/v). After being washed and aired, the samples were observed and photographed using a JEM-100 transmission electron microscope.

Results

Calcium distribution in the stigma

The stigma of lettuce is composed of two dry slivers, and each has a receptive plane, consisting of papillae cells covered by a layer of keratin, and a non-receptive plane (Fig. 1B). Before anthesis, few calcium precipitates appeared in the papillae cells. At anthesis, abundant calcium precipitates accumulated between the cellulose wall and keratin of the papillae cells (Fig. 1C), especially in the wall between the papillae cells (Fig. 1D), which is the site of pollen tube penetration of the stigma. After pollination, there were still numerous calcium precipitates located between the cellulose wall and keratin of the papillae cells. The amount of precipitates increased slightly in the papillae cells, especially in its vacuoles (Fig. 1E). In the epidermal cells of the non-receptive portion of the stigma, few calcium precipitates were located in the cell and cell walls, and some small precipitates were present in the vacuoles (Fig. 1F).

Calcium distribution in the style

The style of lettuce is solid and consists of epidermis, parenchyma cells, centrally located transmitting tissue and two vascular bundles located beside the transmitting tissue (Fig. 1G). In early anthesis samples, few calcium precipitates were observed at the top of the style. Precipitates were mainly located in the intercellular space and cell walls of the transmitting tissue, but not within the cells (Fig. 2A). In parenchyma cells, fine calcium precipitates were located in the vacuoles, and no precipitates were observed in the intercellular gaps (Fig. 2B). Few precipitates were present in the inner surface of the tracheae of vascular bundles (Fig. 2C). In the base of the style, the number of precipitates significantly increased, forming a gradient. In the transmitting tissue at the base of the style, more calcium precipitates appeared in the intercellular gap (Fig. 2D), in parenchyma cells (Fig. 2E), and in the inner surface of tracheae (Fig. 2F) than in the corresponding cells (Fig. 2A–C) at the top of the style.

Two hours after pollination, calcium precipitates increased slightly in parenchyma cells in the top of the style. Most of these precipitates were located in vacuoles, with few in the cell walls and intercellular gaps (Fig. 3A). However, in parenchyma cells at the base of the style, calcium precipitates were mainly concentrated in the cell walls and intercellular gaps (Fig. 3B). There was no change in the calcium distribution and gradient in the trachea elements. A few calcium precipitates were located in the inner surface of the tracheae at the top of the style (Fig. 3C) but more were present in the base (Fig. 3D).One hour after pollination, the pollen tubes had grown into the intercellular gap of the transmitting tissue at the top of the style. Because of its higher electron density, the pollen tube was easy to identify. Most calcium precipitates were located on the surface of the tube wall, with only a few located in the cells of the transmitting tissue (Fig. 3E). The number of calcium precipitates increased in the vascular bundle, especially in the inner surface of the tracheae at the top of the style (Fig. 3F), suggesting that pollination induces a calcium increase.

Discussion

Calcium has long been known to play an important role in pollen germination *in vitro* (Brewbaker & Kwack 1963) and it displays spatial and temporal features during many processes of sexual reproduction in flowering plants (Ge *et al*.

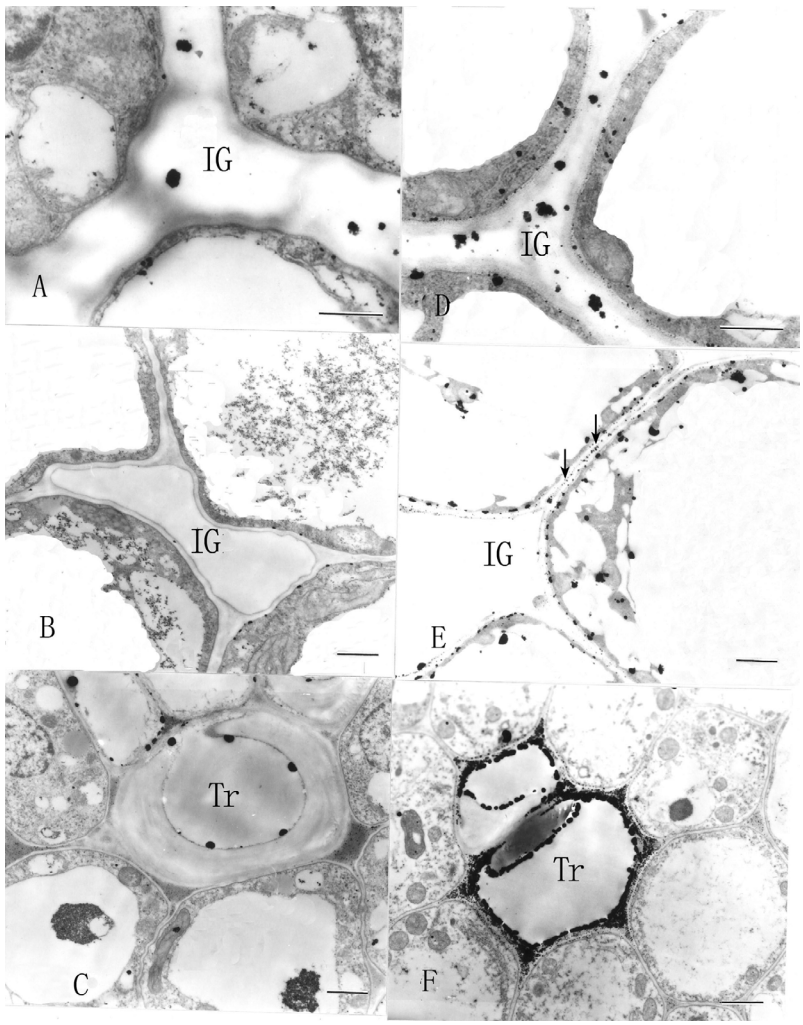

Fig. 2. — **A**: Nearly at anthesis, a few calcium granules in the intercellular gap (IG) of upper transmitting tissue of style. Bar = 0.5 μm. — **B**: A few calcium granules in IG of upper parenchyma tissue of style, some small ones in the vacuoles. Bar = 1 μm. — **C**: A few calcium precipitates in the trachea (Tr) of upper vascular bundle of style. Bar = 1 μm. — **D**: Same stage as in Fig. 1C, more calcium granules accumulated in IG of basal transmitting tissue of style, displaying a gradient distribution of calcium. Bar = 0.5 μm. — **E**: Numerous calcium granules accumulated in IG of basal parenchyma tissue of style (arrows), showing a gradient distribution of calcium. Bar = 1 μm. — **F**: Numerous calcium granules accumulated in Tr of basal vascular bundle of style, also showing a gradient distribution of calcium. Bar = 1 μm.

2007). In the present study, we observed that calcium accumulates in the papillae cell walls of the receptive plane in the lettuce stigma at anthesis. The rich-calcium environment in papillae cells aids pollen germination, matching the *in vitro* results. Our *in vivo* results indicate that more calcium is concentrated in the pollen tube track than in the surrounding tissue.

Gradient distribution of calcium in the style of higher plants has been disputed. Although the studies on *Petunia hybrida* (Lenartowska *et al*. 1997) and lily (Zhao *et al*. 2004) detected calcium precipitates in the transmitting tissue and pollen tube track, there was no mention of a gradient distribution of calcium in the style. However, both papers indicated that pollination should induce increases of calcium in the style, suggesting a developmental change in calcium distribution. Recently, gradient distribution of calcium in the style of tobacco (*Nicotiana taba-*

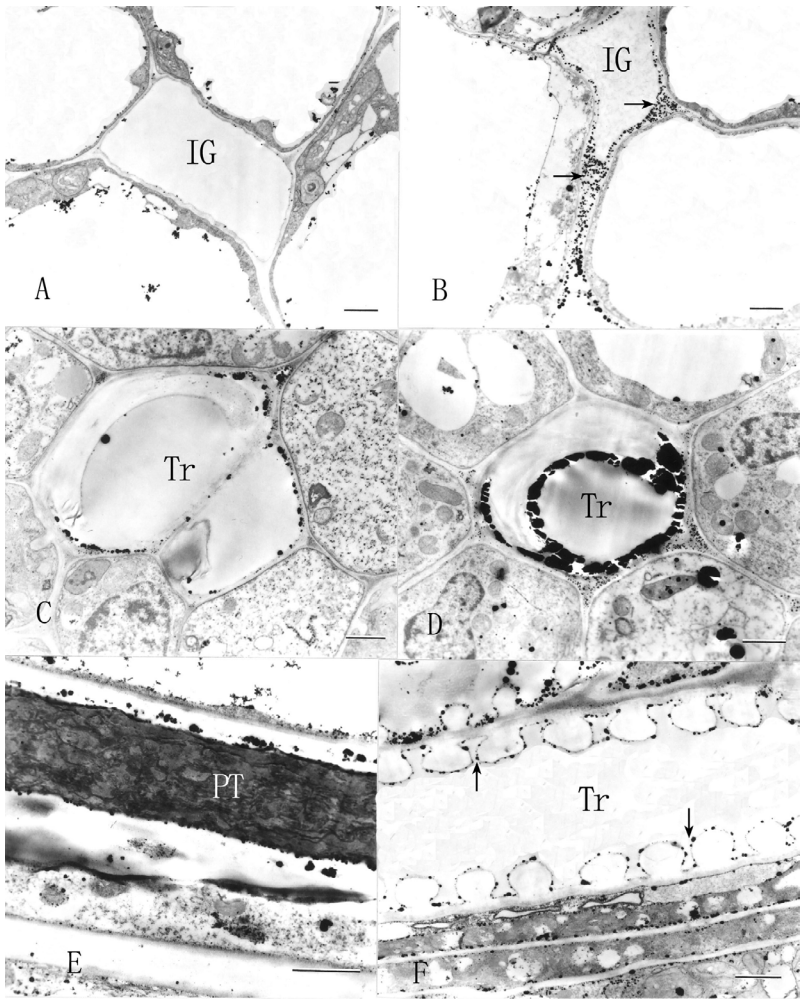

Fig. 3. — **A**: 1 h after pollination, a few calcium precipitates in the intercellular gap (IG) of upper parenchyma tissue of style. Bar = 1 μm. — **B**: Numerous calcium precipitates accumulated in IG of basal parenchyma tissue of style (arrow). A gradient distribution of calcium was maintained 1 h after pollination. Bar = 1 μm. — **C**: Same stage as in Fig. 3B, calcium granules slightly increased in trachea (Tr) of upper vascular bundle of style. Bar = 1 μm. — **D**: Calcium granules increased in trachea (Tr) of basal vascular bundle of style. Bar = 1 μm. — **E**: A longitudinal section of pollen tube (PT) growing in style, numerous calcium precipitates accumulated on tube wall. Bar = 1 μm. — **F**: A longitudinal section of a trachea (Tr), showing numerous calcium granules on its inner surface. Bar = 1 μm.

cum cv. Daqingue) was reported (Ge *et al*. 2009). This is the second antimonite precipitation based report after 45 years that the calcium gradient exists in the styles. We found that calcium gradient in lettuce styles also formed at anthesis. After pollination, the style calcium content increased slightly, and the calcium gradient was preserved. Investigations using more accurate methods are needed to determine the details of gradient distribution of calcium in the styles.

Acknowledgements

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