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Formation of membrane-bound inclusions and their associations with cytoplasmic channels in early prophase male meiocytes of *Althaea rosea* (L.) Cavan

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Abstract

To characterize the cytoplasmic structure reorganization during plant meiosis, the male meiocytes of *Althaea rosea* (L.) Cavan were examined under the combination of light and electron microscopy. Light microscopic observation of the toluidine blue-stained thick resin sections of young anthers revealed that the meiocytes of sporogenous cell stage were extremely voluminous and variable in shape and division plane. The cell walls (CWs) between some meiocytes were discontinuous at one or several site(s). These discontinuous portions varied between 0.2 and 3.0 μm in length. In addition, it was found that some meiocytes were able to produce protuberances that extended into another meiocyte. When transversally sectioned, the protuberance extending to another cell looked like a small cell lying in another cell. Transmission electron microscopy (TEM) showed that there were many long flat ER cisternae that were actively wrapping around a portion of cytoplasm in the male meiocytes at the sporogenous cell stage. During pre-meiosis interphase and early prophase I, a number of huge (0.5–1.0 μm diameter) spherical membrane-bound inclusions (MBIs) lined by single or double layer(s) of membrane were formed, each membrane actually representing one tightly appressed endoplasmic reticulum (ER) cisterna. The MBIs contained many granular, lamellar and fibrillar structures, and even small MBIs. Moreover, it was found that the MBIs could associate with the cytoplasmic channels (CCs) on CWs to release their contents into the cytoplasm of the opposite cell or directly extend from one cell to another through the CC. Taking all the data together, it is suggested that association of the MBIs and other organelles with CCs possibly functions in eliminating the non-identity of cytoplasm of the male meiocytes caused probably by the random asymmetric division observed at sporogenous cell phase, so as to ensure production of a large number of identical functional male gametes required for successful fertilization.

Keywords: Membrane-bound inclusion; Cytoplasmic channel; *Althaea rosea*; Male meiocyte

1. Introduction

Meiosis is a pivotal node in the life cycle of plants, controlling not only the transition of sporophyte to gametophyte, but also providing an opportunity for genetic reassortment. The aperture pattern (number and arrangement) of the pollen grain, one of the major taxonomic divisions of angiosperms, was determined before tetrad formation, so the knowledge of the link between meiosis and aperture pattern determination is a preliminary step toward understanding the genetic basis of development and system evolution of angiosperms (Ressayre et al., 2002).

Meiosis consists of many highly coordinated nucleus and cytoplasm events, any one of which occurring at the wrong time and location will lead to a profound impact on the

Abbreviations: CC, cytoplasmic channel; CW, cell wall; MBI, membrane-bound inclusion; Pd, plasmadesma; ML, middle lamella; PW, primary wall; ER, endoplasmic reticulum; DUM, double unit membrane.

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fertility of progenies in plants (Clandinin and Mains, 1993; Liu et al., 1993; He et al., 1996; Wolfe and Liu, 1999). The cellular events taking place in the cytoplasm involve the formation of cell plate that will be converted to new cell wall and partition of organelles between daughter cells during meiosis, thus being probably involved in controlling cytoplasm transmission (or inheritance), the sporo-gametophyte transition and the ontogeny of aperture pattern.

However, among many previous investigations concerning plant male meiosis, most have focused on nuclear events. There were a few papers (Bal and De, 1961; Maruyama, 1968; Feijo and Pais, 1988; Rashid et al., 1982; Heslop-Harrison and Dickinson, 1967; Dickinson and Heslop-Harrison, 1970; Williams et al., 1973; Dickinson, 1981; Bird et al., 1983) dealing with cytoplasm reorganization. These studies were based on very few plant species, i.e. lily, *Tradescantia* (Bal and De, 1961; Maruyama, 1968), tobacco (Rashid et al., 1982) and *Ophys lutea* (Feijo and Pais, 1988), and the observations differed with regard to plant and/or author. In lily, for example, the ribosome population was reduced at prophase and restored at the tetrad stage (Heslop-Harrison and Dickinson, 1967; Dickinson and Heslop-Harrison, 1970; Williams et al., 1973). Plastids were associated with membrane and/or particulate materials before meiosis, and converted to inclusions with little identifiable content after entering meiosis (Dickinson, 1981; Bird et al., 1983), while mitochondria changed from orthodox into small polymorphic form without internal cisternae (Bird et al., 1983). In *Ophys lutea*, starch was observed to accumulate in plastid at the beginning of prophase I, and later was gradually lost from the plastid (Feijo and Pais, 1988).

Recently, a new phenomenon of one or several plastids crossing the cell wall (CW) to form a bridge between the connected male meiocytes through a cytoplasmic channel (CC) was seen in the male meiocytes of tobacco, lily and onion (Wang et al., 2006). To check whether this case occurs in other plant species, the cytoplasm structure reorganization of the male meiocytes of *Althaea rosea* (L.) Cavan was ultrastructurally investigated in the present paper.

Althaea rosea (L.) Cavan (hollyhock) is a perennial herb of the *Malvaceae* that is of current interest, being used in phytotherapy for the treatment of irregular menstrual cycles, dry cough and catarrhal inflammation of throat and oesophagus (Monika and Papiez, 2004). Phytoestrogens of flavonoids and β-sitosterol groups, considered effective for healing menstrual absence and irregular menstrual cycles, have been isolated from its flowers (Ozarowski and Jaroniewski, 1989; Matlawska, 1990). Previous studies were mostly involved in its biochemistry and pharmacology, little being known about its reproductive biology, especially the cytology of its germ cells.

2. Materials and methods

Althaea rosea (L.) Cavan was cultivated in the garden, growing to 2 m, with alternative single leaves consisting of broad-long crenate blades with stellate pubescences and about 15-cm-long petioles and terminal raceme. This plant has deep rose or wine coloured bell-shaped flowers with many stamens combined into columns.

In the flowering season, the flower buds of 4–5 mm long were collected; 1–2 mm long anthers were carefully excised from receptacle and immediately pre-fixed in 0.1 mol/L sodium phosphate buffer (pH 7.2) containing 3% glutaraldehyde (Sigma, USA) and 0.2% tannic acid (Sigma) for 6 h at room temperature (RT), with one change in the middle. They were post-fixed overnight in the same buffer containing 1% OsO_4 (Sigma) at 4 °C. After rinsing thoroughly with the same buffer and double distilled water, they were dehydrated with 30–95% ethanol series at 4 °C for 15 min in each grade and then with 100% ethanol for 60 min at RT, with a change every 20 min. Later, they were soaked in propylene oxide (PO) for 30 min, and in mixtures of PO and resin (Epon 812, Sigma) with different ratios (3:1, 1:1, 1: 3, v/v) for 2 h each. Finally, they were infiltrated and embedded in pure resin mixture.

The polymerized samples were thick-sectioned first with a common rotation microtome (AO-820, USA), mounted with a glass knife and then thin-sectioned with an ultra-thin microtome (LKB-8800, Sweden). Sections (approx. 2 μm thick) were stained with 0.5% toluidine blue in 2.5% sodium carbonate, and photographed under a light microscope (Olympus, Japan) mounted with a motic B5 digital camera. The thin sections of 70–90 nm were stained with 2% (w/v) aqueous uranyl acetate for 1.5 h and then 2.6% (w/v) lead citrate for 10 min. Finally, they were examined under a transmission electron microscope (TEM) (JEOL-1230, Japan) at an accelerating voltage of 100 kV.

3. Results

Light microscopic observation revealed that the anthers of *A. rosea* consisted of two instead of the normal four sporangia, one per lobe (data not shown). Viewed from transversal sections, each sporangium contained up to four spherical, hemispherical and/or shape-irregular male meiocytes (Fig. 1A,B). On longitudinal sections, 1–2 rows of male meiocytes could be observed in each sporangium, which looked spherical, hemispherical, crescent, rectangular, or irregular. The meiocytes were very large, about 10–20 fold greater than the wall cells, and considerably variable in size and shape. The nucleus was centrally located or pressed against the plasmalemma, with less dense chromatin and a single prominent nucleolus. The meiocytes at the end of sporangium were smaller and more irregular than those in the middle portion (Fig. 1C). The cells at the middle were rectangular (Fig. 1H) or trapeziform (Fig. 1I). The long sides (walls) of the rectangular cells were smooth and vertical to the anther walls, and that of the trapeziform cells were zigzagged and parallel to the anther walls. In addition, it was found that (i) some meiocytes produced a protuberance which extended into the inside of another meiocyte—when