Abstract  Sperm cells released from in vivo-in vitro grown pollen tubes of tobacco are associated in pairs and initially enclosed by the plasma membrane of the pollen tube. When the sperm cells are placed together, using glass microinjector needles, in an enzymatic solution, up to half undergo cellular fusion with subsequent nuclear fusion. The frequency of sperm cell fusion decreases with time during the elongation of the pollen tube, suggesting that mechanisms inhibiting self-fusion of sperm cells may develop as the pollen tube elongates through the style toward the ovule. This tendency may play an important role in inhibiting fusion of the two sperm cells inside the calcium-rich synergid where the male germ unit dissociates and sperm cells are transported to their target cells - the egg and central cell.

Key words  Fusion · Nicotiana tabacum · Male germ unit · Sperm cell

Introduction

Flowering plant sperm cells are structurally simple haploid cells that are essentially entirely dependent on the surrounding cytoplasm for nutrition and transport. Although these cells closely resemble ordinary protoplasts, in vitro manipulation of flowering plant gametes has only begun to be explored, despite the potential attractiveness of reproductive cells for plant cell engineering (Keijzer et al. 1988). Using the special biological and genetic characteristics of sperm cells, new methods of plant breeding may be possible in the future that allow double fertilization mechanisms to be better understood.

Materials and methods

To obtain the highest quality sperm cells from the bicellular pollen of tobacco, pollen tubes were grown using the in vivo-in vitro technique (Shivanna et al. 1988). Styles were pollinated in vivo, and pollen tubes were allowed to grow for a predetermined amount of time. Then styles were cut near the growing pollen tubes at predetermined positions on the style (Fig. 1) and floated in a culture medium of 0.01% (w/v) H3BO3, 0.01% (w/v) KH2PO4, 0.01% (w/v) CaCl2·2H2O and 15% (w/v) sucrose for several hours until tubes emerged from the cut tips (Tian and Russell 1997b). Normally, pollen tube growth through the 4-cm style of tobacco requires 2 days from pollination to fertilization (Tian and Russell 1997a). Four stages were sampled to examine maturational changes in sperm cells over time. Style lengths of 1, 2, 3 and 4 cm (cut at 13, 20, 27 and 34 h after pollination, respectively) were grown in culture medium 4–8 h, until numerous pollen tubes emerged from the cut end of a style. The style was then immersed in a 9% (w/v) mannitol solution to burst the pollen tubes and release the paired, intact sperm cells into the solution.

Sperm cell fusion was induced using solutions containing 0–2% (w/v) cellulase (Onozuka R-10), 0–2% (w/v) pectinase (Serva), 0–25 mM CaCl2, 1 mM MgSO4, 5 mM KH2PO4, 3 mM 2[N-morpholino]ethanesulfonic acid and 1% (w/v) bovine serum albumin, pH 5.4. Osmolality of the fusion solution was adjusted to

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520–580 mOsmol/kg H₂O using 6.5–7.5% (w/v) mannitol. Between 30 and 50 µl of fusion solution was placed on a slide and covered with mineral oil. Sperm cells were collected and transferred into the fusion solution with a microinjector and brought into contact for fusion. In some cases, 5% PEG (molecular weight 3350) was added to induce fusion. Fusion products were maintained in a KM8p medium (Kao and Michayluk 1975).

Results

Newly isolated sperm cells

Release of sperm cells from emergent pollen tubes occurs nearly as soon as the style is transferred to the 9% mannitol solution. Of the two sperm cells, the one initially associated with the vegetative nucleus is almost always the smaller. The larger sperm cell is appressed to the smaller one and is not directly associated with the vegetative nucleus (Fig. 2a).

Within several minutes of isolation, the vegetative nucleus usually broke down; however, the two sperm cells remained associated, with pollen cytoplasmic material apparently wrapped around the two sperm cells, maintaining their ellipsoidal to elongated shape in the 9% mannitol solution (Fig. 2b). Achieving adhesion of the two sperm cells from a single pollen tube was apparently inhibited by this surrounding material.

Sperm cells from different pollen tubes have the additional pollen plasma membrane that has to be removed before adhesion. Sperm cells from the same pollen tube are already in contact with each other within the pollen membrane, although at this stage they do not fuse. When the pairs of sperm cells were transferred to the enzyme-containing solution, however, the adhesion between the two sperm cells almost immediately disappeared, the two sperm cells detached, and they quickly assumed a rounder shape (Fig. 2c). If paired sperm cells were kept in the