African Journal of Agricultural Research Vol. 7(17), pp. 2679-2682, 5 May, 2012
Available online at http://www.academicjournals.org/AJAR
DOI: 10.5897/AJAR11.1734
ISSN 1991-637X ©2012 Academic Journals

Full Length Research Paper

Distribution of nuclei and microfilaments during pollen germination in *Populus tomentosa* Carr.

Yuan Cao, Rui-Zhi Hao, Mei-Qin Liu, Xin-Min An and Yan-Ping Jing*

National Engineering Laboratory for Tree Breeding, NDRC, College of Biological Science and Biotechnology, Beijing Forestry University, Tsinghua East Road No. 35, Beijing, 100083, P.R. China.

Accepted 28 March, 2012

Pollen tubes transport nuclei to the ovules for fertilization. The distribution of microfilaments and the nuclei were investigated by fluorescent phalloidin labeling and DAPI (4', 6-diamidino-2- phenylindole) during pollen germination and pollen tube growth of *Populus tomentosa* Carr., a Chinese native tree species. The coexistence of trinucleate and binucleate pollen was confirmed, which had been previously considered binucleate. Three distinct typical microfilament structures were found, and F-actin was present at the periphery of both the vegetative and the sperm nuclei. The investigation indicated that movement of sperm nuclei and vegetative nucleus is related to the microfilament system.

Key words: Generative nucleus, vegetative nucleus, actin cytoskeleton, pollen tube growth.

INTRODUCTION

Pollen germination and pollen tube growth are two of the most important physical activities in the sexual reproduction of plants, and both are complicated dynamic process. Pollen germination on a compatible stigma involves the pollen cell extending to form the pollen tube, which functions to send the sperm nuclei to the embryo sac (Higashiyama et al., 2003).

Microfilaments form a substantial component of the cytoskeleton and play important roles in pollen germination and pollen tube growth (Taylor and Hepler, 1997; Cai et al., 2000). The movement of the generative cell and the vegetative nucleus are mediated by myosin and transported along microfilaments (Vidali and Hepler, 2001). The dynamic structure of microfilaments was reported to be closely related to the movement of the vegetative and generative nuclei and the generative and sperm cells at different stages in pollen development (Zee et al., 2003).

Populus as an economically important timber tree in China is a model plant for tree research. Research on pollen germination and pollen tube growth in *Populus* is significant for its contributions to plant reproductive the movements of the nucleus and microfilaments in biology and to tree breeding. We know of no reports on *Populus* pollination. In this study, we used live fluorescent labeling and *in vitro* culturing of *Populus* pollen to investigate the movements of nuclei and microfilaments during pollen germination and pollen tube growth in *Populus tomentosa* Carr., Chinese white poplar, which belongs to the genus *Populus*, and is an important fast-growing timber species native to China.

*Corresponding author. E-mail: ypjing.bjfu@gmail.com. Fax: 86-10-62336248.

MATERIALS AND METHODS

Pollen collection and preservation

Batches of branches of LM-50, a male clone of *P. tomentosa* Carr from Guan County, Shandong Province, were cut off in January, 2009, 2010 and 2011 and grown hydroponically in a greenhouse. Pollen was collected from each batch and preserved in sterilized dry glass bottles containing silica gel at -20°C.

Pollen germination and pollen tube growth *in vitro*

Pre-hydrated pollen grains were cultured at a concentration of0.01g/mL in liquid germination fluid (0.8 mmol/L MgSO4, 1 mmol/L KNO_3, 6 mmol/L $Ca(NO_3)_2$, 4 mmol/L H3BO3, 15% PEG-4000, 10% sucrose), which was determined by our earlier studies in a shaker (100 rpm) at 26°C for 6h. Culture was sampled for staining every 30 min.

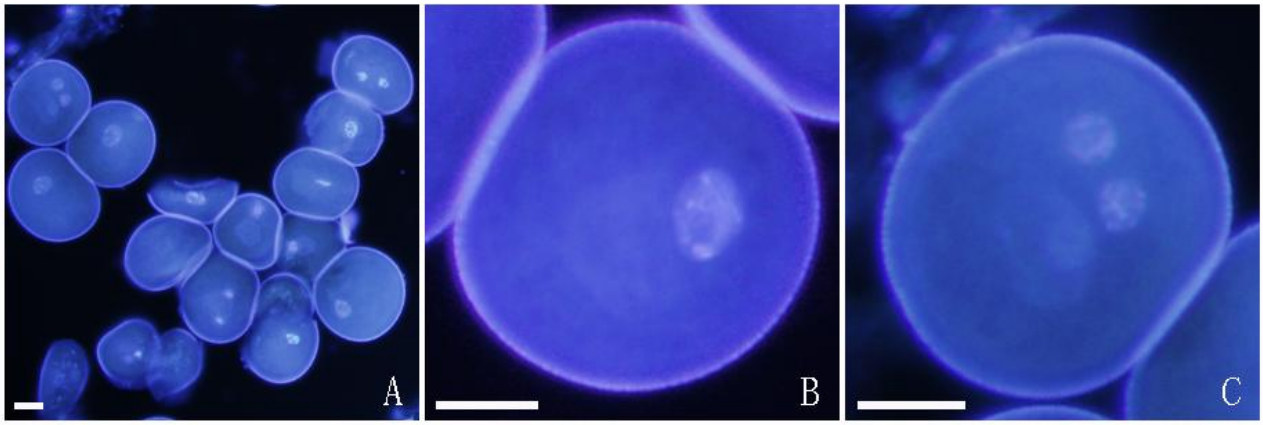

Figure 1. Mature pollen grains in anthers before dehiscence (A-C); bar = 10 µm; A) Binucleate and trinucleate pollen grains in the loculus; B) Binucleate pollen; and C) Trinucleate pollen.

Fluorescent observation of microfilament and nuclei

Anthers before dehiscence were fixed with Carnoy fixative, then paraffin sections were stained with 1 µg/ml DAPI for 5 min after dewaxing and rehydration followed by washing twice with phosphate buffer (137 mmol/L NaCl, 2.7 mmol/L KCl, 8 mmol/L Na_2HPO_4, 1.5 mmol/L KH_2PO_4, pH 7.2). The slide was sealed with 50% glycerol and observed with a fluorescence microscope (OLYMPUS BX51, Tokyo, Japan).

Referring to Li et al. (2001), we used fluorescent phalloidin to investigate actin arrangement. At room temperature, microfilaments in living pollen and pollen tubes were labeled in the dark for 15 min with 165 nmol/mL Alexa Fluor® 488 phalloidin dye (Invitrogen, California, USA) containing 1.5% DMSO and 0.01% NP-40. Nuclei were stained with 0.5 µg/mL DAPI for 5 to 10 min. After staining, one drop of culture liquid was placed on a slide sealed with 50% glycerol and examined with a 3D laser section using the LSCM system (Laser Scanning Confocal Microscope TCS SP5, LEICA, Buffalo Grove, IL, USA).

RESULTS AND DISCUSSION

Mature pollen that was observed before dehiscence contained not only binucleate pollen which had a brighter generative nucleus and a larger paler vegetative nucleus (Figure 1A and B), but also trinucleate pollen, which had two smaller brighter sperm nuclei produced by the division of the generative nucleus and a paler vegetative nucleus (Figure 1A and C). Prior studies have suggested that the pollen of *Populus* is binucleate. However, trinucleate pollen has ever been found in several *Populus* species such as *Populus yunnanensis* (Hamilton and Langridge, 1976). We also found not more than 40% trinucleate pollen in the anther before dehiscence (Figure 1A), in Chinese special local tree species- *P. tomentosa Carr*. Further investigation is necessary to determine the reasons for the coexistence of binucleate pollen and trinucleate pollen in *P. tomentosa Carr*.

Under appropriate *in vitro* culture conditions, the generative cell of binucleate pollen is divided into two sperm cells within the pollen grain prior pollen germination. The dynamic assembling of microfilaments is critical for pollen germination and pollen tube tip growth (Fu et al., 2001; Lee et al., 2008). The sperm nuclei and vegetative nucleus were each surrounded with short fragments of microfilament (Figure 2A). As germination progressed, large amounts of actin assembled to form a microfilament meshwork throughout the pollen grain and the vegetative and sperm nuclei were enveloped by a dense network of microfilaments (Figure 2B). As the pollen tube emerged, the three nuclei moved towards the base of the pollen grain opposite the pollen tube orientation (Figure 2C); subsequently, they began to move out of the grain and into the growing pollen tube (Figure 2D). The pollen tube continued to extend, and ultimately all three nuclei entered the elongated pollen tube (Figure 2E1, E2 and G). At this point, the parallel bundles of microfilaments were distinctive, especially, around the nuclei. Throughout the germination process, microfilaments were always observed around the vegetative nucleus (Figures 2C and E2). There were also microfilaments around the sperm nuclei except in the early germination stage when the nuclei moved towards the base of the pollen grain, when there was no microfilament staining (dark areas) around the sperm nuclei (Figure 2C).

Subapically, the microfilaments usually formed a network (Figure 2H) or a cortical fringe (Figure 2I). Apically, long actin filaments were lacking; instead there were very short fragments (Figure 2H) or a dark area with no fluorescence (Figure 2I). But some abnormalities were observed, such as only one sperm nucleus has entered the pollen tube, while the other sperm nucleus and the vegetative nucleus remain in the pollen grain (Figure 2F). In the pollen tube, the microfilaments around the sperm nucleus appear thin and end-arranged, while the microfilaments in the grain where the two nuclei will move towards disorderly (Figure 2F). However, it has been

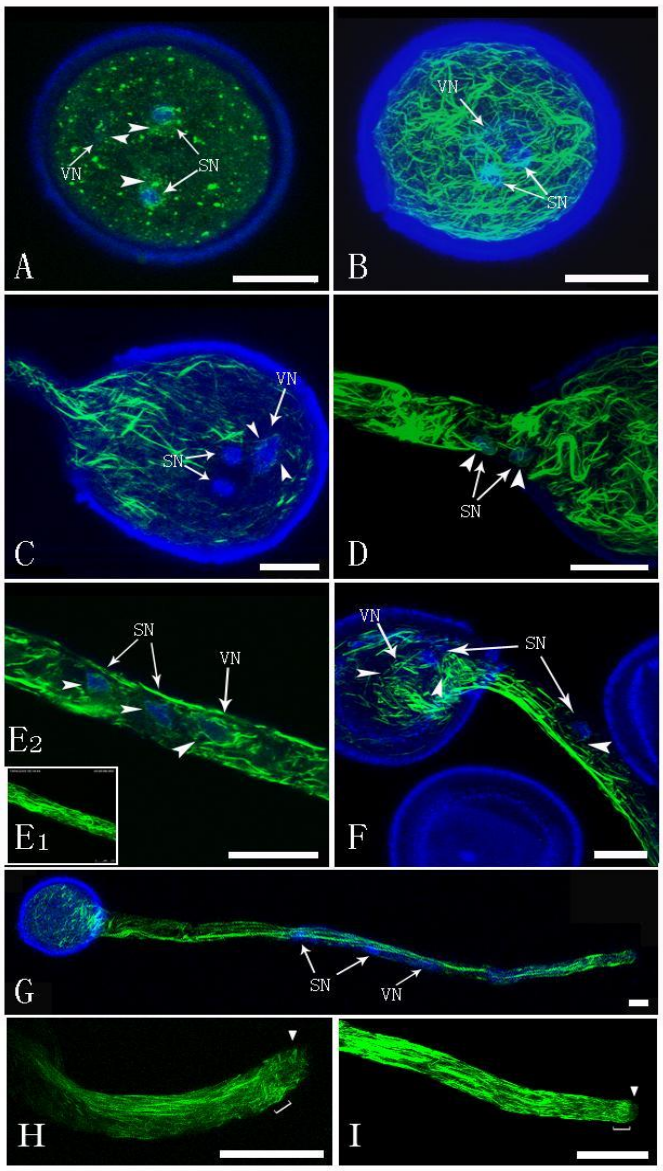

Figure 2. F-actin and the movement of nuclei during pollen germination and pollen tube growth in *P. tomentosa Car.*; bar = 10 μm; Arrow head: microfilament; arrow: nucleus; A) Microfilaments forming spindle- and needle-shaped granules around the nuclei (after hydration); B) Dense network of microfilaments around the nuclei (before germination); C) Nuclei positioned basally in the pollen grain, with distinct microfilaments around the vegetative nucleus (early germination); D) Microfilaments around the sperm nuclei as they moving into the growing pollen tube; E1) 3D image of end-arranged microfilaments near the nuclei in a long pollen tube; E2) Merged image from two channels at one single layer of pollen tube showing microfilaments around the nuclei at the same position shown in E1; F) Microfilaments around the sperm nucleus in the pollen tube appear thin and end-arranged, while microfilaments in the paths of the nuclei movement within the grain were disorderly; G) End-arranged microfilaments in a pollen tube containing three nuclei. SN, sperm nuclei; VN, vegetative nucleus. H-I) Structure of microfilaments in pollen tube; note the parallel bundles in the shanks of the pollen tubes; H, bracket: subapical F-actin network, arrow head: apical short fragments; I, bracket: subapical cortical fringe of microfilament fragments, arrow head: no visible apical F-actin.

found that microfilaments enveloped the vegetative nucleus of mature pollen, while there were no microfilaments around the generative cell (Gervais et al., 1994; Hause et al., 1992) and the movement into the pollen tube of the vegetative nucleus and sperm cells was thought to depend on the microfilament-associated myosin motive system (Heslop-Harrison and Heslop-Harrison, 1989). Our results of microfilaments around both the vegetative and sperm nuclei (Figure 2A to G) indicated that the movement of the nuclei in *P. tomentosa Carr.* is also dependent on the microfilament-associated myosin motive system and the existence of microfilaments in the sperm cells may differ among species. Our result also suggests that thedisruption of microfilament distribution and/or structure could prevent the nuclei from entering the pollen tube (Figure 2F).

The *in vitro* pollen germination ratio of *P. tomentosa Carr.* was relatively low, and stored pollen grains do not live long. Thus, not all aspects of the movement of vegetative and sperm nuclei have been revealed. Furthermore, could the disruption of the microfilament network be a component of incompatibility in *Populus*? Further research, including *in vivo* reporter gene strategies and real-time dynamic observation should be carried out to investigate these issues.

Conclusion

Our investigation confirms the coexistence of trinucleate and binucleate pollen instead of only binucleate pollen in Chinese native species *P. tomentosa Carr.* which indicated that trinucleate pollen, is more widely in *Populus* than is previously acknowledged. As in other species, pollen tubes in *P. tomentosa Carr.* have three distinct microfilament regions. The observation of nuclei during pollen germination and pollen tube growth supports the argument that movement of sperm nuclei in the pollen tube is related to the microfilament system similar to the movement of the vegetative nucleus. We therefore, propose that a disruption in the arrangement of microfilaments in *P. tomentosa Carr.* pollen may adversely affect the normal transportation of nuclei from the pollen grain to the embryo sac, and may be a cause of incompatibility. More attention should be paid to this new aspect of tree breeding as microfilaments are important to the regulation of pollen tube growth and to signal transduction.

ACKNOWLEDGMENT

This study was supported by the Nature Science Foundation of China (30700636, 31170631).

REFERENCES

Cai G, Del Casino C, Cresti M (2000). Cytoskeletal basis of organelle trafficking in the angiosperm pollen tube. Ann. Bot-London, 85:69-77.

Fu Y, Wu G, Yang Z (2001). Rop GTPase–dependent dynamics of tip-localized F-actin controls tip growth in pollen tubes. J. Cell Biol., 152:1019-1032.

Gervais C, Simmonds DH, Newcomb W (1994). Actin microfilament organization during pollen development of Brassica napus cv. Topas. Protoplasma, 183:67-76.

Hamilton D, Langridge P (1976). Trinucleate pollen in the genus Populus. Cell Mol. Life Sci., 32:467-468.

Hause G, Hause B, Van Lammeren A (1992). Microtubular and actin filament configurations during microspore and pollen development in Brassica napus cv. Topas. Can. J. Bot., 70: 1369-1376.

Heslop-Harrison J, Heslop-Harrison Y (1989). Myosin associated with the surfaces of organelles, vegetative nucleus and generative cells in angiosperm pollen grains and tubes. J. Cell. Sci., 94: 319-325.

Higashiyama T, Kuroiwa H, Kuroiwa T (2003). Pollen-tube guidance: beacons from the female gametophyte. Curr. Opin. Plant Biol., 6: 36-41.

Lee YJ, Szumlanski A, Nielsen E, Yang Z (2008). Rho-GTPase–dependent filamentous actin dynamics coordinate vesicle targeting and exocytosis during tip growth. J. Cell Biol., 181: 1155-1168.

Li Y, Zee SY, Liu YM, Huang BQ, Yen LF (2001). Circular F-actin bundles and a G-actin gradient in pollen and pollen tubes of Lilium davidii. Planta, 213: 722-730.

Taylor LP, Hepler PK (1997). Pollen germination and tube growth. Annu. Rev. Plant Biol., 48: 461-491.

Vidali L, Hepler PK (2001). Actin and pollen tube growth. Protoplasma, 215: 64-76.

Zee S, Ye X, Wang L, Yau CP, Yip WK (2003). F-actin visualization in generative and sperm cells of living pollen of rice using a GFP-mouse talin fusion protein. Acta Bot. Sin., 45(008): 949-958.