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POLLEN TUBE GROWTH OF *PAEONIA TENUIFOLIA* L. (PAEONIACEAE) *IN VITRO* AND *IN VIVO*

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Abstract

In vitro and *in vivo* studies on pollen germination of *Paeonia tenuifolia* L. (Paeoniaceae) revealed that pollen grains are shed at two-celled stage. Normal and abnormal pollens were observed. Pollen viability was recorded between 55 and 75%. *In vitro* studies revealed 85% germination and usually the germination was monosphonic. Some pollen tubes with swollen tube tip and undulations were found. Styles and stigma were found to contain many pollen tubes 24 hrs after pollination.

Cytological properties of anther wall, divisions of tapetum cells, micro-, macrosporogenesis (Dane 1997), early embryogeny (Dane and Olgun 1997), endosperm (Dane and Olgun 1998) in *P. tenuifolia* (Ranunculaceae) were previously studied. In this organisms some abnormalities were observed during micro- and macrosporogenesis (Dane 1997). The aim of this study is to examine the pollen morphology, viability, capacity of *in vitro* and *in vivo* pollen germination and cytological properties of pollen tubes in *P. tenuifolia.*

Paeonia tenuifolia L. (Paeoniaceae) was collected from the natural population near Edirne of European part of Turkey in May 2004. Pollen grains were collected after 24 hrs of pollination, which usually takes place early in the morning. Stigmas became receptive almost one day after the flower opened, and lasted for 24 hrs.

Pollen grains were stained following Mulugeta *et al*. (1994). Fertile (stained) and sterile (non-stained) pollens were counted from randomly chosen 30 regions. A total of 1923 pollen grains were counted. The cytological properties of generative and vegetative nuclei of pollen grains were investigated.

Hanging drop technique was used for culturing the pollen grains in liquid medium containing 10% sucrose and 0.01% boric acid (Vasil 1960). Cultures were incubated in room temperature (25^{o} C) and in diffuse laboratory light. The percentage of germinated pollen grains was counted after 12 hrs incubation in triplicate randomly from 100 - 200 grains (in groups of 25 or more from different fields on the slide).

Flowers were collected after 24 hrs of opening to determine the quantity of pollen grains on the pistil and *in vivo* pollen tube growth. After fixation in Carnoy (3 : 1), the pistils were kept in 70% ethanol. The pistils were hydrolized with 1 N HCI for 15 min at 60° C in an oven. They were stained with Feulgen reagent for two hours in darkness at 25° C. Then they were squashed and stained with aceto-orcein. Also they were examined with lactophenol-anilin blue, IKI and resorcin blue.

Pollen grains were prolate, tricolporatae, exine reticulatae and shed during the two-celled stage. The generative cell and vegetative nuclei were cresent and spherical, respectively (Fig. 1). Pollen viability was between 55 and 75% and showed variation in size as well as the degree of tube length. Pollens were 25.5 - 27.2 µm long and 22.1 - 25.5 µm broad. Sterile pollen grains were smaller in dimension.

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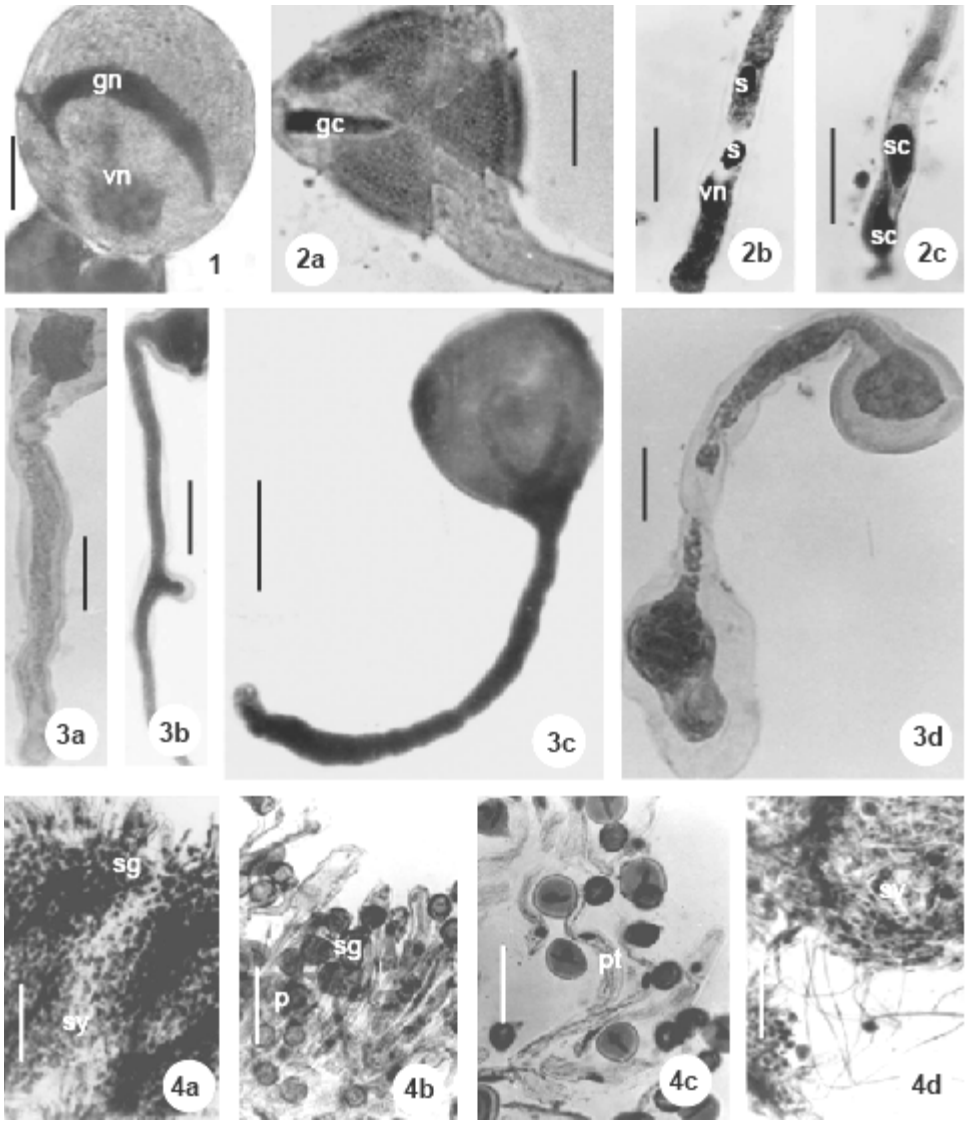

Figs 1-4. Pollen grain and *in vitro* and *in vivo* germination of *Paeonia tenuifolia* L. 1. Pollen grain. 2a. Newly generated pollen tube. 2b. Vegetative nucleus and sperm nuclei in the pollen tube. 2c. Sperm cells. 3a-d. Abnormal pollen. 4a-d. *In vivo* pollen germination. (gc, generative cell; s, sperm; sc, sperm cells; vn, vegetative nucleus, p, pollen; pt, pollen tube; sg, stigma; sy, style). Bars in Figs 1, 4a, d = 10 µm; 2a-c, 3a-d, 4b = 20 µm, 4c = 15 µm.

In vitro pollen germination was slow and started between 20 and 30 min after transfer in the medium. It was revealed that 85% of pollen grains were germinated usually with only one pollen tube (Fig. 2a). Some abnormal pollen tubes were observed with swollen tube tips and weak and undulating tubes (Fig. 3a-d). These abnormalities were found in 10 - 20% pollens.

Vegetative and generative nuclei were observed easily within the growing pollen tube where generative nucleus divides into two sperm nuclei (Fig. 2b). Sperm cells were also seen (Fig. 2c).

The pollen tube germination was generally normal (Fig. 4a-d). Many pollen tubes were recorded on the stigma and styles but a few in the ovary chamber. *In vivo* studies showed that there was no self-incompatibility. These observations are similar to that reported in *P. jishanensis* (Zhou *et al.* 1999).

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