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Pollen viability of common buckwheat (*Fagopyrum esculentum* Moench.) in *in vitro* germination and its application to male gametophyte isolation

Sun-Hee Woo*, Hong-Sig Kim[2], Chul-Won Lee[2], Sang-Il Park[2], Seok-Young Son[2], Young-Mok Park, Taiji Adachi[1], Seung-Keun Jong[2]

Proteome analysis team, Korean Basic Science Institute, Daejeon 305-333, Korea [1]Lab. of Plant Genes and Physiology, Osaka Prefecture University, Sakai, Osaka, 599-8531 Japan [2]Dept. of Agronomy, Chungbuk National University, Cheongju, 361-763, Korea

Objectives

The aim of the present study was to develop a series of simple techniques about an *in vitro* method for the germination and viability of common buckwheat pollen and for isolation of viable protoplasts from male gametophytic cells and to explore the viability under enzymatic treatments.

Materials and Methods

1) Material: Common buckwheat
2) Methods: We were used pollen germination, pollen viability, sperm cells isolation and histological analysis techniques for this experiment.

Results and Discussion

In vitro germination, pollen viability and male gametophyte isolation in common buckwheat were examined. Pollen grains were successfully germinated in an modified Murashige and Skoog (MS) medium. Lack of H_3BO_3 inhibited pollen tube formation. Addition of H_3BO_3 and $Ca(NO3)_2$ significantly increased pollen tube formation within 48 h in culture. Maximum pollen viability was found 2 h and 4 h after first light when plants were maintained at 25°C, respectively. Some pollen remained viable for approximately 24 hr in intact flowers, but almost pollen lost their viability in less than an hour when they stored at room temperature without humidity control. Sperm cells were released from germinated buckwheat pollen. After the initial osmotic burst, the slurry is sieved through a nylon mesh and then removed starch grain with sucrose density gradient centrifugation as much as possible. The isolated cells are spherical and approximately 5.0-6.0 μm in diameter. Characterization of sperm cells were checked using DNA fluorochrome DAPI reveal a nuclei nature of the sperm cell.

* Corresponding author: Sun-Hee Woo, TEL:042-865-3424, E-mail:woosh322@kbsi.re.kr