学 位 論 文 内 容 の 要 旨/Dissertation Abstract

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学 位 論 文 題 名/Title of Dissertation

Establishment of the in vitro evaluation system for pollen viability in Japanese rice cultivars
（日本のイネ品種における花粉稔性のin vitro 評価系の確立）

The establishment of an in vitro pollen viability evaluation system helps in crop improvement and breeding in general. Pollen viability is the ability of the pollen to germinate, elongate the pollen tube, and burst its tip; in summary, it is the ability of pollen to perform its function. Various methods are employed to assess pollen quality, such as seed set counting, in vitro pollen germination assays, and staining techniques using specific dyes. Pollen germination tests in vitro involve assessing the germination capacity of the pollen grains, elongation, and bursting of the pollen tube. At the same time, the staining method is used to indicate if the pollen is alive or dead by using stains. The quality of pollen grains has a big correlation with fertilization and seed formation in seed plants. Therefore, pollen quality assessment is crucial for artificial cross pollination to ensure fertilization and seed formation. Culture media composition and conditions for pollen quality evaluation in vitro may vary from one species to another or cultivar to cultivar, and it may interfere with the evaluation of pollen germination and tube growth in vitro. Incubation time and incubation temperature may interfere with pollen germination and pollen tube growth, therefore interfering with the quality evaluation of pollen grains. Culture media for in vitro rice pollen germination had been developed. However, the information on the system for in vitro pollen viability evaluation in Japanese rice cultivars is still limited. In this study, I conducted experiments to determine the suitable sucrose concentrations, incubation temperature, and incubation period for the high pollen germination capacity, pollen tube growth, and pollen tube bursting rates in vitro in three Japanese rice cultivars as the procedures for pollen quality analysis in vitro. Fluorescein diacetate (FDA) pollen staining dye was applied to clarify the suitable method for pollen quality assessment. Additionally, pollen tube growth behaviors and bursting phenomena in vitro were analyzed.

Fresh mature pollen samples from three Japanese rice cultivars, ‘Kitaake’, ‘Nanatsuboshi’, and ‘Nipponbare’, were used. Pollen samples were cultured on liquid culture media with different sucrose concentrations (0%, 10%, 20%, and 25%) to investigate the suitable sucrose concentration for high rice pollen germination rate, pollen tube growth, and pollen tube bursting rate. The optimized medium was used to investigate the effects of incubation temperature and incubation time on pollen germination, pollen tube growth, and pollen tube bursting. The pollen germination, pollen tube growth, and pollen tube bursting rates were evaluated for a maximum of one hour in intervals of 10 min. A pollen viability test using FDA staining was conducted to test whether the pollen grains

were alive or dead. Alive pollen stained green, and dead pollen remained unstained. The FDA staining test validates the presence or absence of esterase enzyme in pollen grain. The staining of pollen grains showing a green color indicates the presence of esterase enzyme (alive), which means pollen can undergo metabolic activity required for the pollen to grow and function, and unstained pollen grains indicate dead pollen grains.

The sucrose concentration of 20% was found to support high pollen germination. Optimal germination condition in vitro was achieved in 20% sucrose concentration at 25°C for 60 min, allowing easy observation of the germination percentage and length of the pollen tubes. The maximum pollen germination rate of about 80% and pollen tube length of 285 μm were observed in 'Nanatsuboshi' within one hour of initial incubation. Pollen tube bursting rate of 92-97% was attained within 60 min in all three rice cultivars. Incubation temperatures of 25°C and 27°C showed no significant difference and were optimal for pollen germination and pollen tube bursting in 'Nanatsuboshi' pollen. The observation of pollen tube behaviors suggested that the lengths of pollen tubes might be a determinant for inducing bursting.

This study developed a system for evaluating in vitro pollen viability. The optimal sucrose concentration, incubation time, and incubation temperature were determined for Japanese rice cultivars. This study implies that pollen tube length is a key factor influencing pollen tube bursting. Further studies on the biological processes from pollen germination to bursting may offer an understanding of the mechanisms of pollen tube elongation and bursting.

In this study, an efficient system for evaluating rice pollen viability is established. This system will contribute to estimating the crop yield. Furthermore, this system will be utilized for various crops with modifications and could be used for evaluating the condition of crop cultivation.