B-34: Isolation and characterizatian of pollen protoplasts of tea (*Camellia sinensis* L)

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Pollen protoplast provides a rapid method for haploid production and invaluable source for genetic transformation studies. The present study investigates a technique for isolation of pollen protoplasts and characterization of the nuclear state of pollen protoplast using DNA fluorochrome DAPI (4,6 - Diamidino-2-phenylindole). Flower buds of 4 tea clones were collected and surface sterilized with 3% Sodium hypochlorite for 10min followed by 3 rinses with sterile distilled water. The isolated pollen were germinated on a medium consisting of 10% w/v sucrose, 0.01w/v boric acid and 3% agar. The germinated pollens were incubated in different enzyme solutions (0.5% β 1-3-1 glucanase and 1% cellulase, 1% β 1-3-1 glucanase and 0.05% pectyolase, 0.5% β 1-3-1 glucanase, 0.1% β 1-3-1 glucanase and 0.2% β 1-3-1 glucanase) to isolate the pollen protoplast. The effect of pollen tube length on protoplast yield was observed by using the pollen after 1, 2 and 3 h of incubation period and treated with 0.2% β 1-3-1 glucanase and 0.5M sorbitol. The protoplasts were purified using centrifugation at 500rpm for 5 min with washing medium supplemented with 0.5M sorbitol, 125 mM$CaCl_2.2H_2O$, 154 mM NaCl and 0.5 mM KCl. A discontinuous sucrose gradient (10%, 20%, and 30%) centrifugation was used to separate the pollen protoplast. The nuclei of protoplast were stained with 1-2 drops of DAPI 0.02μg/ml solution. The pollen tubes with 250-300 μm length produced maximum number of protoplasts.

The pollen tubes longer than 500μm did not form protoplasts. The diameter of the protoplasts varied (10μm-40μm). About 40% of protoplast were nucleate. Pollen protoplast contained only generative nucleus or vegetative nucleus or both nuclei. Among the enzyme mixtures tested, the highest yield was obtained in 0.2% β 1-3-1 glucanase. This has been the first preliminary investigation on isolation and characterization of pollen protoplast in genus *Camellia.*