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Isolation of protoplasts from embryogenic cell suspension of *Camellia sinensis* L (Tea)

Tea is a woody plant a long term breeding program is required for the development of new cultivars with desired traits. Somatic hybridization through fusion of protoplasts provides a convenient method breeding. The present study investigates the optimum conditions for isolation of viable protoplasts from embroyogenic cell suspension of tea.

Suspension cells were harvested after 4,6,8, and 12 days after subculture and incubated in different enzyme mixtures for isolation of protoplasts. The enzymes used were

cellulose Rs, pectyolase Y23 and macerozyme R10. The ionic solution (CPW) was used to prepare the enzyme mixtures. Different concentrations of (3%,5%, &% and 11%) of mannitol and sorbitol were used to determine the best osmoticum for isolation of protoplasts.

The enzyme mixtures were filter sterilized at pH 5.8. For each isolation 3 g (fw) of packed cell volume of cell suspension was incubated in 14 cm Petri dishes containing 10 -12 mL enzyme solution and kept on a rotary shaker at 60 rpm in dark at at 27 ^{o}C. The incubation period varied 3,4,6,8 and 12 h. The protoplasts were filtered through a 45 µm nylon mesh after incubation. The filtrate was centrifuged at 600 rpm. For 5 min. and rinsed with CPW 9 M solution which contained 9% mannitol.

The viable protoplasts were separated using CPW solution containing 21% sucrose and were resuspended in CPW 9% mannitol solution. The protoplast counts were made using a Fush Rosanthal hamecytometer and viability was assessed using fluorescein diacetate.

Suspension cells in the logarithmic growth phase (8days after sub culture) produced the highest viability in isolated protoplasts. The quality, age and subculture period of the cell suspension directly influenced the yield and the viability of protoplasts. The enzyme mixture consists of 2% cellulose, 0.2% macerozyme RS and 0.02% pectyolase and 6 h incubation period showed the highest yield [6x104 g (fw0] of viable protoplasts. Among the osmoticums tested the best results were achieved with 9% mannitol. The optimum conditions elucidated for isolation of protoplasts will be used for the standardization of a protocol for the isolation and culture of protoplasts in tea.