Isolation and culture of protoplasts from leaf tissue of *Capsicum annnum* var. *accumnatum* Fingerh and *C. frutescens* L. [Syn. *C. minimum* Roxb.] (Bird chilli)

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Abstract

Isolation and culture of leaf protoplasts from two chilli cultivars (*Capsicum annuum* var. *accumnatum* and Bird chilli) were developed to enhance selection process in the somatic hybridization programmes. In order to isolate the protoplasts from leaves of these two chilli cultivars different incubation periods (3, 5 and 10 hours) were tested with combinations of enzyme mixtures containing cellulase and macerozyme. Leaves were incubated on three enzyme mixtures (2% cellulase + 0.4% macerozyme, 1% cellulase + 0.2% macerozyme and 0.5% cellulase + 0.1% macerozyme in 13% mannitol) at 251oC in the dark. Three hours of incubation using 2% cellulase and 0.4% macerozyme was the best for the protoplast isolation of both chilli cultivars tested. The yield was 5 x 108protoplasts/ml/g leaf tissue in both chilli varieties. It was found that in the mixed nurse method using Nagata and Takebe (NT) medium supplemented with 1.0mg/l 2,4-D, NAA and BAP with 0.5M mannitol and 1.2% Sea Plaque agarose is the best medium for protoplast culture. Protoplasts of *Capsicum annum* var. *accumnatum* were alive for 14 days forming cell walls and initiating cell division.