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# Protoplast fusion between *Brassica oleracea* and cytoplasmic male-sterile radish (*Raphanus sativus*)

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## Objectives

Cytoplasmic male steriliy (CMS) is a convenient method for the production of hybrid seeds, but its use in *Brassica* crops has been limited.　In this study, we have tried to make somatic hybrids through protoplast fusion between *Brassica oleracea* and a male-sterile radish line NWB-CMS (*Raphanus sativus*).

## Materials and Methods

1. Plant: HNA line (hypocotyls) of *Brassica oleracea*; NWB-CMS line (leaf) of *Raphanus sativus*

2. Protoplast isolation:

   - Hypocotyls and leaves of 5~21 day old seedlings

   - Enzyme treatment: Cellulase onozuka RS (SERVA) 1~2% / Macerozyme (SERVA) 0.1~0.5%
   　　　　　　　　　 / Pectolyase (YAKULT) 0.1%

3. Protoplast fusion: PEG method (M.W. 8000, 25%, 10 min)

4. Protoplast culture:

   - MS liquid medium + 2% glucose + 7% mannitol + BA 1 / NAA 1 / 2,4-D 0.25 (mg/l)

   - Micro-calli transferred to MS semi-solid medium + kinetin 2 / 2,4-D 0.5 (mg/l)

5. Shoot induction: MS + zeatin 2 / GA 0.035 (mg/l) + agar 1.6%

6. PCR and RAPD analysis: Use of NWB-CMS marker and UBC primers

## Results and Discussion

Protoplasts from cabbage and radish were isolated and fused by PEG treatment.　From the mixture of high concentrated protoplasts, lots of micro-calli were obtained.　The micro-calli grew to normal calli and shoots were regenerated from the calli.　A total of 24 shoots were regenerated, but none of them contained the NWB-CMS marker indicating that the cytoplasmic fusion for the radish NWB-CMS character into cabbage did not occurred.　However, based on the RAPD analysis revealed that nuclear fusion among protoplasts from radish and cabbage was formed in 3 out of 24 shoots.

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