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武庫川女子大紀要（自然科学）

Isolation and Fusion of Protoplasts from *Basella rubra* Leaf and Stem Cultures

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Isolation and fusion of protoplasts from *Basella rubra* leaf and stem were examined. In preparation of protoplasts, the enzyme solution gave a high yield of protoplasts. The diameter of protoplasts induced ranged from 30μm to 120μm. Cell division into 2 cells was observed after 24 hours of culture, then micro colonies formed after 7 days, followed by colony formation within 2 weeks.

Introduction

The leaves and stems of *Basella rubra* L. are used as a healthy vegetable. And also the leaves and stems are used as a medicine for fever.

In this study we isolated protoplasts from the leaf and stem of *B. rubra* and fused the protoplasts.

Materials and Methods

Isolation of protoplasts

Protoplasts were prepared from the fully expanded young leaf and stem. The leaf and stem were cut into small pieces, about 1 to 3mm wide, with a surgical knife. About 0.2g (fresh weight) of leaf and stem blocks were incubated in centrifuge tubes with 5ml of enzyme solution for 1 to 6 hours at 30℃ with shaking (90 reciprocation/min).

For protoplast isolation, enzyme solution of Koyama *et al.*[1] was modified and used in this experiment. Cellulase "Onozuka" RS was used instead of Cellulase "Onozuka" R-10 which had been used by Koyama *et al.* Furthermore, 1% Meiselase P1 and Hemicellulase were added originally to the solution. The isolated protoplasts were washed three times with 0.6M D-mannitol solution by centrifugation at 100 xg for 5 min. The number of protoplasts was counted on a hemacytometer(Thoma counting chamber). Yields of protoplasts were determined by averaging triplicates in each treatment.

Fusion equipment

A Shimadzu Somatic Hybridizer SSH-1 (Shimadzu Co., Japan)was used as a fusion apparatus and the fusion process was observed under an Olympus phase contrast microscope BHS-323, equipped with an Olympus camera OM-1. In order to observe the process of fusion of *B. rubra* protoplasts, a fusion microchamber, which was constructed from a slide glass with two parallel(1mm apart)aluminum foil (15μm thick)electrodes, was used.

Culture and viability of protoplasts isolated from leaf and stem

Protoplasts were cultured with MS[2] liquid medium containing 0.01μM of kinetin, 0.01μM of 2, 4-D and 0.6M D-mannitol without adding saccharose in micro plates(Corning Cell Wells)at 25℃ in the dark. The density of protoplasts was finally adusted to 1.0 X 10^5 ml^{-1}. Protoplasts were observed using an Olympus phase contrast

microscope BHS-323 (Olympus Co., Japan) during the culture.

Results and Discussion

Isolation of protoplasts

The diameter of protoplasts induced ranged from 30μm to 120μm. Protoplasts containing red pigment were isolated from stem tissues (Fig. 1.), whereas that were not isolated from leaf tissues (Fig.2.). Protoplasts were isolated more easily from leaf tissues.

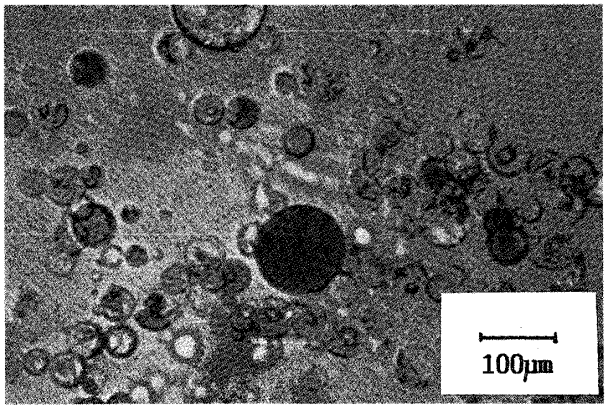

Fig. 1. Fresh protoplasts isolated from stem.

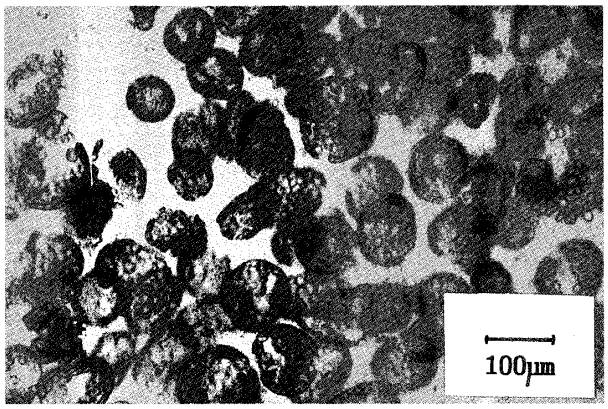

Fig. 2. Fresh protoplasts isolated from leaf.

Protoplast fusion

The concentration of protoplasts, the intensity of the alternating electric field and the time for dielectrophoresis were fixed at 1 X 10^5 protoplasts/ml, 100 V/cm strength, 1 MHz frequency and 60 seconds, respectively. Figure 3 show the fusion process of the protoplasts adhered dielectrophoretically.

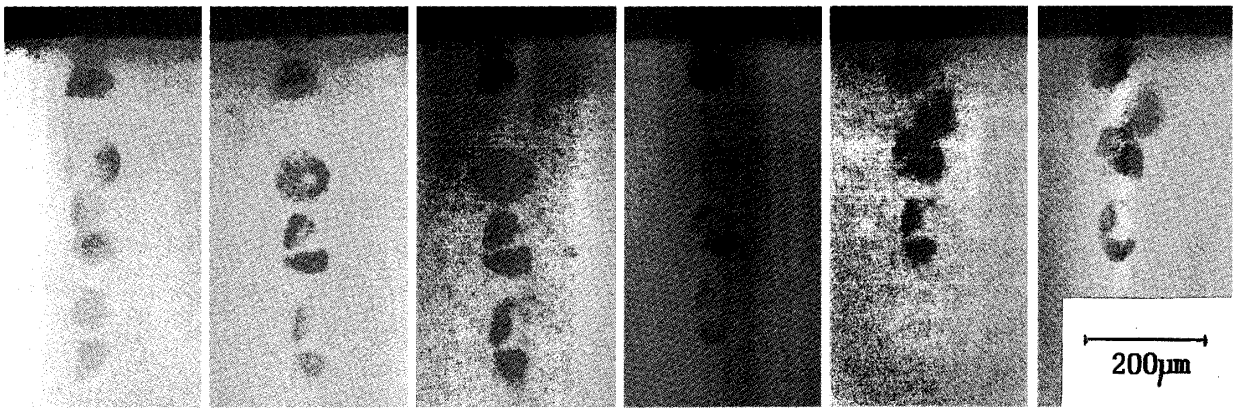

Fig. 3. Fusion process of *B. rubra* protoplast.

Culture and viability of protoplasts isolated from callus

After protoplast culture for 24 hours, several masses composed of two cells showing first cell division were observed, and 4-cell-stage cells appeared after 2 days of culture. Then, many micro colonies were observed after 7 days of culture. Furthermore, colony formation was observed within 2 weeks.

Protoplasting is an important technique for somatic hybridization by cell fusion and for genetic improvement by gene transfer. In this

study, determining the conditions of protoplast isolation and culture is the first step in transformation research of *B. rubra* although further investigations are still needed to establish the appropriate culture conditions for plantlet regeneration from protoplast derived leaves and stems.

Acknowledgments

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References

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2) Murashige, T. and Skoog, F., *Physiol. Plant.*, **15**, 473–497(1962).