

SHORT COMMUNICATION

CULTURE OF PROTOPLASTS FROM CHIMERAL PLANT TISSUE OF NATURE

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Received July 3, 1975

There are many chimeral plants induced by somatic cell mutation in nature. It can be expected that protoplasts from somatic cell mutants of chimeral plants are useful materials for plant breeding and genetics, because various mutant plants could be obtained from mutant cells.

This investigation was initiated to determine if *in vitro* protoplast cultures could be utilized to obtain mutant plants from cells of chimeral tissues and to study the interaction between normal cells and mutant cells. In the experiment, leaf protoplasts of variegated geranium, chlorophyll deficiency of the leaf margin (Fig. 1), were cultured in order to clarify what type of plants could be induced from cells of normal green and mutant white parts in the leaf.



Fig. 1. The variegated geranium with chlorophyll deficiency of the leaf margin. $\times 0.5$.

The expanding leaf was wiped with absorbent cotton containing 70% ethanol and dipped in the solution of HgCl_2 (0.1%) for 10 minutes. After then, the leaf was washed with sterile water twice. After drying in incubator (30°C) for 2 hours, removing epidermis, the leaf was divided into green and white parts. Protoplasts were prepared from each of the parts according to aseptic procedures described previously (Kameya and Uchimiya 1972; Kameya 1973). Protoplasts were suspended in culture medium

containing Murashige and Skoog's (1962) diluted medium of inorganic salts with various concentrations (1, 1/10, 1/100, 1/1000), 10% mannitol, 0.2% sucrose, 3-indoleacetic acid (1 ppm) and 6-benzyladenin (1 ppm). 3 ml of protoplast suspensions (protoplast, 2×10^4 /ml) were cultured in culture bottle (volume, 5 ml) under a fluorescent lamp (3000 lux) at 25° C.

Protoplasts from green part possess many large chloroplasts but ones from white part have a few small plastids (Fig. 2). Cultured protoplasts were investigated whether cell wall formation and cell division occurred or not according to the previous method (Kameya, 1973) after 10 and 20 days. As the result, cell wall formation was observed on 70-80% of protoplasts cultured in all of the medium. Frequency of cell division was much different among the medium with various concentration of inorganic salts; In 1/10 diluted medium, the ratio of the divided cells to total cells was 40-50%, and in 1, 1/100, and 1/1000, it was 10-15%, 20-25% and 10-15% respectively. However, the second division was not observed in the all mediums even after 25 days. So, protoplasts were cultured in 1/10 diluted medium for 10 days and then they were plated on the agar medium (agar, 0.8%) containing Murashige and Skoog's medium, 2% sucrose, 5% mannitol, 3-indoleacetic acid (1 ppm) and 6-benzyladenin (1 ppm). In the result, the

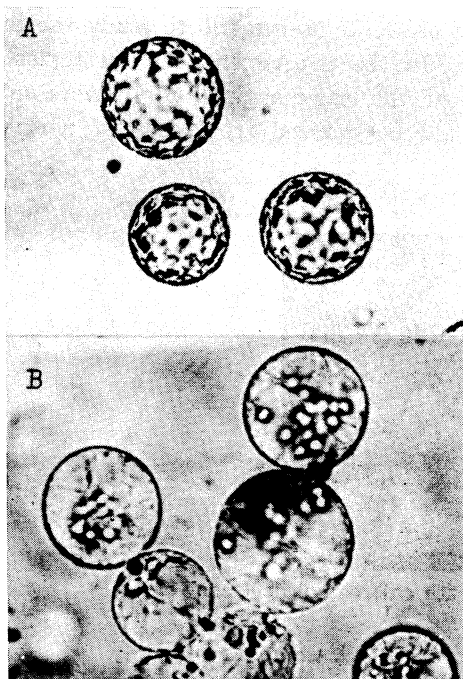


Fig. 2. The leaf protoplast at the time of isolation. A, protoplasts from green part. B, protoplasts from white part. $\times 400$.

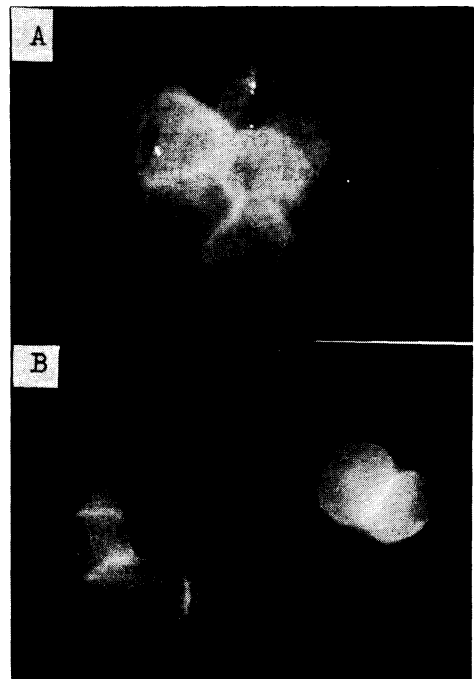


Fig. 3. Fluorescence micrograph of cultured protoplasts stained with Calcofluor White after 15 days. A, the division of protoplasts from green part. B, the division of protoplasts from white part. Protoplasts from green divided more quickly than ones from white part. $\times 400$.

cells developed into many colonies (size, about 1.0 mm) after 40-50 days. In the process, the cells derived from green part had a tendency to develop into colonies more quickly than those from white part (Fig. 3). After isolating larger ones from the colonies, they were cultured on the same agar medium omitted 5% mannitol. Two monthes latter, they developed into calluses but shoots did not differentiate. When the calluses were transplanted on the medium without 3-indoleacetic acid and 6-benzyl-adenin, plantlets began to differentiate after 20-30 days. Plants differentiated from the calluses derived from protoplasts of green part in the leaf were green and ones from white part were albino, but the original variegated plants were not obtained from both green and white parts (Figs. 4, 5).

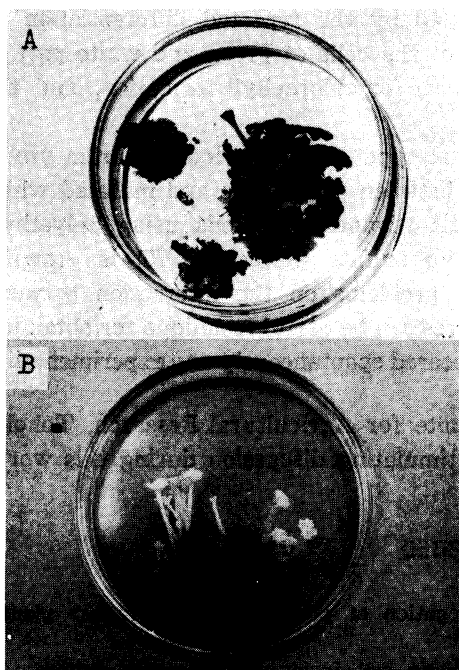


Fig. 4. Regeneration of plants from calluses. A, green plants differentiated from green part protoplasts. B, albino plants differentiated from white part protoplasts. $\times 0.75$.

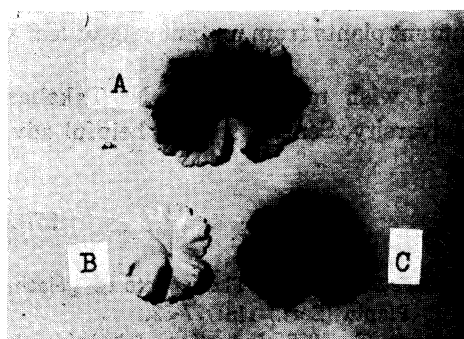


Fig. 5. The leaves of plants from culture of variegated leaf protoplasts. A, original variegated leaf. B, leaf of plants from white part. C, leaf of plant from green part. $\times 0.5$.

In recent years, plants from protoplasts have been regenerated in several species. Geranium can be added to the list. In the present experiment, cell wall formation occurred frequently in all medium but cell division was much different among the medium with different concentration of inorganic salts. And the second cell division did not occur in the liquid medium containing 10% mannitol but occurred on the agar medium containing 5% mannitol. This may mean that cell division is dependent on osmotic pressure in the medium, and it seems to be important for protoplast culture.

It is naturally observed that green shoots appear from the variegated geranium more quickly than those from white part. The cultured protoplasts derived from green

part also developed into colonies more buickly than those from white part. This may mean that the division capacity of wild cells is superior to one of mutant cells.

The plastome (Renner 1929, 1934) comprises all the extranuclear genetic factor which appear localized to the plastids and control their phenotype. When a wild type plastome and a mutated plastome occur together in a plant, the two plastome types segregate at the cell divisions and by this process homoplastomatic bleached and normal green cell lineages are produced (Michaelis 1957). According to Stewart *et al.* (1974), in herbceous plants such as geranium, plastogene mutants provided clear and precise markers of cell lineages in the mature plant, and the part of stem, leaf, or other structure derived from each of the mutant and wild apical layers was clearly apparent on a cell-to-cell basis. From the present experiment, it is also said that the mutant cells are stable and persistent, as indicated by the repeated differentiation of white plants from white part of leaf and each of the cells of green and white part in the variegated leaf possesses own genetic factor(s) for chlorophyll formation, and the leaf cells are homoplastomatic.

In future, interesting problems may be what type plants are induced from protoplasts of the apical layers and whether hybrids between green cells and mutated white cells through somatic cell fusion with the recent advanced methods using polyethylenglycol (Kao and Michayluk 1974) or dextran sulfate (Kameya 1975) is growing normally or not. Such a work may clarify the problems on the interaction between nucleus and plastid. Moreover, protoplast cultures can be useful technique for obtaining mutant plants from mutant cells of leaf which appeared spontaneously and experimentally.

I wish to thank Dr. N. Takahashi, Institute for Agricultural Research, Tohoku University, Sendai, for his helpful advice and stimulating discussion during this work.

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