Hybrid Cell Lines Produced by Protoplast Fusion between *Luffa cylindrica* ROEM. and *Gynostemma pentaphyllum* MAKINO

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Hybrid cell lines were produced by the intergeneric protoplast fusion between *Luffa cylindrica* ROEM. (*L. cylindrica*) and *Gynostemma pentaphyllum* MAKINO (*G. pentaphyllum*). Protoplasts derived from the cotyledons of *L. cylindrica* and protoplasts derived from the cultured cells of *G. pentaphyllum* were fused according to the method of MENCZEL and WOLFE. Four fusion products were confirmed as hybrid cells based on isozyme analysis.

KEY WORDS: Cucurbitaceae, *Luffa cylindrica*, *Gynostemma pentaphyllum*, protoplast, fusion

Introduction

The family Cucurbitaceae includes a number of important vegetables, fruit crops and traditional herbs. *L. cylindrica* have been used as a source of cosmetic material and traditional medicine. *G. pentaphyllum* have been also used as a traditional herb.

TAKEMOTO *et al.* (1983, 1984) found that *L. cylindrica* and *G. pentaphyllum* contained the same saponins as found in *Panax ginseng* C.A. MEYER.

In this family, there are several reports on the culture of protoplasts and the protoplast fusion (MATSUMOTO and TAKEBE 1987, MATSUMOTO *et al.* 1987, Tabei *et al.* 1989, YAMAGUCHI and SHIGA 1989, OOSAWA *et al.* 1989).

This study was conducted to produce hybrid cell lines by the intergeneric protoplast fusion between *L. cylindrica* and *G. pentaphyllum*.

Materials and Methods

Cotyledons from the axenic 7 days old seedlings of *L. cylindrica* and cultured cells from the cell suspension of *G. pentaphyllum* were used. The cultured cells were light brown and friable.

The cell suspension of *G. pentaphyllum* have been subcultured at 10 day intervals for more than a year using MURASHIGE and SKOOG (MS, 1962) medium supplemented with 0.5mg/l picrolam, 0.1mg/l kinetin and 0.2% casamino acid at 25°C with 12hr light (2,000 lux) period.

Cotyledon strips of *L. cylindrica* (0.5g) were incubated overnight at 25°C in 20ml of enzyme mixture containing 0.3% Meicelase (Meiji Seika Kaisha, LTD), 0.05% Macerozyme R-10 (Yakult Honsha CO., LTD) and 0.4M mannitol, pH 5.5.

To cells (1g) of *G. pentaphyllum* harvested 6 days after subculture of the cell suspension was added 20ml of enzyme mixture containing 1.0% Cellulase ONOZUKA R-10 (Yakult Honsha CO., LTD), 1.0% Driselase (Kyowa Hakko Kogyo CO., LTD), 0.5% Macerozyme R-10 and 0.4M mannitol, pH 5.5. The mixture was incubated at 25°C for 2hr.

Protoplasts of *G. pentaphyllum* were adjusted to a cell density of 2×10^6 cells/ml.

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Protoplasts of *L. cylindrica* were suspended in W5 solution including 10mM iodoacetoamide (IOA) (MENCZEL and WOLFE 1984) and incubated at 4°C for 10 min. The inactivated protoplasts were then washed twice with W5 solution and adjusted to a density of 2×10^6 cells/ml.

The IOA-treated protoplasts of *L. cylindrica* and untreated protoplasts of *G. pentaphyllum* were mixed together in equal volumes and fused according to the method of MENCZEL and WOLFE (1984).

Fused protoplasts were cultured in the medium for protoplasts of *L. cylindrica*; modified MS medium (half strength salts) supplemented with 1.0mg/l α -naphthaleneacetic acid (NAA), 0.5mg/l 6-benzyl-aminopurine, 1% sucrose and 0.3M mannitol at a cell density of 2×10^5 cells/ml. After 2 weeks of culture the osmotic pressure of the medium was reduced by adding the same medium supplemented with 0.2M mannitol.

All cultures were maintained at 25°C with 12hr light (2,000 lux) period.

Hybridity of the cell lines was determined by glucose-6-phosphate dehydrogenase (GDH) analysis utilizing native polyacrylamide gel electrophoresis (1mm thick gels, 7.5% acrylamide). Gels were stained for GDH activity according to a modified method of SING and BREWER (1968).

Chromosome number was observed microscopically according to the flame drying method of KURATA and OMURA (1978).

Results and Discussion

In this intergeneric fusion between protoplasts derived from cotyledon of *L. cylindrica* (Fig. 1A) and those from cultured cells of *G. pentaphyllum* (Fig. 1B), about 15 percent of the surviving population were fusants. After the fusion treatment, heterokaryons (Fig. 1C) had a green part from *L. cylindrica* and a colourless part from *G. pentaphyllum*. The heterokaryons started cell division 5 days after protoplast culture. IOA-treated protoplasts derived from cotyledon of *L. cylindrica* and untreated protoplasts derived from cultured cells of *G. pentaphyllum* did not divide in the used medium at all.

After 6 weeks of culture, small colonies (0.5-1mm diameter) were recovered. They were cultured on the agar medium for another a month. Finally 6 small calli were obtained.

These calli were named CF1, CF2, CF3, CF4, CF5 and CF6, respectively. CF3, CF4 and CF6 were yellow friable calli, and CF5 was a white friable callus.

However, the CF1 and CF2 were green compact calli, which looked alike those derived from protoplasts of *L. cylindrica* cotyledon. This morphological characteristic corresponded with the result of GDH isozyme analysis (Fig. 2). Varied isozyme changes of hybrid cells and gradual chromosome elimination of one parent had been reported (KAO 1977, WETTER 1977). In this study, it could be considered that the CF1 and CF2 gradually lost chromosomes of *G. pentaphyllum* after protoplast fusion.

Four calli (CF3, CF4, CF5, CF6) after 3 months of culture were confirmed as hybrid cells since they possessed parental homodimeric isozymes in GDH isozyme analysis (Fig. 2).

These calli had parental characteristics for phytohormone sensitivity, too.

L. cylindrica had a chromosome number $2n=26$ (Fig. 3A). *G. pentaphyllum* had a chromo-

Fig. 1. Protoplast fusion.

A : Protoplasts derived from cotyledon of *L. cylindrica*

B : Protoplasts derived from cultured cells of *G. pentaphyllum*

C : Heterokaryon (indicated by an arrow)

some number $2n=66$ (Fig. 3B). Almost all of cultured cells of *G. pentaphyllum* were composed of tetraploid (Fig. 3C), but some of them were aneuploids.

Chromosomes of the hybrid cells after 3 months of culture were investigated (Fig. 3D-G). Those hybrid cells had different chromosome numbers and were more than 158 ($26+132$).

The varied chromosome numbers of the hybrid cells might have derived from varied chromosome numbers of the cultures cells of *G. pentaphyllum*.

These four hybrid calli have been maintained by subculturing in MS medium supplemented with 1.0mg/l NAA (or 3.0mg/l IAA), 0.5mg/l kinetin and 0.2% casamino acid for 2 years.

These hybrid calli after 2 years of culture also possessed the parental homodimeric isozymes in GDH isozyme analysis (data not shown). The stability of their chromosome number could not be still confirmed since it was too many to count their chromosomes.

Fig. 2. Isozyme analysis of glucose-6-phosphate dehydrogenase.

G : Cultured cell of *G. pentaphyllum*

L : Callus derived from protoplasts of *L. cylindrica* cotyledon

Mix : G+L

CF1-CF6 : Fusion products

No shoot or root has been regenerated from these hybrid calli.

It was confirmed that these hybrid calli contained a saponin qualitatively. Therefore, we will try to analyze the contents of a saponin.

In conclusion, we produced hybrid cell lines by protoplast fusion between *L. cylindrica* and *G. pentaphyllum*. These cell lines would be useful materials for the study of the cucurbitaceae-breeding and saponin biosynthesis.

Fig. 3. Chromosomes of *L. cylindrica*, *G. pentaphyllum* and hybrid cells.

A : Root tip of *L. cylindrica*

B : Root tip of *G. pentaphyllum*

C : Tetraploid cultured cell of *G. pentaphyllum*

D : CF3 E : CF4 F : CF5 G : CF6

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ウリ科植物ヘチマとアマチャヅルの細胞融合による雑種細胞の作出

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ヘチマ, *Luffa cylindrica* ROEM. とアマチャヅル, *Gynostemma pentaphyllum* MAKINO の体細胞融合を行い, その雑種細胞を作出した. すなわち, 10 mM のヨードアセトアミド処理で不活化したヘチマの子葉プロトプラストと未処理のアマチャヅルの培養細胞プロトプラストを PEG-DMSO 法で融合した. 雑種細胞のみを選抜するために, 未処理のヘチマの子葉プロトプラストがコロニー形成し, かつ, 未処理のアマチャヅルの培養細胞プロトプラストが分裂できない培地を用いて培養した. その結果, 6 個の小カルスを得ることができ, そのうち, 4 個の小カルスが培養開始後 3 ヶ月目のアイソザイム分析において両親のバンドを共有し雑種性を示した (Fig. 2). これらの雑種カルスのうち, 3 個は黄色の柔らかいカルスであり, 残りの 1 個は白色の柔らかいカルスであった. これらの雑種カルスは培養開始後 2 年目のアイソザイム分析においても両親のバンドを共有していた. 雑種性を示さなかった 2 個の小カルスは緑色の硬いカルスで, 形態的にもアイソザイム分析においてもヘチマ子葉プロトプラスト由来のカルスとよく似ていた. 雑種カルスは, ホルモン要求性においても両親の性質を併せ持っていた. これらの雑種カルスの染色体数は非常に多く, また, それぞれ異なっていた (Fig. 3). この多様性は, アマチャヅル培養細胞における染色体数の多様性に依存しているのではないかとと思われる. これらの雑種カルスは根や植物体を再生しなかったが, 定性的にサポニン合成を確認することができた.