Original Papers Plant Tissue Culture Letters, 11(2), 129-133(1994)

Cultivation of *Sesamum indicum* L. Callus Cells at 35°C

Keiichi TAKEBAYASHI*, Akio MIMURA*, Akira ICHIKAWA*,
Mitsuru NIWANO*, Yoshimasa TAKAHARA* and Tosihiko OSAWA**

**Biotechnology Research Laboratory, Kobe Steel Ltd.*, Tsukuba 305, Japan
***Department of Food Science and Technology, Nagoya University*, Nagoya 464, Japan

(Received November 5, 1993)
(Accepted February 26, 1994)

We induced sesame callus from segmens of seedlings. The growth rate was greatly influenced by the incubation temperature. Sesame callus grew most rapidly at 35°C, with the shortest doubling time of 1. 1 days(μ max= 0. 63). The sesame cell extracts showed antioxidative activity.

Introduction

Sesame seeds and sesame oil have been evaluated as one of the familiar health foods ancient times. Sesame seeds contain rich nutrients such as oils, proteins, sugars, fiber, minerals and vitamins. Sesame oil, which occupies 50 to 60% of the seed mass, includes a large amount of unsaturated fatty acid[1].

In sesame seeds, α-tocopherol and sesamol are known to be antioxidative compounds. It is known that several lignan compounds and phenolic compounds have potent antioxidative properties[2-4]. It is considered that these antioxidative compounds play an important role in preventing oxidative damage in sesame seeds[5].

In view of sesame antioxidants, we have been studying the production of antioxidants by using plant cell cultivation technology.

Materials and Methods

Chemicals: Components of Murashige and Skoog's(MS)[6] medium and kinetin, 6-benzyladenine (BA), 2, 4-dichlorophenoxyacetic acid(2, 4-D), 1-naphthaleneacetic acid(NAA)were purchased from Wako Pure Chemical Co.. Sugar and sodium hypochlorite were purchased from Wako Pure Chemical Co.. Rabbit preserved blood was purchased from Kojin-Bio Co.. *tert*-Butyl hydroperoxid was purchased from Katayama Chemical Co.. Trichloroacetic acid and thiobarbituric acid were purchased from Tokyo-kasei Co..

Callus induction: Seeds of *Sesamum indicum* L. were sterilized with 1% sodium hypochlorite for 30 min. and washed five times with sterilized distilled-water. For the seedling, BA(1×10^{-5} M), NAA(1×10^{-5} M), 30 g/l of sucrose and 2 g/l of gellan gum were added to MS medium, and the pH was adjusted to 5. 7(MS solid medium). The seeds were incubated at 27°C for 1 week in the dark. In callus induction from seedling, the segments of seedling were cultured on MS solid medium.

Solid culture: BA or kinetin as cytokinin, NAA or 2, 4-dichlorophenoxyacetic acid(2, 4-D)as auxin were tested as plant growth regulators. The examination of cultivation temperature for optimum cell growth was performed at various temperatures between 20 and 43°C. Three grams

of callus was inoculated on MS solid medium in each culture tube, and incubated for 10 days.

Liquid culture: Liquid media were prepared by combining the modified components of MS basal medium. The effects of sugar as carbon source were tested. Xylose and arabinose were used as pentose. Glucose, fructose, galactose and mannose were used as hexose. Sucrose, maltose, lactose and cellobiose were used as disaccharide. The initial pH of the liquid medium was adjusted to 5.7 by adding 1 N sodium hydroxide solution.

Cells cultured with 60 m*l* modified MS liquid medium in 300 m*l* silicone foam-plugged flask were shaken at 60 strokes/min. using reciprocating shakers. Cells cultured with 30 m*l* modified MS liquid medium in 30 mmϕ silicone foam-plugged culture tube, were rotated at an angle of 15 degrees, 10 rpm, 30 cm-diameter cycle using drum-shape rotary shakers. The seed cells were passed through 1.0 mm (mesh aperture) sieve when sub-cultures were prepared.

Extraction of antioxidative compounds: Fifty grams of fresh cells were soaked in 1 liter of 80% ethanol and agitated using a high-speed homogenizer. The 80% ethanol extract was filtered with suction through paper filters (TOYO No. 2), and the filtrate was concentrated *in vacuo* at 40°C.

Assay of antioxidative activity: Antioxidative activities of the cell extracts were assayed with rabbit erythrocyte membrane[7]. The 4 m*l* reaction mixtures contained 2.25 mg erythrocyte membrane lipid, cell extracts, 0.5 M trichloroacetic acid, 100 μM thiobarbituric acid, and the reaction mixtures were initiated by adding 0.4 μM *tert*-butyl hydroperoxid. The antioxidative activities of the cell extracts were determined by measuring the concentration (μg/m*l*) that inhibits 50% of rabbit erythrocyte lipid peroxidation (IC_{50}).

Results and Discussion

The combination of plant growth regulators for optimum cell growth was tested. The concentration of kinetin or BA as cytokinin was fixed at 1×10^{-5} M. The concentrations of 2, 4-D or NAA as auxin was varied between 0 and 5×10^{-4} M, and combined with cytokinin at the fixed concentration for cell propagation. When 1×10^{-5} M kinetin and 8×10^{-8} M 2, 4-D were added to MS solid

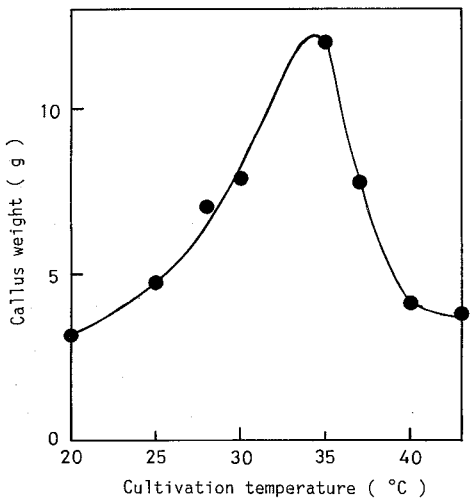

Fig. 1 Effect of temperature on the cell growth of *Sesamum indicum* L.
The incubation temperatures for optimum cell growth was performed at various temperatures between 20 and 43°C. Three grams (fresh weight) of callus was inoculated on MS solid medium in each culture tube. The culture tubes were incubated for 10 days and fresh weight of callus was measured.

medium, the callus cells showed the fastest growth (data not shown).

We then tested the incubation temperature for optimum cell growth. Though the optimum temperature for the growth of plant cells is reported to be between 20 and 30°C[8], the sesame callus cells showed the fastest growth between 34 and 36°C (**Fig. 1**).

The effects of the medium composition for liquid culture were examined by changing the concentration of the components. The components of MS basal medium were separated and the concentration of each component was altered. The altered components were then combined. The modified MS medium was prepared with 2-fold dilution of NH_4NO_3, KNO_3, $CaCl_2 \cdot 2H_2O$, $MgSO_4 \cdot 7H_2O$, KH_2PO_4 as main mineral contents to which was added kinetin (1×10^{-5} M) and 2, 4-D (8×10^{-8} M) and sucrose (final conc. 3%). When the cells were grown in the MS basal medium, the shortest doubling time was 1.7 days. Cells grew faster in the modified MS medium. The shortest doubling time in the modified MS medium was 1.1 days.

Effect of carbon source on the cell growth was examined. Pentose, hexose and disaccharide as carbon sources for cell proliferation were examined. The medium which contained sucrose conferred the fastest growth on the cells (**Fig. 2-a**). Pentose was not effectively metabolized. The cultures with the media which contained hexose such as glucose and galactose exhibited good growth. The optimum sucrose concentration in modified MS for cell growth was 3% (final concentration) (**Fig. 2-b**).

The growth curves of sesame cell culture under various conditions are shown in **Fig. 3**. The culture with MS solid medium at 27°C exhibited the shortest doubling time of 7.2 days. The culture with MS solid medium at 35°C showed the shortest doubling time of 4.2 days. The cells grown in the modified MS liquid medium at 35°C showed the fastest proliferation. The shortest doubling time was 1.1 days (μ max=0.63/day). The sesame cells were found to acquire heat adaptation

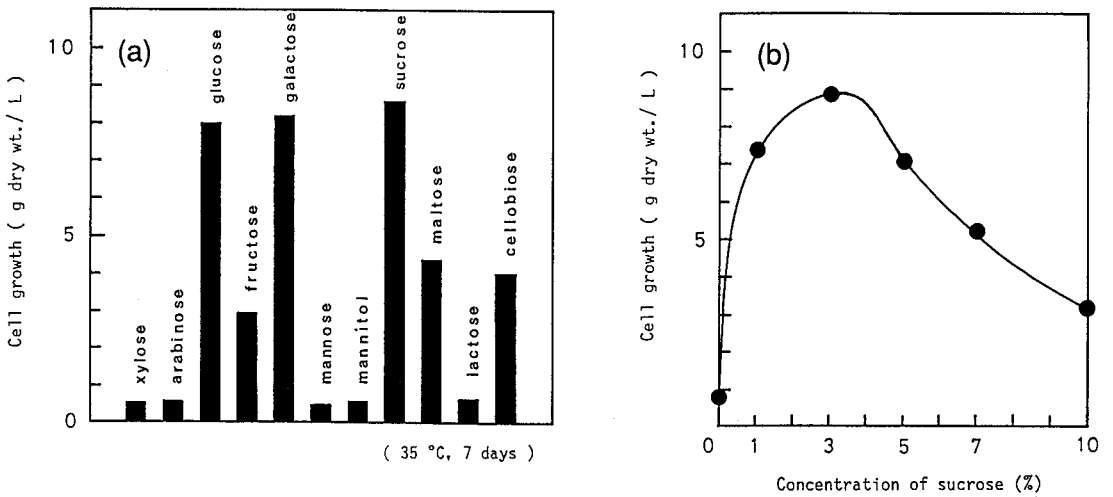

Fig. 2 Effect of sugar on the cell growth of *Sesamum indicum* L.
(a) Effect of various sugar on cell proliferation.
Pentose, hexose and disaccharide were used as carbon source. The cell cultures were performed with modified MS liquid medium which contained 3% sugar (final concentration) at 35°C for 7 days. Cell growth was estimated by measuring the dry weight.
(b) Effect of sucrose concentration on cell proliferation.
The final concentration of the sucrose in the liquid medium was prepared between 0 and 10%. The cell cultures were performed with modified MS liquid medium at 35°C for 7 days. Cell growth was estimated by measuring the dry weight.

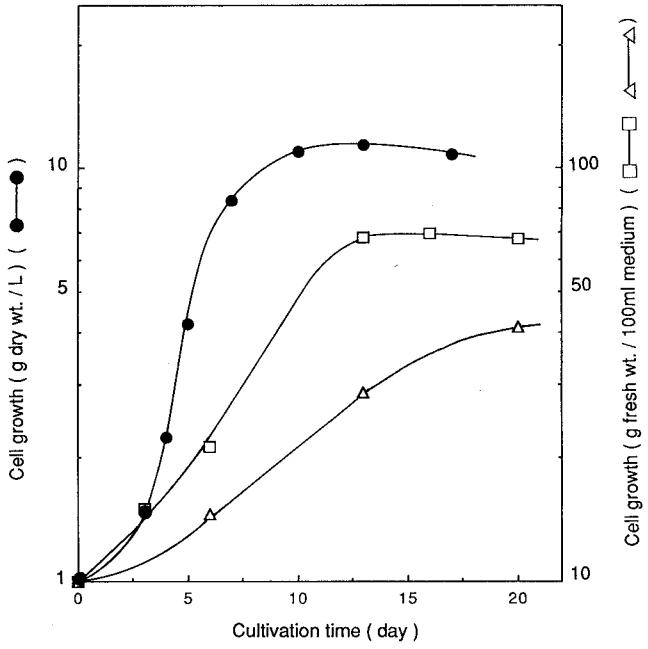

Fig. 3 Growth profiles of *Sesamum indicum* L. callus cell.
Symbols : (open triangle): profile of the cell growth with MS solid medium at 27°C, (open square): profile of the cell growth with MS solid medium at 35°C, (closed circle): profile of the cell growth with modified MS liquid medium in 35°C by reciprocal shaken culture. Cell growth was estimated by measuring the dry weight. Cell dry weight was determined by drying washed cells in a 105°C oven for 16 hr and placed in a desiccator until they reached a constant weight.

after subcultures at 35°C for more than 6 months. They grew rapidly in liquid culture. There have been no studies reporting a shorter doubling time than 1 day in plant cell culture, except tobacco callus cells with 0. 69 days as the stortest doubling time[9]. This is the first report that a plant callus was able to grow at such a high temperature as 35°C.

Antioxidative activities of cell extracts were assayed. Sesame cultured cells were cultivated at 35°C and sampled at the 5th day which was a logarithmic phase, at the 7th day which was a stationary phase and at the 10th day which was a declining phase. Antioxidative activity of each cell extract was assayed using rabbit erythrocyte membrane as a substrate[7]. IC_{50} values of the antioxidative activities of the extracts from the 5th day of culture, the 7th day of culture and the 10th day of culture were 175 $\mu g/ml$, 375 $\mu g/ml$, 625 $\mu g/ml$, respectively. The yields of 80% ethanol extracts were 50%, 55. 1% and 43. 1% of dry cell weight. In terms of dry cell weight, IC_{50} values were 350 $\mu g/ml$, 680 $\mu g/ml$, 1450 $\mu g/ml$, respectively. The antioxidative activity was the highest when the callus cells were exponentially growing. The antioxidative activities from the seeds, leaves and stems of the *Sesamum indicum* L. were assayed. The IC_{50} value of each ethanol extract was 335 $\mu g/ml$, 195 $\mu g/ml$, 2300 $\mu g/ml$, respectively.

Though sesamol and tocopherols, which have potent antioxidative activity in sesame seeds[2-4], were not detected in callus cells by HPLC analysis(data not shown), antioxidative activity was found in the cell extracts. These results suggested that cultivated sesame cells contained other compounds with potent antioxidative activity.

Though effects of antioxidative activities by cultivation temperature have not been precisely

tested, the antioxidative activity of the extract from cells which were cultivated at 27°C (IC_{50}: 900 $\mu g/ml$) was lower than that at 35°C. Therefore it was suggested that the productivity of antioxidative components increase at higher temperature.

The origin of sesame plant is the African tropical savanna[10] which has an atmospheric temperature of about 30°C throughout a year and sometimes over 40°C in the daytime. All of the wild type sesame were summer crops which were fond of high temperature (above 30°C). Though the optimum growth temperature of the plant has not been precisely examined, it is assumed that its genetic character enabled the plant to adapt to high temperatures. Sesame cultured cells are considered to be protected from injuries caused by external stress which involved temperature, light, UV and oxygen free radicals through the accumulation of antioxidative compounds in the cells.

Acknowledgment

The authors wish to thank Miss Fumi Itoh for assaying antioxidative activities.

References

1) Kobayashi, T., 1977. The Heredity. **31**: 54-64.
2) Osawa, T., M. Namiki, 1981. Bioscience and Biotechnology, **19**: 364-365.
3) Fukuda, Y., T. Osawa, M. Namiki, T. Ozaki, 1985. Agric. Biol. Chem., **49**: 301-306.
4) Osawa, T., M. Nagata, M. Namiki, Y. Fukuda, 1985. Agric. Biol. Chem., **49**: 3351-3352.
5) Osawa, T., 1985. Food Chem., **1**(5): 42-50.
6) Murashige, T., F. Skoog, 1962. Physiol. Plant., **15**: 473-497.
7) Osawa, T., A. Ide, Su, J-De., M. Namiki, 1987. J. Agric. Food. Chem., **35**: 808-812.
8) Harada, H., A. Komamine, 1979. In "Plant Cell Tissue Culture" (eds. by Harada, H., A. Komamine), p. 31, Rikougakushya, Tokyo.
9) Azeti, S., 1982. Cell Technology, **1**(3): 261-265.
10) Nayar, M. M., K. L. Mehra, 1970. Sesame, **24**: 20-30.

《和文要約》

35°C におけるゴマ (*Sesamum indicum* L.) カルス細胞の培養

竹林恵一*・三村精男*・市川　明*・庭野　満*・高原義昌*・大澤俊彦**

* ㈱神戸製鋼所生物研究所
** 名古屋大学農学部食品工業化学科

我々は，黒ゴマ種子から得られた芽生えよりカルス誘導を行い細胞培養を試みた．細胞培養条件の検討を行ったところ，培養温度が細胞の増殖に著しい影響を与えた．ゴマ培養細胞は植物細胞培養では通常行われていない 35°C 付近の高温度で旺盛に増殖し，その最短世代時間は 1.1 日 ($\mu max = 0.63$) であった．また，得られた培養細胞抽出物には抗酸化活性が認められた．