Protoplast liberation, cell wall reconstitution, and callus proliferation in **Coffea arabica** L. callus tissues*[1/]

M. R. SONDAHL**, M. S. CHAPMAN***, W. R. SHARP****

RESUMO

Uma metodologia para liberação e cultura de protoplastos de tecidos de café foi definida a fim de permitir a possibilidade de manipulação genética dentro do genero Coffea. *Uma combinação de 2,5% Pectinase, 3,5 por cento, Driselase, 0,51 molal manitol e 6 mM $CaCl_2$ em pH 5,5 foi considerada ótima para liberação de protoplastos de células de calos de café após 7 horas de incubação a 50 rpm. A preparação de protoplastos foi purificada por filtração através duas peneiras de aço inoxidável de 150 e 38 μm. O filtrato foi coletado em tubos de centrifuga, centrifugado a 100 g por 3 min, o supernadante foi removido e o pellet de protoplastos foi resuspenso em 3 ml de meio de regeneração de parede. Esta rotina foi repetida tres vez para diluir os enzimas e eliminar os debris celulares. Finalmente, o pellet foi resuspenso em 1 - 2,5 ml de meio de regeneração de parede para atiingir uma densidade de 10^5 protoplastos/ml. Suspensões de protoplastos de 0,5 ml foram cultivadas em placas de cultura contendo múltipas divisões em condições de luz difusa e alta humidade. Após 5 dias, o meio de regeneração de parede foi diluído com o meio de crescimento e a regeneração de parede celular em protoplastos foi observada com o uso de Calcofluor 0,1 por cento sob microscópio fluorescente. A regeneração de parede celular e a proliferação de calos foram observados em cerca de 30 por cento das culturas.*

Introducción

THE genus *Coffea* has ca. 70 species of which *C. arabica* is economically the most important. This species accounts for 70 per cent of the coffee in the international trade market (3). Moreover, *C. arabica* is the only self-pollinated tetraploid species in the genus *Coffea*. This makes the transference of genetic traits from wild outbred species of the genus to the cultivated *C. arabica* cultivars difficult.

The potential for making protoplast fusion products between dihaploid *C. arabica* protoplasts and somatic protoplasts of the wild species of *Coffea*, is promising for genetic transfer of traits and the development of new commercial cultivars. The development of plants from such protoplast fusions appears feasible because of the recent success in the induction of high frequency somatic embryogenesis in leaf explant derived callus tissues of five *C. arabica* cultivars ('Bourbon', 'Mundo Novo', 'Catuai', 'Laurina', and 'Purpuracens') as well as three diploid species, *C. canephora*, *C. congensis* and *C. Dewevrei*, (5, 6, 8). This communication pertains to the development of a protocol for protoplast liberation, cell wall reconstitution and induction of callus proliferation in *C. arabica* cv. 'Bourbon' callus tissues.

* Received for publication December 11th, 1979.

1/ Supported by the National Council of Research ann Technology (CNPq), Brasilia, Brazil; College of Arts and Sciences; and the Graduate School, Ohio State University, Columbus, USA.

** Department of Genetics, Instituto Agronômico, P. O. Box 28, 13.000 Campinas SP, Brazil.

*** School of Medicine, Case Western Research University, Cleveland, Ohio, U.S.A.

**** Pioneer Research Laboratory, Campbell Institute for Agricultural Research, Cinnaminson, N. J. 08077, U.S.A.

Materials and methods

Tissues used for protoplast isolation and culture were obtained from *C. arabica* cv. 'Bourbon' 30 - 60 day old callus. Callus tissues originated from mature leaf explants were cultured on a modified Murashige and Skoog (4) medium supplemented with 5 μM of 2,4-dichlorophenoxy-acetic acid (2,4-D), 10 μM of kinetin, 117 mM of sucrose, and 8 g/l of Difco-Agar.

The enzyme solution consisted of pectinase (Sigma), Driselase (Kyowa Hakko Kogyo, 35% Driselase/65% corn starch by weight) with the addition of $CaCl_2$

(6 mM) and mannitol (0 51 molal). The enzyme solution was equilibrated at pH 5.5 and filter sterilized using 0 45 μm Nalgene membranes. Fifteen milliliters of the enzyme solution were dispensed into 250 ml Erlenmeyer flasks containing *ca.* 1.5 g f w. of callus tissue. Thereafter, protoplasts were liberated on a rotatory shaker (50 rpm) at 25°C during 7-8 hs.

The protoplast preparation was purified by filtration through two stainless steel sieves of 150 and 38 μm pore sizes. Non-protoplast material, e.g. cell clumps, explant residues, and intact cells were retained by the sieves, allowing the enzyme solution containing protoplasts and suspended cellular debris to pass. The filtrate was collected in sterile Petri dishes for separation of the cellular debris from the isolated protoplasts and transfered to sterile screw cap centrifuge tubes. The tubes were centrifuged at 100 g for 3 min., the supernatant aspirated off, and the protoplast pellet resuspended in 3 ml of Cell Wall Regeneration Medium (CWRM). The resuspended pellet was centrifuged at 100 g for 3 min and washed in 3 ml of CWRM three times to dilute the enzymes and eliminate the cellular debris. Finally, the protoplast pellet was resuspended in 1.0 ml of CWRM and the concentration density determined using a haemacytometer. Thereafter, the pellet was diluted to a plating density of 10^5 protoplasts/ml in CWRM.

The CWRM consisted of half strength Murashige and Skoog (4) inorganic salts, glucose (0.3 M), xylose (3 mM mannitol (o.35 molal), meso-inositol (550 μM), thiamine (3 μM) pyrodoxine (5 μM), and nicotinic acid (10 μM). This was supplemented with 2.5 μM naphtalene acetic acid (NAA) and 10 μM kinetin. The pH was adjusted to 5 5 before filter sterilization using 0.45 μm Nalgene membranes. Protoplast suspensions of 0 5 ml were cultured in Falcon multiwell culture dishes (1.5 cm diameter), under a 12 hr photoperiod of diffuse light, high humidity and a temperature of 26°C. The dishes were sealed with parafilm and placed inside metal trays containing a layer of sterile distilled water for maintenance of high humidity. After 5 days, the liquid medium was supplemented with four drops of sterile Growth Medium (GM) containing half strength MS inorganic salts, meso-inositol (550 μM), thiamine (30 μM), cysteine (210 μM), sucrose (58 5 mM), 2.5 μM NAA and 10 μM kinetin. The medium was autoclaved for 20 min at 20 psi. After two weeks the cells were transferred to GM agar cultures with sterile Pasteur pipettes.

A Zeiss Universal microscope equipped with a Nikon camera and haemacytometer was used for determining protoplast density. One haemacytometer field was equal to 1 0 mm^2 and 0 2 mm deep. Cell wall regeneration was confirmed by staining with 0 1% solution of Calcofluor and flourescence microscopy (2). Cell cluster were viewed after transfer of callus tissues from the agar surface onto microscope slides. All photographs were taken using Ektachrome tungesten light sensitive of Panatomic-X films.

Results and discussion

A major concern in the development of a protocol for the obtainment of viable coffee callus protoplasts was the determination of the proper enzyme concentration and the osmolality for free cell isolation and protoplast liberation.

Initially the osmolality was kept constant at 990 mOs and the tissues treated with varying concentrations of pectinase to determine the proper concentration and incubation period necessary for cell isolation. Concentrations of mannitol (0 8 M) and $CaCl_2$ (6 mM), the rate of shaking (80 rpm), and callus age were held constant. Data from experiments using callus tissues subjected to 1 - 5% pectinase concentrations are summarized in Table 1. It was apparent that within the first few hours more cells were liberated at the higher pectinase concentrations. However, the cells at 4 and 5% pectinase were deformed, and their viability was questionable. Therefore, an intermediate concenration of 2.5% pectinase was used in future experiments.

Subsequent experiments characterized the concentrations of Driselase for protoplast liberation. Non-friable 8 week-old callus tissues and 7 week-old friable callus tissues were treated with 2.5% pectinase and varying concentrations of Driselase to determine the proper enzyme concentration for protoplast liberation. A yield of 0.2 x 10^6 protoplasts/ml was liberated from the non-friable 8 week old callus tissues incubated in 3 5% Driselase for 5 hr (Table 2). Greater concentrations of protoplasts were found at 5% Driselase, however these were mostly non-viable. Protoplast yields of 1.3 x 10^6 per ml were obtained from 7 week old friable callus tissues after 5 hr of incubation in 3 5% Driselase (Table 2). Friable callus tissues proved to be the most suitable source for high yields of isolated single cells and protoplasts.

Table 1.—Single cell liberation from coffee callus tissues submitted to five different pectinase concentrations after different incubation times. Data expressed in numbers of single cells/ml x 10^6 and represent an average of 15 to 20 counts of haemacytometer fields. All other factors were held constant.

Time (hr)	Pectinase concentration (%)				
	1	2	3	4	5
1	0 00	0 06	0 70	0 40	0 50
2	0 30	0 24	1 00	0 50	0 60
3	0 50	0 50	0 80	0 74	0 90
4	0 60	0 70	1 20	0 90	1 20
5	0 70	0 60	0 60	0 80	1 30
6	1 80	0 90	1 30	1 00	1 30

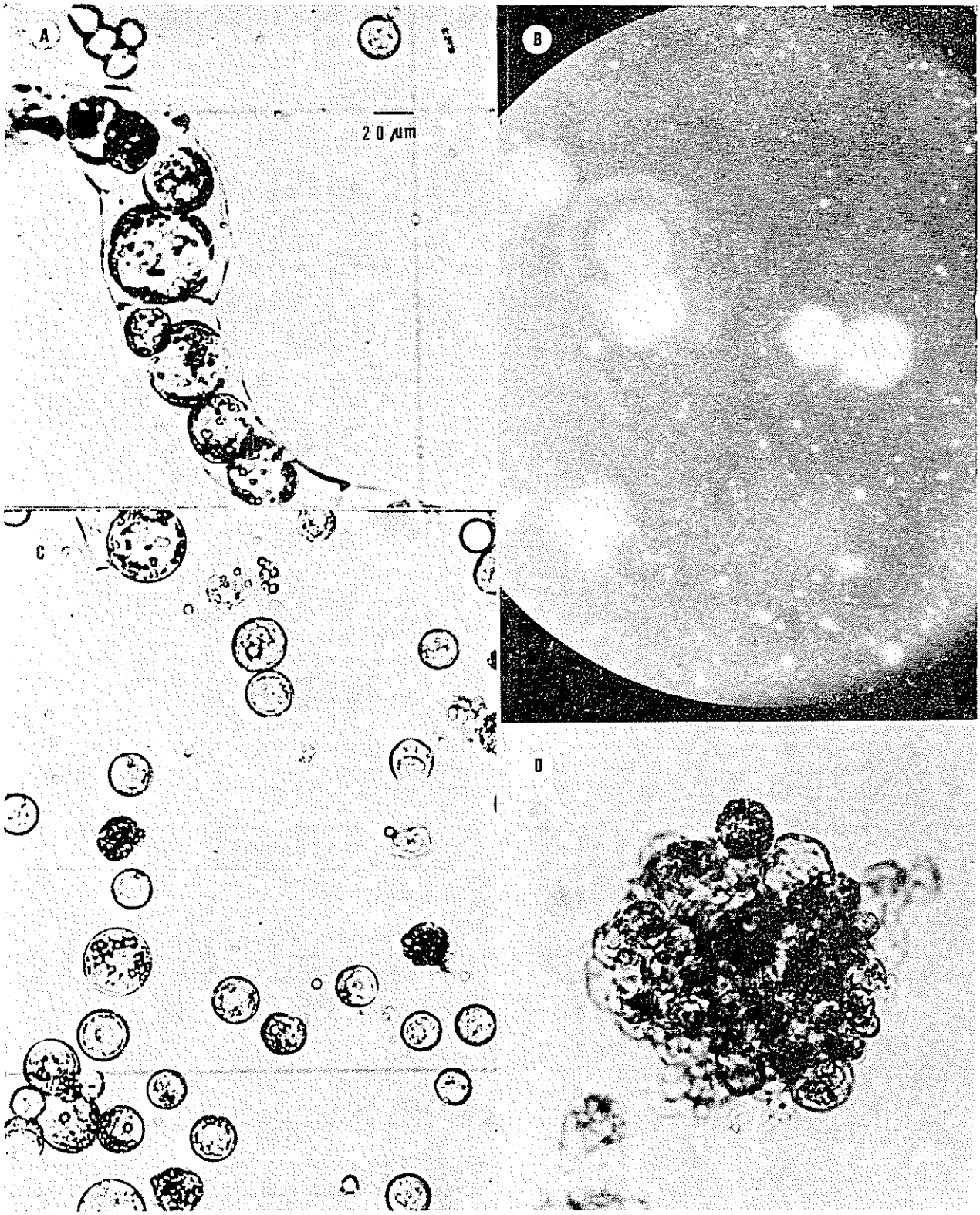

Fig 1—*Protoplasts and regenerated cells from* Coffea arabica *callus tissues (400 X) (A) Sub-propoplast formation at high osmolalities (990 to 1470 mOs); (B) Protoplast isolation following sieve filtration and centrifugation; (C) Cell wall regeneration in protoplasts as demonstrated by Calcofluor fluorescense under U V light, (D) A typical cell cluster originated from protoplast regenerated after 8-week growth period.*

Table 2.—Protoplast liberation from non-friable 8 week old coffee callus tissues and friable 7 week old callus tissues treated with 2,5% pectinase, osmolality 620 mOs, and varying concentrations of Driselase after four incubation periods. Data expressed in number of protoplasts/ml x 10^6 and represent an average of 25 to 30 counts of haemacytometer fields.

	Driselase concentration (%)					
	8-week non-friable callus			7-week friable callus		
Time (hr.)	2.0	3.5	5.0	1.0	3.5	5.0
5	0.04	0.20	0.08	0.10	1.30	1.20
6	0.08	0.20	0.11	0.40	1.20	1.10
7	0.05	0.20	—	0.14	1.14	1.00
8	—	—	—	0.20	1.10	0.60

The ideal osmolality for stabilization was determined prior to the plating of protoplasts. Mannitol concentrations were used to provide 300, 620, 740, 860, 990, 1230 and 1470 mOs at 25°C. Photographs were taken at different time intervals to determine the effects of osmolality on protoplast stabilization. At 990 and 1470 mOs, high frequencies of protoplasts as well as "pinching off" of subprotoplasts (*ca.* 15 μm diameter) were observed (Fig. 1A). Apparently the high osmolality was responsible for protoplast subdivisions and high plasmolysis. The former resulted in the occurrence of nucleated and enucleated subprotoplasts (1). Therefore, osmolalities above 990 mOs were avoided in future experiments. Since an incipient degree of plasmolysis was observed in isolated cells immersed in solutions of 740 mOs and an extensive amount of cellular debris occurred at 500 mOs, solutions containing a total osmolality of 620 mOs were adopted for subsequent experiment.

Table 3.—Measurements of (A) some single cells isolated from 7-week old callus tissues treated with 2% pectinase for 6 hr., and (B) some isolated protoplasts from 7-week old coffee callus tissues at the maximum depth of the microscope field.

	(A) Single Cells		(B) Protoplasts
	Length (μm)	Width (μm)	Diam (μm)
	292.6	46.2	69.3
	231.0	38.5	61.6
	215.6	46.2	57.7
	192.5	38.5	46.2
	154.0	38.8	38.5
	100.1	38.5	38.0
	84.7	30.8	30.8
	69.6	53.9	30.6
	61.6	23.1	27.0
	38.5	38.5	23.1
Mean	144.0	39.3	42.3

Measurements of single cells and protoplasts were made following incubation in pectinase and Driselase to determine the proper sieve size for mechanical separation (Table 3). The average dimensions (length x width) for isolated single cell was *ca.* 144.0 μm x 39.3 μm respectively, while the average protoplast diameter was 42.3 μm. The fact that the average single cell width is slightly smaller than the protoplast diameter did not present a problem with the sieve separation. The single cells were comparatively longer than wide and so only at the perpendicular orientation could they pass through the sieve.

The protoplast incubation solution was sequentially passed through stainless steel sieves of 150 μm, 75 μm, 45 μm, and 38 μm pore sizes. After passage through the 150 μm pore size sieve, the filtrate contained protoplasts, single cells, cellular aggregates and subcellular debris. Sieve sizes of 75 μm and below prevented the passage of undigested cell clumps and the frequency of single cells diminished with decreasing sieve pore size with fewer than 1% single cells passing through the 38 μm sieve. This sieve restricted the passage of large protoplasts, however protoplasts having diameters approaching 50 μm were still able to pass due to flexibility of the plasmalema.

Alternate centrifugation at 100 g, aspiration of the supernatant, and resuspension of the protoplasts in fresh CWRM repeated three times, was found sufficient to eliminate most of the cellular debris following sieve filtration (Fig. 1B). Cell wall regeneration was traced using Calcofluor staining and fluorescence microscopy (Fig. 1C). A typical cell cluster observed following cell wall reconstitution and growth on agar medium for 8 weeks is presented in Fig. 1D).

Conclusions

It is apparent that protoplasts may be obtained from friable callus tissues of *C. arabica*, and they undergo cell wall regeneration and cell proliferation with some success. The conditions found ideal for the isolation of *C. arabica* callus tissue were the following: 3.5% Driselase, 2.5% pectinase, 0.51 molal mannitol, 6 mM $CaCl_2$, shaking rate at 50 rpm, and friable callus tissues aging 4 - 5 weeks.

Summary

A protocol for protoplast liberation and culture of coffee cells was defined in order to allow the possibility of genetic manipulation within the genus *Coffea*. A combination of 2.5% pectinase, 3.5% Driselase, 0.51 molal mannitol, and 6 mM $CaCl_2$ at a pH of 5.5 was found optimal for protoplast liberation of callus coffee cell after 6 - 7 hr. of incubation at 50 rpm. The protoplast preparation was purified by filtration through two stainless steel sieves of 150 μm and 38 μm. The filtrate was collected in centrifuge tubes, centrifuged at 100 g for 3 min, the supernatant aspirated off, and the protoplast pellet resuspended in 3 ml of cell wall regeneration medium (CWRM). This procedure was repeated three times to dilute the enzymes and eliminate the cellular debris. Finally, the pellet was resuspended in 1 - 2.5 ml of CWRM to provide a plating density of 10^5 protoplasts per ml. Protoplast suspensions of 0.5 ml were cultured in multiwell culture dishes under conditions of diffuse illumination and high humidity. After 5 days, CWRM was diluted with Growth Medium (GM) and protoplast cell wall regeneration was detected by 0.1% Calcofluor using flourescent microscopy. Cell wall regeneration and callus proliferation were observed in *ca.* 30% of the cultures.

Resumen

El procedimiento para la liberación y el cultivo de células de café fue definido para permitir manipulación genética dentro del género *Coffea*. Se encontró como combinación óptima, 2,5% de pectinasa, 3,5% Driselasa, 0,51 molal manitol y 6 mM $CaCl_2$ con pH de 5,5 para liberación de protoplastos de células de callo de café, después de 6 a 7 horas de incubación a 50 rpm. La preparación de los protoplastos fue purificada mediante filtración a través de cribas de acero inoxidable de 150 μm y 38 μm. El filtrado se colectó en tubos de centrífuga; se centrifugó a 100 g por 3 minutos, se aspiró el líquido supernadante, y se resuspendió el precipitado de protoplastos en 3 ml del medio para la regeneración de paredes celulares (CWRM). Se repitió este procedimiento tres veces para diluir las enzimas y eliminar los restos celulares. Finalmente, el precipitado fue resuspendido en 1 a 2,5 ml de CWRM para conseguir una densidad para el contaje de 10^5 protoplastos por ml. Suspensiones de protoplastos de 0,5 ml fueron cultivados en placas multiseptadas en condiciones de iluminación difusa y alta humedad. Después de 5 días el CWRM fue diluído con medio de crecimiento (GM) y se comprobó la regeneración de paredes celulares mediante 0,1% calcoflúor y microscopía de fluorescencia. Se observó la regeneración de la pared celular y proliferación de callo en aproximadamente un 30% de los cultivos.

Literature cited

1 BINDING, H. and KOLLMANN, R. The use of subprotoplasts for organell transplantation, *In* Cell Genetics in Higher Plants, D. Dudits, G. L. Farkas & P. Maliga (eds.) Proceedings International Training Course, Szeged, Hungary, 1976 pp. 191-206.

2 CONSTABEL, F. Isolation and culture of plant protoplasts. *In* Plant Tissue Culture Methods, O. L. Gamborg & L. R. Wetter (eds.) National Research Council of Canada, Ottawa, 1975 pp. 11-21.

3 MONACO, L. C.; SÖNDAHL, M. R.; CARVALHO, A.; CROCOMO, O. J. and SHARP, W. R. Applications of tissue culture in the improvement of coffee, *In* Applied and Fundamental Aspects of Plant Cell, Tissues and Organ Culture, J. Reinert & Y. P. S. Bajaj (eds.) Berlín. Springer-Verlag, 1977 pp. 109-129.

4 MURASHIGE, T. and SKOOG, F. A revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiologia Plantarum 15: 473-497. 1962.

5 SÖNDAHL, M. R. and SHARP, W. R. Growth and embryogenesis in leaf tissues of *Coffea*. Plant Physiology 59 (6): 1. 1977.

6 SÖNDAHL, M. R. and SHARP, W. R. High frequency induction of somatic embryos in cultured leaf explants of *Coffea arabica*. Zeitschrift fuer Pflanzanphysiologie 81: 395-408. 1977.

7 SÖNDAHL, M. R. and SHARP, W. R. Research in *Coffea* and applications of tissue culture methods, *In* Plant Cell and Tissue Culture Principles and Application, W. R. Sharp, P. O. Larsen, E. F. Paddock & V. Raghavan (eds.) Columbus Ohio State University Press, 1978. (in press).

8 SÖNDAHL, M. R., SPAHLINGER, D. A. and SHARP, W. R. A histological study of high frequency and low frequency induction of somatic embryos in cultured leaf explants of *Coffea arabica* L. Zeitschrift fuer Pflanzenphysiologie 1979 (in press).

Notas y Comentarios

Cultivo de algas para producir sustancias químicas

Los bioquímicos israelís han iniciado una forma de agricultura en agua salada para producir productos químicos importantes a bajo costo. Su "cultivo" es un vegetal unicelular que fue descubierto sólo hace cinco años. Pero su conversión a glicerina, colorantes para alimentos, y a un alimento rico en proteína es ya una realidad comercial.

El alga marina, *Dunaliella bardawil,* fue identificada por el Profesor Mordhay Avron, del Instituto de Ciencias Weizmann y el Dr. Ami Ben-Amotz, de la Israeli Oceanographic Research Co. Crece naturalmente en agua con un alto contenido de sal, tal como la del Mar Muerto o el Gran Lago Salado. Por esta razón, puede ser cultivada fácilmente en regiones áridas que no pueden sustentar otras industrias. Avron dice que, cuando todos los problemas de producción en gran escala hayan sido resueltos, el precio de la glicerina derivada de *D bardawil* sería mucho menos de la mitad de la proveniente del petróleo.

La glicerina es la base de multitud de industrias como resinas alquídicas, estéres, explosivos, plastificantes para celulosas regeneradas, agentes emulsionantes, perfumería, cosméticos, etc. Se fabrica tradicionalmente a partir de las lejías residuales de la saponificación de las grasas y aceites en la industria del jabón y, desde hace unos 40 años, del propileno, por cloración.

El gobierno de Israel está tomando en serio el proyecto. Encarado a una tasa anual de inflación de más de 100 por ciento y a la reciente pérdida de los campos petroleros del Sinaí, vislumbra un futuro en productos químicos baratos procedentes de recursos renovables. Está apoyando a los científicos del Instituto Weizmann en pruebas que están haciendo en el techo de su laboratorio para encontrar las mejores condiciones y nutrimentos para el crecimiento del cultivo.

Mientras tanto, las firmas Koor Foods y la Yeda Research and Development están mejorando el proceso de extracción. Koor está construyendo 20.000 metros cuadrados de pantanos poco profundos cerca de Eliat de los cuales pueden extraer la pasta del alga roja. Cuando estas compañías hayan alcanzado producción en escala plena, el área de pantanos será medida en kilómetros cuadrados.

Una ventaja grande de *D bardawil* como materia prima para la industria es su capacidad de duplicar su volumen en dos días. Avron manifestó a *New Scientist* (3 April 1980, p. 24) que un cuarto del contenido de un pantano podría cosecharse cada día. Aunque pertenece a una familia botánica que contiene glicerina hasta un 40 por ciento de su peso seco, esta especie es el único miembro que contiene β caroteno, un agente colorante usado ampliamente en la industria alimenticia.

El material que queda después del proceso de extracción contiene 70 por ciento de proteína. Es parecido al extracto de soya en sus propiedades nutritivas y Avron ha encontrado que es un alimento eficaz para los animales. Los científicos en varios países han dedicado muchos esfuerzos en tener cultivos de biomasa. Pero la mayor parte de su trabajo se centra en la planta como combustible. Avron indica que el equipo moderno de conversión solar es mucho más eficiente. Cree que las algas, que convierten la energía solar con dos veces la eficiencia de las plantas superiores, formarán la base de una fuente creciente de productos químicos.

Brassinolida un poderoso promotor del crecimiento vegetal

Tomó más de tres años y 500 libras de polen a un equipo de científicos el identificar el ingrediente activo de las brassinas, un extracto de polen de la colza que se sabe produce un crecimiento rápido en una variedad de plantas, (Cf. *Turrialba* 21:375) El encontrar el compuesto clave fue el primer paso para aprender cómo acelera el crecimiento, un conocimiento que podría mejorar, la producción agrícola (*Agricultural Research* vol. 28, N° 2, 1979).

El compuesto activo es un esteroide, el primer esteroide vegetal que se ha encontrado que se comporta como un promotor de crecimiento. "Es un producto químico único, altamente activo biológicamente", dijo el coordinador del proyecto, George Steffens, refiriéndose al hecho de que un nanogramo (10^{-9}) causa que las células se alarguen. Su nombre propuesto es brassinolida.

El aislamiento y la identificación de la brassinolida fue un esfuerzo cooperativo entre científicos de varios lugares. Michael Kozempel y sus colegas en Filadelfia armaron una planta piloto de varios pasos para obtener un extracto de brassinas de una gran cantidad de polen. Entonces, científicos en Beltsville, Md, y en Peoria, Ill, dirigidos por Nagabhushanam Mandava y Michael D. Grove, trabajaron en equipo para aislar y analizar el compuesto activo. Judith Flippen-Anderson, en el Laboratorio Naval de Investigaciones, en Washington, D.C., también constribuyó al esfuerzo usando técnicas de rayos-X para determinar la estructura de la brassinolida. Cuando el laborioso proceso se completó, las 500 libras de polen produjeron sólo unos 15 miligramos de brassinolida.

Ahora que la estructura es conocida, Malcolm Thompson, trabajando con Mandava, ha podido sintetizar varios compuestos estrechamente relacionados que han producido respuestas de crecimiento similares. Los nuevos compuestos sintéticos permitirán a los investigadores evitar el tedioso proceso de extracción y tener bastante material para pruebas en gran escala en una variedad de plantas.

Hace nueve años, cuando John Mitchell, Joseph Worley, y otros científicos de Beltsville, descubrieron que las brassinas promovían el crecimiento vegetal, hubo grandes expectativas de que esta nueva hormona mejoraría grandemente los rendimientos (*Agricultural Research,* July 1970; Cf. *Turrialba* vol. 21, p. 375). Esto sucedió en muchos casos pero los rendimientos han sido inconsecuentes.

Los resultados más conspicuos han ocurrido con la papa. Aplicando una solución de brassinas a los ojos de la semilla de papa, Luis Gregory logró aumentar en un 24 por ciento el número de papas cosechadas. También se observaron aumentos en los rendimientos de rábano y lechugas.

En pruebas de invernadero, la tasa de crecimiento de varias plantas fue mayor cuando el extracto fue asperjado en plántulas muy jóvenes, en crecimiento activo. Esto llevó a la sospecha de que la brassinolida trabaja en conjunción con otras hormonas conocidas, tales como la auxina, para producir un crecimiento rápido. Gregory cree que el esteroide puede actuar como un regulador de la hormona, controlando la acción de otras hormonas en los puntos de crecimiento activo de la planta.

Todavía no se sabe cómo la brassinolida interacciona con las hormonas para acelerar el crecimiento. Sin embargo, se considera que una comprensión básica de cómo la brassinolida, o sus derivados, afectan el crecimiento vegetal será de gran beneficio para la agricultura en el futuro.