MULTIPLICATION OF SUGARCANE BY APEX CULTURE[1] /

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Resumen

Un serio problema enfrentado en los programas de mejoramiento, utilizando los métodos convencionales, es el tiempo que se gasta en la producción de una nueva variedad en larga escala.

Cultivos de tejidos pueden ser utilizados para solucionar este problema. En este trabajo, ápices de caña de azúcar (var. NA56-79) con cerca de 2 mm fueron desarrollados en brotes y después multiplicados en un medio líquido conteniendo citoquinina (MP II), sin agitación.

Los ápices en MP II, desarrollados en brotes con 2-3 por hijos sin cualquier formación de raíces, provocarán una mejoría en la etapa de multiplicación. El número de brotes producidos por medio de este método fue de siete veces más que aquel reportado anteriormente por Lee.

El enraizamiento fue obtenido por medio de transplante directo de los brotes para vermiculita, en invernadero a 24-32°C y humedad relativa de 70-90%. El aparecimiento de raíces se dio, en general, después de una semana, siendo la tasa de sobrevivencia superior a 80%.

Las plantas producidas mediante este método eran similares a las plantas-madres, excepto por el hecho de que las plantas derivadas de culturas de ápices son más vigorosas. Actualmente se están llevando a cabo comparaciones de productividad entre plantas derivadas de culturas de ápices y sus plantas-madres.

Introduction

The rapid multiplication of sugarcane has long been considered a serious challenge in sugarcane breeding programs. Usually after 10 to 15 years of intense work to complete a selection cycle, a new and better variety can be released only several years later, when enough seedcanes are obtained.

The time spent for this multiplication is considered a serious economic loss, principally in view of the higher yields that would be obtained by planting the new variety on a large commercial scale. It is also quite possible that the new variety could be entering its degenerative cycle earlier, because of the continued contamination which occurs during the multiplication stage in the open field.

Tissue culture has been used for multiplication of ornamental plants. The method, known as micropropagation, usually starts with the apex (shoot-tip) of the plant in order to obtain the same genotypical plant as the original. Callus culture, which can also be used to obtain plants through somatic embryogenesis, is not recommended since it has been proven that variation occurs frequently in this process (4).

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Sugarcane micropropagation has been reported by Barba *et al.* (1), Hendre *et al.* (2) and Lee (3). The method reported by Barba *et al.* (1), using callus culture with the addition of 2,4-D, is recommended more as a supplemental method of breeding than as a mass propagation procedure. The methods used by Hendre *et al.* (2) and by Lee (3) were apex culture. The shaking procedure used by Hendre *et al.* (2) is omitted by Lee (3), since this procedure is not economically feasible and the method caused more browning of the material. A comparison of theoretical and actual production of plants through this procedure was also reported by Lee (3).

This paper describes a modified method which proved to be more efficient than the one reported before by Lee (3).

Material and methods

Sugarcane plants, variety NA56-79, were obtained from the experimental station of the Institute of Sugar and Alcohol (IAA), in Araras-SP, Brasil. Single buds were sterilized by 20% QBoa (commercial sodium hypoclorite solution) for 40 minutes, washed with water and planted in vermiculite in a growth chamber at 30°C for 20-30 days.

When the seedlings were about 20-25 cm tall, stem segments of about 3 cm, together with the apex, were excised. They were first immersed in 70% alcohol for one minute and then sterilized in 20% QBoa solution for 20 minutes. The segments were then washed 3 times with water under sterile conditions and the apex, including the meristem tip and 2-3 leaf primordia, were excised with the help of a stereoscope. The size of the dissected apex was 1 mm in diameter and 2-3 mm in length. The apexes were transferred to test tubes containing 20 ml of different liquid media with filter paper as the support (3).

Seven types of media were tested (Figure 1 and Table 1). MP I and MP II media are those described by Lee (3). Half strength MS is a medium without any other additives, and 1/2 MS + Sucrose has an additional 20 g/l of sucrose.

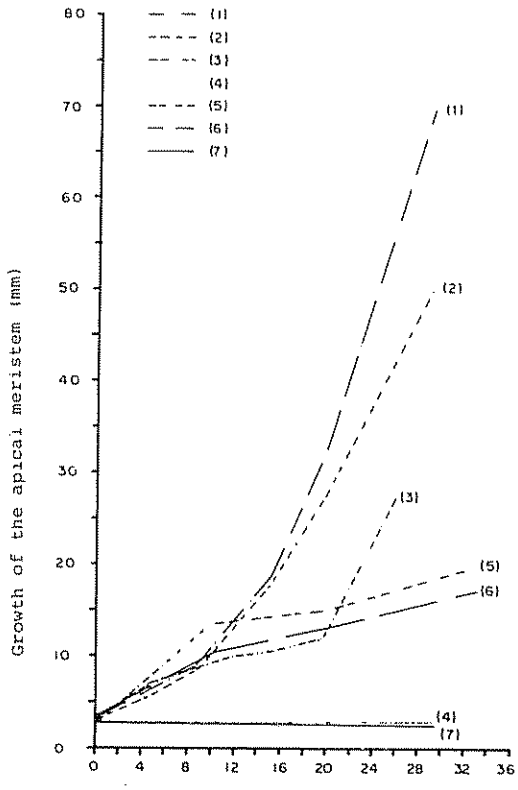
Semi-solid medium was prepared by adding 8 g/l of agar to the liquid medium. All media were adjusted to a pH of 5.8 prior to autoclaving (121°C – 30 minutes).

Multiplication was done using only MP II medium as described previously (3).

Development of the apex in these media was conducted in a growth room with a 16 hour photoperiod and 3 000 lux light intensity measured 20 cm above the cultures with a standard luxmeter. The temperature in this growth room ranged from 24-30°C during a day cycle. Growth of apex was measured at regular intervals. The resulting shoots were transplanted into paper cups containing vermiculite and transferred to the glasshouse under conditions previously described (3, 5). All the data are averages of at least 20 replications.

Results and discussion

As may be seen in Fig. 1 and Table 1, the best development of apex occured in MP I liquid medium while in MP II liquid medium they also grew well, but at a slower rate after 10 days. Medium with 1/2 MS salts was not suitable for apex development, while the addition of sucrose only slightly promoted its growth. Semi-solid medium is definitely detrimental to the development of sugarcane apexes.



(1) MP I-liquid; (2) MP II-liquid; (3) 1/2 MS+ sucrose-liquid; (4) 1/2 MS-liquid; (5) MP I-solid; (6) MP II-solid; (7) 1/2 MS+Sucrose-solid.

Fig 1. Development of the apical meristem of sugarcane in different media

Table 1. Effect of the medium on development of the apex of sugarcane (25 days in medium).

Medium tested		% Apex not developed	% Developed shoots with formed root	% Developed shoots with tillers
1	MP I-liquid	11	83	7
2	MP II-liquid	15	1	71
3	1/2 MS + Sucrose-liquid	59	40	0
4	1/2 MS-liquid	100	0	0
5	MP I-solid	89	0	0
6	MP II-solid	71	0	0
7	1/2 MS + Sucrose-solid	100	0	0

The shoots developed from apexes cultured on both MP I and 1/2 MS + Sucrose medium normally formed roots after 25 days, whereas those cultured on MP II medium usually resulted in shoots with 2-3 tillers but without roots

Table 2 shows the effect of shoot size on multiplication rate In MP I, a shoot size of around 5 cm gives the best results, while in MP II a larger size, around 7 cm, produced the highest rate The developing apex age (shoot age) also influenced the multiplication rate (Table 3). In MP I, younger shoots 25 to 27 days old multiplied better than the older shoots (more than 29 days in age) In contrast to this, in MP II an older shoot around 29 days old gave better results. It was found during this study that shoots with roots multiplied less than those without roots. The greater the number of roots formed, the lower was the multiplication rate.

Table 4 shows the multiplication rate of the apex developed shoots in MP II. Only those shoots derived from MP I and MP II media were used, since these two media gave the best growth rate and survival percentage.

Shoots derived from MP I-developed apexes, gave a multiplication rate of 4 when first transferred to the multiplication medium (MP II). The rate increased to 6 in the second and third passages The apexes which were grown in MP II usually developed into shoots with about 2 tillers. In the first multiplication, each of these apexes developed a stool comprised of about 20 shoots. The multiplication rate is reduced to 9 when each of these 20 shoots was separated and individually multiplied. On the third multiplication stage, the rate became similar between shoots originally developed in MP I and MP II.

The calculated number of shoots produced under these two systems, after 3 multiplication stages, was 144 per apex developed in MP I, compared with 1 080 for MP II. Since the multiplicable shoots are usually 50-60% of the total shoots produced each time, the real number of shoots produced is much less, although the second system again gives about 7 times more shoots after 3 multiplication stages

Shoots produced by these two systems are similar in size after the second multiplication. The rate also becomes similar after this stage At present, a field

Table 2. Effect of shoot size developed in different media on the multiplication rate (var. NA56-79).

Type of medium for apex development	Initial size of shoot (cm)		Multiplication Rate (MP II_{1X})	% Size plant produced (> 5 cm, < 5 cm, initial)		
MP I	0–3	(2 80)	5 57	54	23	23
	3–6	(4 58)	6 00	54	24	22
	6–9	(7 81)	5 00	67	7	26
	9–12	(10 10)	2 66	75	0	25
	12–15	(13 75)	3 00	83	0	17
MP II	0–3	(2 09)	14 30	37	44	19
	3–6	(3 35)	15 50	10	71	19
	6–9	(7 46)	21 66	60	29	11
	9–12	(10 25)	20 00	74	23	3

trial is in progress in order to compare the productivity of the material obtained by the culture system with that of the mother plant

The micropropagation of sugarcane plants described here has several practical advantages over the other culture methods. It precludes the need to use a shaker and averts the frequent changes of medium during the initial stage (2). Furthermore, it was necessary to prepare only one medium both for apex development and for multiplication. Unpublished data by this author also indicate that it is possible to omit the use of coconut milk in the MP II medium.

The importance of rapid propagation of sugarcane was described very clearly by Hendre *et al.* (2) and Lee (3).

The rooting medium which was used by Hendre *et al.* (2) in the last stage, before the shoots were transplanted to the glasshouse, was also omitted. During this work, the unrooted shoots were routinely transplanted directly into vermiculite and transferred to the glasshouse without any problems. The survival rate was usually more than 80% (3, 5).

Table 3: Effect of shoot age developed in different media on the multiplication rate (var. NA56-79).

Type of medium for apex development	Shoot age (Days)	Multiplication rate (MP II_{1x})	% Size plant produced (> 5 cm, < 5 cm, initial)		
MP I	25	5.90	63	9	28
	27	6.70	52	25	22
	29	4.00	62	19	19
	36	3.35	58	15	27
	38	3.00	67	25	8
MP II	25	9.00	52	30	18
	27	12.60	64	22	14
	29	20.81	44	44	12
	36	11.25	51	36	13
	38	8.00	63	30	7

Table 4. Multiplication of sugarcane, variety NA56-79.

Culture stage	Interval per passage (days)	Multiplication rate		Calculated No. of shoots recovered		% Multiplicable shoots*		Minimum actual No. shoots recovered	
		MP I (1)	MP II (2)	MP I (1)	MP II (2)	MP I (1)	MP II (2)	MP I (1)	MP II (2)
Apex developed in MP I and MP II	30	1 : 1	1 : 1**	1	1	100	100	1	1
1st Multiplicat.	15	1 : 4	1 : 20	4	20	53	44	2	9
2nd Multiplicat	15	1 : 6	1 : 9	24	180	51	51	6	41
3rd Multiplicat	15	1 : 6	1 : 6	144	1 080	59	58	21	143

* Multiplicable shoots signifies shoots with a size equat to or larger than 5 cm, which could be used immediately for the multiplication process

** The multiplication rate in the first stage, when the apex is still developing, actually is about 3. Since the shoots were not separated individually when passed through the first multiplication, the multiplication rate was considered as 1 : 1 instead of 1 : 3

1 Apex developed into shoot in MP I.

2 Apex developed into shoot in MP II.

Resumo

Um sério problema enfrentado pelos programas de melhoramento, é o tempo gasto na produção de uma nova variedade em grande escala

A cultura de tecidos pode ser utilizada para resolver este problema Neste trabalho, ápices de cana-de-açúcar (var NA56-79) com cerca de 2 mm foram desenvolvidos em brotos e depois multiplicados em meio líquido contendo citoquinina (MP II), sem agitação

Os ápices em MP II, desenvolvidos em brotos com 2-3 perfilhos sem qualquer formação de raízes, provocaram melhoria na taxa de multiplicação O número de brotos produzidos através deste método foi 7 vezes maior do que aquele relatado anteriormente por Lee (3)

O enraizamento foi obtido através do transplantio direto dos brotos para vermiculita, em casa de vegetação a 24-32°C e umidade relativa de 70-90% O aparecimento de raízes deu-se, em geral, depois de uma semana, sendo a taxa de sobrevivência superior a 80%

As plantas produzidas através deste método eram semelhantes às plantas-mãe, exceto pelo fato das plantas derivadas de cultura de ápices serem mais vigorosas Atualmente está-se desenvolvendo comparação de produtividade entre plantas derivadas de cultura de ápices e suas plantas-mãe.

Literature cited

1 BARBA, R C ; ZAMORA, A B ; MALLION, A K ; LINGA, C K 1977 Sugarcane tissue culture research **In** Congress of the International Society of Sugarcane Technologists (16 , 1977, São Paulo, Bra). Proceedings p 843-863

2 HENDRE, R R ; IYER, R S ; KOTWAL, M ; KHUSPE, S S ; MASCARENHAS, A F 1983 Rapid multiplication of sugarcane by tissue culture Sugar Cane No 1:5-8

3 LEE, T S G 1984 Micropapagação de cana-de-açúcar através de cultura de meristema apical Saccharum 7(35):36-39

4 LEE, T S G 1984 Obtenção de variedades melhoradas através da técnica de cultura de tecidos **In** Congresso Nacional da STAB (3, 1984, São Paulo, Bra). Proceedings p 180-182

5 LEE, T S G ; BACCHI, O O S 1984 Improved rooting of differentiated shoots from sugarcane callus tissue Turrialba 34(4):481-484

Notas y comentarios

Plantas que prestan genes a las bacterias fijadoras de nitrógeno

Si la ingeniería genética pudiese descubrir cómo las bacterias convierten el nitrógeno de la atmósfera en amonio, podría entonces ser capaz de proveer a los agricultores con semillas de cultivos que se fertilizan a sí mismos. Hay ciertas bacterias que viven en estrecha armonía con plantas leguminosas. Las plantas suministran nódulos en sus raíces para que las bacterias se aposenten en ellos, y las bacterias (*Rhizobium* y otras de la misma familia) fijan el nitrógeno del aire y así proveen a las plantas con un suministro continuo de fertilizante nitrogenado.

Si tales bacterias pudieran ser persuadidas a asociarse con otras plantas cultivadas, tales como papas y trigo, los agricultores podrían ahorrar mucho en fertilizar sus campos. Desgraciadamente, la simbiosis entre bacteria y hospedante es muy complicada. La planta produce un tipo de hemoglobina llamada leghemoglobina para proveer el oxígeno que el *Rhyzobium* necesita (**Nature**, vol. 321, p. 669), y la bacteria fabrica la enzima nitrogenasa, que convierte el nitrógeno en amonio. Ahora, dos investigadores de los Estados Unidos han encontrado que la relación entre bacteria y planta es todavía más estrecha. La bacteria simbiótica, afirman ellos, ha adquirido algo de su material genético de su hospedante leguminoso.

En el proceso de fijación del nitrógeno, el amonio recién manufacturado es introducido al metabolismo de una bacteria o de una planta mediante una combinación con el aminoácido glutamato. Este paso esencial es catalizado por la enzima glutamina-sintetesa. Todd Carlson y Berry Chelm, de la Universidad del Estado de Michigan, en East Lansing, encontraron que el *Rhizobium* y sus aliados eran únicos en más cosas que su capacidad de formar nódulos en leguminosas. Producen también dos tipos de glutamina-sintetasa (**Nature**, vol. 322, p. 568). Una forma de la enzima se parece a la producida por las bacterias normales, pero el segundo tipo es distinto en su estructura y en la manera en que es modificada por la bacteria.

Chelm y Carson extranjeron la enzima (llamada glutamina-sintetasa II, GS2) de cultivos de *Bradyrhizobium japonicum*, la bacteria simbiótica en la soya (*Glycine max*). Entonces aislaron el gen que codifica a GS2 y determinaron su secuencia en el DNA, lo que les dio la estructura de la proteína. Esta proteína resultó tener una esctructura similar a la glutamina-sintetasa producida por las plantas de soya. Cerca de la mitad de las posiciones de aminoácidos en el *Bradyrhizobium* GS2 son idénticas a aquéllas en las enzimas elaboradas por las leguminosas y otras plantas. Pero el GS2 no se parecía a la glutamina-sintetasa hecha por *Anabaena*, una alga verdiazul que fija también el nitrógeno. Así, este segundo tipo de enzima GS no es esencial a la fijación del nitrógeno.

Carson y Chelm concluyen que el antecesor de las bacterias fijadoras del nitrógeno adquirieron el gen de su segunda glutamina-sintetasa de una planta hospedante. Es interesante que los genes GS de las plantas contienen "intrones", las secciones no codificadoras del DNA, que actúan como espaciadores entre las partes codificadas del gen, mientras que el gen de *Bradyrhyzobium*, al igual que todos los otros genes bacterianos conocidos, no tiene intrones (**New Scientist**, 26 junio 1986, p. 34).

Esta transferencia de un gen de una planta a una bacteria (el primer ejemplo de tal traspaso) fue supuestamente ejecutada accidentalmente, posiblemente por un virus. Sin embargo, la familia entera posee ahora los dos tipos de glutamina-sintetasa. Los investigadores creen que el GS2 no es esencial para la nitrofijación, así que la pregunta es ¿por qué la bacteria la ha conservado durante incontables generaciones? Los autores sugieren que esta enzima "prestada" debe tener alguna significación para la bacteria.

Este hallazgo puede reforzar algo la teoría, sostenida desde hace años por una esforzada científica, Lynn Margulis, de que los primeros organismos multicelulares se originaron de simbiosis de dos organismos monocelulares, lo que había adelantado ya en un artículo titulado "Origen de las células mitóticas" (**J. Theor. Biol.**, 1967) y en su primer libro "Origen de las células eucarióticas" (Yale University Press, 1970). La historia de como sus ideas van adquiriendo aceptación e importancia en el mundo científico es relatado en sendos artículos de Evelyn Fox Keller (**New Scientist**, 3-VI-86, p. 46) y de G. Kite (**New Scientist**, 3-VII-86, p. 50), pero esta fascinante lucha por sus ideas de esta esforzada visionaria de la ciencia no es para contarla en esta breve nota.

Volviendo al tema de la nota, se puede concluir que este descubrimiento sobre las bacterias fijadoras del nitrógeno complica las cosas en vez de simplificarlas. Así que, el que se pueda o no adaptar la fijación del nitrógeno del tipo *Rhizobium* para cultivos no leguminosos, sigue siendo una incógnita abierta. A. G.