IMPROVED ROOTING OF DIFFERENTIATED SHOOTS FROM SUGARCANE CALLUS TISSUE[1] /

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Resumen

*Se describe un método rápido, sencillo, económico y confiable para enraizar plántulas de caña de azúcar (*Saccharum *spp.) producidas a partir de cultivo de tejido transferidas directamente a un medio de vermiculita sin esterilizar. Después del trasplante las hojas son removidas y los potes se mantienen en el invernadero sin ninguna covertura a una temperatura entre 24-32°C y una humedad relativamente 70-90%. Con este método la sobrevivencia de las plántulas es excelente así como la formación de raíces.*

Introduction

Research on tissue culture of sugarcane was initiated in Hawaii in 1961 by Nickell (4). In general, callus can be obtained from almost any sugarcane tissue, and the callus in turn can be made to differentiate into shoots without much difficulty. However, the rooting of these differentiated shoots is generally considered a more difficult task (2, 4, 5, 6). The Hawaii group has suggested several methods for encouraging root production, such as culturing the shoots in water, storing them at 15°C, trimming the leaves, adding a-naphthaleneacetic acid (NAA) or dalapon to the medium, and transferring to a medium consisting of 1% agar and 7% sucrose (2, 6). Liu (4), using a modified Schenk and Hildebrandt (SH) medium for such a purpose suggested, with two basic requirements, a sufficient quantity of medium, and the maintenance of the culture under diffuse sunlight conditions.

The IAA/PLANALSUCAR laboratory at Araras–SP has been using some of these methods, but the performance was considered unsatisfactory for mass production of plants from sugarcane callus. Results of an improved method of rooting are reported here.

Materials and methods

Three sugarcane varieties of commercial interest were cultured from the spindle leaf of young plants grown in vermiculite from single bud cuttings in a growth chamber at 30°C. The culture medium (CM) for callus formation and proliferation was that of Murashige-Skoog, with modifications (4). Shoot differentiation was initiated in a culture room (26°C) on a medium (PM) similar to that described above, but with 1 mg NAA and 1 mg of kinetin instead of 4 mg of 2.4-D per liter. Root formation was induced either in a modified SH medium (RM), directly in vermiculite, or in a soil mixture (soil : sand : filtercake = 2:1:2).

Rooting conditions were introduced when the differentiated shoots in PM were about 8 to 10 cm in length. One group (120 plantlets) of differentiated shoots was transferred to test tubes containing RM. Each test tube received only one plantlet. The second group (120 plantlets) was transferred directly to a disposable plastic cup containing nonsterilized vermiculite, pre-moistened with tap water. Leaves were them trimmed. Each plastic cup also contained only one plantlet. The group of plantlets in vermiculite, together with 30 plantlets in RM, were then transferred to a greenhouse where the temperature varied from 24 to 32°C and the relative humidity,

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from 70 to 90%. The remaining 90 plantlets were kept in the culture room. Another test was conducted by transferring shoots from PM directly to either vermiculite or a soil mixture. About half of each treatment was then transferred to the greenhouse and the other half maintained in the laboratory. The plastic cups, containing a single plantlet in vermiculite or soil, were irrigated inmediately after they had been moved to the greenhouse, but no covering was used. The plantlets in vermiculite or soil received a nutrient solution once a week (either a commercial leaf fertilizer or Hoagland solution). Survival rate and root formation were recorded after two or four weeks of treatment.

Results and discussion

Sugarcane callus normally gives rise to shoots and roots independently. Some varieties develop only shoots from their callus. In such cases, a special effort has to be made to stimulate root production (4). Several alternative methods are now available to improve development of roots in sugarcane, such as those mentioned above. In some situations (e.g. rapessed), the shoots can be directly transferred to vermiculite in a pot and covered with plastic bags to maintain humidity until the plant is well established (3). The results reported in other laboratories, and our own experience with these methods, were generally unsatisfactory for mass production of plants, since the roots are slow to form and are few in number, with little more than 50% of the plants rooted within one month of development in the sterile culture medium (6). The MS_5 medium used by Nadar and Heinz (6) seems good for root initiation, but no data were shown concerning root proliferation. The root medium consisting of 1% agar and 7% sucrose as reported by Hiraki and Maretzki (2) was very satisfactory for root formation. However, as can be seen in Figures 1 and 2, the present method produces considerably better results for root formation and proliferation. The sugarcane shoots can be easily rooted in non-sterilized vermiculite without any covering, thus obviating the sophisticated sterile culture technique. When shoots are transferred to the greenhouse, leaves must be trimmed to prevent wilting.

Greenhouse conditions were better than the culture room for rooting of sugarcane (Tables 1, 2). Under greenhouse conditions, the temperature varied between 24°C and 32°C, and the light intensity was higher (4-14 mW). Such conditions were more favourable for the sugarcane plant than those used in the culture room (constant temperature, 26°C with artificial light, 1.5-2.0 mW). Light appears to be important for rooting (1). If plantlets are kept under poorly lighted conditions, such as in the laboratory, the mortality rate increased. Perhaps light is important for an adequate production of the hormones necessary for root initiation; alternatively, an adequate supply of photosynthates may be necessary at the sites of root initiation. In any case, some light-dependent process would appear to be more important for rooting than the actual medium used.

Table 1 shows that differentiated shoots rooted better in vermiculite than in culture medium (RM), in terms of both the number of rooted plants, and the size of the root. The plantlets maintained in RM in the culture room have a good survival rate, but root formation is poor after 30 days. The ambient conditions apper to be more important than the rooting medium, because shoots rooted in the greenhouse, whether in RM or vermiculite, were always stronger, greener and presented a much more highly developed root system than those in the culture room (Figures 1, 2). Rooting in vermiculite in the greenhouse is the best choice since this combination gives very good results, in terms root formation, proliferation and survival rate. The formation of a root system can be easily noted after one week in vermiculite in greenhouse, while in RM in culture room roots can barely be seen after two weeks. Root initiation in RM in the greenhouse is faster than that in RM in the culture room.

Covering the test tube or flask containing the plantlets in order to maintain humidity or better aseptic conditions is not recommended for the greenhouse, since it inhibits air exchange and causes an undesirable rise in temperature (reaching 38°C at times), which burns the plantlets. A soil mixture is not a good rooting medium (Tables 2 and 3) because of a high mortality rate of the plants caused by microorganism growth on the soil surface and less favourable drainage. Nevertheless, plants that survived did grow better than those in vermiculite, perhaps

Table 1. Total number of plantlets and percent of rooted plantlets in response to the culture medium (RM) and vermiculite for root formation under (culture room and greenhouse) conditions.

Treatment	Total plantlets	Rooted plantlets (%)	Survival rate (%)	Fresh weight per plant (g)
RM – Culture room	90	50	96	0.068
RM – Greenhouse	30	77	77	0.332
Vermiculite-Greenhouse	120	96	96	1.224

* Plantlets were regenerated from tissue culture of var CB40-13. Data were obtained 30 days after treatment.

Table 2. Total number of plantlets and survival rate in response to two different potting media for root formation (vermiculite and soil mixture) under two different conditions (culture room and greenhouse).

Treatment	Total plantlets	Rooted plantlets (%)	Survival rate (%)	Fresh weight per plant (g)	Dry weight per plant (g)
Vermiculite-Culture room	192	90	90	0.148	0.018
Soil mixture-Culture room	189	23	23	0.099	0.017
Vermiculite-Greenhouse	179	99	99	3.439	0.469
Soil mixture-Greenhouse	189	86	86	5.962	1.079

* Plantlets were regenerated from tissue culture of var. Co740. Data were obtained 25 days after treatment.

of Ca concentration of tops (data not shown) and because of a better nutrient supply. The survival rate of plantlets of IAC48-65 in the soil mixture is especially low, since this variety regenerates very weak plantlets even after they have been transferred to RM before being potted in the soil mixture (Table 3).

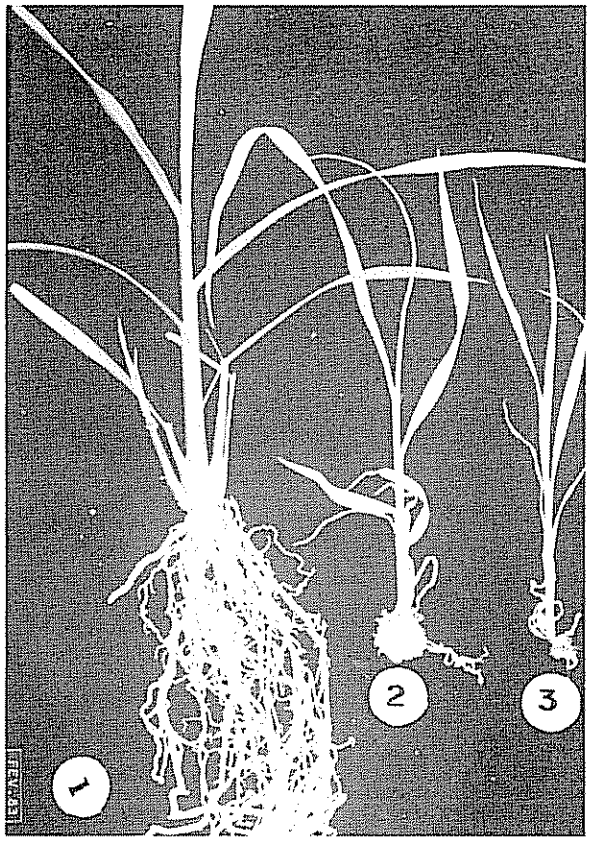

Fig. 1. Root formation (30 days after treatment) on plantlets of variety CB40-13 redifferentiated from callus tissue using

(1) vermiculite in greenhouse
(2) culture medium (RM) in greenhouse
(3) culture medium (RM) in culture room

Rooting sugarcane plantlets in greenhouse in vermiculite without any covering is simple, low-cost, rapid and very reliable. Thus the common procedure of transferring the differentiated shoot of sugarcane first, to a rooting medium in sterile culture and then, to sterilized vermiculite in a greenhouse, can be replaced by the direct transfer of plantlets to unsterilized vermiculite without any covering in greenhouse.

Summary

A rapid, simple, economical and reliable method of rooting the differentiated shoots from sugarcane (*Saccharum* spp.) callus tissue was achieved by the direct transfer of plantlets to moist unsterilized vermiculite. After transplanting, the leaves of the plantlets were trimmed, and the pots maintained in a greenhouse without any form of cover. The greenhouse conditions were: temperature 24-32°C and relative humidity 70-90%. With this method, the survival rate of the plantlets is very high and root formation is excellent.

Table 3. Survival rate of plantlets in response to vermiculite and soil mixture.

Parameters evaluated	Treatment	
	Verm-greenhouse	Soil mixture – greenhouse
Total plantlets	48	48
No. of plantlets dead		
14 days	1	25
15 days	3	1
Total	4	26
Survival rate	92	46

* Plantlets were regenerated from tissue culture of var. IAC48-65. In this case differentiated shoot had been transfered to RM for 40 days and only rooted plantlets were chosen for survival test.

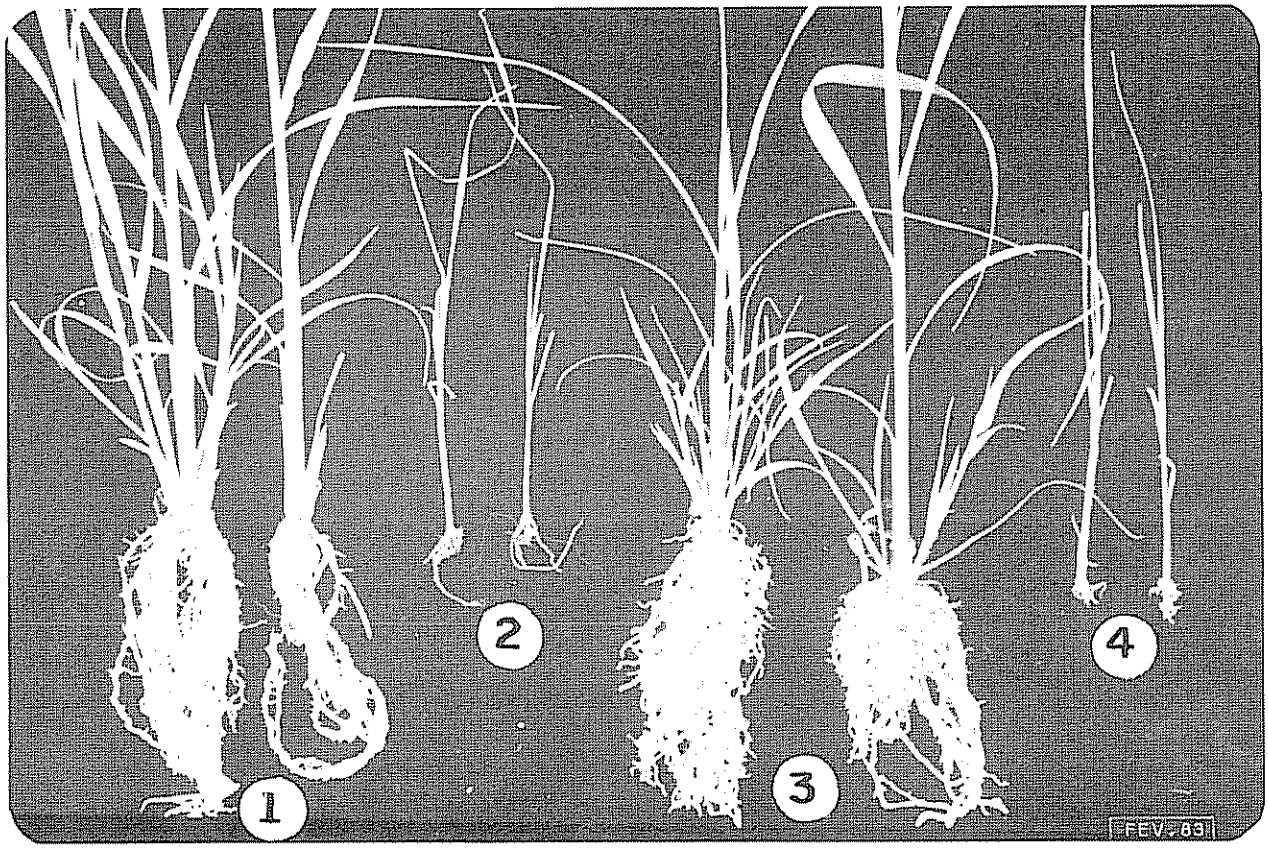

Fig. 2 Root formation (25 days after treatment) on plantlets of variety Co740 redifferentiated from callus tissue using

(1) soil mixture in greenhouse
(2) soil mixture in culture room
(3) vermiculite in greenhouse
(4) vermiculite in culture room

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