SUGARCANE SOMATIC EMBRYOGENESIS FROM CALLUS CULTURES BY SCANNING ELECTRON MICROSCOPY

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RESUMEN. Se obtuvieron callos embriogénicos y no embriogénicos por cultivo de fragmentos de hojas enrolladas de caña de azúcar de la variedad Cuba 87-51 en medio basal de Murashige y Skoog (MS) suplementado con diferentes nutrientes, los cuales se estudiaron comparativamente por microscopia electrónica de barrido. Se tomaron muestras de callos embriogénicos cultivados en mediio de regeneración (MS sin ácido diclorofenoxiacético) a diferentes tiempos, con el objetivo de realizar un análisis secuencial del proceso, también por microscopia electrónica de barrido. En los callos embriogénicos, se observaron células organizadas en embriones, mientras que en los no embriogénicos, se apreciaron células elongadas y desorganizadas. Se describen las características de los embriones durante el proceso de regeneración de plantas. En los embriones de caña de azúcar se observaron los estadíos: globular, globular con muesca lateral y esculeto. Durante el proceso de regeneración de plantas aparecen meristemos apicales, primordios foliares y radiculares y finalmente, hojas y raíces verdaderas.

ABSTRACT. Spindles of CUBA 87-51 sugarcane were cultured in Murashige and Skoog basal medium supplemented with different nutrients. Embryogenic and non-embryogenic callus obtained were studied by scanning electron microscopy for comparing them. Samples of embryogenic callus cultured in regeneration medium (MS without 2,4 dichlorophenoxyacetic acid) were taken at different times for analyzing the sequential process. Distinctive features of two types of callus are shown by scanning electron microscopy: cells organized in embryos are noted in embryogenic callus; while elongated, disorganized cells can be seen in non- embryogenic callus. The characteristics of the embryos during plant regeneration are described. Sugarcane embryoids stages are: globular, globular with lateral notch and scutellum. In this process also appear shoot meristems, leaf and root primordia and finally, real leaves and roots. It is concluded that callus plant regeneration from young leaves segments of sugarcane occur via somatic embryogenesis mainly.

INTRODUCTION

Somatic embryogenesis is a plant regeneration via which allows the production of very large number of plants in a short time and a limited space.

Somatic embryogenesis has been studied in several monocotyledons and some gramineous species from different explants. Young tissues are the ideal sources for the initiation of embryogenic callus culture in *Gramineae*.[1]

Tissue culture methods have been used in sugarcane for obtaining more diseases resistant and sugar rich varieties. Plant regeneration via somatic embryogenesis has been described in some works.[2-6]

In Cuba, where sugarcane is a crop of major economic importance, some morphological and physiological studies of the somatic embryogenesis have been done.[7-12] However, no detailed scanning electron microscopy (SEM) studies have been carried out in order to understand the morphogenesis of plants from callus culture.

In this study, embryogenic and non-embryogenic callusses obtained from spindles of sugarcane were processed for SEM in order to characterize them and to recognize the steps during plant regeneration from callus tissue.

MATERIAL AND METHODS

Plant material and induction of callus

Spindles of the apical verticil of CUBA 87-51 variety of sugarcane plants were used as explants in this study.

Spindles were plated on Murashige and Skoog (MS) medium modified by Heinz[1]; the callus induction medium was supplemented with 2,4 dichlorophenoxyacetic acid (2,4 D) (3 mg/L), kinetin (10mg /L) and indolacetic acid (2,5 mg/L). Callusses were sub-cultured regularly for two months.

Embryogenic and non-embryogenic callusses were chosen macroscopically: white and nodular the former; yellow and friable, the latter.[14] Fragments of the two callus types were prepared for SEM observations.

Plant regeneration

Plant regenerationwas achieved transferringcallus pieces on MS medium without2,4 D. At 3, 6, 10, 12, 14, 17 and 19 d after culture,samples were fixed and procesedas below.

Scanning Electron Microscopy

Specimens were fixed in 3,2 % glutaraldehyde and postfixed in 1 % osmium tetroxide buffered in sodium cacodylate solutions (pH 7,4) for 1h each. Fixed tissues were dehydrated through a 30, 50a nd 70% ethanol series for 10mi n each. (Fixation and dehydration were at 4 ^{o}C). Finally, pieces were passed to absolute ethanol at room temperature. After that, tissues were critical point dried and sputter coated with gold. Observations were made on a JEOL JEM 100 CX II- ASID 4D SEM.

RESULTS

Embryogenic and non-embryogenic callus

There are two types of callusses: a smooth and compact callus (embryogenic) and a friable or soft callus (non-embryogenic). The former consists of small and round cells (Fig. 1) and the latter consists of elongated, highly dissociated cells (Fig. 2).

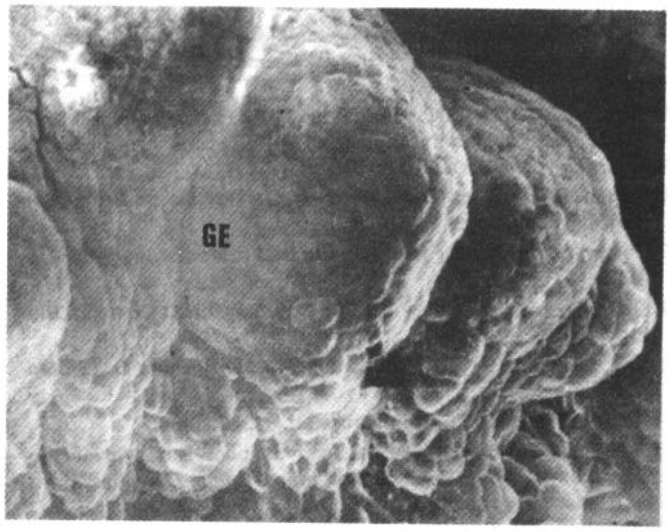

Fig. 1. Globular embryos (GE) in embryogenic callus. X 600

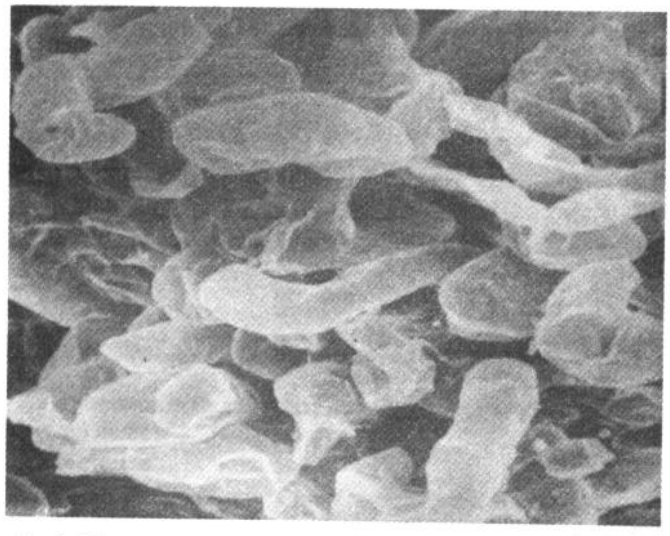

Fig. 2. Elongated and disorganized cells in non-embryogenic callus. X 1000.

Areas where cells have the morphology of non-embryogenic callus were observed in embryogenic callus.

Plant regeneration

At 3 and 6 d after culture the embryogenic callus in regeneration medium, a lot of globular and development somatic embryos with lateral notch were observed (Fig. 3). At 10 d of culture, it was possible to observe embryoids in different stages like globular, globular with lateral notch and early scutellum. Also leaf primordia and areas where there was no apparent organization appeared (Fig. 4). 14 d after culture, root primordia could be seen. At 17 days of culture, short tricomes were noted in the surface of leaf primordia. Finally, 19 d after placement on plantlet regeneration medium, leaves and roots were observed.

Somatic embryogenesis as a plant regeneration via from sugarcane callus has been described formerly and our results allow to corroborate this fact, but the ocurrence of organogenesis in disorganized areas of the callus cannot be forgiven.

DISCUSSION

Embryogenic and non-embryogenic callus

The morphological differentiation between embryogenic and non-embryogenic callusses was in coincidence with what occurs in most cereals and herbs.[15]

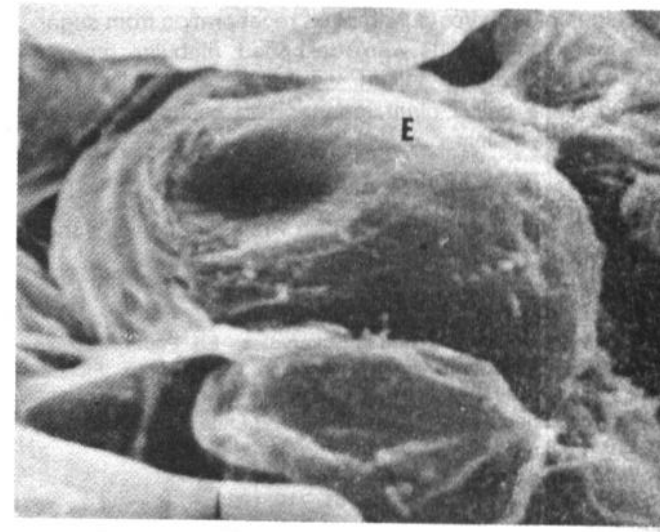

Fig. 3. Embryos (E) with lateral notch at 3 d after culture in plant regeneration medium. X 3200.

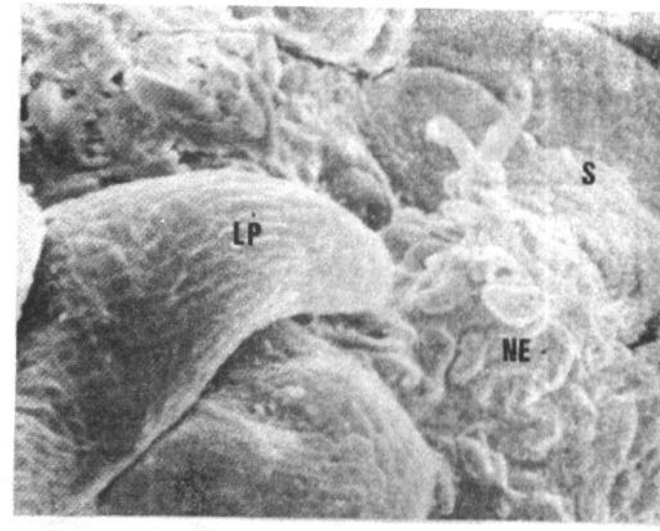

Fig. 4. Embryos in advanced stages of development (scutellum-S) and leaf primordia (LP), 10 d after being transferred to plantlet regeneration medium. A non embryogenic area (NE) is noted. X 1050.

Despite of having been described three and four callus types obtained from sugarcane leaf explants [3-6]; in this work it was possible to identify only two types: a smooth and compact callus (embryogenic) and a friable or soft callus (non-embryogenic).

Areas with non-embryogenic cells within an embryogenic callus have been described formerly.[2,6,16] These cells have been considered senescent or dead cells,[2] while some authors said that they could regenerate via organogenesis.[16]

Plant regeneration

The presence of somatic embryos in different stages of development at the same time demonstrated that somatic embryogenesis is an asyncronic proccess. This fact has been related to the strong tendency of scutellum of in vitro somatic embryos to secondary proliferation, so that, two or more embryos generations could appear simultaneously.[17]

CONCLUSIONS

Embryogenic and non-embryogenic callusses differ in SEM morphology: the former are organized in globular embryos and the latter show disorganized and elongated cells.

Stages of embryos during plant regeneration from sugarcane embryogenic callus were described: globular, globular with lateral notch and scutellum.

Sugarcane callus plant regeneration occur by somatic embryogenesis mainly.

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BIBLIOGRAPHY

1. Vasil I.K. Genetic Engineering in Eukaryotes. Ed. Paul F. Lurquin and Andris Kleinhofs (Plenum Publishing Corporation), Florida, USA, 233-252, 1983.
2. Nadar H.M., Soepraptopo S., Heinz D.J.and Ladd S.L., **Crop Sci., 18**, 210, 1978
3. Ho W.J. and Vasil I.K., **Protoplasma, 118**, 169, 1983.
4. Ho W.J. and Vasil I.K., **Ann. Bot., 51**, 719, 1983.
5. Guiderdoni E. and Demarly Y., **Plant Cell, Tissue and Organ Culture, 14**, 71, 1988.
6. Taylor P.W.J.,Ko H.L., Adkins S.W., Rathus C. and Birch R.G., **Plant Cell, Tissue and Organ Culture, 28**, 69, 1992.
7. Ancheta O., Ramos M.E., de la Rosa M.C. and Kourí J., **Memorias** VIII Seminario Científico. CNIC, La Habana, Cuba, 15 dic., 236, 1982.
8. Torroella T., Ancheta O., de la Rosa M.C., Ramos M.E. and Kourí J., 44 Congreso de la Asociación de Técnicos Azucareros de Cuba, La Habana, Cuba, oct. , 260, 1984
9. Torroella T., Arteaga O. and Ancheta O. **Revista de Protección Vegetal, 2**, 99, 1986.
10. Torroella T. and Kourí J.B., **Revista de Protección Vegetal, 1**, 9, 1986.
11. Díaz P., Ruíz A. and Ancheta O., **Revista CENIC Ciencias Biológicas, 17** , 59, 1986.
12. Díaz E., Korneva S.B., Maribona R.H. and Ancheta O., **Biotecnología Aplicada, 8**, 53, 1991.
13. Heinz D.J. and Mee G.W.P. **Crop Sci. 9**, 346, 1969.
14. Díaz E., Niubó E., Korneva S.B. and Maribona R.H., Adv. in Modern Biothecnology. Book of short reports of the Congress, Havana, Cuba, June 1992, Vol. 1, Abstract 14.13, 1992.
15. Thorpe T.A., ISI Atlas of Sciences: Animal and Plant Sci., 81, 1988.
16. Gnanapragasam S. and Vasil I.K. Plant. Sci., 83, 205, 1992.
17. Wang D. and Vasil I.K. **Plant Sci. Lett., 25**, 147, 1982.