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STUDIES ON THE TISSUE CULTURE OF SUGAR CANE (*SACCHARUM OFFICINARUM* L.) CV. N. CO-310

I. Callus induction and single cell isolation[1]

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Abstract

Axillary buds of *Saccharum officinarum* L. cv. N. CO-310 were excised and cultured. Friable calluses were induced on Murashige & Skoog's medium supplemented with coconut milk (15%), 2,4-D (4 ppm), and kinetin (.4 ppm). With gentle shaking in liquid medium single cells of various shapes as well as cell clusters were obtained.

Introduction

Sterile culture of exised plant tissue had been developed in the late 1930's (White 1939). This technique has been widely adopted as a powerful tool in various fields of basic research as well as applied science. Heinz and Mee (1969) successfully redifferentiated callus tissues of two *Saccharum* species into entire plants. Nickell and Maretzki (1969) obtained cell suspension from explant of sugar cane (var. H50-7209) parenchyma. With traditional methods of sugar cane propagation, it would take many years before enough individuals could be produced from a superior hybrid or mutant cane for commerial cultivation. Inducing the formation of vegetative embryoids from somatic cell suspension culture provides a means of obtaining enormous quantities of genetically identical individuals within a short period (Steward *et al.* 1958). This embryoids production technique should have great potential value to sugar cane breeding and agricultural extension work. Cell suspension is necessary for single cell isolation. The objective of the present study was investigation of the potentiality of sugar cane cultivar N. CO-310 explant de-differentiation into callus tissue and further production of cell suspension from callus tissue.

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Material and Methods

Sugar cane (*saccharum officianurm* L.) cv. N. CO-310, a widely cultivated variety in Taiwan, was used in this investigation.

After removal of the outer bracts, the axillary buds were surface cleaned with detergent. The entire buds (approx. 7×5×4 mm) were then excised from the stem and sterilized by a quick dip in 75% ethyl alcohol followed by soaking in 3% sodium hypochlorite for 10 minutes under reduced pressure. The sodium hypochlorite was washed out by several changes of sterilized distilled water before the buds were transferred into sterile culture media under aseptic condition.

Both Murashige & Skoog's (MS) (1962) and White's (W) (1939) media were used in this study. The media were supplemented by coconut milk (CM) (0 and 15%), 2,4-D (0, 1, 2, 4, and 8 ppm), and kinetin (0, .1, .2, .4, and 8 ppm) in various combinations for callus induction. After induction, the callus tissues were transferred onto fresh media for further growth. One set of media were identical to that on which the particular callus was induced; in the second set of media the CM was replaced by 10 ppm arginine. Cultures were incubated in a 25°C growth chamber supplied with 16 hrs. fluorescent light with a light intensity of approx. 2000 lux.

Cell suspensions were obtained by shaking the callus tissues in liquid medium on a horizontal shaker at 60 rpm under similar temperature and light conditions. The liquid medium used was MS's medium with identical supplements as that in callus induction medium except agar was eliminated. The studies of suspended cells were made under a phase contrast microscope.

Results and Discussion

After three week's incubation, callus tissues were formed on both MS's and W's media supplemented with 15% CM, 4 ppm 2,4-D, and .4 ppm kinetin. Callus growth was some what weaker on W's than MS's medium.

On fresh media, callus tissues grew well on MS's media with or without replacing CM with arginine. Tissues grew weakly and eventually turned brown after being transferred from W's to W's or MS's to W's media. Nickell & Kortschak (1964b) has had success with induction and growth of callus from internodal parenchymatous tissue of Hawaiian sugar can variety H37-1933 on W's medium supplemented with 18% CM and 6 ppm 2,4-D. With our material we found that MS's medium was superior to W's medium as far as callus induction and growth was concerned. Our results on the effect of arginine were in agreement with Nickell's report (1964).

Callus tissues formed by explant of sugar cane cv. N. CO-310 were very

friable. With gentle shaking in liquid medium, the tissue separated readily into single cells of various shapes and sizes as well as cell clusters of various sizes composed by various cell types (Figures a to n).

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甘蔗組織培養的研究

I. 癒合組織的誘導和單細胞的分離

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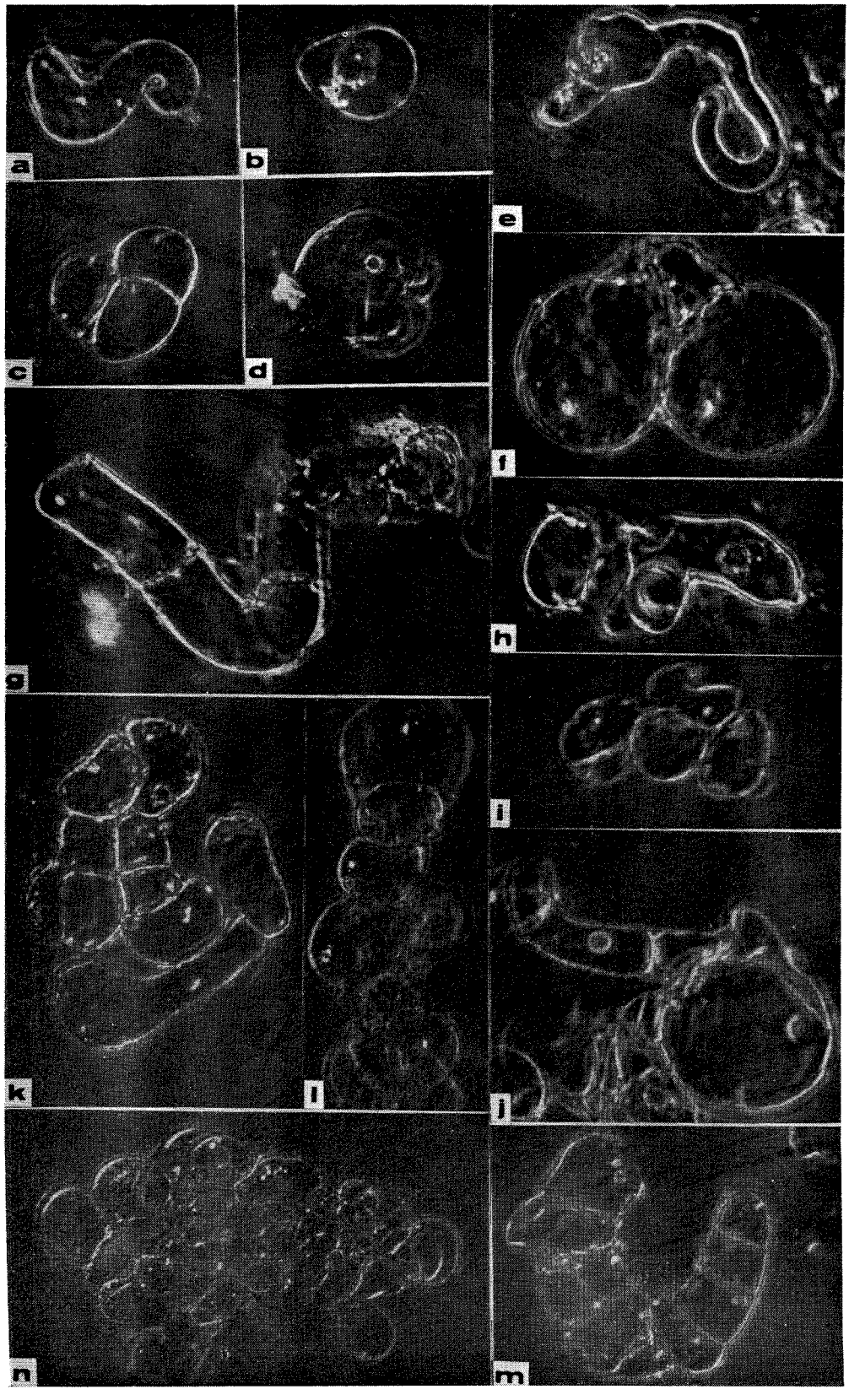
栽培種甘蔗 N. CO-310 莖上的芽在含有 2, 4-D 4 ppm，Kinetin 0.4 ppm，15% 椰子汁的 Murashige and Skoog (M. S.) 和 White (W) 的基本培養基，在 25°C 的恒溫下能誘導癒合組織。將此癒合組織移植到和誘導該組織同樣的培養基，或將該培養基的椰子汁換成 10 ppm 的 Arginine 時，以 M. S. 爲基本的培養基上的生長較 W 爲基本的爲好。癒合組織在 M. S. 之液體培養基中，給以 60 rpm 的等高回轉振盪可使部分癒合組織分離成單細胞。

Figs. a-n. Cells and cell clusters in suspension culture from callus of sugar cane cv. N. CO-310. a) Expanded single cell. b) Round single cell. c, d, i) Small cell clusters. e) Elongated sigle cell. f) Expanded cells. g, h) Elongated-celled clusters. j) Expanded round and elongated cells. k, l, m) Large-celled clusters. n) Large cell-cluster Scale: 100 μ = |————————|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