Kasetsart J. (Nat. Sci. Suppl.) Vol. 22 : 30-36 (1988)

Production of Virus-Free Sugarcane by Tissue Culture

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ABSTRACT

Sugarcane plants of the three commercially grown varieties namely F140, Q83 and Supan I were produced free from sugarcane mosaic virus by the apical meristem culture. Shoot tips, 0.2-0.5 mm. in size, were cultured on MS medium supplemented with 3 mg/L 2,4-D and 15% (v/v) coconut water. Yellowish-white callus developed within 2 months. Plantlets were regenerated from calli cutured on MS medium with 1 mg/L 2,4-D and 15% (v/v) coconut water. The suitable medium for root induction in all cultivars was the MS or MS with 1 mg/L IBA. Detection of sugarcane mosaic virus (SCMV) in plantlets or individual plants by ELISA showed negative result suggesting that they were virus free.

INTRODUCTION

Sugarcanes are the economic important plants of Thailand and other countries in the world. Most are grown and cultured in tropical zone where problems about disease and insect infected particularly sugarcane mosaic virus (SCMV) are common SCMV, the mechanically transmitted and aphid-borne virus, is the most common virus which infected sugarcane plants. Vegetative propagation through cutting of sugarcane planting stock caused the rapid spread out of this viral disease. Recently tissue culture techniques for virus free sugarcane planting stocks production have been conducted using 0.2-0.5 mm. of meristem tip or apical dome. The method of using this explant segment can produce and multiply virus-free stocks of sugarcanes.

The objective of the experiment is to investigate the method of excising and culturing the apical meristem for multiplication stocks of virus free sugarcanes.

MATERIALS AND METHODS

Explants

Three commercially grown sugarcane varieties namely Q83, Fl40 and Supan 1 which were easily infected with sugarcane mosaic virus (SCMV) were selected as tested plants. Apical buds from the shoot of sugarcanes were excised and surface sterilized in 10% commercial clorox (5.25% sodium hypochlorite as the active agent) for 20 min, and 5% clorox for 10 min. respectively and rinsed 3 times with steriled distilled water. Meristematic tissues were taken from the apical regions of the innermost leaf, cut into 0.2-0.5 mm. under stereo microscope.

Callus Induction and Multiplication

Explants were transferred to callus induction medium, MS medium containing 3 mg/L 2, 4-dichlorophenoxy acetic acid (2,4-D), 10-15% (v/v) coconut water and 20% sucrose solidified with 7 gm/L agar. After 2 months, meristematic tissue was subcultured and transferred onto fresh medium (the same formula).

Plant regeneration

Calli were transferred onto MS medium with 1 mg/L 2,4-D, 10-15% (v/v) coconut water and 20% sucrose for shoot induction. Plantlets or shoots of sugarcane were transferred onto MS

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medium with varied concentration of IBA (0, 1, 1.5, 2 mg/L) for root induction.

Transplantation into soil

Before transplanting, the plantlets were soaked into water solution for 7 days to induced new root hair. Water solution was changed every 2 days, the plantets with new root hair established were transferred into sterile vermiculite.

Virus Detection

The Enzyme-linked Immunosorbent Assay (ELISA) technique was applied from standard ELISA (Clark and Adams, 1977) for SCMV detection in the extract of calli and plantlets of sugarcanes. ELISA was tested using the conjugate of anti-rabbit IgG alkaline phosphatase diluted to 1:1000 and p-nitrophenyl phosphate as the substrate. The absorbance was measured 1 hr. after the addition of substrate at the wavelength of 405 nm.

RESULTS

Meristem culture of sugarcanes

Meristematic tissue of sugarcanes variety namely F140, Q83 and Supan 1 were cut, 0.2-0.5 mm. in length, under stereo microscope and transferred onto modified MS medium containing 3 mg/L 2,4-D and 10% (v/v) coconut water. After culturing for 2 months, the meristematic tissues were developed and a group of white cells called callus sized 0.2-0.5 cm. was found.

Q83 variety was developed to calli formation better than another varieties (Table 1). Calli were maintained by subculture at every month on fresh medium (same formula). Calli (0.5-1 cm) subcultured on fresh medium were nearly doubled in size within 30 days. Such callus cultures developed green pin-head-sized structures scattered among the mass of white calli.

Shoot formation and plantlet regeneration

1-month-old-callus transferred to modified MS with 1 mg/L 2,4-D and 10% coconut water for 1 month initiated both somatic embryogenesis and shoot formation. At this stage numerous green dots emerged on the surface of calli. Newly green dots formed shoot and embryogenic callus. Mass of white callus size 1 cm. in length formed embryogenics and plantlets with 8-10 shoots. The shoot apices were elongated and formed leaf-like coleoptile structure. Shoots and somatic embryos formed complete plantlets within 4 weeks. Plantlets were subcultured into big bottle to produced plants about 8-10 cm. in height 4-5 leaves. They were individually separated and kept in the rooting medium, MS media containing varied concentration of IBA (0, 1, 1.5 and 2 mg/L). All variegated plantlets were sucessfully rooted for 1 month in solidified MS media and MS media plus 1 mg/L IBA. The MS media added with 1.5 and 2 mg/L IBA induced short roots and swelled root tips. Three varieties sugarcanes responded to rooting medium. Using low concentration of IBA such as 1 mg/L or on IBA induced many vigorous roots. Increasing IBA concentration, abnormal and short roots occurred.

Transplantation into soil

The healthy plantlets accomplished successfully were transferred into sterile vermiculite and they were established and grown within 14 days. The highest survival sugarcane plantlets percentage were about 70.

Virus detection

A total of 90 plantlets vareity of Supan 1 were derived from meristematic tissue sector, 80 of those plantlets had negative result when tested for virus infection, and only 10 plantlets had positive result (Table 2). From Table 2, the total average percentage of virus free is about 88.88.

This experiment indicates that the tissue culture propagation system using meristem tip will insure the plantlets free of specified pathogen especially virus infection.

Table 1 Callus and shoot formations of callus tissue on modified MS media

Medium	Variety	Callus formation	Shoot formation
MS + 3 mg/L 2,4-D	Fl40	+ +	−
+ 10% cw	Q83	+ + +	−
	Supan	+ +	−
MS + 1 mg/L 2,4-D	Fl40	+	+ +
+ 10% cw	Q83	+	+ + +
	Supan	+	+ +

Callus and shoot formation rating
+ low
+ + medium
+ + + high

Table 2 Virus detection in plantlets of sugarcane derived from meristem tissue

variety Supan	No. plants	No.plant with negative test	No.plant with positive test	%plants indexing negatively	%plants indexing posittively
No. 1	34	30	4	11.76	88.23
2	11	8	3	27.27	72.72
3	25	24	1	4	96
4	16	14	2	12.5	87.5
5	4	4	-	-	100
total	90	80	10	11.11	88.88

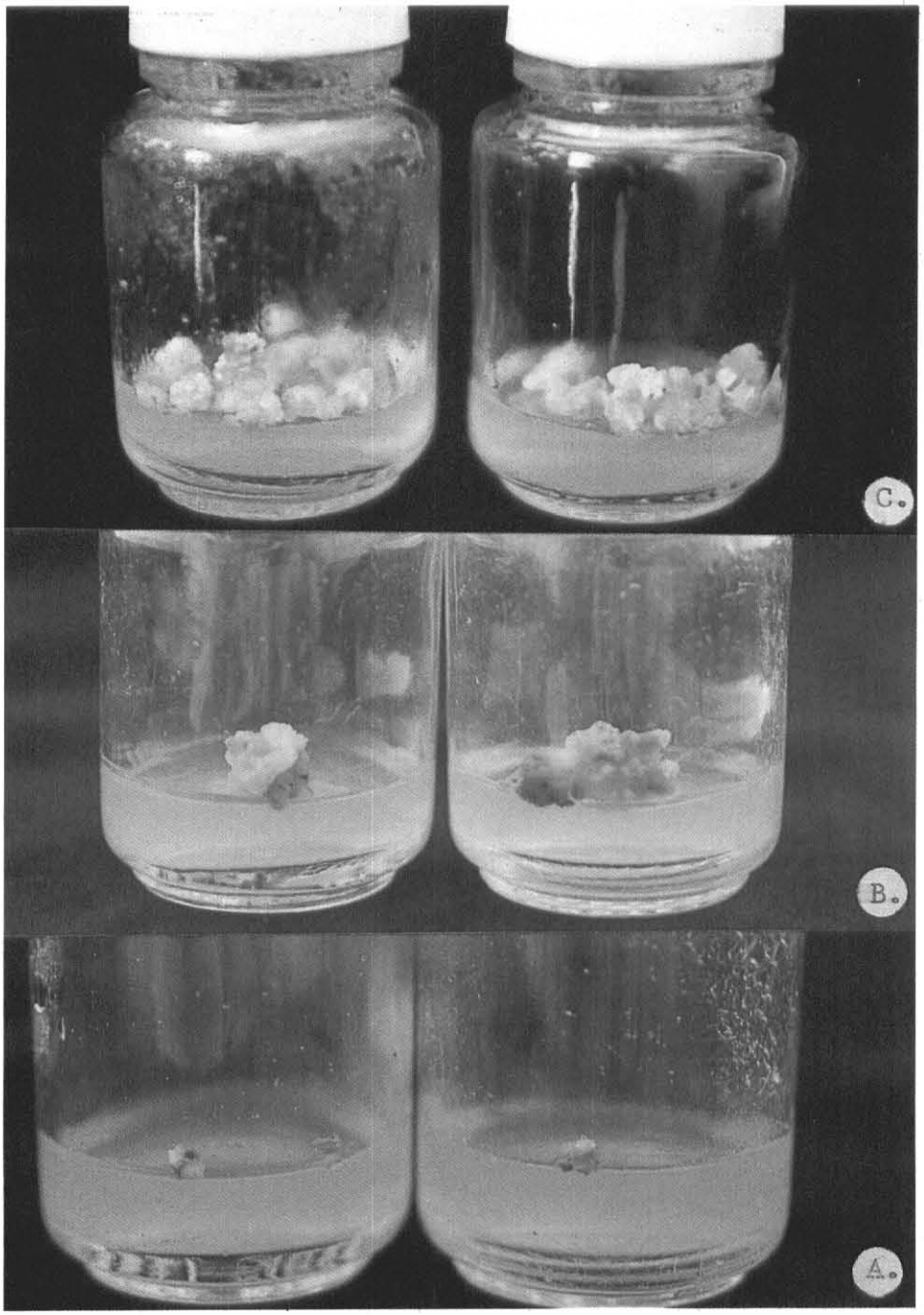

Figure 1 Meristematic tissue development of Q_{83} sugarcane to calli on modified MS media-MS + 3 mg/1 2,4 – D + 15% cw (v/v) within 2, 3 and 4 months (A – C)

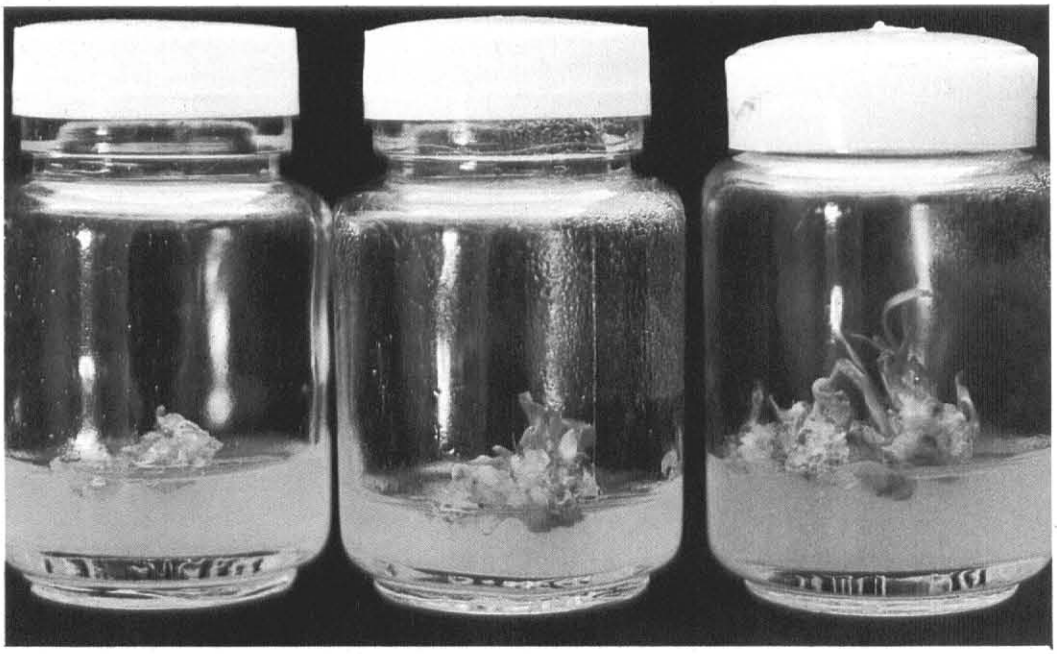

Figure 2 Callus differentiation and shoot formation in Q_{83} cultured on MS medium + 1 mg/1 2,4 – D + 15% cw (v/v) within 2 months.

Figure 3 The plantlets were transfered on MS medium or MS medium + 0.5 mg/1 IBA for root induction within 1 month.

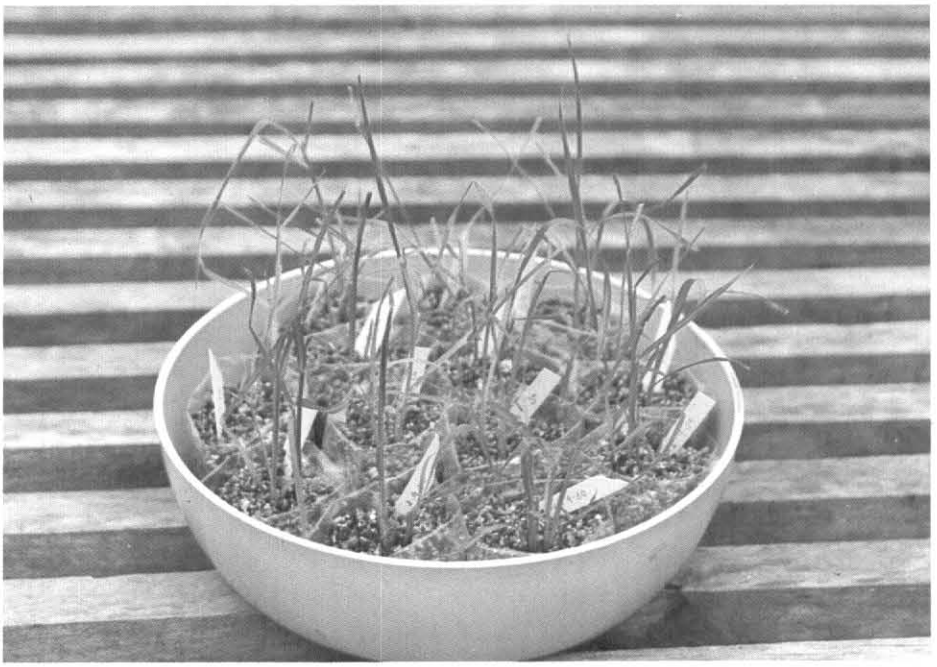

Figure 4 **The plantlets were successfully transferred into steriled vermiculite, afterward estabished plantlets will be grown in the field.**

DISCUSSION

The suitable media for callus induction is MS media with 3 mg/L 2,4-D and 10% coconut water similarily to the experiment of Langhans *et at.*, 1977;Mellor and Slace-Smith 1977;Quak, 1977; Dean, 1982 who previously studied this diseasefree plants techniques through tissue culture propagation. High auxin concentration such as 3 mg/L 2,4-D would promote good callus induction when auxin concentration was decreased to 1 mg/L 2,4-D and with 10% cw was added, calli differentiated to embryoid and occurred green spot on calli corresponding to the experiment of Nadar *et al* (1978) who reported that lower auxin concentration may be required for advanced embryogenesis. Plantlet differentiation occurred only in the absence of auxin. The multiplication of the embryogenesis depended on other factors. One factor was auxin concentration or growth hormone and the other was the increase of enzymes activity such as glutamine synthetase and glutamate synthase. The activity of these enzymes during the corresponding period in the non-shoot-forming was found to decline (Dwivedi *et al;* 1984) as well as the light intensity in the experiment. The most favourable light intensity for shoot differentiation was reported to be 700 ft-candle (Morales; 1977).

The suitable medium for root induction was MS basal media and MS containing 1 mg/L IBA corresponding to the experiment of Maretzki and Hiraki (1981) who reported that root induction was rapid in half strength MS medium with 7-9% sucrose. The plantlets were transferred into the water solution for the increasing of root hair induction which absorbed the content of water and had high survival rating after being transferred in soil. Sreenivasan and Jalaja (1983) reported that profuse rooting occurred in White's liquid medium and the survival was 81% to 90%

from liquid medium to soil. Leaf trimming resulted in vigorous root growth. (Nadar and Heinz; 1977)

Virus detection in sugarcane plantlet showed that most plantlets have high percentage of virus free plant about 88%. This may be due to the use of meristem tissues which were the smallest explant that infected virus cannot spread to this sector or having a little virus. Explant bigger than 0.2-0.5 mm. would have the opportunity to retain much virus infection in plant. Langhans *et al.* (1977) reported the success of using the explants of shoot tip 0.3-0.6 mm. in height was practical. Heat treatment was a valuable technique in establishing a virus free clone including meristem culture technique. After long period at high temperature some parts of the plant would be virus free eventhough others were still infected. Dean (1982) reported that meristem culture and a combination of heat therapy had been used to free sugarcane of sugarcane mosaic virus (SCMV). From this study we might conclude again that shoot tip culture could be a useful tool to completely eliminate bacteria and fungi infection in addition to virus (Baker *et al.* 1982)

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