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Detection of Sugarcane Mosaic Virus in Sugarcane Plantlets derived from Meristem Culture

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ABSTRACT

Meristem culture of five cultivars of sugarcane, namely, Supan, Nigro, Macos, E-Heudang and CO were cultured on Marashige and Skoog media with 3 mg/l or 1 mg/l 2,4-D inculding 10% (v/v) coconut water. The plantlets obtained were detected for the presence of sugarcane mosaic virus by using the enzyme-linked immunosorbent assay (ELISA), with alkaline phosphatase and *p*-nitrophenyl reaction system. Results were evaluated by automatic reading machine and percentage of virus-free stock were determined. It showed that sugarcane plantlets derived from meristem culture provided upto 80-100% virus-free plants which could be mass propagated for furthur plantation in the field.

INTRODUCTION

Sugarcane is one of the economic important plant of Thailand. Export sugar value was about 75% of the total sugar produced in the country. (Tanaboriboon *et al*, 1985). Sugarcane are susceptible to several major diseases and insects and this reduces the yeild and effects the throughout cycle of sugar process, as well. Therefore, sugarcane improvement for disease and insect resistance besides for good quality is considered to be valuable. One of the strategies for protection sugarcane from disease and insect is to use tissue culture technique, especially meristem culture to provide disease-free planting stock for plantation. Disease-free planting stocks can then be delivered to farmers to replace those disease infected planting stocks whose yeilds are rather low, and may result in contaminated planting fields.

This report is a part of research project under KU-Japan project phase II. The objective is concentrated on the detection of sugarcane mosaic virus in sugarcane plantlet derived from meristem culture.

MATERIALS AND METHODS

1. Meristem culture of sugarcane :

Meristems were excised from apical buds from the shoot of five cultivars of sugarcane, namely, Supan, Nigro, Macos, E-heudang and CO. They were surface steriled and cultured on Murashige and Skoog (MS) medium contained 3 mg/l or 1 mg/l 2,4-D and 10% coconut water (v/v). Growth and development of calli, and shoot primordia were observed every 2 months.

2. Detection of sugarcane mosaic virus (SCMV) in sugarcane plantlets derived from meristem culture.

Sample preparation : Weighed 0.25 g of sugarcane leaf cut from plantlets and ground in 0.5 ml of 0.01 M phosphate buffer, pH 7.4 containing 0.05% tween 20, 2% polyvinylpyrolidone (PVP) (Futrakul, 1989). After centrifugation of crude extract to get rid of plant debris, 1 ml of supernatant was pipetted into a microfuge tube (1.5 ml) to be used as sample stock solution. Each sample was diluted with 0.01 M sodium carbonate buffer to make 1:500 dilution, and this was used for virus detection by enzyme-linked immunosorbent assay (ELISA).

Antibody for virus detection : The gamma-globulin (IgG) was purified from antiserum against SCMV as the method perivously described (Clark and Adams, 1977). After ammonium sulfate precipitation, IgG was furthur purified by DEAE-cellulose 32 ionexchange column chromatography, and was used

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as an antibody for virus detection by ELISA. The purified IgG was adjusted to be 5 µg/ml with 0.01 M phosphate buffer, pH 7.4 containing 0.85% NaCl (PBS).

SCMV detection by ELISA : Virus detection was performed according to the indirect double antibody sandwich method of ELISA. Firstly, each well of a plastic microtiter plate was filled with 200 µg of plant sap. Each sample had 3 replications. After incubation of a plate at 37°C for 4 h, plant sap was drawn out from each well and the plate was washed three times with PBS (3 - 5 min). The wells were added with 200 µl of 5 µg/ml IgG solution, and incubation was done at 4°C for 18 h. The plate was washed as above and 200 µl of second antibody (1:1,000 dilution of antirabbit-goat IgG alkaline phosphatase) was added to each well. The plate was kept at 37°C for 4 h, and subsequently washed as above. Substrate solution (200 µl of 0.1 mg/ml p-nitriphenyl in 10% diethanolamine) was added to the well, and the plate was incubated in the dark at 14°C for 30 min-1 h. ELISA values were determined by ELISA reading machine and the results were analysed statistically t-test of CRD.

Healthy and diseased sugarcane leaves were used as negative and positve control for all experiments. Disease-free plantlets were justified according to the significant differences of the positive control.

RESULTS AND DISCUSSION

Meristem culture of sugarcane varieties :

Meristematic tissue of five cultivars of sugarcane developed white callus after culturing on MS containing 3 mg/l or 1 mg/l 2,4-D for 2 months. In the next stage, there were numerous green dots emerging on the surface of calli and some of them formed shoot and embryoid. This was corresponding to the result in an experiment of Nedar *et al.* (1978). Dwivedi *et al.* (1984) reported that multiplication of the embryogenesis depended on other factors such as auxin concentration or growth hormone and increase of enzyme activity such as glutamine synthetase and glutamate synthase in the callus.

In our experiment calli from CO and Nigro varieties can grow better than other varieties. Calli proliferation and green shoots formation could be increased by culturing in the same media for one month. Shoot and somatic embryoes formed completely within 2-3 months showing 4-5 cm height plantlets (Fig. 1).

Efficiency of ELISA method for detecting SCMV : In priliminary test using IgG concentration of 5 µg/ml, SCMV was detected from infected sugarcane leaf tissue when plant sap was diluted to 1:100-1:5,000 and enzyme-substrate reaction was left for at least 30 min. In subsequent experiment, the same condition was used except that sap dilution was 1:500

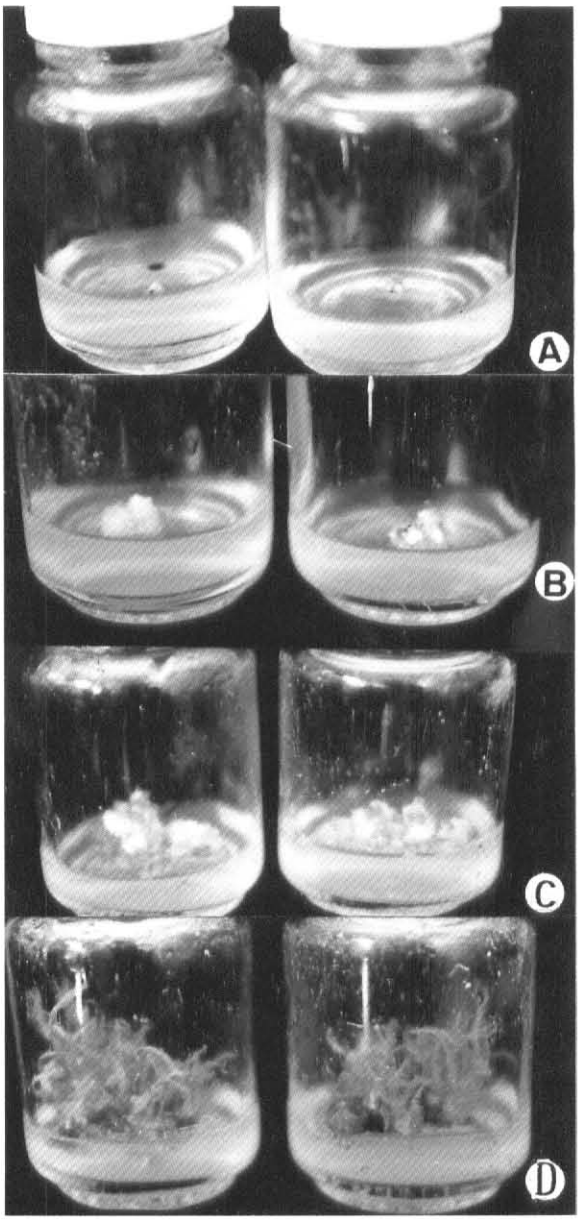

Figure 1 Calli differentiation of sugarcanes to shoot-forming embryoid and plantlets cultured on MS medium added with 1 µg/l 2,4-D and 10% coconut water (v/v) for 1, 2, 3 and 4 month, respectively.

in order to provide high absorbance value suitable for our evaluation (Table 1).

Virus detection in tissue culture : Statistical analysis indicated that about 80-100% disease-free plantlets could be obtained from meristem culture process (Table 2). It is likely that the particular meristem part of about 0.2-0.5 mm long is free from viurs, corresponding to the report that the meristem tip is a region where systemic plant virus was not found, although the plant is infected (Dean, 1982). Moreover, it was suggested that meristem tip not larger than 0.6 mm should be free from virus infection (Langhans *et al.* 1977). In addition, including of hot water treatment with meristem tip culturing would make virus-free plant propagation successful (Dean, 1982). In this study, meristem tip culture of sugarcane variety Nigro yielded 100% disease-free plant, while the

Table 1 Evaluation on statistically analysis of ELISA value ($O.D._{405}$) to justify healthy and diseased sugarcane plantlets.

Plant sap (dilution 1:500)		ELISA value ($O.D._{405}$, $\bar{X}$)	Difference at 95%[1] level in DMRT
Healthy leaf tissue	1	0.3263	a[2]
	2	0.3187	a
	3	0.3187	a
	4	0.3940	a
	5	0.3533	ab
	6	0.3860	a
Diseased leaf tissue	7	0.9337	c
	8	0.9050	c
	9	0.8877	c
	10	1.2740	e
	11	1.2410	e
	12	1.2137	de

1) AOV for CRDDMRT :

SOV	DF	SS	MS	F
TREATMENTS	11.0000	5.2461	0.4769	667.5061**
ERROR	24.0000	0.0171	0.0007	
TOTAL	35.0000	5.2633		

cv. = 3.74%
* = SIGNIFICANT AT 95% LEVEL
** = SIGNIFICANT AT 99% LEVEL
ns = NON SIGNIFICANT AT 95% LEVEL
2) = a, b were justified as healthy samples
c, d, e were justified as diseased samples

Table 2 Virus detection of sugarcane plantlets derived form meristem culture.

Cultivars	No. of tested samples	No. of sample With SCMV	Percentage of virus-free plant
1. Supan	309	57	81.55
2. Nigro	84	0	100.00
3. Macos	45	3	93.33
4. E-heudang	84	18	78.58
5. CO	45	6	86.67

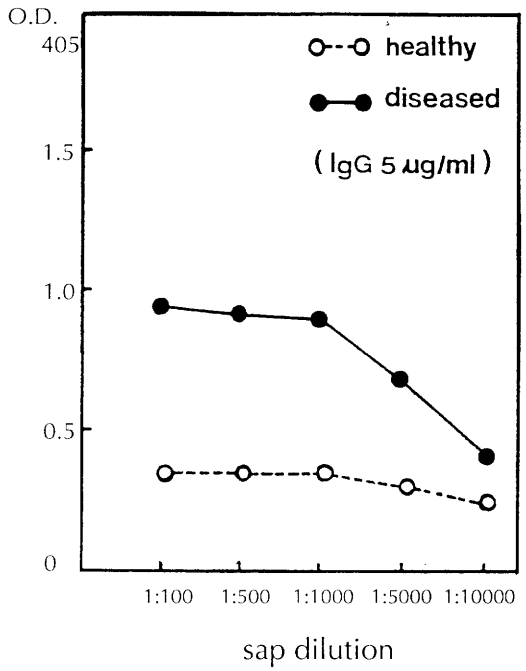

Figure 2 **Efficiency of ELISaAmethod for detection of SCMV sugarcane leaves, using IgG concentration at 5 mg/ml. Absorbance value at 405 nm wavelength of 1:100-1:5,000 diluted sap are 0.6-0.9.**

E-Heudang gave 78.58% which were the highest and the lowest proportion in our experiment, respectively. Higher percentage of virus-free plant is believed to be due to carefully collection of plants avoiding those with viurs symptom on the leaves.

CONCLUSIONS

The ELISA method provides good choice for virus detection in plant derived from tissue culture, although it seems to be a labourious work. Our data on ELISA test showed significant difference between healthy and diseased leaf tissues, enables acceptable standardization to discriminate virus infected plant. The detection scheme here showed about 80-100% disease free stock plants from meristem culture technique. In the previous report (Futrakul, 1989) SCMV was abundantly detected in callus and plantlet from infected sugarcane by using ELISA as well as dot-immunobinding assay methods. Therefore, in order to distribute virus-free plantlets derived from meristem culture to farmer for furthur mass propagation of sugarcane stock by conventional cutting method, those plantlets with SCMV contamination should be certainly discarded. Only clean stock will be succeedingly propagated. The virus detection program for releasing plant stock is considered to be an essensial step to be performed.

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