ERADICATING SUGARCANE YELLOW LEAF VIRUS IN SUGARCANE VARIETY N32 THROUGH TISSUE CULTURE

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Abstract

Yellow Leaf Syndrome of sugarcane is caused by Sugarcane Yellow Leaf Virus (SCYLV) and/or Sugarcane Yellows Phytoplasma (SCYP). Using tissue blot immunoassay to determine the presence of SCYLV, leaf samples of sugarcane variety N32 taken from a propagation plot at Mount Edgecombe all tested positive. In this study, two methods of tissue culture were used in an attempt to eradicate SCYLV from N32. In the first method, meristems were excised and cultured on a medium containing 1 mg kinetin/litre, which caused the direct production of shoots (organogenesis). Although a moderate number of shoots were produced from the cultured meristems, the virus remained. The second method involved culturing immature leaf-roll discs on media containing 0.6 and 3 mg 2,4-D/litre for the production of callus (indirect somatic embryogenesis). An average of 58.8 plants per stalk were produced from discs cultured on the medium containing 0.6 mg 2,4-D/litre, whereas 31.5 plants per stalk were produced from the medium containing 3 mg 2,4-D/litre. Of the 202 plants regenerated, one plant tested positive for SCYLV. The culture medium containing 0.6 mg 2,4-D/litre produced the highest number of virus-free plants and thus proved the most efficient method of production.

Keywords: sugarcane, sugarcane diseases, tissue culture, Sugarcane Yellow Leaf Virus, SCYLV, Yellow Leaf Syndrome, YLS, mosaic, SCMV

Introduction

SCYLV causes yellowing of the leaf midrib, leaf necrosis, an imbalance in the metabolism of carbohydrate and the accumulation of sucrose in the leaf midrib (Fitch *et al.,* 2001). The virus is transmitted by the aphid *Melanaphis sacchari* (Shenck and Lehrer, 2000), and is phloem-specific. Due to this specificity the virus cannot be transmitted mechanically, through surface injury to foliage or by short test probes made by aphids into epidermal cells (Gildhow, 1999). The virus can be detected using tissue blot immunoassay (TBIA) (Shenck *et al.,* 1997) or immuno capture-reverse transcription polymerase chain reaction (IC-RT-PCR) (Berry *et al.*, 2002).

Plant tissue culture, or micropropagation, is a technique used to grow whole plants from a small piece of the mother plant by methods such as organogenesis and somatic embryogenesis. Indirect somatic embryogenesis occurs when the explant is exposed to an auxin, which causes the formation of callus from which plantlets can be regenerated (Ho and Vasil, 1983).

Materials and Methods

Leaves of the sugarcane variety N32 were collected from a propagation plot at the South African Sugar Association Experiment Station at Mount Edgecombe, to identify SCYLV-infected plants. All N32 plants tested were positive for SCYLV.

Plants with symptoms of Sugarcane Mosaic Virus (SCMV) were recorded, and it was subsequently confirmed by RT-PCR that all the plants in the propagation plot were also infected with SCMV. RT-PCR for SCMV was performed according to Smith and van de Velde (1994) and Yang and Mirkov (1997). Tissue culture was then carried out in an attempt to eliminate both SCYLV and SCMV.

Stalk tops were stripped of leaves and swabbed with absolute ethanol to remove bacteria and fungi from the surface. Discs were excised from the immature leaf-roll and placed on Murashige and Skoog medium containing 3 or 0.6 mg 2,4-D/litre. The remaining leaf-roll was then removed to expose the meristem, which was excised and cultured on a medium containing 1 mg/L kinetin. Subculturing was done each week, unless the culture had been contaminated. When the regenerated plantlets were sufficiently large, they were placed on a rooting medium containing 1 mg/L kinetin and 2 mg/L indole-3-butyric acid. Root development took place over the following month, after which the plantlets were potted in sterile mix for hardening-off over the following three weeks. The hardening-off process began with sealing the plantlets in a plastic bag for the first week. In the second week, the bag was removed for one hour on day one and two hours on day two, so increasing the exposure time of the plantlets each day. In the third week the bags were removed. This process ensured controlled humidity while the plantlets were adapting to the new environment. When sufficiently large, a portion of leaf was excised from each plantlet and tested for SCYLV using IC-RT-PCR as described by Berry *et al.* (2002).

Results and Discussion

A moderate number of plants (average 12.3/meristem) were produced from meristem culture through organogenesis. The plants all retained SCYLV and SCMV after micropropagation (Table 1).

Table 1. History of meristems cultured through organogenesis for study on the eradication of Sugarcane Yellow Leaf Virus in cane variety N32.

Meristems		Shoots		Plants			
No. cultured	No. contaminated	No. on rooting medium	No. contaminated	No. hardened off	No. surviving hardening off	No. positive SCYLV	No. positive SCMV
20	4	257	54	203	196	196	196

It would appear that the size of the explant containing the meristem influences whether or not a virus will be eliminated. Chatenet *et al.* (2001) were able to eradicate SCYLV from 62 varieties, with a 92% success rate, by using meristem culture. An excised meristem that is too large may carry residual virus. Many of the plantlets obtained from meristem culture showed symptoms of SCMV, and all regenerated plants tested positive for mosaic using RT-PCR.

An average of 2.8 plants per leaf-roll disc were produced using 0.6 mg 2,4-D/litre, whereas an average of 1.5 plants per disc were produced on the medium containing 3 mg 2,4-D/litre. An average of 21 discs per stalk could be cultured, thus producing 58.8 plants per stalk on the medium containing 0.6 mg 2,4-D/litre, and 31.5 plants per stalk on the medium containing 3 mg 2,4-D/litre. A total of 208 plantlets were regenerated from the 123 surviving discs, of which 202 survived the hardening-off process and were planted out in the field.

SCYLV

Three months after planting, all plants were tested for SCYLV, using IC-RT-PCR, and all were found to be negative. Six months after planting, the plants were again tested for SCYLV, on this occasion using TBIA. Of the 202 plants, one tested positive for SCYLV (Table 2).

Table 2. History of 140 leaf-roll discs cultured on medium containing 0.6 and 3 mg 2,4-D/litre, for a study on the eradication of Sugarcane Yellow Leaf Virus in cane variety N32.

Stages and reactions	2,4-D per litre medium	
	0.6 mg	3 mg
Contaminated discs on 2,4-D and regeneration media	42	63
No. dead (phenolics) on 2,4-D and regeneration media	28	24
No. of discs on regeneration medium	70	53
Average no. plantlets produced per disc	2.8	1.5
No. plantlets transferred to rooting medium	196	83
No. plantlets contaminated on rooting medium	38	33
No. plantlets dead during hardening-off	6	0
No. plants tested negative for SCYLV, 3 months after planting (IC-RT-PCR)	152	50
No. plants tested positive for SCYLV, 6 months after planting (TBIA)	1	

SCMV

After three months in the field plots, the plants showed no symptoms of SCMV and appeared healthy. However, after six months some of the plants showed symptoms of mosaic. TBIA for SCMV was performed on all the plants according to Comstock (pers. comm.), with 10 testing positive. None of the plantlets had shown symptoms of mosaic during hardening-off. The plants that tested positive for SCMV tested negative for SCYLV. Most of the mosaic infection occurred in plants that were situated close to the outer edge of the field, and five of the 10 infected plants were next to each other, suggesting that SCMV was transmitted through aphids and not through tissue culture.

Conclusion

The culturing of discs from immature leaf-roll on a medium containing 0.6 mg 2,4-D/litre proved to be the most efficient method of producing a large number of virus-free plants when compared with the culturing of discs on a medium containing 3 mg 2,4-D/litre and meristem organogenesis.

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