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Effect of Red Rot Phytotoxin on *in Vitro* Shoot Differentiation of Sugarcane Variety CoC671

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ABSTRACT

The sugar industry in India is well developed with a consumer base of more than a billion people. It is also the second largest producer of sugar in the world. The early maturing, high-yielding sugarcane variety CoC671 is very susceptible to red rot disease caused by *Colletotrichum falcatum* Went. During the present investigation, a red rot toxic metabolite (phytotoxin) was isolated using sugarcane host extract medium and solvent extraction. Partially purified phytotoxin was incorporated into modified MS medium supporting the differentiation of shoots from callus of var. 'CoC671' at different concentrations: 0.05 to 0.5% (v/v). The phytotoxin stimulated the emergence of shoot buds from callus at 0.05 and 0.1% but significantly inhibited shoot differentiation at 0.5%. The growth of regenerated shoot buds was stimulated at lower levels of phytotoxin (0.05, 0.1%) and inhibited at 0.4 and 0.5%. Use of the phytotoxin would help in the selection of sugarcane clones resistant to *C. falcatum*.

Keywords: callus, *Colletotrichum falcatum*, phytotoxin

INTRODUCTION

Sugarcane, an important cash crop worldwide, is cultivated in nearly 70 countries spread over five continents and contributes to 65% of the world's sugar production (Vedamurthy 1999). In most cane-growing countries, especially the tropical and sub-tropical regions, red rot disease, caused by *Colletotrichum falcatum* Went, continues to be a threat, resulting in severe loss in cane yield. India ranks second in the world for sugarcane production with 315 million tonnes of cane production, at an average yield of 70-80 tonnes/ha. However, most of the commercial sugarcane varieties are susceptible to this disease which contributes to approximately 20% loss in yield (Mohanraj *et al*. 2004; Ali 2006).

Plant tissue culture technologies have been used as an effective tool to select disease resistant/tolerant sugarcane lines (Liu 1981; Chandrika *et al*. 1984). Methodologies to screen for red rot susceptibility in the initial stages of field multiplications have been reported by Rana and Gupta (1964). However, research efforts towards successfully developing disease-resistant lines against red rot is lacking due to the complexity of the host-pathogen interaction. In a previous study we isolated, identified and extracted phytotoxin from *C. falcatum*. Somaclones were screened against different levels of the phytotoxin during the shoot regeneration phase (Naik and Vedamurthy 1997). In the present investigation we report the sensitivity tests conducted to determine the limits of tolerance of sugarcane (early maturing var. 'CoC671') callus to the toxic metabolite, produced by *C. falcatum*.

MATERIALS AND METHODS

Sugarcane var. 'CoC671' was obtained from Aland sugar factory, Gulbarga (Karnataka, India). These canes were broken into pieces, each having three eye-buds, and treated with organo-mercuric compounds for 10 min at 0.1% (w/v). They were planted in a field plot measuring 6 × 6 m in the Botanical Garden of Gulbarga University, Gulbarga. Standard agronomic practices were followed to obtain healthy seedlings. 3-4 month old disease-free plant materials were used for *in vitro* culture experiments. All media were prepared as described by Gamborg and Shylok (1981). In the present study, Murashige and Skoog (1962; MS) medium were used for initiation and regeneration of callus tissue (Heinz and Mee 1969; Liu 1981).

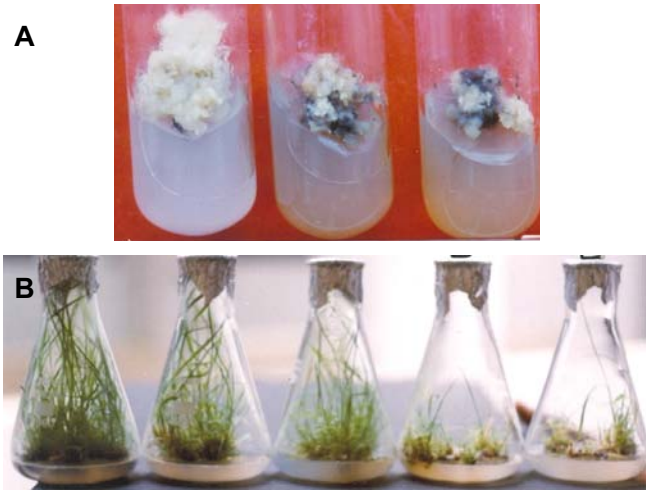

Fig. 1 *In vitro* response of sugarcane callus (**A**) and shoots (**B**) of var. 'CoC671' at different levels of red rot phytotoxin. From left to right: (**A**) Control, 0.2%, 0.5%. (**B**) Control, 0.05%, 0.1%, 0.2%, 0.5%.

Young leaf explants (2-3 cm from the node) were excised with a sterile blade. Explants were sterilized with 70% ethanol followed by 0.1% mercuric chloride, 3 min for each treatment. The explants were then thoroughly washed with sterile distilled water three times. The sterilization procedure was performed under aseptic conditions (Sateesh 2006). The young leaves (inner-most whorl) were excised and used for callus induction. The leaf explants were

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Short Communication

inoculated immediately on MS medium to avoid polyphenol exudation from cut ends of the explants, which would inhibit callus initiation and growth. The culture tubes were incubated at 25 ± 2°C in the dark for 4-6 weeks, after which the callus mass was sub-cultured onto fresh medium for further callus proliferation.

The actively growing callus was sub-cultured onto modified MS shoot regeneration medium supplemented with increasing levels of phytotoxin from 0.0-0.5% (**Table 1**). The culture tubes were incubated under low light intensity (40 µE/m²/s) with a 12-h photoperiod in a culture room.

The values are expressed as mean ± standard error and statistical analysis was carried out by using SPSS 15.0 software by adapting one-way analysis of variance (ANOVA) at $P = 0.05$. Each treatment contained five replications and the experiment was repeated three times.

RESULTS AND DISCUSSION

Actively proliferating sugarcane callus, after sub-culture onto MS-based shoot regeneration medium formed numerous green globular structures (1-1.5 mm) on the callus surface in the control and 0.05, 0.1 and 0.2% phytotoxin-supplemented medium after 2 weeks (**Fig. 1A**). While calculating the number of differentiated shoot buds and their height in each tube, only continuously growing regenerated shoots were considered. The cells near the growing apex of the callus mass often differentiated slowly into leafy shoots within 4 weeks. Phytotoxin at 0.05 and 0.1% stimulated shoot emergence (**Fig. 1B**), while at 0.2 and 0.5% it was significantly inhibited (**Fig. 1B**). Leafy shoots initiated in 0.05 and 0.1% phytotoxin-supplemented medium were allowed to grow for 6 weeks. The shoots growing in 0.05 and 0.1% phytotoxin-supplemented medium were narrow and green while those in 0.2 and 0.5% displayed broad chlorotic and succulent leaves, which wilted soon. The control plants had narrow green leaves (**Table 2**). The number of multiple shoots in each tube and their average height varied in the different treatments (**Table 2**).

Many fungi produce toxins in infected plants as well as in culture medium (Agrios 2005; Amusa 2006). These fungal phytotoxins are deleterious to plant growth and are effective at very low concentrations (Agrios 2005). Vedamurthy *et al*. (2008) reported that *C. falcatum* toxin affected sugar metabolism in sugarcane callus. Phytotoxins affect the host cells by changing permeability of the cell membrane or by inactivating or inhibiting enzymes and subsequently interrupting the corresponding enzymatic reactions (Sateesh *et al*. 1997). Thus, they increase the virulence of the pathogen. Hence the present study was carried out to understand the effect of *C. falcatum* phytotoxin on host (CoC671) callus growth and differentiation. Similar results were reported in coffee and mango (Nyange *et al.* 1995; Jaisankar *et al*. 1999).

Table 1 Shoot regeneration response in sugarcane cultivar CoC 671 to different concentration of red rot phytotoxin on MS-S medium.

Phytotoxin conc. (%)	No. of tubes inoculated	No. of tubes showing shoot initiation		
		2 weeks	4 weeks	6 weeks*
0.0	21	21	21	21 a
0.05	21	15	19	19 a
0.1	21	11	14	14 b
0.2	21	11	15	17 b
0.4	21	2	4	4 c
0.5	21	0	0	0 d

* Means followed by different letters differ significantly when subjected to DMRT ($P \leq 0.05$)

Table 2 Growth characters of regenerated shoots in sugarcane cultivar CoC 671 at different concentration of phytotoxin after 8 weeks.

Phytotoxin conc. (%)	No. of multiple shoots ± standard error in each tube	Height of multiple shoots ± standard error (cm)
0.0	9 ± 4a	4.0 ± 0.8a
0.2	12 ± 3a	3.8 ± 1.2a
0.4	2 ± 1b	2.3 ± 1.1b

* Means followed by different letters differ significantly when subjected to DMRT ($P \leq 0.05$)

CONCLUSION

In vitro selection of crop plants for disease resistance has been employed by using microbial phytotoxic metabolites (e.g., Daub 1986; Nyange *et al*. 1995; Svabova and Lebeda 2005; Amusa 2006). Such optimized parameters will help in rapid selection for disease resistance/tolerance.

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