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RESEARCH ARTICLE

DEVELOPMENT OF RED ROT RESISTANT SOMA CLONES OF SUGARCANE VARIETY COS8436 THROUGH *IN vitro* CULTURE

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ABSTRACT

To develop red rot resistant soma clones from a susceptible sugarcane variety, leaf sheath explants of sugarcane variety CoS8436 (susceptible to red rot disease) were inoculated on MS medium containing different concentrations of 2,4-dichlorophenoxyacetic acid (2,4-D). Maximum callus induction and growth was recorded on medium supplemented with 4.0 mg/lit 2, 4-D. Four week old actively growing calli were treated with sodium azide and transferred on fresh MS callus medium supplemented with various concentrations (1.0-5.0 %) partially purified toxin (culture filtrate) of *Colletotrichum falcatum* Went, the causal organism of red rot. The calli surviving on medium containing the highest concentration of toxin were considered to be toxin resistant and were multiplied further for next 4 weeks through subcultures. Well grown toxin resistant calli were then transferred to shoot regeneration medium comprising MS salts + BAP + Kinetin (0.5 mg/l each). After shoot regeneration, the shoots were rooted on half strength MS medium, hardened for four weeks in the green house and finally transplanted to the field. Forty eight canes randomly selected from somaclonal population were inoculated with a mixture of spore suspension of isolate of *C. falcatum* isolated from naturally infected canes of sugarcane variety CoS8436 using plug method of inoculation. The inoculation was carried out in August at 7 month crop age. The results showed that out of 48 clones tested 25% were found resistant, 50% were moderately resistant and remaining 25% were moderately susceptible.

Key **W**ords : *C. falcatum*, CoS8436, Red rot, Soma clones, Sugarcane, Tissue culture**.**

One of the reasons of low productivity in sugarcane and sugar in the country is the incidence of red rot disease, commonly known as cancer of sugarcane. This disease affects both grower and miller. Red rot is new widely distributed and has been reported from more than 68 sugarcane growing countries. Many popular and high yielding varieties are struggling for their existence due to occurrence of new pathotypes. The breeding methods require a period of over 8 years to develop a new variety and a period of further 8-10 years to reach the seed of new varieties to the growers in remote villages for general cultivation. To develop disease resistant varieties in comparatively shorter period of time, *in vitro* techniques seem to be the only possible solution to the problem. Somaclonal variation is being employed to develop resistant clones of sugarcane for various diseases (3). The present study was undertaken to investigate the red rot behavior in soma clones of sugarcane variety CoS8436 (susceptible).

MATERIALS AND METHODS

Callus cultures were initiated from leaf sheath explants of sugarcane (*Saccharum* species complex) hybrid variety CoS8436 on MS medium (2) containing agar (8 g/l), sucrose (20 g/l) and various concentrations of 2,4-dicholophenoxyacetic acid (1.0-5.0 mg/l) (Fig. 1). Maximum callus induction and growth was recorded on medium supplemented with 4.0 mg/l 2,4-D. Four week old actively growing calli were treated with sodium azide and transferred

Table-1. Red rot behavior of somaclones of sugarcane variety CoS8436 against the mixture of 2 isolates of *C. falcatum*.

	Resistant (0-2.0)	Moderately resistant (2.1-4.0)	Moderately susceptible (4.1-6.0)	Susceptible (6.1- 8.0)	Highly susceptible (above 8.0)	Total
No of somaclones	12	24	12	-	-	48
Donor (CoS 8436)	25%	50%	25%	100%		

on fresh MS callus medium supplemented with various concentrations (1.0-5.0 %) of partially purified toxin (culture filtrate) of *Colletotrichum falcatum* Went, the causal organism of red rot. The calli surviving on medium containing the highest concentration of toxin were considered to be toxin resistant and were multiplied further for next 4 weeks through subcultures on the fresh medium of the same composition. Well grown toxin resistant calli were then transferred to shoot regeneration medium comprising MS salts + BAP + Kinetin (0.5 mg/l each). After shoot regeneration, the shoots were transferred on half strength MS rooting medium containing 5.0 mg/l NAA and 50g/l sucrose at pH 6.0 (Fig. 3). The rooted plantlets were taken out of the culture vessels, washed with water and hardened for four weeks in the green house. Finally the plantlets were transplanted in the field at 45 cm spacing in rows 90 cm apart. Forty eight canes randomly selected from somaclonal population were inoculated with a mixture of spore suspension (10^6 spores/ml) of isolate of *C. falcatum* isolated from naturally infected canes of sugarcane variety CoS8436 (Fig 5), using plug method of inoculation (Fig 4). The inoculation was carried out at 7 month crop age.

RESULTS AND DISCUSSION

Observations made on 48 randomly selected clones showed that the soma clones varied in a number of morphological traits like stalk height, stalk thickness, number of millable canes, shape and size of internodes, length and width of leaves, bud and bud characteristics as compared to conventionally raised plants of the donor variety (CoS8436). The results regarding red rot behavior of soma clones tested by plug method of inoculation against 2 isolates of test pathogen has been presented in Table 1. It is apparent from the data that out of 48 soma clones tested, 12 soma clones (25% of total) were found resistant, 24 (50%) were moderately resistant and remaining 12 (25%) clones were moderately susceptible to red rot. These soma clones were better in red rot resistance than the donor variety CoS8436 which was found susceptible to red rot.

The recovery of moderately susceptible soma clones regenerated even from toxin resistant callus indicated that the variations for disease resistance could arise at any stage during *in vitro* shoot regeneration. The wide variations observed in a number of agronomical traits along with red rot behavior in the somaclonal population, was possibly due to complex genetic nature and existence of chromosomal mosaicism in commercial hybrid varieties of sugarcane (1, 4). Here, we conclude in the present study that red rot resistant somaclones can be regenerated from susceptible variety of sugarcane through *in vitro* technique.

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