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 Shyllok (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).

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 inoculated on petri plates containing modified media with the following concentrations of phytotoxin: Control, 0.2%, 0.5%. (B) Control, 0.05%, 0.1%, 0.2%, 0.5%.

![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%.

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

<table>
<thead>
<tr>
<th>Phytotoxin conc (%)</th>
<th>No. of tubes inoculated</th>
<th>No. of tubes showing shoot initiation after 2 weeks</th>
<th>4 weeks</th>
<th>6 weeks*</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.0</td>
<td>21</td>
<td>21</td>
<td>21</td>
<td>21 a</td>
</tr>
<tr>
<td>0.05</td>
<td>21</td>
<td>15</td>
<td>19</td>
<td>19 a</td>
</tr>
<tr>
<td>0.1</td>
<td>21</td>
<td>11</td>
<td>14</td>
<td>14 b</td>
</tr>
<tr>
<td>0.2</td>
<td>21</td>
<td>11</td>
<td>15</td>
<td>17 b</td>
</tr>
<tr>
<td>0.4</td>
<td>21</td>
<td>11</td>
<td>15</td>
<td>17 b</td>
</tr>
<tr>
<td>0.5</td>
<td>21</td>
<td>0</td>
<td>0</td>
<td>0 d</td>
</tr>
</tbody>
</table>

* Means followed by different letters differ significantly when subjected to DMRT (P ≤ 0.05).

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

<table>
<thead>
<tr>
<th>Phytotoxin conc (%)</th>
<th>No. of multiple shoots ± standard error in each tube</th>
<th>Height of multiple shoots ± standard error (cm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.0</td>
<td>9 ± 4a</td>
<td>4.0 ± 0.8a</td>
</tr>
<tr>
<td>0.2</td>
<td>12 ± 3a</td>
<td>3.8 ± 1.2a</td>
</tr>
<tr>
<td>0.4</td>
<td>2 ± 1b</td>
<td>2.3 ± 1.1b</td>
</tr>
</tbody>
</table>

* Means followed by different letters differ significantly when subjected to DMRT (P ≤ 0.05).

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CONCLUSION

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

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