An improved technique of inoculation for the artificial production of sugarcane smut and development of uniform diseased cane material

S.K. DUTTAMAJUMDER
Division of Plant Pathology, Indian Institute of Sugarcane Research, Lucknow 226 002

ABSTRACT: *Ustilago scitaminea* Sydow, the causal organism of whip smut of sugarcane is basically a disease of meristematic tissue and it proliferates in the young and actively growing tissues. It enters the healthy sugarcane plant through lateral buds, overcoming the barriers of bud scales. With the germination of an infected bud the fungus also gets activated and grows along with the apical meristem and later on manifests itself by converting the apical meristem in a very characteristic manner i.e., by producing a typical whip like smutty structure. Screening of sugarcane genotypes against smut is mainly done either by inoculating the setts (planting material) by dipping in the spore suspension of smut or by painting the buds with the spore suspension at the time of planting. Inoculated setts are planted in the field for germination and symptom expression. Every sugarcane pathologist faces problems of getting enough uniform smut infected sugarcane planting material (setts) for carrying out other experiments. Taking the analogy from the grassy shoot disease (GSD), where the mother shoot feeds the grassy lanky albinoid tillers, this technique was developed by inoculating the underground buds of the cane at the time of tillering, taking advantage of cane's own physiology of tillering and grand growth. In a highly susceptible variety like CoLk 7901 the success rate of infection was to the tune of 83% with the new method as compared to the common sett inoculation method that produced an infection level of 36% only. Moreover, the systematically infected tillers produced at the grand growth stage of sugarcane were found to be excellent experimental material and thus could circumvent the lack of uniform infecting material for conducting other studies.

**Keywords:** Inoculation technique, smut, *Ustilago scitaminea*, sugarcane, screening

*MATERIALS AND METHODS*

Fresh smut spores collected from diseased canes of varieties like CoLk 7901 and Co1158 were air dried in shade. A suspension of such freshly collected smut spores ($\times 10^6$ spores/ml) was made in sterile water. Viability of the smut spores was also tested and a collection showing a viability of >70% was taken for the inoculation purpose.

*Raising of crop*

Two smut susceptible sugarcane genotypes, viz., Co 1158 and CoLk 7901 as well as other sugarcane genotypes with variable susceptibility/resistance to smut
were selected for this experiment. In the month of March, three budded setts obtained from the healthy canes were planted in rows, 90 cm apart with recommended doses of fertilisers. Normal cultural operations were carried out as per the recommendations. In the third year of experiment a number of sugarcane genotypes were also planted to test the efficiency of the proposed technique. By the first fortnight of May, germination was complete and tillering started (about 50-55 days of planting). At this time the field was irrigated and requisite interculture operation followed.

**Inoculation of underground buds**

In the last week of May, when some amount of growth had taken place after germination and tillering had started, soil around a clump was carefully dug out, 4-5 basal leaves were removed carefully to expose the very young buds unhurt. Usually, in the mother shoot, 2-3 buds were inoculated by painting the inoculum on the young buds with the help of an airbrush. Inoculation was carried in the afternoon to avoid the scorching sun. Immediately after the inoculation, soil around the clump was filled and pressed slightly to avoid any uprooting of the young cane plant. After the inoculation (in a week), the field was irrigated.

**Monthly inoculations (above ground buds)**

Monthly inoculations were made in the standing canes after the recession of monsoon rains. Inoculations were made in the month of September, October, November and December. Canes were detopped 24 h prior to inoculation to increase the activity of the buds. Inoculation was carried out by placing a cotton swab, soaked in the inoculum, on the bud and wrapping it with moistened cotton/parafilm to prevent moisture loss. All the above ground buds of a cane were inoculated. Inoculations were carried out in the afternoon.

**Control (Check)**

For comparison, suitable controls were planted. One set was planted following the standard sett inoculation and the other set was left uninoculated. For inoculation, setts were dipped in the smut spore suspension for 30 min, air dried and then planted in the field.

**RESULTS AND DISCUSSION**

**Standing cane inoculation - I (Above ground buds)**

As early as 1938 Luthra et al. (1938) successfully inoculated canes by applying the smut spore suspension on the buds of standing canes. It was observed that in monthly inoculation experiment, canes inoculated in September started producing whips in the month of November (took about 50 days). The success rate of infection in September inoculation varied from 7-16%. Upper buds (young buds) were found more susceptible to smut infection than the lower buds. Susceptibility of young buds to smut infection has also been observed by the other workers (Byther and Steiner, 1974; Singh, 1977). However, most of the inoculations made in the month of October-November did not produce any whip in the month of December/January. By the month of March a flush of whip appeared. Appearance of whips continued in a staggered fashion till the harvesting of the crop in the month of May. Young buds caught infection early and produced maximum number of whips. Wrapping the node with either polythene or parafilm, after placing the spore soaked cotton swab on the bud, helped in better retention of moisture and also greater success of infection than wrapping with only moistened cotton. Parafilm was found superior to polythene strip as a wrapping material and also for the retention of moisture. However, no significant difference could be observed in the success rate of infection and production of whips. Prevailing temperature played a dominant role in the infection and expression of smut symptoms (Fig. 1) as the October, November and December inoculations did not result in the whip formation within 60 days of inoculation. However, inoculations during winter months produced a flush of whips after the return of normal temperature in February/March. The percentage of bud showing smut whip formation never went beyond 26% even in the highly susceptible genotype CoLk 7901. Inoculation of standing canes in winter months of November/December did not produce enough growth of side shoot that can be used for other experimental purposes. However, this provided sufficient amount of fresh smut inoculum for carrying out inoculations in the month of March and also for the inoculations in the month of May.

**Standing cane inoculation - II (Young underground buds)**

The incidence of secondary infection depends largely on the number of successful infection that takes place during the growing season and it depends on several factors like age of shoots, moisture, temperature and spore load. The resultant infection hyphae produced after germination and anastomosis penetrate the host directly and infection takes place in under 8h when temperature and humidity are favourable (Bock, 1964). The increased susceptibility of germinating buds is associated both with swelling of the bud scales and with an increase in the area within the bud which can
Table 1. Response of two sugarcane genotypes against smut disease inoculated with both sett inoculation and the proposed method

<table>
<thead>
<tr>
<th>Genotypes</th>
<th>Sett inoculation</th>
<th></th>
<th>Young bud inoculation</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>First Year</td>
<td>Second Year</td>
<td>First Year</td>
<td>Second Year</td>
</tr>
<tr>
<td>Co 1158</td>
<td>150</td>
<td>150</td>
<td>150</td>
<td>150</td>
</tr>
<tr>
<td>CoLk 7901</td>
<td>49</td>
<td>55</td>
<td>52</td>
<td>60</td>
</tr>
<tr>
<td></td>
<td>13</td>
<td>20</td>
<td>14</td>
<td>22</td>
</tr>
<tr>
<td>Smut incidence (%)</td>
<td>26.5</td>
<td>36.4</td>
<td>26.9</td>
<td>36.7</td>
</tr>
<tr>
<td>Infected bud (%)</td>
<td>8.7</td>
<td>13.3</td>
<td>9.3</td>
<td>14.7</td>
</tr>
</tbody>
</table>

Table 2. Response of different sugarcane genotypes against smut inoculated by the new method

<table>
<thead>
<tr>
<th>Genotypes</th>
<th>No. of buds inoculated</th>
<th>No. of buds germinated</th>
<th>No. of buds infected</th>
<th>% of infected buds</th>
<th>Smut incidence (%)</th>
<th>Disease** ratings</th>
</tr>
</thead>
<tbody>
<tr>
<td>CoLk 7901</td>
<td>30</td>
<td>30</td>
<td>25</td>
<td>83.3</td>
<td>83.3</td>
<td>HS</td>
</tr>
<tr>
<td>Co 1158</td>
<td>20</td>
<td>20</td>
<td>12</td>
<td>60.0</td>
<td>60.0</td>
<td>HS</td>
</tr>
<tr>
<td>Co1 64</td>
<td>24</td>
<td>24</td>
<td>14</td>
<td>58.3</td>
<td>583</td>
<td>HS</td>
</tr>
<tr>
<td>CoS 767</td>
<td>24</td>
<td>24</td>
<td>12</td>
<td>50.0</td>
<td>50.0</td>
<td>HS</td>
</tr>
<tr>
<td>Co 1148</td>
<td>20</td>
<td>20</td>
<td>2</td>
<td>10.0</td>
<td>10.0</td>
<td>MR</td>
</tr>
<tr>
<td>CoLk 8102</td>
<td>18</td>
<td>18</td>
<td>2</td>
<td>11.1</td>
<td>11.1</td>
<td>MR</td>
</tr>
<tr>
<td>BO 91</td>
<td>31</td>
<td>31</td>
<td>19</td>
<td>61.3</td>
<td>61.3</td>
<td>HS</td>
</tr>
<tr>
<td>NG 78</td>
<td>18</td>
<td>18</td>
<td>11</td>
<td>61.1</td>
<td>61.1</td>
<td>HS</td>
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<tr>
<td>N85-808</td>
<td>20</td>
<td>20</td>
<td>12</td>
<td>60.0</td>
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<td>HS</td>
</tr>
<tr>
<td>Co 62399</td>
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<td>21</td>
<td>6</td>
<td>28.6</td>
<td>28.6</td>
<td>S</td>
</tr>
<tr>
<td>UP 5</td>
<td>18</td>
<td>18</td>
<td>12</td>
<td>66.7</td>
<td>66.7</td>
<td>HS</td>
</tr>
<tr>
<td>CoH 72</td>
<td>24</td>
<td>24</td>
<td>3</td>
<td>12.5</td>
<td>12.5</td>
<td>MR</td>
</tr>
</tbody>
</table>

**  
0 0%  R  5  13-25%  MS  
1 1-3%  R  6  26-35%  S  
2 4-6%  R  7  36-50%  HS  
3 7-9%  MR 8  51-65%  HS  
4 10-12% MR 9  66-100% HS  

be penetrated by infection hyphae from germinating spores. The susceptible period is brief, and young shoots become very resistant to infection when only 4 cm in length, even in highly susceptible varieties (Bock, 1964; Byther and Steiner, 1974). This happens because the mature outer bud scales and rapidly elongating inner scales increase protection to the inner meristematic regions of the bud (Waller, 1970). The tillering rate (the rate at which these young shoots appears) is consequently a measure of the amount of infectable tissue present in the crop at any given point of time. There is a progressive decrease in the production of new tillers and in the amount of infectable tissue with a consequent rise in resistance. Probably quick and high tillering habit of the genotype CoLk 7901 renders it highly vulnerable to smut infection. The inoculation of settlings by this new method of inoculation at the time of tillering gave better success of infection than any other method, as it registered as high as 83% infection of buds and production of whips.

In the sub-tropical India where the germination of sugarcane buds is limited to 30-45%, the success rate of smut infection as well as its repeatability is not very high. Taking 40 % as the average germination and 30% incidence as susceptibility (0.4x0.3 = 0.12) it becomes a successful infection of only 12 buds out of 100 buds inoculated with sett inoculation method. Although, the sugarcane smut is one of the oldest recorded diseases of sugarcane, screening against smut is yet to be standardised properly. This is evidenced by the scale of grading for resistance used by different authors in different countries (Satyavir and Beniwal 1978; Nasr, 1977; Singh, 1977; Ferreira et al., 1980; Byther and Steiner, 1974) at different point of time to suit their special need. Nowadays a 0-9 scale (Hutchinson, 1969) is followed worldwide but workers differ widely in their assessment of infection percentage to disease rating. The resistant reaction ranges from 0-15% with most workers judging 10% to be the dividing line between resistance and an intermediate reaction.
For susceptibility, the dividing range is from 16-40% with a mode of about 26%. In establishing guidelines for evaluating disease reactions, there is a constant debate among pathologists and breeders in distinguishing between acceptable level of resistance and susceptibility (Ferriera et al., 1980).

Two highly susceptible genotypes viz., Co 1158 and CoLk 7901 were used for the evaluation of this technique against the traditional method of dip inoculation of setts, as control, in the spore suspension before planting. It was observed that in sett inoculation success rate in case of CoLk 7901 was 36% whereas, it was 26% in case of Co 1158. In case of the proposed technique the smut incidence was to the tune of 83% in CoLk 7901 and it was about 59% in case Co 1158 (Table I, Fig. 1). CoLk 7901 appeared more susceptible as it produced >60% of smut whip in 60 days of inoculation whereas Co 1158 took much longer time for the expression of symptom and took much longer time to achieve that level.

Taking the analogy from the expression of grassy shoot disease (GSD) in nature, where the mother shoot feeds the grassy lanky albinoid tillers arising from the basal buds of cane, this technique was developed. This was done by inoculating the young underground buds of the cane at the time of tillering phase, taking advantage of cane's own physiology of tillering and grand growth. As the mother shoot does not catch infection, it provides most of the nourishment to the infected tillers. The response of different genotypes against smut infection when inoculated with this technique is furnished in Table 2. From the result, it is apparent that this new technique is superior to all other prevailing techniques for artificial reproduction of whip smut in sugarcane.

**The main advantages of the proposed technique are outlined as follows:**

(i) Inoculation of the standing crop - selective inoculation is possible. Saves the loss of inoculum, planting material, land etc. This inoculation can be done in the potted cane plants. As the mother shoot remains healthy, it provides nourishment to the infected tillers. Due to this available nourishment, the tillers grow much longer and form canes and large sized whips. The cane - formed in these well-developed tillers is systematically infected by smut fungus. These infected tillers can act as an excellent experimental material and thus circumvents the longstanding problem of getting uniform systematically smut-infected material for carrying out other experiments.

(ii) The mother shoot can be tested against other diseases like red rot at the same time. Currently sugarcane breeding programmes in India are targeted only against these two diseases i.e., red rot and smut.

(iii) This technique is especially suitable for screening tissue culture raised somaclones at the very early stage and effectively reduces a year and avoids handling of bulk cane material. Screening at early generation is also possible in case fluff (true seed) raised seedlings.

Although this method is more labour consuming, the high success rate of infection is definitely rewarding. It also provides an opportunity of inoculation after the establishment of the cane and thus provides better opportunity of symptom expression. Moreover, as the main shoot is not infected, only the inoculated buds gets the infection, this provides an excellent opportunity of advancing the screening of sugarcane germplasm to the early generation where seed cane is the limiting factor. Due to this fact, one can test for smut resistance in the somaclones directly without going for a full crop cycle and thus saves a year.
REFERENCES


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