SHORT NOTE

Studies on smut disease (*Ustilago scitaminea* Syd.) of sugarcane: Effect of some substrates on the germination of teliospores*

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Sugarcane smut disease is incited by the fungus *Ustilago scitaminea* Sydow (syn. *Sporisorium scitamineum* (Syd.) M. Piepenbring). The disease is very easily recognized in the field by a characteristic long whip-like smut sorus produced at the apex of infected cane plants. The fungal spores are well adapted to long distance dispersal by either wind or irrigation water. Planting of cane cuttings with latent bud infection can effectively perpetuate the smut disease. The fungus is known to be an obligate parasite, thus it requires a living plant to produce spores (Croft *et al*., 2000). Therefore, the importation of infected sugarcane seed materials could introduce the disease.

U. scitaminea Syd. teliospores have been reported to survive for long periods under different conditions (Luthra *et al*., 1938; James, 1969; Alexander and Ramakrishnan, 1978). Leu (1969) reported that spores can survive for 2-3 months in moist soil, but they can survive for longer periods in dry soil or other dry environments. The influence of temperature and humidity on teliospore germination has been well documented (Appalanarasayya, 1964; Saxena and Khan, 1971; Gul, 1989). Smut teliospores have also been reported to lose viability very rapidly when stored under high humidity conditions or in wet soils (Luthra *et al*., 1938; Gul, 1989; Hoy and Geaghan, 1992). The reasons for this rapid loss in viability under such conditions have not, however, been clearly understood.

Therefore, to further understand the germination characteristics of U. scitaminea Syd. teliospores, a laboratory trial was initiated and conducted at Guneid Sugarcane Research Centre, (lat. 15°N, long. 33°E) for two seasons 2007/08 and 2008/09. The objective was to evaluate the suitability of some natural substrates on the germination of sugarcane smut teliospores.

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Collection and preparation of smut teliospores

Smut teliospores for the trials were extracted from freshly collected smut whips from the sugarcane cane variety NCO 376 after air drying for 2-3 days by a series of 200 mm diameter sieves of 106, 250 and 500 μ aperture mounted on an EFL 2000 Endecott sieve shaker. The extracted teliospores were then maintained in sealed polythene bags in the laboratory and used when needed.

Preparation of the substrates

The substrates used were prepared by two methods:

(a) Root extract of the sugarcane variety CO 6806: Roots were dug out from healthy looking cane stools and washed in tap water to remove soil particles. Then, 10 g of roots were blended in 100 ml water for 30 seconds in a blender, filtered through cheesecloth and Whatman filter paper No.1.

(b) Sucrose solution was prepared at 1, 2 and 3% concentrations. One-gram of smut teliospores was added to 100 ml of each substrate which is equivalent to about $10^6 – 10^7$ spores/ ml (Bock, 1964). Thereafter, 10 ml of this suspension were then added to 9-cm-diameter petri-dishes and incubated in the laboratory at room temperature ($25 \pm 5C^0$) for 24 hrs. The germination percentage was determined after 24 hrs by adding a drop from the test substrates separately onto microscope slides and examining them under the light microscope.

Germination on sugarcane internodes/rind surfaces

Four single pieces of internodes were taken from the 6^{th} internodes of cane varieties NCO 376 (HS); CO 527 (MS) and CO N52-219 (HR). The internode pieces were then sprayed to run-off with a smut spore suspension at a concentration of 1g spores/ 100 ml water (equiv. 10^6 -10 spores/ ml) to which 1-2 ml commercial liquid detergent Sheek® brand was added to improve wetting. The treated internode pieces were placed on wet filter papers or commercial tissue paper Fine® brand in 800-1000 ml beakers and sealed with aluminum foil to maintain humidity. This was incubated overnight at room temperature.

Evaluation of spore germination percentage

About 80-100 spores were counted for each slide mount; this was repeated three times (3 replicates) for each extract/substrate tested. A spore that remained intact was considered un-germinated and that with a protrusion of germ tube (regardless of its length) was considered germinated. Germination percentage (GP) of smut teliospores was calculated according to the following equation:

$$GP = \frac{\text{Number of germinated teliospores}}{\text{Total number of counted teliospores}} \times 100$$

The percentage data were arcsine transformed prior to analysis of variance and DMRT was used for the separation of treatments' means. For sugarcane internode/rind surfaces, **t**he teliospore germination percentage was assessed as follows:

(a) The treated internodes were removed and placed on clean petri-dishes and allowed to dry for 10 minutes. When the rind surfaces were completely dry, they were sprayed with acetone. Pieces of self adhesive transparent tapes of approximately 1x2.5 cm size were placed over the sprayed areas and left to dry for 10 minutes.

(b) The adhesive tape was then peeled off and mounted in water or commercial glycerin Raga® brand and observed under the microscope. To assess the percentage of smut teliospore germination, at least 80 to 100 spores were counted for each internode of each variety or replicate. James (1969) and Shetty and Safeeulla (1979) reported that the peel technique removed at least 50% of the original spores sprayed on the internode. The spore germination percentage was calculated as mentioned above.

Results of the percentages of smut teliospore germination for the different substrates are shown in Table 1. The percentages of germinated spores were found to be highest (51.9%) for the 3% sucrose solution which was significantly superior (P=0.05) to all the tested substrates. The 1% and 2% sucrose solutions and internode/rind surfaces of the highly susceptible sugarcane variety NCO 376 which recorded percentages of germinated spores of 40.4%, 45.3% and 42.1% were inferior to the 3% sucrose solution but, superior to distilled water, rind surfaces of sugarcane varieties N 52-219 (HR), CO 527 (MS) and root extracts of cane variety CO 6806 (HR). Also, Table 1 showed that distilled water (21.8%), root extracts of cane variety CO 6806 (23.8%), rind surfaces of cane varieties N52-219 (23.5%) and CO 527 (26.5%) had the lowest germination percentages and were thus, significantly (P=0.05) inferior to the other tested substrates. Differences in the germination percentages in root extracts of sugarcane variety CO 6806 (HR), rind surfaces of varieties N 52-219 (HR), NCO 376 (HS) and CO527 (MS) remains unclear, however, it is thought that some chemical substances/ exudates especially glycoproteins which are known to induce homotypic adhesion and inhibit teliospore germination could be responsible (Schenk, 1999). This finding is supported by the work of Stakman (1913) and Saxena and Khan (1971) who also reported low germination percentage values of 16.2% and 1.7% for distilled water and sugarcane root extracts, respectively, for readings taken at 12 hr. They did not, however, specify the reaction type and cane variety used. It is, therefore, evident that sucrose solution at 3% concentration offers a suitable substrate for routine germination studies of smut teliospores in the laboratory.

In conclusion, the present study has demonstrated that:

1. *U. scitaminea* (Syd.) teliospores germinated readily in all the substrates tested including distilled water but with variable percentages.
2. The highest spore germination percentage was recorded for sucrose at 3% concentration.
3. The lowest germination percentage was recorded for distilled water, sugarcane root extracts of the highly smut resistant cane variety CO 6806, internode surfaces of sugarcane varieties N52-219 and CO 527 consecutively.
4. Germination in sucrose at concentrations of 1% and 2%, and, internodes surfaces of the highly susceptible cane variety NCO 376 was intermediate.
5. Sucrose at 3% concentration is therefore, suitable for routine germination studies of smut teliospores.

Table 1. Effects of some substrates on teliospore germination of *U. scitaminea* Syd. after incubation for 24 hrs at room temperature (25± 5^0C).

Substrate or extract	Germination (%)
Distilled water (control)	(21.87) 27.9 c
1 % sucrose solution	(40.43) 39.5 b
2 % sucrose solution	(45.13) 42.2 b
3 % sucrose solution	(51.93) 46.1 a
Sugarcane root extracts (CO 6806)	(23.80) 29.2 c
Internode surfaces of some sugarcane varieties	
NCO 376 (HS)	(42.13) 40.5 b
N 52-219 (HR)	(23.53) 29.0 c
CO 527 (MS)	(26.50) 30.9 c
S.E. (±)	1.69
C.V. (%)	9

Means followed by the same letter(s) in a column are not significantly different according to Duncan's Multiple Range Test. Data were Arcsine transformed and each value is a mean of three replicates of about 80 to 100 spore counts. Actual data are in parenthesis. HS=highly susceptible; HR=highly resistant; MS=moderately susceptible

REFERENCES

Alexander, K.C. and K. Ramakrishnan. 1978. Studies on the smut disease *Ustilago scitaminea* (Syd.) of sugarcane: Longevity and viability of teliospores. Indian Journal of Sugarcane Technology 1: 47-49.

Appalanarasayya, P. 1964. Some physiological studies on sugarcane smut *Ustilago scitaminea* (Syd.). Indian Phytopathology 17: 284-286.

Bock, K.R. 1964. Studies on sugarcane smut, *U. scitaminea* (Syd.) in Kenya. Transaction of the British Mycological Society 47: 403-417.

Croft, B., J. Irawan and N. Berding. 2000. Screening Australian sugarcane clones for smut reaction in Indonesia: Initial results. In: Proceedings of Australian Society of Sugar Cane Technologists 22: 170-177.

Gul, F. 1989. Analysis of viability of sugarcane smut *Ustilago scitaminea* (Syd.) spores in nature. Pakistan Sugar Journal 3(3): 5-6.

Hoy, J.W. and J.P. Geaghan. 1992. Longevity of teliospores of *Ustilago scitaminea* (Syd.) in soil. Plant Disease 77: 393-397.

James, G.L. 1969. Viability of sugarcane smut spores in soil. Sugarcane Pathologists Newsletter 3: 10-12.

Leu, L.S. 1969. Smut of sugarcane in Taiwan (I). Recurrence of smut in Taiwan. Plant Protection Bulletin 11 (3): 91-99.

Luthra, J.C., A. Sattar and S.S. Sandhu. 1938. Life history and modes of perpetuation of smut of sugarcane *Ustilago scitaminea* (Syd.). Indian Journal of Agricultural Science 8: 849- 861.

Saxena, K.M.S. and A.M. Khan. 1971. Effect of host diffusates on germination of chlamydospores of *Ustilago scitaminea* (Syd.). Journal of Indian Botanical Society 50: 53-54.

Shetty, H.S. and K.M. Safeeulla. 1979. Stimulation of teliospore germination in smut fungi. Proceedings of the Indian Academy of Sciences 88 (6): 479-486.

Schenk, S. 1999. Molecular aspects of the sugarcane smut disease pathogen, *Ustilago scitaminea*, pp.131. In: G.P. Rao, A. Bergamin-Filho, R.C. Magarey and L.J.C. Autrey (eds.). Sugacane Pathology. Science Publishers Inc, Enfield, New Hampshire, USA.

Stakman, E.C. 1913. Spore germination of cereal smuts. Minnesota Agricultural Experimental Station Bulletin No. 133.

دراسة فى مرض التفحم السوطى فى قصب السكر: تأثير بعض المواد العضوية فى نمو الجراثيم التاليدية للفطر *Ustilago scitaminea* (Syd.)

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خلاصة

أجريت تجربة معملية فى مركز بحوث قصب السكر-الجنيد فى خلال الموسمين 2007/ 2008 و 2009/2008 على التوالى بهدف تحديد بعض المواد العضوية المناسبة لنمو جراثيم الفطر المسبب للمرض *Ustilago scitaminea* Syd. وتحديد نسبة النمو تحت الظروف المعملية . أوضحت النتائج أن محلول السكروز 2% و 3% أعطى نسبة نمو عالية 45.13% و51.59%على التوالى بإحتمال 5% وقد أعقب هذا محلول سكروز 1% والنمو على سطح سلاميات قصب السكر الصنف NCO 376 بنسب نمو 40.43% و42.13% على التوالى باحتمال 5% . أدنى نسبة نمو سجلت فى الماء المقطر(21.9 %) وعلى سطح سلاميات قصب السكر الصنف N52/219 (23%) والصنف CO 527 (26.5 %)، وخلاصة جذور قصب السكر فى الصنف O 6806 (23.8%). أثبتت الدراسة أن محلول السكروز فى مستويات تركزه المختلفة مفضل لنمو جراثيم الفطر. وأن سطح سلاميات الأصناف المقاومة من قصب السكر للتفحم السوطى إرتبط بإنخفاض نسبة نمو جراثيم الفطر .