

# A Simple Method for Long-term Preservation of Cultures of *Colletotrichum graminicola* and *C. gloeosporioides* Causing Anthracnose in Sorghum

Kusum Mathur<sup>1</sup>, R P Thakur<sup>2</sup>, and V P Rao<sup>2</sup>

(1. Department of Plant Pathology, Udaipur Agricultural University, College of Agriculture, Udaipur 313 001, Rajasthan, India 2. Genetic Resources and Enhancement Program, International Crops Research Institute for the Semi-arid Tropics (ICRISAT), Patancheru 502 324, Andhra Pradesh, India)

Long-term preservation and maintenance of pathogen isolates is essential for studies on pathogenic variability and virulence monitoring. Once the pathogen isolates are appropriately characterized for their morphologic, pathogenic, and genetic diversity they become reference isolates for future studies. Frequent subculturing of necrotrophic pathogens is not only laborious, but it can also induce spontaneous natural mutations in actively growing cultures. There are numerous reports of reduced viability and pathogenicity of isolates due to repeated subculturing on synthetic media. In our studies on pathogenic variability in *Colletotrichum graminicola* (Ces.) Wils. and *C. gloeosporioides* (Penz.) Sacc., the causal agents of anthracnose of sorghum (Mathur et al. 1998), we faced the problem of adequately maintaining a large number of isolates (>200) by repeated subculturing on oatmeal agar (OMA) at monthly intervals. We found the process laborious and observed visible change in the growth pattern and morphology of isolates due to repeated subculturing. In some cases reduced spore viability and likely reduced infectivity were also observed. In order to study pathogenic variability among isolates by artificial inoculation on a set of sorghum differential lines under greenhouse conditions, we needed a cost-effective, long-term, and simple way to preserve a large number of isolates in a form readily retrievable for retesting and evaluation. A method for the long-term preservation of cultures of *C. graminicola* and *C. gloeosporioides* that is a modification of the method described by Latterell and Rossi (1986) for *Pyricularia oryzae* Cavara, the rice blast pathogen is described.

Pure cultures of *Colletotrichum graminicola* and *C. gloeosporioides* were raised from diseased leaves and grains of sorghum collected from different states of India, and stored at 4°C. Single-lesion bits (20-25 from each infected leaf) were cut, washed twice with distilled steril-

ized water (DSW), surface sterilized with 0.1% HgCl<sub>2</sub> solution for 2 min, rinsed with DSW and placed on OMA in petri dishes. Infected sorghum grains showing acervuli were soaked in water for 20 min, then in HgCl<sub>2</sub> for 2 min, rinsed twice with DSW, placed on sterilized moist blotting papers in petri dishes and incubated at 25°C under continuous fluorescent light for 3 days. The spores that developed on these grains were streaked onto OMA. The plates were incubated at 25°C under continuous fluorescent light for 7 days, then colonies of *Colletotrichum* spp were picked off and transferred to fresh OMA plates. Isolates of *C. graminicola* and *C. gloeosporioides* thus obtained were purified by hyphal-tip culturing. The isolates were tested for pathogenicity on a set of differential sorghum lines under greenhouse conditions.

Leaves of the anthracnose-susceptible sorghum line IS 18442 were used to preserve the cultures. Third and fourth leaves from pot-grown, 21-day-old plants were used. The leaves were thoroughly washed in running tap water, gently dried with a cotton swab, and cut into 6-cm long pieces. Each piece (abaxial side up) was placed in a petri dish lined with double-layered moistened blotting paper, and autoclaved. The leaf-pieces were aseptically inoculated with either a conidial mass or an agar disc removed from the periphery of an actively growing culture of individual isolates of *C. graminicola* and *C. gloeosporioides*. The petri dishes were sealed and incubated at 25°C under continuous fluorescent light for 10 days till good sporulation occurred on the leaf pieces. The petri dishes were then transferred to a laminar flow bench and their lids were left open for about 6 h to dry the cultures. The dried cultures (on pieces of leaf together with blotting papers) were aseptically transferred to autoclaved brown paper seed envelopes (18 x 10 cm), that were then sealed, labeled, and placed in plastic bags. The plastic bags, each containing 10-12 envelopes of dried cultures, were stored at -20°C.

To retrieve a culture, a sliver of freshly poured OMA from a petri dish was removed aseptically, lightly touched on the surface of the dried culture, and transferred to the center of an OMA plate. The plates were sealed and incubated at 25°C under continuous fluorescent light. Growth of *C. graminicola* and *C. gloeosporioides* occurred within 24 h and the isolates were retested for pathogenicity on a susceptible sorghum line.

Currently we have about 150 isolates stored this way, some of which have been tested at monthly intervals for viability, typical cultural characteristics, and pathogenicity and found to remain true to their types for up to 2 years. In the methods described by Latterell and Rossi

(1986) they used rice nodes from rice straw in DSW in culture tubes and corn leaf pieces as media, inoculated with the pathogen isolates, and incubated at 24-27°C and stored at -18°C. Some of these cultures were reported to remain viable for up to 20 years. We believe the method should be equally effective for *C. graminicola*. The storage takes very little space, and a pure culture of any isolate can be revived within 2 days. The method is simple, economical, and effective, and with minor modifications could be used for other foliar pathogens.

## References

Latterell, F.M. and Rossi, A.E. 1986. Longevity and pathogenicity of *Pyricularia oryzae*. *Phytopathology* 76: 231-235.

Mathur, K., Thakur, R.P., and Rao, V.P. 1998. A strain of *Colletotrichum gloeosporioides* causing anthracnose on yellow sorghum. *International Sorghum and Millets Newsletter* 37: 69-70.

## A Simple Technique for Preserving *Sphacelia sorghi* Honeydew Inoculum

A H Rajasab, and B M Veerabhadraswamy (Plant Pathology Laboratory, Department of Botany, Gulbarga University, Gulbarga 585 106, Karnataka, India)

A simple technique that can be used to store the honeydew inoculum of *Sphacelia sorghi* MacRae has been developed. The stored inoculum can subsequently be used to recover the isolate as and when required. This technique involves the use of cellophane strips and petri dishes for the long-term preservation of honeydew inoculum under aseptic conditions.

Cellophane strips (7 cm x 1.5 cm) were cut to fit into the lower part of petri dishes and sterilized by swabbing with absolute alcohol. Petri dishes were sterilized by heating in a hot air oven (75°C for 24 h). The lower parts of the petri dishes were divided into equal halves with vertical sterilized paper barriers. Two cellophane strips were positioned one in each portion of the petri dish. *Sorghum bicolor* (L.) Moench panicles (variety 'Bilijola') with ergot symptoms at the honeydew stage were brought to laboratory and a portion of the honeydew mass was placed on the cellophane strips at three points using a sterilized scalpel (Fig. 1). The petri dishes were closed

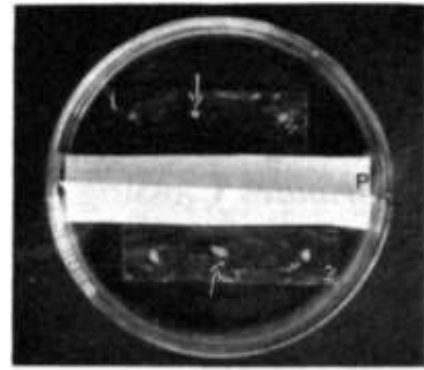


Figure 1. Method developed to store *Sphacelia sorghi* honeydew inoculum under aseptic conditions. Arrows indicate the masses of honeydew placed on cellophane strips (1 and 2) that are separated by a paper barrier (P).

and sealed with parafilm. The sealed petri dishes were stored under laboratory conditions at temperature ranging from 26°C to 30°C and relative humidity ranging from 75% to 80%.

The viability of the conidia from the stored honeydew mass was periodically tested at 10-day intervals. For each test, a honeydew mass was removed from the cellophane strips and inoculated onto potato dextrose agar (PDA) medium and observed for the development of colonies of *Sphacelia sorghi*. The conidia present in the honeydew mass were considered viable as long as inoculation resulted in the production of fresh colonies *S. sorghi*.

Under the laboratory storage conditions, the honeydew mass produced fresh colonies of *Sphacelia sorghi* for up to 9 months (Table 1), indicating that the conidia they contained were viable (Futrell and Webster 1966). After 9 months in storage, the pathogen showed a declining trend in producing fresh colony growth on PDA, indicating their loss of viability.

The technique described here is very simple and helpful in storing pathogen inoculum up to 9 months. Small pieces of conidial mass from pure cultures of *S. sorghi* can also be transferred onto cellophane strips and stored in the same way. The technique could possibly also be used to store the inocula of fungi that produce their spores/conidia in a mucilaginous mass [e.g., *Colletotrichum graminicola* (Ces.) Wils., *C. gloeosporioides* (Penz.) Sacc, *Ramulispora sorghi*. (Ellis & Ev.) Olive & Lefebvre, *Gloeocercospora sorghi* Bain & Edgerton ex Deighton *Septoria nodorum* (Berk.) Berk, and *Cercospora* spp etc.]. However, this possibility needs to be tested. The *S. sorghi* pathogen can be revived on culture media as and when required for use in epidemiological studies. The technique described is useful in maintaining pathogen virulence, that is often lost when pathogens are maintained through repeated subcultures.