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Seed Pathology

DEMONSTRATION OF SEED TRANSMISSION OF DOWNY MILDEW OR GREEN EAR DISEASE, *SCLEROSPORA GRAMINICOLA*, IN PEARL MILLET, *PENNISETUM TYPHOIDES*

A PRELIMINARY COMMUNICATION

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Seed transmission of downy mildew or green ear disease, *Sclerospora graminicola*, in pearl millet, *Pennisetum typhoides*, has been demonstrated. Dormant mycelium was present in different parts of the seed, including the embryo; inoculum from oospores borne on the surface of the seed was eliminated by treatment with mercuric chloride.

For many years it has been generally accepted that downy mildew or green ear disease of pearl millet (bajra), *Sclerospora graminicola* (Sacc.) Schroet., is commonly associated with seed of its major host, *Pennisetum typhoides* (Burm.) Stapf & C. E. Hubb. It has, however, been a matter of considerable controversy whether the pathogen is transmitted from the seed, thus giving rise to primary infections in the field.

The disease is very destructive and although it is known to occur throughout the pearl millet growing areas in Africa and Asia there is the possibility that pathogenic races may spread internationally with seeds moving in trade and during exchange of genetic resource.

The pathogen may be present with seed in two ways: (1) as *oospores* borne on the surface of the seed, first suggested by Butler (1918), later observed by Safeeulla who has routinely tested hundreds of seed samples of pearl millet for the National Seeds Corporation of India for such contaminations: (2) as *mycelium* carried in the tissues of the seed (Suryanarayana, 1962), sometimes in the embryo (Arya & Sharma, 1962; Singh, 1974; Safeeulla, 1976*b*), or in the endosperm (Safeeulla, 1976*b*), more often perhaps in the pericarp (Singh & Pushpavathy, 1965; Safeeulla, 1976*b*).

Attempts have been made to demonstrate transmission from the seed. Suryanarayana (1962) obtained infected seedlings when seed coated with oospores were sown in pots with garden soil but sporulation on the seedlings was not established, and when he used grains carrying mycelial infection he did not get infected seedlings. Williams (pers. comm., 1977) did not obtain infected plants when about 1,000 mature dry seeds from green malformed panicles and normal panicles were grown on Hoagland agar. Also Govindu (pers. comm., 1975) and Lambat (National Seeds Corporation, India, pers. comm., 1976)

obtained negative results by using seeds from infected mother plants. Safeeulla and co-operators (Safeeulla, 1976*b*), showed mycelium in young seedlings grown from seeds of infected mother plants. Tiwari and Arya (1966) obtained infected plants from seed collected from heavily infected earheads. Sundaram, Ramasastry and Nayar (1973) found circumstantial evidence for seed transmission when they recorded severe infections in the fields at the Indian Agricultural Research Institute, New Delhi, where pearl millet had not been grown for 10 years.

At the Institute of Seed Pathology in Copenhagen we are carrying out investigations on the issue of seed transmission under conditions that exclude possibilities of contamination from air-borne and soil-borne inoculum. Our preliminary experiments have shown that seed transmission of downy mildew, *Sclerospora graminicola*, may take place from mycelial infection borne in the seed.

Seeds collected on the 27th March, 1976 from downy mildew diseased plants of cv. HB-3 of pearl millet, grown at the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), Hyderabad, India, were used. The seeds were kept at room temperature, 20-25°C, and sown on the 10th January, 1977 in pots with peat-soil mositened with tap water, held in an environment controlled room at 23-25° C and submitted to 12/12 hr alternating cycles of darkness and artificial daylight (Philips TLF 40W/34). Before sowing, the seed was pretreated with 2% chlorine for 10 min followed by 5 min treatment in 0.2% $HgCl_2$, and finally washed in tap water for 2-3 min. Surface-borne oospores were collected from the pretreated seed as well as from untreated seed and subjected to the tetrazolium viability test. The test indicated that all oospores were killed in the pretreated seed whereas 6.5% of the oospores from the untreated seed were positively stained thus indicating viability.

A subsample of the seed under investigation was examined for mycelial infection. The technique used for detection of mycelium of loose smut, *Ustilago tritici*, in wheat was used with some modification, and about 2,500 seeds were examined. 7.5% of the seed contained the coenocytic mycelium of the fungus, 0.4% of the seed in the embryo, located in the scutellum region.

After sowing, each pot was covered with plastic bags for two weeks. The downy mildew symptoms were observed in 3 plants out of about 1,000 plants on the 17th, 18th and 23rd February subsequently in 3 different pots in the 4th-5th leaf stage, and onwards. The infected plants were stunted, and the leaves chlorotic with water soaked specks (Fig. 1a). Infected leaf pieces were macerated, cleared and stained with cottonblue, and the characteristic coenocytic mycelium was observed, spreading inter and intracellularly, parallel with the vascular bundles, the hyphae growing in the direction towards the stomata (Fig. 1b). An infected leaf of each of the three plants was cut into 2-3 cm pieces, and these were put on wet blotter in petri dishes with the abaxial side up and incubated in darkness for 5-6 hours. Sporangiophores and sporangia (Fig. 2), and the liberation of the zoospores were noticed under the compound microscope. Prior to the experiments reported above several thousand plants were grown under different growing conditions from seeds harvested from diseased fields. The conditions used in the present study may have been critical in triggering off infection. Further work is in progress to identify the critical factors.

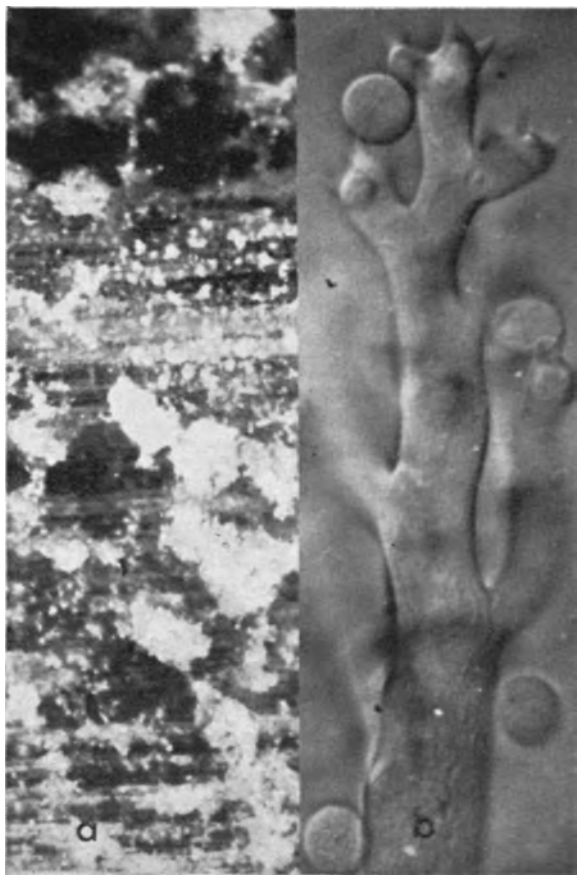

FIG. 1. (a) Pearl millet leaf, healthy (left) and diseased (right); (b) *Sclerospora graminicola* mycelium accumulated at the stomata of the leaf. 200 X.

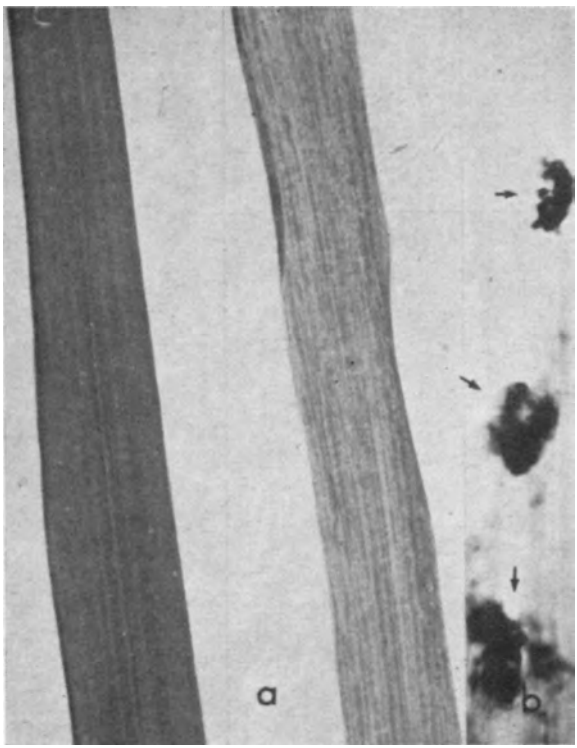

FIG. 2. (a) Infected leaf showing growth of sporangiophores and sporangia of *Sclerospora graminicola*. 50 X; (b) Sporangiophore and sporangia. 750 X.

CONCLUSION

In the present experiments it has been shown that transmission of *Sclerospora graminicola* may take place from dormant mycelium carried in the seed of pearl millet, *Pennisetum typhoides*, after storage of the seed for 10 months under dry conditions. The evidence of transmission from the mycelium is supported by experiments of Safeeulla (1976*a*) which showed that the downy mildew mycelium present in the seed is alive; he placed surface sterilised seeds on modified White's medium and obtained pure cultures of the fungus several times.

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