698

110

Indian J. Plant Prot. 19:101-103, 1991

A REPEATABLE METHOD OF GERMINATION OF OOSPORES OF *SCLEROSPORA GRAMINICOLA* AND ITS SIGNIFICANCE IN DOWNY MILDEW DISEASE

S.D. PANCHBHAI, M.S. REDDY and S.D. SINGH**
Department of Plant Pathology, College of Agriculture, Andhra Pradesh Agricultural University, Rajendranagar, Hyderabad - 500 030, Andhra Pradesh.

Downy mildew disease of pearl millet (*Pennisetum glaucum* (L.) R. Br.) caused by *Sclerospora graminicola* (Sacc.) Schroet, is quite destructive in Africa and in the Indian subcontinent. The oospores (Nene and Singh, 1976; Safeeulla, 1976) or the internal seed borne mycelium (Shetty *et. al.*, 1980; Sundaram *et al.* 1973) cause primary infection in the crop. Several workers (Evans and Harrar, 1930; Huira, 1930; Pande, 1972; Sundaram and Gurha, 1977; Suryanarayana, 1956) have reported the *in vitro* germination of oospores, but the lack of repeatability of these methods (Nene and Singh, 1976) indicates that oospores are defunct bodies and that the internal seed borne mycelium is the sole cause of primary infection. We report here a simple, repeatable method of oospore germination developed in 1986 and also demonstrated at ICRISAT, Hyderabad. This breakthrough work was also mentioned in ICRISAT Research Highlights of 1989 (ICRISAT, 1990). The implications of these results are also discussed.

Oospores bearing leaves were ground to powder and the powder was soaked in distilled sterile water at 10 mg oospore powder in 5 ml water (pre soaking) for 9 h at 30±1°C; the water was drained-off and the oospores suspended in 5 ml of 2.5% NaOcl (Sodium hypochlorite - (Chlorax) in test tubes which were incubated at 30±1°C for 12 h in dark; the NaOcl was drained off and the oospores were resuspended in 5 ml water (germinal-tion medium) and incubated for germination at 25±1°C. The pH of water was maintained at 6.5. During the three treatments, the concentration of oospores was maintained at about 10.8×10^4 oospores ml^{-1}. An oospore was counted as germinated when the germ-tube was as long as the diam of the oospore. Percentage germination was based on 1200-1500 oospores counted in three time replications. This method was also used to study the effects of various factors on germination.

Although germination was observed after 1 h of sodium hypochlorite treatment, maximum germination was seen after 12 h. Germination began with the development of a wedge - shaped structure in the inner wall (endosporium) (Fig. 1a), which later emerged through the outer wall (exosporium). The content of the oospores continued to flow into the germ-tubes until the oospores were fully emptied. The germ tubes were coenocytic, light brownish to transparent (Fig. 1b) measuring 191.3-1760 μm (av. 776 μm) in length and 3.9×19.43 μm (av.11 μm) in width and invariably produced terminal appressoria. Appresoria were mostly globular, subglobular, rarely ovate, reniform and measured 13.39 - 74.29 μm (av. 31.8 μm)×

* Part of Ph.D. thesis of senior author submitted to Andhra Pradesh Agricultural University, Hyderabad.

** Cereals Programme, ICRISAT.

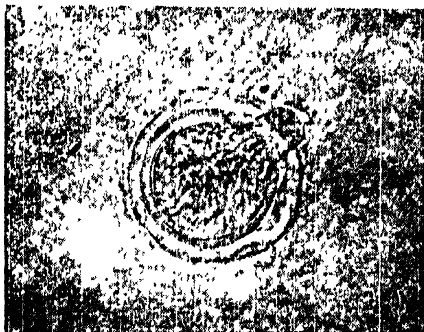

Fig.1a An oospore pushing out a germ tube through an opening in the exosporium.

Fig.1b An oospore showing a long germ tube with uneven thickness.

19.4 - 118.6 μm (av. 38 μm). These results could be reproduced by soaking the oospores in NaOcl by Agarwal *et al.* (1989) based on a personal communication by the third author of this publication.

Germination was influenced by oospore age, NaOcl concentration, oospore concentration and temperature. Oospres retained germinability upto 13 years. Germination was maximum in one year old oospores (76%), decreased gradually reaching less than 10% in 6 year old oospores. Newly formed oospores showed 40% germination and those stored under laboratory conditions (20-40°C) for 13 years gave 10.02% germination. Oospores treated with 2.5% sodium hypochlorite for 12 h. followed by their incubation for germination in distilled water gave the highest germination. Germination was 40% in oospores with a concentration of 0.2 mg oospores/10 ml water, increased gradually and reached to 60% in a concentration of 2 mg oospore/10 ml, followed by a sharp decrease with little or no germination at a concentration of 14 mg oospores/10 ml or more. Highest germination occurred at 25°C.

With the successful germination of oospores, it is now possible to detect the longevity of oospores and the factors that affect their survival. The information may be utilized for the effective control of the disease by cultural and chemical methods. This information will also be useful (1) in the seed production plots where the absence of the disease is essential; (2) in seed imports and exports to check the introduction of a more aggressive race/pathotype of the pathogen to a new area/country; and (3) to exclude the role of externally seed carried oospores from the internally seed borne mycelium in seed transmission studies. Reliable laboratory/greenhouse screening procedure with oospores can now be developed. This in turn will be helpful: (i) to identify effective resistance to oospores, (ii) to differentiate between the oosporic and sporangial resistance, and (iii) to combine the resistance together; cultivars possessing such resistance should withstand the onslaught of the disease for a longer period.

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Received : 15-10-90

Revised : 22-2-91