Annals of Arid Zone 28 (3&4) : 325-329, 1989

PRESENCE AND POSITION OF MYCELIUM OF *SCLEROSPORA GRAMINICOLA* (SACC.) SCHROET: IN THE SEEDS OF PEARL MILLET *[PENNISETUM AMERICANUM* (L.) LEEKE*]* AND ITS ROLE IN DISEASE DEVELOPMENT

R.L. AHUJA[1], J.K. DANG AND J.N. CHAND

Haryana Agricultural University, Hisar-125 004

Sclerospora graminicola (Sacc.) Schroet., the causal agent of downy mildew of pearl millet, is primarily soil borne in nature (Suryanarayana, 1962). Seeds have been reported to carry oospores adhering on the surface (Safeeulla, 1976) or mycelium in the tissue (Arya and Sharma, 1962; Singh and Pushpavathy, 1965 and Shetty et al., 1977). Conclusive information is not available in the literature as to where does the mycelium in the seed survive. Therefore, detailed histopathological and embryological studies were carrid out to establish the location of survival of mycelium of *S. graminicola* in the seeds of pearl millet.

Seeds collected at different developmental stages from healthy and infected ears of cultivar NHB 3 of pearl millet from the fields of Haryana Agricultural University, Hisar were used for the study. The material was fixed in F.A.A. Sections 8-20 microns thick of the seeds of infected ears were cut with microtome as per standard procedure and stained in 0.5% Toluidine blue for light microscopy studies (Feder and O'Brien, 1968).

For electron microscopy studies, one mm piece of seed stalk was fixed in 2% gluteraldehyde for four hours at room temperature and then kept at 4°C for 22 h. Two to three washings with phosphate buffer (pH 7.0) at an interval of one hour each were given. Material was then kept in 2% osmium tetraoxide at room temperature for 2 h and washed with two changes of buffer for 15 min. each. After dehydration, it was infiltrated, using ethyl alcohol propylene oxide series and finally embedded in araldite. Sections were cut on LKB ultratome III with glass knives. Golden to silver sections were lifted on uncoated grids. After washing in 50% alcohol, the grids were stained with lead citrate for 15 min. (Reynold, 1963). These were washed in 0.02 N NaOH, followed by water, and then dried.

Embryos were separated from the seeds of infected ears and observed for the presence of mycelium as per method given by Pawar and Williams (1979).

[1]Government College, Hisar-125 004

The seeds collected from healthy and partially infected ears were thoroughly washed in water and surface sterilised with 0.1% mercuric chloride for 3 min to inactivate the external inoculum. A part of the surface strilised seeds were treated with hot water (52°C±1°C) for ten minutes. The seeds were sown in double sterilised soil in pots in three replications. Each replicate had ten pots and in each pot ten seedlings were maintained. Observations on downy mildew incidence was taken 20 days after sowing.

The seeds of different developmental stages from heavily infected ears were apparently normal. The anatomy of such seeds did not show any marked differences from healthy ones except for the presence of mycelium in a few.

A longitudinal section of rachillus of developing caryopsis from the infected ear showed the presence of intercellular mycelium in the peripheral ground tissue. The electron microscopy showed that the hyphae were triangular in cross section. Numerous mitochondria and ribosomes were observed indicating young and actively growing mycelium in the rachillus (Fig. 1).

Fig. 1. Transverse section of fungus from seed showing triangular hyphae (x 7500. Al-Aleurone layer HP-Hypocarp EP-Epicarp F-Fungus)

The light microscopic histopathological studies of three hundred seeds from infected earheads revealed that 25% of the seeds carried the mycelium in funicle, ovary wall and, occasionally, in the integuments and nucellus in the initial stages of development. In later stages of development, the hyphae were observed in the

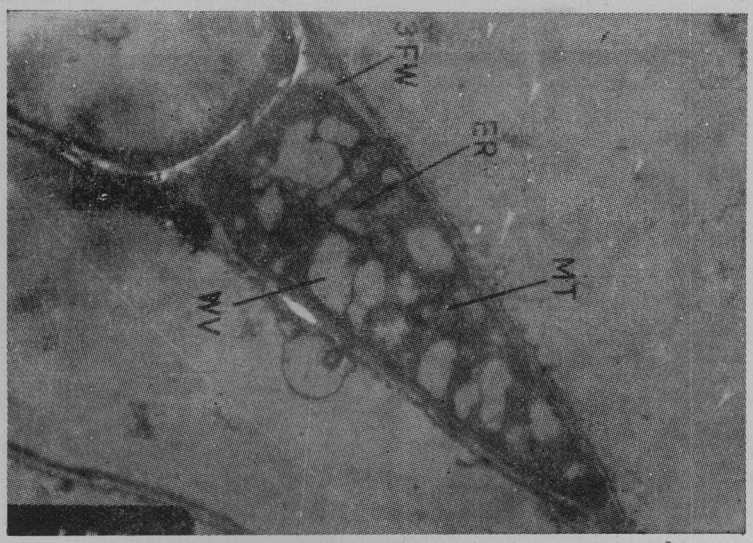

Fig. 2. Longitudinal section of diseased seed showing fungus in the pericarp (x 540, ER-Endoplasmic reticulum, MT-Mitochondria, FW-Fungal Wall, WV-Wall Vesice

degenerating nucellus, integuments, mesocarp and hypocarp but not in the endosperm and the developing embryo (Fig. 2). A transverse section of funicular regions of infected seeds depicted that the fungal hyphae were mainly located in the mesocarp. Embryo isolation technique also revealed that out of 500 seeds,

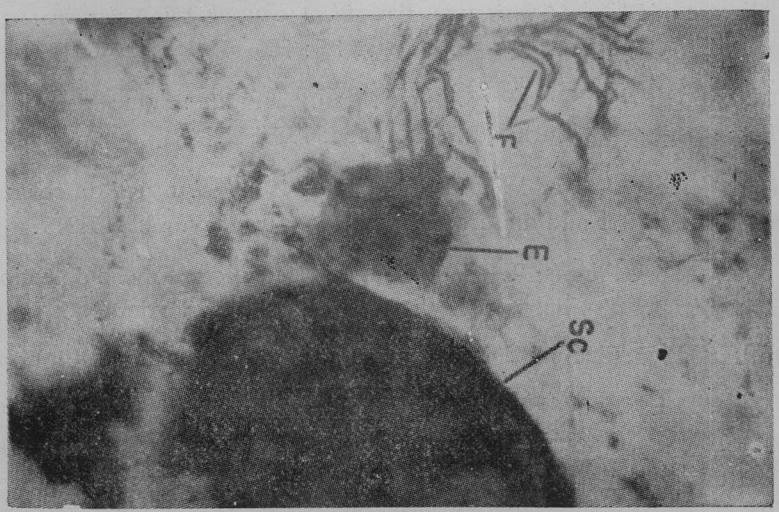

Fig. 3. Fungal mycelium and embryo from the infected seed (x 620. ER-Endoplasmic reticulum, Sc-Scutellum

50% showed the presence of thick walled mycelium only in the funicular region (Fig. 3).

The absence of fungal mycelium in the embryo and endosperm could be due to rapid cell division which somehow checks the fungal penetration into them during their developmental stages. Further, aleurone cells, due to their outer thick walls, dense protoplasm and high density of aleurone grains, may be acting as a barrier to the penetration of fungus in the mature endosperm and embryo as opined by Jones et al. (1972) in case of corn seeds affected by *Sclerospora sorghi*. It seems that mycelium survives in partly degenerated layers of pericarp cells of the funicular region in the seed and this can infect the emerging seedling, if viable.

In pot experiment, washed and surface sterilised seeds from infected heads produced 0.6% infected seedlings whereas seedlings from surface sterilized and hot water treated seeds were disease free. Though the disease transmission through surface sterilised seeds is very less, yet it may help in the build up of the inoculum and spread of disease if the environmental conditions are favourable.

Thus, the study revealed that the mycelium survives in the pericarp cells of funicular region in the seed and it has a possible role in the disease development and its further spread.

REFERENCES

Arya, H.C. and Sharma, R. 1962. On the perpetuation and recurrence of the 'green ear' disease of bajra *(Pennisetum typhoides)* caused by *Sclerospora graminicola* (Sacc.) Schroet. Indian Phytopathology. 15 : 166-172.

Feder, N. and O'Brian, T.P. 1968. Plant Microtechnique : Some principles and new methods. American Journal of Botany. 55: 133-142.

Jones, B.L., Leeper, J.C. and Frederikson, R.A. 1972. *S. sorghi* on corn: its location in carpellate flower and mature seeds. Phytopathology. 62: 817-819.

Pawar, M.N. and Williams, R.J. 1979. A rapid technique for the detection of fungal mycelium in peral millet seeds. ICRISAT Newsletter, Hyderabad, India.

Reynold, E.S. 1963. The use of lead citrate at High pH as an election opague strain in electron microscopy. Journal of Cell Biology. 17: 208.

Safeeulla, K.M. 1976. Serious epidemic of downy mildew on pearl millet in India. Indications of seed transmission. Seed Pathology News. 9: 3-4.

Shetty, H.S., Neergard, P. and Mathur, S.B. 1977. Demonstration of seed transmission of downy mildew or green ear disease, *Sclerospora graminicola* in

pearl millet, *Pennisetum typhoides*. Proceedings of the Indian National Science Academy. 43 : 201-206.

Singh, H. and Pushpavathy, K. M. 1965. Morphological and histological changes induced by *Sclerospora graminicola* (Sacc.) Schroet. in *Pennisetum typhoides* Stapf. et Hubbard. Phytomorphology. 15: 338-353.

Suryanarayana, D. 1962. Occurrence of an unknown fungal mycelium inside the sound grains, produced on partly formed green ears of bajra plants. Science and Culture. 28: 536.