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SCANNING ELECTRON MICROSCOPY OF INVASION PROCESS BY *FUSARIUM MONILIFORME* ON SOYBEAN SEED

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Abstract: *Fusarium moniliforme* J. Sheld was observed as important seed-borne pathogen of soybean (*Glycine max*) having a great potentiality to cause seed-borne infection after establishing a rapid pathogenesis. The infection process was studied with scanning electron microscopy (SEM). Infection hyphae grew directly from the side or near the end of the conidium and entered the seed coat through cuticular cell juncture with or without forming appressorium. After invasion of the fungus, rapid degradation of cell wall occurred followed by intercellular and intracellular development of the fungus. Finally, tissues of the seed lost their integrity and identity and seemed as rotten mass covering with dense mycelium.

Key words: *Fusarium moniliforme,* invasion process**,** scanning electron microscopy and soybean seed

INTRODUCTION

Seed-borne pathogens cause enormous losses to crops in the world as well as in Bangladesh. The presence of pathogenic propagules in a seed lot is pivotal because infected seed may fail to germinate, cause infection to seedlings and growing plants. Out to 16% annual crop losses due to plant diseases, at least 10% loss is incurred because of seed-borne diseases (Fakir 1983).

Species of *Fusarium* occur frequently on plant material including seed, some being serious pathogens, others weakly pathogenic while still others appear to be merely saprophytes (Malone and Muskett 1964). Different *Fusarium* species cause seed rot, pre-emergence and post-emergence seedling damage as well as wilting of growing plants of various crops viz. vegetables, cereals, pulses, oilseed crops etc at home (Fakir 2001) and abroad (Richardson 1979). Nowadays, soybean, a promising oil seed crop, is cultivated extensively especially in southern region of Bangladesh. Ten different species of *Fusarium* were detected by Richardson (1979) as seed-borne fungi of soybean such as *Fusarium anguioides, F. equiseti, F. fusarioides, F. moniliforme, F. martii* var. *minus, F. semitectum, F. solani, F. oxysporum, F. orthocerus* and *F. poae* which were responsible for wilt, root-rot and foot-rot of soybean.

In this paper, infection process by *F. moniliforme* in soybean seed was studied by scanning electron microscopy (SEM) to provide information on the penetration and establishment of infections of *F. moniliforme* in soybean seed.

MATERIALS AND METHODS

Inoculum preparation

F. moniliforme was isolated from infected seed which was previously incubated on moist blotter for 7 days. Pure culture was prepared using isolated hyphal tips *F. moniliforme*. The pure culture of 7-days-old fungal pathogen was taken as a source of inoculum. The spores and mycelium was harvested by rinsing with sterilized water and rubbing gently with a clean brush to float the conidia or mycelial fragments for better harvest. The suspension was sieved to remove agar lumps from the suspension and transferred to a mechanical rotary blender for 1–1½ minutes. Thus, fungal suspension was prepared by blending the collected spores and mycelial mixture.

Seed inoculation

Soybean seeds were surface sterilized with mercuric chloride (0.01%), washed repeatedly with distilled water and inoculated by placing single drop of spore suspension within the area of 0.5 sq. cm on the seed surface. Inoculated seeds were incubated at 20±2°C in seed incubator for further studies.

SEM observations

Samples were prepared following the method of Hayat (1981) for SEM. Observation was done four times. First two times at 6 hours and 12 hours and last two times at 24 and 48 hrs after inoculation.

The specimens were dehydrated with normal dry air and adhered onto aluminium specimen mounts with

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sticky carbon tape. Then the specimens were coated with platinum for 30 sec. and photographed on a JEOL (JSM6490 LA) SEM.

RESULTS AND DISCUSSION

The fungus formed pink coloured growth on the inoculated seed surface after 48 hours of inoculation. Microconidia were hyaline, fusiform to clavate with slightly flattend base, single celled and size in the range of 5–12 x 1.5–2.5 µm (Fig. 1A–C).

This study showed that one germ tube emerged from the side on near the end of the conidium (Fig. 1B–D), Germination of the conidia initiated within 6 h after inoculation. The infection hyphae directly developed from the germinating conidium within 6 hr on the surface of the soybean seed. After 12 hours of inoculation, the infection hyphae entired directly through cuticle via cell juncture without forming appressorium (Fig. 1E) and with forming appressorium (Fig. 1F). Mycelium grew subcuticularly and intercellularly between and below the cuticular layer of the seed coat. The fungus frequently sporulated soon after invasion the surface of the seeds. Sometimes infection haphae formed a long coil without invading the host tissue in nearest position (Fig. 1G). Mycelium growth increased rapidly and after 48 hours from inoculation it covered the whole seed (Fig. 1H, I).

Mycelium ultimately invaded and killed all the tissues of the seed. The tissues soon lost their integrity and identity. The seed then seemed as rotten mass.

During our observation of SEM studies on infected soybean seed, appressoria formation by *F. moniliforme* during infection period is consistent with the findings of Nair and Corbin (1981) and contradictory to the findings of Waller *et al.* (1993). Direct penetration of cuticle via infection pegs might also be the common mode of attack, as was evident from SEM study. It is a significant finding that the above observation is supported by the findings made by Chau and Alvarez (1983) on *Colletotrichum gloeosporioides* on papaya fruit, Das and Bora (1998) on *C. acutatum* on guava fruit, Huang *et al.* (1999) on *Botrytis cinerea* on alfalfa pollen and Mims (1991) in some plant pathogenic fungi.

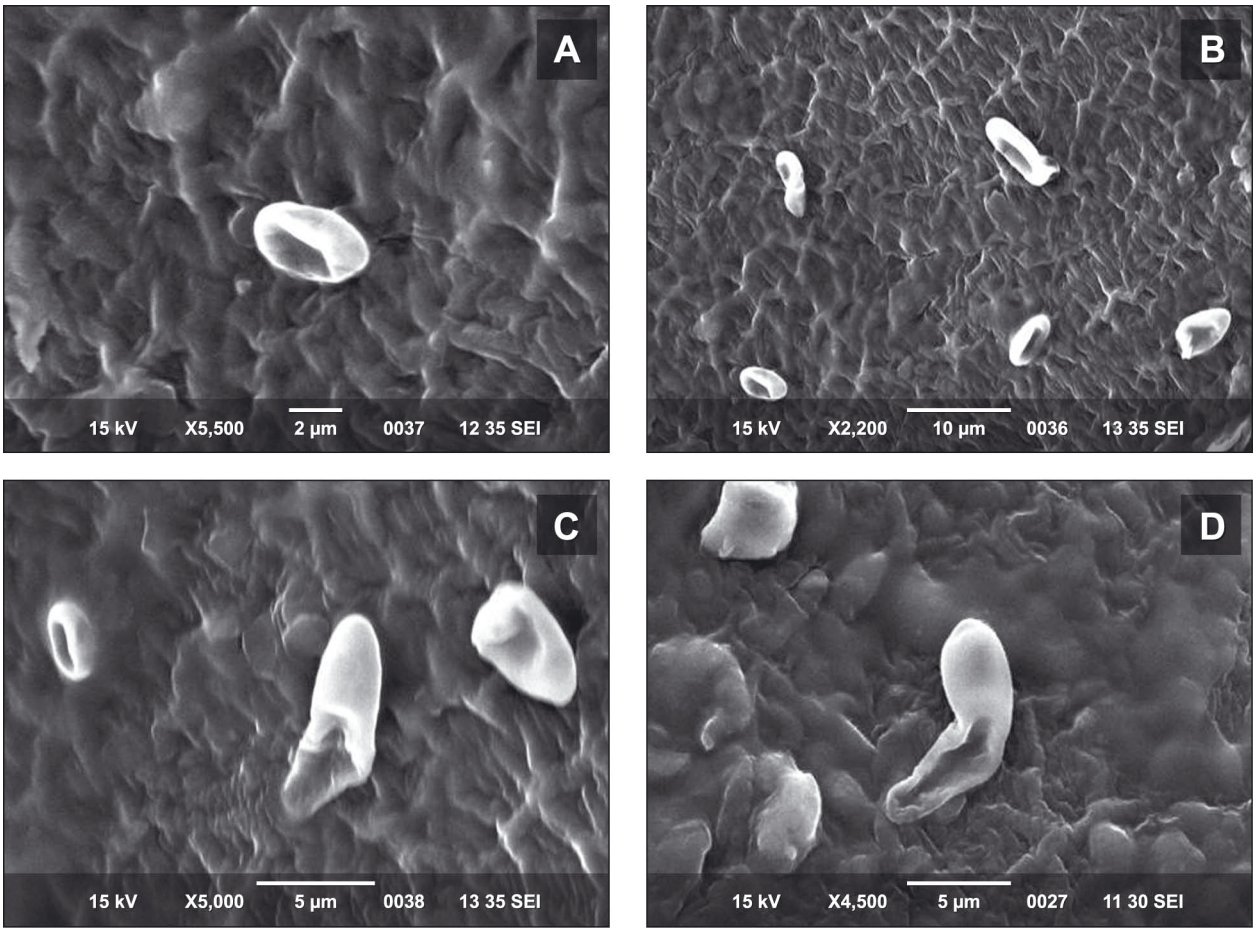

Fig. 1A. Single conidium adhered with rough surface on soybean seed

Fig. 1B. Ungerminating and just germinating conidia after 6 hours of inoculation

Fig. 1C–D. Elongation of germtube after 6 hours of inoculation

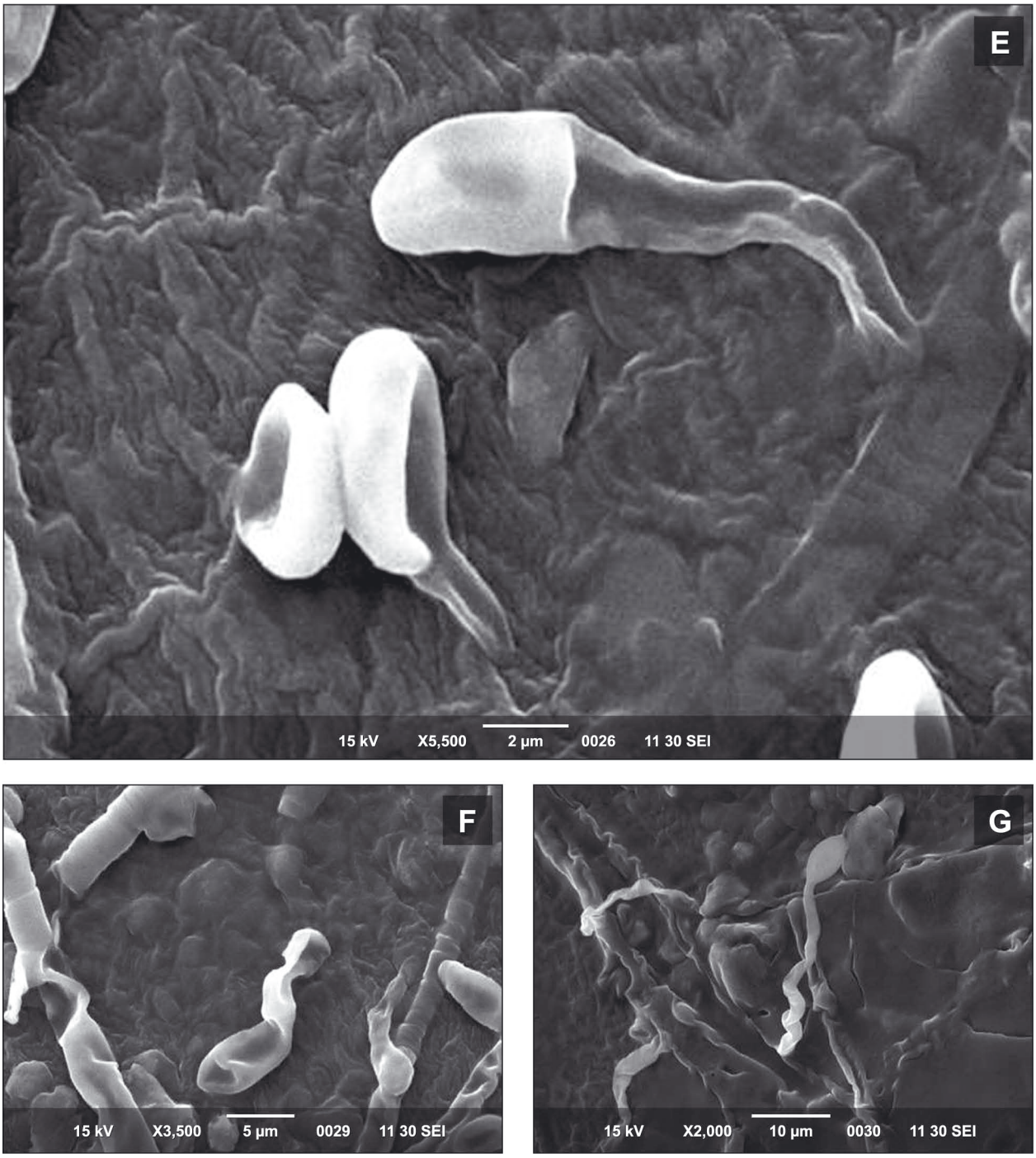

Fig. 1E. Direct invasion by infection hyphae into seed coat through cuticular cell juncture after 12 hours from inoculation

Fig. 1F. Invasion of infection hyphae with forming appressorium after 12 hours from inoculation

Fig. 1G. Coil formation of infection hyphae

The absence of mechanical damage and the appearance of dense mycelial areas in the infection sites prove the possibilities of enzymatic action of the pathogen on soybean seed tissue supported by the studies of Chau and Alvarez (1983) in papaya fruit infected by *C. gloeosporioides* and Kunoh *et al*. (1988) in barley leaf pathogenesis by *Erysiphe graminis*. During our course of investigation by SEM, macrococonidia were not found. This result is in agreement with the description of Mathur and Kongsdal (1994).

The ultrastructural observation in the present study suggested that the infection hyphae of *F. moniliforme* invade the host tissue with or without forming appressorium. Further detailed study on the infection hypha after its entry will be of great importance.

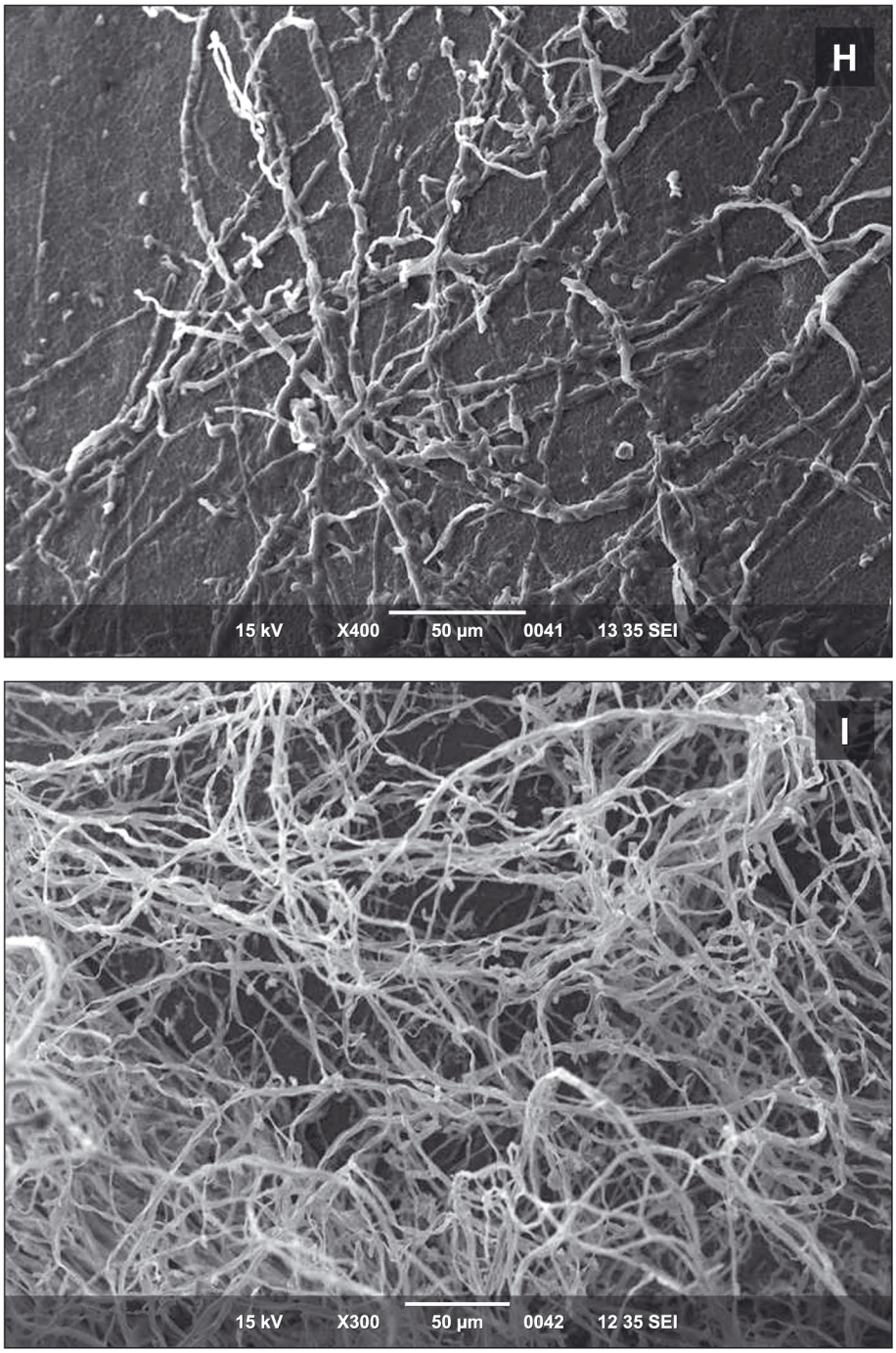

Fig. 1H. Growing of mycelium on seed surface after 24 hours from inoculatioin

Fig. 1I. Growth of mycelium on soybean seed surface after 48 hours from inoculatioin

REFERENCES

Chau K.F., Alvarez A.N. 1983. A histological study of anthracnose on *Carica papaya*. Phytopathology 73: 1113–116.

Das M., Bora K.N. 1998. Ultra structural studies on infection process by *Colletotrichum acutatum* on guava fruit. Indian Phytopathol. 51 (4): 353–356.

Fakir G.A. 1983. Teaching, research and training activities on seed pathology in Bangladesh. Seed Sci. Technol. 11: 1345–1352.

Fakir G.A. 2001. List of Seed-borne Diseases of Important Crops in Bangladesh. Seed Pathology Laboratory, Department of Plant Pathology, BAU Mymensingh, 22 pp.

Hayat N.I.A. 1981. Principles and Techniques of Electron Microscopy. University Park Press, Baltimore, 412 pp.

Huang H.C., Kokko E.G., Erickson R.S. 1999. Infection of alfalfa pollen by *Botrytis cinerea.* Bot. Bull. Acad. Sin. 40: 101–106.

Kunoh H., Yamaoka N., Yoshioka H., Nicholson R.L. 1988. Preparation of infection court by *Erysiphe graminis*. Exp. Mycol. 12: 325–335.

Malone G.P., Muskett A.E. 1964. Seed-borne fungi. Description of 77 fungal species. Proc. Int. Seed Test. Assoc. 29 (2): 180–182.

Mathur S.B., Kongsdal O. 1994. Seed Mycology. Danish Govt. Institute of Seed Pathology for Developing Countries. Copenhagen, Denmark: 156–157.

Mims C.W. 1991. Using electron microscopy to study plant pathogenic fungi. Mycologia 83 (1): 1–19.

Nair J., Corbin J.B. 1981. Histopathology of *Pinus radiata* seedling infected by *Colletotrichum acutatum* f. sp. *pinea*. Phytopathology 71 (8): 777–783.

Richardson M.J. 1979. An Annotated List of Seed-borne Diseases. ISTA as ISTA Seed Health Testing Handbook and CMI. Commonwealth Agricultural Bureaux, 87 pp.

Waller J.M., Bridge P.D., Black R., Hakiza G. 1993. Characterization of the coffee berry disease pathogen *Colletotrichum kahawae* sp. nov. Mycol. Res. 97 (8): 989–994.

POLISH SUMMARY

SKANINGOWA MIKROSKOPIA ELEKTRONOWA PROCESU INWAZJI NASION SOI PRZEZ *FUSARIUM MONILIFORME*

Fusarium moniliforme jest ważnym patogenem przenoszonym przez nasiona soi (*Glycine max*), mogącym je porażać po zakażeniu i szybko postępującej patogeniczności. Proces infekcji badano przy użyciu skaningowego mikroskopu elektronowego (SEM). Strzępki infekcyjne wyrastały bezpośrednio z boku lub blisko wierzchołka konidium i przedostawały się do okrywy nasiennej przez komórki kutikuli, z lub bez wytwarzania przycistki. Po inwazji grzyba następowała szybka degradacja ściany komórkowej, po czym wewnątrzkomórkowy i międzykomórkowy rozwój grzyba. W końcu – tkanki, nasiona traciły swoją integralność oraz tożsamość, i wyglądały jak zgniła masa pokryta gęstą grzybnią.