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Short communication

DOWNY MILDEW OF SOYBEAN (*GLYCINE MAX (L.) MERR.*) — A DREADED SEED TRANSMITTED DISEASE HITHERTO NOT REPORTED FROM INDIA

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Soybean, *Glycine max* (L.) Merrill having both protein and oil, is an important source of low-cost food. Its oil is at top ranking position in the world oil production. Soybean is grown to greater or lesser extent in most parts of the world and occupies more than 62 million h area under cultivation. The total production of soybean in the world is more than 136 million m ton (FAO, 1995). The importance of soybean in India has become overwhelming in view of the shortage of edible oils in the country. In late seventies, the Government of India decided to import soybean seed on large scale for sowing purposes to boost up oil production in the country. At present there is a large area under soybean cultivation in India, particularly in Bihar, Gujarat, Himachal Pradesh, Karnataka, Madhya Pradesh, Maharashtra, Rajasthan and Uttar Pradesh. During 1994, the total area under cultivation in India was 3.95 million h and total production was 3.3 m tons (FAO, 1995). Cultivation of soybean to the required extent has not taken place though it has augmented edible oil resource of India by about 0.6 million tons per year, contributing to the foreign exchange to the extent of US $ 500 million per year (Ali, 1996).

Seed is the most important source of survival and long distance dissemination of plant pathogens and therefore, there is always a risk of introducing exotic pests and pathogens alongwith seed imports. Some of the major plant pathogens reported to be seed-borne in soybean are : *Alternaria tenuissima, Arkoola nigra* (Black leaf blight), *Ascochyta sojicola* (leaf spot), *Aspergillus quercinus, Botrytis cinerea, Cercospora kikuchii* (purple blotch, purple seed stain), *C. sojina* (frog eye leaf spot), *Colletotrichum dematium, C. truncatum* (seedling blight, anthracnose), *Colletotrichum* spp., *Corynespora cassiicola* (target spot), *Diaporthe phaseolorum* var. *batatatis* (stem canker, pod and stem blight), *D. phaseolorum* var. *caulivora, D. phaseolorum* var. *sojae* (pod and stem blight),

Fusarium spp. (Fusarium wilt), *Glomerella cingulata* (anthracnose), *Macrophomina mame, M. phaseolina* (charcol rot), *Melanopsichium nepalense, Peronospora manshurica* (downy mildew), *Phialophora gregata* (Brown stem rot), *Phytophthora megasperma* var. *sojae* (root and stem rot) and *Pleosphaerulina sojicola* (Phyllosticta leaf spot), *Rhizoctonia leguminicola* (black patch), *R. solani* (Damping off, foot and basal stem rot), *Sclerotinia sclerotiorum* (Sclerotinia stem rot), *Sclerotium* sp., *Septoria glycine* (brown spot), *Thilavia basicola, Bacillus subtilis, Corynebacterium* sp. (Seedling wilt, stunt), *Curtobactrium flaccumfaciens* (wilt) *Pseudomonas solanacearum* (wilt), *Pseudomonas syringae* pv. *glycinea* (Bacterial blight), *P. s.* pv. *tabaci* (Wild fire), *Xanthomonas campestris* pv. *glycines* (Bacterial pustule), Arabis mosaic virus, Bean pod mottle virus, Cacao necrosis virus, Cherry leaf roll virus, Cowpea mild mottle virus, Cucumber mosaic virus, Grapevine fan leaf virus, Mulberry ring spot virus, Raspberry ring spot virus, Soybean mild mosaic virus, Soybean mosaic virus, Soybean stunt mottle virus, Tobacco ring spot virus, Tobacco streak virus, Tomato black ring virus and Tomato ring spot virus (Richardson, 1990). Among these, *P. manshurica* (downy mildew) is of highest quarantine significance for India as the disease does not occur in the country and also due to destructive nature of the pathogen, and its existing races.

Geographical distribution

The disease was first reported from United States in 1923 (Sinclair, 1982) and is now occurring in Argentina, Australia, Brazil, Bulgaria, Burmuda, Canada, China, Colombia, Czechoslovakia, Denmark, England, France, Hungary, Iran, Isreal, Italy, Japan, Kora, Mexico, New Zealand, Nigeria, Philippines, Poland, Rhodesia, Romania, South Africa, Sweden, Taiwan, Thailand, Turkey, U.S.A., U.S.S.R. and Yugoslavia (CMI, 1979; Roongruangsree *et al.*, 1988; Kingsley, 1960).

Pathogen

The hyphae of *P. manshurica*, are intercellular in host tissues, coenocytic, and 7-10 µm wide. Sporangiophores are slender (240-984 x 5-9 µm) and tree like, with erect "trunks" grey to pale violet, emerging singly or in clusters from stomata on lower surface of leaf and 2 to 10 times dichotomously branched. The terminal branchlets or sterigmata (9-13 × 2-3 µm) are pointed and more or less straight. Sporangia (usually 19-24 µm) are subglobose without an apical papilla and germinate in water from an indeterminate point on the side. The antheridia fertilize oogonia leading to the development of light brown or yellow oospores with smooth or variously marked outerwall. The oopspores become visible in mounts stained with cotton blue. Oospores are 30-50 µm with an outer wall which is pale yellow and evenly ridged. Oospores

develop on the seed surface and appear as milky white crust consisting of a mass of the hyaline spherical resting spores.

P. manshurica reduced yield by 45% in Bulgaria (Nedelchev, 1978).

Detection of oospores

Johnson and Lefebvre (1942) were the first to detect crusts of oospores of *P. manshurica* on soybean seeds and indicated that the disease was seed-borne. Seed transmission was established by Jones and Torrie (1946). Pathak *et al.*, (1978) detected *P. manshurica* on seeds of soybean by dry seed examination and washing test and also described the tetrazolium test for spore viability. *Peronospora manshurica* forms encrustations of mycelium and oospores on seeds, which then appear milky white (Pietkiewiez, 1959). Infected seeds may be smaller and lighter in weight than the normal ones. Mycelium is found primarily in cell layers in seed coat. Infected seeds may not germinate or may produce systematically infected seedlings (Poonpolgul and Pupipat, 1978; Rosca, 1975; Inaba *et al.*, 1982; Zad, 1989). Marcinkowska (1987) reported that the oospore-encrusted seeds serve as source of primary infection and seedling emergence may be delayed by 2 weeks in the infected seeds. Ovchinnikova and Potlaichuk (1980) developed an indirect oospore detection technique by placing seeds into hollows of sterilized ceramic tiles filled with sterilized water, then covered with glass and incubated at 24- 26°C and reported the development of *P. manshurica* symptoms on seeds after 4 days. Roongruangsree *et al.*, (1988) also developed a Fuelgen technique for detection of mycelium and oospore crust on seed.

Quarantine interception

Soybean downy mildew has not yet been reported from India. However, it was detected (intercepted) on seeds of soybean imported from different countries (Mukewar, *et al.*, 1980; Ram Nath *et al.*, 1981, Agarwal *et al.*, 1990, Majumdar *et al.*, 1991, Agarwal *et al.*, 1996). It has also been intercepted on seeds imported from Malaysia and Indonesia where it is not yet reported (Agarwal and Khetarpal, 1985, Anitha *et al.*, 1993). Butler (1918) reported that *P. trifoliorum* de Bary causing downy mildew in wild species of lucerne and barseem, also attacks soybean (*Glycine hispida Maxim.*) in Kashmir. He reported that *P. trifoliorum* attacking *G. hispida* agreed in its general characters with *P. viciae* on peas; the oospores measuring 24-31 µm in diameter with a thick smooth light brown wall. He further emphasized that a variety of the fungus i.e. *P. trifoliorum* var. *manshurica* Naum. described on soybean from Russian manshurica differed greatly from *P. trifoliorum* on *G. hispida,* in having longer condiophores, nearly round conidia and longer oospores (36-45 µm in diameter). Observations of Butler (1918), and upto date literature survey do not suggest

the occurrence of downy mildew of soybean in India. Hence a constant vigil against the introduction of such dreaded disease through the imported material is of utmost importance. Pathak *et al.* (1978) detected oospores on seed samples from 17 countries and Roongruangsree *et al.* (1988) on seed samples from 5 countries. Rosca (1976) intercepted *P. manshurica* from Romania. Agarwal and Singh (1996) reported from India that while processing of 10,336 samples during 1978-1995 downy mildew (*P. manshurica*) was intercepted in 1514 samples from 15 countries.

Physiological races

Thirty three physiologic races of *P. manshurica* have been reported from U.S.A. alone by Dunleavy (1977) and Lim *et al.* (1984). Morcinkowska (1987) reported 11 physiologic races in Poland and later, on characterization he added 7 races (34-40) for the first time (Marcinkowska, 1991). Li *et al* (1992) collected samples from 9 different countries and identified 3 new races of *P. manshurica* and designated as Zhong 1, 2 and 3. Two soybean cultvars viz., Kanrich and Mendota are resistant to all races in U.S.A. This resistance to all downy mildew fungus is controlled by a single gene Rpm (Lim *et al.*, 1984).

Viability of oospores

The oospores of *P. manshurica* could retain viability from 1 to 8 years (Kovetskii, 1970; Naumova and Obtemperanskaya, 1988; Pathak *et al.*, 1978). Roongruangsree *et al.*, (1988) reported that portions of the mycelium of *P. manshurica* may survive even on the surface of dry, mature seeds.

Dunleavy and Snyder (1962) developed a germination technique for the viability test of oospore and reported 12.6-29.8% germination. The International Seed Testing Association (ISTA, 1976) recommended the use of 2, 3, 5-triphenyl tetrazolium chloride for determining the viability of oospores after 1 year of storage. Pathak *et al.* (1978) reported 30-39% viability in 1-2 year old and 20% in 8 year old oospores by tetrazolium test. They also reported 6-11% viability in oospores collected from 1 year old captan treated seeds. Roongruangsree *et al.* (1988) reported Phloxin B as a promising stain for determination of oospore viability.

Control

Seed is the important source of long distance dissemination and survival of the pathogen. Hence during exchange of seed material, there is always a risk of introduction of the pathogen into new areas where it is not known to occur. Several workers have reported the effective control of the disease by spraying the crop in field as well as by seed treatment. Fundazol, Ridomil, Previcur S 70, Apron 35 SD (Metalaxyl) and Sandofan M (Oxadixyl + mancozeb)

have been reported to be the effective fungicides in controlling the downy mildew disease in soybean (Anonymous, 1978; Dragoescu *et al.*, 1979; Zaggerini *et al.*, 1986) under field conditions. However, methods developed so far are not good enough to eliminate the pathogen from seed. Research aspects on various means of complete elimination of the pathogen from seed (both oospores on the seed and mycelium in the seed) has to be undertaken to eradicate the pathogen by salvaging the infected/contaminated material and making the disease free seed available to the users/breeders for utilization in crop improvement programmes.

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