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DOWNEY MILDEW OF PEARL MILLET AND IT'S MANAGEMENT#

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Abstract

Downy mildew is the most devastating disease of pearl millet particularly on susceptible and genetically uniform hybrids. Its biology, epidemiology and management aspects are complex as compared to the other major pathogens infecting pearl millet. In the present paper current status of knowledge about the symptoms of disease, pathogen variability, to understand biology and epidemiology of downy mildew pathogen and host-pathogen interaction that have helped in development of resistant cultivar and disease management through biological control of the disease.

Keywords- Downy mildew, epidemiology, biological control, resistant cultivar

#General Article

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Introduction

Pearl millet originated in Africa and was subsequently introduced into India. Some researchers believe that millets were the first cubtivated crops to be used for human food in prehistoric times (Singh, SD and King SB 1988). The world area cropped to pearl millet is about 26 million hectares. The crop is grown on the poorest soils and under harsh ctimatic conditions where no other crop can grow. Therefore, pearl millet is the food of the poorest of the poor. Although the crop is quite hardy, it still suffers from various biotic stresses. One of the major biotic yield-reducing factors is the disease downy mildew of pearl millet *[Pennisetum glaucum* (L.) R. Br.], caused by *Sclerosporu gruminicolu* (Sacc.) Schroter, is the most serious disease in major pearl millet growing areas of the world. The economic significance of the crop has become more accentuated than ever before, especially in relation to the high yielding cultivars (Shetty, 1990; Bangar and More, 1993; Singh *et al.,* 1993a,b; Shetty *et al.,* 1995; Singh, 1995; Sreedhara et *al.,* 1995).

The first epidemic of downy mildew occurred in 1971 on the first popular pearl millet hybrid, HB 3, resulting in severe grain loss of about 4.6 million metric tonnes (Singh SD. 1995; Singh SD, *et al.,* 1993). On the basis of a few localized estimates in India the average annual yield losses can reach up to 40%, whereas 10-50% losses have been reported from Nigeria. However, worldwide annual grain yield losses may not exceed 20% (Hash CT, *et al.,* 1999, Nene YL, Singh SD, 1976, Singh SD*, et al.,* 1993, Upadhyaya HD, *et al.,* 2007.). Information on extent of losses caused by DM in pearl millet is rather scanty. Severe and systemic infection reduces fresh weight of the shoot along with the number of basal and nodal tillers (Gupta GK, Singh D. 1996b).

Symptomatology

Two types of symptoms, downy mildew and green ear. are produced. Downy mildew symptoms may appear on the first leaf, but genwlIy on the second and third leaves in the form of chlorosis of the leaf lamina beginning at the base of the infected leaf. The chlorosis progresses to successively higher leaves, covering the entire lamina on the third or fourth leaf. Under high humidity (> 95% RH) and moderate temperature (20 to 25^0C), chlorotic areas on leaves produce abundant asexual spomlation. generally on the abaxial surface of leaves, giving them a downy appearance. Severely infected plants remain stunted and do not produce panicles. The half-leaf symptom, shown by a distinct margin between the diseased (basal) portion and the nondiseased areas toward the tip. is a characteristic symptom of the disease. After leaf symptoms develop, all the subsequent leavcs and the panicle have symptoms due lo the systemic nature of the disease, except in the case of recovery resistance, where plants outgrow the disease (Rachie, KO and Majmudar, JV 1980.). Green ear symptoms appear on panicles due to the transformation of floral parts into leafy structures.

Pathogen

There is no disagreement on the taxonomy of the downy mildew causing fungus. *Sclerospora* is now monotypic, the sole representative of Ito's eusclerospora group. The very name *Sclerospora* is drawn from the thick-walled oospore with its darkwalled exosporium and adherent oogonial envelope. It appears that there are remote chances of

a change in the name of *Sclerospora graminicola* (Nene YL, Singh SD, 1976, Spencer DM. 1981.). Phylogenetic relationship among sequences from Indian and African samples of *S. graminicola* was examined using a nested PCR technology. All the samples from India, Mali, Nigeria and Niger, with the exception of Niger 4, formed a monophyletic group with the oomycetes (Upadhyaya, H.D. *et al.,* 2007.).

Diagnosis of the fungus is based on two types of spores produced by *S. graminicola*. Asexual spores (sporangia, zoospores) are thin walled, hyaline, ellipsoid or elliptic and papillate measuring 15-22 x 12-21 µm. Under natural conditions sporangia are produced in abundance during the night (03.00 h). Normal temperature (20 to 25ºC) and high relative humidity (95-100% RH) favour sporangial production (Gupta GK, Singh D. 2000, Singh SD, *et al.,* 1993). Sporangia are actively ejected and germinate immediately, producing zoospores, or else die within a few hours. Under suitable conditions sporangia form in great abundance on the under surface of the diseased leaf (and, when conditions are favourable, also on the upper surface) forming a conspicuous and characteristic white 'downy' growth (Francis SM, Williams RJ. 1998). Oospores are the sexual spores, which can survive for 14 years under laboratory conditions, but their viability is reduced after 4 years of storage. Oospores germinate in multiple ways; by vesicle-like structures, by both vesicles and germ tubes, by typical irregular structures different from germ tubes and vesicles, by germ tubes and by extrusion of small round bodies/sporangia-like structures (Lukose C, Dave HR. 1995.). The optimum temperature for oospore germination is 28±2°C. Highest germination is observed in sterile distilled water after 24 h. Dry oospores are more resistant to steam sterilization than wet oospores. To ensure the total destruction of oospores from soil, sterilization of wet soil for more than 2 h is needed (Singh SD, Navi SS. 1996.). Oospore density in the soil is highly correlated with disease incidence at 90 days after sowing, indicating the importance of oospores in disease epidemiology (Gilijamse E, *et al.,* 1997). The oospores play an important role as far as transmission of the disease is concerned. Unsterilized seeds produce 23.4% disease incidence when sown in sterilized soil. This suggests that seed borne spores are important in inciting downy mildew (Nagaraja A, Siddiqui MR. 1994, Sheoran RK, *et al.,* 2000). However, there is no significant correlation between the mean incidence of downy mildew and oospore production (Rao VP, Thakur RP. 2004).

Pathogen Variability

S. graminicola reproduces asexually by means of sporangia and sexually through oospores. The fungus is largely heterothallic with two mating types. These characteristics of the fungus make it highly variable and adaptable to diverse environmental conditions. Similarly, its host pearl millet is a highly outcrossing crop species. The information generated for the past few years on genetics of resistance, availability of host differentials, and development of molecular techniques, has made it easier to understand *S. graminicola*- pearl millet interactions. Single cross F1 hybrids have greatly contributed to increasing productivity of pearl millet. Early maturity, uniform crop stand and high harvest index of these hybrids have made them popular among farmers. As a result hybrid cultivars cover about 55% of the total 10 million ha area under pearl millet in India with the cultivation of around 50 hybrids (Thakur RP, *et al.,* 1998, Thakur RP, Rao VP. 1997.). Several races or pathotypes of *S. graminicola* have now evolved in India. Stability of

resistance in pearl millet lines developed at ICRISAT was studied through a collaborative International Pearl Millet Downy Mildew Virulence Nursery (IPMDMVN). The reactions to downy mildew of 11 pearl millet lines at 17 locations in India, Burkina Faso, Mali, Niger, and Nigeria from 1995 to 1999 were recorded (Thakur RP, *et al.,* 1998, Thakur DP. 1987; Thakur RP, Rao VP. 1997; Thakur RP, *et al.,* 2003; Thakur RP, *et al.,* 1998). Seven pearl millet lines (IP 18292, IP 18293, 700651, P310-17, P7-4, MBH 110 and 852B) provided differential reactions permitting classification of the 23 populations into 15 putative pathotypes at the global level. The existence of a highly virulent pathotype of *S. graminicola* is reported from Jodhpur, India (Thakur RP, *et al.,* 2003).

Disease Cycle

Oospores, present in soil, serve as primary source of inoculum, and infect the underground parts of plants, mostly at the seedling stage. The thick impermeable walls of the oospores protect them from desiccation. *S. graminicola* oospores mostly germinate directly by germ tubes. There are also reports of indirect oospore germination by the liberation of zoospores. However, so far there is no microscopic or photographic evidence of direct oospore germination or indirect mode by releasing the zoospores. The infection process begins with the formation of a germ tube which produces an appressorium (Fig-1). This may be located at the junction of epidermal cells, directly over the epidermal cells or over stomata, depending upon the plant organ involved and the stage of development or maturation. Infection from oospores (whether direct by germ tubes or indirect by zoospores) may occur in the coleorhiza, radicle, lower portions of the coleoptile of seedlings, roots and underground portions of stem bases in older plants. Following the infection, pathogen grows intercellularly towards the meristem region. Systemic symptoms appear when the pathogen invades the developing leaves or inflorescence at the growing point. Without exception, younger the host plant, greater the susceptibility to systemic colonization by *S. graminicola*. Young seedlings are easily penetrated as they are succulent with their apical meristems protected only by a coleoptile, and are in close proximity to the soil. However, in older plants the apical meristem is encased by up to several leaf sheaths, making penetration to the meristematic region difficult. The systemic disease caused by *S. graminicola* is characterized by the invasion of the host apical meristem region by the pathogen and the appearance of symptoms when the organs that have been colonized during the process of tissue differentiation grow out and unfold. Thus symptoms appear sometime after the critical infection and colonization processes. Under humid conditions, systemically infected leaves produce abundant sporangia on the abaxial surface. Sporangia are important for the secondary spread of the disease within and among fields if environmental conditions are suitable (Singh and Williams, 1980). These sporangia release zoospores which germinate, penetrate the epidermis or stomata, and cause infection in successive manner. Plant inoculated at coleoptile stage produces systemic symptoms in young leaves in 4-7 days. If the environment is suitable, infected leaves continue to produce sporangia until the tissues become necrotic or senesce. Oospores are not always found in systemically infected leaf tissue, presumably because only one mating type is present and homothallism is inoperative. *S. graminicola* survives as oospores in the soil along with infected leaf residue, and cause primary infection in the subsequent years. Oospores are transmitted on the seed surface, in soil, by wind, or by water.

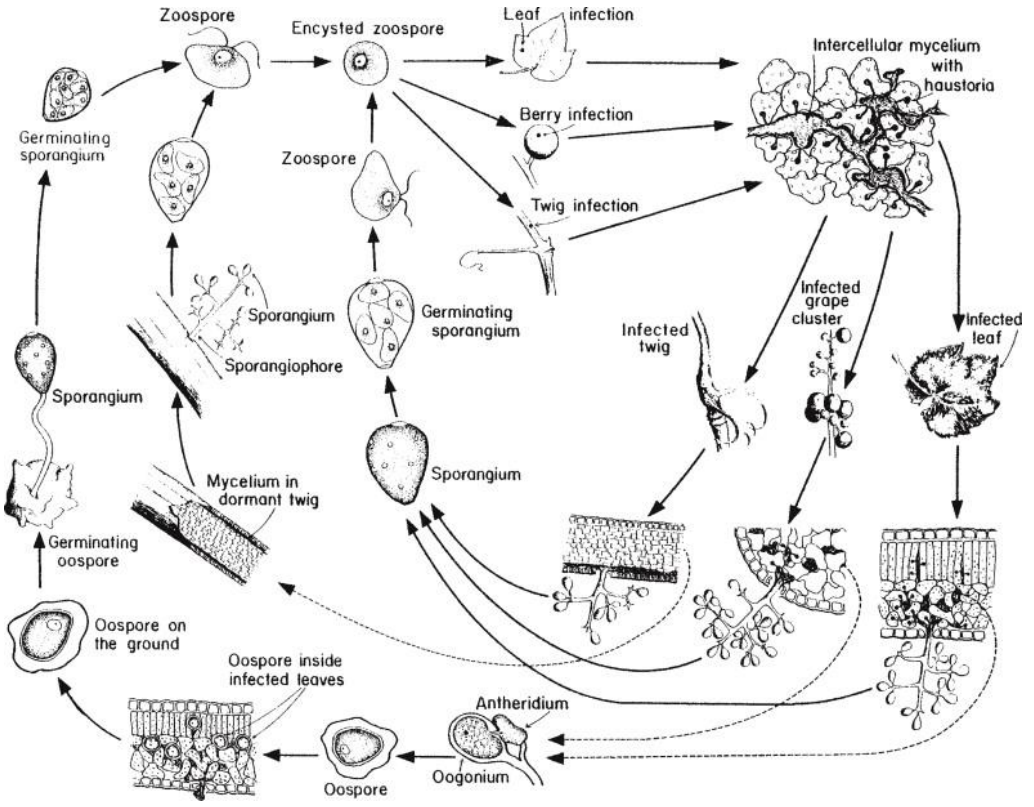

Fig.: 1. Disease Cycle of Downey Mildew of Pearl Millet

Chemical Control

Use of chemicals in the form of fungicides is most favored disease management practice. In case of pearl millet, fungicide use is less popular because of difficulty to take up spray in vast areas by the farmers. Moreover, downy mildew pathogen being an oomycete organism is not controlled by the fungicides normally recommended for other fungal diseases. Seed treatment is more desirable since it can be applied before sowing very easily. General fungicides recommended for pearl millet seed treatment are Thiram and Captan. These fungicides act as seed protectants. For oomycetes pathogen like downy mildew, a systemic fungicide metalaxyl is quite effective; but it is narrow spectrum oomyceticide. Metalaxyl, $C_{15}H_{21}NO_4$, methyl N-(2, 6-dimethylphenyl)-*N*-(methoxyacetyl) DL alanine, is a oomyceticide with a unique combination of residual and systematic properties, is highly active both *in vitro* and *in vivo* against pearl millet downy mildew (Singh and Shetty, 1990). The mode of action of metalaxyl includes the inhibition of protein and ergosterol synthesis, by interference with the synthesis of ribosomal RNA. This action is systemic with protective and curative action, absorbed through the leaves, stems and roots. Metalaxyl is available in the form of Apron 35 SD, Ridomil MZ 72, Master 72% WP (Metalaxyl 8% + Mancozeb 64%). Seed treatment with Metalaxyl 35% WS at 6 g kg-1 seed controls the disease excellently for about the first 35 days after sowing. Foliar

application of the Ridomil MZ 72 at 3 g l-1 arrests further development of the disease in systemically infected plants. Use of metalaxyl to control disease has been, however, found more economical in seed production plots than in commercial crop. Private seed companies are also using this chemical in treatment of commercial seed before selling to farmers. Several new generation chemicals are also being tested for control of downy mildew. Safe and eco-friendly strobilurin group of fungicides/oomyceticides - Flint (Trifloxystrobin), Amistar (Azoxystrobin) and Stroby (Kresoxim-methyl) have been tested and evaluated which are effective in control of downy mildew. Similarly, Cyazofomid and Iprovalicarb were evaluated and found to be promising. Amistar is an effective strobilurin fungicide/oomyceticide for control of downy mildew. Other strobilurin fungicides/ oomyceticides have been widely tested and are found to be very effective in controlling downy mildew disease (Deepak *et al*., 2004; Sudisha *et al*., 2004; Sudisha *et al*., 2007). Presently, different biopolymers are mixed with the fungicide/oomyceticide and used as seed treatment to increase the efficacy of the compound in controlling the disease and also to increase the vigor of the seedlings. In this direction, various biopolymers derived from plants like *Acacia arabica*, neem, drum stick, papaya, atrocarpus and mimosops have been tested and recommended for pearl millet seed treatment at concentration of 1:2 w/v in combination with half dosage of Metalaxyl i.e., 3 g kg 1 which is highly effective in managing pearl millet downy mildew (Sudisha *et al*., 2009).

Biological Control

With increasing environmental awareness the focus of managing plant diseases has been shifted towards viable and sustainable alternatives (Arun-Kumar, 2008). The importance of biological control for managingb downy mildew disease has been discussed. The lack of durable resistance, existence of pathogenic variability, and concerns about fungicide resistance opened the way for searching alternative and eco-friendly methods of DM control by activating the plant's own defense mechanisms with specific biotic or abiotic elicitors. The scientific basis of induced resistance has emerged with the identification and characterization of genes and biochemical pathways governing resistance in plants against invading pathogens. In this regard, operability of induced systemic resistance is gaining importance, as some biotic and abiotic agents have conferred protection against the disease (Arun-Kumar, *et al.,* 2005., Arun-Kumar, *et al.,* 2004, Raj SN, *et al.,* 2005, Raj SN, *et al.,* 2003a, Raj SN, *et al.,* 2003b, Shetty, H. S. (1990)). The efficacy of cerebrosides (glycosphingolipids extracted from various plant pathogens), as resistance elicitors against DM of pearl millet has been reported (Deepak SA, *et al.,* 2003.). β-Aminobutyric acid (BABA) treatment of pearl millet seeds influenced seedling vigour and protected the seedlings from downy mildew disease. Seeds treated with 50 mM for 6 h resulted in the maximum of 1428 seedling vigour and showed 23% disease incidence in comparison with the control, which recorded seedling vigour of 1260, and 98% disease incidence i.e. 75% protection from disease (Shailasree S, *et al.,* 2007.). A combination ofraw cow's milk as seed treatment and *Gliocladium virens* as seed and soil treatments resulted in the lowest disease incidence and highest number of tillers of the crop (Arun-Kumar, *et al.,* 2004). The protection by raw cow's milk and *G. virens* in managing DM is ascribed to induced systemic resistance based on an increase in resistance related enzymes (PPO, CA and PO) and metabolites (phenols, ODP) in the treated plants (Arun Kumar and Purohit AK. 2012.).

Recently, polyphenol oxidase (PPO) activity was analyzed in seedlings of 10 resistant and susceptible pearl millet cultivars with or without inoculation of *S. graminicola*. Seedlings of resistant varieties had greater PPO activity than susceptible seedlings, and inoculated seedlings had significantly higher PPO levels than un-inoculated seedlings. PPO activity was positively correlated with levels of downy mildew resistance in different pearl millet cultivars under field conditions. Native PAGE staining showed four isoforms of PPO, which were differentially induced in relation to the time of appearance and intensities in the un-inoculated seedlings, whereas a fifth PPO isoform appeared after inoculation with *S. graminicola*. PPO activity was significantly higher in the shoot and leaves of pearl millet than in the root. The enzyme was predominantly expressed after pathogen inoculation and was localized in the epidermal and vascular regions (Raj SN, *et al.,* 2006). The importance of integrating indigenous knowledge of using raw cow's milk with biocontrol agents has been emphasized as a logical strategy to induce resistance in crops of low economic value largely grown by resource-poor semi-arid tropical (SAT) farmers (Arun-Kumar 2008, Gupta AK. 2006). A commercial preparation, Elexa, (an aqueous chitosan formulation) has been tested under field conditions. A combination of seed treatment and foliar spray to 7- day-old seedlings recorded 69% protection. The nature of disease control is ascribed to induction of systemic resistance (Shailasree S, *et al.,* 2007). Methanolic extracts of plant species such as *Clematis gouriana, Evolvulus alsinoides, Mimusops elengi, Allium sativum* and *Piper nigrum*, commonly growing across India were reported to be having antisporulant activity against *S. graminicola*. The extracts of 11 species (*Agave americana, Artemisia pallens, Citrus sinensis, Dalbergia latifolia, Helianthus annuus, Murraya koenigii, Ocimum basilicum, Parthenium hysterophorus, Tagetes erecta, Thuja occidentalis and Zingiber officinale*) also exhibited remarkable anti-sporulant effect even after 10-fold dilution of the crude extracts and were found commensurable to that of the marketed botanical fungicides (Deepak SA, *et al.,* 2005.).

Integrated Disease Management

Integrated disease management (IDM) is gaining popularity in all crops. Integration of host resistance, fungicides/oomyceticides and bioagents, and other microorganisms to manage biotic and abiotic stress is also possible in pearl millet. It is therefore recommended that host plant resistance deployed in genetically uniform hybrids be backstopped with appropriate management practices (crop and cultivar rotation, and use of appropriate prophylactic seed dressings chemicals) to extend the economic life of the hybrid (Hash *et al*., 1997; Witcombe and Hash, 2000; Hash *et al.*, 1999; Hash and Witcombe, 2002). This approach is also being practiced under AICPMIP programme. IDM module that includes host plant resistance (moderate level), half dose of metalaxyl (3 g kg-1 of seed), PGPR strain of *Bacillus pumilus* INR 7 (8 g kg-1 of seed) and Chitosan (2.5 g kg-1 of seed) is being tested at various AICPMIP locations for the management of pearl millet downy mildew.

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