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HISTOPATHOLOGICAL STUDY OF *Macrophomina phaseolina* CAUSING CHARCOAL ROT OF SORGHUM

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ABSTRACT

Charcoal rot of sorghum stem blight by *M. Phaseolina* (Tassi) Goid, is favoured by warm dry growing conditions and is often associated with drought stress although charcoal rot disease has been found under humid tropic conditions *M. Phaseolina* can infect a braod array of major crops including common bean, maize, soya bean, cotton, seasame. The common name of the disease caused by *M. Phaseolina* derived from the symptoms present in adult plants where stem tissues show the growth of numerous microsclerotia and pycnidia. The severity of the disease is directly related to the population of viable sclerotia in the soil. So the study of Histopathology in Rajasthan area were carried out on healthy and diseased sorghum roots and stem pieces in order to find out the precise position of pathogen, its mode of penetration into host tissue, and damage caused as a result of its invasion in plants. Histopathological changes taking place within the tissues and vascular region of root and stem were also recorded. *Sorghum bicolor* (L) Moench is a monocot plant belonging to the Poaceae (Graminae) family. The anatomical characters of the root and stem are typical of Monocot plant.

KEYWORDS : *M.phaseolina,* Charcoal Rot, Histopathological studies

Sorghum is a genus with many species and sub species and there are several types of sorghum, including grain sorghum, grass sorghums (for pasture and hay) sweet sorghum (for syrups) and Broom Corn. It ranks fifth among the cereal grains in extent of production after wheat, Rice, Maize & Barley. It is mainly cultivated in the states Maharashtra, Karnataka, Andhra Pradesh, Madhya Pradesh, Gujarat, Chennai, Rajasthan, & U.P. Sorghum is grown primarily for the use in human food (either directly or after brewing) and as a grain for animal feed. In the world about 500 million people depend upon sorghum for food. Increasing pressures of population growth on food supplies necessitate the attention towards realizing fully the potential of this crop which fills a unique and highly significant place in many social, ecological and climatic situation. It may be the only or atleast the principal viable alternative for providing the staple food for local diet. (Mayek et al. ,1997, 1997c, 2002a & 2002c).

Sorghum requires less moisture for growth than other cereals. It is grown where rainfall ranges 500 to 1000 mm and temperature, between 26°c to 32°c respectively. Sorghum requires 90-140 days to mature but most of the developed hybrids and varieties requires around 120 days.

It is affected by many micro organism *M. Phaseolina* is one of the destructive disease found in India and has a great destructive potential as a disease in most sorghum growing regions during Rabi and Kharif season. First report of the disease was given who found the association of *M. Phaseolina* in lodged plants in Rabi Sorghum.

The pathogen of charcoal rot is a soil borne fungus often known by two district stage in its life cycle. First stage is pycrideal and second stage is sclerotial. Pycnidial stage is known as *M. Phaseolina* (Tassi) Goid (imperfect stage) and sclerotial stage as *Rhizoclimia batalicola* (Haigh,1930). An Interconnection between these two stages has been very well established and cited the evidence that Pycnidial state is capable of infecting and producing symptoms in host. The fungus can also thrive as saprophytes and become parasitic on living tissue of susceptible hosts.

Charcoal rot occur in almost all stages of plants growth but the symptoms are more spectacular in older plants even though the infection starts much earlier. It has been noticed that it develops very rapidly in fully matured plants. It is an important disease of polygenic character. The affected part of seedling shown sunken spots around the collar region which rapidly spread to rest of the parts, finally results in death of seedling. In adult plants symptoms appear much later. The ear do not form properly plants ripens prematurely, the stems break easily and infected

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tissues become fibrous and hollows and result in lodging of diseased plants. The diagnostic characteristics of charcoal rot are deterioration of pith and production of large number of tiny black mustard tike bodies in the tissues called microscterotia which serves as primary source of inoculums. Sclerotia produced in plant tissue are released into the soil as crop residue and remain viable for 2-15 years depending on the environment conditions (Cook et al.,1973; Cloud & Rupe, 1991). These are responsible for overcoming the seasonal adversity pycnidia and pycnidiospores are reported on aerial parts such as leaves.The tap roots may appear light gray or silvery in appearance when the appear light gray or from roots and stem base, small black specks can be seen. These specks are so numerous that they appear grayish black and hence the name of disease is charcoal rot

The present investigation is therefore undertaken to study the histopathology of the organism in the disease, so that it will be increase the production of sorghum, result of which are incorporated herein. (Mayek et al.,1977, 1997c, 2002a & 2002c).

MATERIALS AND METHODS

Histopathological studies were undertaken to determine.

1) Mode of penetration of pathogen (*M. Phaseolina*) in sorghum.
2) Histopathological development of causal fungus in different sorghum parts viz roots, stem, leaves, earheads, seeds etc.
3) To observe any pathological changes taking place in the cells of infected parts.

Different parts of systematically infected plants i.e. roots and stem were collected. The materials were fixed in formalin acetic acid solution of following concentration.

Schedule of paraffin method followed.

1. Fixation of diseased as well as healthy stem and root pieces in FAA
2. Removal of fixative and water by passing the material through an ascending dehydration series of ethyl alcohol. (70, 85, 95, 100) and finally bringing it to absolute alcohol.
3. Transferring the materials to absolute alcohol and toluene series. The materials is passed through an ascending series of absolute alcohol and toluene mixture which were prepared as follows (85-15, 70-30, 50-50, 30-60, 10-90, 100% toluene) and finally passing the material dissolved paraffin.
4. Giving three to four changes in liquid paraffin till materials are completely free from toluene.
5. Preparation of paraffin blocks and sectioning at 15, 20 and 30 µ thickness with a hand operated rotary microtome.
6. Fixing of ribbons on slides and drying over the hot plate.
7. Removal of paraffin from slides by passing the sections through a descending series of xylene and alcohol.
8. Staining the sections with safranin and picro aniline blue as well as safranin and fast green following the methods of Johansen and Curl,(1972).
9. Dehydration of the stained slides by passing it through a mixed series of alcohol and xylene.
10. Mounting the slide with DPX.

INFILTRATION

Bits of paraffin was put in specimen tubes having xylene and bits and the tubes were kept in oven at 65°c for 48 hours about ¼ of molten paraffin was replaced with fresh molten paraffin. These changes were repeated four times at an interval of 4 hours. Finally the specimen tubes containing bits were left uncovered for 12 hours making paraffin free of xylene. Blocks were prepared and ribbons of 15, 20, & 30 µm thickness were cut by hand operated rotary microtome. The ribbon were fixed on clean glass slides using hawkers gelatin advesive gelatin dissolved in 100 ml. distilled water at 30°c followed by 2g phenol crystals and 15 ml. glycerine and 3% of formaline solution.

Staining

Staining of the sections was done using safranine, picro aniline blue as well as safrnine and fast green following the method of Johnson and Curl,(1972).

Clearing Techniques

To soften and clear the infected tissue a mixture of absolute alcohol and lectophenyl was used in the ration of 4:1 Diseased stem pieces and epidermal peating were placed in this solution. After seven days, cleared pieces

were taken out, sectioned stained with cotton blue and finally mounted in lectophenol.

Histopathology of Infected Root

The diseased roots showed the following pathological changes.

The fungus show inversion on the external surface and produce mycelium and sclerotia due to which the epiblem show dis integration and browning of tissues.It shows splitting browning and disintegration of cells here and there due to which irregular, large spaces are formed in the cortial region. The mycelium and sclerotia could be also traced at a few places.No note worthy changes except darkening, thickening & black endodermis and pericycle.In the vascular region the pathological changes were quite prominent. Thefungal mycelium could be traced in both xylem and phloem. At certain places the lumen of metaxylem vessels was blocked by the deposition of metabolic and by the physical presence of the mycelium and sclerotia.The pith region also showed similar changes as found in the cortial region i.e. blackening of cell walls, decomposition of cellular contents and disintegration of the parenchyma cells giving, rise to large spaces in the centre thus rendering the root a hollow structure. It addition to this, large number of sclerotia were also noticed within the pith region and near the vascular element.

Histopathology of Infected Stem

The diseased internodes showed external reddish to dark brown or black discolouration. When these internodes were split open, the entire inner tissues showed blackening coupled with formation of innumerable sclerotic over the fibro vascular bundles and segregation of individual vascular bundle owing to total disintegration of pith was also noticed. Thus the histopathological changes in the infected sorghum appeared quite prominent which involved disintegration of different tissue decomposition of individual cells, blackening of intra cellular materials remification of the mycelium and formation of sclerotia. The pathogen caused physical blocking of lumen of sieve tubes, tracheids and vessels.

The L. S. of stem also demonstrated as to how destruction of different tissues as well as blocking of the conducting system had occurred. In comparison to the peripheral vascular bundles the central bundles showed greater blackening of the vessels & tracheids.

RESULTS AND DISCUSSION

Histopathological study was conducted for both healthy as well as infected sorghum plants involving root and stem parts. The results showed that in the young plants the infection occurred through the soil born inoculums and the roots were the first target of attack for the pathogen. In the healthy plants the roots were intact, yellowish and undamaged and histopathologically they showed a normal monocot root structure. In the infected roots their anatomy was rendered abnormal. Such roots not only showed external discolouration but also internal discolouration in cells and tissues coupled with invasion of the entire region by the pathogen. The physical presence of the fungus was clearly demonstrated in the form of thick walled brown hyphae and black sclerotia. This structures were detected in all the tissues eg. Epidermis, cortex, phloem, xylem and pith. This shows how the vascular bundles have clearly reveled and how the fungus blocked the passage of water nutrients in plants.

Similarly a comparatively study of the healthy and infected stem had reveled that the healthy stem was green in colour and possessed a well formed monocot type anatomy. However, the infected stem showed darkened, shrunken internodes and presence of sclerotia externally on epidermis.

The T.S. of these stems have clearly demonstrated that the fungus invaded all the tissue i.e. (epidermis, hy ear headermis, ground tissues and the fibro vascular bundle) and produced hyphal branches and sclerotia. Another important effect was total disintegration of ground tissues and the separation of fibro vascular bundles. Innumerable sclerotia were formed around them and also in the lumen of vessels and tracheids.

Thus the stem structure got disintegrates and the plant showed bending and lodging under the weight of upper crown.

Needless to stress that the present histopathological study has thrown considerable light on the made of infection of sorghum plants. It has also explained the cause and the enzymatic destruction of soft tissue where individual cells have decomposed and irregular spaces have

been created. In this respect the ground tissue was most badly affected.

It has also explained the reason for lodging and banding of plants, poor ear head formation and poor grain setting in infected plants which obviously occurred due to failure of water and mineral supply to the growing plants. This failure was caused by blackening of the conducting system and by bending & lodging of the mature plants.

Further it was also observed that plants grown in artificially inoculated soils got infected through the roots region only and the infection processed internally in the growing plant through the conducting system. In the matured plants, the fungus progressed radially outward i.e. from xylem and phloem towards the ground tissue and epidermis. The fungal hyphae ultimately reached the epidermis and produced profuse sclerotia on the outer surface. Thus this study clearly established that in sorghum plants the pathogen caused systematic infection leading from root to tip of the plant.

Needless to mention that this is first contribution of its kind on the charcoal rot of sorghum plants in Rajasthan.

However there are few reports on the histopathology of sorghum plant carried out in past. Besides sorghum, there are some reports on infected maize plant also recorded. A preliminary histopathology study of infected plants has been studies and found that they were internally effected by disease.

Similarly detection of disintegration of medulla in the lower internodes sorghum our investigations as reported above amply confirm these studies.

SUMMARY

Histopathology investigation of healthy and diseased root and stem of sorghum plants were undertaken. As expected the infected plants showed darkening, decomposition and disintegration of different tissues in these parts.

The infected roots showed these effects in epidermis cells. Cortex, pith and partly in vascular bundles. The xylem of vascular bundles showed blackening due to aggregation of mycelium and metabolism deposits. The sclerotia formation was also observed in cortex, vascular region and pith.

In the infected stems the presence of sclerotia and mycelium was observed in all tissues. The hypodermal sclerenchyma and the ground tissues showed splitting disintegration and browning. The V. B. showed development of sclerotia aroud them & blocking of the xylem vessels and protoxylem lacuna. In phloem the conducting cells like sieve tubes etc. were also blocked.

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