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Pathogenesis of *Sclerotium rolfsii* Sacc. on wheat seedlings

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Histopathological studies and the study of hydrolytic (polygalacturonase and cellulase) enzymes revealed the possibility of preconditioning or plasticisation by enzymes prior to colonization of wheat seedling hypocotyls by *Sclerotium rolfsii*. Once established, the pathogen rapidly colonises the inter-and intra-cellular cortical tissue that rot and lead to collapse of seedlings.

Key words : Pathogenesis, *Sclerotium rolfsii*, Wheat Seedling

Foot rot of wheat (*Triticum vulgare* L.) caused by *Sclerotium rolfsii* Sacc., reported first in 1953 by Chattopadhyay, is gaining importance in W. Bengal.

Little is known about the mode of penetration by this pathogen although Higgins (1927) reported the penetration of cell wall appeared to be mechanical by the passage of constricted hyphae through very small openings and cell to cell movement of fungus. On the other hand, Paintain (1926) reported that hyphae penetrated cell walls with or without constriction and most penetration may occur by other than mechanical means. Pathogenesis caused by *S. rolfsii* has received considerable attention. Bateman and Beer (1965) suggested that both oxalic acid and pectic enzymes are involved in destruction of host tissue by *S. rolfsii* and that two fungal products acting together are more effective than either alone. Hussain (1957, 1958) recognised the importance of pectic enzymes in separating host cells after infection but also pointed out that the rapid break down and collapse of cell walls might also involve cellulolytic enzymes. Strong cellulase activity was recorded in filtrates of *S. rolfsii* and the pathogen infected hypocotyls (Bateman and Beer, 1965). Polygalacturonases (PG) produced by isolate 14 of *S. rolfsii* showed maximum activity near *p*H 4.0 (Bateman, 1972). The mode

of penetration and mechanism of this pathogen on wheat seedlings are reported in this paper.

MATERIALS AND METHODS

Wilted wheat seedlings covered with white mycelial mat at foot region was collected and fixed in formalin acetic-acid solution (FAA) for studying mode of penetration by the fungus. Materials in FAA were washed 5-6 times in water, dehydrated and wax blocks prepared (Johansen, 1940). Ten micron sections were cut using a microtome. The sections were stained with fast green-safranin (Johansen, 1940) and mounted in canada balsam.

The slides were examined under microscope and mode of penetration was observed.

For studying role of hydrolytic enzymes (PG) in pathogenesis, fungus (3 days old culture on PDA) was grown in glucose-nitrate basal medium (glucose-0.5 g, ammonium nitrate 0.1 g, potassium orthophosphate 0.1 g, magnesium sulphate 0 05 g, distilled water 230 ml fortified with 1.2% of pectin) for 15 days at 28 ± 1°C. The culture filtrate was filtered through folds of cheese cloth and centrifuged at 5000 rpm at 5°C for 15 min. The decant was collected and dialysed in distilled water for a period of 20-24 hr at 4°C. The dialysed extraet was used immediately as enzyme source and enzyme activity was studied at *p*H 5.0, 7.0 and 9.0. For studying PG activity *in vivo* diseased portions of infected wheat seedlings were washed in running tap water and then in distilled water and dried with a blotting paper. The tissue was blended with acetate buffer (*p*H 5 0) in the ratio of 1 : 20 and extract was centrifuged at 5000 rpm at 5°C for 15 min. The decant was dialysed and used as *in vitro* at *p*H 5 0.

For studying cellulase (Cx) activity fungas was grown in glucose-nitrate basal medium fortified with 1% carboxymethyl cellulose (CMC) sodium salt and same procedure was followed as before. Cx activity was studied at *p*H 4.5.

Viscosimetric assay of PG or Cx *in vivo* or *in vitro* was adoptedf ollowing method described by Bell *et al.* (1959) with modifications as suggested by Hancock *et al.*, (1964). Specific enzyme activity was calculated as $1/t \times 1000$ where, t is the time required in min for 50% reduction in viscosity by an enzyme preparation.

RESULTS

Mode of penetration

Tufts of hyphae were seen on the epidermis. Considerable disorganisation of cortical tissue was noted. Hyphae branched profusely in the intracellur regions.

Branching was not so profuse in the intercellular region. Slightly swollen appressoria like structures were also seen at times on cortical cells in longitudinal sections and it was possible that penetration was achieved through these. The collapse of the seedling was apparently through extensive disorganisation of cortex as no damage to vascular region was noted and this collapse was noted also among cells that have not been penetrated by the invading hyphae.

Enzyme activity

The results (Table 1) showed that 50% reduction in viscosity of sodium polypectate takes place in about 720 seconds at *p*H 5.0. $PDFT_{50}$ value was not obtained at other *p*H (*p*H 7 and 9). The specific enzyme activity was found to be 83.33 at *p*H 5.0. In infected tissues on the other hand the specific enzyme activity was found to be 31.25.

Table 1 Assay of hydrolytic enzymes of *Sclerotium rolfsii* by viscosimetric method.

Time (in minutes)	Percent decrease in flow time		
	Polygalacturonase		Cellulase
	In vitro pH 5.0	*In vivo* pH 5.0	*In vitro* pH 4.5
2	31.83	13.42	25.00
5	44.98	28.16	35.60
10	48.44	33.45	46.96
20	54.32	41.39	57.57
30	—	48.58	62 12
40	—	55.76	—
Specific activity	83.33	31.25	82.19

DISCUSSION

Histopathological studies did not reveal the actual mode of penetration. However, the massing of hyphae on the hypocotylar region would indicate a pooling of energy source prior to infection as has been suggested by Garret (1971). Intracellular branching is apparently related with the need for rapid utilisation of available food within the cells.

This might also explain the rapid collapse of seedlings once infection has taken place. PG and Cx enzymes were considered the principal rotting agents produced by *S. rolfsii*.

These studies revealed disorganisation of cells even where hyphae have not encroached. This, accompanied by high PG activity noted, would mean that

certain amount of plasticisation and preconditioning of the cells takes place before actual penetration. One could draw similar conclusions from the extensive work on pectic and cell wall degrading enzymes done by Bateman and Beer (1965), Bateman (1972) and others. It is well known that *S. rolfsii* is a weak pathogen (Aycock, 1966) and its sclerotia does not have sufficient intrinsic energy to penetrate host tissue directly. Under such circumstances, plasticisation of cell walls through cell wall degrading enzymes secreted sequentially (Van Etten and Bateman, 1969 ; Cole and Bateman, 1969) will substantially assist the pathogen in establishing itself. Active secretion of Cx by this pathogen would also appear to serve the same objective, i.e. colonisation of the host tissue and conversion of complex carbohydrates into utilisable monosaccharides that can be readily used by the pathogen for its own benefit.

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