Research Paper :

Deterioration of timber quality of *Dalbergia sissoo* by a die-back pathogen *Lasiodiplodia theobromae*

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SUMMARY

Sissoo (*Dalbergia sissoo* Roxb.) is an important timber tree planted in different regions of our country for its highly valuable wood. Die-back caused by *Lasiodiplodia theobromae* leads to deterioration of timber quality of infected plant. The present investigation was carried out to determine the activities of cellulases, xylanase and lignin peroxidase in degradation of chemical constituents of infected wood. The studies indicated that the pathogen is efficient producer of all these enzymes. Different cellulolytic enzymes showed highest activities from 30th to 40th days after incubation whereas xylanase and lignin peroxidase activities reached a peak on 20th and 30th days after incubation, respectively. The increased activities of these enzymes resulted ultimate decay of the sissoo wood.

Key words : Cellulases, *Dalbergia sissoo*, Die-back, *Lasiodiplodia theobromae*, Lignin peroxidase, Xylanase.

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Dalbergia sissoo Roxb. belonging to the family Papilionaceae is a medium to large deciduous tree with a light crown. The tree is planted for its good quality timber and also valued for decorative and often fragrant wood. Die-back of *D. sissoo*, incited by *Lasiodiplodia theobromae* (Pat.) Griffiths and Maubl. (Syn. *Botryodiplodia theobromae* Pat.), is a dreadful sporadic disease causing deterioration of the quality of timber. The disease is characterized by its symptoms expression as dyeing back from tip to downwards, drying and shedding of leaves and twigs all over the plant, loosening of fibres and dusty appearance of the wood. Cellulose is the major constituent of plant cell wall particularly in the secondary wall and it is a linear polymer of D-glucose units with β-1,4 glycosidic linkages. Fungus producing cellulolytic enzymes and having the capacity to cause degradation of hardened tissues have been reported by a number of workers (Wood *et al.*, 1988; Barkai *et al.*, 1991; Mehrotra, 1995). Most studies relating to enzymatic degradation of hemicellulose have focussed on xylans with the virtual exclusion of galactogluconomannans and glucomannans. Xylans are the major hemicellulose in angiospermic wood. The removal of cellulose and hemicellulose creates a channel wide enough to allow access of the enzymes to the sites of the lignin degradation (Reid, 1995). In this study the activities of different cellulolytic enzymes, xylanase and lignin peroxidase responsible for degradation of chemical constituents of sissoo wood infected by *L. theobromae* was assayed.

MATERIALS AND METHODS

The infected wood was collected from different places of Burdwan district of West Bengal. After being chopped up, the blocks were dried in hot air oven and the substrates were finally prepared from the blocks separately by grinding and sieving through a nylon net (40 mesh). The pathogen, *L. theobromae* was also isolated from infected plant.

Flasks containing the wood blocks were incubated individually with agar discs (7mm in diameter) cut from the margin of 7 days old actively growing colonies of the test fungus on PDA medium and incubated at $25^0 \pm 1^0$C under gyratory shaking at 70 rpm.

The cultures were harvested at 10 days intervals and filtered through an ice-cold G_4 sintered glass filter. The filtrates so obtained served as the enzyme sources to assay the enzyme activity.

Exoglucanase activity was determined by incubating 0.25 ml of culture filtrate with 0.75

ml of Sodium acetate buffer (0.2M) at pH 5.0 along with 50 mg Whatman No. 1 filter paper discs at 50^{0}C for 60 minutes. Endoglucanase activity was assayed following the method of Mahadevan (1991). The activity was determined by incubating 0.5 ml of culture filtrate with 0.5 ml of sodium acetate buffer (0.2M) at pH 6.0 and to it 1 ml of carboxymethyl cellulose (1%) dissolved in acetate buffer at pH 6.0 was added. The reaction mixture was subjected to incubate at 50^{0}C for 30 minutes.

In both the cases (exoglucauase and endoglucauase) after a specific period of incubation, reactions were terminated by adding 3 ml of dinitrosalicylic acid (DNS) reagent, boiled for 5 minutes, cooled at room temperature and the absorbance read at 540 nm in a spectrophotometer (Sicospec 100).

β-glucosidase activity was determined following Mahadevan (1991). The reaction mixture containing 1 ml of p-nitrophenyl-β-D glucopyranoside solution in acetate buffer (0.1M, pH 4.8), 1.8 ml of acetate buffer (0.1M, pH 4.8) and 0.2 ml of enzyme solution was incubated at 50^{0}C for 30 minutes. After the incubation period, 2 ml of Na_2CO_3 (1.0 M) was added to this mixture and its absorbance was measured at 410 nm in the same spectrophotometer. An enzyme and a reagent blank were maintained as usual. A standard curve was prepared with p-nitrophenol standard solution (20 mg/100ml). One unit of β-glucosidase activity is defined as the amount of culture filtrate, which is capable of liberating 1 μM of p-nitrophenol per minute from the respective substrates under the conditions of assay. The results are graphically presented in Fig. 1.

Xylanase (hemicellulase) activity was measured following Ghosh and Bisaria (1984). The reaction mixture containing 1 ml of xylan solution and 1 ml of crude enzyme extract was incubated at 30^{0}C for 30 minutes. After incubation, reactions were terminated by adding 3 ml of DNS reagent, boiled for 5 minutes in a hot water bath. After cooling, its absorbance was measured at 540 nm in a spectrophotometer. A standard curve was prepared with xylose solution in acetate buffer (0.2M) at pH 5.4. One unit of xylanase is defined as the amount of culture filtrate, which is capable of liberating 1 μM equivalent of xylose from xylan in 1 minute (Fig. 2).

Estimation of lignin peroxidase activity was done by modified method of Putter (1994). 0.5 ml of freshly prepared pyrogallol solution (0.1 M) and 2 ml of phosphate buffer and 1 ml of enzyme source were mixed in a cuvette in a spectrophotometer and the mixture was immediately adjusted to zero absorbance. 0.1 ml of H_2O_2 (0.1%) was added to it and the content was mixed by inverting the tube. The reaction was initiated by the addition of H_2O_2 and was recorded the change of absorbance at 460 nm per minute in a spectrophotometer. Unit was expressed as the change in the absorbance at 460 nm per minute. An increase in the absorbance of 0.01 in one minute was taken as one unit of peroxidase activity. A control set with boiled enzyme source was also kept. The results are graphically represented in Fig.3.

RESULTS AND DISCUSSION

It is evident from the result (Fig.1) that the pathogen is capable of producing all the unit fractions of cellulolytic enzyme *viz.*, exoglucanase, endoglucanase and β-glucosidase *in vitro*. The die-back pathogen, *L. theobromae* showed the highest activity of exoglucanase on 40th day of incubation in the host plant. Endoglucanase, however, showed a steady rise in its activity upto 30th day in the hosts and thereafter it decreased gradually. β-glucosidase activity showed its peak on 40th day of incubation. It may be concluded that with days of incubation, the different components of cellulolytic enzyme differed significantly in their activities under *in vitro* condition and the activity remains significantly higher in case of endoglucanase than other fractions of the enzyme. Production of various extracellular components of cellulolytic enzyme in cultural condition as obtained in the present study insight into the cellulose degradation process and indicated cellulose degrading ability of *L. theobromae*.

The presence of exoglucanase activity indicates that the pathogen is capable of degrading natural cellulose at the so called crystalline regions thereby corroborating the findings of Sharma *et al.* (1996). Masaply and Levanon (1992) reported the first peak of β-glucosidase appearing at a time when glucose reached its lowest concentration and concluded that this might be related to the decrease

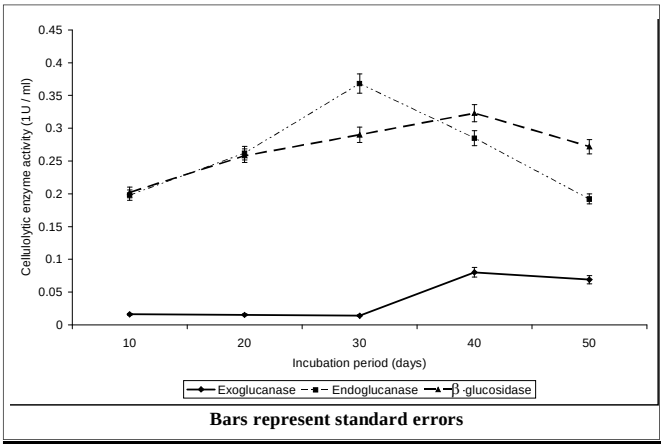

Fig. 1: Cellulolytic enzyme activity (in terms of U/ml) in *D. sissoo* by *L. theobromae* at different incubation periods.

in glucose respiration on cellulose degrading activity that included β-glucosidase. Cellulolytic enzyme activities showing simultaneous appearance and rise in the present findings might be due to synergistic action of the enzymes. Lowering of cellulase activities in the latter stages of incubation may probably be due to catabolic repression of cellulase as soluble reducing sugar gradually increased in the medium.

The result (Fig. 2) shows that the xylanase exhibited a clear rise in its activity shown by the pathogen up to 20th day following incubation and after that the activity decreased gradually. In order to attack the different

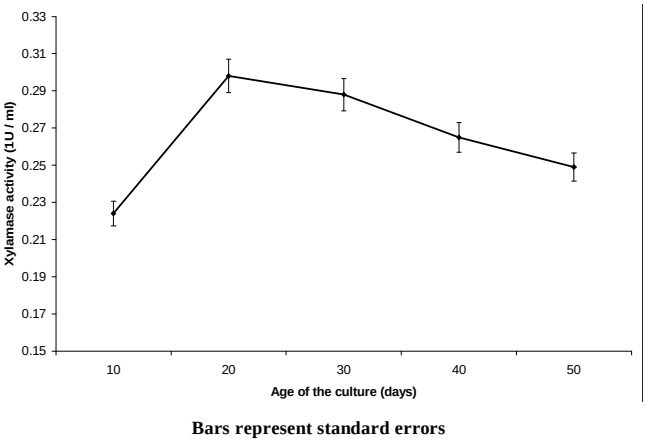

Fig. 2: Xylanase activity (in terms of U/ml) in *D. sissoo* by *L. theobromae* at different incubation periods. Bars represent standard errors

linkages in hemicellulose efficiently, fungus needs to evolve hemicellulolytic enzyme systems consisting of a number of different specificities. Production of different extracellular degradative enzymes like cellulases, hemicellulases (xylanase) suggest that the pathogen is capable of degrading all major components of lignocelluloses *viz.*, cellulose, hemicellulose and lignin which have also been reported by Saxena and Rai (1992).

It may be noted from the result (Fig.3) that *L. theobromae* showed enhancement in lignin peroxidase activity up to 30th days of incubation and then the activity decreased gradually. Lignin peroxidase can directly oxidize non-phenolic lignin. Production of significant amount of lignin peroxidase in host tissues suggests that the pathogen has a unique mode of action in degradation of lignin. Native lignin being hydrophobic, insoluble and sequestered in a dense matrix of polysaccharides and it restricts access of enzymes to only the surface of the cell wall. Physical contact between enzymes and lignin is known to be a limiting factor in lignin biodegradation. The

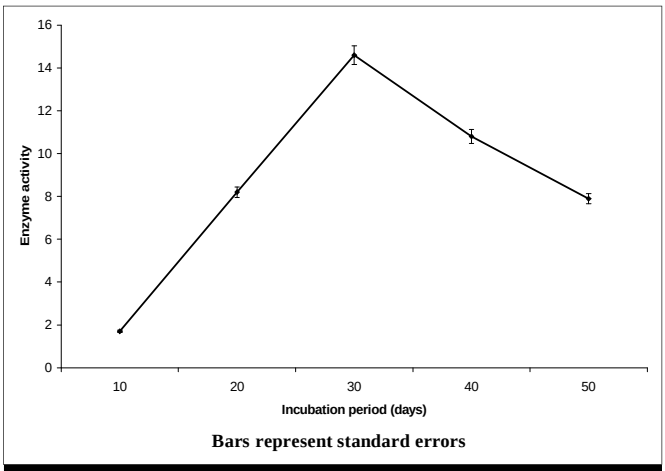

Fig. 3: Lignin peroxidase activity in *D. sissoo* by *L. theobromae* at different incubation periods. Bars represent standard errors

removal of cellulose and hemicellulose creates a channel wide enough to allow access of the enzymes to the sites of the lignin degradation (Reid, 1995) and ultimately wood looses its timber quality and durability.

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