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CHANGES IN CELLWALL CONSTITUENTS OF *LATHYRUS SATIVUS* DUE TO *FUSARIUM*-WILT DISEASE

BY

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The role of the host cell-wall components of *Lathyrus sativus* in *Fusarium*-wilt disease was investigated. The experimental data revealed that galacturonic acid is the main component of the host cell-wall which was degraded by the pathogen more effectively. The slight increase in xylose, arabinose, mannose, glucose and glucorunic acid might be due to break down of other constituents. All these were quantitatively determined by gas chromatography.

INTRODUCTION

During establishing itself on host the pathogen first encounters the host cell-wall as barrier. To overcome this barrier, the pathogen alters the chemical constituents of the host cell-wall by its various types of enzymes. Albershium *et al* (1969) have elaborately reviewed the biochemistry of the cell-wall in relation to infection processes of the pathogen. Bateman and Beer (1965) has reported that *Fusarium solani* and *Fusarium phaseoli* are able to degrade the pectic substances of the host cell-walls. Cooper *et al* (1974, 1975, 1978) have reported *Verticillium albo-atrum* produces a range of inducible polysaccharidases capable of degrading cell-walls of tomato vascular tissue in which conditions should be favourable for enzyme synthesis and activity during colonisation by the pathogen. Their activity could cause many of the symptoms of vascular wilt diseases. Hancock (1968) has also reported similar degradation of host cell wall by *F. solani f.* sp. *cucurbitae* and stated that the degree of polymerization of the rasidual pectic substances in infected tissues is approximately one half that of pectic substances extracted from the healthy tissue.

In the present investigation an attempt has been made to determine qualitatively and quantitatively the cell wall components of *Lathyrus sativus* due to the infection by *Fusarium* ortheceras *f. lathyri*.

MATERIALS AND METHODS

A. *Isolation of Cell walls* :- Cell walls of *Lathyrus sativus* were prepared from frozen tissue obtained from 30 days old plants following the method of

English *et al* (1971) using 100 m*M* potassium phosphate buffer pH-7.0. The starch level in tissue was reduced by placing the plants in dark for 30 hours prior to harvest.

B. *Preparation of culture filtrates of F. orthoceras, F. lathyri for determination of enzyme activities*

The fungus was grown on Richard's medium in conical flask containing different sources as substrates. The inoculated flasks were incubated at 27° ± p. 05° C for 10 days. The mycelium was harvested by centrifugation at 5,000 g and of Buchner funel and partial vacuum. The filtrate was placed in cellophone dialysis tube and dialysed in a large volume of deionised distilled water for 24 hours at 2°C. The dialysed filtrate was used for enzyme analyses.

C. *Cell wall degradation assay* :—Reaction mixture for the determination of cell wall degradation of *Lathyrus* plants by *F orthoceras* var *lathyri* contained the following 10 mg. of cell wall, 0.2 ml. dialysed culture filtrate, 0.2 ml. inhibitor, 10 ml. of a 10 mM solution of $MnCl_2$ and $MgCl_2$ in water, the volume of 50 mM sodium acetate, pH 5.2 to make the final volume 2.0 ml. In control experiment, with the help of buffer, the final volume was prepared. The entire set was incubated at 30°C for 6 hours. At the end of the incubation period, 2.0 ml. distilled water was added to each reaction flask and the cell wall materials was isolated by centrifugation. The supernatant fluid was removed. The cell wall washed twice with 2 ml. distilled water. The composition of the washed cell wall of the enzyme treated and controlled sets were determined by gas chromatography.

Several inhibitors were used to the normal assay mixture and the enzymatic assays were carried out, obviously the inhibitors were different for different enzymes.

RESULTS AND DISCUSSION

The data obtained are presented in the following Tables 1 & 2.

The polysaccharide composition of the cell wall of 100 *mM Lathyrus sativus* before and after washing with water were quantitatively determined by gas chromatography. The data have been given in Table 1. It reveal that galacturonic acid is the main component of the cell wall. The degradation of cell wall polysaccharide of the *Lathyrus sativus* tissues by *F. orthoceras*, F. *lathyri* after 6 hours enzymolysis was also quantitatively determined by gas chromatography. The result has been given in Table 2. The data have clearly indicate that about 70% of galacturonic acid present in the healthy cell wall and was removed by the pathogens' enzyme present in dialysed culture filtrates. Rhamnose and galactose are also partially removed by the pathogen. The slight

Table 1. *Composition of cell wall polysaccharide fraction of* Lathyrus sativus

Preparation analyzed	Percentage of cell wall constituents							
	Rhamnose	Xylose	Arabinose	Mannose	Galactose	Glucose	Galacturonic acid	Glucouronic acid
Walls prepared with 100 mM phosphate buffer	0.8	6.1	2.7	0.8	4.1	2.6	15.7	0.6
Walls prepared with 100 mM phosphate buffer and then washed with water.	0.7	6.0	2.5	0.6	4.0	2.2	11.6	0.4

Cell wall constituents were determined by Gas Chromatography. Cell walls composition are expressed as mg/100 mg of cell wall after correction for starch contamination.

Table 2. *Degradation of 30 days old* Lathyrus sativus *cell walls by* F. orthoceras f. lathyri *culture filtrate enzymes*

Lathyrus cell walls and treatment	Percentage of cell wall constituents							
	Rhamnose	Xylose	Arabinose	Mannose	Galactose	Glucose	Galacturonic acid	Glucorunic acid
Untreated wall	0.7	6.1	2.9	0.9	4.4	3.2	15.8	0.5
Treated wall residue after 6 hours enzyme action	0.5	6.3	3.2	1.7	3.7	5.2	4.9	0.9

10 mg of sample of 100 mM prepared cell walls from 30 days old Lathyrus plants were suspended in reaction mixture as described previously. Composition is expressed as mg/100 mg of cell wall material in each case.

increase in xylose, arabinose, mannose, glucose and glucorunic acid might be due to breakdown of other constituents.

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