

STUDIES ON WILT DISEASES OF PULSES :
II. PARASITISM OF SELECTIVE PATHOGENECITY
OF *FUSARIUM* SPECIES ASSOCIATED WITH
WILT DISEASES OF PULSES

BY

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Histopathological studies of the roots of gram (*Cicer arietinum*), Lentil (*Lens esculentus*) and pigeon pea (*Cajanus cajan*) inoculated with isolates of *Fusarium oxysporum* f. *ciceri*, *F. oxysporum* f. *lentis* and *F. oxysporum* f. *udum* showed that at the initial stages of attack, progress of the pathogen inside both susceptible and non susceptible host tissues was similar. Fungal hyphae colonized first in the epidermal cells and later in the cortical cells. Advancement of hyphae beyond cortical cells did not take place in non susceptible hosts and there was no invasion of xylem vessels. In the host susceptible to the attack of a particular forma species of *F. oxysporum*, hyphae proceeded beyond cortex, through endodermis and pericycle into xylem vessels which were blocked.

Exudates from the roots of gram, lentil and pigeon pea differentially influenced growth of isolates of *F. oxysporum* f. *ciceri*, *F. oxysporum* f. *lentis* and *F. oxysporum* f. *udum*. Root exudates of gram and lentil enhanced the growth of isolates of *F. oxysporum* f. *ciceri* and *F. oxysporum* f. *lentis*, but the effect was more pronounced with exudates from the susceptible hosts. The root exudate of pigeon pea inhibited the growth of isolates of the both *F. oxysporum* f. *ciceri* and *F. oxysporum* f. *lentis*, but enhanced the growth of isolates of *F. oxysporum* f. *udum*.

Paper chromatographic analysis of the exudates of root of gram, lentil and pigeon pea differences in amino acid and organic acid pattern were noted.

INTRODUCTION

Wilt diseases of gram (*Cicer arietinum* L.), Lentil (*Lens esculentus* Moench.), and pigeon pea (*Cajanus cajan* L. Millsp.) caused by *Fusarium oxysporum* f. *ciceri* Padwick emend Snyder and Hans. (= *F. orthoceras* var. *ciceri* Padwick), *F. oxysporum* f. *lentis* Vasudeva and Srinivasan emend Snyder and Hans. (= *F. orthoceras* var. *lentis*) Vasudeva and Srinivasan) and *F. oxysporum* f. *udum* Butl. emend Snyder and Hans. (= *F. udum* Butl.) respectively are of common occurrence in West Bengal. The authors have shown earlier that although these *Fusaria* morphologically belong to the same species, yet they are specific in pathogenicity to their respective hosts, i.e. they can not induce wilting in any host other than its natural ones as evident from cross inoculation studies.

The present paper deals with the study of parasitism of selective pathogenicity in the these three forma species of *F. oxysporum* attacking gram, lentil and pigeon pea. The investigation has been confined to two aspects, namely histopathological studies regarding behaviour of different isolates of three forma species of *Fusarium oxysporum* to their susceptible and non-susceptible hosts and the influence of host root exudates on growth of the isolates of different forma species of *F. oxysporum* and paper chromatographic analyses of the root exudates.

MATERIAL AND METHODS

Histopathological studies : Isolates of *Fusarium oxysporum* f. *ciceri*, *F. oxysporum* f. *lentis* and *F. oxysporum* f. *udum* isolated from the roots of wilted gram, lentil and pigeon pea respectively were used for inoculation on gram, lentil and pigeon pea in all the cases. The inocula were multiplied in sand maize medium and added to the previously sterilized soil in 4" sterilized earthen pots. Seeds of both susceptible and non-susceptible hosts were sown in these pots.

After germination of the seeds, seedlings were taken out at 10 days intervals, roots washed in distilled water, cut into small bits and fixed in chro-acetic acid-alcohol for 24 hours, dehydrated and fixed in paraffin following the method described by Johanson (1950). 10 μ thick microtome sections of the materials were cut and stained by Stoughton's (1930) triple stain for microscopic observation. Sections were also cut from uninoculated roots for comparison.

Study on the effect of host exudates : Nine isolates of different *Fusarium oxysporum* f. *ciceri* isolated from wilted gram plants, five of *F. oxysporum* f. *lentis* from wilted lentil and seven of *F. oxysporum* f. *udum* from wilted pigeon pea as well as cultures of *F. oxysporum* f. *ciceri*, (*F. orthoceras* var. *ciceri*), *F. orthoceras* var. *lentis* and *F. oxysporum* f. *udum* obtained from Indian Agricultural Research Institute New Delhi, were included in this study.

Root exudates were collected from germinating seeds of gram, lentil and pigeon pea on sterilized filter papers as per method described by Andal *et al* (1956). These filter papers were then poured aseptically in flasks containing Richard's agar which was previously melted and held at a temperature of about 45°C and properly shaken for thorough mixing of the contents of the filter papers in the medium. In the control sterilized filter paper was added. The medium was subsequently poured in petridishes and inoculated with different isolates of *Fusaria* and incubated at 25°C.

For paper chromatographic analysis of the amino acids and organic acids the root exudates of gram, lentil and pigeon pea were collected as per method suggested by Andal *et al* (1956).

A descending unidimensional chromatographic technique was employed using n-butanol acetic acid water (4 : 1 : 1 :: V/V/V) as the solvent mixture 0.2% ninhydrin solution was used as the developer in case of amino acids and 1% bromo-phenol blue in case of organic acids. The Rf values of the unknown amino acids and organic acids were calculated and the amino acids or organic acids involved were worked out by comparing with Rf values of known amino acids and organic acids obtained by running chromatograms with known amino acids or organic acids under identical conditions.

EXPERIMENTAL RESULTS

Histopathological studies : The microscopic observations on the distribution of fungal hyphae of different isolates of *Fusarium* in the tissues of the susceptible as well as nonsusceptible host at different time intervals are presented in a summarized form below.

The hyphal invasion may be divided into two phase namely (a) epidermal and cortical invasion, and (b) invasion of endodermis, pericycle and xylem vessels. Progress of hyphae of the different isolates of different forma species of *Fusarium oxysporum* within the cells of susceptible both and non-susceptible hosts was similar in the first phase.

The hyphae of the isolates at first colonized on the outer surface of the epidermal cells and adhered firmly to the roots. Hyphae then gradually invaded the epidermal cells. Mechanism of invasion appeared to be purely mechanical. Appressorial formation was observed. After entry into the epidermal cells at a few points, hyphae gradually spread into other epidermal cells and grew inside the epidermal cells and in many cases the cells were completely filled up with hyphae. Gradually hyphae invaded the cells of the cortex and the cells of the outermost layers of the cortex were gradually filled with hyphae. In case of both susceptible and the non-susceptible hosts the mode of invasion was intracellular and hyphae were intracellular. In non-susceptible hosts, hyphae progressed into the cells innermost layer of cortex, in the former (susceptible host) spread was a little rapid and the quantum of hyphal invasion was more. In non-susceptible hosts, isolates did not progress further and hyphal invasion appeared to be arrested near the endodermal layer.

In respect of susceptible hosts, hyphae gradually invaded endodermal and pericyclic tissues and then attacked the xylem vessels. Both proto-and metaxylem vessels were attacked. They were filled with hyphae. Xylem vessels were also observed to be collapsed due to attack of the pathogen. Phloem tissues were observed to be unaffected. Dissolution of cell walls of the affected host tissues was not noted at any stage and there was no evidence of loss of cohesion of the cells.

Study on the effect of host-root exudates :

Linear growth of the different isolates of different form a species of *F. oxysporum* in different host root exudates was recorded on the 7th day after inoculation. The data are presented in Table I.

Table 1 : Data showing the linear growth in mm. (after 7 days of inoculation at 22°C) of isolates of different form a species of *Fusarium oxysporum* in the root exudates from gram, lentil and pigeon pea.

Isolates	Root exudates of Lentil root exudate			Control
	Gram	Lentil	Pigeon pea	
<i>F. oxysporum</i> f. <i>ciceri</i> .				
FG 2	69.6	64.3	58.0	61.3
FG 4	62.3	55.6	50.6	52.3
FG 8	64.6	60.0	56.3	56.0
FG 8A	58.6	54.6	49.6	50.6
FG 12	51.6	47.6	43.0	42.3
FG 12A	58.0	54.6	50.6	52.0
FG 12B	65.6	61.3	58.6	58.0
FG 13	66.0	62.3	57.0	58.6
FG 13A	69.6	65.0	57.3	60.3
<i>F. orthoceras</i> var. <i>ciceri</i> of I.A.R.I.				
	68.3	66.3	61.3	64.0
<i>F. oxysporum</i> f. <i>lentis</i> .				
FL 1	65.0	69.6	55.3	60.6
FL 1A	60.0	65.3	56.6	58.6
FL 2	62.3	68.0	59.0	60.6
FL 3	45.6	50.3	40.0	40.3
FL 3A	32.3	42.6	29.3	29.0
<i>F. orthoceras</i> var. <i>lentis</i> of I.A.R.I.				
	46.0	51.0	38.0	40.6
<i>F. oxysporum</i> f. <i>udum</i> .				
FA 1	47.3	47.3	53.6	47.0
FA 2	46.3	41.3	50.6	46.0
FA 2A	47.6	48.0	56.0	50.3
FA 3	52.3	51.0	58.3	51.0
FA 3A	55.3	57.6	64.0	58.3
FA 3B	58.0	57.3	63.3	60.0
FA 4	54.6	52.0	61.0	55.6
<i>F. udum</i> of I.A.R.I.				
	50.3	48.0	57.3	50.6

From Table 1 it may be observed that the exudates from roots of gram, lentil and pigeon pea influenced differentially the linear growth of *Fusaria* isolated from these hosts. Exudates from gram and lentil accelerated the growth of the isolates from each of these two hosts, *F. oxysporum* f. *ciceri* and *F. oxysporum* f. *lentis*, but the rate of growth

was much faster when the exudate was from its own host. The pigeon pea root exudate either retarded the growth of *Fusarium* isolates from gram (*F. oxysporum* f. *ciceri*) and lentil (*F. oxysporum* f. *lentis*) or had no effect at all. The growth of pigeon pea isolate (*F. oxysporum* f. *cajani*) was accelerated by pigeon pea root exudate, but was retarded by root exudates from gram or lentil.

To find if the differential reactions of the root exudates on the *Fusarium* isolates from different host plants was due to chemical composition of the root exudates, the exudates were analysed chromatographically for the presence of different amino acids and organic acids. The results are presented in Table 2, which shows that some differences existed in the amino acid and organic acid patterns of the root exudates of gram, lentil and pigeon pea. Maximum number of amino acids could be detected in the exudates of lentil roots (8) and minimum in the pigeon pea roots (5). The amino acid pattern of the root exudates of gram and lentil was almost identical except that lysine, which is exuded by lentil roots was not noted in gram root exudate. Tryptophane was absent in gram and lentil root exudates but was present in exudate of pigeon pea roots, which however lacked glutamic acid, aspartic acid and lysine.

Contrary to amino acids, organic acids were more in number in pigeon pea root exudates than in the exudates of gram and lentil roots. Oxalic acid, citric acid and tartaric acid were present in all three exudates. Malic acid, which was present in pigeon pea root exudate could not be detected from root exudates of gram and lentil.

Table 2. Data showing amino acids and organic acids in the root exudates of gram, lentil and pigeon pea.

Exuded amino acids and organic acids	Root exudates of		
	Gram	Lentil	Pigeon pea
<i>Amino acids :</i>			
Lucine	+	+	+
Phenylalanine	+	+	+
Valine	+	+	+
Tyrosin	+	+	+
Tryptophane	-	-	-
Alanine	+	+	-
Glutamic acid	+	+	-
Aspartic acid	+	+	-
Lysine	-	+	-
<i>Organic acids :</i>			
Oxalic acid	+	+	+
Citric Acid,	+	+	+
Tartaric Acid	+	+	+
Malic acid	-	-	+

+ = Present ; - = Absent.

DISCUSSION

It was evident that the behaviour of different isolates of forma species of *F. oxysporum* attacking, gram, lentil and arhar towards both susceptible and nonsusceptible hosts was similar in the initial stages of attack. Penetration which was noticed in all the cases of study were mechanical as could be evident from appressorial formation and non-dissolution of cell walls. All the isolates could invade upto cortical cells though the invasion was comparatively limited and the pathogens showed their selectivity while passing from cortical cells to endodermis and pericycle to vascular bundles. In the case of susceptible host-pathogen combination, fungal invasion of endodermis, pericycle and ultimately xylem vessels took place. In case of non-susceptible host-pathogen relationship, the fungus was arrested in cortical region and there was no invasion of xylem vessels. These observations are in similar lines with that of Smith and Walker (1930). Delimitation of the fungus in non-susceptible hosts was not manifested by any anatomical changes but it was effectively eliminated.

Various workers suggested the role of host root exudates on the pathogenic behaviour of the soil microorganisms. From the findings of Table 1 and Table 2, a correlation can be drawn on the composition of the amino acids and organic acids exuded by the roots of gram, lentil and pigeon pea and the growth rate of the *Fusaria* isolated from wilt infected roots of gram, lentil and pigeon pea. The exudates from gram and lentil accelerated the growth of the isolates from each of these two hosts, but pigeon pea root exudate retarded them. On the otherhand the growth of the isolates from pigeon pea was accelerated by pigeon pea root exudate, but retarded by gram and lentil root exudates. Buxton (1957 a, b) similarly observed that exudates from root of three cultivars of pea.

When the exudates from the roots of gram, lentil and arhar were chemically analysed for amino acids and organic acids, more or less similar pattern of distribution of these acids was detected in gram and lentil root exudates. In respect to amino acids, leucine, phenylalanine, valine, tyrosine, alanine, glutamic acid and aspartic acid were detected in exudate from both the sources, while lysine was found to be present only in lentil exudates. In respect to the organic acids, however, oxalic, citric and tartaric acids were found to be present in both the cases.

The amino acid and organic acid composition of pigeon pea root exudates were found to differ from that of gram and lentil exudates. Among the amino acids found in the latter group, glutamic acid, aspartic acid and lysine were not detected and in contrast to that group, tryptophane was detected in this case. In respect of the organic acids arhar root exudate has been found to contain malic acid in addition to the acids found in gram and lentil exudates.

Penetration and invasion of cells took place in both susceptible and non-susceptible hosts, inspite of differences in composition and influence of different root exudates on growth of different form a species of *F. oxysporum*. The effect of root exudate if any will be in the prepenetration phase. So it may be concluded that the selective pathogenecity of different forma species of *F. oxysporum* cannot be explained on the basis of root exudates. The reaction at the inner most host cells between the host and the pathogen appears to determine whether there would be any successful attack or not.

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