
Differential effects of root exudates and extracts from tomato cultivars on *Fusarium oxysporum* f. sp. *lycopersici* in relation to their disease reaction

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Root exudates from three tomato cultivars (Patharkuchi, Pusa Bonny and Pusa Ruby), showing variable reactions to vascular wilt disease incited by *Fusarium oxysporum* f. sp. *lycopersici*, differentially affect both spore germination and hyphal growth of the pathogen, their *in vitro* toxicity showing direct relation to host resistance. While the exudate from susceptible cv. Patharkuchi, which develops severe symptoms and shows extensive vascular colonization by the pathogen, stimulates both spore germination and hyphal growth, that from resistant cv. Pusa Ruby showing limited symptoms and restricted colonization considerably inhibits both. Pusa Bonny plants show intermediate reactions in all the above respects. Such differential activity is also shown by the methanolic extracts of the roots of cvs. Patharkuchi and Pusa Ruby, but the differences are less pronounced than those recorded from root exudates. Significant toxicity is also noted in root extracts from susceptible cv. Patharkuchi plants in seed treatments with ferric chloride, zinc chloride and manganese sulphate that induce variable levels of resistance in them. Results indicate that constitutive toxic compounds present in tomato roots contribute towards host resistance to *F. oxysporum* f. sp. *lycopersici* through their inhibitory action on the pathogen, both at the portals of infection following exudation and also subsequently inside the host after penetration.

Key words : Tomato cultivars, Root exudates, Root extracts, *Fusarium oxysporum* f. sp. *lycopersici*, Induced resistance, Induced fungitoxicity.

INTRODUCTION

While most defence mechanisms in plants are pathogen elicited host responses and are induced only after contact with the pathogen, sometimes physiological interaction between the host and pathogen start even before the contact. This is particularly relevant in the soil environment. In the effective survival and

biological success of soil-borne fungal pathogens, host root exudates often play a decisive role (Rovira, 1965; Mitchell, 1976). By influencing the behavioral pattern of fungal propagules, root exudates may regulate the inoculum potential of the pathogen in the given soil environment and also its infection potential at the root-soil interface. In interactions involving various *formae specialis* of *Fusarium oxysporum*, the wilt pathogen, and their specific hosts, reports on the differential effects of root exudates from susceptible and resistant cultivars on the pathogen are available for pea (Buxton, 1957), banana (Buxton, 1962), pigeonpea (Kotasthane and Gupta, 1985) and chickpea (Satyaprasad and Ramarao, 1983), that from resistant cultivars exerting strong inhibitory effect on the pathogen. This is often followed by constitutive inhibitory effect in the resistant host tissue on the pathogen. However, the evidence is not unequivocally so. The present paper reports the results of investigations on this aspect of interactions involving tomato cultivars and *F. oxysporum* f. sp. *lycopersici*.

MATERIALS AND METHODS

Tomato cultivars investigated were Patharkuchi, widely grown locally, Pusa Bonny and Pusa Ruby, known to have different levels of disease reaction to *F. oxysporum* f. sp. *lycopersici*. Seedlings raised on garden soil in wooden boxes were transplanted to 15 cm earthen pots at the age of 2 weeks. These were inoculated a week later by mixing with the top soil in pots 100 g mixture of inoculum grown on sandmaize meal medium and sterilized soil (1:1). There were three pots per treatment, each with five plants.

Leaf symptoms involving yellowing and/or wilting were assessed at intervals for each leaf of a plant on a 0-4 scale (0=no yellowing or wilting; 1=incipient yellowing and/or wilting in one leaf; and 4=all leaves completely wilted or dead or shed). Mean disease index was computed on leaf basis. Both disease incidence and mortality percentage were also recorded, but only the final results have been shown.

For collection of root exudate, 50 surface sterilized and germinated seeds were placed on sterile nylon net stretched on top of a 100 ml glass beaker with 80 ml of sterile distilled water, and the whole set was placed in a 250 ml beaker with sterile water that touched the rim of net and kept it wet. The large beaker was closed on top with a petri dish and covered on sides with black polythene to cut off light. These beakers, three per treatment, were kept at 25°C under 12 hrs illumination. Radicles soon grew down and developed branched root systems in beaker water which was collected after 7 days. Pooled exudates were concentrated in vacuum at 40°C and finally made into the desired final

volume with sterile distilled water keeping a relation to root dry weight (50 ml g^{-1}) and then sterilized by passing through millipore filter before use in bioassay.

Root extracts were obtained from 3-week-old plants by using organic solvents. Fresh root tissue weighing 4 g was extracted thrice with 15 ml organic solvent each time. These extracts were pooled together and evaporated to dryness at 40°C . The residue was dissolved in sterile distilled water to make a final volume of 0.5 ml g^{-1} fresh weight of root tissue.

Both root exudates and extracts were bioassayed against spore germination of the pathogen by slide germination technique (Chakraborty and Sinha, 1984). Other methods are given later.

RESULTS

Disease reaction of tomato cultivars

When artificially inoculated at the age of 3 weeks, three different tomato cultivars exhibited differential disease reaction (Table 1). Symptoms could be noticed

Table 1: Disease responses of three tomato cultivars to artificial inoculation with *F. oxysporum* f. sp. *lycopersici* recorded over 5 weeks*.

Cultivar	Mean disease index/leaf (0-4)				Disease incidence (%)	Mortality (%)
	2 wk	3 wk	4 wk	5 wk	5 wk	5 wk
Patharkuchi	0.5	1.5	2.6	3.6	91.6	50.6
Pusa Bonny	0.3	1.0	1.7	2.3	66.6	16.6
Pusa Ruby	0	0	1.1	1.6	41.6	Nil

* Results represent the averages of 15 plants

within 2 weeks in cvs. Patharkuchi and Pusa Bonny, but in cv. Pusa Ruby the onset was much delayed. In Patharkuchi plants, symptoms developed rapidly over the next 3 weeks to reach a severe state of damage, as reflected in the disease index of 3.6/4.0. At this stage, disease incidence was as high as 92% and mortality 50%. On the other hand Pusa Ruby plants had a disease index of 1.6, 42% disease incidence and no mortality. Here, not only the onset of symptoms was delayed, but the progress was much slower. Based on above observations, Patharkuchi plants appear to be highly susceptible and Pusa Ruby resistant to *Fusarium* wilt. Pusa Bonny plants with intermediate responses in all respects appear to be moderately susceptible.

Vascular colonization by the pathogen

The above results were confirmed in a subsequent experiment, when tomato plants of three varieties were grown in both sterilized and non-sterilized soil and inoculated as usual. Though initially symptom development was slightly faster in non-sterilized than in sterilized soil, not much difference in disease index existed between these two sets 5 weeks after inoculation. In sterilized soil, mean disease index per leaf after 5 weeks was Patharkuchi-3.4/4.0, Pusa Bonny-2.0 and Pusa Ruby-1.6. Transverse hand sections prepared at this stage from 12 plants of each cultivar at the region of root (middle of tap root), collar region, and lower and upper stem were suitably stained with picro-aniline blue and observed under microscope for a study of vessel colonization.

Differences in the extent of fungal colonization in three tomato cultivar becomes evident from Table 2. In all three, the pathogen spread up to 8 cm above the

Table 2 : Fungal colonization of vessels in three tomato cultivars, recorded 5 weeks after inoculation with *F. oxysporum* f. sp. *lycopersici**

Cultivar	Percentage of vessels with hyphae at different locations			
	Root portion	Collar region (C.R.)	Basal stem (C.R.+4 cm)	Upper stem (C.R.+8 cm)
Patharkuchi	20.1	34.9	40.0	17.6
Pusa Bonny	18.5	29.5	18.5	5.6
Pusa Ruby	13.5	28.5	15.0	2.2

* Results represent the averages of 12 plants

collar region, but with a difference. In the root and collar region, though Patharkuchi plants had hyphae in most vessels, Pusa Ruby plants in the least and Pusa Bonny plants in between, the differences between them do not appear to be of much significance. However, at 4 cm above the collar region, the differences between Patharkuchi and other varieties appear to be significant and more so further 4 cm above. Though Pusa Ruby plants recorded lower values than Pusa Bonny plants at all points, the differences between them are only marginal in nature.

Effect of tomato root exudates on the pathogen

Root exudates collected from three tomato cultivars, concentrated and sterilized as described before, were bioassayed against germination of conidia and mycelial growth of the pathogen following slide germination technique. On each of the two grooves of a clean glass slide, one drop of exudate mixed with spore

suspension ($c. 5 \times 10^5$ conidia/ml) in 19 : 1 ratio was placed. There were two slides for each treatment. After 16 hrs incubation in moist chamber at room temperature in complete darkness, one drop of cotton blue in lactophenol was added to each groove to fix and stain the germinated spores, and these were observed under microscope to determine the extent of spore germination.

The same exudate was added in 0.5 ml portion to 4.5 ml of half-strength Richard's medium in culture tubes and inoculated with one drop of spore suspension. After incubation for 14 days at 28°C, mycelial growth was separately collected from each tube on pre-weighed dry filter paper and then dried to a constant weight. The results of both spore germination and mycelial growth are presented together in Table 3.

Table 3 : Effects of root exudates from three tomato cultivars on conidial germination and mycelial growth of the wilt pathogen, *F. oxysporum* f. sp. *lycopersici*.

Cultivar	Spore germination (%)*	Mean germ tube length (μ m)**	Mean mycelial dry wt. (mg)***
Water (Control)	62.9	172 $\pm$ 9.0	13.3
Patharkuchi	76.4	204 $\pm$ 4.1	24.7
Pusa Bonny	55.6	167 $\pm$ 3.8	21.6
Pusa Ruby	37.7	112 $\pm$ 1.4	10.6

* Mean of 400 spores

** Mean of 80 germlings

*** Mean of 3 replicates

It becomes evident from the results that conidia of *F. oxysporum* germinate well and show good germ tube growth even in the medium of distilled water. While the root exudate from cv. Patharkuchi appreciably stimulates both spore germination and germ tube growth, that from cv. Pusa Ruby causes considerable inhibition in both respects. The exudate from cv. Pusa Bonny has only marginal inhibitory effects. In case of mycelial growth, it was seen that the exudates from cvs. Patharkuchi and Pusa Bonny plants cause considerable stimulation but that from cv. Pusa Ruby plants has only a moderate inhibitory effect.

Effects of root extracts on the pathogen

Results obtained with tomato root exudates indicate the presence of some fungitoxic inhibitory compounds in that from resistant cv. Pusa Ruby and imply that such compounds must be constitutively present in its roots. To resolve this, root extracts were obtained from 3-week-old cvs. Patharkuchi and Pusa Ruby plants using organic solvents such as acetone and methanol, evaporated

to dryness, and finally brought into aqueous medium as described before. These extracts were assayed for fungitoxicity following the same procedure as used for root exudates. Results are shown in Table 4.

Table 4 : Effects of acetone and methanol extracts of root tissue of two tomato cultivars on conidial germination of *F. oxysporum* f. sp. *lycopersici*

Source of extract	Spore germination* (%)	Mean germ tube** length (μ m)
Patharkuchi (Acetone)	64.2	60.9 $\pm$ 2.2
Pusa Ruby (Acetone)	53.2	49.1 $\pm$ 2.1
Patharkuchi (Methanol)	68.2	68.7 $\pm$ 3.0
Pusa Ruby (Methanol)	48.3	50.8 $\pm$ 2.4

* Mean of 400 spores

** Mean of 80 germlings

In both acetone and methanol extracts of cv. Pusa Ruby roots, spore germination and germ tube growth were appreciably less than those in comparable extracts from cv. Patharkuchi roots. However, the difference was more significant with methanol extracts. It appears from these observations that the root tissue of cv. Pusa Ruby has some inhibitory constituents which are not present in cv. Patharkuchi roots.

Relation between induced resistance in plants and fungitoxicity in root extracts

In separate experiments, a group of chemicals had been found to be effective in wet seed treatment in providing susceptible tomato plants (cv. Patharkuchi) with different levels of protection from *Fusarium* wilt disease. In the context of these observations and those just reported, it became of some interest to investigate if such treatments besides strengthening dynamic defence potential of the host would also cause to develop in them some inhibitory factors. Three chemicals selected for this study were ferric chloride, zinc chloride and manganese sulphate, known for their differential effects against the above disease. While ferric chloride had strong effect, zinc chloride was somewhat less effective, and manganese sulphate showed the least and only moderate effect. Seeds were given pre-sowing treatment by soaking for 24 hrs in 10^{-4} M solutions of these chemicals or in water for the control treatment. Root extracts were made with methanol from 3-week-old plants, and these were bioassayed as before. Table 5 shows that seed treatment with all three chemicals caused to develop in wilt susceptible tomato plants (cv. Patharkuchi) significant levels of toxicity, and their effects in this respect bear some relation to the protection they are known to provide to such plants. In ferric chloride treatment with strong protective

Table 5 : Effects of root extracts (in methanol) from 3-week-old tomato plants, having received seed treatment with three chemicals, on spore germination of *F. oxysporum* f. sp. *lycopersici*

Treatment	Spore germination* (%)	Mean germ tube length** (μ m)
Water (Control)	71.5	135 $\pm$ 5.3
Manganese sulphate (10^{-4} M)	49.5	129 $\pm$ 0.6
Zinc chloride (10^{-4} M)	35.5	78 $\pm$ 3.6
Ferric chloride (10^{-4} M)	32.8	56 $\pm$ 2.6

* Mean of 400 spores

** Mean of 80 germlings

effect, the extract also shows strong fungitoxic action against the pathogen both in respect of spore germination and germ tube growth. The extract from manganese sulphate treatment, which provides only moderate protection, affects spore germination moderately and germ tube growth only marginally. Zinc chloride with fairly strong protective action also induced fairly toxicity in the root extract, but it was not as strong as that from ferric chloride treated plants.

DISCUSSION

Fungitoxicity displayed by the root exudates from three tomato cultivars towards spore germination and hyphal growth of *F. oxysporum* f. sp. *lycopersici*, the wilt pathogen, shows a fair degree of correlation to their disease reaction, such toxicity being directly related to host resistance. While the root exudate from susceptible cv. Patharkuchi, that shows fairly extensive vascular colonization by the fungus, supports both good germination of its spore as well as hyphal growth *in vitro*, that from resistant cv. Pusa Ruby, which does not apparently show any extensive colonization, causes significant levels of inhibition in all respects. Moderately susceptible cv. Pusa Bonny exhibits intermediate reactions in all respects. Such observations indicate the presence of constitutive toxic compounds in cv. Pusa Ruby plants, and the inhibitory effects of its methanolic root extracts only confirms this. Accumulated evidences suggest that through differential action of their root exudates, *Fusarium* wilt susceptible and resistant tomato cultivars may create in the soil, at the portals of infection, conditions that respectively favour or restrict the pathogen and regulate its inoculum as well as infection potential thus promoting or limiting the chances of a successful infection. Even after penetration, the pathogen is further checked in its activity by the toxic compound(s) present in resistant root tissue, hence its failure to spread and limited pathogenic potential therein.

In conclusion, this is not to suggest that resistance of tomato cultivars to *F. oxysporum* does not depend on the dynamic defence mechanism as in most other host parasite interactions. Nevertheless, the present series of observations strongly indicate that constitutive toxic compounds in a resistant tomato cultivar have a role in it and contribute to it through action both outside the plant roots, after exudation, and later inside it, after penetration, and thus supplement the dynamic defence mechanism, if any. The presence of induced toxicity in the root exudates from susceptible chickpea plants in different treatments and its correlation with levels of resistance induced in them provides good experimental support to this view.

REFERENCES

- Buxton, E. W. (1957). Differential rhizosphere effects of three pea cultivars on physiologic races of *F. oxysporum* f. sp. *pisi*. *Trans. Brit. Mycol. Soc.* 40 : 305-317.
- Buxton, E. W. (1962). Root exudates from banana and their relationship to strains of the *Fusarium* causing Panama wilt. *Ann. Appl. Biol.* 50 : 269-282.
- Kotasthane, S. R., Gupta, O., Tiwari, D. P. and Khare, M. N. (1985). Studies on root exudates of susceptible and resistant cultivars of pigeonpea. *Jawaharlal Nehru Krishi Viswavidyalaya Res. Jour.* 17 : 141-144.
- Mitchell J. E. (1976). The effect of roots on the activity of soil-borne plant pathogens. In 'Physiological Plant Pathology' (ed.) R. Heitefuss and P. J. Williams, Springer Verlag, P. 104-128.
- Rovira, A. D. (1965). Interactions between plant roots and soil microorganisms. *Ann. Rev. Microbiol* 19 : 241-266.
- Satyaprasad, K. and Ramarao, P. (1983). Effect of chickpea root exudates on *F. oxysporum* f. sp. *ciceri*. *Indian Phytopath.* 36 : 79-81.
- Chakraborty, D. and Sinha, A. K. (1984). Similarity between the chemically and biologically induced resistance in wheat seedlings to *Drechslera sorokiniana*. *Z. Pflkrankh. Pflschutz.* 91 : 59-64.

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