Eur J Plant Pathol (2011) 131:355–369
DOI 10.1007/s10658-011-9813-3

Pathogenicity of indole-3-acetic acid producing fungus *Fusarium delphinoides* strain GPK towards chickpea and pigeon pea

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Accepted: 16 May 2011 / Published online: 7 June 2011
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Abstract An indole-3-acetic acid (IAA) producing fungal strain was isolated from chickpea grown rhizospheric soil samples. Based on morphological and Internal Transcribed Spacer (ITS) region sequence analysis the new isolate was identified as *Fusarium delphinoides*. The *Fusarium delphinoides* strain produces and secretes IAA *in-vitro* as identified by HPLC and Mass spectrometry. The IAA production is dependent on tryptophan (Trp) as a nitrogen source in the medium. The IAA production is influenced by growth conditions such as pH of the medium, concentration of Trp and the nature of the carbon source. Additional nitrogen sources repress Trp dependent IAA production. Glucose and Trp served as the best carbon and nitrogen sources respectively. Pathogenicity of *Fusarium delphinoides* towards the plants was tested by electrolyte, nutrient leakage analysis and also by scoring the disease symptoms. Two cultivars of chickpea (ICCV-10 and L-550) and two cultivars of pigeon pea (Maruti and PT-221) were assessed for the pathogenicity by inoculating with spores of *Fusarium delphinoides*. The inoculation induced symptoms of *Fusarium* wilt as in the case of *Fusarium oxysporum f. sp. ciceris* (FOC), a known pathogen causing *Fusarium* wilt in chickpea. Electrolyte and nutrient leakage from the infected plants were used to assess the resistance, tolerance (moderately resistance) and susceptibility of the plants to the infection. Based on the results, both the pigeon pea cultivars (Maruti and PT-221) were rated as resistant, and ICCV-10 was rated as a tolerant cultivar of chickpea. However, chickpea cultivar L −550 was found to be a susceptible host for infection by *Fusarium delphinoides*. These results suggest that *Fusarium delphinoides,* which belongs to the *Fusarium dimerum* species group, is an IAA producing plant pathogen and causes wilt in chickpea. Further, along with pathogenicity tests, electrolyte and nutrient leakage analysis can be used to assess the pathogenicity of pathogenic fungi.

Keywords Chickpea · Electrolyte leakage · *Fusarium delphinoides* · IAA · Pathogenicity

Abbreviations

IAA	Indole-3-acetic acid
Trp	Tryptophan
FOC	*Fusarium oxysporum f. sp. ciceris*
FDG	*Fusarium delphinoides* strain GPK
HPLC	High Performance Liquid Chromatography
PCR	Polymerase Chain Reaction

Introduction

IAA is the most abundant member of the auxin family of phytohormones (Bartel 1997). It plays a role in root

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initiation and elongation as well as number of other processes concerned with the differentiation and proliferation of plant tissues (Arshad and Frankenberger 1998). Many bacteria and fungi are capable of synthesizing auxins using several pathways (Chung et al. 2003), which increase the potential for forming associations with plants. Tryptophan (Trp) serves as a physiological precursor for the biosynthesis of auxin in higher plants and in microbes (Frankenberger and Arshad 1995). Root exudates are natural sources of Trp for the rhizosphere microflora, which may enhance auxin biosynthesis in the rhizosphere (Martens and Frankenberger 1994). The most efficient auxin producers are found among fungal inhabitants of the plant rhizosphere and phyllosphere (Tsavkelova et al. 2005). The amount of IAA produced depends on the composition of the medium and the cultural conditions like temperature, pH, and substrate concentration (Tsavkelova et al. 2005). The physiological role of auxins in fungi is not fully understood. One of the roles suggested for fungal produced IAA is to mediate the fungal-plant interaction. The high concentration of IAA can inhibit the hypersensitive response (Jouanneau et al. 1991). Furthermore, the disease symptoms caused by some fungal pathogens are similar to the symptoms caused by high concentrations of IAA on plants, such as epinasty, tumour formation and plant organ deformation (Tudzynski and Sharon 2002). However, the direct evidence for the involvement of IAA in plant disease is available only for plant pathogenic bacteria (Patten and Glick 1996).

The *Fusarium* species consists of plant endophytes that colonise roots without causing disease symptoms (Fravel et al. 2003) as well as plant pathogens causing two major plant diseases: *Fusarium* wilt and *Fusarium* root rot. The wilt-causing strains are specific with regard to their host plants. More than 150 host specific *formae speciales* have been described in the *Fusarium oxysporum* complex associated to one species or a narrow range of plant species, each of them consisting of one or more vegetative compatibility groups and often distinct pathogenic races (Baayen et al. 2000). A well known example is *Fusarium oxysporum f. sp. ciceris* (FOC) causing *Fusarium* wilt in chickpea (*Cicer arietinum*) (Nene et al. 1989). Chickpea is one of the most important legumes grown worldwide, especially in dry areas of the Indian subcontinent with annual production exceeding 7 million metric tons (FAO 2005). Chickpea production has however remained virtually stagnant over recent decades because of its susceptibility to diseases such as wilt caused by *F. oxysporum f sp. ciceris* and root rot. *Fusarium* wilt is widespread in India, Iran, Pakistan, Nepal, Burma, Spain, Mexico, etc. (Nene et al. 1989). Wilted plants show marked discolouration of the vascular tissue (Haware and Nene 1982) and involves a directed growth of the fungus towards the stele and a preferential colonization of the xylem vessels where sporulation occurs (Di Pietro et al. 2003).

The fungus *Fusarium delphinoides* belongs to the *Fusarium dimerum* species group (FDSG) and is an inhabitant of soil particles or soil-associated debris (Vismer et al. 2002). Recently *F. delphinoides* was isolated from the stem lesions of *Hoodia gordonii* (Schroers et al. 2009), a succulent plant from the Apocynaceae family; prior to this strains of *F. delphinoides* were considered as saprophytes of decaying plant substrata or soil fungi and their role as pathogens was not known. The host range and the biological potential of this fungus have to be determined (Schroers et al. 2009). Since soil-borne pathogens are difficult to control, the study of their biology and of their interaction with host plants are important steps for the development of crop protection strategies. Thus the main objectives of the study were to screen the pathogenicity (by pathogenicity tests, electrolyte and nutrient leakage analysis) of IAA-producing *F. delphinoides* strain GPK and to establish a system for studying the disease and resistance in these host plants. As *Fusarium delphinoides* was isolated from chickpea-grown agricultural soil samples, *F. oxysporum f. sp. ciceris* (known to cause *Fusarium* wilt in chickpea) was used as a positive control pathogen.

Materials and methods

Chemicals

IAA (Sigma number: I2886-5G and chemical grade: plant cell culture tested, crystalline) and Trp (Sigma number: T0254-25G - and chemical grade: reagent grade, ≥98% (TLC)) were procured from Sigma Chemicals Co. (St. Louis, USA); all other chemicals were of analytical grade and purchased from Himedia Laboratories Pvt. Ltd (Mumbai, India).

Microorganisms

Fusarium delphinoides strain GPK (FDG) was isolated from chickpea grown agricultural soil samples from Gulbarga region, Karnataka, India. *Fusarium oxysporum f. sp. ciceris* (FOC), used as a positive control for pathogenicity tests, was a generous gift from Agriculture Research Station (ARS) Gulbarga, Karnataka, India. FOC was previously isolated from infected chickpea plants grown in a naturally infected field at ARS Gulbarga. Stock cultures were grown on potato dextrose agar (PDA) medium for 15 days at room temperature (28°C±2) and subsequently maintained at 4°C.

DNA isolation, PCR and DNA sequencing

Mycelium of FDG for DNA extraction was grown in 25-ml tubes filled with 2 ml of liquid complete medium (Pontecorvo 1953). Mycelium was dried on a sterile Whatman filter paper after which DNA was extracted using FastDNA®Kit. ITS4 (5′ TCC TCC GCT TAT TGA TAT GC3′) and ITS5 (5′ GGA AGT AAA AGT CGT AAC AAG G 3′) primers were used for PCR amplications. The following PCR reaction conditions were maintained: initial denaturation at 95°C for 5 min; denaturation at 95°C for 1 min; annealing at 56°C for 1 min; extension at 72°C for 1 min; and a final extension at 72°C for 10 min. Each 25 µl PCR reaction mixture contained 16.8 µl H_2O, 2.5 µl PCR buffer (10X), 1 µl dNTP's (200 µM), 0.2 µl Taq polymerase (1 U μl^{-1}), 1 µl ITS4Primer (10 pM μl^{-1}), 1 µl ITS5 Primer (10 pM μl^{-1}) and 2.5 µl Template (10 ng μl^{-1}). The PCR products were checked on 1.2% agarose gel (Tarson horizontal gel electrophoresis unit) and photographed using the BIORAD gel documentation system. The PCR products were cleaned with a Axygen PCR Cleanup kit and DNA was quantified with a NanoDrop spectrophotometer. Subsequently, PCR sequencing with ITS5 primer was performed with a Big Dye Terminator Cycle sequencing kit (Applied Biosystems) and analyzed on an automated ABI Avant prism 3100 (Applied Biosystems) DNA sequence. Sequences obtained were manually edited for inconsistency and a NCBI BLAST search was conducted to get the identity.

Growth medium

FDG was grown in the mineral salt medium consisting of K_2HPO_4 1 g, $MgSO_4$ 0.4 g, NaCl 0.2 g, $CuSO_4$ 0.003 g per l^{-1}, pH 6 along with Trp (1 mg ml^{-1}) and glucose (1.5 mg ml^{-1}) as the sources of nitrogen and carbon respectively. The medium was inoculated with 1 ml of spore suspension (1×10^6 spores ml^{-1}) prepared from a 15 day old culture of FDG and incubated at room temperature. The determination of spore concentration was performed by using haemocytometer. For pathogenicity tests, including the electrolyte leakage analysis, inocula of FDG and FOC were generated in mineral salt medium by inoculating respective cultures and incubating at 28°C±2°C in natural light. The culture medium containing conidiospores and growing hyphae was separated by filtration using 8 layers of cheese cloth followed by filtration through glass wool. Then spore suspension was added to 100 ml of sterilized distilled water so as to achieve the desired spore concentration.

Estimation of IAA

For the IAA analytical assay, the mycelium from a 15-day-old grown culture was separated by filtration using Whatman No. 2 filter paper and the supernatant was used for the IAA estimation according to the method of Glickmann and Dessaux (1995). Briefly, 2 ml of Salkowski reagent (4.5 g $FeCl_3$ per litre in 10.8 M H_2SO_4) was added to 1 ml of supernatant and the mixture was left in the dark for 30 min at room temperature. Development of pink colour indicates IAA production. Absorbance was read at 530 nm using a UV-visible spectrophotometer (Analytic Jena Specord 50). The concentration of IAA in the spent medium was calculated by using authentic IAA as a standard.

HPLC and mass spectral analysis

To confirm the presence of IAA in the culture supernatant the components were separated by HPLC and analyzed by liquid chromatography followed by mass spectroscopy. The fungal culture was cultivated in a mineral salt medium supplemented with 2 mg ml^{-1} of Trp and then centrifuged. Supernatants were concentrated to 10 ml under vacuum and adjusted to pH 2.8 with 1 N HCl. HPLC analysis was performed by injection of 10 µl aliquots into a Sunfire C18 (300×5 µ) column connected to an HPLC pump (Waters). Absorbance was monitored at 254 nm. The mobile phase consists of water:

acetonitrile: acetic acid (40: 60: 1), filtered through 0.2 mm membrane filters (Pall Life Sciences, India). The flow rate was 1 ml min^{-1}, and the operating pressure was 4,000 psi. Peak retention time was compared to that of authentic IAA added to the same mineral salt medium.

The LC system was synchronized with Mass Q traps 4000 MS-MS (Applied Biosystems MDS-SCIX). Total Ion Chromatogram (TIC) was performed for 30 min. EMS spectra were generated from the TIC. The Mass conditions for the data acquisition were as follows. The spectrometer was operated at vacuum, 3.5 e^5 Torr pressure; curtain gas, 20 arbitrary units (a.u.); source gas 1, 50 a.u.; source gas 2, 50 a.u.; collision associated disruption gas medium; interface hater, on; source temperature, 400°C. The mass spectrometer was tuned and calibrated according to the manufacturer's recommendations.

Optimization of cultural conditions for IAA production

The effect of the incubation period was studied by inoculating 1 ml of spore suspension (1×10^6 spores ml^{-1}) prepared from 15-day-old fungal culture into 100 ml of the mineral salt medium supplemented with Trp (1 mg ml^{-1}) and incubated for 15 days at 28±2°C. Samples were withdrawn every 24 h and the IAA concentration was determined as described earlier. The effect of pH on IAA production was studied by growing the fungus in mineral salt medium of varying pH (4, 5, 6, 7, 8 and 9) and keeping all other conditions similar. As Trp is believed to be the substrate for IAA biosynthesis, the effect of substrate concentration on IAA production was studied. Varying concentrations of Trp (0.5 mg ml^{-1} to 2.5 mg ml^{-1}) were added to the growth medium as a sole source of nitrogen and its effect on IAA production was studied. The medium without Trp served as a control. The effect of additional nitrogen sources on IAA production was studied by supplementing KNO_3, $(NH_4)_2SO_4$, NH_4NO_3, $NaNO_3$, $NaNO_2$, peptone and yeast extract at different concentration ranging from 0.5 mg ml^{-1} to 2.0 mg ml^{-1} along with Trp (2 mg ml^{-1}) and glucose (1.5 mg ml^{-1}). The medium with only Trp as a sole source of nitrogen served as control. To study the effect of carbon sources on IAA production, glucose in the mineral salt medium was replaced by different monosaccharides like mannose, fructose, and arabinose, and the disaccharides such as sucrose, and lactose. The medium with glucose served as control. In all the experiments the amount of IAA was estimated after 13 days, which was the optimum incubation period for the IAA production.

Fungal biomass determination

In all the optimization experiments fungal biomass was determined in terms of mycelial wet weight. 100 ml of fungal culture grown in the respective medium were filtered through pre-weighed Whatman No. 2 filter paper and the filter paper was reweighed. The biomass was expressed as mycelial wet weight (g/100 ml).

Plants

Two cultivars of chickpea (*Cicer arietinum*), accessions ICCV-10 and L-550, moderately resistant and susceptible to *Fusarium* wilt caused by FOC respectively, and two cultivars of pigeon pea (*Cajanus cajan*), Maruti and PT-221 resistant and susceptible to *Fusarium* infection respectively, were obtained from ARS, Gulbarga, India.

Pathogenicity tests

Chickpea and pigeon pea cultivars were used to screen pathogenicity of FDG. Pathogenicity tests were carried out in several experiments by using the seed treatment method, water-culture and pot-culture inoculation methods. For all the experiments, inocula of FDG and FOC were generated in a mineral salt medium containing Trp and glucose. The culture medium containing conidiospores and growing hyphae was separated by filtration using 8 layers of cheese cloth followed by filtration through glass wool. Then spore suspension was added to 100 ml of sterilized distilled water so as to achieve the spore concentration of 3×10^6 spores ml^{-1}. For the seed treatment method all the four cultivar seeds were surface sterilized using 1% sodium hypochlorite for 5 min, rinsed with distilled water and soaked in 20 ml of respective inocula (3×10^6 spores ml^{-1}) for 1 h. Then the seeds were germinated and planted (10 seeds/pot) in 15 cm diameter clay pots containing autoclaved (twice at 121 lb for 20 min) potting

mixture (clay loam and sand; 1:1, vol/vol). Plants were grown in growth chambers adjusted to a 12 h photoperiod of fluorescent light at 12,000–15,000 lux. Temperature and relative humidity respectively were maintained at 28±2°C and 70–90% during the light period and 24±2°C and 80–100% during the dark period. Plants were watered daily and observed for disease symptoms.

For water culture experiments, a modified method of Pande et al. (2007) was used. Briefly, plants grown for 7 days in sterile potting mixture were removed, washed and transferred (without intentional wounding) to 6 cm diameter cylindrical glass bottles (four per bottle) each containing 200 ml of either FOC (positive control) or FDG (test fungus) inocula at a concentration of 3×10^6 spores ml^{-1}. Sterile mineral salt medium containing Trp and glucose was used as an uninoculated control. The plants were held in position by strips of adhesive paper and the bottles were incubated on a rotary shaker at 30°C for 24 h and then planted (10 seedlings/pot) in 15 cm diameter clay pots containing autoclaved (twice at 121 lb for 20 min) potting mixture. The afore-said growth conditions were maintained.

For the pot culture method, 20 ml of the respective inocula prepared in mineral salt medium were poured over the pots containing sterilized potting mixture. Uninoculated mineral salt medium was used as a control. Surface sterilized seeds of all the four cultivars were planted (10 seeds/pot) in 15 cm diameter clay pots. All pots were randomized in growth chambers. Plants were observed daily for symptoms development. Severity of disease infection caused by the pathogens was assessed by scoring the disease index on a scale from 0 to 4 based on the percentage of foliage with yellowing or wilting in acropetal progression (0 = no symptoms, 1 = 25% wilt, 2 = 50% wilt, 3 = 75% wilt and 4 = 100% wilt/all dead plants) at 5, 10, 15 and 20 days after inoculation by the water culture and seed treatment methods and at 20 days after inoculation by the pot culture method. All the experiments had two replicates in a randomized block design. This study was conducted in the lab using young plants and not under field conditions.

Electrolyte leakage study

In laboratory experiments, roots and shoots were detached from 7-day-old healthy plants of all the cultivars, rinsed thoroughly in running tap water followed by four rinses in distilled water. The same number of root pieces (8, each 25 mm long) and shoot pieces (12, each 50 mm long) were weighed and placed in sterile 250 ml Erlenmeyer flasks containing respective inocula. Each flask contained 1 g of plant sample (root/shoot) suspended in 50 ml of sterilized distilled water containing FDG spores at a concentration of 1×10^6 spores ml^{-1}. In the negative control treatment, plant samples were suspended in 50 ml of sterilized distilled water containing equal amount of mineral salt medium that was used to prepare FDG inoculums. In the positive control treatment, plant samples were suspended in 50 ml of FOC spores at a concentration of 1×10^6 spore ml^{-1}. FDG and FOC control treatments each containing 50 ml of sterilized distilled water having either FDG or FOC spores at a concentration of 1×10^6 spores ml^{-1} were also maintained to check the leakage of electrolytes from the fungal cultures. The flasks were incubated in an incubator shaker at 30°C for 5 days. Electrolyte leakage (conductivity) from both inoculated and control plant samples were measured at every 24 h interval using a conductivity bridge (Model 371, Systronics, India) and a conductivity cell (K=0.01) and expressed as microsiemen (μS) per gram fresh weight of plant sample.

Nutrient leakage analysis

Analysis of different nutrients leached from the plant samples were conducted as follows: total carbohydrates by the Anthrone method; total phosphorous by the method of Fiske and Subbarow (1925);protein concentration by the method of Lowry et al. (1951) using bovine serum albumin as standard; and total phenols by the Folin-Ciocalteau Reagent (FCR) method (Bray and Thorpe 1954).

Statistical analysis

All optimization experiments were carried out in duplicate to check the reproducibility of results. The results presented here are the average values of duplicate determinations ± SD. The values of the electrolyte and nutrient leakage tests are mean ± S. E. M (standard error mean) of triplicate determinations and analyzed by ANOVA and post hoc Tukey's *t*-test for multiple comparison. Differences between groups were considered significant at $p<0.05$ levels.

Results

Microorganisms

The IAA-producing fungus was isolated from soil samples collected from chickpea-grown agricultural fields. Based on direct sequencing of ITS regions the fungus was identified as *Fusarium delphinoides* and designated as *Fusarium delphinoides* strain GPK (FDG). The sequence was deposited in NCBI gene bank (accession number GU828007.1). The strain FDG showed 100% homology with *Fusarium delphinoides* EU926244.1.

HPLC and mass spectra analysis

IAA was identified in the medium by HPLC and mass spectral analysis when the fungus was grown *in-vitro* in Trp-containing medium. The results from the HPLC analysis showed the peak at retention time of 3.6 min corresponding well with the peak obtained for standard IAA having same retention time (Fig. 1a and b). Mass spectral analysis confirmed the presence of IAA in the extract prepared from the culture supernatant of FDG. Results of mass spectral analysis and fragmentation pattern are represented in Fig. 2a and b. The molecular ion peak of IAA was observed at $[M]^+$ m/z 175.8, which corresponded to the molecular weight of IAA. The molecular ion further fragmented into $[M]^+$ m/z 129.1, $[M]^+$ m/z 116.9, $[M-2]^+$ m/z 101.7 and $[M-1]^+$ m/z 77.4 which corresponds to 3-methylene indole, indole, benzo azete and benzene respectively. However, the fragmented peak of the molecular ion observed at m/z 148.4 could not be determined.

Optimization of cultural conditions for IAA production

The incubation period had a significant effect on IAA production. During the initial days of incubation, the growth of the fungus was very slow along with low IAA production (Fig. 3). Significant amounts of IAA production (14 μg ml^{-1}) started from the 6th day of incubation and thereafter a linear increase in IAA production was seen with time. Maximum production of IAA (29.6 μg ml^{-1}) was observed on the 13th day and then a marginal decline in IAA production was observed. Since maximum IAA production was observed on the 13th day, in further experiments the amount of IAA was measured at this incubation period. pH is an important parameter for fungal growth and IAA production. The effect of initial pH of the growth medium on IAA production and fungal biomass is shown in Fig. 4. Variation in pH of the growth medium had a significant effect on fungal biomass and Trp dependent IAA synthesis. Although the fungus showed growth along with IAA production over a wide range of pH, the maximum growth and IAA production (29.2 μg ml^{-1}) was noted at pH 7.0. Trp concentration in the growth medium had a considerable effect on the IAA production. As shown in the Fig. 5, the fungus did not produce IAA in the growth medium lacking Trp as the source of nitrogen, indicating that IAA production is only through a Trp dependent pathway. A significant increase in growth of fungus and IAA production was seen when the concentration of Trp in the growth medium was increased from 0.5 mg ml^{-1} (23 μg ml^{-1}) to 2.0 mg ml^{-1} (40.4 μg ml^{-1}). With further increase in concentration of Trp (2.5 mg ml^{-1}) there was a decrease in IAA production by the fungus; however there was a steady increase in fungal biomass. The effect of nitrogen source on fungal growth and IAA production was studied by supplementing different nitrogen sources in varying concentrations (0.5 mg ml^{-1} to 2 mg ml^{-1}) along with the Trp (2 mg ml^{-1}). The results are depicted in Fig. 6 and it is evident that there was a decrease in IAA production when any of the additional nitrogen sources was used. The production of IAA further decreased when the concentration of the additional nitrogen sources was increased from 0.5 mg ml^{-1} to 2.0 mg ml^{-1}; however there was increase in fungal biomass. These studies revealed that the supplementation of additional nitrogen sources, such as $(NH_4)_2SO_4$, KNO_3, NH_4NO_3, $NaNO_2$, $NaNO_3$, yeast extract and peptone, support fungal growth but not IAA production. The effect of different carbohydrates serving as carbon sources on the production of IAA and fungal biomass is shown in Fig. 7 When carbohydrates were supplied individually, the maximum biomass and IAA production were observed in the presence of glucose followed by fructose, lactose and mannose. On the other hand, when glucose was replaced with sucrose, arabinose, galactose and maltose, a decrease in both fungal biomass and IAA production was observed. This clearly indicates that the glucose is the best source of carbon for fungal growth and IAA production.

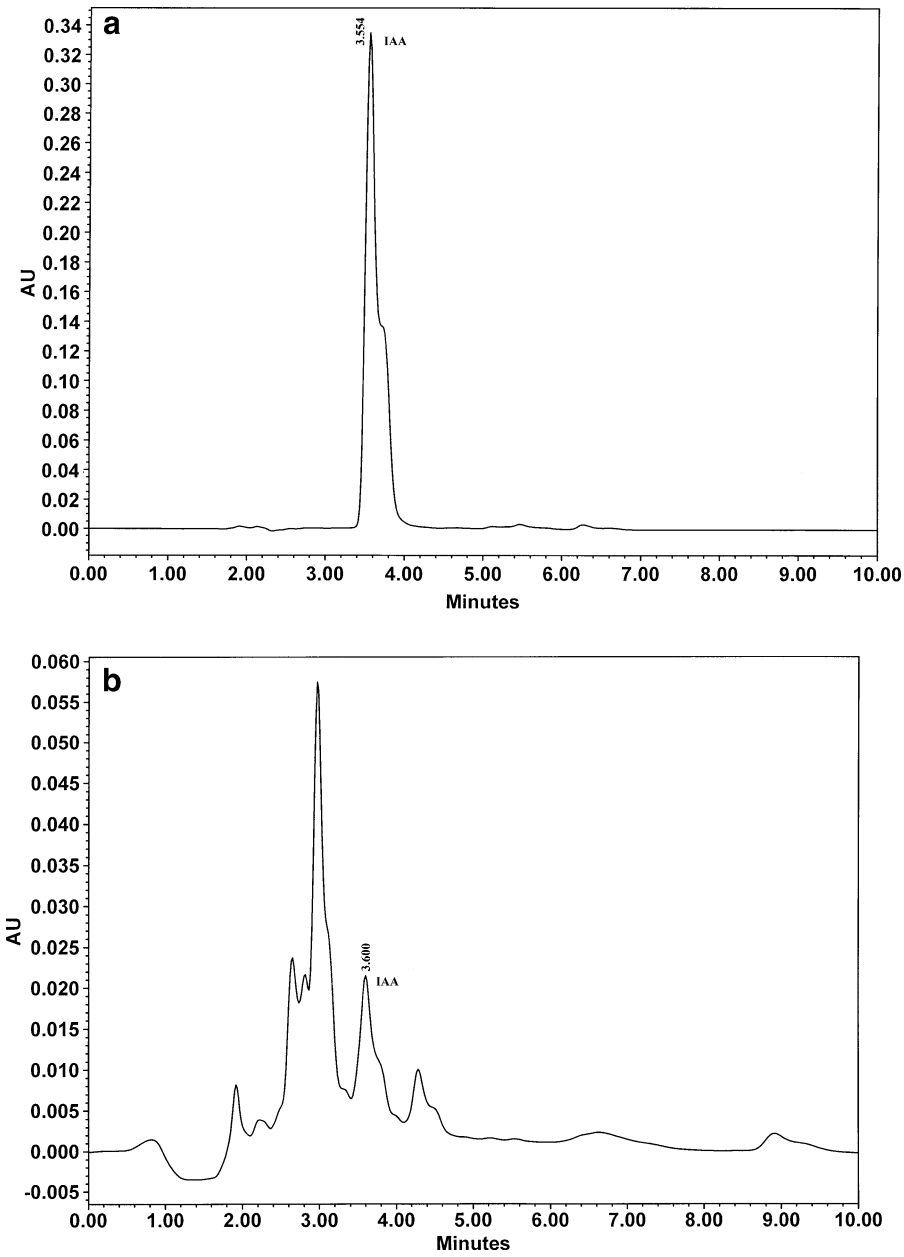

Fig. 1 HPLC analysis of IAA. HPLC elution profile obtained from **a**: authentic IAA and **b**: IAA from the culture supernatant of *Fusarium delphinoides* strain GPK. Chromatographic conditions: Column, Sunfire C18 (300×5 μ): The mobile phase, Isocratic containing water: Acetonitrile: Acetic acid (40: 60: 1), filtered through 0.2 mm membrane filters (Pall Life Sciences, India). The flow rate was 1 ml min^{-1}, and the operating pressure was 4,000 psi

Pathogenicity tests

To check the pathogenicity of *F. delphinoides* strain GPK, Chickpea and Pigeon pea plants were used. These are the major pulse crops cultivated abundantly in Gulbarga region, Karnataka, India. The FOC inoculated chickpea cultivars ICCV-10 and L-550 showed the symptoms of wilt with infected plants collapsing within 10–12 days of treatment in the water culture and seed treatment method, compared with 20 days for the pot culture method. Adult plants showed typical wilt symptoms of flaccidity of leaves and succulent shoots, followed by discolouration and chlorosis of leaves, desiccation and death. Cultivar ICCV-10 showed a lesser disease severity when compared with L-550. In the same way, the plants treated with FDG showed similar symptoms to that of Fusarium wilt with a slow fading of green colour in the beginning, followed by chlorosis and ultimately death of the plants. However, inoculated

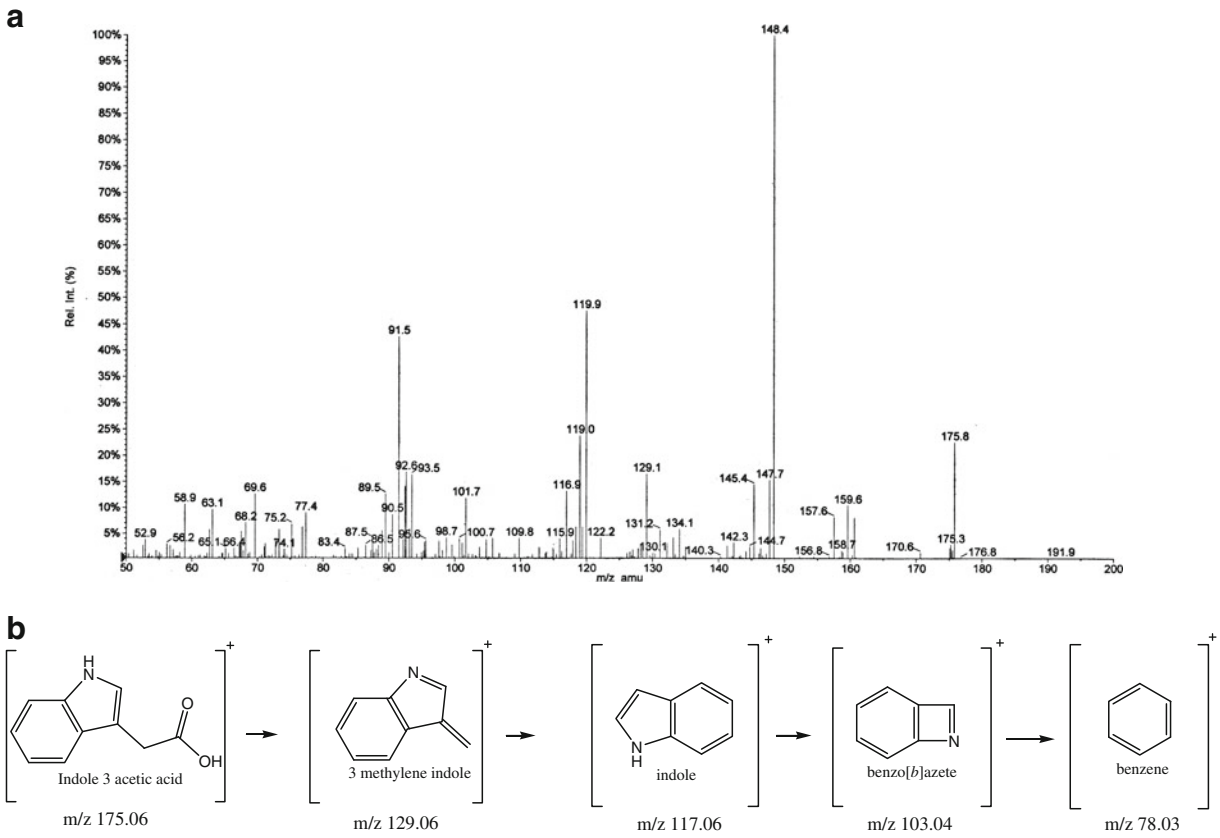

Fig. 2 Mass spectral analysis of **a**: IAA from the culture supernatant of *Fusarium delphinoides* strain GPK. **b**: Mass spectral ions arising from the fragmentation of the IAA. The Mass conditions for the data acquisition were: The spectrometer was operated at vacuum, 3.5 e^{5} Torr pressure; curtain gas, 20 arbitrary units (a.u.); Source gas 1, 50 a.u.; source gas 2, 50 a.u.; collision associated disruption gas medium; interface hater, on; source temperature, 400°C. The molecular ion peaks observed at m/z 175.8, m/z 129.1, m/z 116.9, m/z 101.7 and m/z 77.4 corresponds to IAA, 3-methylene indole, indole, benzo azete and benzene respectively

pigeon pea cultivars did not show any disease symptoms (Table 1).

Electrolyte leakage study

The loss of electrolytes (occurring due to the disruption of cell membrane) is the most common effect in pathogen infection in plant tissues. Electrolyte leakage was studied in the laboratory to confirm the pathogenic effect of the fungus by inoculating *F. delphinoides* strain GPK to different cultivars of chickpea and pigeon pea. There were leakages of electrolytes in the FDG and FOC control treatments. This may be due to the release of electrolytes from the fungus into the medium along with electrolytes present in the medium itself. There was also a steady increase in electrolytes leakage from the non inoculated plant samples over a period of 120 h (Table 2a and b). However electrolyte leakage in inoculated plant samples was much higher. Chickpea cultivars with FDG and FOC treated root and shoot samples showed much higher significance ($P<0.001$ or $P<0.01$) in electrolyte leakage than their controls. FDG and FOC treated pigeon pea plant samples showed slight increases in electrolyte leakage ($P<0.05$) than their controls (Table 2a and b). This small increase in electrolyte leakage from pigeon pea cultivars may be due to the electrolyte leakage from the treated fungus along with electrolyte content of the medium itself. However, either FOC or FDG treated L-550 plants showed higher electrolyte leakage ($P<0.001$) than ICCV-10 ($P<0.01/P<0.05$), with infected L-550 plants completely decaying after 120 h of incubation. In laboratory experiments, L-550 variety was the most

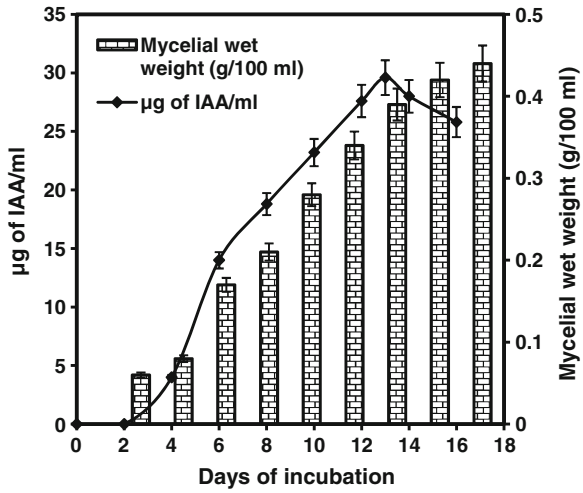

Fig. 3 Effect of incubation time on fungal growth and IAA production. Cultural conditions: Trp (1 mg ml^{-1}) and glucose (1.5 mg ml^{-1}), pH 6, temperature 28°C±2°C. Spent medium was filtered and the supernatant was taken to determine the IAA production and mycelial wet weight is determined as described in material and methods

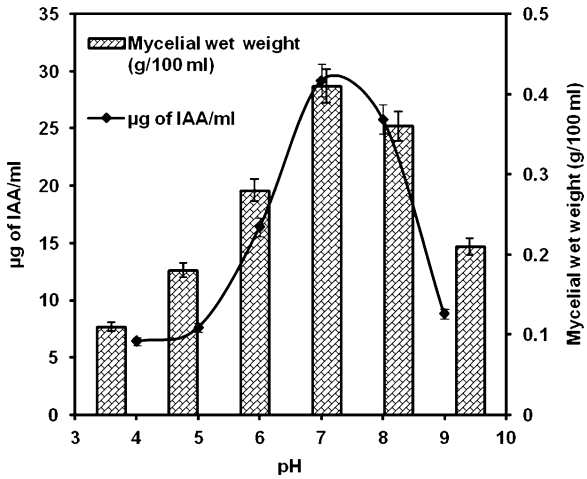

Fig. 4 Effect of pH on fungal growth and IAA production following the growth in mineral salt medium of different pH for 13 days. Spent medium was filtered and the supernatant was taken to determine the IAA production and mycelial wet weight is determined as described in material and methods

susceptible cultivar to infection by FDG followed by ICCV-10.

Nutrient leakage analysis

Leachates such as phosphates, total phenols, proteins and carbohydrates were higher in pathogen treated plant samples. However, the leakage of nutrients was highest in FOC treated L-550 cultivar of chickpea, where all the nutrients showed significant leakage ($P<0.001$). However FDG treated L-550 plant samples showed comparatively lesser electrolyte leakage than FOC treated plant samples with ($P<0.01$) for protein leakage and ($P<0.05$) for phenolic, carbohydrate and phosphate leakage (Tables 3 and 4) .However FDG treated ICCV-10 plant samples showed lesser electrolyte leakage than FDG treated L-550 plants with significant differences occurring only in protein and phosphate leakage. As shown in Tables 3 and 4, FDG treated PT-221 and Maruti cultivars of pigeon pea showed less nutrient leakage with no significant difference among respective controls and their pathogen treated groups. The carbohydrates showed the highest leakage in root and shoot samples of L-550 and ICCV-10 in comparison to their controls, followed by phenols, phosphates and proteins. In contrast different results were obtained for pigeon pea: where in Maruti cultivar, phenols showed highest leakage followed by proteins, carbohydrates and phosphates; and in PT-221, carbohydrates showed highest leakage followed by phenols, proteins and phosphates when compared to their controls.

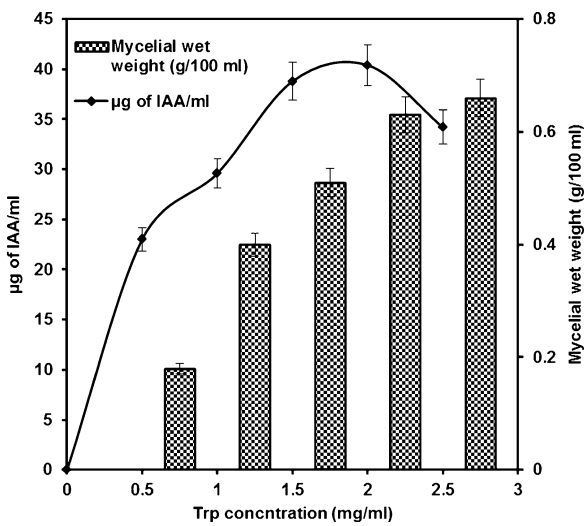

Fig. 5 Effect of Trp concentrations on fungal growth and IAA production by *Fusarium delphinoides* strain GPK. Fungus was grown in mineral salt medium containing different concentrations of Trp (0.5 mg ml^{-1} to 2.5 mg ml^{-1}) at pH 7 and temperature 28°C±2°C

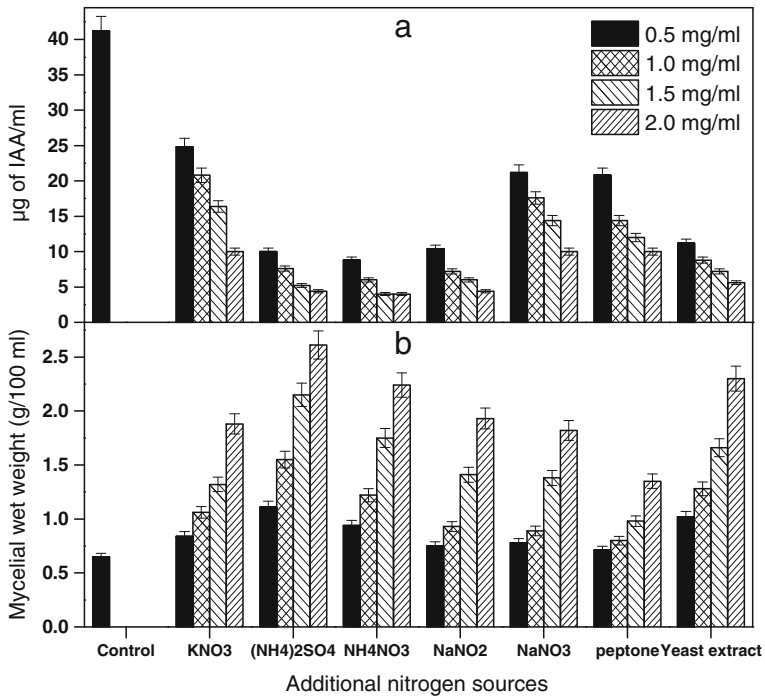

Fig. 6 Effect of varying concentrations of different organic and inorganic nitrogen compounds as additional nitrogen sources on **a**: IAA production by *Fusarium delphinoides* strain GP*K* grown in mineral salt medium containing Trp. and **b**: Fungal biomass in the presence of different additional nitrogen sources

Discussion

In the present work we describe the first study on IAA production from the soilborne fungus FDG and its interaction with chickpea and pigeon pea cultivars, with an aim to establish a system for the study of disease and resistance in these plants. IAA is synthesized by plants and a few microbes (Woodward and Bartel 2005). IAA plays a key role in root and shoot development of plants. In this study, we have shown the presence of IAA in culture supernatants of FDG by preliminary studies using spectrophotometric assays. The presence of IAA in the supernatant is further confirmed by HPLC elution profile showing a peak with a retention time of 3.6 min corresponding with that of authentic IAA and by the fragmentation pattern obtained by mass spectral analysis. To our knowledge this is the first report on the demonstration of IAA production in this microorganism. Trp is generally considered as IAA precursor, as its addition to IAA-producing microbial cultures promotes an increase in IAA biosynthesis (Costacurta and Vanderleyden 1995). The elimination of Trp from the growth medium resulted in the absence of IAA in the culture filtrates of FDG, indicating that IAA production by the fungus is through a Trp dependent pathway. Further, we have optimized the cultural conditions for the production of IAA by this fungus. Optimum pH for most of the fungi is in the range of 5–6. However, FDG showed the maximum growth, as determined by fungal biomass, and activity for IAA production at pH 7.0. Increasing concentration of Trp increases the IAA production along with fungal growth. However at a higher concentration of Trp (2.5 mg ml^{-1}) there is an increase in fungal biomass

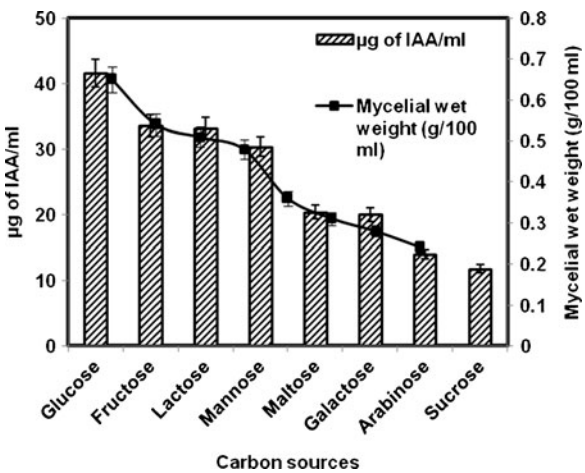

Fig. 7 Effect of different carbon sources on fungal growth and IAA production by *Fusarium delphinoides* strain GPK. Fungus was grown in mineral salt medium containing different mono and disaccharides at pH 7 and temperature 28°C±2°C. The medium containing Glucose served as control

Table 1 Pathogenicity of *Fusarium delphinoides* strain GPK to different cultivars of chickpea and pigeon pea in comparison with *Fusarium oxysporum sp. ciceris*. A- Seed treatment, B- Water culture, C-Pot culture. FDG- *Fusarium delphinoides* strain GPK, FOC- *Fusarium oxysporum sp. ciceris*, ND- Not detected

Treatment	Symptoms	Severity of disease											
		ICCV-10			L-550			Maruti			PT-221		
		A	B	C	A	B	C	A	B	C	A	B	C
Control	ND	–	–	–	–	–	–	–	–	–	–	–	–
FOC	Wilt	1.5	2.0	1.0	3.0	3.5	2.5	ND	ND	ND	ND	ND	ND
FDG	Wilt	0.5	1.0	ND	1.5	2.5	1.0	ND	ND	ND	ND	ND	ND

Table 2 Electrolyte leakage from a: Root and b. Shoot of different plant cultivars followed by the pathogen infection. Control: without pathogen treatment, FDG test: *Fusarium delphinoides* strain GPK treatment, FOC test: *Fusarium oxysporum sp. ciceris* (positive control) treatment, FDG control: *Fusarium delphinoides* strain GPK control and FOC control: *Fusarium oxysporum sp. ciceris* control. Values are mean ± S. E. M (standard error mean) of triplicate determinations

Cultivar	Treatment	24 h	48 h	72 h	96 h	120 h
(a) Electrolyte leakage (μS) ± S. E. M in root samples						
L 550	Control	32.6±0.66	43.5±0.54	57.7±1.24	61.3±1.10	66.7±1.08
	FDG	46.6±0.85[c, d]	62.6±1.47[c, d]	80.5±1.53[c, d]	90.5±2.06[c, d]	94.7±1.46[c, d]
	FOC	52.7±1.18[c, d,b, e]	71.2±1.39[c, d,b, e]	88.5±2.01[c, d,a, e]	97.6±0.86[c, d,a, e]	107.0±1.86[c, d,b, e]
ICCV	Control	34.1±1.36	43.5±1.94	49.9±1.56	55.5±1.88	59.0±1.56
	FDG	40.7±1.65[a, d]	50.3±1.82 [a, d]	57.7±1.92[a, d]	63.4±1.72[a, d]	70.0±2.32[a, d]
	FOC	46.3±1.47[b, d]	57.5±1.72[b, d]	64.8±1.65[b, d]	69.9±1.82[b, d]	76.4±2.19[b, d]
Maruti	Control	26.3±1.33	34.6±1.56	47.2±1.56	53.8±1.50	60.1±1.61
	FDG	28.5±1.48	40.7±1.68	52.7±1.47	60.9±2.17	66.8±0.95[a, d]
	FOC	34.5±1.39[a, d]	46.6±1.53[b, d]	56.3±1.53[a, d]	64.4±1.71[a, d]	70.8±1.71[b, d]
PT 221	Control	30.3±1.30	37.5±1.27	44.6±1.53	49.6±1.71	53.0±1.82
	FDG	35.5±1.30	43.9±1.39[a, d]	53.3±1.79[a, d]	59.1±1.82[a, d]	60.6±1.53[a, d]
	FOC	39.5±1.25[b, d]	49.6±1.69[b, d]	56.3±1.99[b, d]	61.9±1.62[b, d]	65.2±1.62[b, d]
(b) Electrolyte leakage (μS) ± S. E. M in shoot samples						
L 550	Control	36.6±1.05	72.5±1.59	85.4±2.28	90.7±1.82	99.0±2.36
	FDG	49.8±1.36[c, d]	84.6±1.5[a, d]	104.0±2.18[b, d]	126.0±3.03[c, d]	135.0±2.80[c, d]
	FOC	67.6±1.00[c, d,c, e]	102.0±2.58[c, d,b, e]	112.0±3.15[c, d]	136.0±2.72[c, d]	151.0±2.83[c, d,a, e]
ICCV	Control	26.4±0.95	65.4±1.59	72.6±1.86	80.8±1.70	90.4±1.70
	FDG	29.3±1.07	70.2±1.59	79.0±2.07 [a, d]	88.7±1.88[a, d]	95.4±1.61
	FOC	34.9±1.30[b, d,a, e]	75.8±1.70[b, d]	83.6±1.62[a, d]	92.9±2.17[a, d]	103.0±2.77[a, d]
Maruti	Control	21.5±1.07	54.3±1.39	60.8±1.50	65.8±1.30	72.9±1.47
	FDG	23.6±1.11	61.6±1.11[a, d]	68.5±1.02[a, d]	73.8±1.10[a, d]	80.7±1.77[a, d]
	FOC	25.6±1.07	63.8±1.36[a, d]	71.5±1.25[b, d]	78.0±1.04[b, d]	83.8±1.50[b, d]
PT 221	Control	23.7±1.18	57.6±1.33	65.5±1.62	75.6±1.82	78.4±1.86
	FDG	27.5±1.27	63.5±1.25[a, d]	73.5±1.48[a, d]	82.5±1.77	84.6±1.68
	FOC	30.3±1.19[a, d]	67.3±1.07[a, d]	76.8±1.39[b, d]	87.4±1.56[b, d]	91.5±2.48[b, d]
	FDG control	6.3±0.31	6.7±0.33	6.9±0.34	7.4±0.37	7.7±0.38
	FOC control	9.4±0.47	9.7±0.48	10.2±0.51	10.8±0.54	11.2±0.56

[a] $P<0.05$, [b] $P<0.01$, [c] $P<0.001$, [d] When compared with control of the respective cultivar. [e] When compared with FDG of the respective cultivar

Table 3 Nutrient leakage from root samples of different plant cultivars followed by the pathogen infection. Control: without pathogen treatment, FDG test: *Fusarium delphinoides* strain GPK treatment, FOC test: *Fusarium oxysporum sp. ciceris* (positive control) treatment. Values are mean ± S. E. M (standard error mean) of triplicate determinations

Cultivar	Time (h)	Nutrient leakage from root samples											
		Protein (μg ml^{-1})			Phenolics (μg ml^{-1})			Carbohydrates (μg ml^{-1})			Phosphates (μg ml^{-1})		
		Control	FDG test	FOC test	Control	FDG test	FOC test	Control	FDG test	FOC test	Control	FDG test	FOC test
L-550	24	52.0±2.31	94.7±3.53$^{c, d}$	100.0±2.31$^{c, d}$	2.0±0.0	4.67±0.66$^{a, d}$	8.67±0.66$^{c, d,b, e}$	2.0±0.0	4.67±0.66$^{a, d}$	6.67±0.66$^{b, d}$	3.73±0.13	4.53±0.13$^{a, d}$	8.13±0.13$^{c, d,c, e}$
	48	72.0±2.31	101.0±3.53$^{b, d}$	121.0±3.53$^{c, d,a, e}$	2.67±0.66	5.33±0.66	9.33±0.66$^{c, d,a, e}$	3.33±0.66	6.0±1.15	8.67±0.66$^{a, d}$	4.67±0.13	5.33±0.13$^{a, d}$	9.47±0.13$^{c, d,c, e}$
	72	98.0±2.00	127.0±3.53$^{b, d}$	145.0±2.91$^{c, d,b, e}$	4.67±0.66	7.33±0.66	10.7±0.66$^{b, d,a, e}$	4.0±0.0	8.0±1.15$^{a, d}$	12.0±0.0$^{c, d,a, e}$	6.27±0.13	7.07±0.13$^{a, d}$	12.4±0.23$^{c, d,c, e}$
	96	120.0±2.31	144.0±2.31$^{b, d}$	175.0±3.53$^{c, d,c, e}$	5.33±0.66	8.0±0.0$^{b, d}$	12.0±0.0$^{c, d,c, e}$	5.33±0.66	9.33±1.76	22.0±1.15$^{c, d,b, e}$	7.20±0.23	8.13±0.13$^{a, d}$	14.0±0.23$^{c, d,c, e}$
	120	136.0±2.31	153.0±3.53$^{a, d}$	192.0±2.31$^{c, d,c, e}$	6.0±0.0	8.67±0.66$^{a, d}$	13.3±0.66$^{c, d,b, e}$	6.67±0.66	10.7±1.33	24.7±1.76$^{c, d,c, e}$	7.47±0.13	10.5±0.13$^{c, d}$	14.5±0.26$^{c, d,c, e}$
ICCV-10	24	42.7±1.33	54.7±1.33$^{b, d}$	72.0±2.31$^{c, d,c, e}$	4.67±0.66	5.33±0.66	6.0±0.0	3.33±0.66	7.33±0.66$^{a, d}$	10.7±0.66$^{c, d,a, e}$	2.53±0.13	3.73±0.13$^{b, d}$	5.60±0.23$^{c, d,c, e}$
	48	60.0±2.31	65.3±1.33	89.3±1.33$^{c, d,c, e}$	6.0±0.0	7.33±0.66	8.67±0.66$^{a, d}$	5.33±0.66	11.3±0.66$^{b, d}$	15.3±0.66$^{c, d,a, e}$	3.73±0.13	5.60±0.23$^{c, d}$	6.67±0.13$^{c, d,a, e}$
	72	74.7±1.33	82.7±1.33$^{a, d}$	119.0±1.33$^{c, d,c, e}$	7.33±0.66	10.0±1.15	10.0±1.15	10.7±0.66	12.7±0.66	18.7±0.66$^{c, d,b, e}$	5.20±0.23	6.27±0.26$^{a, d}$	8.47±0.17$^{c, d,b, e}$
	96	100.0±2.31	109.0±1.33$^{a, d}$	132.0±2.31$^{c, d,c, e}$	9.33±0.66	12.7±0.66$^{a, d}$	14.7±0.66$^{b, d}$	13.3±0.66	14.7±0.66	21.3±1.33$^{b, d,b, e}$	5.73±0.13	7.40±0.20$^{c, d}$	10.5±0.13$^{c, d,c, e}$
	120	107.0±1.33	120.0±2.31$^{a, d}$	145±2.67$^{c, d,c, e}$	11.3±0.66	14.7±0.66$^{a, d}$	17.3±0.66$^{b, d}$	14.7±0.66	17.3±0.66	24.0±1.15$^{c, d,b, e}$	6.40±0.23	8.53±0.13$^{c, d}$	11.9±0.13$^{c, d,c, e}$
Maruti	24	10.7±1.33	14.7±1.33	17.3±1.33$^{a, d}$	2.0±0.0	2.0±0.0	2.0±0.0	2.0±0.0	2.0±0.0	6.0±0.66$^{b, d,b, e}$	1.73±0.13	1.87±0.13	2.13±0.13
	48	20.0±2.31	24.0±2.31	29.3±1.33$^{a, d}$	2.0±0.0	2.67±0.66	4.0±0.0	2.67±0.66	3.33±0.66	7.33±0.66$^{b, d,a, e}$	2.53±0.13	3.07±0.13	2.93±0.13
	72	26.7±1.33	33.3±1.33$^{a, d}$	38.7±1.33$^{b, d}$	2.67±0.66	4.0±0.0	4.67±0.66	5.33±0.66	5.33±0.66	10.7±0.66$^{b, d,b, e}$	3.73±0.13	4.13±0.13	4.53±0.13$^{a, d}$
	96	33.3±1.33	40.7±2.91	42.7±1.33$^{a, d}$	4.0±0.0	4.67±0.66	6.0±0.0 $^{a, d}$	7.33±0.66	9.33±0.66	12.7±0.66$^{b, d,a, e}$	5.20±0.23	5.20±0.23	6.40±0.0$^{b, d,b, e}$
	120	37.3±1.33	41.3±1.33	46.7±1.33$^{b, d}$	4.67±0.66	6.0±0.0	6.67±0.66	10.0±0.0	11.3±0.66	13.3±0.66$^{a, d}$	5.60±0.23	6.13±0.13	6.93±0.13$^{b, d,a, e}$
PT-221	24	22.7±0.66	24.7±1.33	28.7±1.33$^{a, d}$	2.0±0.0	2.67±0.66	2.0±0.0	2.0±0.0	3.33±0.66	5.33±0.66$^{a, d}$	0.66±0.13	1.07±0.13	1.33±0.13$^{a, d}$
	48	34.7±1.33	38.7±1.33	42.7±1.33$^{a, d}$	2.67±0.66	4.0±0.0	4.0±0.0	4.67±0.66	7.33±0.66	8.67±0.66$^{a, d}$	1.20±0.23	2.27±0.13$^{b, d}$	2.0±0.0 $^{a, d}$
	72	46.7±1.33	53.3±1.33$^{a, d}$	57.3±1.33$^{b, d}$	4.0±0.0	5.33±0.66	5.33±0.66	8.67±0.66	10.7±0.66	12.0±1.15	1.60±0.23	3.33±0.13$^{c, d}$	3.73±0.13$^{c, d}$
	96	52.0±2.31	56.0±0.0	66.7±1.33$^{b, d,b, e}$	4.67±0.66	6.0±0.0	6.0±0.0	10.7±0.66	12.0±0.0	14.0±1.15	2.93±0.13	3.73±0.13$^{a, d}$	4.27±0.13$^{c, d}$
	120	56.0±2.31	61.3±1.33	69.3±1.33$^{b, d,a, e}$	6.0±0.0	6.67±0.66	7.33±0.66	12.7±0.66	14.7±0.66	18.0±1.15$^{a, d}$	3.73±0.13	4.13±0.13	5.07±0.13$^{c, d,b, e}$

[a] $P<0.05$, [b] $P<0.01$, [c] $P<0.001$, [d] When compared with control of the respective cultivar. [e] When compared with FDG of the respective cultivar

Table 4 Nutrient leakage from shoot samples of different plant cultivars followed by the pathogen infection. Control: without pathogen treatment, FDG test: *Fusarium delphinoides* strain GPK treatment, FOC test: *Fusarium oxysporum sp. ciceris* (positive control) treatment. Values are mean ± S. E. M (standard error mean) of triplicate determinations

Cultivar	Time (h)	Nutrient leakage from shoot samples											
		Protein (μg ml^{-1})			Phenolics (μg ml^{-1})			Carbohydrates (μg ml^{-1})			Phosphates (μg ml^{-1})		
		Control	FDG test	FOC test	Control	FDG test	FOC test	Control	FDG test	FOC test	Control	FDG test	FOC test
L-550	24	84.0±2.31	105.0±3.53 [b, d]	125.0±3.53[c, d,a, e]	6.0±0.0	8.67±0.66[a, d]	10.7±0.66[b, d]	4.0±0.0	5.33±0.66	13.3±0.66[c, d,c, e]	6.27±0.13	9.87±0.13[c, d]	12.4±0.23[c, d,c, e]
	48	121.0±3.53	145.0±3.53 [a, d]	176.0±4.62[c, d,b, e]	7.33±0.66	11.3±0.66[a, d]	15.3±0.66[c, d,a, e]	7.33±0.66	11.3±0.66[a, d]	30.0±1.15[c, d,c, e]	9.47±0.13	12.0±0.23[c, d]	14.7±0.13[c, d,c, e]
	72	172.0±2.31	175.0±4.06	263.0±3.53[c, d,c, e]	10.0±1.15	12.7±0.66	18.7±0.66[c, d,b, e]	11.3±0.66	22.0±1.15[b, d]	49.3±1.33[c, d,c, e]	11.7±0.13	16.7±1.33[c, d]	22.5±0.26[c, d,c, e]
	96	205.0±3.53	261.0±2.91[c, d]	328.0±4.62[c, d,c, e]	11.3±0.66	16.0±0.0[b, d]	22.7±0.66[c, d,c, e]	22.0±1.15	29.3±1.33[a, d]	62.7±1.33[c, d,c, e]	14.3±0.13	18.4±0.23[c, d]	32.4±0.23[c, d,c, e]
	120	215.0±3.71	293.0±3.53[c, d]	375.0±4.06[c, d,c, e]	13.3±0.66	17.3±0.66	24.7 ±1.76[b, d,b, e]	25.3±1.33	35.3±1.33[b, d]	70.0±1.15[c, d,c, e]	17.1±0.13	22.3±0.13[c, d]	34.8±0.23[c, d,c, e]
ICCV-10	24	69.3±1.33	86.7±1.33[b, d]	105.0±2.67[c, d,b, e]	5.33±0.66	7.33±0.66	11.3±0.66[b, d,a, e]	6.0±0.0	11.3±0.66[b, d]	14.7±0.66[c, d,a, e]	5.20±0.23	7.73±0.13[c, d]	13.3±0.13[c, d,c, e]
	48	120.0±2.31	120.0±2.31	140.0±2.31[b, d,b, e]	10.7±0.66	11.3±0.66	15.3±0.66[b, d,a, e]	11.3±0.66	18.0±1.15[a, d]	29.3±1.33[c, d,c, e]	6.80±0.23	10.3±0.13[c, d]	16.7±0.13[c, d,c, e]
	72	141.0±1.33	143.0±1.33	172.0±2.31[c, d,c, e]	15.3±0.66	18.0±1.15	21.3±0.66[b, d]	14.7±1.33	26.7±1.33[b, d]	54.0±1.15[c, d,c, e]	9.07±0.13	14.0±0.23[c, d]	19.6±0.23[c, d,c, e]
	96	165.0±1.33	203.0±2.67[c, d]	245.0±3.53[c, d,c, e]	19.3±0.66	22.7±0.66[a, d]	26.7±0.66[c, d,a, e]	27.3±1.76	34.0±1.15[a, d]	65.3±1.33[c, d,c, e]	12.3±0.13	15.3±0.13[c, d]	24.4±0.23[c, d,c, e]
	120	191.0±1.33	224.0±2.31[c, d]	271.0±3.53[c, d,c, e]	22.0±1.15	26.7±0.66[a, d]	30.7±0.66[c, d,a, e]	34.7±1.33	42.7±0.66[a, d]	72.7±1.76[c, d,c, e]	13.9±0.13	17.1±0.13[c, d]	25.6±0.23[c, d,c, e]
Maruti	24	60.0±2.31	66.7±1.33	72.0±2.31[a, d]	6.0±0.0	7.33±0.66	8.67±0.66[a, d]	11.3±0.66	11.3±0.66	15.3±0.66[a, d,a, e]	5.73±0.13	6.13±0.13	8.27±0.13[c, d,c, e]
	48	80.0±2.31	96.0±2.31[b, d]	103.0±1.33[c, d]	7.33±0.66	8.67±0.66	10.0±0.0[a, d]	14.0±0.0	14.7±0.66	17.3±0.66[a, d,a, e]	9.07±0.13	9.33±0.13	10.5±0.13[c, d,b, e]
	72	116.0±2.31	119.0±1.33	139.0±2.67[c, d,b, e]	8.67±0.66	11.3±0.66	13.3±0.66[b, d]	16.7±0.66	20.7±0.66[b, d]	22.0±0.0[b, d]	12.4±0.23	12.8±0.23	14.3±0.13[b, d,b, e]
	96	133.0±1.33	144.0±2.31[a, d]	160.0±2.31[c, d,b, e]	10.7±0.66	12.7±0.66	15.3±0.66[b, d]	20.7±0.66	23.3±0.66	24.7±0.66[a, d]	14.9±0.13	15.6±0.23	15.7±0.13[a, d]
	120	152.0±2.31	159.0±1.33	179.0±1.33[c, d,c, e]	12.7±0.66	14.0±0.0	17.3±0.66[b, d]	24.0±0.0	26.7±0.66[a, d]	28.7±0.66[b, d]	18.4±0.23	19.7±0.13[b, d]	20.4±0.23[b, d]
PT-221	24	68.0±2.31	73.3±1.33	81.3±3.53[a, d]	5.33±0.66	7.33±0.66	9.33±0.66[a, d]	6.67±0.66	8.67±0.66	9.33±0.66	5.47±0.13	6.0±0.23	6.27±0.13[a, d]
	48	103.0±1.33	111.0±1.33[a, d]	113.0±1.33[b, d]	7.33±0.66	9.33±0.66	11.3±0.66[a, d]	10.7±0.66	12.0±0.0	15.3±0.66[b, d,a, e]	6.93±0.13	7.33±0.13	6.93±0.13
	72	145.0±1.33	152.0±2.31	164.0±2.31[b, d,a, e]	10.7±0.66	12.0±1.15	13.3±0.66	19.3±0.66	20.0±1.15	24.7±0.66[a, d,a, e]	7.73±0.13	8.40±0.23	10.7±0.13[c, d,c, e]
	96	184.0±2.31	195.0±1.33[a, d]	207.0±1.33[c, d,b, e]	12.7±0.66	13.3±0.66	15.3±0.66	22.7±0.66	23.3±0.66	33.3±0.66[c, d,c, e]	8.93±0.13	10.5±0.13[b, d]	12.0±0.23[c, d,b, e]
	120	193.0±1.33	205.0±1.33[b, d]	217.0±1.33[c, d,b, e]	13.3±0.66	15.3±0.66	16.0±3.06	28.7±0.66	34.0±1.15[a, d]	36.7±0.66[b, d]	9.87±0.13	11.3±0.13[c, d]	13.3±0.13[c, d,c, e]

[a] $P<0.05$, [b] $P<0.01$, [c] $P<0.001$, [d] When compared with control of the respective cultivar. [e] When compared with FDG of the respective cultivar

with decreased IAA production. This might be explained by the fact, as Patten and Glick (1996) observed in bacteria, that they often utilize more than one biosynthetic pathway for IAA production. Probably this might be the reason for decrease in IAA production at higher concentrations in FDG. The supplementation of additional nitrogen sources along with Trp increases the fungal growth but decreases the IAA production. The highest IAA production is seen in the medium lacking the additional nitrogen sources. A decrease in IAA production upon the supplementation of additional nitrogen sources could be attributed to less utilization of Trp in the presence of easily available nitrogen from the additional nitrogen source.

The results from the electrolyte leakage study and nutrient leakage analysis conducted on the plant samples in the laboratory corroborated the disease index data measured at whole plant level in the growth chambers. The data showed that a direct relationship between the amount of electrolyte and nutrient loss and the degree of susceptibility to *Fusarium* infection. Electrolyte and nutrient loss in the control treatment was mainly due to physical wounding of the plant tissue. The most commonly observed effect in infection is the loss of electrolytes due to disruption of the cell membrane, which may occur via pathogen enzymes or toxins and often leads to a dramatic increase in ion movement especially K^+ and H^+ through the cell membrane (Agrios 1997). The quantification of cell and tissue electrolyte leakage was used reliably to screen avocado rootstocks for resistance to phytophthora root rot (Zilberstein and Pinkas 1987). This technique was also employed to compare and identify disease tolerant and susceptible citrus infected with two *Phytophthora* species (Widmer et al. 1998). Along with the electrolyte leakage assay, nutrient leakage analysis can also be used to judge the susceptibility of the host plant to a pathogen. Black and Wheeler (1966) reported the significant loss of phosphates from victorin-treated oat tissues. Electrolyte leakage (as measured by conductivity) and nutrient leakage in chickpea and pigeon pea infected with either FOC or FDG also proved reliable as a rapid technique to screen and identify susceptible and resistance host plants for the pathogens. Chickpea wilt caused by FOC is reported from many Asian, African, Mediterranean and South American countries, with yield losses ranging from 10 to 90% (Jimenez-Diaz et al. 1989; Ratnaparkhe et al. 1998). Different pathogenic races of FOC have been identified. Races 1, 2, 3 and 4 were reported from India (Haware and Nene 1982), but there are no reports on the chickpea wilt caused by *Fusarium delphinoides*, previously considered as a saprophyte of decaying plant substrata or soil fungus. The role of this fungus as a pathogen was not known until it was isolated from stem lesions of *Hoodia gordonii.* In our studies we were able to show disease symptoms in chickpea caused by FDG. The present pathogenicity tests confirmed the resistance of pigeon pea cultivars (Maruti and PT-221) and tolerance (moderate resistance) of chickpea cultivar ICCV-10 towards infection caused by FDG. However chickpea cultivar L-550 reacted as a susceptible host to the infection. The resistance of the pigeon pea cultivars might explain the fact that pigeon pea is known to be a symptomless carrier of the pathogen (Haware and Nene 1982).

Thus the present investigation showed the ability of the soil fungus FDG to produce a significant amount of IAA from Trp under favourable cultural conditions. This is the first report of an attempt to study the interaction between FDG, a member of the *Fusarium dimerum* species group, and chickpea in order to establish a system for the study of disease and resistance in these plants.

Acknowledgments Authors wish to thank University Grants Commission (UGC) for the financial support through Major Research Project and SAP programme. One of the authors Mr. Guruprasad B K wishes to thank Gulbarga University Gulbarga for providing University fellowship. We are also thankful to Dr. Ramchandra D. Gaikwad, Ecocert India Pvt. Ltd. for statistically analyzing the experimental data and to Agharkar Research Institute, Pune, India for their help in sequencing studies.

References

Agrios, G. N. (1997). *Plant pathology* (4th ed., p. 635). San Diego: Academic.

Arshad, M., & Frankenberger, W. T. (1998). Plant growth-regulating substances in the rhizosphere: Microbial production and functions. *Advances in Agronomy, 62*, 34–151.

Bartel, B. (1997). Auxin biosynthesis. *Annual Review of Plant Physiology and Plant Molecular Biology, 48*, 51–66.

Baayen, R. P., O'Donnell, K., Bonants, P. J. M., Cigelnik, E., Kroon, L. P. N. M., & Roebroeck, E. J. A. (2000). Gene genealogies and AFLP analyses in the *Fusarium oxysporum* complex identify monophyletic and nonmono-

phyletic formae speciales causing wilt and rot disease. *Phytopathology, 90*, 891–900.

Black, S. H., & Wheeler, H. (1966). Biochemical effects of victorin on oat tissues and mitochondria. *American Journal of Botany, 53*, 1108–1112.

Bray, H. G., & Thorpe, W. V. (1954). Analysis of phenolic compounds of interest in metabolism. *Methods of Biochemical Analysis, 1*, 27–52.

Chung, K. R., Shilts, T., Esturk, U., Timmer, L. W., & Ueng, P. (2003). Indole derivatives produced by the fungus *Colletotrichum acutum* causing lime anthracnose and postbloom fruit drop of citrus. *FEMS Microbiology Letters, 226*, 23–30.

Costacurta, A., & Vanderleyden, J. (1995). Synthesis of phytohormones by plant associated bacteria. *Critical Reviews in Microbiology, 21*, 1–18.

Di Pietro, A., Madrid, M. P., Caracuel, Z., Delgado-Jarana, J., & Roncero, M. I. G. (2003). *Fusarium oxysporum*: Exploring the molecular arsenal of a vascular wilt fungus. *Molecular Plant Pathology, 4*, 315–325.

FAO (2005). From FAOSTAT on-line statistical database. *Food and Agricultural Organization of the United Nations*

Fiske, C. H., & Subbarow, Y. (1925). The colorimetric determination of phosphorus. *The Journal of Biological Chemistry, 66*, 376–400.

Frankenberger, W. T., & Arshad, M. (1995). *Phytohormones in soil: Microbial production and function*. New York: Marcel Dekker.

Fravel, D., Olivain, C., & Alabouvette, C. (2003). *Fusarium oxysporum* and its biocontrol. *The New Phytologist, 157*, 493–502.

Glickmann, E., & Dessaux, Y. (1995). A critical examination of the specificity of salkowski reagent for indolic compounds produced by phytopathogenic bacteria. *Applied and Environmental Microbiology, 61*, 793–796.

Haware, M. P., & Nene, Y. C. (1982). Symptomless carriers of the chickpea wilt *Fusarium*. *Plant Disease, 66*, 250–251.

Jimenez-Diaz, R. M., Trapero-Casas, A. T., & Cabrera de la Colina, J. (1989). Races of *Fusarium oxysporum f. sp. ciseris* infecting chickpea in southern Spain. In E. C. Tjamos & E. H. Beckman (Eds.), *Vascular wilt diseases of plants, vol H28*. Berlin: Springer.

Jouanneau, J. P., Lapous, D., & Guern, J. (1991). In plant protoplast, the spontaneous expression of defense reactions and responsiveness to exogenous elicitors are under auxin control. *Plant Physiology, 96*, 459–466.

Lowry, O. H., Rosebrough, N. J., Farr, A. L., & Randall, R. J. (1951). Protein measurement with the folin phenol reagent. *The Journal of Biological Chemistry, 193*, 265–275.

Martens, D. A., & Frankenberger, W. T. (1994). Assimilation of exogenous 2-C_{14}-indole acetic acid and 3-C^{14}-tryptophan exposed to the roots of three wheat varieties. *Plant and Soil, 166*, 281–290.

Nene, Y. L., Haware, M. P., Reddy, N. M. V., Philips, J. C., Castro, E. L., & Kotasthane, S. R. (1989). Identification of broad based and stable resistance to wilt and roots in chickpea. *Indian Phytopathology, 42*, 499–505.

Pande, S., Narayana Rao, J., & Sharma, M. (2007). Establishment of chickpea wilt pathogen *Fusarium oxysporum f. sp. ciceris* in the soil through seed transmission. *The Plant Pathology Journal, 23*(1), 3–6.

Patten, C. L., & Glick, B. R. (1996). Bacterial biosynthesis of indole-3-acetic acid. *Canadian Journal of Microbiology, 42*, 207–220.

Pontecorvo, G. (1953). The genetics of Aspergillus nidulans. In M. Demerec (Ed.), *Advances in genetics* (pp. 141–238). New York: Academic.

Ratnaparkhe, M. P., Santra, D. K., Tullu, A., & Muehlbauer, F. J. (1998). Inheritance of inter-simple-sequence-repeat polymorphisms and linkage with a *Fusarium* wilt resistance gene in chickpea. *Theoretical and Applied Genetics, 96*, 348–353.

Schroers, H. J., O'Donnell, K., Lamprecht, S. C., Kammeyer, P. L., Johnson, S., Sutton, D. A., et al. (2009). Taxonomy and phylogeny of the *Fusarium dimerum* species group. *Mycology, 101*(1), 44–70.

Tsavkelova, E. A., Cherdyntseva, T. A., & Netrusov, A. I. (2005). Auxin production by bacteria associated with orchid roots. *Microbiology, 74*, 55–62.

Tudzynski, B., & Sharon, A. (2002). Biosynthesis, biological role and application of fungal phytohormones. In H. Osiewacz (Ed.), *The Mycota: A comprehensive treatise on fungi as experimental systems for basic and applied research* (pp. 183–211). Berlin: Springer-Verlag.

Vismer, H. F., Marasas, W. F. O., Rheeder, J. P., & Joubert, J. J. (2002). *Fusarium dimerum* as a cause of human eye infection. *Medical Mycology, 40*, 399–406.

Widmer, T. L., Graham, J. H., & Mitchell, D. J. (1998). Histological comparison of fibrous root infection of disease tolerant and susceptible citrus host by P*hytophthora nicotianae* and *P. palmivora*. *Phytopathology, 88*, 389–395.

Woodward, A. W., & Bartel, B. (2005). Auxin: Regulation, action, and interaction. *Annals of Botany, 95*, 707–735.

Zilberstein, M., & Pinkas, Y. (1987). Detached root inoculation- a new method to evaluate resistance to phytophthora root rot in avocado trees. *Phytopathology, 77*, 841–844.