The future Journal of Biology
2019;3:44-53

EFFECT OF *Trichoderma harzianum* AND *Aneurinobacillus migulanus* APPLICATIONS ON AUXIN, GIBBERELLIC ACID AND ABSCISIC ACID CONTENT OF GLADIOLUS CORMS INFECTED WITH *Fusarium oxysporum* f. sp. Gladioli

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RESEARCH PAPER ***Accepted:*** *10 June 2019* ***published online:*** *25 August 2019*

ABSTRACT

Growing gladiolus corms in soil-less culture fertilized by soluble nutrients raised the question of how both *Trichoderma harzianum* and *Aneurinobacillus migulanus* enhance plant growth and provide protection against infection by *Fusarium oxysporum* f. sp. *gladioli*. Changes in the concentrations of the plant growth regulators (PGRs) gibberellic acid (GA_3), indole acetic acid (IAA) and abscisic acid (ABA) were determined in gladiolus corms following treatment with *T. harzianum*, *A. migulanus* and *F. oxysporum* f.sp. *gladioli*. The presence of these PGRs in corms was confirmed using liquid chromatography-mass spectrometry (LC-MS/MS) and the compounds quantified using high performance liquid chromatography (HPLC). Concentrations of both GA_3 and IAA increased linearly in all corms over the 10 days after treatments; lowest concentrations of both PGRs were consistently found in corms inoculated with *F. oxysporum* f. sp. *gladioli*. In contrast, highest concentrations of both GA_3 and IAA occurred in corms treated with *T. harzianum* alone. Highest concentrations of ABA, however, were found in corms inoculated with *F. oxysporum* f. sp. *gladioli*, regardless of the presence of *T. harzianum* or *A. migulanus*. Treatments with *T. harzianum* or *A. migulanus* in the absence of *F. oxysporum* f.sp. *gladioli* maintained ABA concentrations at the same levels as control corms. These results indicate that promotion of Gladiolus growth by *T. harzianum* or *A. migulanus* may be partly mediated through stimulation of the production of GA_3 and IAA in corm tissues. Increases in these PGRs may also be a mediator in the biological control activity shown by *T. harzianum* or *A. migulanus.*

Key words: GA_3, IAA, ABA, *Trichoderma harzianum*, *Aneurinobacillus migulanus*, Gladiolus

INTRODUCTION

Plant growth regulators (PGRs) are organic compounds naturally occurring in plants, which regulate many processes in plant development. Since the discovery of PGRs and their varying effects, it has become evident that these compounds play an important role in plant-pathogen interactions **(Arteca, 1996)**.

Gibberellins influence various developmental processes, including stem elongation, germination, dormancy, flowering, sex expression, enzyme induction, and leaf and fruit senescence. Gibberellic $acid_3$ (GA_3) helps in promoting plant growth and cell elongation and also stimulates the cells of germinating seeds to produce mRNA coding for hydrolytic enzymes.

Indole-3-acetic acid (IAA) is the main auxin in plants, controlling many important physiological processes including cell enlargement and division, tissue differentiation, and responses to light and gravity **(Leveau, and Lindow, 2004)**. IAA and phenol compounds are known to play a key role in Fusarium wilt banana **(Fernandez-Falcon *et al*., 2003).**

Abscisic acid (ABA) is crucial in plant protection in a number of ways (1) it causes the stimulation of stomata closure (water stress brings about an increase in ABA synthesis); (2) inhibits shoot

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ISSN:2572-3006(Print)2572-3111(Online)
http://www.futurejournals.org

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growth; (3) induce the synthesis of storage proteins in seed, and (4) antagonizes the stimulatory effect of gibberellins on α-amylase and has some effect on the induction and maintenance of dormancy. ABA also induces gene transcription particularly for proteinase inhibitors in response to wounding which may explain an apparent role in defense against pathogen attack **(Davies, 1987; Hansen and Grosmann, 2000).** ABA is also produced in the roots in response to stresses, including pathogen attack **(Davies, 1987).** ABA is involved in the wound-induced activation of the proteinase inhibitor II gene **(Davies, 1987)**, a hypothesis compatible the model suggesting that this hormone is the mediator of the systemic wound response in tomato and potato **(Gemishev *et al*., 2005)**. The role of ABA on plant resistance pathways is more complicated. ABA has been implicated in both biotroph and necrotroph susceptibilities. Therefore, it seems that this hormone acts as a negative regulator of plant defense. In addition, ABA plays a crucial role in response to abiotic stress. The two opposite roles of ABA on biotic and abiotic stresses lead to the hypothesis that plants prioritize the response towards abiotic stress over the response towards biotic stress. It is worth noting that ABA has also been associated with disease resistance. However, our understanding of its role in defense is rather limited **(Lopez *et al*., 2008).**

Plants respond to pathogen attack via a complex and integrated set of responses encompassing both constitutive and induced factors. Interactions between plants and pathogens initiate a series of events in plants, including production of signaling compounds such as ethylene, salicylic acid, jasmonic acid, and/or reactive oxygen species **(Dowd *et al*., 2004)**. The response also clearly involves gene activation in the host plant, sometimes at sites remote from the point of wounding or infection **(Pēna-Cortés *et al*., 1989)** Disease affects plant growth and development of the plant by impacting on, amongst other regulatory processes, the PGRs through several possible actions including interfering with synthesis, degrading the PGRs or producing the PGRs directly **(Agrios, 1997).** The nature and rate of PGR production by a pathogenic micro-organism, however, may differ in vitro compared to in vivo. Information on the abilities of pathogens to produce PGRs under *in vitro* conditions, therefore, may give little insight into the situation occurring within the diseased plant **(Agrios, 1997)**.

Some fungal pathogens are able not only to induce increasing IAA synthesis in their respective hosts, but also to produce IAA which may be directly released into the soil **(Agrios, 1997)**. When plants encounter an invading pathogen, not only responses signaled by defense hormones are activated to restrict pathogen invasion, but also the modulation of additional hormone pathways is required to serve other purposes, which are equally important for plant survival, such as re-allocation of resources, control of cell death, regulation of water stress, and modification of plant architecture. Notably, pathogens can counteract both types of responses as a strategy to enhance virulence **(Lopz *et al* 2008)**.

Trichoderma species have long been recognized as biological control agents (BCAs) for the control of plant diseases and for their ability to increase plant growth and development. These fungi are known to be involved in complex interactions with host plants and resident microbial communities. The mechanisms involved in the antagonism of Trichoderma toward phytopathogenic fungi include competition, antibiosis, direct mycoparasitism, and induction of systemic resistance to pathogens in planta. **(Harman and Kubicek, 1998)**.However, the mechanisms by which *Trichoderma* sp. stimulate plant development remain unclear, but may involve the production of growth-promoting metabolites released close to the plant root

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system **(Chang *et al*., 1986; Windham *et al*., 1986)** and an increase in the uptake of nutrients by the roots as a result of the plant–*Trichoderma* interaction **(Altomare *et al*., 1999; Yedidia *et al*., 2001).** *T. harzianum* appears to use several mechanisms in the enhancement of plant growth, including (1) solubilization of MnO_2, metallic zinc, and rock PO_4^{2-} (mostly Ca_2PO_4); (2) production of organic acid; (3) chelation of Iron and (4) production of PGRs-like metabolites **(Altomare *et al*., 1999).**

Plant hormones play important roles in regulating developmental processes and signalling networks involved in plant responses to a wide range of biotic and abiotic stresses. The roles of other hormones such as abscisic acid (ABA), auxin, gibberellin (GA), cytokinin (CK) and brassinosteroid (BL) in plant defense are less well known. Much progress has been made in understanding plant hormone signalling and plant disease resistance. However, these studies have mostly proceeded independently of each other, and there is limited knowledge regarding interactions between plant hormone-mediated signalling and responses to various pathogens **(Robert-Seilaniantz, *et al*., 2007).**

There is little information on the literature on the role of plant growth regulators during the multitrophic interactions involved in the biological control of plant diseases. Most previous studies on the mechanisms of action of BCAs focus on the ability of these beneficial organisms to increase nutrient solubility and on the enzymes induced in host plants by BCAs **(Fernández-Falcón *et al*., 2003; Pryce-jones *et al*., 1999)**. These activities are related to plant growth stimulation but, given the role of PGRs in plant growth and development, it is pertinent to determine the impacts of beneficial microorganisms on concentrations of these substances in plant. The aim of the work reported here, therefore, was to determine the effects of *T. harzianum* and *A. migulanus* on PGR concentrations in *Gladiolus grandiflorus*.

MATERIALS AND METHODS

Preparation of pathogen and antagonist inoculate

The isolate of *F. oxysporum* f. sp. *gladioli*, G010 was maintained on PDA (Oxoid, Basingtoke, Hants, UK) at 22° C and routinely sub–cultured at 15 day intervals. Subcultures were prepared by inoculating PDA with 1 cm diam. disks of colonized PDA plus mycelium, cut from the edge of an actively growing, 7 day old colony.

Trichoderma harzianum isolate T22, obtained from CBS, was cultured on PDA. Petri dishes were sealed with Parafilm, and incubated at 22° C, with routine sub–culturing at 15 day intervals. Spore suspensions were obtained by flooding 7 day old cultures on PDA with 5 ml sterile distilled water, gently agitating the surface with a wire loop and passing the suspension through two layers of washed sterile muslin cloth directly into 50 ml centrifuge tubes. Spores were centrifuged at 1700 x ***g*** in a Thomson–MSE Mistral bench top centrifuge for 10 min, the spore pellets rinsed twice in sterile distilled water, with repeated centrifuging and spore concentrations adjusted to 8×10^6 ml^{-1} using repeated haemocytometer counts.

The isolate of *A. migulanus* was obtained from laboratory stocks and maintained on nutrient agar (NA; Oxoid, Basingstoke, Hants, UK) at 35° C, with routine sub-culturing at 15 day intervals. Cultures of *A. migulanus* were prepared by transferring approximately 1 ml of cell suspension from a 24 hour old liquid culture in tryptic soya broth (TSB; Oxoid, Basingstoke, Hants, UK) to 20 ml fresh TSB in 250 ml conical flasks. Flasks were incubated at 37° C on a rotary shaker at 150 rpm for 24 h. The suspension was centrifuged at 1700 x ***g*** for 10 min, resuspended in 15

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ml quarter-strength Ringers solution (Sigma, UK) and washed 3 times in fresh Ringers solution by re-centrifuging, as described above. Spore densities were estimated as colony forming units on NA following by serial dilutions to 5×10^8.

Pathogen inoculation

Gladiolus corms were inoculated *F. oxysporum* f. sp. *gladioli* by removing 10 mm- diam. and 5 mm- depth piece of tissue from the side of the corm and replacing with a plug of inoculated PDA + fungal mycelium of the same dimensions, this method was modified from **Cappelli and Minco (1998).** The lesion area was calculated based on the height and width of the lesion. The lesion criteria were determined by the rotting and softening of the corm.

Antagonist inoculation

T. harzianum or *A. migulanus* were inoculated according to a modified method from **(Elad *et al*., 1982)**. Ten corms were prepared for each treatment. Corms were submerged in *T. harzianum, A. migulanus* spore suspensions for 30 min. For the interaction treatments, corms were suspended in the antagonist suspension, blotted dry on sterilized filter paper under aseptic conditions in a laminar flow cabinet. The combination between *T. harzianum* and *A. migulanus* was prepared by mixing the same volume of suspension of antagonist in beaker, immersing surface sterilized corms in the mixed suspension for 30 min and inoculating with the pathogen, as described above. Control corms were immersed in sterilized distilled water for the same time. Inoculated corms were incubated at 25° C.

Corms inoculation

Gladiolus corms were inoculated under aseptic conditions and incubated at 25° C. Five replicated from each treatment were collected after 2 days of inoculation and the samples were collected at 2 days intervals.

Extraction of Plant growth regulators

Extractions of plant growth regulators from the infected Gladiolus corms were performed with modified methods of **Yeang *et al*. (2001)** and **Durgbanshi *et al*. (2005)**. Briefly, 5 replicated of the treated corms were collected at 2-day intervals for 10 days. The tissue was frozen at -80° C until required. Frozen tissues were homogenized in liquid N_2 using a chilled mortar and pestle. The weight of each replicate was adjusted to be 20 g/ replicate. The weighted and homogenized tissue were added to 250 ml glass flasks and three volumes of dichloromethane (50 ml) were added and the extract sonicated for 30 min using a Bransonis 2510 sonicator (Branson Co., Danbury, USA). The extract was filtered twice through muslin cloth and the organic phase separated from the aqueous phase in a 100 ml separatign funnel. The pH of the organic phase was adjusted to 3.5 using 1N HCl and dried using a rotary evaporator (Buchi Rotavapor R-200, Switzerland) at 40°C. The resulting residue was re-dissolved in 1.0 ml of methanol and kept frozen at -20° C until analysis.

Liquid chromatography-mass spectrometry (LC-MS)

One sample from each treatment was subjected to (LC-MS) was obtained from a Thermo Instruments MS system (LTQ XL/ LTQ Orbitrap Discovery) coupled to a Thermo Instruments HPLC system (Accela PDA detector, Accela PDA autosampler and Accela Pump). The following conditions were used: capillary voltage 45 V, capillary temperature 260 ℃, auxiliary gas flow rate 10-20 arbitrary units, sheath gas flow rate 40-50 arbitrary units, spray voltage 4.5 kV, mass range 100 – 2000 amu (maximum resolution 30000).

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High Performance Liquid Chromatography

High performance liquid chromatography (HPLC) was carried out on a C18 HD analytical column (250 mm x 4 mm; Agilent, USA) using an Agilent Series 1100 LC pump, connected to a Jasco MD-910 multiwave length detector (Jasco International Co. Ltd.) at room temperature. Samples (200 µl) were eluted in a linear gradient of 20 - 80% acetonitrile and water acidified with 0.1% trifluoroacetic acid (Sigma- Aldrich) over 30 min..GA_3, ABA, IAA were detected by monitoring absorbance of the eluate at 208, 265 and 280 nm, respectively, at a flow rate of 1 ml/ min.T he retention times of GA_3, IAA, ABA were 12, 17 and 21 min, respectively.

Quantitation of Plant Growth Regulators

To prepare calibration curves, a modified method of **Chiwocha *et al*. (2003)** was used. PGRs were dissolved in methanol to prepares stock solution. 100 mg of each PGRs were dissolved in 1 ml methanol to prepare stock solutions for standard curve calibration. in to 5-ml volumetric flasks. The standard solutions were made up to 5 ml with 1:1 (v/v) acetonitrile – water containing 5% v/v glacial acetic acid. Each of the five standards was dried using a continuous air flow. The standards were reconstituted in 1000 µl methanol, sonicated and centrifuged at 1700 x ***g*** in a Thomson–MSE Mistral bench top centrifuge for 10 min. Aliquots of(20, 50, 100, 150, and 200 µl) of each PGR was injected into the HPLC system and the peak area was calculated automatically by Agilent 1200 software. Calibration curves were created for the three PGRs by plotting concentration against peak area.

Statistical analysis

Experiments were organized in complete block designs. Statistical analyses were conducted using the general linear model procedures of SPSS version 15. Experiments were analyzed using analysis of variance (ANOVA). Significance was evaluated at $P<0.05$ for all tests. Mean separation was tested using the Tukey HSD test.

RESULTS

GA_3 concentrations increased linearly after inoculation of corms with *T. harzianum* and *A. migulanus*. In contrast, corms inoculated with *F. oxysporum* f. sp. *gladioli* contained significant less in GA_3 compared with other treatments (Figure, 5.1; $P< 0.001$). Corms treated with both *T. harzianum* and *A. migulanus* had significantly higher contents of GA_3. Between days 2 and 8, GA_3 concentrations in corms treated with *T.harzianum* or *A. migulanus*, followed by inoculation with *F. oxysporum* f. sp. *gladioli* were significantly higher than in corms treated with *F. oxysporum* f. sp. *gladioli* alone, or in control corms ($P< 0.001$). The GA_3 concentrations in corms treated with *A. migulanus* followed by inoculation with *F. oxysporum* f. sp. *gladioli*, however, were significantly lower than in those inoculated with *A. migulanus* alone ($P>0.05$). Corms treated with both antagonists prior to inoculation with *F. oxysporum* f. sp. *gladioli* had a significantly higher GA_3 concentration than those treated with *A. migulanus* alone ($P<0.001$).

IAA concentration increased linearly with time in all treatments (Figure 5.2; $P> 0.001$). Corms treated with *T. harzianum* alone had the highest IAA contents throughout the time course of the experiment (Figure 2 & 4; $P< 0.001$). In contrast, concentrations of IAA were lowest in corms inoculated with *F.oxysporum* f. sp. *gladioli* alone, compared with all other treatments ($P<0.001$). Treatment of corms with *T. harzianum* or *A. migulanus* followed by inoculation with *F. oxysporum* f. sp. *gladioli* lead to significantly higher concentrations of IAA than in corms inoculated with *F. oxysporum* f. sp. *gladioli* alone. Corms treated

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with both antagonists prior to inoculation with *F. oxysporum* f. sp. *gladioli* also had higher IAA concentrations compared with those treated with *A. migulanus* alone prior to pathogen inoculation.

ABA concentrations remained nearly constant in all treatments during the 10 days incubation period. Corms inoculated with *F. oxysporum* f. sp. *gladioli* alone contained significantly greater concentrations of ABA compared with all other treatments ($P < 0.001$). ABA concentrations in corms treated with *T.harzianum* or *A. migulanus* followed by inoculation with *F. oxysporum* f. sp. *gladioli* were significantly lower than in corms inoculated with *F. oxysporum* f. sp. *gladioli* alone ($P>0.001$). In contrast, however, ABA concentrations in corms treated with *A. migulanus* followed by inoculation with *F. oxysporum* f. sp. *gladioli* were significantly higher than those in corms treated with *A. migulanus* alone. No significant differences were observed between corms treated with *T. harzianum* alone, *A. migulanus* alone, *T. harzianum* and *F. oxysporum* f.sp. *gladioli* or the controls ($P>0.05$).

DISCCUSION

This is the first paper to report the impacts of the application of *Trichoderma harzianum* and/or *Aneurinobacillus migulanus* on the concentrations of plant growth regulators in host plant tissues. Most previous work on the use of *Trichoderma* spp. as a biological control agent has concentrated on its efficiency as an antagonist against soil-borne and aerial pathogens (e.g. **Elad *et al.*, 1981; Elad, 2000)**, without considering the effects on host plant physiology. Work on *A. migulanus* has focused on its ability to control pathogens of aerial plant parts, such as mildews and grey mould **(Edwards, and Seddon, 1992).**

Knowledge of the actual mechanisms involved in the reduction of disease incidence following corm treatment with Trichoderma spp. is still incomplete. In the past, most studies have focused on microbial interactions in the rhizosphere, not on the possible impacts of the BCAs on the host plant, although increased plant growth response with penetration of T. harzianum into the root system has been demonstrated **(Yedida et al., 1999)**. The results of the present work support the previously reported mechanisms responsible for the influence of Trichoderma treatments on plant development, such as the production of plant growth regulators **(Inbar *et al.*, 1994)**. Additional mechanisms of action include an improvement in the solubility of minor nutrients in Trichoderma-treated soils **(Harman and Bjorkman, 1998)**, which can support an increase in the uptake and translocation of less available minerals and improvements in plant growth. Also, this work results support the findings of **O'Donnell *et al.*, (2003),** and **Schmelz *et al.*, (2003)** when quantified the hormone content in Arabidopsis after challenge with two virulent pathogens: Xanthomonas campestris pv. campestris and Pseudomonas syringae pv. tomato DC3000 (PstDC3000). They found an increase of the usual stress hormones such as SA, JA and ET, they found rapidly elevated levels of auxin and induced auxin biosynthetic genes in comparison with mock-inoculated plants. They showed that in the same conditions, the same pathogen also induced abscisic acid (ABA). The induction of auxin or auxin-related phenomena in a susceptible interaction is not an isolated case or a consequence of plant cell disorder.The ability of *A. migulanus* on increasing Trifolium repens L. growth and inhancing the plant Cd tolerance. Indole acetic acid produced by B. brevis may be related to its ability for improving root growth, nodule production and AM fungal intra and extraradical development

ISSN:2572-3006(Print)2572-3111(Online)
http://www.futurejournals.org

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Different regulatory pathways play roles in the plant-pathogen interaction. For example, when plants encounter an invading pathogen, not only are responses signaled by defense regulators activated to restrict pathogen invasion, but also modulation of additional regulatory pathways occurs for other purposes, of equal importance for plant survival, such as re-allocation of resources, control of cell death, regulation of water stress and modification of plant architecture **(Lopez *et al*., 2008)**. Pathogens may counteract both types of responses as a strategy to enhance virulence **(Lopez *et al*., 2008)**. Pathogens may alter the production and signaling responses of plant growth regulators during infection, and also produce PGRs themselves which also modulate plant responses **(Lopez *et al*., 2008)**. The findings of **Lopez *et al*. (2008)** indicate that growth regulator signaling is a relevant component in plant-pathogen interactions, and that the ability to dictate hormonal directionality is critical to the outcome of an interaction.

The results reported in this Manuscript shows the complex interaction effect of T. *harzianum* and *A. migulanus* on gladiolus growth and stimulating plant defense through different mechanisms , improving plant growth and plant health are one of them, which in agree with **Harberd, (2003)** when reported GA seems to have an opposite effect on plant defense. GA promotes plant growth by inducing the degradation of the DELLA proteins, which are negative regulators of plant growth. The recent studies demonstrated that loss-of-function mutations in DELLA proteins render the plant more resistant to PstDC3000 through potentiation of the SA-dependent defense pathway **(Harberd, 2003).** So, Increases in plant growth and development following treatment with BCAs may result from control of deleterious root microflora, including those not causing obvious disease **(Roco *et al*., 2001)**, direct production of growth-stimulating factors (*i.e*., PGRs or other growth factors), increased nutrient uptake through enhanced root growth or promoted availability of essential nutrients. Also, **Elad *et al*., (1986)** reported the plant growth promotion by bacteria and fungi may be involved in the control of minor pathogens in the rhizosphere or production of PGRs **(Cheng *et al*., 2002)** or nutrient release from soil or organic matter **(Christian *et al*, 1998)**, or increased uptake and translocation of minerals **(Lo *et al*., 1997).**

T. harzianum and *A. migulanus* had strong stimulatory actions on Gladiolus growth and flower production. Although the mechanisms of this stimulation are not known, it is likely that the alterations in growth regulator concentrations shown in the work reported in this chapter are involved in the enhanced growth response. The efficiency of *T. harzianum* in promoting root and vegetative growth has been demonstrated previously in other crops. For example, **Altomare *et al*. (1999)** reported that an isolate of *T. harzianum* increased both root and shoot growth in Maize. The enhanced growth and flower quality observed in Gladiolus may result from a similar improvement of root development factors, stimulated by auxin production.

The efficiency of *Trichoderma* spp. as a plant growth promoting agent has been reported in several studies on various plant species, although efficiency was not always consistent **(Ousley *et al*. 1994; Avni *et al*., 1994, and Dissevelt and Ravensberg 2002),** which in agree with the results reported in chapter 8 and could explain the results obtained in this chapter about the role of *T. harzianum* and *A. migulanus* in PGRs stimulation. The production of plant growth hormones or analogues is another mechanism by which strains of Trichoderma can enhance plant growth. 162 species of fungi have been reported to produce auxins, which are key hormones effecting plant growth and development that can be produced by fungi in both symbiotic and pathogenic interactions

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with plants **(Gravel *et al*., 2007; Losane and Kumar, 1992; Patten and Glick, 2002).** Sixty percent of the Trichoderma strains we studied produced IAA or analogues **(Hoyos-Carvajal *et al*., 2009).**

ABA has also been associated with disease resistance. ABA-deficient mutants have been found to be insensitive to b-aminobutyric acid (ABA)-induced resistance. The mechanism by which ABA induces resistance against a pathogen is still unclear.ABA treatment mimicked the priming effect of BABA-induced resistance **(Ton and Mauch-Mani, 2004).** One possible explanation for the results obtained in this chapter and in chapter 8 is the timing of the infection. ABA controls stomatal closure and therefore controls the entry of the bacteria into the plant. Inside the plant, pathogenic bacteria need to maintain a high water potential in order to establish disease. The other well established role of ABA in water stress conditions or diseases conditions may also explain the effect of this hormone on plant susceptibility **(Ton and Mauch-Mani, 2004)**. **de Torres-Zabala *et al*., (2007)** showed that bacterial effectors delivered into plant cells enhanced susceptibility by increasing ABA production and activating of ABA-responsive genes. In these studies ABA enhanced bacterial growth by attenuating basal defense, and ABA induction and suppression of basal defense transcripts could be mimicked by the in planta expression of the bacterial effector avrPtoB. ABA may thus have different actions at different infection steps, favoring resistance during pre-invasion, and susceptibility at later stages of colonization.

The network of interactions between hormones cannot be overlooked to fully understand the tight interaction between a host and its pathogen. Often a coordinated range of hormones is necessary to achieve the proper response **(Schenh *et al*., 2000)**. **Nemhauser *et al*., (2006)** found the time course of the experiment will probably dictate the hormonal response observed and that could be a reason in this study. Pathogen-induced hormone disruption should be viewed as an imbalance in a network where every component will be affected, rather than as an interaction between two pathways. Much work is needed to understand the impact of each hormone on the disease pathway and to discover the nodes where the interactions occur

The current work used plants grown in a soilless culture system, where soluble nutrients were provided in a recycled solution. The results of this study, however, have furthered understanding of how treatment with *T. harzianum* (and *A. migulanus*) enhanced growth of *Gladiolus*. But the mechanisms of action of these BCAs in this respect require further investigation, including work on the relationship between the effect of *F. oxysporum* f. sp. *gladioli*, *T. harzianum*, and *A. migulanus* secondary metabolites on PGR production by *Gladiolus*.

REFERENCES

Agrios, N. (1997). Plant Pathology. Fifth edition, Academic Press, New York, USA. 922 pp. ISPN 0-12-044564-6.

Altomare, C.; Norvell, W.; Bjorkman, T. and Harman, G. (1999). Solubilization of phosphates and micronutrients by the plant-growth-promoting and biocontrol fungus *Trichoderma harzianum* Rifai 1295-22. Applied and Environmental Microbiology, **65:** 2926-2933.

Arteca, R. (1996). Plant growth substances: principles and applications. Kluwer Academic Pub. USA. 332pp. ISPN 0-412-03911-7.

Avni, A.; Bailey, B.; Mattoo, A. and Anderson, J. (1994). Induction of ethylene biosynthesis in Nicotiana tabacum by a *Trichoderma viride* xylanase is correlated to the accumulation of 1-aminocyclopropane-1-carboxylic acid (ACC) synthase and ACC oxidase transcripts. Plant Physiology, **106:**1049-1055.

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Bailey, A.; Dean, D. and Anderson, D. (1990). An Ethylene Biosynthesis-Inducing Endoxylanase Elicits Electrolyte Leakage and Necrosis in *Nicotiana tabacum* cv Xanthi Leaves 1. Plant Physiology, **94:**1849-1854.

Barea, M.; Azcón, R. and Azcón-Aguilar, C. (2002). Mycorrhizosphere interactions to improve plant fitness and soil quality, Antonie van Leeuwenkoek, **81** :343–351.

Chang, C,; Baker, R.; Kleifeld, O. and Chet, I. (1986). Increased growth of plants in the presence of the biological control agent *Trichoderma harzianum*. *Plant Disease*, **70**:45–148.

Christian,L.; Harman, E. and Ondik , K. (1998). *Trichoderma* and *Gliocladium* for plant disease control and plant growth enhancement, p. 229–265. *In* C. K. Kubicek and G. E. Harman (ed.), *Trichoderma* and *Gliocladium*. Taylor and Francis, London, England.

Davies, J. (1987). The Plant Hormones: Their Nature, Occurrence, and Functions. *In*: Davies, P.J. (Ed.), Plant Hormones and Their Role in Plant Growth and Development. Martinus Nijhoff Publishers, USA.

de Torres-Zabala, M.; Truman, W.; Bennett, M.H.; Lafforgue, G.; Mansfield, J.W.; Rodriguez Egea, P.; Bogre, L. and Grant, M. (2007). *Pseudomonas syringae* pv. *tomato* hijacks the Arabidopsis abscisic acid signalling pathway to cause disease. EMBO Journal, **26**:1434-1443.

Dowd, C.; Wilson, W. and Mcfadden, H. (2004). Gene expression profile changes in cotton root and hypocotyl tissues in response to infection with *Fusarium oxysporum* f. sp. *vasinfectum*. *Molecular Plant-Microbe Interactions*, **17:**654-667.

Durgbanshi, A.; Arbona, V.; Pozo, O.; Miersch, O.; Sancho, J.V. and Gomez-Cadenas, A. (2005). Simultaneous Determination of Multiple Phytohormones in Plant Extracts by Liquid Chromatography− Electrospray Tandem Mass Spectrometry. J. Agric. Food Chem, **53:** 8437-8442.

Edwards, S.G. and Seddon, B. (1992). Bacillus brevis as a biocontrol agent against Botrytis cinerea on protected Chinese cabbage. In: Verhoeff K, Malathrakis NE and Williamson B (eds) Recent Advances in Botrytis Research. (pp. 267-271) Pudoc Scientific Publishers, Wageningen. The Netherlands.

Elad, Y. (2000). Biological control of foliar pathogens by means of *Trichoderma harzianum* and potential modes of action. Crop Protection, 19: 709-714.

Elad, Y.; Chet, I. and Henis, Y. (1981). Biological control of *Rhizoctonia solani* in strawberry fields by *Trichoderma harzianum*. Plant and Soil, 60: 245-254.

Fernández-Falcón, M.; Borges, A. and Borges-Pérez, A. (2003). Induced resistance to Fusarium wilt of banana by exogenous applications of indoleacetic acid. *Phytoprotection*, **84:**149-153.

Gemishev, O.T.; Vaseva, I.I. and Atev, A.P. (2005). Abscisic acid and ethylen influence on endo-1,4-B-glucanase activity in trichoderma reesei I-27. Biotechnology and Biotechnological Equipment, **19**; 106-112.

Gravel, V.; Antoun, H. and Tweddell, R.J. (2007). Growth stimulation and fruit yield improvement of greenhouse tomato plants by inoculation with Pseudomonas putida or Trichoderma atroviride: possible role of indole acetic acid (IAA). Soil Biology and Biochemistry, 39: 1968–1977.

Hansen, H. and Grossmann, K. (2000). Auxin-induced enthylene triggers abscisic acid biosynthesis and growth inhibition. Plant Physiology, **124**: 1437–1448.

Harberd, N. P. (2003). Botany. Relieving DELLA restraint. Science, 299:1853-1854.

Harman, G. E. and Kubicek, C. P. (1998). Trichoderma *and* Gliocladium Vol. 2 (Taylor and Francis, London, 1998).

Hoyos-Carvajal, L.; Orduz, S. and Bissett, J. (2009). Growth stimulation in bean (*Phaseolus vulgaris* L.) by Trichoderma. Biological Control, **51**: 409-416.

Inbar, J. and Chet, I. (1997). Lectins and biocontrol. Critical Reviews in Biotechnology, **17:**1–20.

Inbar, M.; Abramsky, D.; Cohen, J. and Chet., I. (1994). Plant growth enhancement and disease control by *Trichoderma harzianum* in vegetable seedlings grown under commercial conditions. European Journal of Plant Pathology, **100**: 337-346.

Leveau, J. and Lindow, S. (2004). Utilization of the Plant Hormone Indole-3-Acetic Acid for Growth by *Pseudomonas putida* Strain 1290. Applied and environmental microbiology, **71**: 2365–2371.

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2019;3:44-53

Lo, C. (1997). Improved biocontrol efficiency of *T.harzianum* for foliar Phases of Turf Disease by Use of Spry Applications. Plant disease, **81**: 1132-1138.

Lopez, M.A.; Bannenberg, G. and Castresana, C. (2008). Controlling hormone signaling is a plant and pathogen challenge for growth and survival. Current Opinion in Plant Biology, **11**(4), 420-427.

Losane, B.K. and Kumar, P.K.R. (1992). Fungal plant growth regulators. In: Arora, D.K., Elander, K.G., Mujerji, K.G. (Eds.), Handbook of Applied Mycology: Fungal Biotechnology, vol. 4. pp. 565–602.

Nemhauser, J.L.; Hong, F. and Chory, J. (2006). Different plant hormones regulate similar processes through largely nonoverlapping transcriptional responses. Cell, **126**(3):467-475.

O'Donnell, P.J.; Schmelz, E.A.; Moussatche, P.; Lund, S.T.; Jones, J.B. and Klee, H.J. (2003). Susceptible to intolerance —a range of hormonal actions in a susceptible Arabidopsis pathogen response. Plant Journal, 33:245-257

Patten, C.L. and Glick, B.R. (2002). Role of *Pseudomonas putida* indoleacetic acid in development of the host plant root system. Applied and Environmental Microbiology, **68**: 3795–3801.

Pēna-Cortés, H.; Sánchez-Serrano, J.J.; Mertens, R.; Willmitzer, L. and Prat, S. (1989). Abscisic acid is involved in the wound-induced expression of the proteinase inhibitor II gene in potato and tomato. Proceedings of the National Academy of Sciences of the United States of America, **86:**,9851.

Pryce-Jones, E.; Carver, T. and Gurr, S.J. (1999). The roles of cellulase enzymes and mechanical force in host penetration by Erysiphe graminis f. sp. hordei. Physiological and Molecular Plant Pathology, **55:** 175-182.

Robert- Seilaniantz, A.; Navarro, L.; Bari, R. and Jones, J. (2007). Pathological hormones imbalances. Plant Biology, **8**: 372-379.

Roco, A. and Pérez, M. (2001). In vitro biocontrol activity of *Trichoderma harzianum* on Alternaria alternata in the presence of growth regulators. Electronic Journal of Biotechnology, **4:**1-2.

Schenk, P.M.; Kazan, K.; Wilson, I.; Anderson, J.P.; Richmond, T.; Somerville, SC. and Manners, J.M. (2000). Coordinated plant defense responses in Arabidopsis revealed by microarray analysis. Proceedings of the National Academy of Sciences, **97**:11655-11660.

Schmelz, E.A.; Engelberth, J.; Alborn, H.T.; O'Donnell, P.; Sammons, M.; Toshima, H. and Tumlinson, J.H. (2003). Simultaneous analysis of phytohormones, phytotoxins, and volatile organic compounds in plants. Proceedings of the National Academy of Sciences, **100**:10552-10557.

Ton, J. and Mauch-Mani, B. (2004). B-amino-butyric acid-induced resistance against necrotrophic pathogens is based on ABA-dependent priming for callose. Plant Journal, **38**:119–130.

Van, K.; Hulzink, R.; Barense, M. and Wullems, J. (2001). The expression of tgas118, encoding a defensin in Lycopersicon esculentum, is regulated by gibberellin. Journal of Experimental Botany, **52**:1427–1436.

Vivas, A.; Barea, J.M. and Azcon, R. (2005). Interactive effect of Brevibacillus brevis and Glomus mosseae, both isolated from Cd contaminated soil, on plant growth, physiological mycorrhizal fungal characteristics and soil enzymatic activities in Cd polluted soil. Environmental pollution (Barking, Essex : 1987), 134:257-266.

Windham, T.; Elad, Y. and Baker, A. (1986). mechanism for increased plant growth induced by *Trichoderma* spp. Phytopathology, **6**:518–521.

Yeang, J.; Zhang, J.; Wang, Z.; Zhu, Q. and Wang, W. (2001). Hormonal changes in the grains of rice subjected to water stress during grain filling. Plant Physiology, **127:** 315-323.

Yedidia, I.; Srivastva, K.; Kapulnik, Y. and Chet, I. (2001). Effect of *Trichoderma arzianum* on microelement concentrations and increased growth of cucumber plants. Plant Soil, **235**:235–242.

Yedidia, N.; Benhamou, I. and Chet, 1. (1999). Induction of Defense Responses in Cucumber Plants (*Cucumis sativus* L.) by the Biocontrol Agent *Trichoderma harzianum.* Applied and Environmental Microbiology, **65:** 1061–1070.