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Induced Systemic Resistance and Plant Responses to Fungal Biocontrol Agents

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Abstract

Biocontrol fungi (BCF) are agents that control plant diseases. These include the well-known *Trichoderma* spp. and the recently described *Sebacinales* spp. They have the ability to control numerous foliar, root, and fruit pathogens and even invertebrates such as nematodes. However, this is only a subset of their abilities. We now know that they also have the ability to ameliorate a wide range of abiotic stresses, and some of them can also alleviate physiological stresses such as seed aging. They can also enhance nutrient uptake in plants and can substantially increase nitrogen use efficiency in crops. These abilities may be more important to agriculture than disease control. Some strains also have abilities to improve photosynthetic efficiency and probably respiratory activities of plants. All of these capabilities are a consequence of their abilities to reprogram plant gene expression, probably through activation of a limited number of general plant pathways.

INTRODUCTION

BCF: biocontrol fungi

Biocontrol fungi (BCF) are beneficial organisms that reduce the negative effects of plant pathogens and promote positive responses in the plant. Recent data indicates that their abilities to control plant diseases are only a subset of their capabilities. They do control diseases and in addition have other benefits, including amelioration of intrinsic physiological stresses in seeds and alleviation of abiotic stresses. They can also improve photosynthetic efficiency, especially in plants subjected to various stresses. Finally, several fungi also increase nitrogen use efficiency in plants. As a consequence, plants treated with beneficial fungi may be larger and healthier and have greater yields than plants without them. Mechanisms by which these changes occur are becoming known.

Most of the early work on biocontrol of plant diseases by *Trichoderma* spp. revolved around the direct ability of these fungi to interact with soil pathogens. The specific mechanisms described were mycoparasitism, production of antibiotics, and competition for nutrients in the rhizosphere (23, 44, 51, 122). During the process of mycoparasitism, the fungi first locates target hyphae by probing with constitutively produced cell wall degrading enzymes (CWDEs) coupled with very sensitive detection of cell wall fragments released from target fungi (51, 109, 122, 137). Expression of fungitoxic CWDEs is induced, and these diffuse toward the target fungi and attack even before physical contact (16, 124, 137). This detection stimulates increased and directional growth toward the target fungus (16, 124, 137). Once the fungi come into contact, *Trichoderma* spp. attach and may coil around and form appresoria on the surface of the host (62). Enzymes and antibiotic substances are produced that kill and/or degrade the target hyphae and permit penetration of the *Trichoderma* strains. Both the enzymes and the antibiotics are strongly antifungal and are synergistic in their action (24, 58, 73, 92).

However, more recent findings indicate that a primary method of pathogen control occurs through the ability of the fungi to reprogram plant gene expression. As a consequence, induced systemic resistance (ISR) occurs. Genetic reprogramming also induces mechanisms in the plant that alleviate physiological and abiotic stresses and to improve plant nitrogen use efficiency (NUE). Significant progress in understanding how BCF interact directly with the plants has been achieved. This review describes new knowledge regarding BCF's abilities and the molecular mechanisms for induction of plant responses.

FORMATION OF THE INTERACTION AND LOCAL PLANT RESPONSES

Many *Trichoderma* strains colonize plant roots of dicots and monocots (55). During this process *Trichoderma* hyphae coil around the roots, form appresoria-like structures, and finally penetrate the root cortex (132). *Trichoderma* grows intercellularly in the root epidermis and cortex and induces the surrounding plant cells to deposit cell wall material and produce phenolic compounds. This plant reaction limits the *Trichoderma* growth inside the root (132). *Piriformospora indica*, the model system for *Sebacinales* fungi, has root colonization characteristics different from that of *Trichoderma* spp. These axenically culturable mycorhiza-like fungi (120) colonize the root elongation zone mainly intercellularly. However, the root differentiation zone is heavily infested by inter- and intracellular hyphae, and the majority of the hyphae are present in dead rhizodermal and cortical cells, which become completely filled with chlamydospores (30). *P. indica* seems to induce cell death by interfering with the host cell death machinery (30) and not by releasing cytotoxic molecules (91). Nevertheless, this does not provoke root-tissue necrotization and does not resemble pathogen-derived programmed cell death (30, 90, 91, 95, 126). Unlike *Trichoderma*, growth of *P. indica* within the root cortex does not induce visible cell wall reinforcement (91, 111, 127). *P. indica* may even induce

production of gibberellins as part of modulation of plant defenses in the roots (91). Other mechanisms are employed by the plant to restrict the growth of this fungus within roots, and recent studies implicate a β-glucosidase, PYK10, and perhaps germines in this process (91, 101), as well as salicylic acid (111). When inside plant roots, fungi have access to plant nutrients, which allow them to proliferate. Moreover, they significantly enhance root growth in many cases (34, 47, 49, 54, 83, 116), thus providing more niches for growth of the fungi. The plants benefit from this relationship through increased root and shoot growth, increased macro- and micronutrient uptake, and protection from diseases (39, 47, 50, 51, 95, 100, 107, 132, 135). This interaction of BCF with the plant results in reprogramming plant transcriptome and proteome (3, 75, 91, 94, 104, 105, 127). Hence, this interaction is mutually beneficial. Given that *Trichoderma* spp. and other fungi are also capable of living freely in soil, they should be considered as opportunistic plant symbionts.

FUNGAL COMPOUNDS INVOLVED IN INDUCTION OF PLANT RESPONSES

Studies revealed many classes of compounds that are released by *Trichoderma* spp. into the zone of interaction and induce resistance in plants. The first class is proteins with enzymatic or other activity. Fungal proteins such as xylanase, cellulase, and swollenin are secreted by *Trichoderma* species (5, 38, 74, 76) but seem to induce only localized plant reactions and necrosis (8, 15, 76). *Trichoderma* endochitinase can also enhance defense, probably through induction of plant defense–related proteins (55, 73). Other proteins and peptides that are active in inducing terpenoid phytoalexin biosynthesis and peroxidase activity in cotton, e.g., the small protein, SM1, which has hydrophobin-like properties, were found to be produced by strains of *T. virens* (32, 33, 41). Another hydrophobin-like protein produced by T22 that induces both enhanced root development and disease resistance was identified (89). Another group of proteins that induce defense mechanisms in plants are the products of avirulence-like (Avr) genes (129, 130). These are not only produced by a variety of fungal and bacterial plant pathogens but also by BCF. They usually function as race- or pathovar-specific elicitors of hypersensitive and other defense-related responses in plant species that contain the corresponding resistance (R) gene. At least some of these fungal elicitors of plant defense response could be identified by plants as microbe-associated molecular patterns (MAMPs). This recognition plays a key role in innate immunity (11).

A different group of metabolites that induce plant defense mechanisms against pathogens are peptaibols. Peptaibols are a class of linear short-chain length (≤20 residues) peptides of fungal origin produced by the nonribosomal peptide synthase. The biological role of peptaibols has been demonstrated in few systems, and antimicrobial activity was reported (20, 25, 88, 92, 112). However, a growing number of reports indicate that peptaibols can elicit plant defense responses (21, 36, 123).

Another class of elicitors of plant defense includes oligosaccharides and low-molecular-weight compounds. These are released from fungal or plant cell walls by the activity of *Trichoderma* enzymes (51, 129, 130). Other small secondary metabolites produced by different *Trichoderma* strains were also isolated and shown to induce expression of pathogenesis-related (PR) proteins when applied to plants as well as reduce disease symptoms systemically (121). Less-characterized metabolites produced by other BCF induce resistance, induce lignifications at the site of pathogen infection, and elicit generation of reactive oxygen species (ROS) (67). Plant responses were also recorded for a cell wall extract from *P. indica*. However, these extracts promote growth but not defense responses (117). It appears that modulation of Ca^{2+} signal perception as well as H^+-signaling are an early step of plant cells response to the interaction with BCF metabolites (37, 80, 117).

Microbe-associated molecular pattern (MAMP): a motif or domain with conserved structural traits typical of whole classes of microbes but not present in their host

Reactive oxygen species (ROS): partially reduced or activated derivatives of oxygen [singlet oxygen ($^1O_2$), superoxide anion (O_2^-), hydrogen peroxide (H_2O_2), and hydroxyl radical ($HO^\cdot$)]

Systemic acquired resistance (SAR): salicylic acid mediated and triggered by necrotizing pathogens

Induced systemic resistance (ISR): mediated by jasmonic acid and ethylene signaling pathways and triggered by nonpathogenic microbes

INCREASED DISEASE RESISTANCE

Although mycoparasitism was considered to be highly important in many systems, antibiosis was the accepted mechanism for others such as the biocontrol of *Rhizocontia solani* by *T. virens* (58). However, a series of mutations in *T. virens* resulting in deficiency of mycoparasitic ability and/or inability to produce antibiotics had no effect on the biological activity of these strains (59). Instead, there was a very strong correlation between the abilities of these strains to induce terpenoid phytoalexin defense compounds in cotton seedlings and control of *R. solani*. Another classical biocontrol using *Trichoderma* spp. has been the control of seedrotting *Pythium* spp. by *T. harzianum* strain T22 (52, 61), in which mycoparasitism was considered the primary mechanism. However, it was recently demonstrated that control of *P. ultimum* on *Arabidopsis* seedlings by T22 required the *NPR1* gene, which is a key gene involved in disease resistance (F. Mastouri & G.E. Harman, unpublished data). These examples clearly demonstrate the importance of induction of plant responses by BCF. In other plant-pathogen systems, the effect clearly is via plant systemic response because BCF and pathogen are spatially separated (in dicotyledonous and monocotyledonous plants). Thus, any effect must be via systemic resistance. A range of pathogens were found to be controlled from fungi to oomycetes to bacteria and even one virus (2, 12, 28, 51, 54, 67, 72, 95, 111, 126, 127, 134). Systemic changes are frequently associated with enhanced levels of PR proteins and/or with accumulation of phytoalexin-type compounds (2, 32, 54, 70, 91, 127, 133, 134). *Trichoderma* spp. are not the only well-documented fungi that induce systemic resistance. *P. indica* has very similar capabilities (111, 127). Essentially, the data regarding induced resistance have dealt with disease control, but there is a good prospect that these systems may also increase resistance to, or enhance predation of, insect pests, especially because the ethylene/jasmonate pathway is involved in plant resistance to insects (93, 114). Similar pathways, such as the jasmonate/ethylene pathway of induced resistance, are induced by insect herbivory, so if this effect was enhanced by the presence of *Trichoderma*, then greater insect control would probably result. In addition, as described above, *Trichoderma* spp. have abilities to limit nematode damage (44, 98, 99). However, *Sebacinales* may increase growth performance at the expense of herbivore resistance (10).

PLANT SIGNALING PATHWAYS INDUCED BY BCF LEADING TO DISEASE RESISTANCE

Contact with pathogenic and nonpathogenic microorganisms triggers a wide range of defense mechanisms in plants. Two main mechanisms are recognized: systemic acquired resistance (SAR) and induced systemic resistance (ISR). SAR is usually triggered by local infection, provides long-term systemic resistance to subsequent pathogen attack, is correlated with the activation of PR genes, and requires the involvement of the signal molecule salicylic acid (SA) (35). ISR is known to result from colonization of roots by certain nonpathogenic rhizosphere bacteria (119). ISR is not SA-dependent, but rather requires components of the jasmonic acid (JA) signaling pathway followed by the ethylene signaling pathway.

The molecular mechanisms activated by *T. asperellum* in cucumber have been particularly well studied. Colonization of *T. asperellum* on roots induces resistance to *Pseudomonas syringae* pv. *lachrymans* (*Psl*) on foliage. During the process of the *Trichoderma* interaction with the plant, SA content did not differ from that of control plants, even though *Psl* infection did increase salicylate concentrations (106). However, the biocontrol activity of the organism was strongly reduced by diethyldithiocarbamic acid (DIECA), an inhibitor of JA production, or silver-thiosulfate (STS), an inhibitor of ethylene activity. These data strongly suggested that both JA and ethylene are required for the biocontrol activity of the fungi. Neither treatment affected colonization of roots by the fungus. However, ethylene content did not

differ between control and *Trichoderma*-inoculated plants, suggesting that although ethylene signaling is required, total ethylene levels did not change (106). This is similar to the finding that JA and ethylene are involved in ISR induced by rhizobacteria (84).

Further evidence for the involvement of JA and ethylene in transducing the signals from *Trichoderma*-inoculated roots to the leaves comes from gene expression studies. In roots, real-time reverse transcription polymerase chain reaction (RT-PCR) indicated that *Lox1*, which encodes a lipoxygenase involved in jasmonate synthesis and controls a feed-forward loop in jasmonate synthesis, was upregulated by inoculation with *T. asperellum*. *Lox1* is induced in the roots as early as 1 h post *Trichoderma* inoculation. A second peak was observed 24 h postinoculation, possibly resulting from the initiation of the octadecanoic pathway and the synthesis of JA. Another gene found to be upregulated by *Trichoderma* inoculation is *Pal1*, which encodes for phenylalanine ammonia-lyase (PAL) (104, 106). Activity of PAL was also shown to increase in sunflower by *Trichoderma* inoculation (70). *Pal1* is considered to be activated by JA/ethylene signaling during plant defense response. It catalyzes the first step of phenylpropanoid pathway, leading to production of phenolic compounds, including phytoalexins. The transient activation of this gene by *Trichoderma* could contribute to the accumulation of phytoalexins, leading further to a better defense of the plants against *Psl* infection.

Hydroperoxide-lyase (HPL) is another enzyme in the octadecanoic pathway. It utilizes some of the LOX products as its substrates, shifting them toward production of antimicrobial and wound-related compounds [also called green leaf volatiles (GLVs)] (134). *Trichoderma* induced *hpl* expression in leaves, but expression was much higher when *Psl* infection followed *Trichoderma* inoculation than with *Psl* infection only (134). This is similar to the priming effect described in the past for rhizobacteria-induced ISR (84, 85).

Ethylene response is considered to be downstream of JA response in rhizobacteria-mediated ISR. ETR1 and CTR1 proteins work together to negatively regulate the ethylene response pathway in the absence of ethylene (60). Ethylene binding to the receptor (ETR) downregulates the activity of this complex and results in derepression of the response pathway. In leaves of *Trichoderma* root-inoculated plants, there was a transitory increase in ETR1 expression followed by a reduction to below control levels, and the expression of CTR1 was almost abolished in plants inoculated with both organisms (106). This may enhance ethylene sensitivity in the leaves, leading to higher defense response in subsequent pathogen challenges. Thus, even though ethylene production did not increase after *Trichoderma* inoculation, the increased sensitivity to the ethylene signal could be the key for activation of this pathway. Interestingly, in roots both genes are induced by *Trichoderma*, suggesting ethylene response is inhibited. This local silencing of plant defense response probably enables symbiotic interactions, as has been observed for other symbiotic systems. Downregulation of the PR protein, PRMS, in roots by *Trichoderma* inoculation is in agreement with local silencing of defense to allow fungal growth into the roots (22). *P. indica* also seems to be able to silence root defense mechanisms and probably for the same reason. Shortly after barley root colonization with *P. indica*, defense-related genes were upregulated (91), but three weeks later no induction of defense-related genes was observed (127). However, ethylene signaling is required for *P. indica* colonization of the roots, as *Arabidopsis* mutants in ethylene signaling were less colonized (111).

Priming: activation of plant defense prior to contact with a challenging microbe

Further evidence for the induction of JA and ethylene signaling pathways by *Trichoderma* inoculation comes from other studies (68, 94) and from the study of *Trichoderma* elicitors. Engelberth et al. (36) showed that emission of ethylene, JA, and volatile compounds related to the octadecanoic signaling pathway are induced in lima bean plants treated with the peptaibol alamethicin from *T. viride*. In addition, Viterbo et al. (123) showed that the 18-residue peptaibols-induced expression of phenylalanine

ammonia-lyase (*pal*), hydroperoxide lyase (*hpl*), and peroxidase, which are involved in production of antimicrobial compounds, concomitant with a systemic increase in antimicrobial compounds content in the plant. The SM1-ISR was associated with notable induction of JA and GLV-biosynthetic genes (33), whereas genes involved in SAR were not induced. In the *T. asperellum*–cucumber system as well, the PR proteins chitinase, β-1, 3 glucanase, and peroxidase, which are known to be induced by SA, were not induced by *Trichoderma*, suggesting SAR is not involved. However, if leaves were subsequently inoculated with *Psl*, the expression of these PR genes was much higher than if the pathogen or the *Trichoderma* were used singly (106, 134), again exemplifying the priming phenomenon. This increase in enzyme level was also associated with an increase in phenolic glycoside levels; the aglycones of these materials are strongly antibiotic to a wide range of microorganisms, and *Psl* cell numbers in leaves from plants with both organisms were dramatically decreased compared with plants without *Trichoderma* treatment. Thus, the presence of *T. asperellum* primes the systemic resistance system, but the entire pathway is not constantly turned on. In a subsequent pathogen attack, the plant will react more strongly and/or more rapidly and hence will be more resistant. This implies that certain upstream regulatory genes are activated to provide a much more rapid response than would occur in its absence. The priming mechanism has also been reported in rhizobacteria-mediated ISR and by plant inoculation with *P. indica* (84, 85, 111, 127). However, it should be noted that in maize plants inoculated with *T. harzianum* strain T22, at least some PR proteins were constitutively turned on in the presence of the fungus even in the absence of any pathogen (104). Thus, priming may not occur universally in plant-*Trichoderma* interactions.

There is a striking similarity between plant response to *Trichoderma* spp. and *P. indica*. Recent studies show that *P. indica* may induce ISR through the JA/ethylene signaling pathway as well. *P. indica* root colonization reduced powdery mildew infection in *Arabidopsis* wild type and NahG mutant (unable to accumulate SA). However, two jasmonate signaling mutants were fully compromised in *P. indica*–mediated powdery mildew resistance even though their root colonization level did not differ from control plants (111). This indicated that systemic resistance response was independent of SA signaling, but required JA signaling for the process. In addition, *P. indica* colonization of barley roots exhibited no induction of SA biosynthesis genes, but enzymes involved in production of JA and ethylene, such as lipoxygenases and ACC oxidase, were induced (91). Priming was also exemplified in the *P. indica*–plant system for some genes (vsp1 and PR17b) (111, 127). The priming effect was even demonstrated for the pathogen-induced alkalinization response (37).

Recently, it was found that although wild-type strains of *Arabidopsis* were protected against *Pythium* seedling blight in the presence of *T. harzianum* T22, no protection was conferred to *npr1* mutants by T22 (F. Mastouri & G.E. Harman, unpublished data). Like T22, *P. indica* requires functional *NPR1* to induce resistance (111).

Transcription analysis of plant interaction with *T. hamatum* failed to detect induction of ISR markers, and only one marker of SAR (PR5) was upregulated (3). Therefore, it may be that some *Trichoderma* species use other mechanisms to induce plant defense.

PERCEPTION OF THE SIGNAL AND ACTIVATION OF MAPK SIGNALING CASCADE

The mechanisms by which these pathways are regulated are no doubt controlled by the interaction of the signal molecules from the BCF with particular plant receptor molecules in the interaction zone, which further activates a MAPK signaling cascade. Proteomic studies have shown that plant interaction with *Trichoderma* results in induction of NBS/leucine-rich repeat resistance protein–like proteins (75, 104). These are known to be part of the

specificity determinants of plant immune response, and when activated they trigger a cascade of signal transduction, which results in resistance response. In addition, *P. indica* failed to deliver its effects to *Arabidopsis* plants mutated in a gene coding for a leucine-rich repeat protein, and another receptor protein is required for this process (97). Thus, receptor proteins like these detect the fungus and further deliver the signal of perception.

One plant MAPK protein that is essential to signal transduction in the *T. asperellum*–cucumber system has been identified (102). This protein has been named *Trichoderma*-induced MAPK (TIPK). The gene is homologous to *WIPK*, *MPK3*, and *MPK3a* (from tobacco, *Arabidopsis*, and parsley, respectively). *TIPK* is induced by *Trichoderma* in the roots and leaves of cucumber plants. *TIPK* is also activated by pathogen challenge, but its expression was primed when plants were inoculated with *Trichoderma* prior to the pathogen challenge. A unique attenuated virus vector, zucchini yellow mosaic virus (ZYMV-AGII) was used to overexpress TIPK protein and antisense RNA. Plants overexpressing *TIPK* were more resistant to pathogenic bacterial attack than control plants, even in the absence of *T. asperellum* preinoculation. Conversely, plants expressing *TIPK*-antisense revealed increased sensitivity to pathogen attack. Moreover, *Trichoderma* preinoculation could not protect these antisense plants against subsequent pathogen attack. These results demonstrate that *T. asperellum* exerts its protective effect on plants through activation of the *TIPK* gene. Application of JA or SA or inhibitors of JA and ethylene revealed that *TIPK* operates upstream to the JA/ethylene signaling pathways. Using similar systems, it will be possible to discover regulatory proteins that act earlier in the system and link between *Trichoderma* elicitors interacting with plants receptors to the plant defense pathways activated.

Interestingly, the signal for growth requires *mpk6* because *Arabidopsis mpk6* mutants showed no growth enhancement in response to *P. indica* inoculation (115). However, the signal for defense response post *Trichoderma* inoculation is delivered through the *mpk3* homolog (102). Whether these two signaling pathways are destined for different plant responses or are different pathways activated by different fungi needs to be determined.

Induction of MAPK signaling cascade is known to induce regulatory proteins. Indeed, many proteomic and transcriptomic studies demonstrate systemic upregulation of regulatory genes and proteins, including RNA binding proteins, elongation factors, GTP binding proteins, and transcription factors (3, 7, 94, 104).

SYSTEMIC INDUCTION OF DEFENSE-RELATED GENES AND PROTEINS

Using proteomic analysis of maize plants inoculated with *T. harzianum* T22, a total of 205 differentially expressed spots, over both roots and shoots, were identified with more differences in the shoots than in the roots, even though T22 is present on roots (104, 105). Many proteins of defense/stress-related functions were upregulated. Stress response enzymes such as oxalate oxidase and superoxide dismutase (SOD) were upregulated in roots. In shoots, methionine synthases is highly upregulated. Methionine synthase forms methionine that can further be transformed into S-adenosyl-L-methionine, the precursor of ethylene, which provides additional evidence that ethylene-regulated systems are important in the plant-*Trichoderma* interaction. Other proteins that were upregulated in shoots include glutathione-S-transferase and glutathione-dependent formaldehyde dehydrogenase (FALDH), which act as detoxifying enzymes; peroxidase, a scavenging enzyme controlling the amount of damage resulting from the oxidative burst; heat shock proteins, which are also a known stress protein; oxalate oxidase, which was found to be involved in stress and defense responses and is probably involved in producing the oxidative burst of hydrogen peroxide; and others (7, 94, 104). Several PR proteins were also upregulated (75, 133).

Six-day-old maize seedlings grown from *T. harzianum* T22–treated seeds had elevated levels of proteins and increased activity levels of chitinase and β-1, 3 glucanase in both shoots and roots. Mostly, these activities were increased further when plants were coinfected with *Pythium ultimum* (54). Not only was total chitinase activity increased but also specific chitinase isozymes were specifically affected by root-inoculation with *Trichoderma* (103). Comparison of the interaction between plants and *Trichoderma* and plant-*Trichoderma*-pathogen indicated the activation of specific response to the biocontrol agent (75).

In T22-inoculated maize plants infected with *P. ultimum*, isoflavone reductase, which is involved in phytoalexin production, was also upregulated (22). The observed induction of phytoalexin accumulation in cucumber and sunflower (70, 134) and the upregulation of proteins involved in the process in maize (22, 104) indicate that this is a common response of defense induction by BCF.

A cell wall biosynthesis enzyme was upregulated in roots, which is not surprising considering that cell wall is being deposited at the site of *Trichoderma* inoculation. In shoots, cell wall metabolism is also activated: Sucrose synthase, UDP-Glc dehydrogenase, and UDP-glucuronate decarboxylase were upregulated, and all are part of the same metabolic pathway for production of cell wall material (hemicelluloses). This may benefit plant resistance by strengthening physical barriers in the shoots (104). Other cell wall metabolism–related proteins were differentially regulated by *Trichoderma* inoculation. Genes encoding extensin-like proteins were upregulated in tomato plants (3) but downregulated in cacao seedlings by several *Trichoderma* isolates (7). Extensin is also involved during rhizobia-mediated ISR. These proteins are involved in cell wall changes, and their metabolism is part of plant mechanism for defense control.

In addition, enzymes that provide protection against oxidative stresses were also upregulated, including glutathione reductase and glutathione S-transferase (3, 6, 7, 104). As noted below, protection against oxidative stress is important in the abilities of BCF to reduce abiotic stress and also to ameliorate the destructive actions of plant pathogenic fungi (66).

Altogether, a whole array of stress- and defense-related proteins are upregulated or primed in plant shoots post *Trichoderma* inoculation of the roots, thus rendering plants to be more resistance to subsequent pathogen attack. Most expression studies of the *P. indica*–plant interaction focus on root response. However, systemic induction of a few defense-related genes/proteins was shown (111, 127), suggesting a similar mechanism.

BCF INDUCE RESISTANCE TO ABIOTIC STRESS

Recently, several BCF, as well as some plant growth–promoting rhizobacteria (PGPR), have been shown to efficiently help plants overcome abiotic stresses, such as salinity and drought, in both field crops and trees (1, 6, 9, 78, 101, 126, 136). Among the most important stress factors in the field is water deficit. *T. harzianum* added as seed treatment (tomatoes) or as a soil treatment (*Arabidopsis*) largely improved the germination at osmotic potentials of up to 0.3 MPa (F. Mastouri, T. Björkman, G. Harman, unpublished data). Plants grown from these *Trichoderma* treatments are much more resistant to water deficit conditions. Effects are quite large and probably account for at least a substantial part of the increase in growth of *Trichoderma*-treated versus untreated plants in the field. The ability of maize plants grown from seeds treated with *T. harzianum* to resist water deficit has been demonstrated in the field, and the enhanced deep rooting clearly contributes (47). Moreover, in *Trichoderma*-inoculated cacao seedlings, drought-induced changes such as stomatal closure and reduction of net photosynthesis were delayed under drought compared with noninoculated plants, allowing plants to continue growing (6). In maize, it has been shown that in addition to induction of carbohydrate metabolism and photosynthesis-related proteins, the

starch content of the leaves was higher in *Trichoderma*-inoculated plants (104). This could be beneficial for plants under drought, especially if stress is prolonged enough to result in carbon starvation due to prolonged stomatal closure. Water deficit stress induces changes in photosynthetic efficiency, and *T. harzianum* alleviates these effects, described below. Similarly, *P. indica* inoculation of *Arabidopsis* increased drought tolerance (101). Inoculated plants exposed to drought continued to grow, whereas in uncolonized controls, growth was inhibited. Inoculated plants had higher chlorophyll content and higher photosynthetic efficiency under drought-stress.

The ability of BCF to alleviate salt-stress damage to plants was also tested. Growth of most plant species is inhibited by salinity. Salinity affects plants via alterations of water relations in the tissue, disturbances of ion balance, and secondary-induced stresses such as oxidative stress (79). Fresh weight of squash plants was significantly higher in T22-inoculated plants than controls under salinity (136). Similarly, *P. indica* inoculation abolished the leaf chlorosis and reduced growth that was observed in noninoculated plants under salinity (9, 126). In addition, the rate of metabolic activity increased in leaves of *P. indica*–inoculated plants after salt treatment, suggesting that BCF overcompensate the salt-induced inhibition of leaf metabolic activity (9).

Trichoderma also increased potassium content of plants (135, 136). Salt stress is well known to reduce potassium uptake, and in several systems increasing potassium uptake ameliorated salt-induced damage (96). Potassium serves as a compatible solute and hence is important to osmotic adaptation. It is also important in stomatal closure control. Therefore, increased potassium uptake can improve a plant's tolerance to water or salt stress–induced osmotic stress. In general, *Trichoderma* can alter the nutritional status of plants (78, 135). Given that salinity negatively affects the plant nutritional status (81), it could be that *Trichoderma* treatment can ameliorate the salt stress–induced growth inhibition through affecting the plant nutritional status. Salinity also reduces calcium content in plants (27, 81), and *Trichoderma* inoculation increased calcium content under salinity compared with nonsaline conditions (136).

A number of other stresses are also alleviated. *T. harzianum* has recently been shown to improve resistance to heat and cold (seedlings of tomato were imbibed at 25°C for 1 day, then exposed to either 10°C or 35°C, and then returned to 25°C). Seedlings were much less damaged by the temperature extremes in the presence of *T. harzianum.* (F. Mastouri, T. Björkman, G. Harman, unpublished data). Similarly, maize plants with *Trichoderma*-colonized roots were 70% larger at all durations of cold treatment (14).

Trichoderma can greatly induce tree growth in soil that has been used for disposal of building material and sewage sludge (1). After 12 weeks growth, willow saplings grown with T22 in the contaminated soil produced 39% more dry weight biomass and were 16% taller than the noninoculated controls. In addition, plants inoculated with *Trichoderma* are more tolerant to pathogen attack under salinity stress (78).

T22 provides benefits to seeds under stresses. Seed germination is the first stage of plant growth that must perform well. It is very important to note that effects that occur early in the life of the plant continue throughout the life of at least annual plants (42, 47, 49). Seeds exposed to abiotic stresses, including osmotic-, salt-, heat-, and cold stresses, in the presence of T22 have much higher percentages of germination and improved seedling vigor (Mastouri F, Björkman T, Harman G). Tomato seed lots with reduced vigor caused by various aging regimes exhibit higher percentages of germination and improved seedling vigor compared with nontreated seeds (Mastouri F, Björkman T, Harman G). Conidia of T22 added as a seed treatment benefits the seed by an increase in phase III imbibition (cell elongation, followed by radicle protrusion). The seed response is rapid and appears to begin before the fungus penetrates into the living portions of the seed.

A volatile elicitor from T22 that enhances tomato seedling growth at 400 pg L^{-1} has been identified. These data suggest that T22 produce volatile elicitors that enhance plant performance even at a distance. Other volatiles such as 6-n-pentyl-6H-pyran-2-one that induce plant growth have been isolated from different *Trichoderma* strains (121).

ALLEVIATION OF DAMAGE BY REACTIVE OXYGEN SPECIES

Under severe stress, ROS production can exceed the scavenging capacity and accumulate to levels that can damage cell components, e.g., via lipid peroxidation (77). It seems that endophytic fungi on roots can modulate the damaging levels of ROS, thus symptoms of biotic and abiotic stress can be limited. Roots and leaves of *P. indica*–inoculated plants showed increased levels of antioxidant compounds and antioxidative enzymes and reduced levels of hydrogen peroxide (9, 90, 126). *Trichoderma* spp. also enhances protection against ROS possibly by increasing ROS scavenging abilities. Proteomics of roots inoculated with *Trichoderma* identified increased levels of SOD as well as increased levels of peroxidase, glutathione-reductase and glutathione-S-transferase (GST), and other detoxifying enzymes in leaves (104). A peroxidase gene was also primed in pathogen-infected cucumber plants inoculated with *Trichoderma* (106). Seeds that were subjected to oxidative stress had much reduced vigor, but subsequent treatment with *Trichoderma*-T22 restored vigor (14). In a recent study, treating seeds of tomato with *T. harzianum* T22 enhanced germination percentage under osmotic stress (Mastouri F, Björkman T, Harman G). We found an increase in lipid peroxide content in young seedlings with increase in the water potential of media, whereas T22-treated seedlings had significantly less lipid peroxide than untreated seedlings (Mastouri F, Björkman T, Harman G). Similarly, root colonization by *P. indica* attenuated the salt-induced ROS damage (9). Altogether this suggests that BCF alleviates stress damage through controlling ROS damage.

INDUCTION OF GENES INVOLVED IN PLANT RESPONSE TO ABIOTIC STRESS

Various proteomic and transcriptomic studies published also provide clues for BCF ability to induce abiotic stress tolerance. *Trichoderma* induce various proteins/genes involved in stress response in different plants, including antioxidative response such as peroxidase and H_2O_2 producers such as oxalate-oxidase and glucose-oxidase (7, 94, 104). FALDH and GST were upregulated (7, 104). Among their many activities, they have a broad role in protecting cells from oxidative injuries by detoxifying compounds that would otherwise damage plant cells. The heat shock protein group of chaperones was also upregulated (94, 104). Interestingly, ornithine decarboxylase, a primary control point in polyamine biosynthesis, was upregulated in cacao seedlings by several *Trichoderma* isolates (7). Polyamines have been associated with abiotic stress, and modulation of their biosynthetic pathway confers tolerance to drought or salt stress (18, 69). In addition, tonoplast intrinsic protein, a member of the major intrinsic proteins (MIPs), was downregulated in cacao seedlings by several *Trichoderma* strains (7). Members of the MIP superfamily in plants function as membrane channels that selectively transport water out and between cells, and their expression declines in response to drought (63, 110). The repression of MIP expression may reduce membrane water permeability and encourage water conservation during periods of drought (110). Moreover, changes in drought-induced gene expression in leaves were delayed by three days in *Trichoderma*-inoculated cacao seedlings (6). This may be the result of increased water content in these seedlings, which may have caused a delay in drought response. Additionally, *Trichoderma* induced osmotin-like, salt-induced proteins in tomato plants (3). Drought stress–related genes were upregulated sooner and to a higher extent in *P. indica*–inoculated plants (101). These included marker genes involved in drought stress tolerance, *RD29A* and *ERD1*, and other drought stress–related genes encoding

for proteins involved in signaling, protein degradation, and control of gene expression. Also included in those genes is a histone acetyl transferase (HAT), which may be involved in chromatin histone acetylation. Chromatin acetylation serves as a general transcriptional regulation mechanism; hence it may imply that BCF can control gene expression more generally by regulating factors involved in histone acetylation, but this needs to be studied further. Overall, modulation of different biochemical pathways involved in adaptation of plants to abiotic stress by BCF inoculation can result in the observed enhancement of abiotic tolerance in different plants.

PLANT GROWTH ENHANCEMENT BY BCF INOCULATION

It has long been known that BCF can enhance plant growth. *Trichoderma* and *Sebacinales* species inoculation induce root and shoot growth (10, 47, 49, 51, 54, 83, 86). *Trichoderma* even promoted growth of trees (1, 6). BCF also increase percentages of germination and rates of germination of seeds (10, 14, 19, Mastouri F, Björkman T, Harman G). The application of *Trichoderma* led to an increase in dry matter content, starch, total and soluble sugars, and a reduction in sugar content in leaves of different plants (1, 70, 104). *P. indica* can promote adventitious root formation in cuttings and may thus be a good candidate for biological hardening of micropropagated plantlets (34, 107). More importantly, the effect of BCF on plant growth has a long duration and even last for the entire life of annual plants (10, 47, 51, 126). Other nonpathogenic root colonizing fungi also have similar abilities (71).

It is possible that BCF affect growth by counteracting deleterious root-associated microflora. However, *Trichoderma* and *Sebacinales* species were shown to induce growth under sterilized and nonsterilized conditions (54, 71, 83, 86, 107, 135), suggesting a direct mechanism through plant response.

The activation of direct defense reactions has a metabolic cost that reduces plant fitness (31, 56, 57, 113). However, rather than reducing growth, BCF typically either have no effect or substantially increase plant growth. Van Hulten et al. (118) have shown that priming has a smaller effect on fitness than directly induced defense. Thus, priming reduces the energetic costs of plant readiness status to resist pathogens. In other cases, defense mechanisms and PR expression are induced (94, 103, 104), yet growth is not compromised, probably because of increased energy supply to cover the energetic cost (104, 105). However, in some systems the metabolic costs cannot be covered by the growth enhancement, as has been demonstrated for *Sebacina vermifera* (10).

This greater energy supply must ultimately come from photosynthesis, and probably needs to be accompanied by greater respiratory rates (105). This indeed appears to occur. Recent progress in elucidating the direct effect of *Trichoderma* on plant growth was obtained from a proteomic study of the T22-maize system, which demonstrates highly reproducible growth promotion (104). Of the 205 differentially expressed proteins in the presence of *Trichoderma*, the most commonly affected were those involved in carbohydrate metabolism, especially those in the glycolytic, tricarbixylic acid (TCA) or respiratory pathways, and most of them were upregulated in the shoots (104, 105). Some of the carbohydrate- and respiratory-related proteins were also found to be upregulated in studies involving other *Trichoderma* species (17, 94). In addition, several photosynthesis-related proteins were upregulated in plants by the interaction with *Trichoderma*. Maize plants inoculated with T22 have higher leaf greenness than noninoculated plants (47). Together, this suggests that *Trichoderma* can increase photosynthetic capacity of the plants.

IMPROVED PHOTOSYNTHETIC ABILITIES

In addition to increased levels of photosynthetic apparatus, photosynthesis can also be

improved by higher photosynthetic efficiency. When measuring fluorescence kinetics and using $F_{variable}/F_{maximum}$ ratio (F_v/F_m) as a measure of photosynthetic efficiency (13), no difference was found between control- and *P. indica*–inoculated plants under nonstress conditions (83), nor was there any difference in chlorophyll content. However, fast chlorophyll fluorescence kinetics (O-J-I-P) used to analyze photosynthetic efficiency demonstrated that the yield for electron flow was substantially increased by root colonization with the fungus and that electron transport per trapping center was strongly enhanced. This improvement in photosynthetic efficiency was strongly related to plant height and root colonization by *P. indica* (87).

Transferring *Arabidopsis* seedlings adapted to low light to higher light conditions severely damaged seedling growth and foliages turned red. However, no obvious damage was observed in plants inoculated with *Trichoderma*-T22. The F_v/F_m of control plants was reduced to 0.45, but this value for T22-treated plants was 0.78 (F. Mastouri & G.E. Harman, unpublished data). The value for unstressed *Arabidopsis* leaves is approximately 0.80 (101). Thus, the photosystem in *Arabidopsis* in the presence of T22 under light stress was near optimal (Mastouri F, Björkman T, Harman G).

Rapid chlorophyll fluorescence transients analysis is a nondestructive and sensitive method that can detect water deficit stress before irreversible wilting and damage occur (82), whereas the F_v/F_m parameter decreases only at approximately the time that plants suffer irreversible water deficit stress (128). Oukarroum et al. (82) identified a performance index (PI) based on the measured parameters that accurately calculated the intrinsic resistance to water deficit and that differentiated between barley cultivars varying in resistance to water deficit. Using this method, we showed that *T. harzianum* substantially reduced effects of water deficit even after two weeks of withholding irrigation, when plants were at or approaching the permanent wilting point. Even after just one week, the PI was significantly different: In plants grown from seeds treated with T22, PI $= 0.601 \pm 0.071$ (mean $\pm$ SE), and in plants grown from untreated seeds, PI $= 508 \pm 0.067$, whereas other parameters such as F_v/F_m were not changed at the early stages of water deficit stress. These results demonstrate that *Trichoderma* strains reduce effects on photosynthetic systems as they increase water deficit tolerance in a manner similar to that demonstrated by differentially resistant barley lines (82).

Altogether, BCF enhance plant growth at least in part because respiratory systems and carbohydrate metabolism are upregulated and thereby increase energy and sugar supply to the growth of the plant. This is probably maintained by the increase in photosynthesis. In a study with tomato plants inoculated with *T. hamatum*, the expression of stress-, cell wall–, and RNA metabolism–related genes was also upregulated, demonstrating similarities of plant responses to *T. harzianum* (3). However, in this system carbohydrate metabolism–related genes were not upregulated, and no positive growth response was recorded. This strengthens our suggestion that there is a direct connection between the ability of *Trichoderma* to induce energy metabolism and its ability to induce growth response. A summary of plant responses is depicted in **Figure 1**.

EFFECT ON ROOT DEVELOPMENT AND PERFORMANCE

Inoculation of plants roots by *Trichoderma* or *Sebacinales* species results in changes of root development. *Trichoderma*-inoculated roots are deeper and more robust (47). Main and secondary roots of maize increased in size, and the area of the root hair was greater with *Trichoderma*-T22 inoculation (53). *P. indica* also induced root developmental changes. Promotion of root growth and increased length of root hairs were detectable even before notable root colonization (83). Root branching can improve soil exploitation and hence result in plant growth promotion. An effector hydophobin-like protein from T22

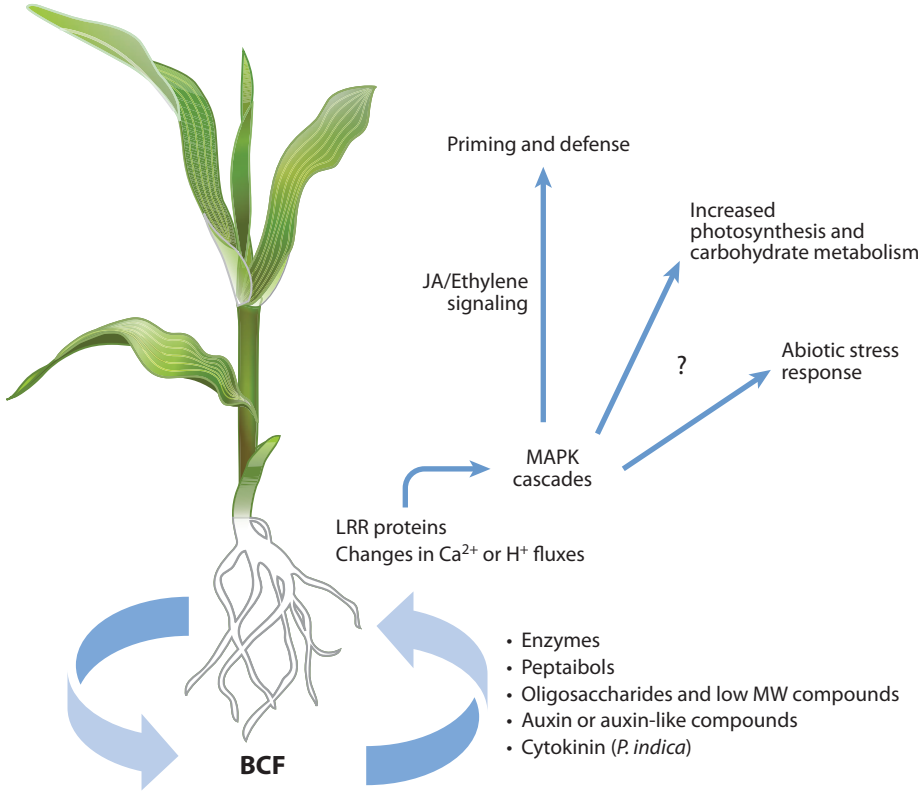

Figure 1

Biocontrol fungi (BCF) grow to interact with the roots. By forming this interaction, the BCF and the plant exchange signals. BCF releases elicitors into the zone of chemical communication (both outside and inside root tissue) and activates a mitogen-activated protein kinase (MAPK) cascade in the plant. The jasmonic acid (JA)/ethylene signaling pathway is being activated, which results in priming and/or increase of plant defense genes that ultimately increase plant resistance to pathogens. In addition, increase of carbohydrate metabolism and photosynthesis change the source-sink relationship resulting in more energy and carbon source to the growing plant, leading to the observed enhanced growth response. At least for *Trichoderma*, we know that there are strains that induce defense but not growth and vice versa, suggesting that the signaling pathways leading to these plant responses are different. Whether these signaling pathways also differ from the one leading to abiotic stress responses needs to be determined. MW, molecular weight.

has been identified that mimics the effect of the fungus (89). *Arabidopsis* root colonization by *P. indica* resulted in a stunted but highly branched root system, which is probably mediated by low amounts of auxins produced by *P. indica* (108). Several auxin-like secondary metabolites produced by *Trichoderma* strains were able to induce plant growth and are required for development of lateral roots in *Arabidopsis* (26, 121). However, a recent study implicates cytokinins in plant growth promotion (116). *P. indica* induces relatively high levels of cytokinins, and the cytokinin levels are higher in colonized roots compared with uncolonized controls. Although root colonization was not affected in cytokinin biosynthesis or receptor mutants, no growth response was recorded in mutants possessing decreased levels of *trans*-Zeatin cytokinins. This indicated that cytokinins are required for the plant growth response but not the root interaction with the fungus (116).

Although *P. indica* infects mainly the differentiation zone of the roots, from which

it can spread, and causes localized cell death, *Trichoderma* spp. have the abilities to rapidly colonize seeds and immediately confer benefits to seeds and seedlings even before radicle protrusion occurs (Mastouri F, Björkman T, Harman G). This allows *Trichoderma* spp. to be used widely as seed treatments, which is a very cost-effective method of application. Given that *Trichoderma* strains can grow with and keep up with the developing root system, long-term protection (45–47, 131) occurs even though the initial application rate is only one gram or less per hectare.

Another component of growth induction could be due to increase in nutrient uptake. *Trichoderma* spp. have significant abilities to solubilize a range of plant nutrients, such as phosphorus and micronutrients including iron, copper, zinc, and manganese, thus rendering them available for plants (4). In addition, even in hydroponics with fully soluble and available nutrients, the presence of *T. asperellum* on cucumber roots increased the uptake of a similar range of plant nutrients (135). *P. indica* can also mediate solubilization and translocation of minerals to plants (39, 83, 107), although there are some contradicting results (10). When interaction of plants with *P. indica* was underbalanced, nutrient-supply, no-shoot growth enhancement was observed, whereas under low nutrient or poor soil, *P. indica* induced plant growth (83, 95, 108). It could be that the balanced nutrient supply obscured the advantage conferred by root branching, and hence growth can be promoted by *P. indica* under suboptimal conditions. However, even under near-optimal conditions, *Trichoderma* seed treatment of maize sometimes gives improved yields (47, 54, 55). Thus, BCF can solubilize plant nutrients (indirect effect) and also induce plants to uptake more nutrients (direct effect). There is no doubt that the increased root development associated with colonization of plant roots by BCF contributes to this and other benefits to plants. BCF treatments therefore have the potential to improve overall crop yield and may be particularly important in suboptimal field conditions.

ENHANCED NITROGEN USE EFFICIENCY

BCF increase nitrogen use efficiency (NUE) in plants (47, 50, 87, 100). This effect was first noticed with *T. harzianum* T22 in maize field trials in the late 1990s (42, 47). Plants grown under conditions of low soil nitrogen from seeds treated with T22 were larger and darker green (47). Plants generally respond to increasing nitrogen fertilizer levels with increased yield and growth up to a point when increasing nitrogen fertilizer no longer increases yields. In the presence of T22, this yield plateau was reached with 40–50% less nitrogen fertilizer than in its absence (42, 47, 50). This increase in plant NUE is a long-term effect and can be induced by a seed treatment whose effect persists for the productive lifetime of the crop. Although, in maize, a few genotypes respond negatively to *Trichoderma* (48), seed treatment of wheat with this fungus is highly effective, nearly always giving improved yields (49). This ability is being exploited in the United States, and approximately 0.3 million hectares of wheat are being planted with seeds treated with *T. harzianum* strain T22. Our new understanding of the mechanisms of action of *Trichoderma* strains, and their broad capabilities, permitted improved screening protocols, and new strains were selected and compared for NUE improvements with the former strain *T. harzianum* T22. The new strains *T. harzianum* RR17Bc and *T. atroviride* WW10TC4 were selected because they had better growth induction capabilities than T22, and *T. harzianum* F11Bab was selected because it was improved over T22 in inducing systemic disease resistance (49). Nitrogen rate ranging trials were conducted in large pots in the greenhouse, which are the only way to fully define the yield/nitrogen uptake effects of added *Trichoderma* (F. Mastouri & G.E. Harman, unpublished data). Nitrogen content increased in *Trichoderma*-treated plants, but moreover, correlation analyses over several strains and nitrogen levels revealed that nearly all of the yield variation could be explained by the nitrogen variation levels in plants. Thus,

the strains improve yields by increasing NUE. Strain WW10TC4 of *T. atroviride* appeared to be the most effective. Moreover, both *Trichoderma* and *P. indica* induce expression of nitrate reductase in plants (6, 100) that convert nitrate to ammonium ions (the required form for nitrogen metabolism) (100), and hence they may be involved in nitrogen accumulation through the symbiotic association. NUE in *P. indica*–colonized plants is associated with a marked increase in nitrate reductase.

SIMILARITIES OF BENEFICIAL EFFECTS OF DISTANTLY RELATED ENDOPHYTIC PLANT MICROBES: AN EXAMPLE OF CONVERGENT EVOLUTION?

Throughout this review, we have pointed out the similarities in effects and mechanisms of disparate endophytic microorganisms, including *Trichoderma* spp., which are ascomycetous fungi; *P. indica*, which are basidiomycetous fungi; and various PGRPs, which are Eubacteriales. These effects and mechanisms are summarized in **Table 1**. Although we have until recently considered these and similar microorganisms as BCFs, it is clear that their benefits are much greater than just the control of plant pathogens. These organisms are very distant from one another and represent dissimilar genetic lineages.

Each organism mediates systemic resistance, mostly by ISR, although other mechanisms may also be involved (it probably depends on which elicitors are involved). Where it is known, they also induce a considerable amount of tolerance to plant abiotic stresses, which is a recently discovered advantage of these organisms. Further, some increase NUE in plants, which is perhaps the most significant advantage of these organisms because it can reduce environmental

Table 1 Comparison of three distantly related root-colonizing microbes (endophytic plant symbionts) on plant growth and biochemical mechanisms

Plant effect or mechanism	*Trichoderma* spp.	*Piriformaspora indica*	Plant growth promoting rhizobacteria	References
Internal root colonization	+	+	+	51, 65, 90
Improved plant shoot and root growth	+	+	+	47, 65, 126
Systemic resistance to disease	+	+	+	51, 64, 127
Induced resistance to abiotic stresses	+	+	+	53, 64, 126
Enhanced root development/adventitious root formation	+	+	/+	34, 49
Enhanced general uptake or solubilization of plant nutrients	+		+	4, 40
Increased plant nitrogen use efficiency	+	+	–/?	47, 100
Enhancement of performance of physiologically impaired seeds	+	–	–	Mastouri F, Björkman T, Harman G
Mechanisms				
Induced systemic resistance	+[a]	+[a]	+[a]	64, 65, 104, 106, 111
Amelioration of oxidative damage induced by stress	+	+	+	40, 66, Mastouri F, Björkman T, Harman G
Enhanced activity of nitrogen reductase in plants	?	+	?	100
Enhanced photosynthetic capability and/or efficiency especially in plants under stress	+	+	+	40, 47, 87, 105

[a]Other mechanisms may also be induced.

pollution and enhance food security, especially in developing countries. Nitrogen is expensive and thus is limited in less wealthy regions of the world, and thereby food production is reduced.

The organisms that appear in **Table 1** have demonstrated abilities to reduce accumulation of damaging levels of ROS during plant stress. This is probably associated with an increase of photosynthetic efficiency, especially during stressful conditions.

T. harzianum strain T22 appears to have an additional benefit. It alleviates physiological stresses in seeds caused by poor seed quality (14, Mastouri F, Björkman T, Harman G). These effects happen very quickly; when seeds are treated with the conidia of the organism, the conidia germinate only 18–20 h after imbibition begins and then must traverse the seed coat before reaching the living portions of the seeds. However, effects are noted by the time radicle protrusion occurs (48–96 h after imbibition begins), which means that the effects of the fungus had to occur earlier, probably in stage 2 of germination, approximately 24–36 h after the start of imbibitions. These data suggest the benefit happens before the living portions of the seeds are colonized, which suggests the involvement of highly effective elicitors. We are currently investigating volatile *Trichoderma* metabolites for this role (Mastouri F, Björkman T, Harman G).

It is unlikely that these diverse organisms trigger their beneficial mechanisms in the same way. Indeed, given the range of elicitors produced by *Trichoderma* spp., there probably are different mechanisms/triggering molecules produced by these fungi. Nonetheless, similar responses occur in a diverse range of plants, and the basic mechanisms seem to be similar. The abilities of these fungi to elicit similar end responses may suggest that these mechanisms are the ones that are most likely to benefit plants across a range of species and climatic conditions.

Of course, the organisms discussed here are those whose lifestyles are benefited greatly from large numbers of healthy roots. This provides nutrients and protection of these fungi against competitors. Certainly with *Trichoderma* spp., greater numbers of the fungi are found when large numbers of healthy roots are present (53). This is further evidence for the symbiotic nature of the relationship between these organisms and plants. It is probable that the elicitors and specifics of the specific elicitation of responses differ between the microbes, and this ought to be a fruitful area to consider for the nongenetic improvement of plant performance. The authors are working to provide widescale systems for use with *Trichoderma* spp. to improve plant productivity and plant health (for resistance to biotic and abiotic stresses) and to use NUE to increase food and fiber production, while reducing environmental damage.

CONCLUSION

Although general mechanisms regarding plant response to BCF inoculation can be derived from the studies described above, it is important to remember that natural variations do occur. For example, some *Trichoderma* species induce growth in addition to ISR (49), whereas others do not (3). Biocontrol *Trichoderma* strains were also reported that do not induce resistance or enhance growth despite their endophytic abilities (29). Bailey et al. (7) have demonstrated that plant gene expression profiles depend on the *Trichoderma* isolate colonizing the plant. The responses depend not only on the BCF used but also on the plant species or cultivar. *Trichoderma*-treated maize has an average yield increase of approximately 5%, but there are significant varietal differences, with some maize lines giving neutral or even negative growth responses (48). However, yield responses of T22-treated wheat appears to be extremely robust in the field (55). Different *Arabidopsis* ecotypes also respond differently to *P. indica* or *Trichoderma* spp. (83). Once the specific control mechanisms of the BCF-plant interaction are known, then very specific genetic lines that have favorable outcomes can be readily identified and used. Moreover, knowledge of specific critical gene products that are associated with favorable outcomes will permit rapid assays of the expression of critical proteins or genes even

on a field scale. This will provide a major management tool that will afford reliable assessment of the interaction. We believe that the abilities of these fungi to (*a*) induce resistance to biotic stresses such as disease and abiotic stresses such as drought and salinity and (*b*) increase NUE make them extremely useful tools with which to increase plant productivity, improve food security, and improve the environment. Specifically, these fungi's ability to produce NO compounds from unused fertilizer can reduce nitrogen fertilizer application, thereby reducing nitrate pollution of waterways and air pollution (40, 64, 65).

DISCLOSURE STATEMENT

G.H. has an equity share in some of the companies that funded research in his lab.

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