 doi:10.20944/preprints202103.0303.v1

Biological Seed Treatments

Gary E. Harman

Cornell University

geh3@cornell.edu

Abstract

There are many types of seed treatments, including dry treatments, liquid treatments, water-based treatments, and oil-based treatments. There are also biorational treatments, which are based on metabolites from fungi or bacteria. Bacteria and fungi are both used in biological seed treatments. While all have potential uses, some organisms are more widely and successfully used than others. Shelf life is an important consideration. For this reason, organisms that lack cell walls are more difficult to use than ones with long-lasting spores. *Bacillus* and *Trichoderma* are both widely effective, have good shelf life, and are frequently used. Even though Rhizobiacae lack cell walls, which is a limitation, they are widely used because their symbiosis with legumes facilitates nitrogen fixation which is an important factor that provides economic, agricultural and environmental sustainability. It is crucial to store cultures properly to avoid phenotypic changes, and mechanisms of variability and methods for storage are included in (Samuels and Hebbar 2015). For all organisms, proper formulation of microbial agents is critical for success; this is especially true for Rhizobiacae and other gram-negative bacteria. There are several specialized processes used to deliver microbial agents or to enhance their biological activity, such as solid matrix priming. Both living organisms and biorationals provide benefits to plant agriculture by controlling diseases and increasing resistance to abiotic stresses such as drought, temperature, salt, and flooding. They also can improve photosynthesis. For these applications, the most effective bacteria and fungi colonize roots internally and provide season-long benefits. These endophytes have been shown to induce systemic changes in plants' gene expression and encoding of proteins.

Keywords: Fungi; Bacteria; Holobionts; Delivery

Introduction

This review will consider:

- seed treatments and formulations,
- biorational treatments,
- benefits of seed treatments,
- the importance of, and methods for, long-term maintenance of cultures,
- endophytic and rhizospheric competence,

 doi:10.20944/preprints202103.0303.v1

- maintenance of internal cellular functions,
- effects on photosynthesis,
- control of plant diseases and pests,
- effects on biotic and abiotic stresses,
- value economically and to sustainable agriculture.

• Seed treatments and formulations

There are many types of seed treatments. Some are suitable for biological agents, while others are not. **Dry seed treatment** powders have been used with a variety of microorganisms, and originally they were the only ones used. This was particularly true for gram negative bacteria, since the absence of a cell wall makes them very vulnerable to wetting and drying. Dry powder treatments have limited use in commercial seed treatments.

Liquid seed treatments are usually used in commercial seed treatments because they are convenient to use and are well-suited to high-throughput systems. They contain both pesticides and formulary materials and are lethal to microorganisms when applied in the tank. However, most microorganisms are compatible with biologicals if the organisms are applied after the seeds are treated. Liquid seed treatments may be water-based or oil-based.

Water-based treatments suspend the organisms in water. With this preparation, there must be some method to avoid growth of the organisms because if they grow, they are likely to die. Moisture content is the percentage of moisture in a material. On the other hand, water activity is a measure of the water that is available to react or attach itself to another material. Water activity (aw) = 1 indicates pure water, while aw = 0 is completely dry material. The permanent wilting point of plants occurs at about aw = .95, while fungi such as *Trichoderma* can grow at about aw = .92 and can survive at values even lower than this. Very halophilic organisms can grow at about aw = .80. This differential is very helpful in formulating seed treatments. Water activity meters are available, see https://www.metergroup.com/food/products/aqualab-4te-water-activity-meter/. For a comprehensive treatment of water relations see (Leopold 1986).

Oil-based seed treatments are also available. Fungi are well-suited for such preparation. Many contain proteins (hydrophobins) on the surfaces of their cell walls. Hydrophobins have a polar end and a polar end. Many fungi, such as *Trichoderma,* can be either hydrophobic or hydrophilic. Dry *Trichoderma* spores placed initially in oil readily suspend in the oil and are not miscible with water. However, if they are initially placed in water, they become immiscible in oil. The different portions of the molecule reorient depending upon the medium in which they are suspended.

Other types of seed treatments are used including encrustments and pelleting. These differ according to the thickness of the treatment (Afzal et al. 2020).

 doi:10.20944/preprints202103.0303.v1

Biorational treatments are microbial metabolites. These can be used as seed treatments. Examples derived from *Trichoderma* include 1-octen-3-ol, 6-pentyl-a-pyrone and harzianic acid. Seed treatments with 1-octen-3-ol increase yield in corn at very low concentrations (Harman and Uphoff 2019a). Treatment of grapes with 6-pentyl-a-pyrone or harzianic acid increases nutrient availability and induces resistance to powdery mildew (Pascale et al. 2017).

Solid matrix priming

Seeds that have been primed will germinate more quickly. For this process, seeds are placed in a material such as humate or clay which has a moisture level below that required for the seed to germinate. Some organisms such as *Trichoderma* or *Enterobacter* may be able to grow at these low moisture levels (Taylor and Harman 1990; Taylor et al. 1988).

These organisms potentially can control pathogens that rot the seed, but they are ineffective against *Pythium* spp. *Pythium* responds very rapidly to the presence of seeds, often infecting seeds in as little as 1 hour (Stasz et al. 1980). Biocontrol organisms are unable to grow that quickly. However, if seeds have been primed, they can grow at these low-moisture levels. Once they are actively growing, these organisms can protect seeds against *Pythium* and other pathogens (Harman and Taylor 1988; Harman et al. 1989).

- **The importance of, and methods for, preserving viability for microbial agents**

The issues of strain stability are critical. For example, in the 1980's, a large international trial was initiated, and it failed. The researcher indicated that the strain used was not the same one used at the start of trial. If cultures are transferred repeatedly, the organism used will differ from the original. Several mechanisms for this are known. For example, *Nectria haematococca* is a pathogen of pea. The fungus contains a small unstable chromosome that encodes a gene for degradation of pisatin. If this gene is lost, the strain is no longer pathogenic (Miao et al. 1991). Another mechanism is gene silencing, which is "the regulation of gene expression in a cell to prevent the expression of a certain gene" (Wikipedia 2021). Additionally, modifications of chromatin structure in the regulatory regions results in changes in translation of proteins (Jaskiewicz et al. 2011).

Further, many fungi cells contain multiple nuclei, ranging from one to several thousand. These nuclei may be dissimilar. These nuclei may exchange genetic material, and upon sectoring, different phenotypes may be obtained (Roper et al. 2011; Kubicek and Harman 1998; Harman et al. 1998). These mechanisms give rise to variability in the strains derived from them. Another source of variability are transposons. In *Magnapothe oryzae* has highly unstable ends of telomeres that undergo frequent genetic rearrangements (Rahnama et al. 2020). Given this genetic instability, changes in phenotype of strains are inevitable. Therefore, it is essential that strains not be serially transferred.

Methods to maintain strains in an inactive form are required. The American Type Culture Collection lyophilizes cultures at low temperatures (American Type Culture Collection 2021) Another method uses silica gel beads (without colorant). Spores are mixed with skim milk, and the granules are added to vials and then sterilized (Samuels and Hebbar 2015). Cultures can also be preserved by storage in liquid nitrogen containing 10-20% glycerol (Samuels and Hebbar 2015). Cultures of fungi can be preserved by placing spores with Wa=.03-0.5 at 0◦C.

My colleagues and I never successively transfer cultures, and always start with inoculum that has been stably preserved by one of the methods just described. For many fungi, the silica gel procedure is convenient. To start a new culture, a few beads are scattered over an appropriate medium from which the fungi grow across the plate. Using this method, we have stably stored *Trichoderma* for a decade.

- **Formulations**

Formulations are essential components of seed treatments. Dry formulations may contain clays, peat moss, and other materials along with adhesives, etc. Water-based liquid formulations typically contain various binders, stickers, and/or colorants. For microbial agents, growth in the liquid environment must be avoided. Water activity becomes important. The most effective method of avoiding growth of microbial agents is through addition of an osmoticant that can increase the osmolality of the solution.

Various materials can potentially be used. In considering which material to use, shelf life and stability of the microbial agents need to be considered. Various salts provide the requisite water activity (wa=.90-.94), but they are usually toxic when stored for long periods. Organic osmoticants allow growth of microorganisms and so cannot be used. Perhaps the most useful material is glycerol, which is non-toxic and most microorganisms cannot use it as a food source.

For liquid formulations, emulsifiers are usually needed. For living microorganisms, the emulsifiers must be non-toxic and compatible with the other ingredients used. For biorational materials such as 1-octen-3-ol that are volatile, oil-water emulsions are very useful. They help keep the material in suspension and are readily mixed with other seed treatment components.

- **Endophytic and rhizopheric competence**

Endophytic and rhizospheric competence can be defined as the ability to grow within the root interior and on the root surface, respectively. In many cases, the organisms persist and live for at least the life of an annual crop. *Trichoderma* added as a seed treatment grows rapidly from the seed onto the plant root radical and then grows and becomes established in the interior of the root. The fungal endophyte usually is limited to the root and does not become established in the above-ground portions of the plant.

Many other fungi and bacteria are endophytic and can potentially be used for seed treatment. These include fungi in the genera *Trichoderma* (Harman 2011; Woo et al. 2014; Doni et al. 2017), *Aspergillus* (Ismail et al. 2020), *Penicillium* (Ikram et al. 2018a), *Clonostachys* (Shafia et al. 2001), *Piriformaspora* (Sherameti et al. 2005), and *Yarrowia lipolutoca* (Franza et al. 2019), as well as bacteria in the genera *Pseudomonas* (Konappa et al. 2020) and *Bacillus* (Konappa et al. 2020; Brannen 1997), and in the family Rhizobiacae (Chi et al. 2015).

- **Benefits of these seed treatments**

These microorganisms have a variety of functions—they can be used to increase nitrogen fixation, control diseases, ameliorate abiotic stress, increase plant yields, improve nutrient utilization and for plant nutrient efficiency (Marra et al. 2019), and to increase photosynthesis.

- **Nitrogen fixation**

Rhizobiacae are well-known for their ability to fix nitrogen. The complex interactions between plants and these bacteria are well documented in (Jones et al. 2007). Recently, a land race of maize was identified that fixes nitrogen in a process supported by mucilage-associated diazotrophic microbiota; this is expected to reduce the maize plants' requirement for nitrogen fertilizer (Van Deynze et al. 2018). Nitrogen fixation requires an absence of oxygen since oxygen inactivates the enzymes responsible for nitrogen fixation. In legumes, leghaemogobin binds tightly to oxygen and permits nitrogen fixation to proceed within the nodules affixed to plant roots.

Rhizobiacea do not only colonize legumes, but can also become beneficially endophytes in the roots and even in the canopies of cereal grains (Chi et al. 2005). While they colonize the roots initially, they can move throughout plants and are found in the upper parts of plants. It is not known whether these bacteria colonize other plants than cereal grains because this has not been studied.

- **Maintenance of internal cellular functions**

Plant may be damaged by Reactive Oxygen Species (ROS). ROS damage nearly all functions within cells (Mittler 2002). Many endophytic bacteria and fungi can mitigate the damage caused by ROS; these include *Trichoderma*, which may be applied as a seed treatment. They alleviate biotic, abiotic, and physiological stresses in germinating seeds and seedlings (Mastouri et al. 2010). These microorganisms include mycorrhizal fungi that regulate plant growth, photosynthesis, antioxidation, and osmosis (Mo et al. 2016); *Piriformospora indica*, which stimulating the expression of drought stress-related genes in leaves (Sherameti et al. 2008); *Azospiillum*; and *Rhizobium*, which can mitigate salinity stress (Fukami et al. 2018).

ROS may result from either biotic or abiotic stresses and from over-excitation of the photosynthetic machinery (Nath et al. 2013). We have proposed the term 'Optimization of Internal Redox Environments' (OIRE) to describe systems where the alleviation of damaging effects from ROS is associated with the by overexpression of certain genes and of the proteins that they encode (Harman and Uphoff 2019b). This is one of the important effects of these beneficial organisms.

- **Photosynthesis**

Photosynthesis is essential to all life on Earth, providing fixed carbon for plants and ultimately for all other organisms. This energy is necessary for processes such as plant growth, resistance to biotic and abiotic stresses, nutrient uptake, and establishing and maintaining OIREs at the cellular level.

Photosynthesis having been reviewed elsewhere will just be summarized here (Harman et al. 2019). Endophytic microorganisms alter gene expression and the proteins that they encode (Doni et al. 2019; Chi et al. 2005; Chi et al. 2010). The systems up-regulated include light-harvesting, regulation of the dark reactions, and synthetic functions for photosynthetic processes. These are summarized in (Harman et al. 2019). In a recent study, transcriptomic study of plants treated with *T. asperellum* identified 335 transcripts differentially expressed; of this total, 301 were up-regulated. Of these up-regulated genes, 238 were related to the functioning of the thylakoid membranes in chloroplasts, and 192 were directly related to photosynthesis (Doni et al. 2019; Harman et al. 2019).

Endophytic *Trichoderma* induce higher density of stroma (the network and chloroplasts related to higher photosynthetic capabilities) (Paradiso et al. 2017) and increased levels of photosynthetic pigments (Vitti et al. 2016; De Palma et al. 2016; Pelhivan et al. 2017). Mitigation of ROS damage is also related to changes in gene expression (Mastouri et al. 2010). Mycorrhizal fungi increase drought-tolerance through greater protection of photosynthetic systems, a stronger root system, and an increase in anti-oxidative systems (Mo et al. 2016). Seed treatment with two rhizobacterial strains provided greater photosynthetic activity and chlorophyll content, and also increased water use efficiency (Stefan et al. 2013). In endophytic *T. virens,* a sucrose invertase is crucial for root colonization, for plant disease control, and for enhancing photosynthetic activity (Vargas et al. 2009).

- **Increased plant yield and growth**

Many endophytic microorganisms and biorational materials increase plant growth and yield. *B. subtilis* has increased plant growth including roots of cotton, and suppressed diseases caused by *Rhizoctonia* and *Fusarium* (Brannen 1997). *Rhizobium* seed inoculants have increased crop yields (Thilakarathna and Raizada 2017; Thelen and Shulz 2007; Yanni et al. 1997). *Glomus mossea* increased yield under drought conditions, but not under well-watered ones (Mo et al. 2017; Tyagi et al. 2017). *Piriformaspora indica* induced gene reprogramming that resulted in greater plant growth and resistance to abiotic stresses (Varma et al. 1999). *Trichoderma* spp. increased plant growth in vegetables (Inbar et al. 1994) in greenhouse ornamental plants (Harman 2000; Harman et al. 2004a) and in cereal crops (Harman 2004a), in corn (Vargas et al. 2009), and in bean (Mayo-Prieto et al. 2020). Seed treatments with *Trichoderma, Trichoderma + Bacillus amyloiquifaciens,* or the biorational 1-octen-3-ol markedly increased root growth in maize (Fig. 5).

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 11 March 2021 doi:10.20944/preprints202103.0303.v1

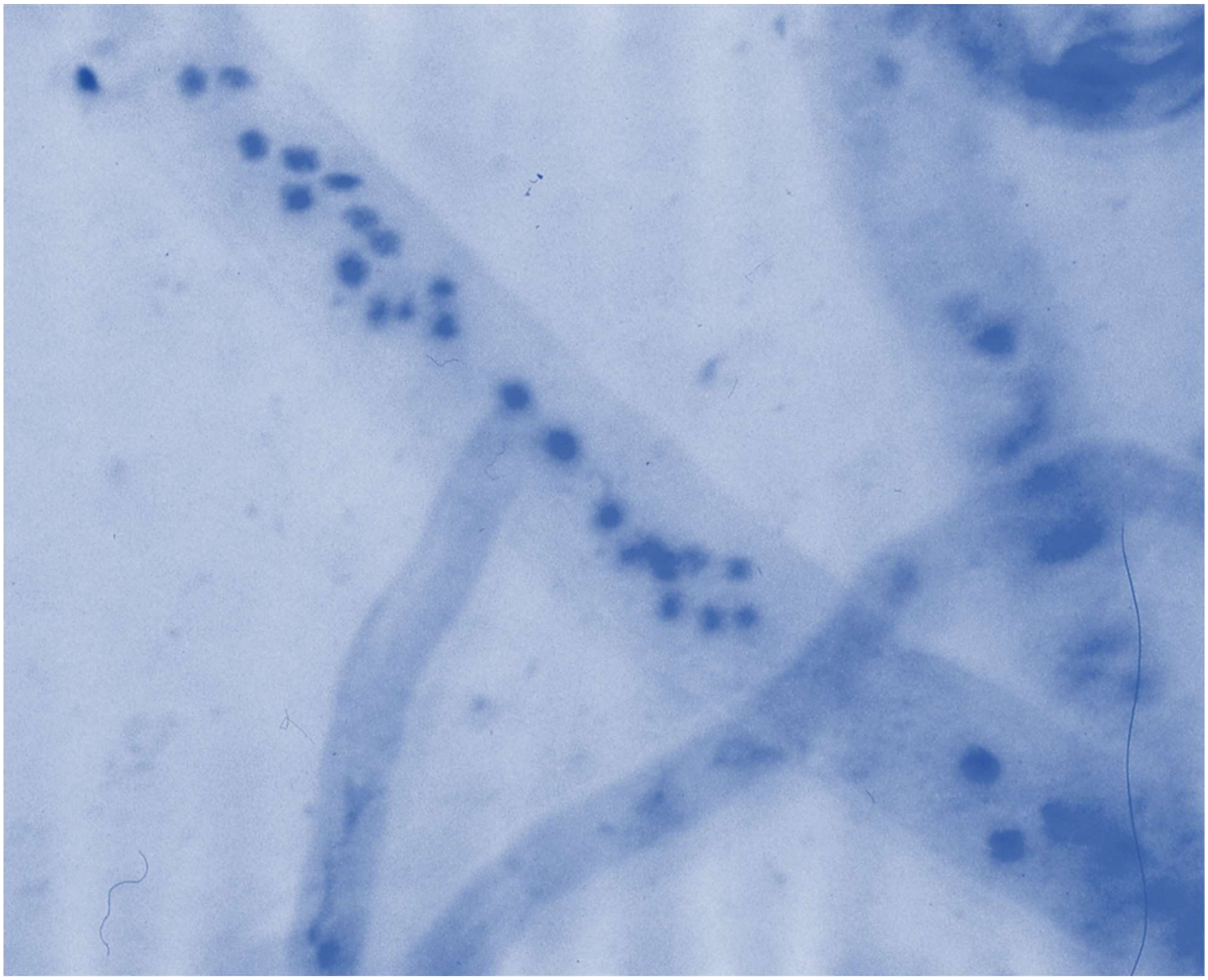

Fig. 1. A hyphae of *T. afroharzianum* showing multiple nuclei per cell. A similar photograph was published in (Harman et al. 1998).

- **Control of plant diseases and pests**

Endophytic fungi and bacteria minimize various types of plant diseases. Indeed, *Trichoderma* were first proposed as agents for biological control almost 100 years ago (Weindling 1932; Weindling and Fawcett 1936). There are many reports of the ability of these fungi to control disease in both aerial and below-ground plant parts (Harman 2000).

Even though most endophytes colonize only roots, and may be applied as seed treatments, there is evidence also of their control of above-ground diseases occurs, such as of anthracnose on corn (Harman et al. 2004b) and powdery mildew on cucurbits (Fig. 2). These are examples of systemic responses that occur throughout the plant even if the location of endophytes is in the roots. Many endophytes exhibit this behavior, including *P. indica* (Gill et al. 2016), which reprograms plant cells' gene expression (Waller et al. 2005), as do most of the other

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 11 March 2021 doi:10.20944/preprints202103.0303.v1

endophytes described here. Endophytic microorganism have been used to control plant diseases (Bonanomi et al. 2018).

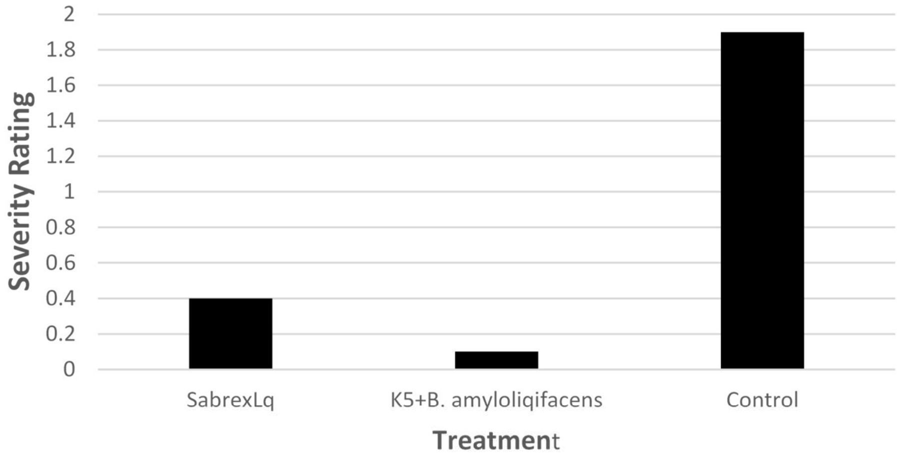

Fig. 2. Control of powdery mildew with *Trichoderma* applied as a seed treatment

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 11 March 2021 doi:10.20944/preprints202103.0303.v1

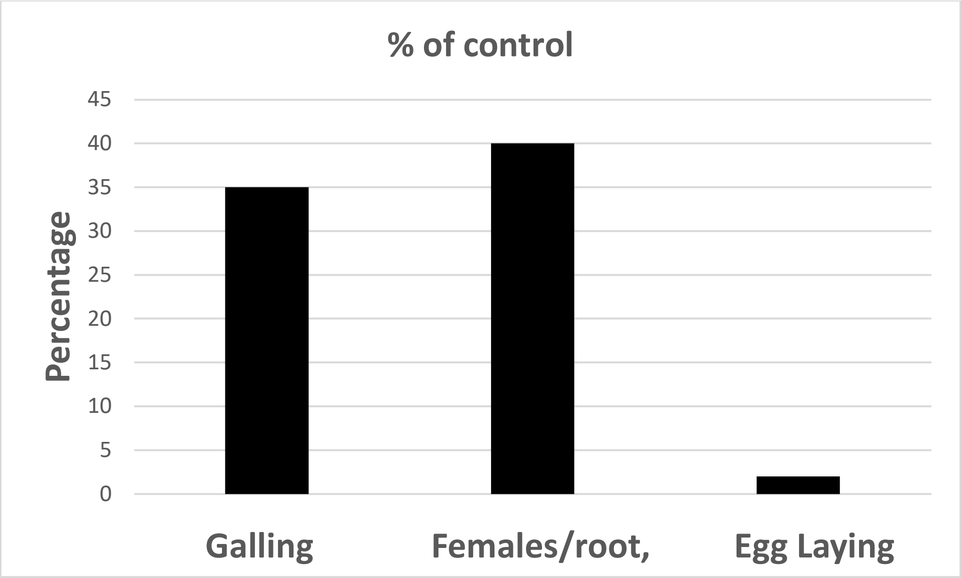

Figure 3. Nematode control on tomato with *T. atroviride* strain K5

Nematodes may be controlled by endophytic *Trichoderma* spp. A selected strain of *T. atroviride* when applied to tomatoes reduced egg-laying by about 90% (Fig. 4). This result was confirmed with corn, although the number of eggs laid was not reduced as much was with tomatoes. An important consideration is that this effect is strain-specific. *T. atroviride* strain K5 was the only effective one. Other *Trichoderma* stains, including another strain of *T. atrovide*, were found to be ineffective, as the number of adult or juvenile nematodes was not reduced.

Fig. 4: Ears of corn grown under drought conditions. The upper photo shows ears from corn treated with *Trichoderma* plus fungicides, while the lower photo shows ears treated only with fungicides.

This capability is systemic, i.e., it is plant-wide. Results of split-root experiments to assess nematode control have shown a reduction in egg-laying to occur on roots in soil that had only nematodes (no *Trichoderma*), when *T. atrovide* was applied only to the soil around the other root (Harman et al. 2018). Frequently, *Trichoderma* spp. control plant diseases through reprogramming of genes involved in systemic resistance (Poveda et al. 2019; Shoresh et al. 2006), and similar effects have been induced by *Piriformaspora indica* (Waller et al. 2005).

The entomopathogen *Beauveria bassinia* is endophytic and can be applied as seed coating, seed drenches, or soil drenching and may result in control of aphids (Ramakuwela et al. 2020). These effects are mediated by endophytic effects on the systemic expression of genes related to disease-resistance pathways (Bastias et al. 2017).

Pseudomonas spp. when applied as seed treatments has suppressed *Fusarium* wilt of carnation, induced resistance to *Colletotrichum obiculare* in cucumber, and to angular leaf spot caused by *P. syringae*; it also suppresses damping-off caused by *Pythium ultimum* or *Rhizoctonia solani* (Weller 2007).

Pennicillium roqueforti and *T. reesei* produce antibiotic compounds active against several bacterial plant pathogens (Ikram et al. 2018b). *P. itrinum* and *Aspergillus terrus* minimize stem rot caused by *Sclerotium rolfsii* through the induction of systemic resistance (Wargas et al. 2015).

- **Alleviation of stresses**

Plants experience a variety of stresses, including temperature, salt, and drought. Endophytic microorganisms provide protection to these production constraints.

- **Drought stress**

Many endophytes confer on plants resistance to drought stress. Grasses in coastal and geothermal areas require symbiotic fungi for salt- and heat-tolerance (Rodriguez et al. 2008). Mycorrhizal fungi are protective against heat stress (Mo et al. 2016). A commercial product containing *Trichoderma* has been shown to confer resistance against drought (Fig. 4).

 doi:10.20944/preprints202103.0303.v1

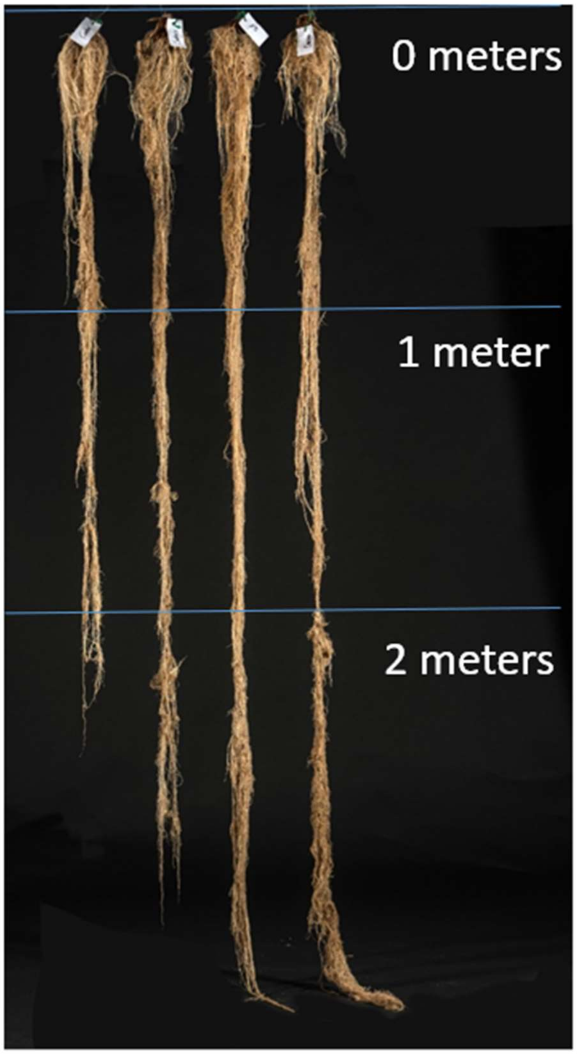

Fig. 5. Root growth of corn after seed treatment with nothing, with *Trichoderma afroharzianaum* and *T. atroviride* strain K4*,* with *T. atroviride* strain Kt plus *Bacillus amyloliquifacies,* or with 1-octen-3-ol (right to left). To facilitate root recovery plants were grown in split plastic pipes. Upon harvest, the two halves of the pipes were separated and roots and soil removed.

There are at least three mechanisms for buffering drought stress. The first is alleviation of the damaging effects of ROS, already discussed. Accumulation of osmolytes such as proline improve plants' water management systems (Ngumbi and Kloepper 2016). Accumulation of ethylene can be damaging to plants, and some endophytes produce the enzyme 1-aminocyclopropane-1-carboxylate (ACC) deaminase which confers protection (Glick 2014; Glick et al. 2007).

- **Salt stress**

Numerous endophytes Induce resistance to salt and other stresses by the mechanisms just described. *P. indica* is known to confer tolerance to salt stress (Baltruschat et al. 2008;

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 11 March 2021 doi:10.20944/preprints202103.0303.v1

Abdelaziz et al. 2019). *Bacillus amyloliquefaciens* increases the growth of plants under salt stress, with evidence of increased expression of several stress-related genes (Nautiyal et al. 2013). *T. asperellum* treatment of plants prior to exposure to salt stress gave increased seed germination, and there were changes in expression of 137 stress-response genes (Brotman et al. 2013). Other organisms that confer resistance to salt stress include *Dietzia natronlimnaea* (Bharti et al. 2016), *Enterobacter* (Kim et al. 2014), and *Yarrowia lipolytica* (Faranza et al. 2019). *Piriformaspora indica* alleviated salt stress through reprogramming of barley genes (Waller et al. 2005).

- **Temperature stress**

Heat and cold stress limit agricultural production and cause significant economic loss. Cold stress limits growth by causing membrane damage (Yadav et al. 2014). *Trichoderma, Burkholderia*, and *Pseudomonas* have frequently been used to alleviate temperature stress in plants (Mishra et al. 2011; Subramanian et al. 2015). Treatment with *Pseudomonas* increases growth and chlorophyll content and reduces electrolyte leakage (Mishra et al. 2009). *Trichoderma* strains increase the tolerance of tomato to cold stress first through mitigation of ROS damage and increased proline content and also by changes in the regulation of gene expression of transcription factors *NAC1* and dehydrin *TAS14* (Ghorbanpour et al. 2018). *A. niger* has been shown to be effective in alleviating heat stress by controlling ROS damage and increasing chlorophyll contents (Ishmail et al. 2010).

Summary

In this paper we discuss seed treatment methods; the importance of, and methods for preservation of microbial agents. In addition, we consider rhizospheric and endophytic competence. An economical way to apply these is through seed treatments. We also discuss benefits of seed treatments, including nitrogen fixation, maintenance of cellular functions, and improvements in photosynthesis. We also consider control of plant diseases and pests, increased yield and plant growth and alleviation of various types of plant stresses.

Seed treatments can be valuable. For example Rhizobacae fix nitrogen, so they decrease the amount of fertilizer required and are applied on legumes everywhere to the kind of microbe. This means that their value is many millions of dollars.

Another example is *Trichoderma* which has been used widely. Globally, the value of *Trichoderma* developed by the author and his colleagues in Israel has a net present value of greater than 600 million dollars (Kapulnik 2020).

Soil biology provides value by better managing nutrients, by avoiding runoff of polluted water with high levels of nitrates and nitrites, by increasing microbial diversity, and by increasing resistance to biotic and abiotic stresses, as discussed above, increasing plant productivity and alleviating heavy metal stresses. They have value in all these roles (Eid et al. 2019).

References

Abdelaziz ME, Abdelsattar M, Abdeldaym EA, Atia MAM, Mahmoud AWM, Saad MM, Hirt H (2019) *Piriformospora indica* alters Na+/K+ homeostasis, antioxidant enzymes and LeNHX1 expression of greenhouse tomato grown under salt stress. Scientia Horticulturae (Amsterdam) 256:108532. doi:10.1016/j.scienta.2019.05.059

Afzal I, T. J, Amirkhani M, Talor AG (2020) Modern seed technology: seed coating delivery systems for enhancing seed and crop performance. Agriculture 10:526; 510.3390/agriculture101526

American Type Culture Collection (2021) Lyophilization https://www.atcc.org/support/faqs/147c6/Lyophilization%20vs%20freezedrying-342.aspx

Baltruschat H, Fodor J, Harrach BD, Niemczyk E, Barna B, Gullner G, Janeczko A, Kogel K-H, Schaefer P, Schwarczinger I, Zuccaro A, Skoczowski A (2008) Salt tolerance of barley induced by the root endophyte *Piriformospora indica* is associated with a strong increase in antioxidants. New Phytologist 180 (2):501-510

Bastias DA, Martínez-Ghersa MA, Ballaré CL, Gundel PE (2017) Epichloë fungal endophytes and plant defenses: not just alkaloids. . Trends Plant Sci 22:938-948

Bharti N, Pandey SS, Barnawal D, Patel VK, Kalra A (2016) Plant growth promoting rhizobacteria Dietzia natronolimnaea modulates the expression of stress responsive genes providing protection of wheat from salinity stress. Sci Rep 6:1-16

Bonanomi G, Lorito M, Vinale F, Woo SL (2018) Organic amendments, beneficial microbes, and soil microbiota: toward a unified framework for disease suppression. Annu Rev Phytopathol 56:1-20

Brannen PM, Kenney, D. S. (1997) Kodiak: A successful biological-control product for suppression of soil-borne plant pathogens of cotton. J Industr Microbiol Biotechnol 19:169-171

Brotman Y, Landau U, Cuadros-Inostroza A, Takayuki T, Fernie AR, Chet I, Viterbo A, Willmitzer L (2013) Plant Root Colonization: Escaping Early Plant Defense Responses and Activation of the Antioxidant Machinery for Saline Stress Tolerance. PLoS Pathogens 9 (3):e1003221. doi:10.1371/journal.ppat.1003221

Chi F, Shen S-H, Cheng H-P, Jing Y-X, Yanni YG, Dazzo FB (2005) Ascending migration of endophytic rhizobia, from roots to leaves, inside rice plants and assessment of benefits to rice growth physiology. Appl Envir Microbiol 71 (11):7271-7278. doi:10.1128/aem.71.11.7271-7278.2005

Chi F, Shen S-H, Cheng H-P, Jing Y-X, Yanni YG, Dazzo FB (2015) Ascending migration of endophytic rhizobia, from roots to leaves, inside rice plants and assessment of benefits to rice growth physiology. Appl Environ Microbiol 71:7271-7278

Chi F, Yang P, Han F, Jing Y, Shen S (2010) Proteomic analysis of rice seedlings infected by *Sinorhizobium meliloti* 1021. Proteomics 10 (9):1861-1874. doi:10.1002/pmic.200900694

De Palma M, D'Agostino N, Proietti S, Bertini L, Lorito M, Ruocco M, Caruso C, Chiusano ML, Tucci M (2016) Suppression Subtractive Hybridization analysis provides new insights into the tomato (*Solani lycopersica* L.) response to the plant probiotic microorganism *Trichoderma longibrachiatum* MK1. Journal of Plant Physiology 190:79-94. doi:10.1016/j.jplph.2015.11.005

Doni F, Fathurrahman F, Mispan MS, Suhaimi M, Yussof WMW, Uphoff N (2019) Transciptome profiling of rice seedlings inoculated with symbiotic fungus T*richoderma asperellum* SL2. J Plant Growth Reg https://doi.org/10.1007/s00344-019-09952-7

Doni F, Zaln CRCM, Isahak A, Faturrahaman F, Anhar A, Mohamad W, Yusoff WMW, Uphoff N (2017) A simple, efficient, and farmer-friendly Trichoderma-based biofertilizer evaluated with the SRI rice management system. Organic Agric https://doi.org/10.1007/s13165-017-0185-7. doi:10.1007/s13165-017-0185-7

 doi:10.20944/preprints202103.0303.v1

Eid AM, Salim SS, Hassan E-DH, Isrmail MA, Fouda A (2019) Role of endophytes in plant health and abiotic stress management. In: Kumar V (ed) Microbiome in Plant Health and Disease. Springer Nature Pte Ltd., Singapore, pp 119-144

Faranza GF, Muahammad H, Hussain A, Gul J, Amjad L, Aman K, In-Jung L (2019) Am endophytic isolate of the fungus *Yarrowia lipolytica* produce metabolites that ameliorate thenegative impact of salt stress on the physiology of maize. BMC Microbio 19:3 http:/doi.org/2866-2013-2866

Franza GJ, Muhammad H, Hussain A, Gul J, Aman K, In-Jung L (2019) An endophyte isolate of the fungus *Yarrowia lipolytica* produce metabolite that ameliorate the negative impact of salt stress on the physiology of maiaze. BMC Microbiol 19:3 http:/doll.org/2866-2018-1374-2866

Fukami J, de la Osa C, Javier Ollero F, Megias M, Hungria M (2018) Co-inoculation of maize with Azospirillum brasilense and Rhizobium tropici as a strategy to mitigate salinity stress. Functional Plant Biology 45 (3):328-339. doi:10.1071/fp17167

Ghorbanpour A, Salimi A, Ghanbary MAT, Pirdashti H, Dehestani A (2018) The effect of T*richoderma harzianum* in mitigating low temperature stress in tomato (*Solanum lycopersicum* L.) plants. Sci Hort 230:134-141

Gill SS, Gill R, Trivedi DK, Anjum NA, Sharma KK, Ansari MW, Ansari AA, Johri AK, Prasad R, Pereira E, Varma A, Tuteja N (2016) Piriformospora indica: Potential and Significance in Plant Stress Tolerance. Front Microbiol 7:332. doi:10.3389/fmicb.2016.00332

Glick BR (2014) Bacteria with ACC deaminase can promote plant growth and help to feed the world. Microbiological Research 169 (1):30-39. doi:10.1016/j.micres.2013.09.009

Glick BR, Cheng Z, Czarny J, Duan J (2007) Promotion of plant growth by ACC deaminase-producing soil bacteria. European Journal of Plant Pathology 119 (3):329-339

Harman GE (2000) Myths and dogmas of biocontrol. Changes in perceptions derived from research on *Trichoderma harzianum* T-22. Plant Dis 84:377-393

Harman GE (2011) *Trichoderma*--not just for biocontrol anymore. Phytoparasitica 39:103-108

Harman GE, Cadle-Davidson M, Nosir W (2018) Patent application WO2017192117A1. Highly effective and multifunctional microbial compositions and uses. USA Patent,

Harman GE, Doni F, Khadka RB, Uphoff N (2019) Endophytic strains of *Trichoderma* increase plants' photosynthetic capability. J Appl Microbiol https://doi.org/10.1111/jam.14368

Harman GE, Hayes CK, Ondik KL (1998) Asexual genetics in *Trichoderma* and *Gliocladium*: mechanisms and implications. In: Kubicek CP, Harman GE (eds) *Trichoderma* and *Gliocladium,* Vol. 1. Taylor and Francis, London, pp 243-270

Harman GE, Howell CR, Viterbo A, Chet I, Lorito M (2004a) Trichoderma species---opportunistic, avirulent plant symbionts. Nature Rev Microbiol 2:43-56

Harman GE, Petzoldt R, Comis A, Chen J (2004b) Interactions between *Trichoderma harzianum* strain T22 and maize inbred line Mo17 and effects of these interactions on diseases caused by *Pythium ultimum* and *Colletotrichum graminicola*. Phytopathology 94:147-153

Harman GE, Taylor AG (1988) Improved seedling performance by integration of biological control agents at favorable pH levels with solid matrix priming. Phytopathology 78:520-525

Harman GE, Taylor AG, Stasz TE (1989) Combining effective strains of *Trichoderma harzianum* and solid matrix priming to improve biological seed treatments. Plant Dis 73:631-637

Harman GE, Uphoff N (2019a) Advantages and methods of using symbiotic microbes to enhance plant agriculture and the environment. Scientifica

Harman GE, Uphoff N (2019b) Advantages and methods of using symbiotic microbes to enhance plant agriculture and the environment. Scientifica https://doi.org/10.1155/2019/9106395

Ikram M, Ali N, Jan G, Jan FG, Rahman IU, Iqbal A, Hamayun M (2018a) IAA producing fungal endophyte *Penicillium roqueforti* Thom., enhances stress tolerance and nutrients uptake in wheat plants grown on heavy metal contaminated soils. PLoS One 13 (11):e0208150. doi:10.1371/journal.pone.0208150

Ikram M, Jan G, Jan FG, Rahman IU, Iqbal A, Hamayun M (2018b) IAA producing fungal endophyte *Penicillium roquefortI* Thom enhances stress tolerance and nutrient uptake in wheat plants grown on heavy metal contaminated soils. Plos One 13:e0208150

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

Ishmail HM, Hamayun M, Hussain A, Iqbal A, Khan SA, Lee I-J (2010) *Aspergillus nige*r boosted heat stress tolerance sunflower and soybean via regulating their metabolic and anitioxidant system. J Plant Interact 15:223-232

Ismail, Hamayun M, Hussain A, Iqbal A, Khan SA, Lee I-J (2020) *Aspergillus nige*r boosted heat stress tolerance in sunflower and soybean via regulating their metabolic and antioxidant system. J Plant Interact 15 (1):223-232. doi:10.1080/17429145.2020.1771444

Jaskiewicz M, Peterhansel C, Conrath U (2011) Detection of Histone Modifications in Plant Leaves. Jove-J Visualized Experi (55). doi:10.3791/3096

Jones KM, Kobayashi H, Davies BW, Taga ME, Walker GC (2007) How rhizobial symbionts invade plants: the *Sinorhizobium-Medicago* model. Nature Rev Microbiol 5 (8):619-633. doi:10.1038/nrmicro1705

Kapulnik Y (2020) Letter from the binational agriculture and research development fund.

Kim K, Jang YJ, Lee SM, Oh BT, Chae JC, Lee KJ (2014) Alleviation of salt stress by *Enterobacter* sp. EJ01 in tomato and *Arabidopsis* is accompanied by up-regulation of conserved salinity responsive factors in plants. Mol Cells 7:109-117

Konappa N, Krishnamurthy S, Arakere UC, Chowdappa S, Ramachandrappa NS (2020) Efficacy of indigenous plant growth-promoting rhizobacteria and *Trichoderma* strains in eliciting resistance against bacterial wilt in a tomato. Egyptian J Bioll Pest Control 30 (1):106. doi:10.1186/s41938-020-00303-3

Kubicek CP, Harman GE (1998) *Trichoderma* and *Gliocladium*, Vol. 1. Basic Biology, Taxonomy and Genetics. Taylor and Francis, London

Leopold AC (ed) (1986) Membrarnes, Metabolism and Dry Organisms. Comstock, Ithaca, NY

Marra R, Lombardi N, d'Errico G, Troisi J, Scala G, Vinale F, Woo SL, Bonanomi G, Lorito M (2019) Application of *Trichoderma* Strains and Metabolites Enhances Soybean Productivity and Nutrient Content. Journal of Agricultural and Food Chemistry 67 (7):1814-1822. doi:10.1021/acs.jafc.8b06503

Mastouri F, Bjorkman T, Harman GE (2010) Seed treatments with *Trichoderma harzianum* alleviate biotic, abiotic and physiological stresses in germinating seeds and seedlings. Phytopathology 100:1213-1221

Mayo-Prieto S, Campelo MP, Lorenzana A, Rodriguez-Gonzalez A, Reinoso B, Gutierrez S, Casquero PA (2020) Antifungal activity and bean growth promotion of Trichoderma strains isolated from seed vs soil. European Journal of Plant Pathology 158 (4). doi:10.1007/s10658-020-02069-8

Miao VP, Covert SF, Van Etten HD (1991) A fungal gene for antibiotic resistance on a dispensable ("B") chromosome. Science 254:1773-1776

Mishra PK, Bisht SC, Bisht JK, Bhatt JC (2011) Cold-Tolerant PGPRs as Bioinoculants for Stress Management. In: Maheshwari DK (ed) Bacteria in Agrobiology: Stress Management. Springer, Berlin, pp 95-118

Mishra PK, Bisht SC, Ruwari P, Selvakumar G, Bisht JK (2009) Enhancement of chilling tolerance of inoculated wheat seedlings with cold tolerant plant growth promoting Pseudomonads from N.W. Himalayas. Arch Microbiol 193:497-513

Mittler R (2002) Oxidative stress, antioxidants and stress tolerance. Trends in Plant Sci 7:405-410

Mo Y, Wang Y, Yang R, Zheng J, Liu C, Li H, Ma J, Zhang Y, Wei C, Zhang X (2016) Regulation of plant growth, photosynthesis, antioxidation and osmosis by an arbuscular mycorrhizal fungus in watermelon seedlings under well-watered and drought conditions. Front Plant Science 7. doi:10.3389/fpls.2016.00644

Nath K, Jajoo A, Poudyal RS, Timilsina R, Park YS, Aro E-M, G. NH, Lee C-H (2013) Towards a critical understanding of the photosystem II repair mechanism and its regulation under stress conditions. FEBS Letters 587:3372-3381

Nautiyal CS, Srivastava S, Chauhan PS, Seem K, Mishra A, Sopory SK (2013) Plant growth-promoting bacteria *Bacillus amyloliquefaciens* NBRISN13 modulates gene expression profile of leaf and rhizosphere community in rice during salt stress. Plant Physiol Biochem 66:1-9

Ngumbi E, Kloepper J (2016) **B**acterial-mediated drought tolerance: current and future prospects. Appl Soil Ecol 105:109-125

Paradiso R, Arena C, De Micco V, Giordano M, Aronne G, De Pascale S (2017) Changes in Leaf Anatomical Traits Enhanced Photosynthetic Activity of Soybean Grown in Hydroponics with Plant Growth-Promoting Microorganisms. Frontiers in Plant Science 8:674. doi:10.3389/fpls.2017.00674

Pascale A, Vinale F, Manganiello G, Nigro M, Lanzuise S, Ruocco M, Marra R, Lombardi N, Woo SL, Lorito M (2017) *Trichoderma* and its secondary metabolites improve yield and quality of grapes. Crop Protection 92:176-181. doi:10.1016/j.cropro.2016.11.010

Pelhivan N, Yesilyurt AM, Durmas N, Karaoglu SA (2017) *Trichoderma lixi*i ID11D seed biopriming mitigates dose dependent salt toxicity in maize. Acta Physiol Plantarum 39

Poveda J, Hermosa R, Monte E, Nicolás C (2019) he Trichoderma harzianum Kelch protein ThKEL1 plays a key role in root colonization and the induction of systemic defense in Brassicaceae plants. Front Plant Sci 10:1478. doi:doi: 10.3389/fpls.2019.01478

Rahnama M, Novikova O, Starnes JH, Zhang S, Chen L, Farman ML (2020) Transposon-mediated telomere destabilization: a driver of genome evolution in the blast fungus. Nucleic Acids Res 48 (13):7197-7217. doi:10.1093/nar/gkaa287

Ramakuwela T, Hatting J, Bock C, Vega FE, Wells L, Mbata GN, Shapiro-Ilan D (2020) Establishment of *Beauveria bassian*a as a fungal endophyte in pecan (*Carya illinoinensis*) seedlings and its virulence against pecan insect pests. Biol Cont 140:104102. doi:doi: 10.1016/j.biocontrol.2019.104102

Rodriguez RJ, Henson J, Van Volkenburgh E, Hoy M, Wright L, Beckwith F, Kim Y-O, Redman RS (2008) Stress tolerance in plants via habitat-adapted symbiosis. ISME Journal 2 (4):404-416. doi:10.1038/ismej.2007.106

Roper M, Ellison C, Taylor JW, Glass NL (2011) Nuclear and Genome Dynamics in Multinucleate Ascomycete Fungi. Current Biology 21 (18):R786-R793. doi:10.1016/j.cub.2011.06.042

Samuels GJ, Hebbar PK (2015) *Trichoderma*. Identification and Agricultural Properties. The American Phytopathological Society, St. Paul MN

Shafia A, Sutton JC, Yu H, Fletcher RA (2001) Influence of preinoculation light intensity on development and interactions of *Botrytis cinerea* and *Clonostachys rosea* in tomato leaves. Canadian J Plant Pathol 23 (4):346-357

Sherameti I, Shahollari B, Venus Y, Altschmied L, Varma A, Oelmueller R (2005) The endophytic fungus *Piriformospora indica* stimulates the expression of nitrate reductase and the starch-degrading enzyme glucan-water dikinase in tobacco and *Arabidopsis* roots through a homeodomain transcription factor that binds to a conserved motif in their promoters. J Biol Chem 280 (28):26241-26247

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 11 March 2021 doi:10.20944/preprints202103.0303.v1

Sherameti I, Tripathi S, Varma A, Oelmueller R (2008) The root-colonizing endophyte *Pirifomospora indica* confers drought tolerance in *Arabidopsis* by stimulating the expression of drought stress-related genes in leaves. Molecular Plant-Microbe Interactions 21 (6):799-807

Shoresh M, Gal-on A, Liebman D, Chet I (2006) Characterization of a mitogen-activated protein kinase gene from cucumber required for *Trichoderma*-conferred plant resistance. Plant Physiol 142:1169-1179

Stasz TE, Harman GE, Marx GA (1980) Time and site of infection of resistant and susceptible germinating pea seeds by *Pythium ultimum*. Phytopathology 70:730-733

Stefan M, Munteanu N, Stoleru V, Mihasan M, Hritcu L (2013) Seed inoculation with plant growth promoting rhizobacteria enhances photosynthesis and yield of runner bean (*Phaseolus coccineus* L.). Scientia Hort (Amsterdam) 151:22-29. doi:10.1016/j.scienta.2012.12.006

Subramanian P, Mageswari A, Kim KL, Y., Sa T (2015) Psychrotolerant endophytic *Pseudomonas* sp. strains OB155 and OS261 induced chilling resistance in tomato plants (*Solanum lycopersicum* Mill.) by activation of their antioxidant capacity. MolecPlant Microbe Interact 28:1073-1081

Taylor AG, Harman GE (1990) Concepts and technologies of selected seed treatments. Ann Rev Phytopathol 28:321-339

Taylor AG, Klein DE, Whitlow TH (1988) Smp: Solid Matrix Priming Of Seeds. In: Scientia Horticulturae, vol 37. pp 1-11

Thelen K, Shulz T (2007) Soybean seed applied inoculation. http://msueanrmsuedu/new/soybean_seed-applied_inoculum

Thilakarathna MS, Raizada MN (2017) A meta-analysis of the effectiveness of diverse rhizobia inoculants on soybean traits under field conditions. Soil Biol Biochem 105:177-196. doi:10.1016/j.soilbio.2016.11.022

Tyagi J, Varma A, Pudake RN (2017) Evaluation of comparative effects of arbuscular mycorrhiza (*Rhizophagus intraradice*s) and endophyte (*Piriformospora indica*) association with finger millet (*Eleusine coracana*) under drought stress. Europ J Soil Biol 81:1-10. doi:10.1016/j.ejsobi.2017.05.007

Van Deynze A, Zamora P, Delaux P-M, Heitmann C, Jayaraman D, Rajasekar S, Graham D, Maeda J, Gibson D, Schwartz KD, Berry AM, Bhatnagar S, Jospin G, Darling A, Jeannotte R, Lopez J, Weimer BC, Eisen JA, Shapiro H-Y, Ané J-M, Bennett AB (2018) Nitrogen fixation in a landrace of maize is supported by a mucilage-associated diazotrophic microbiota. PLOS Biology 16 (8):e2006352. doi:10.1371/journal.pbio.2006352

Vargas WA, Mandawe JC, Kenerley CM (2009) Plant-derived sucrose is a key element in the symbiotic association between *Trichoderma virens* and maize plants. Plant Physiol 151:792-808

Varma A, Verma S, Sudha, Sahay N, Butehorn B, Franken P (1999) *Piriformospora indica*, a cultivable plant-growth-promoting root endophyte. Appl Environ Microbiol 65:2741-2744

Vitti A, Pellegrini E, Nali C, Lovelli S, Sofo A, Valerio M, Scopa A, Nuzzaci M (2016) *Trichoderma harzianum* T-22 Induces Systemic Resistance in Tomato Infected by Cucumber mosaic virus. Front Plant Science 7:1520. doi:10.3389/fpls.2016.01520

Waller F, Achatz B, Baltruschat H, Fodor J, Becker K, Fischer M, Heier T, Hueckelhoven R, Neumann C, von Wettstein D, Franken P, Kogel K-H (2005) The endophytic fungus *Piriformospora indica* reprograms barley to salt-stress tolerance, disease resistance, and higher yield. PNAS 102 (38):13386-13391

Wargas M, Khan AL, Hamayun M, Shahzad S, Khang S-M, Kim S-H, Lee. L-*J* (2015) Endophytic fungi promote plant growth and mitigate the adverse effecs of stem rot: an example of *Penicillium citrinum* and *Aspergillus terrus*. J Plant Nutr 42:2600-2062

Weindling R (1932) *Trichoderma lignorum* as a parasite of other soil fungi. Phytopathology 22:837-845

 doi:10.20944/preprints202103.0303.v1

Weindling R, Fawcett HS (1936) Experiments in the control of Rhizoctonia damping-off of citris seedlings. J Agric Sci , CA Agric Exp Station 10:1-16

Weller DM (2007) *Pseudomonas* biocontrol agents of soil borne pathogens: Looking back over 30 years. Phytopathology 97:250-257

Wikipedia (2021) Gene Silencing. https://enwikipediaorg/wiki/Gene_silencing

Woo SJ, Ruocco M, Vinale F, Marra R, Lombardi N, Pascale A, Lanzuise S, Manganiello G, Lorito M (2014) *Trichoderm*a-based and their widespread use in agriculture. Open Mycology J 8:71-126

Yadav SK, Chen YH, Sant'anna SMR, Voldeng HD, Fedak G, Savard ME, Savard ME, Zhang JX (2014) Comparison of in vitro regereation efficiency leaf explants in response to different cyckinins and assessment of genetic unifromity of regenerated plantlets of *Solanum surattense* Burm.f. African J Biotech 9:8891-8997

Yanni YG, Rizk RY, Corich V, Squartini A, Ninke K, Philip-Hollingsworth S, Orgambide G, De Bruijn F, Stoltzfus J, Buckley D, Schmidt TM, Mateos PF, Ladha JK, Dazzo FB (1997) Natural endophytic association between Rhizobium leguminosarum bv. trifolii and rice roots and assessment of its potential to promote rice growth. Plant and Soil 194 (1-2):99-114. doi:10.1023/a:1004269902246