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Mechanism of Drought Tolerance in Plants Using *Trichoderma spp.*

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Abstract: ***Drought is a major stress limiting the increase in the demand for food crops yet the world population is rapidly growing. Trichoderma spp. has been found to be associated with almost all natural ecosystems. They exist either in the rhizosphere or on plant roots. They form a symbiotic relationship with the host plant. In the existence of the relationship, the proteome and transcriptome of plants change as a consequence of the interaction of Trichoderma metabolites or plant colonization. Alleviation of damage by reactive oxygen species (ROS), Water-use efficiency and Secretion of phytohormonal analogues are the three mechanisms employed by the fungi in enhancing plant growth under drought stress. Globally, studies clearly reveal that, the use of Trichoderma spp. under drought stress can effectively augment plant growth.***

Keywords: Drought stress, *Trichoderma spp*. and plant growth

1. Introduction

FAO (2009) points out that one major challenge towards global agriculture includes production of 70% more food crop for an extra 2.3 billion people by 2050 worldwide. Drought is a major stress limiting the increase in the demand for food crops. Global climate change has been witnessed over the past decades and is anticipated to continue in the future (IPCC, 2007). Rainfall patterns cannot be foretold with much assurance and therefore, extreme weather conditions are becoming common. In that, some regions are experiencing overflows while others are affected by deficiency of precipitation. Water deficit limits growth and productivity of main crop species, reducing yields to less than half. On the other hand, *Trichoderma spp*. is a cosmopolitan fungi found in agricultural, forest, desert soils. Also, they colonize roots of various plants found in different ecosystems. They have been defined as plant symbiont opportunistic avirulent organisms, able to colonize plant roots and to produce compounds that stimulate growth and plant defense mechanisms under suboptimal conditions (Harman *et al.*, 2004). For the past many years, *Trichoderma spp.* has been mostly used as biocontrol agents. However, in the recent years, they have become popular as plant growth promoter (Hermosa et al., 2012).

Studies show that, *Trichoderma spp.* form a symbiotic relationship with the host plant as early as the third phase of seed germination and this association lasts till the plant matures. Once the association has been established, systemic effects are induced in the host plant, so that while only roots are typically colonized, the effects also occur in leaves and in stems. The proteome and transcriptome of plants change as a consequence of the interaction of Trichoderma metabolites (Marra et al., 2006) or plant colonization (Bae et al., 2011). Thus, the fungi reprogramme plant gene expression, resulting in alteration of plant responses to their environment.

Donoso et al. (2009) points out that there exists a very little information concerning the way in which the fungus enhances plant growth to water deficit. Mastouri (2009) made an assumption that, since the interaction between the plant and the fungus happens largely at the rhizosphere; therefore, such a mechanism is probable connected to an increase in the water absorption effectiveness, due to the increased root capacity, hence, increased water absorption. However, *Trichoderma* spp. has been shown to enhance drought tolerance in plants as early as germination phase. Therefore, different explanations on the mechanisms employed by the fungus are worth investigating. In that connection, the present paper seeks to review on the up to date developments on the mechanisms employed by *Trichoderma spp.* in enhancing plant growth under drought stress.

2. Plant- Fungus Interaction

Researchers have shown that plants in nature do not function as independent individuals; however, they house diverse communities of symbiotic microbes. The role played by these microbes in plant development and protection cannot be overlooked. These symbiotic microbial interactions are substantial for the existence of both the host and microbe in both abiotic and biotic stressed environments. A good number of fungi are known to form a symbiotic interaction with various plants without causing disease, for example; mycorrhizas, binucleate Rhizoctonia spp., *Piriformaspora indica*, a range of plant Growth-promoting rhizobacteria and, the focus of this present study, *Trichoderma spp*. (Shoresh et al., 2010). For decades, these organisms have been known as agents that can biocontrol diseases in plants. Nevertheless, recent researches have clearly revealed other useful attributes of these organisms. Harman et al., (2004), described *Trichoderma spp*. as plant symbionts. Many *Trichoderma* strains colonize plant roots of dicots and monocots. During this process *Trichoderma* hyphae coil around the roots, form

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appresoria-like structures, and finally penetrate the root cortex. *Trichoderma* grows intercellularly in the root epidermis and cortex and induces the surrounding plant cells to deposit cell wall material and establish chemical communication with the plant compounds. When inside plant roots, fungi have access to plant nutrients, which allow them to proliferate. Moreover, they significantly enhance root growth in many cases thus, providing more niches for growth of the fungi. On the other hand, the plant benefits from this relationship through increased root and shoot growth, increased macro- and micronutrient uptake, and protection from diseases (Harman et al., 2004).

A quite large number of the chemical communicants (effectors / elicitors) produced by the fungi include, small proteins, peptides and other metabolites, including volatile ones. Once the contact has been initiated, the beneficial responses to plants can last for at least the growing season for an annual plant because the fungi grow and continue to colonize the roots as they, in turn, also grow and increase (Harman, 2000). There exist signalling induces systemic effects in plants, so that while only roots are typically colonized, the effects also occur in leaves and in stems. The proteome and transcriptome of plants change as a consequence of the interaction of *Trichoderma* metabolites (Marra et al., 2006) or plant colonization (Bae et al., 2011). Thus, the fungi reprogramme plant gene expression, resulting in alteration of plant physiology and responses to their environment.

3. Mechanisms Employed by the Fungus

Plants develop a variety of mechanisms to acclimatize themselves to forever changing environments. These mechanisms are facilitated through multiple signal transduction pathways acting in a global signal network (Zhou et al., 2012).

Alleviation of damage by reactive oxygen species (ROS).

Trichoderma spp. colonizes roots and remains restricted to the cortex and outer layers of the root epidermis of host plant. However, it modulates gene expression in both shoots and roots (Yedidia et al. 1999). When plants are subjected to abiotic stresses, the content of reactive oxygen species may increase to toxic concentrations. Several pathways in plants convert toxic ROS to a lesser toxic form (Mittler, 2002). On the other hand, *Trichoderma* strains enhance the activity of these pathways, through enhanced expression of genes encoding the component enzymes (Mastouri et al., 2010). For example, if these pathways are enhanced in the chloroplasts, then it is expected that the photosynthetic efficiency will Increase by reducing damage by the superoxide anion and other reactive species involved in photosynthesis.

Sharma and Dubey (2005) demonstrate that under drought stress condition ROS like superoxide radical, hydrogen peroxide and hydroxyl radicals greatly affect the membrane and DNA of cells. Mittler (2002) reveals that under severe stress, ROS production can surpass the scavenging aptitude and amass to levels that can harm cell components for example, through lipid peroxidation. *Trichoderma* spp. augments protection against ROS perhaps by increasing ROS scavenging capacities. Proteomics of roots inoculated with *Trichoderma* showed an increase in levels of anti-oxidative enzymes mainly Superoxide dismutase (SOD) as well as increased levels of peroxidase, glutathione-reductase and Glutathione-S-transferase (GST), and other detoxifying enzymes in leaves (Shoresh and Harman, 2008). SOD is the main scavenger of superoxide radicals, which converts the toxic superoxide ($O2^{-}$) to hydrogen peroxide and oxygen, through a process called *dismutation reaction*: $2O_2^{-} + 2H+ H_2O_2 + O_2$. The enzyme embodies the first line of cell defense against ROS generated abiotic stresses like drought in plants, therefore, preventing the tissue damage due to oxidative stress. Blokhina et al., (2003) explained that CAT and POD enzymes are able to convert toxic H_2O_2 to water and oxygen. under water stress only elevated SOD activity cannot protect the plants from toxic effect of oxygen free radical hence CAT and POD is needed to remove toxicity of H_2O_2 (Arora et al., 2002).

Rodriguez et al. (2008) revealed that, when both symbiotic and nonsymbiotic plants were exposed to abiotic stress; panic grass and tomato were subjected to heat stress while dune grass and tomato were subjected to salt stress, the results showed that; in the absence of stress, both nonsymbiotic and symbiotic plant leaf tissues for all remained green demonstrating the absence of ROS generation and therefore lack of stress response. On the other hand, when these plants were exposed to stress, nonsymbiotic tissues bleached white indicating the generation of ROS while symbiotic tissues remained green. This suggests that plant symbionts aid either in scavenging ROS or inducing plants to more efficiently scavenge ROS, or prevents ROS production when exposed to abiotic stress

In an earlier experiment conducted by Bjorkman (1998) showed that, those Seeds that were subjected to oxidative stress had much reduced vigor, nevertheless, subsequent treatment with *Trichoderma*-T22 restored vigor. Furthermore, peroxidase gene was primed in cucumber plants inoculated with *Trichoderma* (Shoresh et al., 2005). Another study by Mastouri et al. (2009) clearly reveals that treating seeds of tomato with *T. harzianum* T22 enhances germination percentage under osmotic. The study further found an increase in lipid peroxide content in young seedlings with an increase in the water potential of media, whereas T22- treated seedlings had significantly less lipid peroxide than untreated seedlings. In a recent experiment performed by Gusain, et al., (2014), *T. harzianium* (T-35) benefited rice plants to tolerate severe drought stress through the reduction of oxidative stress by enhancing the production of SOD, CAT and POD anti-oxidative enzymes.

4. Water-Use Efficiency

Research has it that, symbiotic plants spend significantly less water than nonsymbiotic plants. A research on rice, tomato and dune grass shows that these plants used significantly less fluid than their counterpart nonsymbiotic plants (Rodriguez et al., 2008). Moreover, these symbiotic plants achieve increased biomass levels; therefore, decreased water

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consumption suggests more efficient water usage. Decreased water consumption and increased water-use efficiency could provide a unique mechanism for symbiotically conferred drought tolerance.

5. Secretion of Phytohormonal Analogues

Studies show that, numerous *Trichoderma* isolates have revealed the ability for increasing plant growth by improving through their ability to absorb nutrients and the secretion of growth-promoting metabolites (Chowdappa et al., 2013). Linkies et al. (2009) shows that auxin, cytokinin, abscisic acid, and ethylene are major players in plant growth and in fitness. IAA is the most abundant naturally occurring auxin in vascular plants, and it is known to play a major role in lateral and adventitious root initiation and emergence and in shoot development (Simon and Petrasek 2011). In a study conducted by Medina et al., (2014), IAA levels were shown to be increased by the *Trichoderma* isolates that also promoted plant growth. According to Martinez-Medina et al. (2011) another growth-promoting strain *T. harzianum* T-78 is said to have the capacity to increase IAA levels in plant. Another study by Contreras-Cornejo et al. (2009) demonstrated that *Trichoderma virens* promote growth of Arabidopsis thaliana through the classical auxin response pathway, due to production of IAA and auxinlike compounds. A good number of Trichoderma isolates have been reported to produce and release in the culture medium auxin related compounds (Gravel et al., 2007). However, Sofo et al. (2012) did not detect hormone production by *T. harzianum* T-22 in axenic growing media, suggesting that T-22 is able to induce hormone synthesis ex novo in the plants, probably through the up-regulation of plant genes for hormone biosynthesis or the down-regulation of the genes involved in hormone catabolism.

Abscisic acid has been related to plant development and defense against abiotic stress. It is known to modify growth and development under stress conditions, such as cold, drought, and salinity (Christmann et al., 2006). On the other hand, Martínez-Medina et al., (2014) elucidates that *Trichoderma* isolates that promoted plant growth also decreased the concentration of ABA in the melon shoots, therefore, suggesting that the promotion of plant growth provided by these Trichoderma isolates might be accompanied by an improvement in plant fitness, as has been suggested for mycorrhizal associations regarding drought stress (Aroca et al. 2013).

Another major regulator of plant growth and development is ethylene. Martinez-Medina et al. (2014) reports that the concentration of the ethylene precursor ACC was decreased by the *Trichodema* isolates. No wonder Viterbo et al., (2010) reports that some Trichoderma isolates are known to produce ACC deaminase (ACCD), which reduces the availability of the ACC necessary for ET biosynthesis, which might result in plant root growth.

6. Efficacy of Trichoderma in enhancing plant growth under drought stress

Studies show that, *Trichoderna spp*. is utilized worldwide to enhance plant growth even in optimal growth conditions. However, research show that, the response is highly positive under suboptimal conditions. Asaduzzaman et al. (2010) clearly showed that *T. harzianum* IMI 392432 enhanced germination percentage in chili seeds both in laboratory and field conditions. Chaur-Tsuen Lo and Chien-Yih Lin (2002) screened *Trichoderma* strains on plant growth and root growth of bitter gourd, loofah and cucumber and noted that *Trichoderma* strains significantly increased from 26 to 61 % in seedling height, 85-209 % in root exploration, 27-38% in leaf area and 38 to 62 % in root dry weight after 15 days of sowing. Hassan et al., (2013) recently demonstrated that *Trichoderma spp.* significantly increase millet seedling height, root length and root dry weight. Mastouri et al.,(2010), demonstrated that treating tomatoes with *T. harzianum* T22 enhances speed of germination in that, the seed treated with T22 germinated faster and more uniformly compared with untreated seed at all water deficit levels. Also, seedling growth was also improved by T22 treatment. Bae et al. (2009) through an experiment on cocoa seedlings showed that colonization of these seedlings by *T. hamatum* isolate DIS 219b resulted in a delay in many aspects of the drought response. The contributing factor to this was hypothesized to be through enhanced root growth, resulting in an improved water status allowing cacao seedlings to tolerate drought stress. Mastouri et al. (2010) reports that *T. harzianum* when added as seed treatment in tomatoes or as a soil treatment in *Arabidopsis* mainly enhanced the germination at osmotic potentials of up to 0.3 MPa. Ilyas *et al*. (2012) wrapped up his finding by revealing that, those microbial strains isolated from soils with moisture stressed conditions have even more potential to induce tolerance to host plant, when inoculated.

7. Conclusion

Trichoderma spp. is a cosmopolitan fungal organism that has been utilized not only in biocontrol of plant pathogens but also in enhancing plant growth even under suboptimal plant-growth conditions. Several studies have revealed that, these fungi promote plant growth even as early as germination phase. The fungi form a mutualistic relationship with the host plant hence changing the gene expression of the host plant. Alleviation of ROS in plants under drought stress has been reported as one of the mechanisms employed by the fungi. Other mechanisms include; release of metabolites analogous to phytohormones that enhance growth in presence of the drought stress. Water use efficiency has been also proposed as one of the ways employed by these fungi. It is worth noting that, the use of Trichoderma in enhancing plant growth under drought stress is an effective method that has been proved by several studies globally.

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