academicJournals

Vol. 16(50), pp. 2299-2306, 13 December, 2017
DOI: 10.5897/AJB2017.16270
Article Number: 19F099C67135
ISSN 1684-5315

http://www.academicjournals.org/AJB

African Journal of Biotechnology

Review

Trichoderma as biological control weapon against soil borne plant pathogens

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Received 4 October, 2017; Accepted 14 November, 2017

The genus of *Trichoderma* is widely applied for the biocontrol of phytopathogenic fungi in agriculture sector. Moreover, *Trichoderma* species are also excessively exploited in different industrial purposes due to their production of important lytic enzymes such as chitinases, glucanases and proteases. Several genetic improvement trials are carried out for maximizing the role of *Trichoderma* as biological control agents via mutation, protoplast fusion, and genetic transformation. This review highlights the mode of action of *Trichoderma* against pathogenic fungi, potential applications in different fields of life, and the recent genetic improvement trials for increasing the antagonistic abilities of this fungus as biological control agent.

Key words: *Trichoderma*, antagonism, lytic enzymes, genetic improvement.

INTRODUCTION

Farmers around the world need the chemical pesticides to control the plant disease pathogens in order to maintain the quality and redundancy of agricultural products (Junaid et al., 2013). It was estimated that 37% of crop loss is due to pests, of which 12% is due to pathogens (Sharma et al., 2012). On the contrast, the excessive and the misuse of pesticides over the past decades caused environmental pollution and several health problems in addition to their expensive costs for developing countries. Moreover, the long term use of chemical pesticides can lead to development of certain resistant organisms (Naher et al., 2014). Recently, the world attention resort to find sustainable, safe and ecofriendly alternatives. Biological control agents (BCA) refer to the utilization of some living microorganisms to suppress the growth of plant pathogens (Pal and Gardener, 2006). In other words, biological control means the use of beneficial organisms, their genes, and/or products to reduce or suppress the negative effects of plant pathogens (Junaid et al., 2013). Currently, several biocontrol agents have been recognized and are available as bacterial agents for example *Pseudomonas, Bacillus*, and *Agrobacterinum* and as fungal agents such as *Trichoderma, Aspergillus*, *Gliocladium*, *Ampelomyces*, *Candida*, and *Coniothyrium* (Naher et al., 2014). *Trichoderma* is one of the famous filamentous fungi widely distributed in the soil, plant material, decaying vegetation, and wood (Gajera et al., 2013). The

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Trichoderma genus are related to the order of Hypocreales, family of Hypocreaceae and the genus have more than 100 phylogenetically defined species (Kumar, 2013). Among the common species of *Trichoderma* are *Trichoderma harzianum*, *Trichoderma koningii*, *Trichoderma longibrachiatum*, *Trichoderma pseudokoningii*, *Trichoderma viren*, and *Trichoderma viride*.

Trichoderma is considered an excellent biocontrol agent model due its high ability to multiply, spread, easy to isolate and culture (Pandya et al., 2011). *Trichoderma* strains can act as biocontrol agents against fungal phytopathogens such as *Phythium*, *Phytophthora*, *Botrytis*, *Rhizoctonia,* and *Fusarium* through several mechanisms including competition for nutrients and space, antibiosis and induction of plant defensive mechanisms and mycoparasitism (Benítez et al., 2004). It was shown that *Trichoderma* can use one or more of these mechanisms according to type of fungus and the environmental conditions such as temperature, pH, and nutrient concentrations (Gajera et al., 2013). Recently, the number of products based on *Trichoderma* found in the international market is increasing with more than 250 products (Woo et al., 2014). This review highlights the different mechanisms of *Trichoderma* as biological control agents and the genetic tools to improve these activities.

HISTORY OF *TRICHODERMA*

The first description of *Trichoderma* as a fungus was by Persoon in 1794 who described this fungus as appearing like mealy powder enclosed by a hairy covering. It was reported that the genus of *Trichoderma* contain only one species, namely *T. viride* (Bisby, 1939). Then Rifai (1969) distinguished nine species using the analysis of morphological characteristics: *T. harzianum* Rifai*, T. viride, Trichoderma hamatum* (Bonord.) Bainier*, T. koningii* (Oudem.) Duché *&* R. Heim*, Trichoderma polysporum* (Link) Rifai*, Trichoderma piluliferum* J. Webster *&* Rifai*, Trichoderma aureoviride* Rifai*, T. longibrachiatum* Rifai, and *Trichoderma pseudokoningii* Rifai (Błaszczyk et al., 2014). Recently, 104 species of *Trichoderma* have been registered internationally (Pandya, 2011). It must be mentioned that Weindling (1932) referred for the first time the importance of *Trichoderma* as bioagents.

TRICHODERMA BIOCONTROL MECHANISMS

Competition

Since *Trichoderma* strains grow rapidly in the soil due to their natural resistance to many toxic compounds, including herbicides, fungicides, and pesticides; this gave it a superior ability to colonize, take up soil nutrients, and therefore starvation of other organisms from nutrition (Chet et al., 1997; Benítez et al., 2004). Competition for space and for nutrients such as carbon and nitrogen is an important feature of *Trichoderma* antagonism (Vinale et al., 2008). Sivan and Chet (1989) demonstrated that competition for nutrients is the major mechanism used by *T. harzianum* to control *Fusarium oxysporum f. sp. melonis*. It is well known that iron uptake is essential factor for viability of pathogen in soil, so that some *Trichoderma* strains produce highly efficient siderophores to chelate iron and stop the growth of other fungi (Chet and Inbar, 1994). Furthermore, the ability of *Trichoderma* to obtain ATP from the metabolism of several substrates such as cellulose and glucan give it a competitive advantage than the other pathogens. Ferre and Santamarina (2010) showed that colonies of *T. harzianum* inhibited the growth of *Fusarium culmorum* strains in different environmental conditions and the macroscopic analysis of the petri plates revealed that *T. harzianum* competed *F. culmorum* for space and nutrients.

Mycoparasitism

Mycoparasitism is a very complex process in which *Trichoderma* recognizes signals from the host fungus, coils around host hyphae and host penetration. The lytic enzymes of *Trichoderma* such as chitinases, glucanases and proteases degrade the host cell wall and kill them (Sharma et al., 2012). It well known that the cell wall of *Pythium* species is composed of cellulose (Figure 1), while chitin is the main structural component of *Rhizoctonia solani* cell walls (Bartinicki-Garcia, 1968; Farkas, 1990; Sivan and Chet, 1989). Moreover, *Trichoderma* are good producer of chitinases that hydrolyze the glycosidic bonds between the N-acetyl glucosamine residues of chitin (Agrawal and Kotasthane, 2012); also, cellulases which hydrolyse β-1,4 glucans (Nevalainen and Penttilä, 1995) and these enzymes are among the most effective weapons for plant diseases biological control. It was shown that *Trichoderma* species are the most common mycoparasitic and saprophytic fungi that have high ability for colonization and attack a great variety of phytopathogenic fungi responsible for important diseases of major economic crops worldwide (El-Hassan et al., 2012*)*.

Howell (2003) obtained transformants of *T. harzianum* T3 that produce a variety of cellulases, which make this isolate very effective in the control of *Pythium ultimum* on cucumber seedling than the wild type. Furthermore, Limon et al. (1999) obtained transformants of *T. harzianum* strain CECT 2413 that overexpressed chitinase (*chit33*) and these transformants were more effective in inhibiting the growth of *R. solani* as compared to the wild type.

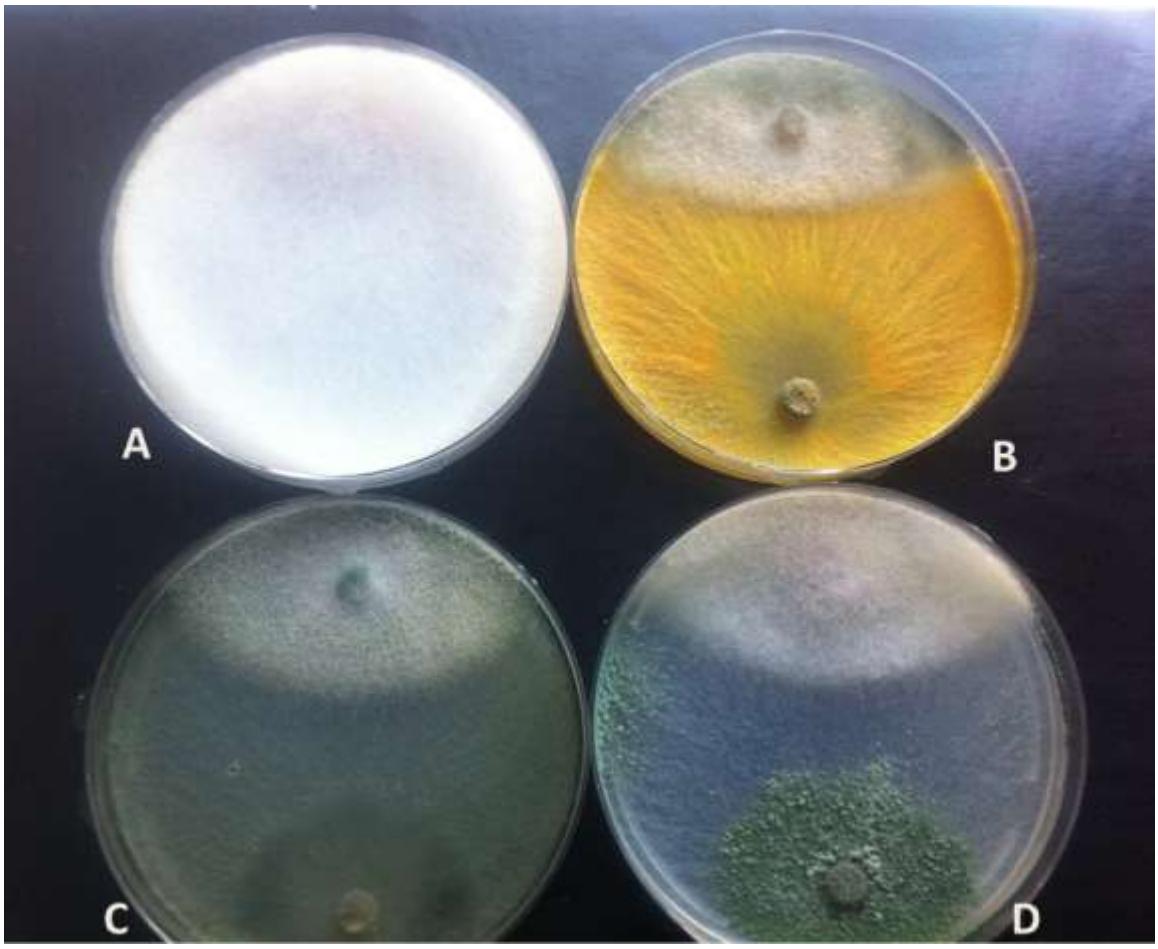

Figure 1. The antagonism of *Trichoderma* spp. against *Pythium* spp. (A) The fungus of *Pythium* spp. while B, C and D different species of *Trichoderma* overgrowth *Pythium* spp.

Antibiosis

Trichoderma can produce low molecular weight diffusible compounds or antibiotics that inhibit the growth of other microorganisms. There are several metabolites or antibiotics secreted from *Trichoderma* against their pathogens such as: harzianic acid, tricholin, peptaibols, 6-penthyl-α-pyrone, viridin, gliovirin, glisoprenins, and heptelidic acid (Gajera et al., 2013). Peptaibols are a large family of antibiotic peptides and *Trichoderma* can synthesize more than 190 of these compounds. Trichokonin VI (TK VI) of *T. pseudokoningii* is one of the peptaibols that can induce an extensive apoptotic programmed cell death in plant fungal pathogens such as *F. oxysporum* (Shi et al., 2012).

Sadykova et al. (2015) tested the antibiotic activity in 42 strains of 8 species of the *Trichoderma* genus (*Trichoderma asperellum, T. viride, T. hamatum, T. koningii, T. atroviride, T. harzianum, T. Citrinoviride*, and *T. longibrachiatum*) isolated from Siberian. It was shown that these species differ in the degree of their antibacterial and antifungal activity and the strain *T. citrinoviride* TV41, exhibited high activity and a wide range of actions against the pathogenic fungi of the *Aspergillus* and *Candida albicans* genus and bacteria, including methicillin resistant *Staphylococcus aureus*. The authors expected that peptaibols are probably the most active compounds in the strain culture extracts according to mass and IR spectrometry data. Vinale et al. (2014) showed that the pyrone 6-pentyl-2H-pyran-2-one is a metabolite purified from the culture filtrate of different *Trichoderma* spp. (*T. viride, T. atroviride, T. harzianum* and *T. koningii*) and has shown both *in vivo* and *in vitro* antifungal activities towards several plant pathogenic fungi. In addition, Ghisalberti et al. (1990) demonstrated that the biocontrol efficacy of *T. harzianum* isolates against *Gaeumannomyces graminis var. tritici* is related to the production of pyrone-like antibiotics. Furthermore, Howell (1999) reported that strains of *Trichoderma virens* (P group) produce the antibiotic gliovirin which is very active against *P. ultimum,* while the Q group of these strains can produce gliotoxin, which is very active against

R. solani.

Induction of plant growth and defense

Trichoderma spp. are well-known for their ability to promote plant growth and defense. *Trichoderma* can increase root development, shoot length, leaf area and therefore crop yield via colonization of plant roots, proliferation of secondary roots and solubilizing several nutrients as P and Fe to plants (Hermosa et al., 2012). The previous studies showed that *Trichoderma* can produce gluconic and citric acids that decrease the soil pH, enhance the solubilization of phosphates, micronutrients, and mineral components such as iron, magnesium, and manganese (Benitez et al., 2004; Harman et al., 2004b; Vinale et al., 2008). It was noted that the bean plants treated with *T. harzianum* T019 always had an increased size respect to control. In addition, this strain induced the expression of plant defense-related genes and produced a higher level of ergosterol, indicating its positive effects on plant growth and defense in the presence of the pathogen (Mayo et al., 2015). Moreover, the roots of maize plants treated with *T. harzianum* strain T-22 were about twice as long compared to untreated plants after several months from treatment (Harman, 2004a).

Saravanakumar et al. (2016) showed that *Trichoderma* cellulase complexes trigger the induced systematic resistance (ISR) against *Curvularia* leaf spot in maize by increasing the expression of genes related to the jasmonate/ethylene signaling pathways. Furthermore, Rao et al. (2015) suggested that treatment of legume seeds (*Cajanus cajan*, *Vigna radiate* and *Vigna mungo*) with *T. viride* induces systemic resistance by reprogramming defense mechanisms in these legumes. Reprogramming alleviated the levels of defense enzymes (PO, PPO and PAL), ROS ($O_2^{\bullet-}$, H_2O_2, OH•), antioxidant enzymes (CAT, SOD), scavenging activity of antioxidant enzymes in response to oxidative stress induced by *F. oxysporum* and *Alternaria alternata*. This mechanism helps in developing resistance in plants and therefore protect from pathogens. *Trichoderma* metabolites may also increase disease resistance by triggering systemic plant defence activity and/or enhance root and shoot growth (Vinale et al., 2014).

OTHER APPLICATIONS OF TRICHODERMA

In addition to their important roles as biocontrol agents, plant growth promoter and defense, there are some other applications for *Trichoderma* in different fields as shown in Figure 2.

Bioremediation of contaminated soils

Trichoderma strains play an important role in the bioremediation of soil contaminated with pesticides and herbicides as consequence of their high abilities to degrade a wide range of insecticides: organochlorines, organophosphates, and carbonates (Kumar, 2013). Moreover, since *Trichoderma* is a potent producer of hydrolytic and industrially important enzymes, like cellulases and chitinases, this make *Trichoderma* spp. highly resistant to a wide range of toxicants, heavy metals, tannery effluents, and harmful chemicals like cyanide (Hasan, 2016). The above advantages make them an ideal fungal genus in bioremediation of toxic pollutants. Previous studies showed that *Trichoderma* spp. can remove and accumulate the various heavy metals such as copper, zinc, cadmium, and arsenic through sorption and biovolatilization (Yazdani et al., 2009; Srivastava et al., 2011; Zeng et al., 2010). Teng et al. (2015) showed that *T. reesei* FS10-C enhances the phytoremediation ability of Cd-contaminated soil by the hyper accumulator *Sedum plumbizincicola* and also increases soil fertility. Moreover, it was reported that each *T. virens PDR-28* and *T. pseudokoningii* increased the dry biomass and Cd accumulation of maize and pearl millet, respectively as compared to the control (Babu et al., 2014; Bareen et al., 2012). Furthermore, Arfarita et al. (2013) reported that the isolate of *T. viride strain FRP3* was able to grow in culture medium containing the herbicide glyphosate as the sole phosphorus source. This was coupled with a decrease in the total phosphorus concentration, indicating that the strain may perhaps possess mechanisms for degradation of glyphosate.

Foods and textiles industries

The *Trichoderma* lytic enzymes such as cellulases, hemicellulases, and pectinases are used as food additives in the production of fruit and vegetable juices and also to improve wine flavor and enhance fermentation, filtration, and quality of beer (Błaszczyk et al., 2014). Cellulases produced by *Trichoderma* are applied also in the textile industry to soften and condition the textiles as well as to produce high quality washing powders. In addition, these enzymes are used in the pulp and paper industry to modify fiber properties and to reduce lignin contents. In parallel, cellulases and hemicellulases produced by *T. reesei* are used in the production of bioethanol from farm wastes via degradation of substrates to simple sugars and then converted them to chemical intermediates such as ethanol (Błaszczyk et al., 2014). It must be mentioned that production of xylanase, cellulase and pectinases of *Trichoderma* account for 20% of the world enzyme market (Polizeli et al., 2005). In the food industry, xylanase enzymes help to break down polysaccharides in the dough of cookies, cakes, and aids in the digestibility of wheat by poultry by decreasing the viscosity of the feed (Harris and Ramalingam, 2010).

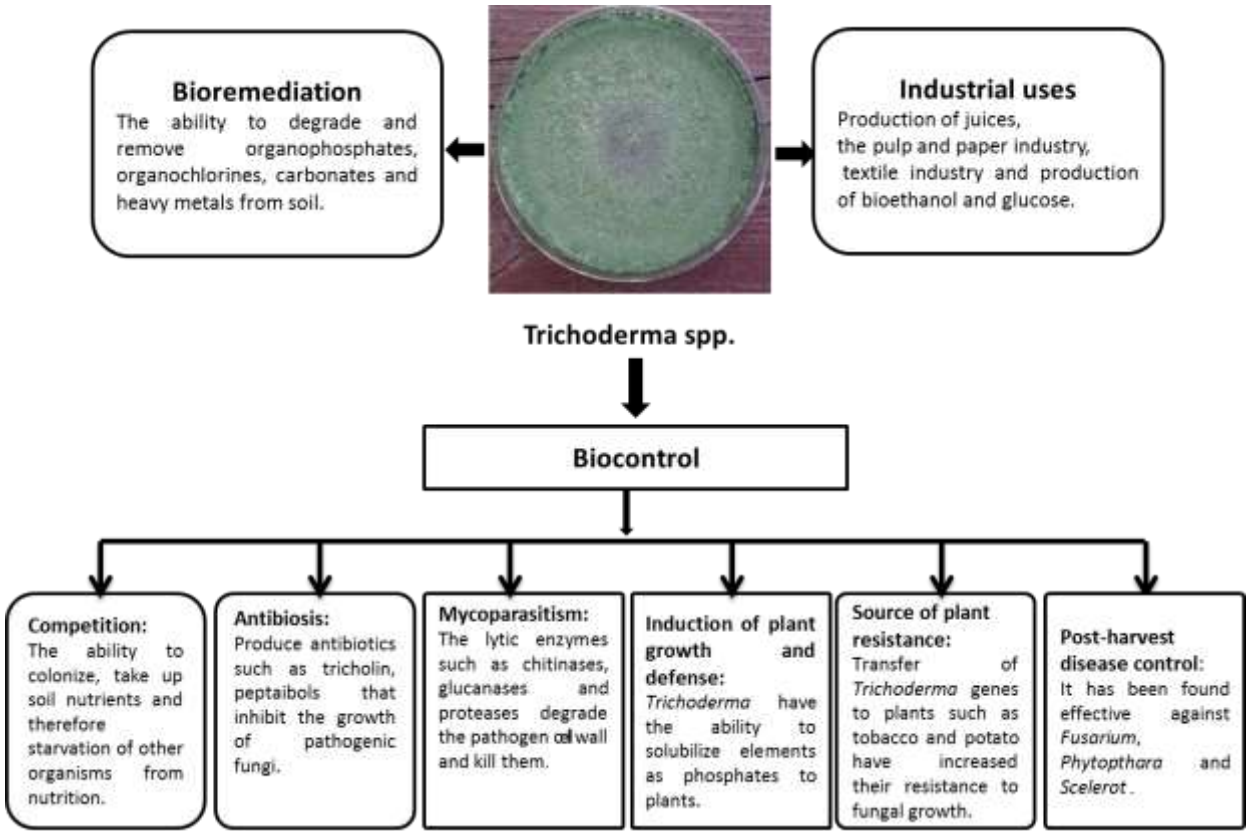

Figure 2. Different uses of *Trichoderma* in several fields.

Trichoderma genes as source of plant resistance

Trichoderma genes involved in pathogen cell wall degradation, such as chitinases and glucanases can be excellent sources for improving plant resistance against fungal pathogens. lorito et al. (1998) transferred the gene encoding a strongly antifungal endochitinase from the mycoparasitic fungus *T. harzianum* to tobacco and potato. High expression levels of the fungal gene were obtained in different plant tissues and this was linked with high resistance to the foliar pathogens *Alternaria alternata*, *A. solani*, *Botrytis cinerea*, and *R. solani*. Similarly, a chi gene from *T. asperellum*, designated *Tachi*, was cloned and transferred to soybean. Transgenic soybean plants with constitutive expression of *Tachi* showed increased resistance to *Sclerotinia sclerotiorum* compared to wild type plants. The overexpression of *Tachi* in soybean increased reactive oxygen species (ROS) level and each of peroxidase (POD) and catalase (SOD) activities. These results suggest that *Tachi* can improve disease resistance in plants by enhancing ROS accumulation and induction activities of ROS scavenging enzymes (Zhang et al., 2016). Moreover, Dana et al. (2006) generated transgenic tobacco (*Nicotiana tabacum*) lines that overexpress the endochitinases *CHIT33* and *CHIT42* from the mycoparasitic fungus *T. harzianum* and evaluated their tolerance to biotic and abiotic stress. The transformed plants with *CHIT33* and *CHIT42* exhibited broad resistance to fungal and bacterial pathogens, salinity, and heavy metals with no obvious effects on their growth. Furthermore, the endochitinase gene (Chit33-cDNA) of *T. atroviride* was overexpressed under the CaMV35S constitutive promoter in canola via *Agrobacterium tumefaciens* transformation. It was reported that lesion sizes of transgenic canola caused by *S. sclerotiorum* were significantly retarded when compared with non-transgenic canola plants (Solgi et al., 2015).

TOOLS FOR GENETIC IMPROVEMENT OF *TRICHODERMA*

Several genetic improvement trials are carried for maximizing the benefits of *Trichoderma* as biological control agents and in different industrial purposes. Recently, the genetic improvement of *Trichoderma* genus has entered a new era with the sequencing of *T. reesei*, *T. atroviride*, and *T. virens* genomes (Seidl and Seiboth, 2010; Mukherjee, 2011). The results indicated that the smallest genome size (34 Mb) was found in *T. reesei*,

while the largest genome (38.8 Mb) was recorded for *T. virens* (Mukherjee, 2011). Here, some of the genetic tools used for improving the biocontrol activity of *Trichoderma* against soil borne pathogens were highlighted.

Mutation

Mutagenesis is an excellent tools for developing *Trichoderma* mutants with enhanced secreted enzymes yields as compared to the parent strains (Seidl and Seiboth, 2010; Singh et al., 2016). Khandoker et al. (2013) employed ultraviolet (UV) irradiation and Ethidium bromide (EtBr) treatments to improve the production of cellulases from *T. viride*. The mutants of *T. viride* treated with UV and EtBr gave the highest cellulase activity with 11.28 and 14.61 U/ml, respectively as compared to 5.52 U/ml for the parent strain. In addition, Abbasi et al. (2014) used gamma rays for obtaining mutants of *T. harzianum* with maximum growth inhibition against *Macrophomina phaseolina*. It was found that the charcoal rot disease of melon reduced with 28% in the treated plants with *Trichoderma* mutants as compared to control. Moreover, N_methyl-N_nitro-N_guanidine (NTG) was used as mutagen for enhancing the antagonistic abilities of *T. harzianum*-1432 and *T. atroviride* against *Sclerotium rolfsii*, the causal agent of chickpea collar rot (Rashmi et al., 2016). The antagonistic capability of *T. harzianum* against *M. phaseolina*, *A. flavus* and *A. parasiticus* as pathogens was improved after exposure to UV-irradiation. Some mutants of *T. harzianum* released higher level of lytic enzymes, chitinases and cellulases (Singh et al., 2010; Patil and lunge, 2012; Walunj and John, 2015). Finally, γ-radiation induced mutants of *T. viride* with high ability to restrict *M. phaseolina* (Baharvand et al., 2014).

Protoplast fusion

Protoplast fusion is an important improvement tool for developing hybrid strains and improving the biocontrol potential of *Trichoderma* where the sexual cycle is difficult (Kowsari et al., 2014). Kowsari et al. (2014a) obtained some of *T. harizianum* fusants that expressed 1.5 fold of *chit42* transcript and exhibited a higher growth inhibition rate against *R. solani* than the parent strain. Moreover, Balasubramanian et al. (2012) carried out protoplast fusion between *T. harzianum* and *T. viride*. The *Trichoderma* HF9 fusant exhibited 3, 2.5 and 1.5-fold increase of total chitinase, specific chitinase and protein, respectively as compared with parent strains. Similarly, Mohamed and Haggag (2010) obtained fusants between *T. koningii* and *T. reesei* and most of these fusants showed superiority in their antagonistic activity against fungal pathogens which cause root-rot and damping-off diseases than their parental strains. Furthermore, Prabavathy et al. (2006) carried out self-fusion of *T. harzianum* strain PTh18 protoplasts and among the fusants, the strain SFTh8 produced maximum chitinase with a two-fold increase as compared to the parent strain. All the self-fusants exhibited high antagonistic activity against *R. solani* than the parent.

Genetic engineering

The huge progress in DNA sequencing techniques and comparative genomics analysis of different organisms has provided large lists of genes and their functions. Modification of *Trichoderma* genome by directly manipulating the DNA sequence of specific genes is considered modern and efficient tool to obtain strains with desired traits. Giczey et al. (1998) cloned a 42-kDa endochitinase encoding gene, *Tham-ch* from *T. hamatum* strain Tam-61. The *Tham-ch* with its own regulatory sequences was reintroduced into the host strain. Most of the transformants expressed higher levels of chitinase activity with 5-fold in comparison with the wild-type recipient strain. Moreover, Mendoza et al. (2003) cloned a mitogen-activated protein kinase encoding gene, *tvk1*, from *T. virens* and examined its role during the mycoparasitism, conidiation, and biocontrol in tvk1 null mutants. The null mutants displayed an increased protein secretion of lytic enzymes in culture supernatant compared to the wild type. Consistently, biocontrol assays demonstrated that the null mutants were considerably more effective in disease control than the wild-type strain or a chemical fungicide. These data suggest that Tvk1 acts as a negative modulator during host sensing and sporulation in *T. virens*.

A chimeric chitinase with improved enzyme activity was produced by fusing a ChBD from *T. atroviride* chitinase 18 to 10 with Chit42 (Kowsari et al., 2014b). The Chit42-ChBD transformants showed higher antifungal activity towards seven phytopathogenic fungal species suggesting that ChBD provides a strong binding capacity to insoluble chitin. In parallel, *T. atroviride* was transformed with *Aspergillus niger* glucose oxidase-encoding gene, *goxA*, under a homologous chitinase (*nag1*) promoter (Brunner et al., 2005). The transgenic strain was more quickly overgrown and lysed the plant pathogens *R. solani* and *P. ultimum* than control.

CONCLUSION

The genus of *Trichoderma* are widely used in agriculture and industry sectors due to its production of important lytic enzymes such as chitinases, glucanases, and proteases. Several genetic improvement trials are carried out for maximizing the role of *Trichoderma* as biological control agents via mutation, protoplast fusion and genetic transformation. Additional efforts must be done for

isolation of new strains with high antagonistic abilities and more violent against soil borne plant pathogens as a safe alternative than pesticides.

CONFLICT OF INTEREST

The author has not declared any conflict of interest.

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