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PJBS

ISSN 1028-8880

Pakistan Journal of Biological Sciences

ANSInet
Asian Network for Scientific Information
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan

Pakistan Journal of Biological Sciences 17 (1): 122-125, 2014
ISSN 1028-8880 / DOI: 10.3923/pjbs.2014.122.125

Enhanced Root and Shoot Growth of Wheat (*Triticum aestivum* L.) by *Trichoderma harzianum* from Turkey

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Abstract: It is well known that *Trichoderma* species can be used as biocontrol and plant growth promote agent. In this study, *Trichoderma harzianum* isolates were evaluated for their growth promotion effects on wheat in greenhouse experiments. Two isolates of *T. harzianum* were used. The experimental design was a randomized complete block with three replications. Seeds were inoculated with conidial suspensions of each isolate. Wheat plants grown in steriled soil in pots. *T. harzianum* T8 and T15 isolates increased wheat length, root dry weight and shoot dry weight according to untreated control. Turkish isolates T8 and T15 did not produce damage in seeds nor in plants.

Key words: *Trichoderma harzianum,* wheat (*Triticum aestivum* L.), growth promotion

INTRODUCTION

Trichoderma species are used as effective against several plant pathogenic fungi (Avis *et al*., 2008; Inbar *et al*., 1994; Papavizas, 1985). *Trichoderma* species have different mechanisms such as competition for space and nutrients, secretion of chitinolytic enzymes, antagonistic activity and production of antimicrobial compounds (Altomare *et al*., 1999; Cutler and Jayno, 1991; Cutler *et al*., 1986; Gravel *et al*., 2007; Haran *et al*., 1996). Many studies have proved the potential of *Trichoderma* spp. as biological agents antagonistic to several soilborne plant pathogens including *Fusarium* spp., *Rhizoctonia solani*, *Gaeumannomyces graminis* var. *tritici* (Inbar *et al*., 1994; Kucuk and Kivanc, 2003; Windham *et al*., 1986; Zimand *et al*., 1996). *Trichoderma harzianum* isolates have produced fungal cell degrading enzymes such as chitinase, glucanase and protease (Kucuk and Kivanc, 2003).

In addition, several researchers reported that *Trichoderma* species have been to plant growth promote (Ousley *et al*., 1993; Yedidia *et al*., 2000; Whipps, 2001; Vessey, 2003) and seed germination (Chang *et al*., 1986; Gravel *et al*., 2007). *Trichoderma* sp. also increased affordability of certain plant nutrition elements in the soil (Altomare *et al*., 1999). Altomare *et al*. (1999) were obtained to solubilize phosphate of *Trichoderma harzianum* isolate.

In the present study, the Turkish *Trichoderma harzianum* T8 and T15 isolates that were determined as effective antagonists to *Gaeumannomyces graminis* var. *tritici* and *Fusarium culmorum* on wheat (Kucuk and Kivanc, 2003), were evaluated for their effects on shoot and root weights and seed germination of wheat in greenhouse trials.

MATERIALS AND METHODS

Plant and soil: Bread wheat (*Triticum aestivum* L.) was employed in the inoculation experiments. Seeds were obtained from the Osmangazi University, Faculty of Agriculture. The day before sowing, pots were filled with sterilized soil. The soil was analyzed for pH, organic material, P, exchangeable K, Electrical Conductivity (EC), $CaCO_3$ and soil texture. Selected some chemical and physical properties of the soil are presented in Table 1. Soil pH was measured with an electrometer. Soil texture was determined by Bouyoucos (1951), organic material by the modified Walkley-Black method (Jackson, 1958), calcium carbonate content was measured by a Scheibler calcimeter (Caglar, 1958). Electrical Conductivity (EC) was determined with an electrical conductivity meter in a 1:5 soil water suspension. P and K were determined in the nitric-perchloric digestion extract, P by the method of Murphy and Riley (1962) and K by flame photometry.

Microorganism: *Trichoderma harzianum* isolates T8 and T15 were obtained from the culture collection of the

Table 1: Characteristics of soil used in this study

Soil properties	Values
pH	7.60
$CaCO_3$	1.02
Soil texture	Sandy loam
Organic mater (%)	2.47
P_2O_5 (kg da^{-1})	18.50
K_2O (kg da^{-1})	104.10
EC	0.064

Department of Biology, Anadolu University and maintained on Potato Dextrose Agar (PDA, MERCK) slants at 4°C till further use. Inoculum was prepared by culturing the fungus on PDA medium for 7 days in petri plates. Conidial suspensions of each isolate were obtained by flooding plates with 0.2% (v/v) Tween 80 in sterile distilled water and the concentration of conidia was adjusted to 1×10^7 conidia mL^{-1}.

Plant growth and inoculation in pots: Seven day old mycelial mats of *T. harzianum* isolates were homogenized added to cooled 1% water agar and poured into petri dishes. A sterile cellophane disk was placed on the surface of the medium (Windham *et al*.,1986). Ten seeds of wheat were placed on top of the membranes. Dishes without isolate served as controls. Seeds were incubated at 25°C and rates of germination determined from the second day to the sixth.

The study of the effect of *Trichoderma harzianum* isolates T8 and T15 on wheat growth was carried out in pot experiments. The experimental design was a randomized complete block with three replications. The day before sowing, pots were filled with 400 g steriled soil. Seeds were immersed in conidial suspensions (10^7 conidia mL^{-1}) of each isolate (100 mL) for 60 min before placed into in 20 cm diameter pots. Diammonium phosphate and ammonium nitrate were added to soil of each pot before sowing. Five seeds of wheat were sown per pot.

After germination plants were thinnned to obtain three plants per pot. In all experiments, seeds were surface disinfested by a 0.5% sodium hypochlorite and 5% ethanol mixture for 1 min. seeds of the untreated control were only washed with sterile distilled water. Plants were grown in pots for 45 days under greenhouse conditions. The soil was moistened with water and maintained at 60% of its moisture holding capacity. The following plant response parameters were measured in each test plant form the various treatment; plant length (only aerial part), plant and root dry and fresh weight. Forty five days after germination shoots and roots were separated and dried 105°C before determining the shoot dry weight.

Statistical analysis: Analysis of variance for each character was performed the the plot means. All data were analysed using of an analysis of variance (Yurtsever, 1984).

RESULTS AND DISCUSSION

Isolates of *T. harzianum* have been shown to produce metabolites with phytotoxic activities such as 6-pentyl-α-pyrone (Zimand *et al*., 1996) and harzianopyridone (Cutler and Jayno, 1991). Sensitivity to these compounds varies with plant species (Cutler and Jayno, 1991; Zimand *et al*., 1996). In the present study, the rate of seed germination was increased compared with controls. The effects of metabolites produced by *T. harzianum* T8 and T15 were tested by germinating of seeds. The time required for germination of wheat was shortened significantly by such treatment in comparison with untreated control. There was no effect on the ultimate level of germination (Table 2). Significant differences occured in the 2nd, 3rd and 4th days for isolates but 5th and 6th days were similar. Treatments (Control, T8, T15) were also significant for all days (Table 2).

Inoculated plants showed a significiant increase in weight of shoots and roots compared with the controls (Table 3). Both T8 and T15 significantly increased the plant length as compared with uninoculated controls. This strongly suggested that T8 and T15 isolates produced a growth regulating factor that increases the rate of seed germination. Similarly, Rabeendran *et al.* (2000), reported that *Trichoderma* species were observed to growth promotion effects on cabbage. *Trichoderma* treatment on rate of seed germination (after six days) were significant. The rate of seed germination response was similar to our isolates T8 and T15 (Table 3).

In our study, average shoot and root dry weights of wheat at 45 days in control pots were 0.36-0.49% and 0.33-0.42% (Table 1). Although, higher values for shoot and root dry weight were recorded for *T. harzianum* isolate T15, shoot and root dry weights significantly increased for both isolates. Recently however *Trichoderma* spp., have been reported to trigger a defence response in plants. Many antagonistic fungi, particularly certain isolates of *Trichoderma* species, can provide plant growth promotion in the absence of any major pathogens (Kleifeld and Chet, 1992). These studies were restricted to simple observations of improved plant growth with no indication of the possible mechanisms involved, although, there are exceptions. For example, *Trichoderma harzianum* 1295-22 was shown to solubilize phosphate and micronutrients that could hence be made available to plant growth (Altomare *et al*., 1999; Yedidia *et al*., 2000).

In the present study, *T. harzianum* T8 and T15 isolate, were applied to seed. The increased growth response in plants is usually determined by measuring their length and dry weight (Inbar *et al*., 1994; Zimand *et al*., 1996; Yedidia *et al*., 2000). Applying *T. harzianum* T8 and T15 as a seed coating treatment to wheat seeds, increased plant length (72.9 and 99%,

Table 2: Effect of *T. harzianum* Isolates on germination and rate of increase (%I) by the mean of the control (%)

	Days									
Treatments	2	%I	3	%I	4	%I	5	%I	6	%I
Control	4		9		10		16		16	
T8	5	25	15	66.7	20	100	30	87.5	30	87.5
T15	17	325	24	166.7	30	200	29	81.3	30	87.5

ANOVA

		Mean square				
		Days				
Source of variation	Degrees of freedom	2	3	4	5	6
Replication	2	0.12	0.34	0.34	0.34	0.1
Treatment (C,T8,T15)	2	17.45**	19.0**	33.40**	20.34**	21.8**
Control (C) and isolates	1	10.93**	24.5**	49.97**	40.47**	43.5**
Isolates (T8, T15)	1	24.0**	13.3**	16.63**	0.13	0.0
Error	4	0.44	0.333	0.158	0.165	0.275

Significant at the **0.01 levels of probability

Table 3: Effect of *T. harzianum* T8 and T15 on various features wheat and analysis of variance

	Parameters[a]									
Treatments	RDW	%I	RFW	%I	SDW	%I	SFW	%I	PL	%I
Control	0.22		1.92		0.303		1.92		13.7	
T8	0.33	50	3.11	61.9	0.360	18.8	3.11	61.9	23.7	72.9
T15	0.42	91	3.97	106.7	0.493	62.7	3.97	106.7	27.3	99.2

ANOVA

		Mean square				
Source of variation	Degrees of freedom	Root Dry Weight (g)	Root Fresh Weight (g)	Shoot Dry Weight (g)	Shoot Fresh Weight (g)	Plant Length (cm)
Replication	2	0.002	0.005*	0.0023	0.084	0.1
Treatment	2	0.03**	1.63**	0.029**	3.18**	150.1**
Control and isolates	1	0.42**	49.40**	0.029*	5.29**	280.0**
Isolates (T8, T15)	1	0.01**	0.70**	0.032**	1.10**	20.2**
Error	4	0.0005	0.10	0.00143	0.034	0.45

Significant at the **0.01 levels of probability, [a]RDW: Root dry weight, RFW: Fresh weight; SDW: Shoot dry weight, SFW: Shoot fresh weight, PL: Plant length and %I: Increase rate

respectively) and dry weight of roots (by 50 and 91%, respectively). Treatments had significant effects on two characters and treatment (control, T8, T15). Significant different were observed for all characters between the control and the isolates (T8, T15) and also between isolates (T8, T15) were significant for all characters. *T. harzianum* T15 caused the highest increases on both characters. The effects of *Trichoderma* preparation on cucumber and peppers have already been reported by Inbar *et al.* (1994). Application of *Trichoderma* preparation to both cucumber and pepper increased seedling length 17-24%, leaf area by 50-96% and dry weight by 29% (Inbar *et al.*, 1994). Most probably other mechanisms also operate to provide such increase in plant growth (Chang *et al.*, 1986; Inbar *et al.*, 1994; Windham *et al.*,1986; Yedidia *et al.*, 2000).

These reactions may be involved in the apparent ability of this fungus to stimulate plant growth as well as to reduce susceptibility to root attacking pathogens. In this study, *T. harzianum* T8 and T15 isolates did not produce any damage in seeds nor in plants. On the contrary, T8 and T15 isolates promoted the plant growth and T15 isolate was more effective than *T. harzianum* T8 isolate in this study.

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