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Induction of water stress tolerance of mustard plants using *Trichoderma* as biological seed treatment

K. K. Sharma[1]*and U. S. Singh[2]

[1]Vivekanand Parvatiya Krishi Anusandhan Sansthan, Almora-263601, (Uttarakhand), INDIA
[2]IRRI, 9th Floor, Aggarwal Corporate Tower, Plot No. - 23, Rajendra Place, New Delhi - 110 008, INDIA
*Corresponding author. E-mail: abhay23p@yahoo.com

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Abstract: Water scarcity is one of the main consequences of changing climate which adversely affects the plant growth and productivity. Enhanced root development results in increased surface area of active absorption for water and nutrient uptake which helps in tolerating abiotic stresses including drought in plants. *Trichoderma* is well known for its biocontrol and growth promontory effect in plants in addition to alleviate abiotic stress. In our study, thirty isolates of *Trichoderma* were grown on sterilized cow dung at different moisture content ranges from 5 to 30 percent to investigate their ability to grow and multiply under water stress condition. Mustard plants were grown under glass house condition by treating seeds with selected isolates of *Trichoderma* subjected to water stress subsequently. All isolates of *Trichoderma* grew upto 20% moisture whereas only eleven isolates exhibited growth at 10% moisture. Isolate PB23 was only isolate which was able to grow and resulted in 1.0 x10^9 cfu/g air dried cow dung even at 5% moisture content and induced the tolerance of mustard plants under water stress conditions when applied as seed treatment before sowing.

Keywords: Mycorrhiza, PGPF, Tensiometer, *Trichoderma*, Water stress

INTRODUCTION

Drought stress is one of the key factor limiting plant growth and productivity in many areas (Gusain *et al.*, 2014). Root size and architecture are the factors which determine yield performance, particularly under conditions of limited water availability (Price *et al.*, 2000). The arbuscular mycorrhizal (AM) and vesicular arbuscular mycorrhizal (VAM) fungi are ubiquitous component of most agro-ecosystems, symbiotically associated with roots of various plants forming a network of fungal mycelium with roots of host plant, increase active absorptive surface area of roots results in enhanced nutrient uptake and drought stress tolerance (Farahani *et al.*, 2008, Naher *et al.*, 2013 and Smith and Read, 2008) in a variety of crops. Among several strategies including mycorrhizal fungi use to improve crop yield under stress conditions, the use of bio-agents such as *Trichoderma* is an effective and easily adaptive strategy (Bailey *et al.*, 2006). For the several reasons like enhancement of overall plant growth including increased nutrient absorption and stress resistance (Hoyos-Carvajal *et al.*, 2009), *Trichoderma* species are also recognized as plant growth promoting fungi (PGPF) (Hyakumachi and Kubota, 2004 and Doni *et al.*, 2013). Zaidi and Singh (2004) and Shukla *et al.* (2012) studied the effect of different moisture levels on growth and multiplication of *Trichoderma harzianum* using cow dung as substrate. *Trichoderma* spp. can improve the early stage of plant development through the enhancement of root growth and root length which is the primary direct effect of *Trichoderma* colonization regardless of water status, which caused delay in the drought responses (Shukla *et al.*, 2012). Interestingly, some genes of *Trichoderma* species have also been reported which can be used to give resistance to the abiotic stresses including drought (Mastouri *et al.*, 2010). A few recent reports demonstrated that PGPF, *Trichoderma* spp. alleviate abiotic stresses and indicates that they may confer tolerance to drought stress at least in part through promotion of deeper root penetration into the soil profile (Daniel *et al.*, 2011).

In the present investigation thirty isolates of *Trichoderma* were subjected to grow on sterilized and air dried cow dung at different moisture levels. Subsequently selected isolates were also tested for their ability to induce the water stress tolerance in mustard plants when used as seed treatment prior to sowing. The reason behind evaluation of drought tolerance in the current strains of plant growth promoting fungi (PGPF), *Trichoderma* was that the stress tolerant strains can be efficiently deployed in extreme environments where they can show better rhizosphere competence and saprophytic competitive ability.

MATERIALS AND METHODS

Experimental materials, *Trichoderma* strains were

isolated from rhizospheric soils of different crops and locations of Uttarakhand (Table 1) and mustard seeds (var. Varuna) were obtained from oil seed pathology lab G.B.P.U.A. & T. Pantnagar respectively. Cow dung was used to study the growth of isolates at moisture stress conditions. The clots were removed and 50 g cow dung was filled in 250 ml Erlenmeyer flasks. Different amount of water was added to maintain 30, 25, 20, 15, 10 and 5 per cent moisture (based on air dried cow dung). The flasks were autoclaved at 15 lbs psi. for 30 minutes and three flasks were maintained for each moisture level. The flasks were inoculated with 2 ml spore suspension of individual *Trichoderma* isolate. The inoculated flasks were incubated at 26±2 ^{0}C for 14 days. Flasks were weighed regularly and loss of moisture was replenished by adding sterilized water and 1 g of air dried samples were estimated for *Trichoderma* population by serial dilution method using TSM medium (Zaidi and Singh, 2004).

On the basis of growth performance at different moisture regimes on cow dung selected isolates were also evaluated for their ability to enhance water stress tolerance of mustard plants moisture stress conditions. Plastic pots (2.5 kg capacity) were filled with 2.0 kg autoclaved soil and saturated with water holding calibrated tensiometer. Seeds were treated with powdered formulations of selected isolates of *Trichoderma* except for control. Treated brassica seeds were sown at 60 percent moisture content in pots holding a tensiometer. Three replications for each treatment including control and finally with five plants per pot were maintained. Moisture was maintained with adding known amount of water until plants attained the age of three weeks and at this point stress was given by stop watering. From the next day observations were taken daily on the basis of readings in tensiometer from initiation of wilting in control till the complete wilting (Shukla *et al.*, 2012).

RESULTS

Growth of *Trichoderma* isolates at different moisture levels: All *Trichoderma* isolates were evaluated for their growth response at six moisture levels (30, 25, 20, 15, 10 and 5%) and results are presented in table 2. PB23 resulted in maximum population (cfu/g air dried substrate) followed by PB 18 at 30 and 25% moisture while minimum population was recorded for PB11. Growth of the isolates PB 2 and 9; PB 16, 27 and 29, PB 1, 8 and 26; PB 3, 7, 15 and 17; PB 4, 5, 6, 25, 28 and 30; PB 29 and 22; PB 14 and 21; PB 10, 12, 13

Table 1. Isolates of *Trichoderma* with source of soil samples.

S. No.	Sample code	Crop	Location	Isolate code
1	R1KG	Rice	Kathgodam-Haldwani	PB1
2	R3H	Rice	Halduchaur-Haldwani	PB2
3	R2LCb	Rice	Lamachaur-Haldwani	PB3
4	R1Da	Rice	Kherna-Almora	PB4
5	R1Db	Rice	Kherna-Almora	PB5
6	R2Da	Rice	Kherna-Almora	PB6
7	R2Db	Rice	Kherna-Almora	PB7
8	SPC1	Rice	SPC-Pantnagar	PB8
9	SPC2	Rice	SPC-Pantnagar	PB9
10	1a	Rice	Rudrapur-U.S. Nagar	PB10
11	1ab	Rice	Rudrapur-U.S. Nagar	PB11
12	1bc	Rice	Rudrapur-U.S. Nagar	PB12
13	3	Rice	Rudrapur-U.S. Nagar	PB13
14	5	Rice	Rudrapur-U.S. Nagar	PB14
15	AM	Apple	Mukteshwar-Almora	PB15
16	BM	Broccoli	Mukteshwar-Almora	PB16
17	PM1	Pea	Mukteshwar-Almora	PB17
18	PM2	Pea	Mukteshwar-Almora	PB18
19	SM	Strawberry	Mukteshwar-Almora	PB19
20	WM	Walnut	Mukteshwar-Almora	PB20
21	RP1	Rice	Premnagar-Dehradun	PB21
22	TR1	Mustard	Premnagar-Dehradun	PB22
23	A	Maize	Dhalwala-Rishikesh	PB23
24	B	Maize	Bhaniawala-Dehradun	PB24
25	B1	Rice	Bhaniawala-Dehradun	PB25
26	C1	Rice	Mazra-Ranipokhri	PB26
27	D	Maize	Geetanagar Rishikesh	PB27
28	D1	Rice	Raipur-Dehradun	PB28
29	F1	Rice	Raiwala-Hardwar	PB29
30	G1	Rice	Nagani, Tehri Garhwal	PB30

Table 2. *Trichoderma* population (cfu/g) at different moisture regimes.

Isolate code	*Trichoderma* population (cfu/g air dried cow dung)						Mean
	Moisture content (%)						
	30	25	20	15	10	5	
PB1	3.7 x10^9	3.3 x10^9	2.7 x10^9	1.7 x10^9	0.3 x10^9	0.0	2.6 x10^9
PB2	4.7 x10^9	4.3 x10^9	3.7 x10^9	2.3 x10^9	0.7 x10^9	0.0	1.8 x10^9
PB3	3.3 x10^9	3.0 x10^9	2.7 x10^9	1.7 x10^9	0.0	0.0	1.2 x10^9
PB4	2.7 x10^9	2.3 x10^9	1.3 x10^9	0.7 x10^9	0.0	0.0	1.3 x10^9
PB5	2.7 x10^9	2.3 x10^9	1.7 x10^9	1.0 x10^9	0.0	0.0	1.1 x10^9
PB6	2.7 x10^9	2.0 x10^9	1.7 x10^9	0.3 x10^9	0.0	0.0	1.7 x10^9
PB7	3.3 x10^9	3.3 x10^9	2.7 x10^9	0.7 x10^9	0.0	0.0	1.9 x10^9
PB8	3.7 x10^9	3.3 x10^9	3.0 x10^9	1.3 x10^9	0.3 x10^9	0.0	2.7 x10^9
PB9	4.7 x10^9	4.7 x10^9	3.3 x10^9	2.3 x10^9	1.3 x10^9	0.0	1.0 x10^9
PB10	1.7 x10^9	1.7 x10^9	1.7 x10^9	1.0 x10^9	0.0	0.0	0.5 x10^9
PB11	1.3 x10^9	1.0 x10^9	0.3 x10^9	0.3 x10^9	0.0	0.0	0.8 x10^9
PB12	1.7 x10^9	1.3 x10^9	1.0 x10^9	0.7 x10^9	0.0	0.0	0.7 x10^9
PB13	1.7 x10^9	1.3 x10^9	1.0 x10^9	0.3 x10^9	0.0	0.0	0.9 x10^9
PB14	2.0 x10^9	1.7 x10^9	1.3 x10^9	0.7 x10^9	0.0	0.0	2.0 x10^9
PB15	3.3 x10^9	3.0 x10^9	2.7 x10^9	2.3 x10^9	0.7 x10^9	0.0	2.4 x10^9
PB16	4.3 x10^9	3.7 x10^9	3.0 x10^9	2.3 x10^9	1.0 x10^9	0.0	1.9 x10^9
PB17	3.3 x10^9	3.3 x10^9	2.7 x10^9	2.0 x10^9	0.3 x10^9	0.0	2.3 x10^9
PB18	5.0 x10^9	4.3 x10^9	2.7 x10^9	1.7 x10^9	0.3 x10^9	0.0	1.2 x10^9
PB19	2.3 x10^9	2.0 x10^9	1.7 x10^9	1.3 x10^9	0.0	0.0	0.6 x10^9
PB20	1.7 x10^9	1.3 x10^9	0.3 x10^9	0.0 x10^9	0.0	0.0	1.0 x10^9
PB21	2.0 x10^9	1.7 x10^9	1.3 x10^9	1.0 x10^9	0.0	0.0	1.0 x10^9
PB22	2.3 x10^9	2.0 x10^9	1.3 x10^9	0.3 x10^9	0.0	0.0	3.7 x10^9
PB23	6.0 x10^9	5.3 x10^9	4.0 x10^9	3.3 x10^9	2.3 x10^9	1.0 x10^9	2.3 x10^9
PB24	4.0 x10^9	3.7 x10^9	3.0 x10^9	2.3 x10^9	1.0 x10^9	0.0	1.3 x10^9
PB25	2.7 x10^9	2.3 x10^9	1.7 x10^9	1.0 x10^9	0.0	0.0	1.8 x10^9
PB26	3.7 x10^9	3.3 x10^9	2.7 x10^9	1.3 x10^9	0.0	0.0	2.4 x10^9
PB27	4.3 x10^9	3.7 x10^9	3.3 x10^9	2.3 x10^9	0.7 x10^9	0.0	1.2 x10^9
PB28	2.7 x10^9	2.3 x10^9	1.7 x10^9	0.7 x10^9	0.0	0.0	1.9 x10^9
PB29	4.3 x10^9	3.3 x10^9	2.7 x10^9	1.3 x10^9	0.0	0.0	1.3 x10^9
PB30	2.7 x10^9	2.3 x10^9	2.0 x10^9	0.7 x10^9	0.0	0.0	1.9 x10^9
Mean	3.1 x10^9	2.8 x10^9	2.2 x10^9	1.3 x10^9	0.3 x10^9	0.0	1.6 x10^9
sem1=0.05				sem2=0.11		sem3=0.28	
cd1 at 5%=0.14				cd2 at 5%=0.31		cd3 at 5%=0.77	

and 20 were at par at 30% moisture. Maximum population was obtained for PB23 followed by PB 2 whereas minimum population was recorded for PB 11 and 20 at 20% moisture. Growth of the isolates PB 9 and 27; PB 1, 3, 7, 15, 17, 18, 26 and 29; PB 8, 16 and 24; PB 5, 6, 10, 19, 25 and 28; PB 4, 14, 21 and 22; PB 12 and 13; PB 11 and 20 were at par at 20% moisture. The growth of all isolates was drastically reduced at moisture below 20%. Isolate PB 20 did not produce any population at 15% moisture. Only 11 isolates exhibited growth at 10% moisture. Interestingly, isolate PB 23 was only isolate which was able to grow and resulted in 1.0 x10^9 cfu/g air dried cow dung at 5% moisture level.

Effect of seed treatment with *Trichoderma* on water stress tolerance of the mustard plants: On the basis of growth performance on cow dung at different moisture level, five isolates (PB2, 8, 9, 18 and 23) from different growth promoting categories were evaluated for their ability to enhance water stress tolerance of mustard plants when used as seed treatment under glass house condition. Results are summarized in Table 3. No sign of wilting was observed in the plants of any treatment at 15-16 centibar soil suction pressure as compare to check 1st day after water stress (DAWS). Incipient wilting was observed in treatment PB2 and PB18 2nd day after water stress at 21-23 centibar soil suction pressure which completely wilted

Table 3. Effect of seed treatment with *Trichoderma* on water stress tolerance of the mustard plants.

Days	Parameters	Treatments					
		PB 2	PB 8	PB9	PB 18	PB 23	Control
1 Day after Water stress	Suction (cb)	15	16	16	15	16	15
	Wilting	-	-	-	-	-	+
2 Days after Water stress	Suction (cb)	21	21	22	21	23	23
	Wilting	+	-	-	+	-	++
3 Days after Water stress	Suction (cb)	33	34	33	34	35	34
	Wilting	++	+	-	++	-	++++
4 Days after Water stress	Suction (cb)	43	44	45	43	45	
	Wilting	++++	++	+	++++	+	
5 Days after Water stress	Suction (cb)		58	56		58	
	Wilting		++++	++		+	
6 Days after Water stress	Suction (cb)			73		75	
	Wilting			++++		++++	

+	<20 % Wilting
+ +	20-40 % Wilting
+ + +	41-70 % Wilting
+ + + +	70-100 % Wilting
−	No Wilting

4th day after water stress at 43 centibar soil suction pressure while the control plants wilted 3rd day after water stress. Plants of treatment PB8 were wilted 5th day after water stress at 58 centibar soil suction pressure. The plants of treatment PB9 and PB23 exhibited incipient wilting 4th and 5th DAWS respectively however plants of both the treatments were wilted 6th day after stress at 73-75 centibar of soil suction pressure and hence both these isolates enhanced the water stress tolerance of mustard plants. Isdolate PB 23 induced maximum tolerance to water stress of mustard plants.

DISCUSSION

Results revealed that all isolates of *Trichoderma* multiplied best on 30% moisture (w/w) with maximum growth ($6x10^9$ cfu/g air dried cow dung). They grew up to 20% moisture with lowest population of $0.3x10^9$ cfu/g however, growth was drastically reduced beyond 20% moisture. At 5% moisture level there was no growth except one isolates (PB23) indicating its good tolerance to moisture stress. The results are in agreement with the findings of Zaidi and Singh (2004) who studied the effects of moisture content (10, 20, 30, 50 and 70% w/w) on the growth and multiplication of *T. harzianum* PBAT-43 on sterilized or unsterilized cow dung and reported the maximum growth of *T. harzianum* was obtained at 30% moisture level of air-dried cow dung but decreasing the moisture level below 10% or increasing to 50% resulted in the drastic growth reduction of *T. harzianum*. Cow dung-neem cake mixture is already a recommended practice for field multiplication of *Trichoderma* (KAU, 2002). The results were also supported by Shukla *et al*. (2012) who reported that out of 43 isolates of *T. harzianum*, only five isolates were able to colonize well on cow dung at low moisture content of 10-20 percent, even though two isolates, Th 56 and Th 75, grew even at 5 percent moisture content.

Out of 30 isolates, only five isolates (PB 2, 8, 9, 18 and 23) of *Trichoderma* were selected to study drought tolerance in mustard plants on the basis of growth performance on air dried cow dung at different moisture regimes. Glass house experiment revealed the drought tolerance of mustard plants was enhanced by treating the mustard seeds with powdered formulation of *Trichoderma*. Isolates PB 9 and PB 23 performed well to stand the plants against water stress even 6th day after stress while plants treated with other isolates showed wilting 2nd day after stress. The results are in accordance with Shukla *et al.* (2012) who investigated the impact of endophytic fungus *T. harzianum* on rice response to drought stress. Among test isolates of *Trichoderma*, Th 56 induced maximum drought tolerance as treated rice plants recorded only 20-40 percent wilting even at 9 DDS. *Trichoderma*-colonized rice seedlings were slower to wilt in response to drought. Rawat *et al*. (2011) observed that *Trichoderma* strains in plants increases root length, which helps in additional water achievement and in that way increasing the plants ability to resist abiotic stresses (drought, salt etc) and uptake of nutrients. Another recent study has evaluated the behavior of endophytic *Trichoderma* spp. (*T. hamatum* strain DIS 219b) that colonize the *Theobroma cacao* tree caused the significant delay in the onset of many drought induced physiological changes (Bae *et al.*, 2009). Our results are in accordance with Doni *et al*. (2014) who

reported that *Trichoderma* spp. significantly enhanced the water use efficiency of rice plants compared to NPK treatment and control and highest water use efficiency was observed for *Trichoderma* sp. SL2 treated plants. Drought tolerance in mustard plants using *Trichoderma* is justified by the fact that enhanced rooting provides increased surface area for absorption and long roots penetrate deeper in soil which can absorb the deep seated water and increase plant stand in water stress conditions. In addition of biocontrol ability of *Trichoderma* spp., the activity that contributes to the enhancement of root growth and distribution was also considered as a key factor to the prolonged photosynthetic activity and the delayed senescence in rice plants (Mishra and Salokhe, 2011). Ghahfarokhy *et al*. (2011) reported that colonization of roots with VAM fungi and *Trichoderma* spp. promoted massive root growth which intern help in improved absorption of nutrients. The physical presence of mycelial mass in rhizosphere is itself would serve as appendages to the normal rhizosphere of plants (Mariola *et al*., 2007) or development of a plant-fungus relationship similar to that described for mycorrhizal fungi (Barea *et al*., 2002). The positive role of *Trichoderma* spp. in ectomycorrhizal sphere has been also elaborated by Wu *et al.* (2005) which is an indirect mode for their plant growth promotional activity. Hence findings of our experiments are in agreement with those of the above mentioned authors.

Conclusion

This study has identified potential drought tolerant strain of *Trichoderma* (isolate PB 9 and 23) when applied as seed dresser. However, our results demonstrate that seeds treated with *Trichoderma* resulted in significantly higher survival of drought-stressed mustard plants and in greater biomass production. With or without exposure to drought, colonization by *Trichoderma* promoted seedling growth, with isolates PB 9 and 23 giving the most consistent effect. This research opens a new way in abiotic stress management in rainfed agro-ecosystems for enhancing crop productivity for the benefit of small and marginal farmers. In this respect, the present study concludes that *Trichoderma* spp. have the potential to induce drought tolerance and enhance physiological processes and growth. The results of the present study serve as base for the mediation of PGPF in enhancing water stress tolerance in plants and need the evaluation of more strains for growth promotion and productivity of various crops under different environmental stress condition.

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