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BIOSTIMULANT EFFECT OF *TRICHODERMA* ON THE DEVELOPMENT OF WHEAT AND BARLEY PLANTS AND ITS SURVIVAL APTITUDES ON THE ROOTS

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Abstract

Inoculation with an isolate of *Trichoderma* sp. has a significant effect on seed germination, growth and yield of wheat plants (hard and soft) and barley. The germination percentage is 100% in seeds treated with *Trichoderma* and 75% in those not treated. After 75 days, the growth and yield parameters in wheat and barley plants from seeds treated with *Trichoderma* are higher than those observed in control plants, respectively the length (66.5 to 65 cm / 50 to 49.6 cm) and the weight of the aerial part (5.6 to 5.5 g / 3.4 to 3.1 g), the length (19.5 to 1.8 cm / 13 to 12.2 cm) and the weight of the root system (3.2 to 2.8 g / 1.4 to 1.2 g), the number of internodes (6/4), the number of leaves (9 to 8 / 6 to 5), the length (18.3 to 18.1 cm / 13.5 to 12.8 cm) and the weight of the spikes per plant (5.5 to 5.1 / 3 to 2.7 g) and the number of grains per spike (70 to 66 / 50 to 48). Re-isolation of *Trichoderma* was positive throughout the crop cycle of wheat and barley plants and its presence was also confirmed by microscopic observations of fine roots. Thus, the mycelium, phialides and spores of *Trichoderma* have been observed at the level of these roots.

Key words: Wheat, barley, seeds, treatment, *Trichoderma*, agronomic parameters, yield parameters, roots, microscopic observations, phialides.

Introduction

With the increasing interest to ensure competitive yields without causing harmful environmental effects, the use of beneficial microorganisms may be an attractive procedure (Singh *et al.,* 2011). Among these, the fungus *Trichoderma* (Teleomorph: Hypocrea, an Ascomycete), is considered a common inhabitant of rhizosphere and symbiont of the plants roots that can promote initial growth and plant development (Harman and Bjorkman, 1998; Qi and Zhao, 2013; Guimarães *et al.,* 2014). The influence of *Trichoderma* species on plant development may include beneficial effects on seed germination, seedling emergence, grain growth and yield (Chagas *et al.,* 2016). Some of them are frequently applied as seed treatments to control diseases (De Algaba *et al*., 1993; Heidi and Abo-Elnaga, 2012; Pereira *et al.,* 2019), enhance seedling emergence and growth (Dabiré *et al.,* 2016; Kamaruzzaman *et al.,* 2016; Sellal *et al.,* 2020) as well as to boost long-term improvements in plant quality (Harman, 2000 and 2006) via various mechanisms (Harman *et al.,* 2004). There also are reports of considerable yield increase when plant seeds were previously treated with spores from *Trichoderma* (Chet *et al.,* 1997). Also, *Trichoderma* may provide major benefit by producing growth factors that increase the rate of seed germination (Benítez *et al.,* 1998).

Root colonization by *Trichoderma* strains frequently enhances root growth and development, crop productivity, the uptake and use of nutrients (copper, iron, phosphorus, manganese, sodium, etc.) from the soil solution (Arora *et al.,* 1992; Yedidia *et al.,* 2001).

However, some authors, indicated that *Trichoderma* isolates and their enzymes can be affected by environmental changes such as temperature and pH (Yedidia *et al.,* 2001). Therefore, it is worth noting that the conditions for using *Trichoderma* isolates can limited its efficiency on promoting growth parameters (Dabiré *et al.,* 2016). Also, Butt and Copping, (2000) suggested that the efficiency of *Trichoderma* was related to the use shape. In addition, it should be taken into account the differences among all plant species responses and their varieties as well as *Trichoderma* species potentials for increased crop yields because the plant growth promoting potential varies amongst different *Trichoderma* species and strains (Martínez-Medina *et al.,* 2014).

The objective of this study was to examine one *Trichodema* isolate as a seed treatment for its efficacy as plant growth promoter of wheat and barley seedlings.

Materials and Methods

Plant material

The seeds (5 g of each variety) of barley, Osama variety, durum wheat, Amjad variety and common wheat, Wafia variety, were previously disinfected by soaking for 5 min in 70% ethanol solution. Afterwards, they were rinsed three times with sterile distilled water and dried on filter paper for 24 hours.

Seed treatment technique

The tested *Trichoderma* isolate, TR-B 98 (3) obtained from the roots of plants growing on sludge from phosphate washing plants (Kribel *et al.,* 2019) was grown on Potato Sucrose Agar medium (PSA: 200 g potato; 20 g saccharose; 15 g agar-agar per 1000 mL distilled water) and incubated at 25°C for 8 days in the dark. The surface of the cultures was then washed with sterile distilled water and the concentration of the conidial suspension has been adjusted to 10^7 conidia / mL.

The used seed treatment solution contained 10% gelatin, 4% superphosphate 0.1% sucrose plus 10 mL of the *Trichoderma* conidial solution. Wheat and barley seeds at a rate of 5 g were soaked in Petri plates containing the treatment solution. Afterwards, these seeds were placed in other Petri plates containing the clay and the contents were gently agitated so that the clay formed a thin layer around the seeds.

The treated seeds were then placed in sterile Petri dish and air-dried for 72 h under ambient conditions. A treatment without *Trichoderma* inoculation were added as control,

Wheat and barley seeds disinfested in the same way without *Trichoderma* inoculation served as controls. The treated seeds were stored at 4°C and at room temperature and their viability has been checked according to the storage time.

The treated and control seeds were individually sown into pots (at a rate of 1 seed per pot) filled with sterile Mamora sandy soil. The pots were then placed in a plastic greenhouse where temperatures varied from 18 to 25°C. The final evaluation of total length, the root length, the number of leaves and fresh weight of the aerial and underground parts of plants using a caliper, a precision scale and a graduated scale was conducted 75 days after sowing.

Root colonization by *Trichoderma*

Colonization capability of *Trichoderma* isolate on the roots of wheat and barley plants was conducted from the 1 month old plants up to 8 months (with the observation interval of 30 days). Observations carried out by pulling out the plants derived from control and coated seeds. Root samples were collected and cut into small fragments (1 cm-long) then surface-disinfected by immersion for 2 min in alcohol at 90°C. Afterwards rinsed thoroughly with sterile water, dried on sterile filter paper and were transferred onto PSA then incubated at 25°C for 4 days in darkness.

The percentage of re-isolation (Pr%) was calculated according to the following formula:

$$Pr = Ns\ Px / NT \times 100$$

Ns Px : Number of segments containing the fungal species x.

NT: Total name of segments.

The existence of *Trichoderma* in the epidermal tissue of roots from 30 days old barley and wheat plants were observed under microscope. The thinner roots were cut, soaked in a solution of lactophenol, in water or colored

Table 1: Effect of seed treatment with *Trichoderma* on the growth of wheat and barley plants.

Treatment	Varieties	% G	Roots		stem		No. of internode	leaves	Spike		NTG
			T	P	T	P			T	P	
Control	Soft wheat Wafia	75^c	13^b	1.3^b	49.8^d	3.4^b	4^b	5^d	$13b^c$	2.7^c	48^d
	Durum wheat Amjad	66.6^d	12.9^c	1.4^b	50^c	3.2^b	4^b	5^d	12.8^c	2.9^c	50^c
	Barley Oussama	75^c	12.2^c	1.2^b	49.6^d	3.1^b	4^b	6^c	13.5^b	3^b	50^c
Treatment with *Trichoderma*	Soft wheat Wafia	100^a	18.9^a	3.2^a	65^a	5.5^a	6^a	8^b	18.1^a	5.1^a	70^a
	Durum wheat Amjad	100^a	19.5^a	2.9^b	66.5^a	5.5^a	6^a	9^a	18.3^a	5.2^a	68^b
	Barley Oussama	100^a	18^a	2.8^b	65.7^a	5.6^a	6^a	9^a	18.3^a	5.5^a	66^a

%G: Percentage of germination. T: size. P : weight. NTG: Total number of grains per spike.
*Two values read in the same column, followed by the same letter, are not significantly different at the 5% threshold.

Table 2: Effect of seed treatment on the biomass of aerial and root parts of wheat and barley.

Treatment	Varieties	Dry weight stem (g)	Dry weight root (g)
Control	Soft wheat Wafia	1.84[c]	0.59[d]
	Durum wheat Amjad	1.91[b]	0.66[c]
	Barley Oussama	2[ab]	0.61[c]
Treatment with *Trichoderma*	Soft wheat Wafia	3.1[a]	2.3[a]
	Durum wheat Amjad	3.12[a]	1.95[b]
	Barley Oussama	3.2[a]	1.97[b]

*Two values read in the same column followed by the same letter are not significantly different at the 5% threshold

with cotton blue and examined afterwards under a microscope.

Statistical analysis

The data processing involved the analysis of variance by the ANOVA order I test. A comparison of the means was carried out by the PPDS test (the smallest significant difference) if a significant difference is registered at the probability threshold of 5%.

Results

The results in table 1 showed the effect of seed treatment with *Trichoderma* on the growth of wheat and barley plants. Seeds treated with *Trichoderma* have shown great germination capacity, it was 100% in treated seeds and varied between 66.6 and 75% in untreated seeds. The average length of wheat and barley plants from coated seeds with *Trichoderma* exceeded those of plants from untreated seeds; it varied between 65 and 66.5 and between 49.6 and 50 cm, respectively. This observed difference in the length of the plants was also reflected in the weight of the stems which was around 5.6 g in plants from treated seeds against 2.7g and 3g in control plants of wheat and barley respectively. The number of internodes was 6 for *Trichoderma*-treated wheat plants compared with that of control plants (4). The application of *Trichoderma* isolate by seed coating improved the number of formed leaves (8 and 9) on the three varieties in comparison with the untreated plants (5 and 6).

The average length of the roots of wheat and barley plants from treated seeds is greater than that of control plants, ranged from 18 to 19.5 cm and 12.2 to13 cm respectively. The weights were of 2.8 and 3.2 g in plants from coated seeds compared to 1.2 and 1.4 g in control plants.

It was also observed that the size of wheat and barley ears from treated seeds were higher (18.1 cm and 18.3 cm) than those of control not exceeding 13.5 cm. Similar results marked the ear weights and grain yields with an average of grains number in the order of per 66 and 70 per spikes in the treated plants against 48 and 50 per spikes in the control plants. The number of empty grains per spike is 2 to 4 in the ears of control and treated plants, respectively.

In relation to stem dry mass of wheat and barley plants from seeds treated with *Trichoderma* a significant increase was noted with respective values of 3.1 and 3.2 g while those of control plant did not exceed 2g. Once again, the control plants showed lower values of root dry weight ranged from 0.59 to 0.66g whereas those of the treated plants varied from 1.95 to 2.3g (Table 2).

It appears from these results that the treatments of wheat and barley seeds with *Trichoderma* had a positive effect on the agronomic and yield parameters of these two plant species. The positive effect is apparent compared to that observed in plants from untreated seeds.

Wheat and barley plants from treated seeds showed increased rates

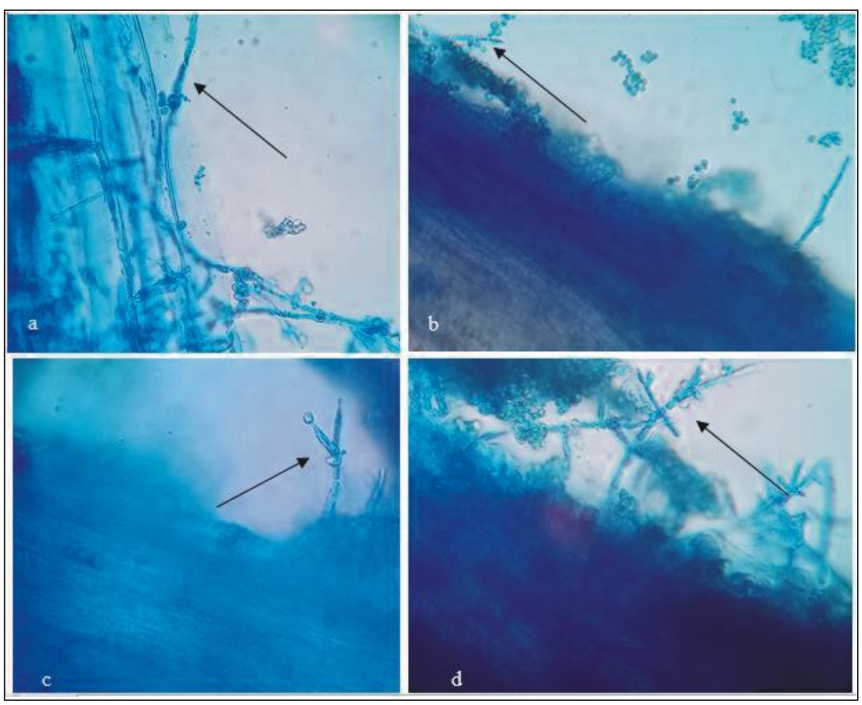

Fig. 1: Microscopic observations of the plant roots obtained from seeds treated with *Trichoderma*. a: mycelial filaments. (b, c, d): phialides with conidia.

Table 3: Colonization percentage of the plants roots obtained from seed inoculated with *Trichoderma* as a function of time.

	Variety	% of root colonization by *Trichoderma*							
		1 month	2 month	3 month	4 month	5 month	6 month	7 month	8 month
Control	Soft wheat Wafia	0[a]	0[a]	0[a]	0[a]	0[a]	0[a]	0[a]	0[a]
Trichoderma		64.25[d]	73.5[c]	80.1[b]	88.9[b]	90.5[a]	86.5[b]	80.7[b]	74.6[c]
Control	Durum wheat Amjad	0[a]	0[a]	0[a]	0[a]	0[a]	0[a]	0[a]	0[a]
Trichoderma		67.5[d]	75.4[c]	82.7[b]	89.8[b]	91.6[a]	88.7[b]	79.9[c]	75.6[c]
Control	Barley Oussama	0[a]	0[a]	0[a]	0[a]	0[a]	0[a]	0[a]	0[a]
Trichoderma		66.5[d]	76.8[c]	83.6[b]	90.4[a]	92.4[a]	87.9[b]	78.6[c]	76.9[c]

*Two values read on the same line, followed by the same letter, are not significantly at the 5% threshold.

of root colonization by tested *Trichoderma* isolate (Table 3). The percentages of root colonization after one month of cultivation varied between 64.25 and 67.5%. After 8 months of sowing, they reached respective rates of 74.6% and 76.9% in roots of wheat and barley plants. Control plant roots had no colonization by any *Trichoderma* isolate within the whole experiment period.

Microscopic examination of the thiner roots of wheat and barley plants from treated seeds made it possible to observe the conidia of *Trichoderma* in the root cortex and mycelial filaments with specific phialides of this fungus (Fig. 1 and 2).

Discussion

The used *Trichoderma* isolate to treat wheat and barley seeds elicited a significantly higher response in plants from these seeds. Regarding seed germination parameter, the present findings were in agreement with those of (Doni *et al.,* 2014) who have reported *Trichoderma* species/isolates as stimulant of the seed germination in some plant species. As for the other growth characters, the stimulator capacity resulted essentially in better axial growth and higher biomass. This stimulation of the biomass concerned not only the aerial part but also the root system marking a noticeable increase. In accordance with results reported here, Jang *et al.,* (1993) reported that cucumber growth was promoted when seeds were coated with conidia of *Trichoderma* and *Gliocladium* isolates. At the same time, the role of our tested *Trichoderma* isolate in leaf number promoting joins the results of Ozbay and Newman, (2004) works in which these authors showed an increase in leaf number and area and shoot length of tomato plants treated with *Trichoderma* isolates, Similar beneficial effects were provided by strains of *T. asperellum* and *T. harzianum* after treatment of wheat seeds (de Oliveira *et al*., 2018) and lettuce seeds (Pereira *et al.,* 2019) or by *Trichoderma* isolates on tomato seeds (Petrisor *et al*., 2019). Similar results were reported by Mouria *et al.,* (2007) and Chliyeh *et al.,* (2014) indicating that the inoculation of tomato plants with *T. harzianum* greatly improved the weight and length of the aerial and root parts of these plants. *T. harzianum* has also shown a significant effect on the growth and diameter of the roots of carob plants (*Ceratonia siliqua* L.) (Talbi *et al.,* 2015). In contrast, Azmani *et al.,* (2011) reported that seed inoculation with *Trichoderma* had no significant effect in leaf number and also in total area of leaf and plant height, root fresh and dry weight. Results of Ortega-Garcia *et al.,* (2015) supported that differences in growth stimulation by *T. asperellum* isolates is related to their ability to produce phytohormones IAA like compounds in presence of precursors.

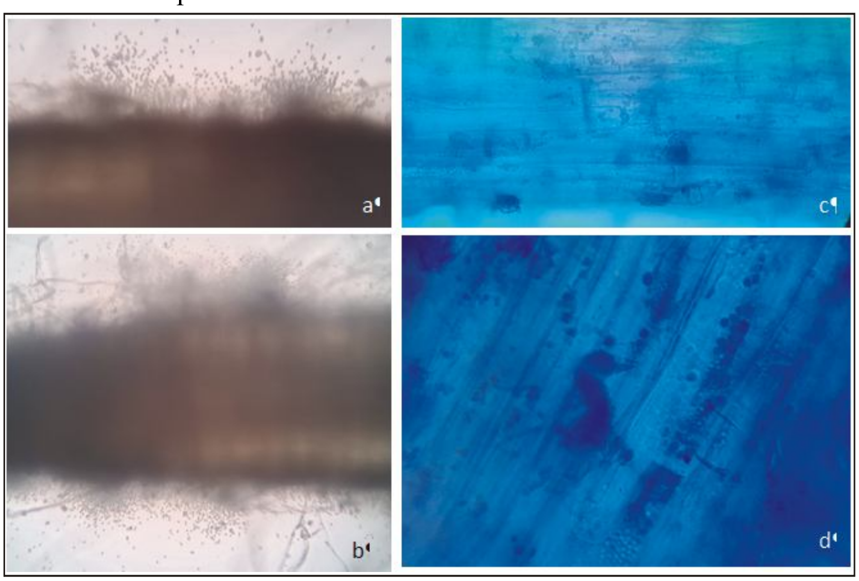

Fig. 2: Microscopic observation of the plant roots obtained from seeds treated with *Trichoderma* (a and b): microscopic observation in cotton blue of different *Trichoderma* structures in the roots of plants from treated seed. (c and d): *Trichoderma* conidia in the cells of the root cortex

In some studies, it was suggested

that the growth promoting effect induced by the *Trichoderma* species can be attributed to their capacity to produce secondary metabolites such as indole-acetic acid (Contreras-Cornejo *et al.,* 2009), a hormone that stimulates growth in meristematic tissues and increases the production of cysteine which modifies root architecture and increases their growth (Samolski *et al.,* 2012). According to Chacón *et al*., (2007), *Trichoderma* inoculation can lead to root proliferation and as consequence an increase in water and nutrient absorption capacity. In addition to aforementioned studies, the higher responses of plants following inoculation with *T. harzianum* have been explained by the ability of this fungus to dissolve insoluble phosphorus (Altomare *et al.,* 1999; Yedidia *et al.,* 2001). The work of Chang *et al.,* (1986), Kleifeld and Chet, (1992) and Paulitz *et al.,* (1986) stated that *Trichoderma* also influences the metabolism and enzymatic activity of plants as well as the defense systems. Kleifeld and Chet, (1992) reported that stimulation of plant growth by strains of *Trichoderma* sp. would be due to the increased transfer of nutrients from the soil to the roots through colonization by *Trichoderma* sp. Hmouni *et al.,* (2006) and Mouria *et al.,* (2007) noted that *Trichoderma* sp. can colonize the epidermis of the roots and the outer cortical layers and release bioactive molecules which induce resistance in plants and stimulate plant growth. In this sense, according to Besnard and Davet, (1993), only the identification of compounds responsible for growth stimulation and the study of their effect on plants grown in cultivation under shelter should conclude on the involved mechanism.

The higher colonization percentages noted in this study revealed that tested *Trichoderma* isolate has maintained its viability and population density at high level after inoculation in the period of 8 months. Similarly, all components of the *Trichoderma* life cycle have been observed under a microscope in the fine roots of barley and wheat plants from treated seeds which proved that it was successfully able to colonize them. The work of Kleifeld and Chet, (1992) showed that root colonization by *Trichoderma* positively influences the transfer of nutrients from the soil to the roots of plants. *Trichoderma* spp. possess a strong colonization ability which, with the growth and extension of the root system, can increase the contact area between root and soil and increase the secretion of extracellular enzymes such as sucrase, urease, phosphatase and organic acids in the rhizosphere, so as to improve the nutrient cycle and enzyme activity in the soil (López-Bucio *et al.,* 2015; Pelagio-Flores *et al.,* 2017).

This study concluded that the *Trichoderma* isolate originating from sites adjacent to the Moroccan phosphate mines showed a great potential in promoting wheat and barley growth. But further experiments under different conditions are needed and recommended.

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