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MICROBIAL INOCULANTS APPLIED AS SEED TREATMENTS AND THEIR EFFECT ON COMMON WHEAT *Triticum aestivum* L.

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Keywords: microbial inoculants, wheat, plant ghowth-promotion

ABSTRACT

Wheat is considered the most wide spread culture in the world regarding the area harvested. In Romania, it is grown on approximately 25% of the arable land and 40% of the cereal grains. Improving wheat productivity and yield quality is a continuous concern. Therefore, plant growth promoting microorganisms with biocontrol potential are of great interest for the farmers. In the present, five beneficial microorganisms, bacteria and fungi, were analysed as agro-inoculants. Their effect on wheat germination and growth initiation was evaluated *in vitro*. According to the biometric analysis best results were obtained when using *Azospirillum brassilense* and *Bacillus amyloliquefaciens* strains, although *Trichoderma pseudokoningii* and *Bacillus endophyticus* also improved wheat vigour indexes, compared to the untreated control. However, *B. endophyticus* 1T2 strain delayed the germination process. The dual culture assay performed against *Fusarium graminearum*, revealed that three strains *B.amyloliquefaciens* OS17, BW, and *T.pseudokoningii* Td85, have also biocontrol potential.

INTRODUCTION

Wheat is considered the most wide spread culture in the world regarding the area harvested, overlaying 218.5 mil ha in 2017. In Romania, it is grown on approx. 25% of the arable land and 40% of the cereal grains. According to the official data, the area harvested had 2 052 920 ha, with an average yield of 4.9 t/ha (www.fao.org). The interest for wheat growth is due to several factors: the grains are the raw material for a large variety of agrifood products, with a balanced ratio between the carbohydrates and protein substances, according to the needs of the human body. Wheat grains have a long shelf life and can be transported without the risk of high degradation. It can also be used as animal feed, either as grains or milled. Wheat straws can also be used as feed or as manure, or in the preparation of organic fertilizers, or as raw material for cellulose. Moreover, wheat can be grown in different climatic zones and at high altitude, due to its high ecological plasticity. From the agro-technical point of view, this crop is fully mechanized, and it enters in almost all agricultural rotation systems, being considered a very good pre-plant.

Considering these, improving wheat growth promotion, productivity and yield quality is a continuous concern. Another important aspect is plant protection from pest and diseases. For the sustainable agriculture the eco-friendly methods that promote plant growth, improve crop productivity or ensure plant protection are a continuous quest (Ekin, 2019). Several natural resources can be used for these purposes, such as humic substances (Anwar et al., 2016), mineral rocks (Manning, 2010), composts and mulches, algae and vegetal extracts (Onofrei et al., 2017) as well as plant growth promoting microorganisms (Kumar et al., 2019).

The aim of this study is to evaluate the influence of several microbial inoculants, applied as seed treatments, on wheat germination and growth initiation. Likewise, we studied the influence of these beneficial

microorganisms against *Fusarium* sp., an important wheat pathogen and mycotoxin contaminant.

MATERIAL AND METHOD

Vegetal material.

The study was carried out on wheat kernel *Triticum aestivum* L. Seed disinfection was carried out in two steps, 2-3 min in 70% ethanol and 25min in 5% sodium hypochlorite, followed by 5 rinses with sterile distilled water.

Beneficial microorganisms.

Five microbial strains were used in this study, four bacteria and one filamentous fungi (Table 1). Routinely, the bacteria were grown on Luria Bertani (LB) at 28°C, and the fungal strain was grown either on Malt Agar (MA), or Potato-Dextrose-Agar (PDA) at 26° to 28°C.

Microbial inoculum preparation.

To obtain the bacterial cell suspension, 48h old broth cultures were centrifuged at 3700rpm, for 20minutes at 10ºC. The pellet was wasted with sterile phosphate saline buffer and resuspended in the same buffer up to 10^8 cfu/ml. Bacterial load was quantified by spectrophotometric analysis at different length waves (620nm, and 600nm) depending on the strain. For the fungal spores suspension, *Trichoderma pseudokonigii* was grown for 10 days on MA, spores were washed in distilled water, supplemented with few drops of Tween 80 to avoid spore clumps. The fungal inoculum was quantified using a Burker-Turk counting chamber, and prepared at 10^7 spores/ml.

Seed treatments.

Disinfected wheat kernels were surface treated with pure-culture microbial-inoculants. Five treatment variants were used in this study, one fungal and four bacterial treatments.To improve the attachment of the beneficial microorganisms on the kernel surface, the inoculum was supplemented with 2% corboxymethil cellulose, in 9:1 v/v ratio. Sterile distilled water was used for the control variant.

Germination assay.

Treated wheat kernels were placed in sterile Petri dishes on filter paper moistened with sterile distilled water, 25 seeds/plate, in four replicates. Plates were kept in complete darkness at room temperature. After 7 days of incubation, the germination percentage was analyzed to determine the effect of delayed germination. Biological parameters such as fresh and dry weight, seedling height (H), number of roots per plant and total length (L) of root system were analyzed.

Plant pathogen.

The phytopathogenic fungi used in this study (Table 1) was acquired from the German Collection of Microorganisms and Cell Cultures GmbH. Routinely, it was grown and maintained on PDA during the study.

Microbial antagonism study.

Direct interaction among the biocontrol microorganisms and the plant pathogenic fungi was studied *in vitro* by dual cultures technique. Both microorganisms, the phytopathogenic fungi and the biocontrol microorganisms, were inoculated simultaneously, in the same plates, at 2cm distance from each other. The fungal inoculum was calibrated as plugs of 6mm in diameter. The beneficial strains were inoculated in spots or strakes, using two days old bacterial biomass. Microbial co-cultivation was performed on PDA medium. Plates were incubated at 28°C and periodically analyzed up to 14 days of co-cultivation.

RESULTS AND DISCUSSIONS

In order to evaluate the effect of different microbial inoculants on wheat germination and growth initiation, aqueous bacterial cells and spores suspensions were applied as seed treatments, before wheat kernel incubation in humid chambers. Tested microorganisms were selected due to their beneficial effects on different plant species, and biocontrol activity against various fungal pathogens. *Azospirillum brasilense* Sp7 (DSM 1690) was used

due to its plant growth promotion activity, atmospheric nitrogen fixation and root colonization of wheat (Deaker & Kennedy, 2001; Gulii et al., 2015). *Bacillus amyloliquefaciens* OS17 and BW strains were used due to their antifungal activity against *Fusarium* spp. pathogens (Sicuia et al., 2012), plant stimulatory effect and plant protection potential against various phytopatogenic fungi (Sicuia, 2013). *Bacillus endophyticus* 1T2 strain was selected due to the bioavailability potential of soil phosphorus that can induce beneficial plant growth effects (Boiu-Sicuia, 2017). *Trichoderma pseudokonigii* strain Td85 (DSM 23661) was used due to its antagonistic activity towards different fungal pathogens, such as: *Botrytis cinerea* (Ștefan et al., 2015), *Fusarium* spp., *Sclerotinia* sp. (Ștefan et al., 2013), and *Rhizoctonia solani* (Petrișor et al., 2016). Moreover it revealed plant stimulating effect on tomato seedlings (Petrișor et al., 2019).

Results regarding microbial inoculants effect on wheat are presented in Table 2. Several biologic parameters were analyzed, such as fresh and dry weight, seedling height (H), number of roots per plant and total root length (L). Based on these observations, the force indices (or Seed Vigor Index – SVI) were determined: SVI-I, calculated according to the size of the plant, respectively SVI-II, calculated according to the dry weight of the plants (Abdul-Baki & Anderson, 1973).

Regarding the germination process, most of the seed treatments applied to the wheat kernels generated a delayed germination compared to the untreated control. Only *A.brassilense* Sp7 and *B.amyloliquefaciens* BW rushed the germination process with one day.

All microbial treatments applied have increased the shoot and root growth in length, biomass quantity, as both fresh and dry weight.

Both seed vigor indexes were improved when the microbial treatments were applied. Best results were obtained with Sp7 and BW, which revealed more than 50% seed vigor augmentation. Several studies reveal that high seed vigor triggers an increased growth and productivity in the agricultural production (Han et al., 2014; Wen et al., 2018).

The antagonistic activity of the beneficial bacterial and fungal strains was evaluated by dual culture technique against *Fusarium graminearum* 183 (Table 3). The antifungal activity was visually evaluated, and different symbols were attributed according to the inhibitory action against *F. graminearum* as follows: „–“ = no inhibition activity; „+“ = slightly inhibition of the pathogen growth, „+ +“ = moderate inhibition; „+ + +“ = good inhibition activity; „+ + + +“ = very good inhibition activity against the phytopathogen.

The biocontrol bacteria *Bacillus amyloliquefaciens* OS17 and BW reduced the pathogenic fungal growth and maintained a clear inhibition zone around their colony. Shi et al. (2014) mentioned other five *B.amyloliquefaciens* strains with good potential of *F.graminearum* inhibition. They obtained a mycelia inhibition rate of 41,4% to 51,5% in the dual culture technique, and 97,8% to 100% in the tip culture assay, associated with DON inhibition rate.

In our study, during the microscopic analysis, fungal modifications were observed in the pathogenic growth when using *Bacillus amyloliquefaciens* OS17 and BW strains. The mycelia was curled, and the fungal cells were thickened, beaded or swollen (Fig. 1A). Baffoni et al. (2015) also mentioned damaged hyphae and conidia in *Fusarium* sp. due to an antagonistic strain of *Bacillus amyloliquefaciens*.

No antagonistic activity was observed for *Bacillus endophyticus* 1T2 against *F.graminearum* 183.

Regarding the interaction between *Trichoderma pseudokonigii* Td85 and *Fusarium graminearum* 183, beside the inhibitory activity on the phytopatogen mycelial growth, a good mycoparasitic activity was observed (Fig. 1B).

The hyperparasitic activity in *Trichoderma* species has been well-documented over time as a biocontrol mechanism. Studies have revealed different genes related to mycoparasitism (Elamathi et al., 2018), like the genes encoding for cell wall degrading enzymes: chitinase (*chit33*), endochitinase (*endo42*), β-1,3-glucanase (*glu*), exochitinase 1 (*exc1*), exochitinase 2 (*exc2*), and the genes related with proteases, such as alkaline proteinase (*prb1*), trypsin-like protease (*Pra1*), subtilin-like serine protease (*ssp*). Gomes et al. (2015) mentioned the cerato-platanin protein Epl-1 as involved in the mycoparasitism mechanism expressed by *Trichoterma* sp. Cytochrome p450 activity involved in secondary metabolites synthesis, related to mycoparasitism, was also detected in *Trichoderma* sp. (Ramírez-Valdespino et al., 2019). Likewise, several other authors described different fungal strains of *Trichoderma* spp. with mycoparasitic activity against Fusarium Head Blight species complex, and other fusaria and plant pathogens (Ghazanfar et al., 2018).

CONCLUSIONS

Five plant beneficial micro-organisms, bacteria and fungi, were analysed for their plant growth promoting effect on wheat and biocontrol potential against *Fusarium graminearum*. All tested microbial inoculants improved wheat root growth and vigour index. Best results regarding germination and seedling growth were obtained with *A.brassilense* and *B.amyloliquefaciens*. However, the bacterial strain *B.endophyticus* 1T2 delayed the germination process compared to the untreated control. Beside plant growth stimulation, the bacterial strains *B.amyloliquefaciens* OS17, BW, and the fungal strain *T.pseudokoningii* Td85 inhibited the growth of *F. graminearum*, which is an important wheat pathogen.

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Table 1.

Microorganisms used in this study

Microorganisms, species and strains	Provenience
Beneficial microorganisms	
Azospirillum brasilense Sp7 (DSM 1690)	DSMZ Collection, Germany
Bacillus amyloliquefaciens OS17	RDIPP – Bucharest collection
Bacillus amyloliquefaciens BW	Faculty of Biotechnology, UASVM – Bucharest collection
Bacillus endophyticus 1T2	RDIPP – Bucharest collection
Trichoderma pseudokonigii Td85	RDIPP – Bucharest collection
Pathogenic microorganism	
Fusarium graminearum 183 (DSM4527)	DSMZ Collection, Germany

Table 2.

Wheat germination and growth parameters

Experimental variant	Germination %	Fresh weight (g)		Dry weight (g)		Plant H (cm)	Root no./ plant	Total root L/plant (cm)	Vigor Index	
		root	Shoot	root	Shoot				SVI –I	SVI-II
0	1	2	3	4	5	6	7	8	9	10
Untreated control	88.3	0.0270 ^{a}	0.0231 ^{a}	0.0038 ^{a}	0.0032 ^{a}	3.42 ^{a}	3.95	21.01 ^{a}	301.99	282.56
Sp 7	92	0.0445 ^{bc}	0.0410 ^{bc}	0.0056 ^{b}	0.0049 ^{b}	5.47 ^{b}	4.06	27.34 ^{b}	503.24	450.80
OS17	80	0.0478 ^{bc}	0.0354 ^{b}	0.0059 ^{b}	0.0046 ^{b}	5.49 ^{b}	4.13	31.03 ^{c}	439.20	368.00
BW	92	0.0403 ^{b}	0.0416 ^{c}	0.0053 ^{b}	0.0047 ^{b}	5.64 ^{b}	4.15	24.98 ^{ab}	518.88	432.40
1T2	77.5	NA	0.0401 ^{bc}	NA	0.0047 ^{b}	5.51 ^{b}	3.92	32.63 ^{c}	427.03	364.25
Td85	86.3	0.0420 ^{b}	0.0340 ^{b}	0.0054 ^{b}	0.0041 ^{b}	5.10 ^{b}	4.30	26.45 ^{b}	440.13	353.83

Legend: NA = value not available. The values corresponding to columns 1÷8 were statistically processed (Duncan test, p <0.05), the letters highlight the significant differences between the treatment variants.

Table 3.

Antagonistic activity against *Fusarium graminearum* 183 plant pathogen

Plant beneficial microorganism	Antifungal activity	Clear inhibition zone (mm)	Mycoparasitism
Bacillus amyloliquefaciens OS17	+ + +	4	N.A.
Bacillus amyloliquefaciens BW	+ + +	3	N.A.
Bacillus endophyticus 1T2	-	-	N.A.
Trichoderma pseudokonigii Td85	+ + +	-	+

Legend: NA = not available

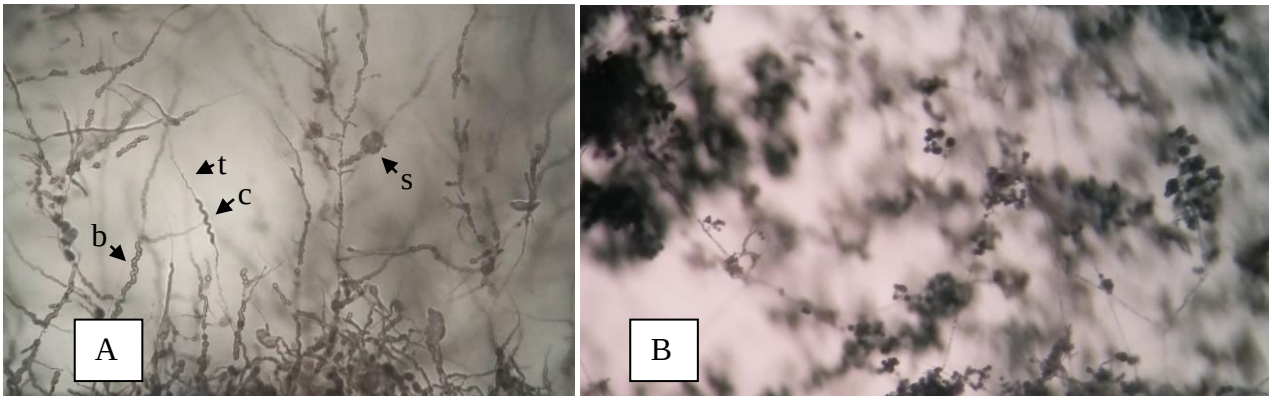

Figure 1. Microscopic aspects captured from the microbial interaction zone. A. B.amyloliquefaciens OS17 effect on Fusarium graminearum, s = swelling, c = curling, and t= thining of the fungal cells, b= beaded fungal cells; B. Mycoparasite mycelia, conidiophores and conidia of T. pseudokonigii Td85 grown on top of Fusarium graminearum