Screening fungal endophytes from a wild grass for growth promotion in tritordeum, an agricultural cereal

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Abstract

Celtica gigantea is a large perennial grass which grows in nutrient-poor sandy soils in semiarid zones of the western Iberian Peninsula. The purpose of this work was to find out if culturable fungal symbionts isolated from roots of this wild grass could have growth promoting activity in tritordeum, a hybrid cereal for human consumption. A survey of fungi from the root endosphere of *C. gigantea* produced an isolate collection consisting of 60 different taxa, mostly ascomycetes. Fungal strains were inoculated into tritordeum plants in order to evaluate their effect in leaf and root biomass, nutrient content, and total antioxidant capacity. Two consecutive screening processes were made to test endophyte effects in plants. In the first screening, 66 strains were inoculated into seedlings by dipping roots in a liquid suspension of inoculum. In the second screening, 13 strains selected from the first screening were inoculated by sowing seeds in a substrate containing inoculum. The inoculation method used in the second screening involved less labor and plant manipulation and improved the quantity and quality of the inoculum, making it more appropriate for big scale experimental inoculation procedures. Several fungal strains promoted leaf or root growth. In particular, a strain belonging to the genus *Diaporthe* caused an increase in leaf and root biomass in both screening processes, suggesting that this endophyte might have a good potential for field application in tritordeum.

Keywords: plant growth promotion, *Celtica gigantea*, inoculation method, microbiome

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1. Introduction

As holobionts, plants possess a complex microbiome composed by fungi, bacteria, viruses, and other microorganisms [1]. Some components of the microbiome have functions related to the habitat adaptation of their host plants. For example, some fungal symbionts of plants adapted to salinity or high soil temperature increase the tolerance of their hosts to these factors [2], and others are known to increase plant tolerance to herbivory or disease [3, 4]. Thus, selection pressures are exerted upon holobionts, and their microbiome acts as an extended source of genes available for plant adaptation [5, 6]. Plant microbiomes from high stress habitats represent an attractive system to search for components useful for agricultural plant improvement. If some of those microorganisms can assist a plant to adapt to a given stress factor present in a natural habitat, they might increase tolerance to that stress in an agricultural species. Thus, from an agricultural point of view, the microbiome could be seen as an accessory source of plant characters, available for crop improvement. In attempts to domesticate "wild" symbiotic fungi, some of them have been successfully transferred from their original hosts to agricultural species, providing benefits to the inoculated plants [7-12].

Celtica gigantea (=*Stipa gigantea,* Poaceae) is a large perennial grass whose leaves measure up to 50 cm in length, forms dense clumps, and its flowering stems can reach up to 2.5 m. It is endemic of the western Iberian Peninsula and North Africa, growing in nutrient-poor sandy soils in semiarid habitats [13, 14]. Based on the large size of plant individuals growing in habitats limited in water and nutrient resources, our hypothesis was that some fungi associated with its roots could have a role in habitat adaptation. Therefore, the objectives of this work were to identify culturable fungi associated with roots of *Celtica gigantea,* and to find out if any of these fungi had the capability of modifying the growth and other parameters of agronomic interest of tritordeum. For this purpose, we made a survey of culturable fungi associated with *C. gigantea* roots, followed by an inoculation-based screening in order to detect strains having a growth promotion effect on tritordeum. Tritordeum is a hybrid grain cereal apt for human consumption which was developed from a cross between durum wheat (*Triticum durum*) and *Hordeum chilense,* a wild barley species native to Chile and Argentina [15].

2. Materials and methods

2.1. Isolation and identification of fungal strains

Roots of *Celtica gigantea* were sampled at four different locations in the province of Salamanca, Spain: Cuatro Calzadas, Monterrubio, Mozárbez, and Ledesma (Table 1). At each location root samples from 10 plants were obtained by digging out part of a clump to a depth of about 30 cm. Soil from the rhizosphere of three plants from each location was mixed in order to obtain one pooled sample per location for chemical analysis.

Root samples were transported to the laboratory and processed for the isolation of fungi the day after they were collected in the field. For this purpose, a sample of about 20 root fragments of 4-5 cm in length was obtained from each plant. Each root sample was washed with tap water in a 50 mL Falcon tube, and later surface-disinfected with a solution of 20% commercial bleach (1% active chlorine) containing 0.01% Tween 80 for 10 min, followed by treatment with a solution of 70% ethanol for 1 min. Finally, the roots were rinsed with sterile water and cut into pieces about 5 mm long. Twenty root pieces from each sample were plated in two Petri plates (9 cm diameter) with potato dextrose agar (PDA) containing 200 mg L^{-1} of chloramphenicol. Plates were kept in the dark at room temperature (20-24 °C). As mycelium emerged from a root fragment into the agar, a sample was transferred to a 5 cm diameter PDA plate and maintained in the same conditions. The root fragment and remaining mycelium were taken out of the original plate to avoid overgrowth. The plates with root samples were checked daily for the presence of fungi for about four weeks.

The fungal strains obtained were grouped according to their culture morphotype, considering growth rate, color and mycelium surface characteristics. Afterwards, one or more cultures of each morphotype were processed for a taxonomic identification based on nucleotide sequences of the internal transcribed spacers (ITS) 1 and 2, including 5.8S rDNA. Primers ITS4 and ITS5 [16] were used to obtain PCR amplicons of this genomic region following the procedure described by Sánchez Márquez et al. [17].

Amplicons were sequenced at the DNA sequencing service of the University of Salamanca. All the nucleotide sequences obtained were clustered, using the Cd-Hit Est program [18], sequences having an identity value of 97% or greater were considered to belong to the same taxon and cluster. Taxonomic identity was assigned to a representative sequence of each cluster by searching for close matches at the RefSeq database of the ITS region from Fungi type and reference material at the National Center for Biotechnology Information (NCBI) using the BLAST algorithm. This database contains sequence data from internal transcribed spacer regions from fungi type and reference material [19]. Further taxonomic information was obtained by means of a phylogenetic analysis made with representative sequences from each taxon. This analysis was made with MEGA6 software using the maximum likelihood method with distances

calculated according to the Tamura 3 parameter model [20]. Tree branch confidence values were estimated by bootstrapping with 1000 replications [21].

2.2. Greenhouse inoculation experiments

A series of inoculation experiments were made in a greenhouse with the purpose of identifying fungal strains capable of increasing the growth, nutrient content, or antioxidant capacity of tritordeum plants.

A first evaluation of 66 fungal strains was made using a liquid inoculation method. To produce inoculum, a PDA Petri plate with an actively growing fungal culture was crushed in a blender at low speed for 10 s with 80 mL of sterile water. Seeds of tritordeum cv. Aucan were germinated in perlite, and the roots of seedlings with a 5-cm-long epicotyl were submerged in the inoculum suspension. After incubation at 25 °C for 24 h, each seedling was transplanted to a 200 mL pot filled with a substrate consisting of a 1:1 (v/v) mixture of peat moss and perlite previously treated at 80 °C for 24 h. When transplanting, 5 mL of the inoculum suspension were added to the substrate at the base of the seedling. Typically, each inoculation experiment consisted of a test of seven different fungal strains, each one inoculated in seven plants (replicates), plus a control treatment of seven uninoculated plants. After six weeks growing in the greenhouse, the plants were harvested, roots were washed to remove substrate, and dry biomass of aerial and underground tissues was measured. Four replicates of each inoculation treatment were freeze-dried for chemical analyses of mineral content and total antioxidant capacity in aboveground plant tissues.

A second evaluation of 13 fungal strains selected from the first screening was made using a different method of inoculation, consisting of the direct sowing of seeds in a substrate containing inoculum. To produce fungal inoculum, 30 g of sugar beet pulp pellet were mixed with 3.0 g $CaCO_3$, 1.5 g $CaSO_4$ and 60 mL of water in 1 L glass jars and autoclaved. Each jar of beet pulp medium was inoculated with a fungal strain by adding four 5×5 mm blocks of mycelium from a PDA culture. The cultures were then maintained at room temperature (20-24 °C) for a period of four weeks [22]. To inoculate plants, one part of beet pulp inoculum was mixed with seven parts (w/w) of a substrate consisting of a 1:1 mixture of peat moss and perlite (v/v) previously treated at 80 °C for 24 h. Before mixing with the inoculum, the dry substrate was hydrated with 1/3 volume of tap water. Three seeds of tritordeum cv. Aucan were sown on 200 mL pots filled with substrate containing inoculum, and after germination only one seedling was left on each pot. Each inoculation treatment and the control, consisting of seeds sown in peat perlite substrate alone, were replicated in 14 pots. After six weeks growing in the greenhouse, the plants were harvested as in the first screening experiment. Six replicates of

each inoculation treatment were freeze-dried for chemical analyses of mineral content and total antioxidant capacity.

2.3. Chemical analyses

The concentrations of P, K, Ca, Mg, S, Mn, Fe, Cu and Zn in freeze-dried and ground plant samples were determined by inductively coupled plasma atomic emission spectroscopy (ICP-OES, Varian 720-ES) after the calcination of samples at 450°C for 8 h and the dissolution of ashes in $HCl:HNO_3:H_2O$ (1:1:8).

Trolox equivalent antioxidant capacity (TEAC) was determined in lyophilized and ground plant material using the Ferric ion Reducing Antioxidant Power (FRAP) assay described by Benzie and Strain [23]. The measurements were made in a multimodal 96-well plate reader (FLUOstart Omega, BMG Labtech).

Soil samples were analyzed for pH, organic matter, C, N, P, Ca and K content by standard methods at the Analysis and Instrumentation Service of IRNASA-CSIC.

2.4. Data analysis

Each screening consisted of several independent inoculation experiments which were consecutively made at different dates. Therefore, to correct for differences in the growth of plants due to seasonal variation among experiments, all parameters were expressed as a percent of variation respect to the non-inoculated control plants of each experiment. A heat-map was made to show the effect of each fungal strain on leaf and root dry weight, mineral content (P, K, Ca, Mg, S, Cu, Fe, Mn, Zn) and TEAC, in inoculated tritordeum plants. Three categories were established: a positive response was considered as an increase greater than 8% respect to controls in the value of a parameter; a neutral effect was a variation between 8% and −8%, and a negative response was a decrease greater than −8%.

A principal component analysis (PCA) was performed to simultaneously analyze all variables measured in inoculated plants, considering a matrix of 66 rows (fungal strains) and 12 columns (variables) consisting of leaf and root dry weight, mineral content (P, K, Ca,Mg, S, Cu, Fe, Mn, Zn) and TEAC. For the results of the second screening, a similar procedure was used with a data matrix obtained from inoculations with 13 fungal strains. IBM SPSS Statistics (version 25) software was used for the multivariate analysis.

3. Results

3.1. Fungi associated with the root endosphere of Celtica gigantea

The soils from the four locations where plants were sampled had sandy texture, acid pH, and low fertility in terms of organic matter, N, P and K content (Table 1).

As a result of sequencing one or more fungal strains from each morphotype, 205 nucleotide sequences of the ITS-rDNA region were obtained. These sequences were grouped into 60 clusters, each cluster composed by one or several sequences having an identity of 97% or greater. A representative sequence from each cluster was used to assign it to a taxon by means of comparing it to sequences of type strains. In several cases BLAST searches with these sequences returned similar identity values for type strains of several species of the same genus. For this reason, we considered that our ITS-rDNA sequences did not contain enough information for a reliable identification to species rank; therefore, fungal strains were identified to genus rank (Table 2). In cases where sequences had an identity lower than 95% with a type strain, assignment to a genus was not reliable, and these sequences were considered to belong to an unknown taxon. As a result, within the 60 distinct taxa identified, 36 were assigned to known genera, and 24 were considered to belong to unknown genera.

Additional taxonomic information was obtained from a phylogenetic tree made with the nucleotide sequences of all the strains listed in Table 2 (Figure 1). In this tree, a clade containing taxa belonging to the Ascomycota was separated from another clade containing basidiomycetes and zygomycetes. Within the Ascomycota, four clades were composed by strains belonging to the Sordariomycetes, Eurotiomycetes, Leotiomycetes, and Dothideomycetes classes. In all cases, the closest database match to strains of unknown genera placed within these clades belonged to the taxonomic class of the clade. For example, the closest database match to unknown strain A41, placed within the Sordariomycetes clade, is *Conioscypha pleiomorpha* (91.2% identity), a sordariomycete.

3.2. First screening: liquid inoculation method

The initial screening in tritordeum was conducted with 66 fungal strains identified in the first part of the study. In terms of biomass, nine strains caused an increase greater than 8% in both leaf and root dry weight with respect to the uninoculated controls. Those were T6, T72, T24, T5, A76, T61, T29, T62 and A60 (Table 3, Figure 2). In particular, *Pseudophialophora* T72 and *Diaporthe* T6 caused an increment greater than 30% in both root and shoot biomass, and positively affected the concentration of four mineral elements (Figure 2, Appendix A Table A1). On the contrary, 19 strains caused a decrease greater than –8% in both leaf and root biomass respect to control plants (Table 3). No strain caused plant death, although extreme effects causing leaf or root dry weight losses greater than 20% occurred with strains like *Penicillium* T21 and leotiomycetes A16 and A12 (Figure 2).

Only 13 strains caused an increase in TEAC above 8% respect to the control plants. Among these, only *Trichoderma* strains A14 and A3 caused an increase in antioxidant activity in parallel with an increase in biomass (Table 3). TEAC decreased with 18 treatments, and for 35 treatments the effect was neutral.

In a principal component analysis of the 12 parameters measured in the inoculated plants, the first two principal components accounted for 29.7% and 27.7% of the total variance, respectively. All mineral elements and TEAC variables were in the positive part of the component I (Figure 3). The most important loading variables in the component I were Ca, S, Cu, Fe and Zn. In the component II the most important loading variables were leaf biomass, root biomass, and Mn. The ordination of all samples on the factorial plane delimited by components I and II showed four clusters (Figure 3). Cluster A included inoculation treatments that mainly increased leaf and root dry weight and in some cases Mn content. Cluster B was composed mostly by strains causing neutral values in most variables and increased TEAC values. Cluster C mainly included those inoculation treatments that reduced the root dry weight and had a neutral or reducing effect on mineral content and TEAC values. Cluster D comprised strains that mostly decreased biomass, increased mineral content, and had a neutral or reducing effect on TEAC (Table 3, Figure 3).

Based on the results of this screening, 13 strains were chosen for the second evaluation. These strains were selected because of their positive effect on both leaf and root biomass (*Coniochaeta* T5, *Diaporthe* T6 and T61, *Collembolispora* T24, *Alternaria* A60 and zygomycete T29); increase in leaf biomass (*Alternaria* T7); increase in root biomass (basidiomycete T40 and zygomycete T80); or increase in the concentration of several nutrients (Dothideomycete T10: P, K, Ca, Cu, Mn, B and TEAC; *Collembolispora* T17: S, Mn, Zn, B, Mo; *Paraconiothyrium*T33: K, Cu, Fe and TEAC; *Diaporthe* T56: Ca, Mg, S, Fe, B).

3.3. Second screening: beet pulp inoculation method

For the second evaluation the number of plant replicates was increased to 14, and an inoculation procedure different from that of the first screening was used. Seeds were directly germinated in a substrate containing the fungal inoculum.

In the second screening only *Diaporthe* T6 increased both leaf and root biomass (Table 4), and this strain also increased Ca, Mg and S leaf content. Strains T80, T40, T10 and T33 increased leaf biomass and the content of several nutrients. Strain A60 had a neutral effect on leaf and root biomass, and produced an increase of most of nutrients (Table 4, Appendix A Table A2). The remaining strains (T17, T56, T24, T61, T5, T29 and T7) decreased leaf and root biomass and increased nutrient content. None of the 13 strains increased TEAC.

Figure 4 shows the results of the PCA of the 12 parameters analyzed in inoculated plants. Components I and II accounted for 52.6% and 22.8% of the total variance, respectively. The variables related to micronutrient content (Mn, Fe, Cu, Zn) had high loading values. Four clusters were differentiated, Cluster A was formed only by the T6 strain, clearly segregated from the other inoculation treatments, and related to high leaf and root biomass, and low Mn values. Cluster B was formed by strains that increased Fe and decreased Mn; in this cluster strains T40, T10 and T33 increased leaf biomass, but strains T56 and T24 had a negative effect on biomass. Cluster C contained strains T80, A60 and T17 which strongly increased Mn content, and Cluster D was formed by strains that increased Fe and Cu contents.

4. Discussion

The purpose of this work was to find out if fungal endophytes isolated from roots of *Celtica gigantea*, a grass adapted to habitats with limited water and nutrient resources, could have growth promoting activity in tritordeum, an agricultural crop.

In the survey of culturable fungi from the root endosphere of *C. gigantea*, 60 different taxa were identified, 36 of these could be assigned to a genus, and the remaining 24 to a class. A conservative approach was used to identify the strain collection. A database of ITS-rDNA sequences of fungal type strains (ITS RefSeq; [19]) was used to assign strains to taxonomic classes or genera, and the latter was the highest taxonomic rank used for strain identification. When genus assignment was uncertain, class assignments made by the previous method could be verified in a phylogenetic analysis. This approach might have helped to minimize errors due to incorrect identifications in entries from less restricted sequence databases.

Most taxa belonged to the Ascomycota, a characteristic of culturable endophyte assemblages from numerous plant species, including grasses [12, 24-26]. Several genera such as *Acephala, Alternaria, Darksidea, Exophiala* and *Microdochium* contain species of dark septate endophytes (DSE), a group of fungi associated with roots of numerous host species, often in arid and semiarid habitats [26, 27]. Some symbiotic DSE might play a role in soil nutrient cycling, thus improving plant nutrient acquisition and growth [28, 29].

The response of tritordeum to inoculation with 66 different strains belonging to 60 different taxa was analyzed in the first screening. Considering as neutral the effects in plants ranging from 8% to −8% respect to controls, 17 strains behaved as plant growth promoters, causing an increase above that threshold in tritordeum leaf and/or root dry weight. The remaining strains had neutral or deleterious effects on these parameters. In terms of leaf and root growth promotion the results from the second screening differed from those of the first for most strains except for *Diaporthe* T6, which promoted leaf and root growth in tritordeum in

both cases. It is interesting that in both screenings most strains which caused a reduction in leaf or root biomass, also increased the content of several nutrients with respect to the controls. This suggests that these fungi could be affecting diverse plant processes that result in a lower efficiency in the use of nutrients, which might accumulate because of not being properly channeled to growth. For instance, *Diaporthe viticola* caused changes in *Arabidopsis* root architecture that might favour nutrient absorption, however, shoot biomass was not affected [30]. In such situation a nutrient accumulation is likely to occur.

In addition to biomass and nutrient content, endophyte effects on antioxidant capability of symbiotic plants were studied. Some endophytes produce antioxidant compounds that may enhance tolerance to oxidative stress resulting from plant defense reactions in fungus-plant interactions [31, 32]. Fungal active antioxidants such as phenolic compounds and sugar alcohols scavenge reactive oxygen species (superoxide or hydroxyl radicals) or can even act as osmoprotectants. However, increased antioxidant capacity was observed only in two treatments causing biomass increments (*Trichoderma* A1 and A3). In the first screening a TEAC increase was more prevalent among inoculation treatments that had a neutral or negative effect on plant biomass, and in the second screening, no strain enhanced the antioxidant capacity of tritordeum. Therefore, TEAC was of little informative value for screening plant growth promoting endophytes under our test conditions. Further experimentation about the timing of TEAC measurements or the environmental conditions used for plant response measurement might be useful to understand if increased antioxidant capacity of endophyte-inoculated tritordeum can be related to improved plant performance under stress conditions like nutrient deficiency, salinity or drought.

For the same strain, the plant response generally differed between both screenings indicating the importance of the inoculation methods used for endophyte screening. In the second screening seeds were directly sown in a substrate containing inoculum, while in the first screening seedlings were dip-inoculated in a liquid suspension of inoculum, and afterwards transplanted to soil. Thus, seedling manipulation was avoided using the solid inoculum. In addition, the solid inoculum can be easily and gently disaggregated into small particles to mix with potting substrate, minimizing damage to the mycelium in comparison to the method used to triturate the mycelium with a mechanical blender in liquid medium. Also, more uniformity among the responses for all parameters of different inoculation treatments was observed in the second than in the first screening, where no two strains caused an identical response. Therefore, for future screenings the inoculation method based on solid inoculum prepared in beet pulp [22]has advantages over the liquid inoculation method.

Differences related to the nutrient input and other characteristics of the medium used to produce inoculum might also affect plant growth. Plant nutrient availability was greater for the second screening. The planting substrate used for the second screening contained 1/8 volume of beet pulp inoculum. Beet pulp contains micro and macronutrients as well as organic compounds [22]. In addition, many fungal species grow profusely in beet pulp medium, for instance, after four weeks about 50% of the dry weight of a beet pulp culture of *Diaporthe* T6 strain is fungal biomass [22]. Thus, the inoculum load and viability were also greater for the second screening.

Strain *Diaporthe* T6 performed equally well using both inoculation methods, and this makes it a candidate of choice for future studies of its effects on tritordeum plant growth in field conditions. *Diaporthe* strain T61 also increased root and shoot biomass in the first screening, but not in the second one. The genus *Diaporthe* contains pathogenic as well as numerous endophytic species [33]. As it occurs with other fungal species, some *Diaporthe* strains might behave as pathogens in some plant species, and as asymptomatic endophytes in others [30, 34]. As an endophyte, *Diaporthe* is a dominant taxon in the microbiome of plant species such as *Festuca rubra*, olive, mangrove, and others [12, 35-37]. *Diaporthe* endophytes are a component of the core microbiome of *Festuca rubra* subsp. *pruinosa*, a halophytic grass from sea cliffs, and can confer salinity tolerance to *Lolium perenne*, a forage grass [12]. Therefore, as components of plant microbiomes *Diaporthe* species could have functions related to host adaptation, and this genus could have a high potential if searched for beneficial endophytes for agricultural applications.

There are several ways by which symbiotic root fungi could promote plant growth (e.g. Nutrient solubilization, organic acid exudation, phytohormone secretion, pH acidification…), but to our knowledge a comprehensive picture of how this occurs is not known for any plant-endophyte system. In our screening experiments, stressors such as drought, herbivores or disease were absent, so we will not consider increased tolerance to biotic or abiotic stress as a factor related to the plant growth promotion observed with some fungal strains. Plant hormone production and improved nutrient acquisition are the factors most cited in relation to plant growth promotion by root endophytes, and these two factors can be related [30, 38-40]. Plant hormones such as gibberellins and indole acetic acid are produced by numerous symbiotic fungi *in vitro*, and in some cases where fungal culture filtrates promoted plant growth, this effect was attributed to phytohormones [38, 40, 41]. Hormone involvement might be associated with improved nutrient uptake, for example, improved P uptake was considered as a main factor associated with increased tillering, grain yield, and P content observed in wheat inoculated with *Piriformospora indica* [42]. This could occur because *P. indica* produces extracellular indole

acetic acid (IAA), which promotes root branching, and thus increased root exploration and nutrient uptake [41]. A similar situation is reported with some strains of *Diaporthe viticola* and *Diaporthe phaseolorum* [30]. When *Arabidopsis* seedlings grew in the presence of those strains, the number of lateral roots and length of root hairs increased, as well as the expression of an auxin-induced gene in root tips. In addition, some of the above mentioned root fungi possess genes coding for P transporters and P processing enzymes similar to those used by plant roots for absorption, and might acidify the rhizosphere, increasing the solubility of inorganic P and other nutrients [30, 43]. Another mechanism for improved nutrient availability is attributed to some DSE, which can accelerate the mineralization of peptides and amino acids to nitrogen forms available for plant roots [29]. We found that the five strains that increased leaf biomass in the second screening also increased nutrient content, suggesting improved nutrient uptake as a possible mechanism. Nevertheless, in the context of this report plant growth is a complex process that goes beyond increased nutrient content, because in the first screening this factor was often linked to reduced plant growth, and only one strain, *Diaporthe* T6, consistently promoted tritordeum growth in both screenings.

The improvement of nutrient content in inoculated plants could be of interest when other parameters than increased plant biomass are sought. For instance, feed supplementation is sometimes necessary to meet animal nutrition requirements, especially when forage does not have an adequate mineral content. The fact that several fungal strains increased macro and micronutrient content make them good candidates for forage improvement. Our research group has found that some of the fungal strains from *Celtica* inoculated in tritordeum have beneficial effects on *Lolium perenne* (unpublished), an important forage grass. Therefore, wild grass endophytes like the ones selected in this work could be tested for forage grass quality improvement.

5. Conclusions

This work departed from the idea of finding out if symbiotic root fungi from a wild grass species adapted to an unhospitable habitat for crop production could be useful to improve the performance of tritordeum, a cereal crop. The results obtained suggest that several components of the *Celtica gigantea* microbiome can increase the root and leaf growth of tritordeum. In particular, a strain belonging to the genus *Diaporthe* consistently promoted root and leaf growth in both screenings. This strain is a good candidate for field testing and further research on the nature of its symbiotic relationship with plants. This work also describes a screening procedure based on inoculum produced in a beet pulp medium which is appropriate for testing relatively large numbers of fungal strains and plants.

6. References

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Table 1. Characteristics of soils from the locations where plants were sampled.

Location	Coordinates	Soil texture	pH (H_2O)	Organic matter (%)	C (%)	N (%)	C/N	P (ppm)	Ca (ppm)	K (ppm)
Cuatro Calzadas	40°49'03.2"N 5°36'47.4"W	Sandy clay	5.32	2.46	1.43	0.099	14.3	5.53	384.0	51.3
Monterrubio	40°44'55.3"N 5°42'32.9"W	Sandy	5.22	0.93	0.54	0.045	11.9	4.44	649.4	96.9
Mozarbez	40°50'14.9"N 5°38'53.3"W	Sandy clay	5.67	1.07	0.62	0.096	6.4	5.54	636.73	128.9
Ledesma	41°06'56.1"N 5°59'57.2"W	Sandy clay	5.85	1.24	0.72	0.058	12.4	11.93	737.1	139.1

Table 2. List of fungal taxa identified in roots of *Celtica gigantea.* The identification proposed for these strains is based on the identity of their ITS-rDNA sequences to those of fungal type strains.

GenBank accession number	Representative strain	Type strain with greatest sequence identity (%)	Proposed taxon
MT645098	T48	*Absidia cuneospora* (98.4)	*Absidia* sp.
MT645099	C12	*Acephala applanata* (98.4)	*Acephala* sp.
MT645100	B10	*Acremonium cavoraeanum* (95.2)	*Acremonium* sp.
MT645101	T69	*Alternaria arbusti* (100)	*Alternaria* sp. A
MT645102	T4	*Alternaria multiformis* (100)	*Alternaria* sp. B
MT645104	A72	*Arcopilus turgidopilosus* (96.0)	*Arcopilus* sp.
MT645105	A76	*Arxotrichum succineum* (96.7)	*Arxotrichum* sp.
MT645151	A37	*Chaetosphaeria ciliata* (95.9)	*Chaetosphaeria* sp.
MT645107	T17	*Collembolispora barbata* (96.6)	*Collembolispora* sp.
MT645109	T5	*Conyochaeta marina* (100)	*Coniochaeta* sp.
MT645112	A128	*Dactylidina shoemakerii* (99.1)	*Dactylidina* sp.
MT645113	T31	*Darksidea delta* (98.5)	*Darksidea* sp.
MT645115	T23	*Diaporthe tuberivora* (100)	*Diaporthe* sp. A
MT645114	A64	*Diaporthe hongkongensis* (96.6)	*Diaporthe* sp. B
MT645118	T41	*Exophiala tremulae* (98.3)	*Exophiala* sp.
MT645119	A66	*Fusarium spp.* (99.8)	*Fusarium* sp. A
MT645120	T2	*Fusarium circinatum*(98.8)	*Fusarium* sp. B
MT645121	A61	*Fusarium dlaminii* (100)	*Fusarium* sp. C
MT645139	B15	*Geomyces destructans* (98.1)	*Geomyces* sp.
MT645123	A26	*Leptobacillium leptobactrum* (98.2)	*Leptobacillium* sp.
MT645126	A8	*Metarrhizium carneum* (99.3)	*Metarrhizium* sp.
MT645127	C16	*Microdochium trichocladiopsis* (100)	*Microdochium* sp.
MT645128	A15	*Mortierella parvispora* (94.7)	*Mortierella* sp.
MT645131	A29	*Paraconiothyrium estuarinum* (97.9)	*Paraconiothyrium* sp.
MT645132	B12	*Parasarocladium radiatum* (97.0)	*Parasarocladium* sp.
MT645117	A39	*Parastagonospora novozelandica* (96.1)	*Parastagonospora sp.*
MT645133	T54	*Penicillium rubefaciens* (99.6)	*Penicillium* sp. A
MT645134	T81	*Penicillium cremeogriseum* (100)	*Penicillium* sp. B
MT645135	A46	*Penicillium jugoslavicum* (95.1)	*Penicillium* sp. C
MT645136	T63	*Penicillium nodositatum* (99.7)	*Penicillium sp.* D
MT645906	T21	*Penicillium* spp. (100)	*Penicillium sp.* E
MT645141	T46,	*Pseudophialophora eragrostis* (96.7)	*Pseudophialophora* sp.
MT645142	T78	*Rhizopus oryzae* (99.8)	*Rhizopus* sp.
MT645143	T35	*Sarocladium kiliense* (99.8)	*Sarocladium* sp. A
MT645144	B17	*Sarocladium strictum* (99.0)	*Sarocladium* sp. B
MT645145	B21	*Talaromyces atricola* (98.4)	*Talaromyces* sp.
MT645147	T8	*Trichoderma caribbaeum* (99.6)	*Trichoderma* sp. A
MT645146	A78	*Trichoderma* spp. (98.4)	*Trichoderma* sp. B
MT645129	A133	*Paecilomyces hepiali* (100)	*Paecilomyces* sp.
MT645130	A143	*Paraconiothyrium thysanolaenae* (94.5)	unknown Dothideomycetes sp. A
MT645137	T10	*Periconia epilithographicola* (92.8)	unknown Dothideomycetes sp. B
MT645116	T1	*Pyrenopora novozelandica* (90.5)	unknown Dothideomycetes sp. C
MT645154	B19	Not found	unknown Dothideomycetes sp. D
MT645124	T11B	Loramyces *macrosporus* (92.1)	unknown Leotiomycetes sp. A
MT645149	T44	*Lachnellula hyalina* (86.3)	unknown Leotiomycetes sp. B
MT645150	A17	*Chrysosporium filiforme* (89.5)	unknown Leotiomycetes sp. C
MT645122	T14	*Hymenoscyphus ohakune* (91.4)	unknown Leotiomycetes sp. D
MT645138	T16	*Neomollisis gelatinosa* (94.7)	unknown Leotyomycete sp. E
MT645156	T26	*Chrysosporium filiforme* (89.6)	unknown Leotiomycetes sp. F
MT645106	T15	*Chalara hyalocuspica* (93.0)	unknown Leotiomycetes sp. G
MT645140	A28	*Pseudophialopora eragrostis* (94.4)	unknown Sordariomycetes sp. A
MT645103	B30	*Anthostomelloides leucospermi* (90.8)	unknown Sordariomycetes sp. B
MT645108	A41	*Conioscypha verrucosa* (91.5)	unknown Sordariomycetes sp. C
MT645110	T34	*Coniochaeta gigantospora* (91.0)	unknown Sordariomycetes sp. D

MT645157	A110	*Funiliomyces biseptatus* (92.1)	unknown Sordariomycetes sp. E
MT645152	B7	*Dactylaria acacia* (88.7)	unknown Sordariomycetes sp. F
MT645153	C10	*Clitocybula albida* (83.5)	unknown Basidiomycota sp. A
MT645125	T40	*Ganoderma sandunense* (89.2)	unknown Basidiomycota sp. B
MT645111	T30	*Crinipellis malesiana* (90.7)	unknown Basidiomycota sp. C
MT645155	C6	Not found	unknown Basidiomycota sp. D
MT645148	T29	*Umbelopsis dimorpha* (91.4)	unknown Zygomycota

Table 3. First inoculation screening: Effect of fungal inoculation of tritordeum plants on dry weight (DW) of leaves and roots, mineral content and total antioxidant capacity (TEAC), expressed as percent of variation respect to controls without inoculation; >8%; between -8% and 8%; <-8%. The strains selected for the second screening are marked with an asterisk.

Taxon (strain)	DW leaf	DW root	P	K	Ca	Mg	S	Cu	Fe	Mn	Zn	TEAC	Cluster PCA #
Diaporthe A (T6)*													A
Pseudophialophora (T72)													A
Collembollispora (T24)*													A
Darksidea (T64)													A
Penicillium B (T53)													A
Coniochaeta A (T5)*													A
Chaetomium (A76)													A
Diaporthe A (T61)*													A
Zygomycete (T29)*													A
Basidiomycete B (T62)													A
Alternaria A (A60)*													A
Basidiomycete B (T40)*													A
Alternaria A (T7)*													B
Leotiomycetes C (T20)													B
Trichoderma A (A14)													B
Trichoderma A (A3)													B
Zygomycete (T80)*													B
Leotiomycetes B (A2)*													B
Diaporthe A (A38)													B
Diaporthe A (T23)													B
Pseudophialophora (T42)													B
Mortierella (A15)													B
Penicillium D (T70)													B
Leotiomycetes D (T68)													B
Trichoderma A (A24)													C
Metarrhizium (A8)													C
Coniochaeta (T34)													C
Leptobacillium (A26)													C
Alternaria A (A50)													C
Paraconyothyrium (A29)													C
Leotiomycetes E (T43)													C
Pseudophialophora (A28)													C
Pseudophialophora (T85)													C
Alternaria A (T73)													C
Parastagonospora (A39)													C
Fusarium C (A33)													C
Leotiomycetes B (A1)													C
Pseudophialophora (A23)													C
Leotiomycetes C (A18)													C
Leotiomycetes C (A12)													C
Leotiomycetes E (T16)													D
Chaetosphaeria (A37)													D
Collembolispora (T17)*													D
Pseudophialophora (T66)													D
Alternaria B (T4)													D
Pseudophialophora (A40)													D
Exophiala (T41)													D
Sarocladium A(T35)													D
Leotiomycetes G (T25)													D
Diaporthe A (T56)*													D
Dothideomycetes (T10)*													D
Darksidea (T32)													D
Leotiomycetes C (A19)													D
Sordariomycetes C (A41)													D
Paraconyothirium (T33)*													D
Leotiomycetes F (T26)													D
Pseudophialophora (T77)													D
Absidia sp. (T48)													D
Pseudophialophora (T74)													D
Alternaria A (T3)													D
Leotiomycetes G (T15)													D
Pseudophialophora (T50)													D
Leotiomycetes C (A16)													D
Penicillium E (T21)													D
Leotiomycetes D (T14)													D
Leotiomycetes C (A10)													D

see PCA Figure 3

Table 4. Second inoculation screening: Effect of fungal inoculation of tritordeum plants on dry weight (DW) of leaves and roots, mineral content and total antioxidant capacity (TEAC), expressed as percent of variation respect to controls without inoculation. >8%; between -8% and 8%; <-8%. The strains are listed in decreasing order with respect to the increase in biomass as compared to controls.

Taxon (strain)	DW leaves	DW root	P	K	Ca	Mg	S	Cu	Fe	Mn	Zn	TEAC	Cluster PCA #
Diaporthe (T6)													A
Zygomycota (T80)													C
Basidiomycota B (T40)													B
Dothideomycete B (T10)													B
Paraconyothirium (T33)													B
Alternaria A (A60)													C
Collembolispora (T17)													C
Diaporthe A (T56)													B
Collembollispora (T24)													B
Diaporthe A (T61)													D
Coniochaeta (T5)													D
Zygomycota (T29)													D
Alternaria A (T7)													D

see PCA Figure 4

Figure 1. Maximum likelihood phylogenetic tree of fungal taxa isolated from roots of *Celtica gigantea* based on nucleotide sequences of the ITS1-5.8SrDNA-ITS2 region. Isolate numbers corresponding to descriptions from Table 1 are shown in parentheses. Bootstrap values indicated at nodes.

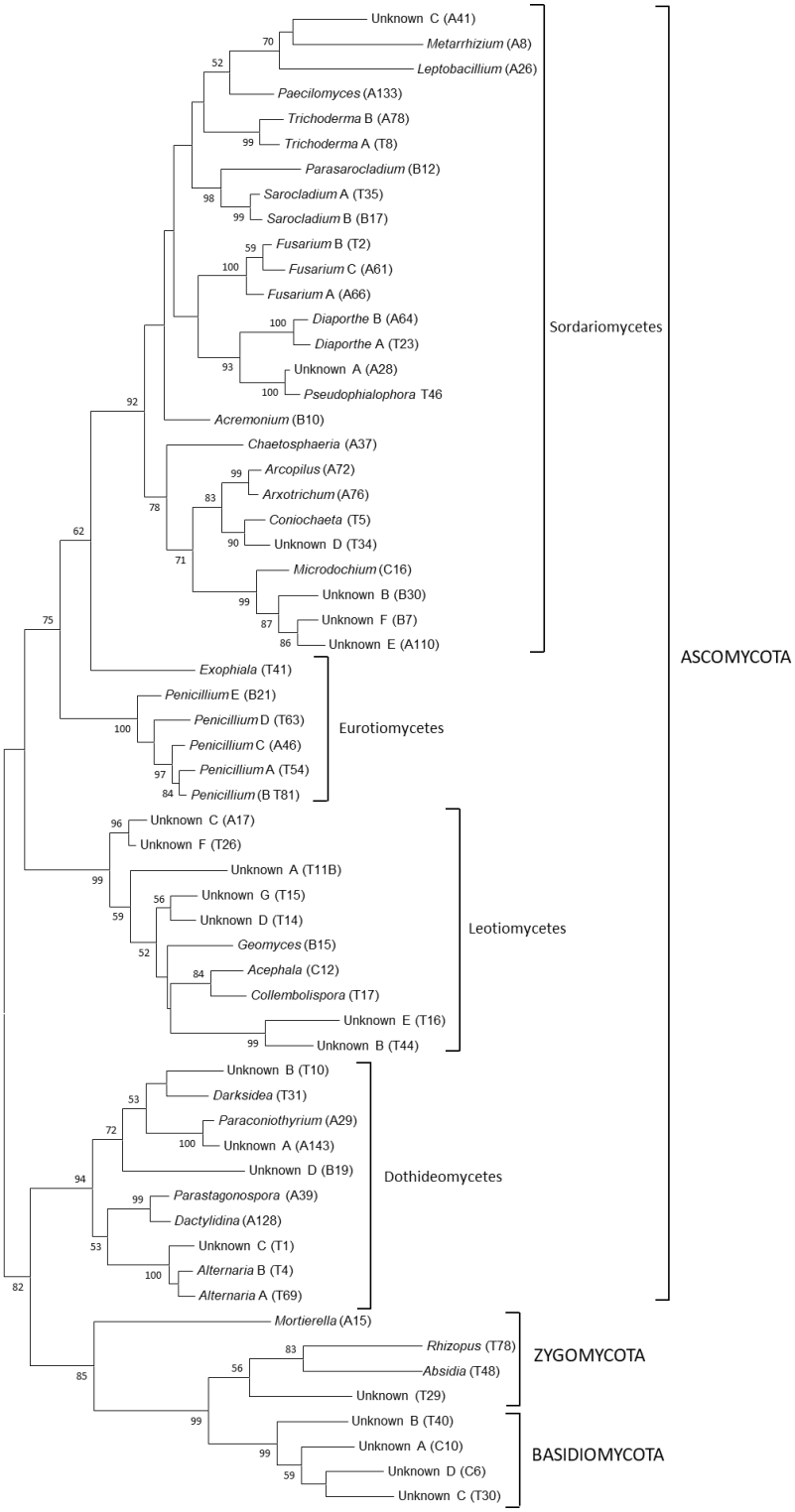


Figure 2. Change in leaf and root weight respect to an uninoculated control observed in plants of tritordeum inoculated with 66 fungal strains from *Celtica gigantea* roots. Strains causing important increments or losses of leaf and root biomass are labeled (See Table 1 for strain identification).

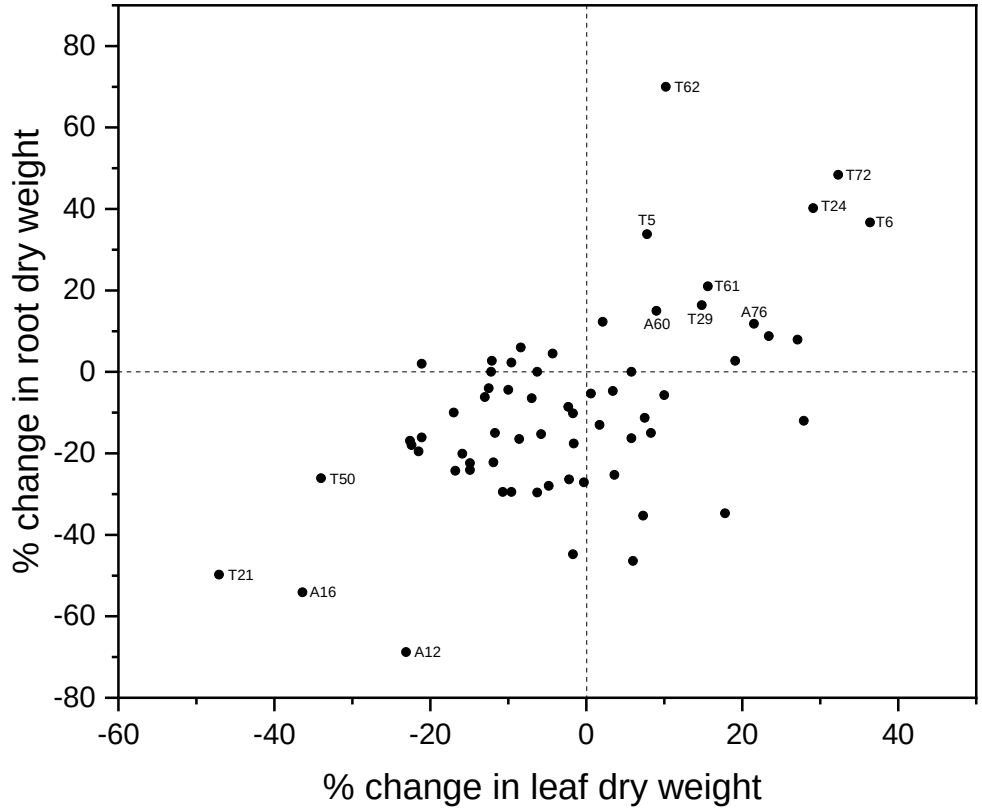


Figure 3. Principal component analysis of the effect of inoculation treatments of the first screening on biomass (DMroot, DMleaves), nutrient content and trolox equivalent antioxidant capacity (TEAC) of tritordeum plants: Dispersion of samples on the plane defined by principal components I and II were gathered in four clusters. Identification of inoculated strains in each cluster is provided in Table 2.

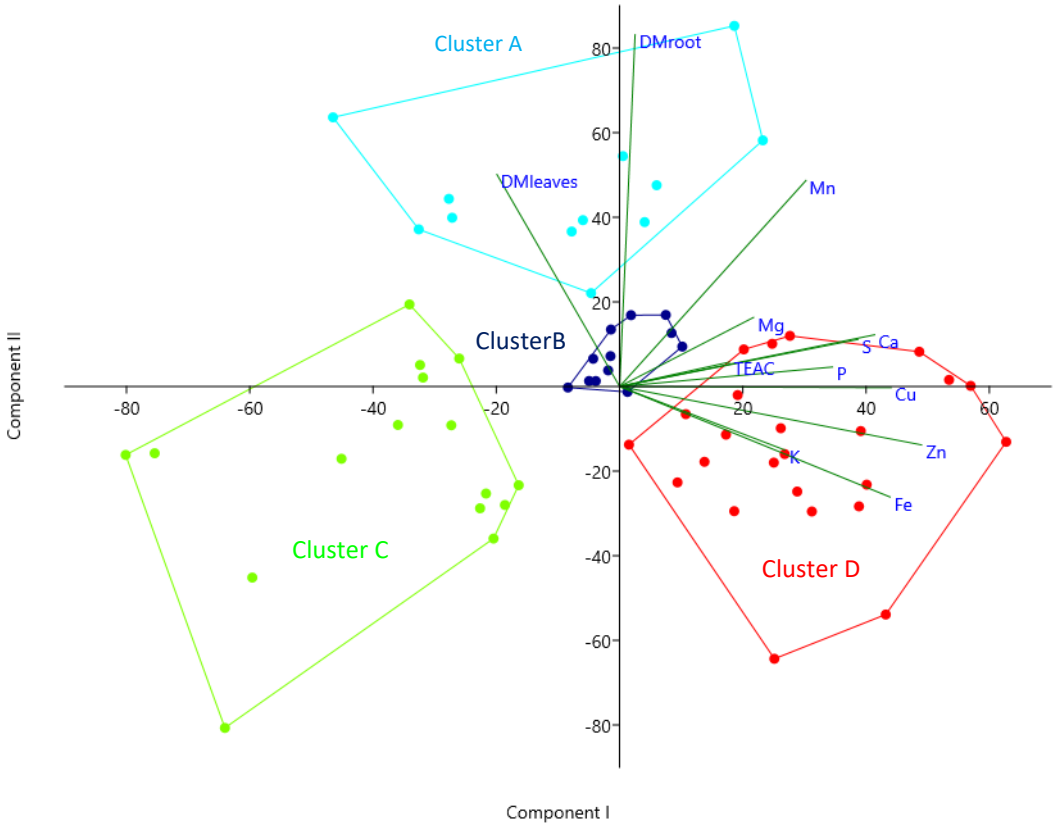
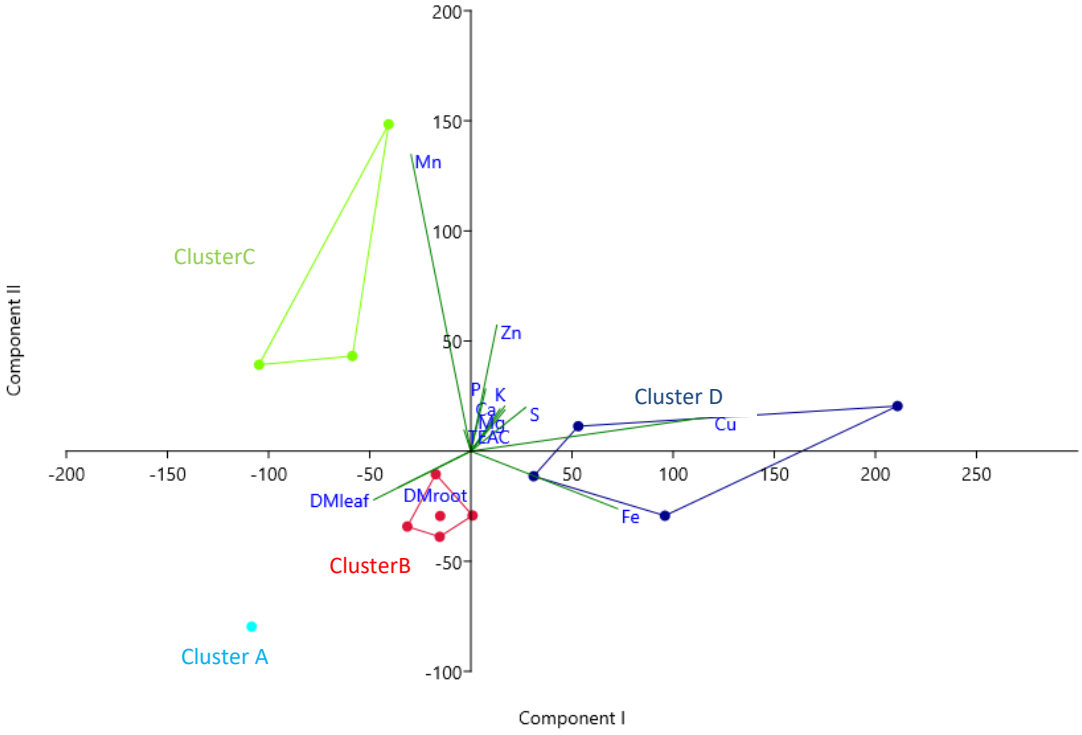


Figure 4. Principal component analysis (PCA) of the effect of inoculation treatments of second screening (beet pulp inoculation method) on biomass (DMroot, DMleaf), nutrient content and trolox equivalent antioxidant capacity (TEAC) of tritordeum plants: Dispersion of samples on the plane defined by principal components I and II were gathered in four clusters.



APPENDIX A

Table A1. First inoculation screening: Effect of fungal inoculation of tritordeum plants on dry weight (DW) of leaves and roots, mineral content and total antioxidant capacity (TEAC), expressed as percent of variation respect to uninoculated controls.

Taxon (strain)	DM leaf	DM Root	P	K	Ca	Mg	S	Cu	Fe	Mn	Zn	TEAC
Diaporthe sp. A (T6)	36.4	36.7	19.2	6.5	7.4	17.3	23.9	-6.5	10.5	5.3	4.6	3.8
Pseudophialophora sp. (T72)	32.3	48.4	5.8	11.5	5.3	13.1	8.1	-7.1	0.0	56.0	-3.0	-7.6
Collembollispora (T24)	29.1	40.2	-1.4	3.0	-9.9	-2.4	-3.0	25.2	-4.6	9.3	-0.4	-19.3
Darksidea (T64)	27.9	-12.0	8.6	-1.1	13.7	5.3	-7.8	-2.7	-0.9	7.5	-18.3	-1.9
Penicillium sp. B (T53)	27.1	7.9	-9.7	-9.0	-2.5	7.6	-12.0	68.2	-24.2	11.6	-5.2	-18.6
Coniochaeta A (T5)	23.4	8.8	-5.5	-11.5	-5.3	8.3	-3.0	5.2	-2.3	19.1	-12.2	2.0
Chaetomium (A76)	21.5	11.8	-7.2	-4.8	-0.3	-1.4	-12.8	-14.2	-27.0	1.1	-21.0	4.1
Diaporthe A (T61)	15.6	21.0	-12.6	-13.4	7.9	1.3	-6.9	-15.9	-14.6	-2.2	-22.7	3.7
Zygomycete sp. (T29)	14.8	16.4	-0.7	0.0	1.1	-4.8	-5.5	13.2	-4.6	42.7	-9.0	-13.7
Basidiomycete (T62)	10.2	70.0	-24.2	-13.7	-18.2	-12.1	-14.2	-13.0	-16.2	-11.6	-26.0	2.3
Alternaria sp. A (A60)	9.0	15.0	11.3	0.9	28.3	22.0	0.3	-19.1	-12.6	-4.5	-30.1	-5.2
Basidiomycete (T40)	7.8	33.8	-8.5	-11.7	2.2	-3.3	-21.0	-22.7	-14.8	4.2	-11.0	3.0
Alternaria sp. A (T7)	19.1	2.7	5.3	6.7	0.4	-1.8	2.1	14.7	0.0	-8.0	3.5	-0.9
Lachnum (T20)	17.8	-34.7	-3.7	-7.3	-1.2	-11.5	5.5	8.4	-2.0	13.3	-12.2	3.0
Trichoderma A (A14)	10.0	-5.7	-5.2	5.2	-25.7	-22.3	-7.7	7.9	-5.8	19.4	41.3	17.6
Trichoderma A (A3)	8.3	-15.0	3.7	8.0	-9.0	-6.0	0.5	-15.1	4.4	-2.5	11.5	12.1
Zygomycete (T80)	2.1	12.3	0.9	-8.2	-7.6	-7.2	4.0	-2.0	1.0	-5.1	12.9	2.0
Leotiomycetes (A2)	1.7	-13.0	0.6	1.0	-19.8	-15.5	22.7	-5.6	-5.5	3.6	0.0	11.1
Diaporthe sp. A (A38)	0.6	-5.3	-5.2	-1.4	-4.7	-4.2	-2.2	-6.4	10.0	-7.3	-2.5	-2.1
Diaporthe sp. A (T23)	-4.3	4.5	-13.0	1.2	-10.1	-6.1	-2.2	5.9	10.1	-4.6	-7.7	0.5
Pseudophialophora sp. (T42)	-6.3	0.0	-8.8	-2.4	-7.1	-2.3	-15.8	8.6	8.6	-14.1	-10.7	8.7
Zygomycete (A15)	-7.0	-6.5	-4.6	11.1	34.3	19.7	0.2	-20.3	-4.5	-10.0	-20.2	-9.3
Penicillium sp.D (T70)	-12.1	2.7	-3.9	2.9	-3.7	-5.5	8.5	-1.2	7.4	15.0	-6.3	3.0
Leotiomycetes (T68)	-12.2	0.0	8.3	-2.0	4.7	0.2	-3.2	-11.0	2.9	10.0	-22.2	9.3
Trichoderma A (A24)	7.3	-35.3	-32.1	-14.7	-34.5	-17.0	-37.8	-16.3	-27.2	-6.6	-28.2	-60.7
Metarrhizium (A8)	5.8	-16.3	-24.5	-17.3	-32.4	-20.6	-29.8	-20.8	-15.0	-41.3	-35.0	-4.5
Coniochaeta (T34)	5.8	0.0	-16.3	-13.3	-9.0	-16.4	-4.1	-7.3	-15.9	2.5	-28.8	3.0
Leptobacillium (A26)	3.6	-25.3	-21.5	-10.1	3.6	-9.4	-6.1	-6.2	-14.2	-10.1	-1.8	-39.5
Alternaria sp. A (A50)	3.4	-4.7	0.1	-2.2	1.6	3.5	-9.3	-20.8	-16.0	-25.0	-23.9	-7.0
Paraconyothyrium sp. (A29)	-0.3	-27.1	-13.5	-4.7	-25.2	-11.3	-25.6	-1.7	-11.1	-15.1	-23.4	-22.8
Leotiomycetes (T43)	-1.6	-17.6	-15.5	-12.2	-5.5	-11.2	3.0	-9.3	-15.7	-4.5	-29.6	-3.0
Pseudophialophora sp. (A28)	-1.7	-44.8	-16.0	1.9	-20.0	-14.4	-18.4	-23.9	-9.8	-52.7	-30.0	-11.6
Pseudophialophora sp. (T85)	-1.7	-10.2	-4.5	-3.1	1.8	-1.9	-12.6	-13.6	-20.8	-7.2	-20.3	6.9
Alternaria sp. A (T73)	-2.2	-26.4	-10.8	-0.1	-23.1	-16.1	-18.1	-13.7	0.8	-24.9	22.0	12.5
Dydimocyrtis sp. (A39)	-5.8	-15.3	-7.6	3.6	-21.5	-7.9	-11.9	-17.4	-10.4	-12.8	-18.7	-17.0
Fusarium sp. C (A33)	-6.3	-29.6	-10.5	-1.0	-2.8	1.2	-12.9	-12.1	-4.1	-20.4	6.6	-12.9
Leotiomycetes (A1)	-9.6	-29.5	-14.3	-1.7	-31.8	-21.9	-18.4	-8.6	-0.3	-24.8	27.7	4.0
Pseudophialophora sp. (A23)	-11.9	-22.2	-12.9	4.8	-10.6	0.0	-12.8	-7.6	5.2	-35.1	-8.4	-13.6

Taxon (strain)	DM leaf	DM Root	P	K	Ca	Mg	S	Cu	Fe	Mn	Zn	TEAC
Leotiomycetes sp. C (A18)	-21.5	-19.5	-1.9	7.0	-9.8	-7.2	-7.7	-13.7	-1.1	-30.3	-20.5	8.4
Leotiomycetes sp. C (A12)	-23.1	-68.8	-19.1	2.5	-41.4	-34.7	-35.7	-23.8	8.3	-45.3	-23.4	-19.5
Leotiomycetes (T16)	7.5	-11.3	6.2	-2.9	9.5	0.9	4.8	8.1	-3.7	4.8	23.4	2.0
Sordariomycetes sp. F (A37)	6.0	-46.4	-3.1	-11.0	27.1	8.7	11.1	0.9	2.8	-6.0	9.5	-11.8
Collembolispora sp. (T17)	-2.3	-8.6	1.4	0.8	-1.5	6.2	8.8	0.3	1.0	24.7	32.7	2.0
Pseudophialophora sp. (T66)	-4.8	-28.0	10.4	10.7	16.0	4.0	13.3	0.3	17.6	-1.8	-16.5	-6.4
Alternaria sp. B (T4)	-8.4	6.0	28.5	29.6	24.2	19.9	23.9	16.5	12.5	-11.8	5.0	-5.3
Pseudophialophora sp.(A40)	-8.6	-16.5	9.7	-6.1	36.7	13.4	18.7	10.0	36.0	20.4	18.2	-23.2
Exophiala sp. (T41)	-9.6	2.3	-12.6	6.8	1.5	-4.5	-11.6	7.7	27.7	-16.4	2.0	20.7
Sarocladium sp. A (T35)	-10.0	-4.4	13.6	19.9	-6.3	-10.1	-3.8	7.6	22.0	-43.6	-1.8	20.8
Leotiomycetes (T25)	-10.7	-29.5	5.2	5.9	5.7	0.3	11.0	11.4	12.9	-2.2	8.6	4.3
Diaporthe sp. A (T56)	-11.7	-15.0	-8.0	6.7	19.6	27.9	23.1	7.0	19.5	-19.7	-3.4	-2.0
Dothideomycetes (T10)	-12.5	-4.0	11.1	18.6	10.6	6.5	-2.4	8.9	0.4	10.3	-8.8	40.1
Darksidea sp. (T32)	-13.0	-6.2	6.8	17.5	7.1	-4.5	-0.3	12.7	4.1	-1.1	1.1	4.0
Leotiomycetes sp. C (A19)	-14.9	-22.4	-1.3	-14.8	11.7	1.4	24.1	37.5	-3.4	30.3	59.2	-11.8
Sordariomycetes sp. C (A41)	-14.9	-24.1	1.4	-12.6	1.9	-3.1	-3.1	-0.5	-13.9	-4.0	24.4	-20.5
Paraconyothirium (T33)	-15.9	-20.1	5.5	12.9	-4.8	-4.1	-12.3	26.1	46.5	-13.9	4.1	19.7
Leotiomycetes sp. F (T26)	-16.8	-24.3	1.7	12.1	-2.2	-3.3	1.7	22.3	10.9	-25.3	10.7	6.6
Pseudophialophora sp. (T77)	-17.0	-10.0	32.1	28.5	19.1	-0.9	17.9	30.1	32.2	-6.3	13.3	9.8
Absidia sp. (T48)	-21.1	-16.1	8.3	12.6	-8.3	-6.4	-9.0	7.6	17.8	10.8	25.9	-2.0
Pseudophialophora sp. (T74)	-21.1	2.0	16.7	15.4	25.0	4.3	6.5	21.3	38.7	-20.5	-8.5	-6.2
Alternaria sp. A (T3)	-22.4	-18.0	-8.1	10.2	-5.1	-9.1	-3.2	9.8	33.0	0.1	50.4	2.0
Leotiomycetes sp. G (T15)	-22.6	-16.9	6.6	23.3	17.6	-3.0	9.1	23.4	27.3	-16.7	0.3	21.3
Pseudophialophora sp. (T50)	-34.0	-26.1	1.7	27.7	12.0	-0.1	-7.7	13.6	23.1	10.8	-8.8	-1.8
Leotiomycetes sp. C (A16)	-36.4	-54.1	1.6	19.3	8.2	3.0	5.0	5.1	24.7	-27.7	17.7	-24.8
Penicillium sp. D (T21)	-47.1	-49.8	6.6	33.1	14.0	-2.8	-3.1	26.5	23.3	1.2	6.7	-7.1
Leotiomycetes sp. D (T14)	-49.9	2.0	48.3	164.3	34.6	11.0	42.9	100.2	5.9	-41.3	42.9	-4.0
Leotiomycetes sp. C (A10)	-76.4	-75.8	169.1	136.1	125.9	2.3	210.2	44.5	496.6	150.8	63.3	-14.3

Table A2. Second inoculation screening: Effect of fungal inoculation of tritordeum plants on dry weight of leaves and roots, mineral content and total antioxidant capacity (TEAC), expressed as percent of variation respect to uninoculated controls.

Taxon (strain)	DW leaf	DW root	P	K	Ca	Mg	S	Cu	Fe	Mn	Zn	TEAC
Diaporthe (T6)	41.3	11.3	-2.7	-6.4	25.8	14.6	23.6	-23.6	-37.3	-51.0	-0.3	-15.0
Zygomycota (T80)	41.0	-14.0	16.7	17.8	21.7	26.5	96.8	-12.0	-54.1	58.2	8.8	-5.2
Basidiomycota B (T40)	33.9	3.7	24.1	39.0	50.4	25.2	153.5	22.3	14.2	-39.1	32.6	-20.0
Dothideomycete B (T10)	16.4	7.6	25.3	32.4	41.5	20.9	67.8	17.7	15.3	-33.4	34.5	0.3
Paraconyothirium (T33)	14.5	0.0	30.9	23.9	32.9	24.2	56.7	28.3	36.2	-10.1	55.5	1.3
Alternaria A (A60)	-6.6	-8.1	44.2	39.0	42.4	39.8	63.5	10.5	-32.3	38.3	31.8	0.5
Collembolispora (T17)	-17.2	-32.8	53.3	46.3	63.4	44.7	96.4	18.5	-27.4	115.6	106.9	-8.5
Diaporthe A (T56)	-19.3	-30.4	35.0	40.1	12.3	6.5	46.0	13.1	27.3	-39.4	34.0	-10.8
Collembollispora (T24)	-19.6	-37.8	32.7	30.4	68.0	54.8	105.2	15.9	10.1	-48.7	24.2	-26.6
Diaporthe A (T61)	-26.4	-37.8	48.5	48.6	75.7	48.0	89.9	51.5	65.5	-23.1	74.8	8.5
Coniochaeta (T5)	-27.2	-61.2	16.7	37.7	34.3	41.3	119.8	114.4	44.3	-52.5	26.9	-21.5
Zygomycota (T29)	-33.2	-57.7	44.7	32.9	65.7	54.4	130.4	31.6	25.2	-38.8	26.9	-22.6
Alternaria A (T7)	-58.9	-57.1	32.4	46.7	54.1	52.2	98.9	232.4	95.0	-16.8	46.3	-11.4