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SOIL & CROP SCIENCES | RESEARCH ARTICLE

Endophytes from the crop wild relative *Hordeum secalinum* L. improve agronomic traits in unstressed and salt-stressed barley

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Abstract: Agricultural crops growing in saline soils produce sub-optimal yields due to the negative effects of excess salts on plant growth and development. Salinity stress for crops is likely to increase as a result of climate change and the consequent salinisation of soils, particularly in coastal areas. In this study, we recovered fungal endophytes from the seeds of the wild barley relative *Hordeum secalinum* and tested their effects on a barley cultivar grown in a controlled environment under four levels of continuously applied salt stress: 0, 75, 150 and 250 mM NaCl. For the unstressed plants and plants stressed with 75 mM NaCl we found an endophyte-associated increase in early growth, grain dry weight, shoot dry weight, root dry weight, number of tillers, number of heads and number of grains. However, this relationship was reversed for the plants grown with 150 and 250 mM NaCl, where the endophytes negatively affected these traits. Plants stressed with the highest concentration of NaCl (250 mM) produced no grain. These results suggest that the endophytes may have potential to benefit field grown barley crops growing on moderately salt-stressed sites.

Subjects: Agriculture & Environmental Sciences; Botany; Plant & Animal Ecology

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Brian R. Murphy is a Research Fellow in the Botany Department at Trinity College Dublin, Ireland. From an Irish perspective, he is largely responsible for the exciting research and associated publications relating to fungal endophyte application in agriculture. He is recognised as a leading expert in endophyte discovery from wild relatives of crops and their application to increasing stress resistance in cereal crops.

Trevor R. Hodkinson is a Professor in Botany and a Head of the Botany Molecular Laboratory, Trinity College Dublin, Ireland. His research is mainly focussed in fields known endophyte biology, molecular systematics and genetic resource characterisation. He has specialist knowledge of the grass family and of forest tree genetics/mycorrhizas.

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PUBLIC INTEREST STATEMENT

The crop plants that feed the world are under increasing threats from global climate change, with damaging events such as severe drought likely to become more frequent. For this study, we have taken a group of microorganisms from inside the seeds of wild crop relatives and used them to improve the performance of barley crops in salt-stressed growing conditions. We also found that using these microorganism allowed us to successfully grow barley in a salt-stressed environment which would be unsuitable for cultivation without the microorganism treatment. This is particularly significant, as changing growing conditions due to global climate change will result in more croplands becoming unsuitable for crops as a result of soil salinisation. The use of this innovative biological crop treatment in salt-stressed crop growing sites will be of great benefit both now and in the future. This type of alternative crop treatment is natural and environmentally friendly and has tremendous potential for agriculture.

| **Keywords:** barley; endophytes; *Hordeum secalinum*; salt stress; yield

1. Introduction

High levels of soil salinity can negatively impact agricultural production, water quality and terrestrial biodiversity (FAO, 2011; Paul & Lade, 2014; Queensland Government, 2016). According to the FAO Land and Plant Nutrition Management Service, more than 6% of the world's land surface is affected by either salinity or sodicity (FAO, 2011). A 20-year study of world soils estimated that globally the total area of saline soils was 397 million ha and that of sodic soils 434 million ha (FAO/UNESCO, 1974). Of the 230 million ha of irrigated land, 45 million ha (19.5%) were salt-affected soils; and of the almost 1500 million ha of dryland agriculture, 32 million (2.1%) were salt-affected soils. Since that ground-breaking study, other researchers have highlighted the impact and scale of saline soils. Oldeman, Hakkeling, and Sombroek (1991) estimated that the total area affected by salinity was over 76 million ha. They did not distinguish between irrigated and rainfed areas. Dregne, Kassas, and Rozanov (1991) estimated that about 43 million ha of irrigated land in drylands were affected by various processes of degradation, mainly waterlogging, salinisation and sodication. Umali (1993) estimated that 1–1.5 million ha were lost to salinisation every year. Ghassemi, Jakeman, and Nix (1995) estimated that salinisation of irrigated lands caused losses of annual income of about US\$ 12 billion globally. Nelson and Mareida (2001) estimated that about 12 million ha of irrigated land may have gone out of production as a result of salinisation. Data from FAO's database Aquastat show that in some countries the area affected by salinity can be as high of 50% of the areas fully equipped for irrigation (FAO, 2016). Soil salinity is predicted to become a larger problem in the coming decades as soil salinisation is reducing the area that can be used for agriculture by 1–2% every year, hitting hardest in arid and semi-arid regions (FAO, 2002). Other studies suggest that salinised areas are increasing at an even faster rate (Jamil, Riaz, Ashraf, & Foolad, 2011). Global warming is likely to increase seawater intrusion onto land, which is already a growing problem as sea levels rise in many parts of the world, and this can deposit a large amount of salts in soils of coastal lands (Rengasamay, 2010).

All soils contain salts, and all irrigation waters contain some dissolved salts. Hence, the process of soil salinisation is dramatically exacerbated and accelerated by crop irrigation. The overall effect of irrigation in the context of salinity is that it adds large quantities of new salts to the soil that were not there before. If the ground water is saline, which it commonly is in semi-arid environments, the surface can become saline owing to the salt concentrating as water evaporates. Other factors also contribute to the salinisation of soils, including low precipitation, high surface evaporation, weathering of native rocks, irrigation with saline water and poor cultural practices. It has been estimated that more than 50% of the arable land would be salinised by the year 2050 (Jamil et al., 2011).

The development of salt-tolerant crops has been a much desired scientific goal but has had relatively little success (Munns & Tester, 2008). Consequently, amelioration has focused on different methods, such as the movement of excess soluble salts from upper to lower soil depths via leaching, the surface flushing of salts from soils, the biological reduction of salts by harvest of high-salt accumulating aerial plant parts and amelioration of saline soils through cropping and leaching (Qadir, Ghafoor, & Murtaza, 2000). These cultural methods can be expensive and may take many years to become effective, so other biological means of alleviating salinity stress in crops could be more cost-effective over a shorter time scale.

Several studies have shown that plant-associated microorganisms, and particularly endophytes, are able to benefit plants growing under salt stress (Bagheri, Saadatmand, Niknam, Nejadstari, & Babaeizad, 2013; Khan et al., 2013; Qin, Druzhinina, Pan, & Yuan, 2016; Shrivastava & Kumar, 2015), but very few have focused specifically on endophytes recovered from a wild relative of the studied crop. Hammami et al. (2016) found that, contrary to expectations, the colonisation rate of roots by endophytes increased with soil salinity, indicating

that under salt stress the endophyte-plant association is promoted. Our own previous studies have shown significant benefits to agronomic traits of barley through the use of fungal endophytes recovered from a wild barley relative (summarised in Murphy, Doohan, & Hodkinson, 2018). But these studies focused on endophytes recovered from only one wild barley species growing on sites specifically targeted for nutrient and drought stress alleviation. Therefore, we hypothesised that endophytes recovered from the seeds of another wild relative of barley growing in a relatively moist coastal salt meadow could improve important agronomic traits in a barley cultivar growing under salt stress.

2. Materials and methods

Seeds from a mature population of *Hordeum secalinum* Schreb. were collected from five widely separated plants growing on a rural coastal marsh site in Ireland (location not given due to intellectual property protection issues). *Hordeum secalinum* is native to the Madeira Islands in Macaronesia, northern Africa and is widespread in Europe (USDA, 2014). It is found in moist, saline, mainly coastal areas or very scattered inland areas in saline or (rarely) freshwater habitats such as meadows and pastures. Environmental variables were recorded at the time of collection and included soil pH, soil salinity (measured as osmotic potential in bars), and soil moisture content. Seeds were de-husked and seeds and husks were surface-sterilised by immersing in 70% EtOH for one minute, placing in 5% NaClO for 5 min, immersing for another minute in 70% EtOH and then rinsing five times with sterile ultrapure water. Ten halved seeds and halved husks from each plant were transferred onto culture plates of potato dextrose agar (Oxoid CM0139) and incubated in the dark at 21°C for 28 days. The powdered medium was mixed to half-strength of the manufacturers' recommendations (to avoid osmotic shock to the endophytes) using ultrapure water, then sterilised by autoclaving. From previous experience, we considered 28 days to be sufficient time to allow recovery of the slowest emerging endophytes. Dishes were inspected daily and those containing seed or husk pieces with surface fungal growth were discarded (i.e. not emerging from the cut tissue area). Emergent endophytes were removed and subcultured on the same medium in the dark at 19°C for a further 14 days. From a total of 76 individual cultures recovered, we selected 14 for DNA sequencing and controlled environment trials based on our selection criteria (early sporulation and high spore yield at room temperature, 18–21°C).

For the DNA analysis, we used the hot CTAB DNA extraction method as detailed in Hodkinson et al. (2007) and performed PCR using the forward primer ITS1F (Gardes & Bruns, 1993) and the reverse primer ITS4 (White, Bruns, Lee, & Taylor, 1990). PCR products were cleaned up using the ExoSAP-IT™ PCR Product Cleanup Reagent (ThermoFisher Scientific) and final products were sequenced using the Sanger Sequencing Service offered by Source Biosciences.

DNA sequences were compared with those in NCBI GenBank (National Centre for Biotechnology Information) and UNITE (Unified system for the DNA based fungal species linked to the classification, <https://unite.ut.ee>) accessions using the Basic Local Alignment Search Tool (nBLAST) for identification. Similarity criteria for assigning taxonomic rank to the endophyte strains was allocated based on an initial survey of existing fungal taxa in UNITE and GenBank, as follows: >97% similarity was assigned to the same species, 90–96% to the same genus, 85–90% to the same order and <85% to no significant match. In all cases, genetic identity assignment was confirmed or further assessed by examination of morphological characters of the fungi using light microscopy and by referencing the taxonomic descriptions found in Cannon and Kirk (2007).

Individual endophyte inoculants were prepared by washing mature culture plates of the endophyte strains with 10 ml ultrapure water to dislodge the spores. The spore solution was transferred to individual 50 ml plastic tubes and further diluted with ultrapure water to give a final concentration of 1×10^6 spores/ml (measured with a haemocytometer). For the inoculant treatment of all 14 endophyte strains, we combined equal aliquots from each of the individual spore solution preparations.

Seeds of the barley cultivar Planet (Goldcrop Ltd., Cork, Ireland) were soaked in warm water for 3 h. Three seeds per pot were sown into John Innes No. 2 compost (Westland Garden Health) at 20 mm depth into three litre plastic pots. Seven pots per treatment were sown and the pots were labelled with a reference number (reference numbers were anonymous to the plant grower). Seeds were inoculated with either 200 μ l (~200,000 spores/seed) of the endophyte inoculant or 200 μ l of ultrapure water for the controls, and the seeds covered with the compost. Pots were randomly distributed in three blocks in controlled environment growth chambers and repositioned every week during the experimental period. The environmental settings of the growth cabinets (Conviron PGR14) were programmed to produce a 16 h photoperiod at a compost surface illumination of 220 μ mol $m^{-2} s^{-1}$, a photoperiod temperature of 20°C reducing to 10°C in the dark period and a constant 65% relative humidity. The plants were given no supplemental nutrients during the experimental period.

Four concentrations of salt treatments, as a dissolved volume of NaCl in 500 ml of water per pot, were applied to the growing barley: 0, 75, 150 and 250 mM NaCl. Salt treatments were applied five times during the experimental period starting at Zadok's growth stage 11 (GS11) when the first leaf was fully unfolded (Zadoks, Chang, & Konzak, 1974), approximately 9 days after seed sowing, and thereafter at two-weekly intervals. The fifth and final salt treatment was applied at GS45-GS47 (booting to flag leaf sheath opening). The total volume of NaCl applied to each plant was 3.66 g for the 75 mM treatment, 7.33 g for the 150 mM treatment and 12.25 g for the 250 mM treatment. All plants were watered as necessary during the experimental period.

The barley plants were harvested after Zadok's growth stage 89 was reached (maturity) at day 100 from sowing. The number of tillers, number of heads and number of grains per pot were recorded. After oven drying in paper bags at 65°C for one week, the grain and shoot dry weight were measured. The pots were dried at room temperature for 1 month before the root dry weight was calculated.

We tested for the presence of the endophytes in seed tissue of the mature plants. Twenty seeds from each treatment were surface sterilised (as above) then aseptically split in half. The prepared seed halves were plated out onto half-strength PDA, with cut side in contact with the agar, and incubated at 19°C.

To test the salinity tolerance of the individual endophyte strains, we inoculated, with single spores, half-strength PDA medium with dissolved salt at the same NaCl concentrations used for the plant growth experiment (0, 75, 150 and 250 mM), replicated three times for each strain, on 90 mm agar plates. Culture plate coverage and number of colonies formed were assessed after 21 days incubation at 19°C. Plate coverage was assessed by recording colony radial growth from a single spore placed in the centre of the Petri dish.

Data analysis was carried out using single factor ANOVA with Bonferroni correction and Pearson's Product Moment correlation statistical analyses supplied with the Data Analysis module within Microsoft Excel© and Datadesk 7.01. In addition, overall totals for each measured trait and treatment were calculated and compared.

3. Results

The site from which *H. secalinum* seeds were collected had a relatively high soil moisture content of 32.2%, with a soil salinity of 1.46 bars (4.05 dS/m) and a soil pH of 7.1.

The 14 endophyte isolates were compared with known GenBank and UNITE database accessions, revealing close matches (98–99% pairwise similarity) with four different species of *Penicillium* (Table 1). Sequence length ranged from 558 to 567 base pairs. The isolate sequences were deposited in NCBI GenBank under accession numbers MH705343–MH705356.

We found a general trend, in the measured agronomic traits, of an endophyte-associated benefit for the unstressed plants and the plants with 75 mM salt stress. However, this relationship was

Table 1. Identity of experimental fungal endophyte strains inferred from GenBank and UNITE database searches using the ITS gene

Endophyte strain ID	GenBank accession	Nearest BLAST Match	% Pairwise similarity
TCDHs16H2	MH705343	<i>Penicillium purpurogenum</i>	99
TCDHs16H5	MH705344	<i>Penicillium crustosum</i>	99
TCDHs16H15	MH705345	<i>Penicillium olsonii</i>	99
TCDHs16H16	MH705346	<i>Penicillium oxalicum</i>	99
TCDHs16H19	MH705347	<i>Penicillium oxalicum</i>	99
TCDHs16H21	MH705348	<i>Penicillium olsonii</i>	99
TCDHs16H25	MH705349	<i>Penicillium oxalicum</i>	99
TCDHs16H35	MH705350	<i>Penicillium olsonii</i>	99
TCDHs16H40	MH705351	<i>Penicillium olsonii</i>	99
TCDHs16H41	MH705352	<i>Penicillium oxalicum</i>	99
TCDHs16S6	MH705353	<i>Penicillium purpurogenum</i>	99
TCDHs16S10	MH705354	<i>Penicillium purpurogenum</i>	99
TCDHs16S16	MH705355	<i>Penicillium purpurogenum</i>	99
TCDHs16S32	MH705356	<i>Penicillium purpurogenum</i>	98

reversed for the higher salt stresses of 150 and 250 mM. This was true for all measured traits (number of tillers, number of grains, grain dry weight, shoot dry weight and root dry weight). Overall totals for each trait most clearly show this relationship (Table 2).

Due to the large variability in the mean values and the relatively high standard errors in some means there was less significance found in this statistic, though the general trend is still apparent (Table 3). For the compared means, root and shoot dry weights were both significantly greater for the endophyte inoculated plants with zero salt stress ($p < 0.05$). The differences in means is best illustrated by presenting the percentage differences in each trait (the effect size) due to endophyte inoculation (Figure 1).

The greatest increase in endophyte-associated mean grain dry weight was found in the plants stressed with 75 mM NaCl, where the overall yield for this set was 39% greater than the control. This compares with an increase of 7% in grain dry weight for the unstressed plants, and a decrease of 65% for the plants stressed with 150 mM NaCl. Salt stress of 250 mM resulted in no grains being produced and extremely low values for the other traits.

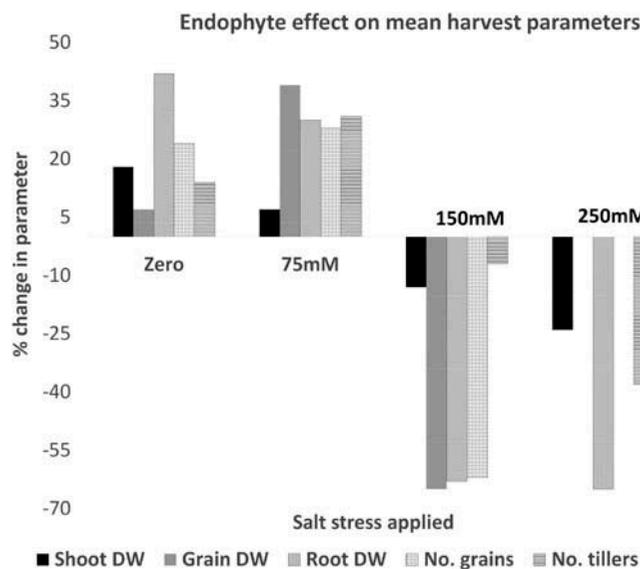
Table 2. Barley harvest parameter totals; figures are totals for seven pots of three plants for each salt stress treatment

NaCl salt stress applied		Number of tillers	Number of grains	Shoot dry weight (g)	Root dry weight (g)	Grain dry weight (g)
Zero	Endophyte	334	2251	236.7	24.5	125.9
	Control	293	1827	200.1	17.2	117.6
75 mM	Endophyte	514	545	145.9	10.0	35.4
	Control	394	429	136.1	7.7	25.3
150 mM	Endophyte	186	92	46.5	2.7	5.6
	Control	204	144	52.4	4.2	9.4
250 mM	Endophyte	59	0	17.7	1.0	0
	Control	77	0	21.9	1.6	0

Table 3. Barley harvest parameter means ± S.E.; figures are means for seven pots of three plants for each salt stress treatment. Figures in bold and marked * are significantly different between the control and endophyte treatments ($p \leq 0.05$) with 95% confidence

NaCl salt stress applied		Number of tillers	Number of grains	Shoot dry weight g	Root dry weight g	Grain dry weight g
Zero	Endophyte	47.7 ± 5.5	321.6 ± 44.4	33.8 ± 4.6*	3.5 ± 0.2*	18.0 ± 2.7
	Control	41.9 ± 3.2	261.0 ± 18.8	28.6 ± 0.4	2.5 ± 0.2	16.8 ± 0.7
75 mM	Endophyte	73.4 ± 2.7	77.9 ± 16.0	20.9 ± 1.8	1.4 ± 0.3	5.1 ± 1.1
	Control	56.3 ± 8.2	61.3 ± 11.7	19.4 ± 5.8	1.1 ± 0.2	3.6 ± 0.8
150 mM	Endophyte	26.6 ± 1.9	13.1 ± 5.2	6.7 ± 0.9	0.4 ± 0.0	0.8 ± 0.4
	Control	29.2 ± 2.2	20.6 ± 10.1	7.5 ± 0.7	0.6 ± 0.0*	1.4 ± 0.7
250 mM	Endophyte	8.4 ± 2.1	0	2.5 ± 0.1	0.2 ± 0.1	0
	Control	11.0 ± 2.5	0	3.1 ± 0.7	0.3 ± 0.1	0

Figure 1. Percentage change of harvest parameters associated with endophyte inoculation.



Root dry weight was the factor which responded most strongly to endophyte inoculation and salt stress, and was particularly negatively impacted at the two highest salinity stresses of 150 and 250 mM NaCl, where endophyte-associated mean root dry weight was reduced by 63% and 65%, respectively. In contrast, root dry weight was greater for the endophyte inoculated plants with zero and 75 mM salt stress, where we found respective increases of 42% and 30%.

When we tested for the presence of the endophytes in seed tissue of the mature plants we were unable to recover any of the inoculated strains, indicating that the endophytes were not being transmitted to the seeds.

After 1 month of room temperature drying, salt crystals were evident on the surface of the compost for the 250 mM salt-stressed set and at the base of rootballs for both 150 and 250 mM sets.

When we tested the salinity tolerance of each endophyte strain *in vitro*, we found that nearly all strains grew equally well on all NaCl concentrations, where we detected no differences in colony

numbers or plate coverage after 21 days for most strains, with only *P. purpurogenum* showing significantly less growth ($p < 0.05$) on the higher salinity stresses of 150 and 250 mM.

4. Discussion

Our study has found that a consortium of 14 fungal endophyte strains recovered from the seed of a wild barley relative growing naturally on a moderately saline site improved agronomic traits in a moderately salt-stressed barley cultivar (Katerji et al., 2006). The endophytes associated with the habitat-adapted symbiosis (Rodriguez et al., 2008) conferred benefits to a close relative growing in a similar saline environment. However, for the more severely salt-stressed plants, endophyte inoculation was associated with a **reduction** in these traits. This “flip” in endophyte effect between plant-beneficial at zero and 75 mM NaCl stress and plant-detrimental at 150 and 250 mM NaCl stress was quite dramatic and totally unexpected.

We propose that this change in relationship is due to competition for resources under more severe salt stress. The plants could tolerate and benefit from endophyte colonisation under zero to moderate salt stress, but the endophytes may have been an insupportable burden at higher salt stresses. The endophytes thus act as endosyms or endosymbionts *sensu* Hodkinson and Murphy (2019) depending on the environmental conditions. After all, the plants were growing in pots and received no supplementary feeding during the experimental period so there was a relatively low level of nutrients available. Barley crops growing under field conditions are supplied with high levels of nutrients and in addition their roots are able to obtain nutrients from a wider and deeper pool in the soil. All of the strains were shown to be salt-tolerant *in vitro* at every concentration tested which indicates that they were better able to tolerate the higher salt stress and out-compete the plants for the scarce resources.

While we did not directly determine the mechanisms involved in the endophyte-induced trait improvements, some inferences can be made. One of the most plausible explanations uncovered to date is that selected endophyte characteristics relieve reactive oxygen species (ROS) activity by enhancing anti-oxidative enzyme systems in host plants (Alikhani et al., 2013; Bu et al., 2012; Gond, Torres, Bergen, Helsel, & White, 2015; Redman et al., 2011; Rodriguez et al., 2008; Zhang & Nan, 2007). Azad and Kaminskyj (2016) also recovered fungal endophytes from plants growing naturally on salinised soil. They found that endophyte-colonised plants had higher photosynthetic efficiency and lower ROS content, implying a mechanism for stress tolerance. They applied one large NaCl treatment (300 or 500 mM) to three week old tomato plants which gave the plants one big shock; in contrast, we applied five treatments at lower NaCl concentrations to maintain a consistent salinity level in the growing medium. This approach more accurately affects growing conditions in a salinity stressed field environment.

As in other studies using endophytes, Azad and Kaminskyj (2016) focused on the use of a single endophyte strain. However, rapid progress in rhizosphere microbiome research has revived the understanding that plants may benefit more from association with interacting, diverse microbial communities (microbiome) than from individual members in a community (Qin et al., 2016); a view with which we concur. Murphy et al. (2018) reasoned that the different modes of action associated with different endophytes, for endophytes obtained from the same plant species, would allow a degree of compatibility when used as a consortium, with each endophyte bringing different functional mechanisms to the target plant in a particular ecological niche space.

Li, Wang, Zhu, Wu, and Qi (2017) found that the development and weight of roots of *Medicago truncatula* co-cultivated with the fungus *Piriformospora indica* was strongly promoted under all degrees of salt stress that they applied (zero, 100 mM, 175 mM, 250 mM). However, as with other studies (Azad & Kaminskyj, 2016; Khan et al., 2011), the salt stress was applied once and the crop was not grown to full maturity, so cannot be directly compared with our study.

Another conceivable mechanism regards an endophyte ability to create phytohormones or to modulate phytohormone biosynthesis of host plants. Empirical data have supported the idea that auxin, gibberellic acid, abscisic acid, salicylic acid and ethylene biosynthesis processes are likely related to the delay of stress responses in hosts (Cheng, Woody, Mc Conkey, & Glick, 2012; Khan, Waqas, & Lee, 2015; Siddikee, Glick, Chauhan, Yim, & Sa, 2011; Straub, Yang, Liu, & Ludewig, 2013; Yaish, Antony, & Glick, 2015).

As far as we are aware, the endophytes' original plant host, *H. secalinum*, has never previously been sampled for fungal seed endophytes. Many grass species contain fungal endophytes in seed tissue which are normally vertically transmitted (Cheplick, 2017; Schulthess & Faeth, 1998). However, we found that none of the barley seeds harvested at the end of this experiment contained any of the original endophyte inoculants; this may be due to the inoculant being applied as a seed dressing, where the endophyte may only have colonised the early root tissue, without subsequent systemic or vertical transmission (Sanchez Marquez, Bills, Herrero, & Zabalgogezco, 2012). This result would have important implications for the potential of these endophytes in agriculture, as it means that the barley grain would not contain any traces of inoculated endophyte. Distributors would benefit by being able to guarantee an uncontaminated product, alleviating safety concerns.

The site from which *H. secalinum* seeds were collected had a relatively high soil salinity of 1.46 bars (4.05 dS/m) so the endophytes that the plants recruited represent a habitat-adapted symbiosis (Redman et al., 2011), which was confirmed by the *in vitro* salinity tolerance exhibited by all of these strains. A saline soil is generally defined as one in which the electrical conductivity (EC) of the saturation extract (EC_e) in the root zone exceeds 4 dS m⁻¹ (approximately 40 mM NaCl) at 25°C (Katerji et al., 2006). So the 75 mM salinity stress treatment we applied in this experiment would be classified as a moderately saline environment (equating to an EC of 6.87 dS m⁻¹), the 150 mM treatment as severely saline and 250 mM as very severely saline. The 150 and 250 mM salt concentrations would be unlikely to occur in most agricultural soils. At the salt concentration of 75 mM, barley crops would be expected to be significantly negatively impacted, so the endophyte-induced improvements in all barley traits at this concentration would indicate that endophyte treatment using our newly discovered strains would be of real benefit to farmers growing on moderately saline soils.

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Competing interests

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