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The Difference between Life and Death: Fungal Endophytes Improve Survival and Increase Biomass in Multiply-Stressed Barley

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The Difference between Life and Death: Fungal Endophytes Improve Survival and Increase Biomass in Multiply-Stressed Barley

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Keywords: *barley, fungal root endophytes, multiple stresses, agronomic traits, climate change.*

I. Introduction

Biotic and abiotic stresses such as extreme temperatures, low water availability, low nutrient availability and pathogenic infections are frequently simultaneously encountered by plants in both natural and agricultural systems(Langridge *et al.* 2006). For example, high temperature and water stress are often co-associated. Abiotic stresses alone are estimated to reduce global crop yields by over a half of that possible under optimal growing conditions (Boyer 1982). Abiotic stresses, in particular, may increase in the future due to global climate change (IPCC 2014) and predicted increases in drought and temperature-related stresses are expected to reduce crop productivity even further (Ciais *et al.* 2005; Larson, 2013). In order to successfully address the challenge of future food security it is necessary to increase yields, find more sustainable farming methodologies and to cultivate additional farmland. Potential exists for further extending farming on to marginal, arid, and semi-arid lands, especially in the developing world (Lantican *et al.*, 2003).The key risk associated with the likelihood of an increase in multiply stressed growing conditions will be reduced crop productivity, with strong adverse effects on regional, national, and household livelihood and food security (IPCC 2014).

These risks will be exacerbated by the exponential growth in the world population(Coleman-Derr & Tringe 2014), and will be most significant for the important global crops, including barley. Barley is grown on 56 Mha worldwide with a 2005 – 2008 mean production of 1.43×10^{11} kg(Newton *et al.* 2011), and while it can be grown profitably on marginal land, future increases in multiple stressors will require new crop varieties and new farming techniques to maintain acceptable crop yields.

Traditional approaches to breeding crop plants with improved stress tolerance have made some progress and wild relatives and landraces of cereal crops still offer great potential for breeding desired traits into crops(Langridge *et al.* 2006).However, conventional breeding practices often neglect the complex ecological context of the soil environment in which the crop is grown(Coleman-Derr & Tringe 2014), and other supplementary techniques are needed to improve barley stress tolerance. A class of microorganisms called endophytes have been shown to enhance biotic and abiotic stress tolerance in plants(Baltrusch at *et al.* 2008; Worchel *et al.* 2012; Hubbard *et al.* 2013; Murphy *et al.* 2014a). Endophytes are microorganisms (bacteria, fungi and unicellular eukaryotes) which can live at least part of their life cycle inter- or intra cellularly inside of plants usually without inducing pathogenic symptoms. This

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can include competent, facultative, obligate, opportunistic and passenger endophytes. Endophytes can have several functions and/or may change function during their lifecycle (Murphy *et al.* 2014a).

The complexities of stress responses essentially limit the predictive relevance of experimental evidence using individual stresses, suggesting that combinatorial studies of stress responses may be the best approach (Mittler 2006; Atkinson & Urwin 2012). For example, with heat stress alone plants can cool their leaves by transpiration, but if heat stress is combined with drought, plants cannot open their stomata to cool their leaves, leading to overheating (Rizhsky *et al.* 2002). Plants activate a specific and unique stress response when subjected to a combination of multiple stresses (Rizhsky *et al.*, 2004), so current techniques for developing and testing stress-tolerant plants by imposing each stress individually may be inadequate (Mittler & Blumwald, 2010). Signalling pathways associated with combinations of biotic and abiotic stresses may act antagonistically, changing the plant response in ways not predictable from individual stresses (Anderson *et al.*, 2004; Asselbergh *et al.*, 2008).

While previous studies have examined the effects of one or two simultaneous stresses on barley, we aimed to test the effects of inoculating fungal root endophytes (hereafter endophytes) derived from a wild barley species, *Hordeum murinum* subsp. *murinum* L.,onto a barley cultivar growing under a combination of heat, drought, pathogen (*Gaeumannomyces graminis* var. *tritici*) and nutrient stress. *Hordeum murinum* is an annual grass and a ruderal of roadsides, rough grassland and waste places (Streeter *et al.* 2009; Stace 2010). As the species generally grows in abiotically-stressed environments (El-Shatnawi *et al.* 1999; Myrna Johnston *et al.* 2009; Murphy *et al.* 2014a), it may have evolved symbiotically-conferred stress tolerance associated with endophyte infection (Rodriguez *et al.* 2008). Endophytes isolated from *H. murinum* may have the potential to benefit cultivated barley in similar stressed conditions.

II. Materials and Methods

Five endophyte isolates - 0401IA76 (GenBank ID: KM492846), 0406050(2)A (GenBank ID: KM492844), 0406050(2)C (GenBank ID: KM492845), 040901(3) (GenBank ID: KM492837) and 040906(4) (GenBank ID: KM492839) - were selected from a previous experiment which characterised endophytes that were isolated from wild populations of *Hordeum murinum* in Ireland (Murphy *et al.* 2014a). The provided nuclear ribosomal internal transcribed sequences (nrITS) DNA sequences for these strains were compared with existing GenBank accessions to establish the identity of the strains.

Untreated seeds of the barley cultivar 'Propino' (Goldcrop Seeds, Cork, Ireland) were used. Seeds were surface-sterilised by soaking in 5% NaClO for 15 min, rinsing three times with 70% ethanol and then rinsing five times with pure water. The growth compost of John Innes No.3 formulation (Westland Horticulture Limited) was placed into 1.5 litre washed and sterilised (soaked for 2 hours in 5% NaClO then rinsed × 5 with tap water) plastic pots. For each of the seven inoculation treatments (including a control), twenty five seeds of barley, in 5 pots containing 5 seeds each, were sown at 30 mm depth and either inoculated with an inoculant solution of 250μl containing one of the five endophytes or an inoculant solution of 250μl containing a combination of all five endophytes (AllEndos). The inoculant solution was prepared by mixing 10 mg of each fungal culture with 8 ml of pure water and stirring with a magnetic bar for 2 mins at 25°C. 250μl of the solution was directly inoculated onto each seed. For the controls, the seeds were inoculated with 250μl pure water. Every seed was also directly inoculated with a 250ul solution of the common and serious barley pathogen *Gaeumannomyces graminis* var.*tritici* ("take-all") (Gen Bank Accession KF018415), prepared as above.

Pots were placed into a controlled environment chamber, then randomly relabelled with a single number by a third party, to produce a double-blind and randomised setup. The pots were moved to a new position within the growth chamber every 3 days. The environmental settings of the growth cabinet (Conviron PGR14) were programmed to produce a 14 hr photoperiod at a compost surface illumination of 220 μmol.m^{-2} s^{-1}, a photoperiod temperature of 33°C reducing to 12°C in the dark period and a photoperiod relative humidity of 45%, increasing to 65% in the dark.

Three covered culture dishes containing five sterilised barley seeds on malt extract agar (Fluka 38954) were kept in the growth chamber during the experimental period to test for seed surface sterilisation success and to monitor any contamination that may be present from seed-produced fungal infection.

The seedlings were thinned to three plants per pot, 12 days after germination, producing 15 individual plant replicates for each treatment. A Germination Index (GI) was calculated using the formula:

$$GI = ((G_t / G_n) / G_n)$$

where G_t is the cumulative number of days to germination for all seeds and G_n the total number of seeds germinated. Soil moisture content at a depth of 50 mm was measured daily using a Delta-T Devices HH2 WET sensor kit (Delta-T Devices, Cambridge, UK) and pots were watered with tap water only when the soil moisture content was between 10% and 15% which was when the barley plants were starting to wilt and showed a drought-associated colour change. The pots were watered until soil moisture content was at field capacity (~45%). Total water input was 4.19 litres per pot. All

pots were given a liquid fertiliser (Bayer Phostrogen®) at each watering. Total nutrient input per plant was: ammoniacal N = 4mg, ureic N = 20mg, Total N = 24mg, P = 20mg, K = 40mg, Mg = 4mg, S = 8mg, Ca = 4mg and traces of Boron, Copper, Iron, Manganese, Molybdenum and Zinc. Inputs for nitrogen (N), phosphorous (P) and potassium (K) were approximately 6%, 17% and 16% respectively of that recommended for spring barley growing on low-nutrient soils (http://www.teagasc.ie/crops/winter/fertilisers/winter_cereals_fertiliser_requirements.pdf) so plants were severely nutrient-stressed. A liquid fertiliser was used because the low water input may have resulted in incomplete nutrient delivery if a solid fertiliser formulation were used.

A further set of 25 plants for each treatment were grown in close to optimal conditions (OC), with the environmental settings programmed to produce a 14 hr photoperiod at a temperature of 21°C reducing to 12°C in the dark period and a constant 70% relative humidity. The seedlings were thinned to three plants per pot, 12 days after germination, producing 15 individual plant replicates for each treatment. A Germination Index (GI) was calculated as above. Plants were watered to maintain the compost at near field capacity and total water input was 6.39 litres per pot. Nutrient input per plant was approximately five times that of the stressed plants (ammoniacal N = 20mg, ureic N = 100mg, Total N = 120mg, P = 90mg, K = 220mg, Mg = 20mg, S = 40mg, Ca = 20mg and traces of Boron, Copper, Iron, Manganese, Molybdenum and Zinc).

The number of days to reach selected Zadoks stages (Zadoks *et al.* 1974) was recorded for each plant. Plants were grown for 90 days (13 weeks) from date of sowing, then harvested and processed in one day. Before processing the plants, four 5 mm pieces of mid-section root from each plant were surface-sterilised and incubated on half-strength malt extract agar at 25°C in the dark to test for endophyte presence. Endophyte emergence was recorded over the next 35 days, and emergents were identified by morphological examination and by sequencing of the internal transcribed region (ITS) of nuclear ribosomal DNA(nrDNA). For the DNA analysis, 20 mg of fungal material was scraped from the agar surface and placed into shaker tubes. DNA was extracted using a Qiagen DNeasy mini kit, following the Qiagen protocol, producing 200 µl of DNA extract for each isolate. PCR was carried out on the DNA extracts using the nuclear ribosomal DNA (rDNA) internal transcribed spacer (ITS) primers ITS1 and ITS4 (White *et al.* 1990). The thermal cycling parameters were programmed to optimise primer annealing, consisting of: 3 min at 95°C; 9 cycles of 1 min at 94°C, 1 min at 56°C, 2 min at 72°C; 20 cycles of 30 sec at 94°C, 1 min at 56°C, 3 min at 72°C; a final extension for 7 min at 72°C. PCR products were cleaned up using Exonuclease (New England Biolabs) and Shrimp Alkaline Phosphatase (ExoSAP ; Roche). Purified PCR products underwent cycle sequencing using the reverse ITS4 primer (4 pmol) or forward ITS1 primer (4 pmol) in separate reactions with the ABI BigDye 3.1 kit (Foster City, CA). The products were further purified using a BigDye XTerminator purification kit and protocol. DNA was sequenced using an Applied Bio systems 3130xL Genetic Analyzer. The recovered sequences from the roots of each treatment were compared to the sequences of the original inoculants.

Pots were selected for processing in random order. Measurements were made for each plant of fresh and dry weights of shoots and roots, mean height of plants to tip of highest leaf and number of tillers. Shoot and root tissue from each plant of the MS treatment was inspected for signs of *Gaeumannomyces graminis* var. *tritici* infection and the degree of infection estimated as a proportion of total tissue showing signs of disease. All plant parts were separately dried in ovens for 7 days at 65°C before dry weights were measured.

Data analysis was carried out using single and two-factor ANOVA with Bonferroni correction and Pearson's correlation statistical analyses supplied with Data desk® 6.1.

III. Results

When we compared the provided nrITS sequences of the endophyte strains with GenBank accessions, we found that they were only distantly related to known fungi (Table 1) with an overall mean pairwise similarity of only 88%, and thus represent relatively novel organisms. It would therefore be unwise to assign these strains to a particular taxon, and we will refer to them throughout using the strain codes.

While two of the multiply-stressed (MS) endophyte treatments, 040605(2)A and 040605(2)C, had a greater germination index (GI) than the control ($P < 0.01$), there was no overall difference in germination index between endophyte treatments and control. We found large and significant differences between optimally grown plants (OC) and plants subjected to multiple stresses (MS) (Table 2). The main difference was that all of the OC control and treatment plants survived until the end of the experiment, whereas only 13% of MS control plants survived. However, over 80% of the endophyte-inoculated MS plants survived. For two of the MS endophyte treatments, 040906(4) and 040605(2)A, all of the plants survived. Although all of the OC plants produced seeds, only 10% of MS plants produced stems with rudimentary flowering structures, and none of these produced any heads with grains. Most measured barley traits showed greater values for the OC plants (Figure 1); the mean height of OC plants was twice that of the MS plants; the mean number of tillers for the OC plants was five times greater than the MS and the mean shoot dry weight for the OC plants was over three times greater than the MS. However, the

mean root dry weight was exactly the same for both OC and MS plants. The root dry weight for the control plants was greater than all endophyte-inoculated plants in the OC treatment, whereas in the MS plants the root dry weight for all endophyte-inoculated plants was greater than the control. Final overall comparisons between control and endophyte inoculated plants revealed a significant overall improvement over the control in agronomic barley traits for the MS plants ($P < 0.01$) but with no detectable differences for the OC plants.

For the MS plants that survived until the end of the experiment, we found significant differences in trait performance between control and endophyte-inoculated plants (Figure 2 and Figure 3).The mean root dry weight differed significantly between treatments (single factor ANOVA, $F_{6,98} = 8.32$, $P < 0.001$), where all of the endophyte treatments had greater root dry weight than the control ($P < 0.001$). The mean plant height, shoot dry weight and number of tillers for the endophyte treatments were all significantly greater than the control ($P < 0.05$), with one exception: there was no detectable difference in shoot dry weight and number of tillers between the control and the endophyte treatment 040901(3). The combined endophyte inoculant (AllEndos) was the treatment that gave the greatest improvement for all harvest traits (number of dead plants, plant height, number of tillers, shoot dry weight, root dry weight) in the multiply-stressed plants ($P < 0.01$ for every trait).

We found no difference between treatments in the MS plants for the proportion of root and shoot tissue displaying signs of *Gaeumannomyces graminis* var. *tritici* infection, where all plants had less than 5% of total root tissue with disease symptoms, but with no visible symptoms on above ground tissue.

At the end of the experiment, the three covered culture dishes containing five sterilised barley seeds on malt extract agar (Fluka 38954) that were kept in the growth chamber during the experimental period produced no evidence of seed-produced fungal infection.

A mean 50% (182) of root pieces that were removed from the MS plants at harvest produced fungal endophyte emergents. When we compared the emergent endophyte ITS sequences with the original inoculants, we found that each of the recovered sequences exactly matched that of the original.

IV. Discussion

Crop growers have long known that it is often the simultaneous occurrence of multiple stresses, rather than a particular stress condition, that is most lethal to crops (Mittler 2006).In this study, we have shown that endophytes derived from a wild relative of barley can reduce the lethal effect of a combination of heat, drought, nutrient and pathogen stress. In fact, very few of the control plants survived to the end of the experiment, and those that did survive performed significantly worse than the endophyte-inoculated plants. While each individual endophyte treatment induced improvements in several agronomic traits, it was the combined endophyte inoculant that improved all barley traits in the multiply-stressed plants most significantly. This suggests that a combination of endophytes may give the best results in a more realistic agricultural environment where the interactions between many competing microorganisms can make the outcome from a single endophyte inoculant uncertain.To our knowledge, this is the first ever study of the effect of endophytes derived from a congeneric wild relative of barley grown under multiple stresses.

An overall analysis of barley traits showed that there was a neutral effect due to the endophyte treatments in the optimally grown plants (OC) compared with a highly significant improvement in all traits for the multiply-stressed plants (MS). This suggests that barley plants may derive the most benefit from endophyte infection in stressful growing conditions (Singh *et al.* 2011; Khan *et al.* 2013; Song *et al.* 2014). However, not all of the measured traits showed improvements induced by endophyte inoculation under both regimes. In particular, root dry weight for the OC plants was significantly higher in the control plants, whereas root dry weight was lower for controls in the MS plants, suggesting that the endophyte infection stimulates greater root activity under the multiple stresses applied here. There have been contradictory reports regarding the relationship between endophyte inoculation and root biomass, with one study showing an endophyte-associated reduction in root weight in drought stressed barley (Murphy *et al.* 2015a) and another showing an endophyte-associated increase in root weight in optimally grown plants (Kumar *et al.* 2012). Murphy *et al.* (2015) report a neutral response in root weight due to endophyte inoculation in nutrient-stressed plants. Taken together, these contrasting results indicate that simultaneous multiple stresses induce a different response in root tissue allocation than a single stress. Since field conditions present multiple rather than single stresses for crop plants, our results may more accurately reflect plant responses to endophyte inoculation in agricultural situations (particularly as we also used a soil-based growing compost).

While there was no obvious endophyte effect on the degree of *Gaeumannomyces graminis* var. *tritic* infection in the post-harvest tissue, this may partly be due to the growing conditions used in this study. In general, this particular cool-temperate strain of *Gaeumannomyces graminis* var. *tritici* prefers much lower temperatures, higher moisture and a longer time to fully develop pathogenicity (Bockus & Tisser at 2000; Mathre 2000; Cook 2003; Mehta 2014). The high temperature and extremely low moisture in our

experiment may have either completely halted development and spread or even killed it off altogether. The barley cultivar that we used, Propino, does have good resistance to foliar disease, and an endophyte effect on this pathogen in the early stages of growth cannot be ruled out.

Plant responses to different stresses are highly complex, and recent evidence shows that plants respond to multiple stresses differently from how they do to individual stresses (Atkinson & Urwin 2012), with the interaction between biotic and abiotic stresses orchestrated by plant hormones and regulatory networks of molecular mechanisms such as transcription factors and reactive oxygen species (Langridge *et al.* 2006). The 'additional interactions' associated with endophyte infection serves to increase this complexity even further. The specific mechanisms associated with the improvements in agronomic traits that we and others have found are presently not known (Singh *et al.* 2011). While there have been studies examining changes in plant responses induced by the model fungal root endophyte *Piriformospora indica* and others (Schulz *et al.* 1999; Waller *et al.* 2008; Molitor & Kogel 2009; Lahrmann & Zuccaro 2012), further work using gene expression arrays and hormone cross-talk may elucidate the particular mechanisms associated with the stress related symbiosis that we have studied. It must also be noted that plant responses such as photosynthetic performance are cultivar-related (Afshari-Behbahanizadeh *et al.* 2014).The endophytes were derived from plants growing in very dry and nutrient poor soils (Murphy *et al.* 2014a) similar in several respects to the experimental conditions, so there may be a habitat-related selection of beneficial endophytes for these particular conditions(Rodriguez *et al.* 2008).

The positive benefits for barley related to endophyte inoculation that we have found has real significance for barley growers, particularly in the light of probable future changes in regional climate associated with global change. Where crop performance may currently only be limited by a single stressor, the addition of another climate change related stress may make the cultivation of the crop unviable. It is also desirable to reduce crop fertiliser inputs, both to save money and relieve environmental damage, but this will be difficult to achieve due to increasing demand for food. Endophyte treatments may provide part of the solution. We have shown for the first time that novel endophytes can even make the difference between life and death for multiply-stressed barley.

V. Conclusions

While controlled environment experiments using single plant stress factors have limited predictive value when applied to complex field conditions, multiple stress experiments with multiple endophyte inoculants more closely reflect the conditions that the cereal crop encounters in real agricultural environments. We have demonstrated that fungal root endophytes, derived from congeneric wild relatives of barley, have real potential in alleviating these stresses and could become particularly important for survival under future climate change scenarios. Future research should focus on translating results from controlled environment experiments using single endophyte inoculants of barley and other crops under single stresses to developing field crop inoculants using multiple endophytes.

Note

A patent for the use of these endophytes has been filed by Trinity College Dublin, and the use of these endophytes for biofertilisation and biocontrol purposes in cereal crop plants by third parties is subject to negotiated agreement with Trinity College Dublin and they may not be used without such permission.

VI. Acknowledgements

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Figure captions

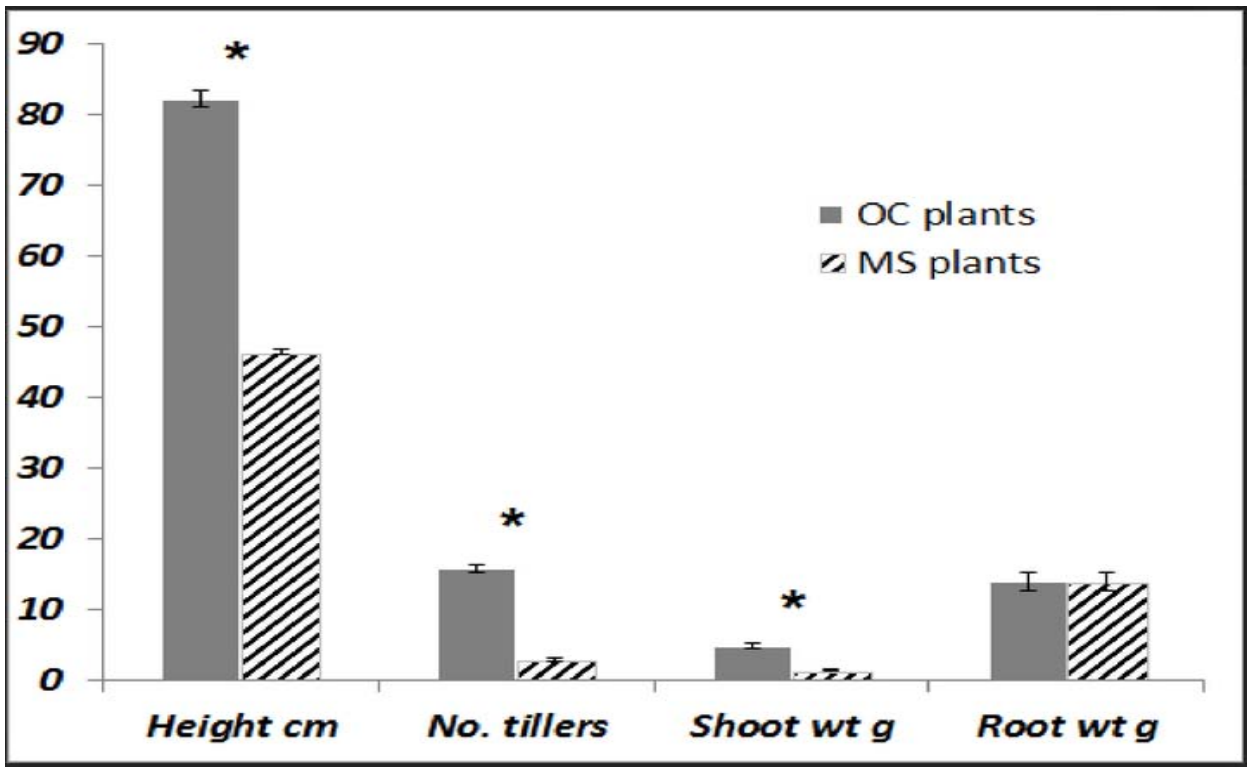

Figure 1 : Mean harvest values for selected barley traits between barley grown under optimal conditions (OC) and under multiple stresses (MS). Items marked with '*' indicate significantly greater values for OC treatment ($P < 0.01$) (n=15)

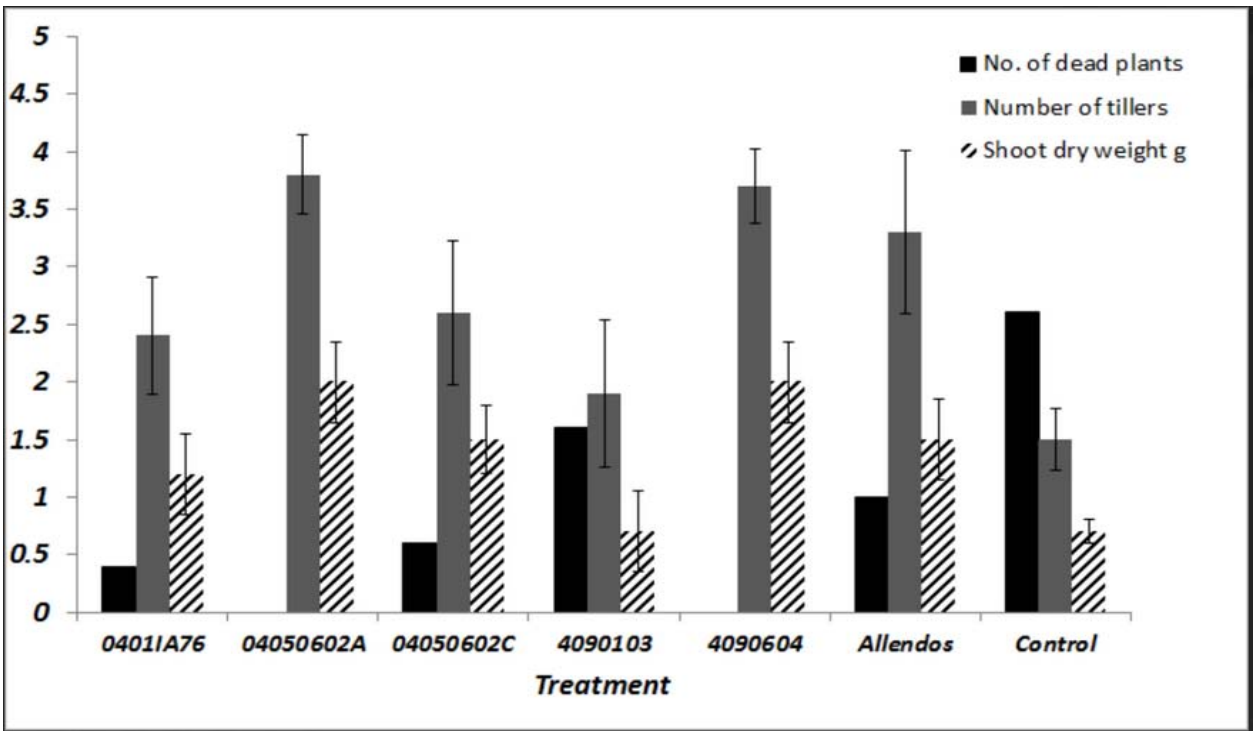

Figure 2 : Number of dead plants per pot of 3 plants, number of tillers per plant ± S.E. and shoot dry weight per plant ± S.E. for barley grown under multiple stresses (MS) (n=15)

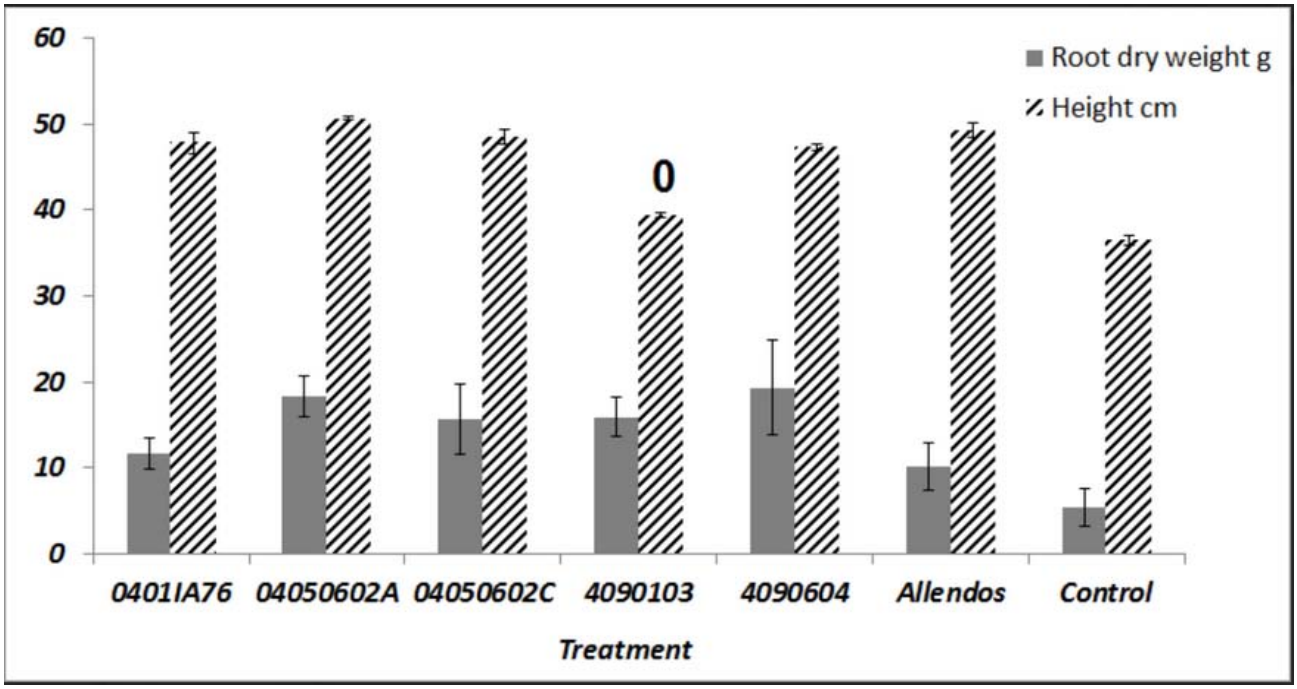

Figure 3 : Mean root dry weight ± S.E.and mean plant height ± S.E.for barley grown under multiple stresses (MS). All values for endophyte treatments, except those marked with '**0**', are significantly greater ($P < 0.05$) than the control (n=15)

Table 1 : ITS sequence relationship of endophyte strains with known GenBank accessions.

Endophyte strain	Gen Bank Accession	Nearest BLAST Match	% pairwise similarity
040605(2)A	KM492844	Uncultured *Cladosporium* clone, JF449686	82
040605(2)C	KM492845	*Penicilliumglabrum*, JN887323	85
0401IA76	KM492846	*Lophiostomacorticola*,HM116751	85
040901(3)	KM492837	*Penicilliumbrevicompactum* EU587331 [1]	95
040906(4)	KM492839	Uncultured *Metarhizium* KC797571 [2]	95

Table 2 : Significant negative (--) and positive (++) differences in barley traits between endophyte-inoculated and control plants grown in optimal conditions (OC) and under multiple stress (MS); 0 indicates no difference, * indicates $p < 0.05$, ** indicates $P < 0.01$ (n=15).

Trait	OC/MS	Endophyte difference, less (--) or greater (++) than control						Overall
		0401IA76	*040605(2)A*	*040605(2)C*	*040901(3)*	*040906(4)*	*Allendos*	
Germination Index	OC	0	0	0	++, *	0	0	0
	MS	0	++, **	++, **	0	0	0	0
Mean number of dead plants per pot	OC	0	0	0	0	0	0	0
	MS	++, **	++, **	++, **	++, **	++, **	++, **	++, **
Mean height	OC	0	++, **	++, **	++, **	++, **	++, **	++, **
	MS	++, **	++, **	++, **	++, **	++, *	++, **	++, **

Mean shoot dry weight	OC	++, **	0	0	--, **	0	0	0
	MS	++, *	++, **	++, **	0	++, **	++, **	++, *
Mean root dry weight	OC	--,*	--,*	--,*	--,*	0	--, *	--,*
	MS	++,**	++,**	++,**	++,**	++,**	++, **	++,**
Mean number of tillers	OC	++, *	++, *	0	++, *	0	++, *	++, *
	MS	++, *	++, *	++, **	0	++, **	++, **	++, *
Overall difference	OC	++, *	++, *	0	0	0	0	0
	MS	++,*	++,**	++,**	++,*	++,**	++, **	++,**

References

1. Afshari-Behbahanizadeh S, A. Akbari G, Shahbazi M, Alahdadi I(2014). Relations Between Barley Root Traits and Osmotic Adjustment Under Terminal Drought Stress. *Journal of Agricultural Science***6**: 112–119.
2. Anderson JP, Badruzsaufari E, Schenk PM, Manners JM, Desmond OJ, Ehlert C, Maclean DJ, Ebert PR, Kazan K(2004). Antagonistic interaction between abscisic acid and jasmonate–ethylene signaling pathways modulates defense gene expressionand disease resistance in Arabidopsis. *The Plant Cell***16**: 3460–3479.
3. Asselbergh B, De Vieesschauwer D, Hofte M(2008). Globalswitches and fine-tuning—ABA modulates plant pathogen defense.*Molecular Plant-Microbe Interactions***21**: 709–719.
4. Atkinson NJ, Urwin PE (2012). The interaction of plant biotic and abiotic stresses: from genes to the field. *Journal of Experimental Botany***63**: 3523–3543.
5. Baltruschat H, Fodor J, Harrach BD, Niemczyk E, Barna B, Gullner G, Janeczko A, Kogel K-H, Schäfer P, Schwarczinger I, *et al.* (2008). Salt tolerance of barley induced by the root endophyte Piriformospora indica is associated with a strong increase in antioxidants. *New Phytologist***180**: 501–10.
6. Bockus WW, Tisserat NA(2000). Take-all root rot. *The Plant Health Instructor*. DOI:10.1094/PHI-I-2000-1020-01.*Updated 2005.*
7. Boyer JS (1982). Plant productivity and environmant. *Science***218**: 443–448.
8. Ciais P, Reichstein M, Viovy N, Granier A, Ogée J, Allard V, *et al*(2005).Europe-wide reduction in primary productivity caused by the heat and drought in 2003.*Nature***437**: 529–533.
9. Coleman-Derr D, Tringe SG (2014). Building the crops of tomorrow: advantages of symbiont-based approaches to improving abiotic stress tolerance. *Frontiers in Microbiology***5**: 1-6.
10. Cook JR (2003). Take-all of wheat. *Physiological and Molecular Plant Pathology***62**: 73–86.
11. El-Shatnawi MKJ, Ghosheh HZ, Shannag HK, Ereifej KI(1999). Defoliation Time and Intensity of Wall Barley in the Mediterranean Rangeland. *Journal of Range Management***52**: 258.
12. Hubbard M, Germida JJ, Vujanovic V (2013). Fungal endophytes enhance wheat heat and drought tolerance in terms of grain yield and second generation seed viability. *Journal of Applied Microbiology***106**:109-122.
13. IPCC(2014).Summary for policymakers. In: *Climate Change 2014: Impacts, Adaptation, and Vulnerability. Part A: Global and Sectoral Aspects. Contribution of Working Group II to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change* [Field, C.B., V.R. Barros, D.J. Dokken, K.J. Mach, M.D. Mastrandrea, T.E. Bilir, M. Chatterjee, K.L. Ebi, Y.O. Estrada, R.C. Genova, B. Girma, E.S. Kissel, A.N. Levy, S. MacCracken, P.R. Mastrandrea, and L.L. White (eMS.)]. Cambridge University Press, Cambridge, United Kingdom and New York, NY, USA, pp. 1-32.
14. Khan AL, Waqas M, Khan AR, Hussain J, Kang S-M, Gilani SA, Hamayun M, Shin J-H, Kamran M, Al-Harrasi A, *et al.* (2013). Fungal endophyte *Penicillium janthinellum* LK5 improves growth of ABA-deficient tomato under salinity. *World Journal of Microbiology &Biotechnology*.**29**: 2133-2144.
15. Kumar V, Sarma MVRK, Saharan K, Srivastava R, Kumar L, Sahai V, Bisaria VS, Sharma AK (2012). Effect of formulated root endophytic fungus Piriformospora indica and plant growth promoting rhizobacteria fluorescent pseudomonads R62 and R81 on Vigna mungo. *World Journal of Microbiology & Biotechnology***28**: 595–603.
16. Lahrmann U, Zuccaro A (2012). Opprimo ergo sum-Evasion and Suppression in the Root Endophytic Fungus *Piriformospora indica*. *Molecular Plantmicrobe Interactions MPMI***25**: 727–37.

17. Langridge P, Paltridge N, Fincher G (2006). Functional genomics of abiotic stress tolerance in cereals. *Briefings in Functional Genomics & Proteomics***4**: 343–54.
18. Lantican MA, Pingali PL, Rajaram S (2003). Is research on marginal lands catching up? The case of unfavourable wheat growing environments.*Agricultural Economics***29**: 353–361.
19. Larson, C (2013). Losing arable land, China faces stark choice: adapt or go hungry. *Science***339**: 644–645.
20. Mathre DE(2000). Take-all disease on wheat, barley, and oats.Online.*Plant Health Progress* doi:10.1094/PHP-2000-0623-01-DG.
21. Mehta YR (2014). Wheat diseases and their management. Heidelberg: Springer International Publishing.
22. Mittler R(2006). Abiotic stress, the field environment and stress combination. *Trends in Plant Science***11**: 15–9.
23. Mittler R, Blumwald E(2010). Genetic engineering for modernagriculture: challenges and perspectives. *Annual Review of Plant Biology***61:** 443–462.
24. Molitor A, Kogel K (2009). Induced resistance triggered by *Piriformospora indica*. *Plant Signaling & Behaviour***4**: 215–216.
25. Murphy BR, Doohan FM, Hodkinson TR(2014). Fungal endophytes of barley roots. *The Journal of Agricultural Science***152**: 602–615.
26. Murphy BR, Doohan FM, Hodkinson TR(2014a). Persistent fungal root endophytes isolated from a wild barley species suppress seed- borne infections in a barley cultivar.*Biocontrol* **60**: 281-292.
27. Murphy BR, Doohan FM, Hodkinson TR (2015).Fungal root endophytes of a wild barley species increase yield in a nutrient-stressed barley cultivar. *Symbiosis***65**: 1-7.
28. Murphy BR, Nieto LM, Doohan FM, Hodkinson TR (2015a).Fungal endophytes enhance agronomically important traits in severely drought-stressed barley. *Journal of Agronomy and Crop Science*. In press.
29. Myrna Johnston B, Alfredo Olivares E, Carolina Calderón E (2009). Effect of quantity and distribution of rainfalls on Hordeum murinum L . growth and development. *Chilean Journal of Agricultural Research***69**: 188–197.
30. Newton AC, Flavell AJ, George TS, Leat P, Mullholland B, Ramsay L, Revoredo-Giha C, Russell J, Steffenson BJ, Swanston JS,*et al.* (2011). Crops that feed the world 4. Barley: a resilient crop? Strengths and weaknesses in the context of food security. *Food Security***3**: 141–178.
31. Rizhsky L, Liang H, Mittler R(2002).The combined effect of drought stress and heatshock on gene expression in tobacco. *Plant Physiology***130**: 1143–1151
32. Rizhsky L, Liang HJ, Shuman J, Shulaev V, Davletova S, Mittler R(2004). When defense pathways collide. The response ofArabidopsis to a combination of drought and heat stress. *Plant Physiology***134**: 1683–1696.
33. Rodriguez RJ, Henson J, Van Volkenburgh E, Hoy M, Wright L, Beckwith F, Kim Y-O, Redman RS (2008). Stress tolerance in plants via habitat-adapted symbiosis. *The ISME Journal***2**: 404–16.
34. Schulz B, Rommert A-K, Dammann U, Aust H-J, Strack D (1999). The endophyte-host interaction : a balanced antagonism ? *Mycological Research***103**: 1275–1283.
35. Singh LP, Gill SS, Tuteja N (2011). Unraveling the role of fungal symbionts in plant abiotic stress tolerance. *Plant Signaling & Behavior***6**: 175–91.
36. Song M, Chai Q, Li X, Yao X, Li C, Christensen MJ, Nan Z (2014). An asexual Epichloë endophyte modifies the nutrient stoichiometry of wild barley (*Hordeum brevisubulatum*) under salt stress. *Plant and Soil*. Doi: 10.1007/s11104-014-2289-0.
37. Stace C (2010). New Flora of the British Isles. Cambridge: Cambridge University Press.Streeter D, Hart-Davies C, Hardcastle A, Cole F, Harper L (2009). Collins Flower Guide. London: HarperCollins Publishers.
38. Waller F, Mukherjee K, Deshmukh SD, Achatz B, Sharma M, Schäfer P, Kogel K-H (2008). Systemic and local modulation of plant responses by Piriformospora indica and related Sebacinales species. *Journal of Plant Physiology***165**: 60–70.
39. Worchel ER, Giauque HE, Kivlin SN (2013). Fungal Symbionts Alter Plant Drought Response. *Microbial Ecology***65**: 671-678.
40. Zadoks JC, Chang TT, Konzak CF (1974). A Decimal Code for the Growth Stages of Cereals.*Weed Research***14**: 415–421.