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Effects of osmotic and matric potential on radial growth and accumulation of endogenous reserves in three isolates of *Pochonia chlamydosporia*

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Abstract

For the first time, the effects of varying osmotic and matric potential on fungal radial growth and accumulation of polyols were studied in three isolates of *Pochonia chlamydosporia*. Fungal radial growth was measured on potato dextrose agar modified osmotically using potassium chloride or glycerol. PEG 8000 was used to modify matric potential. When plotted, the radii of the colonies were found to grow linearly with time, and regression was applied to estimate the radial growth rate (mm/day^{-}). Samples of fresh mycelia from 25 day-old-cultures were collected and the quantity (mg g^{-1} fresh biomass) of four polyols (glycerol, erythritol, arabitol and mannitol) and one sugar (glucose) was determined using HPLC. Results revealed that fungal radial growth rates decreased with increased osmotic or matric stress. Statistically significant differences in radial growth were found between isolates in response to matric stress ($p<0.006$) but not in response to osmotic stress ($p=0.759$). Similarly, differences in the total amounts of polyols accumulated by the fungus were found between isolates in response to matric stress ($p<0.001$), but not in response to osmotic stress ($p=0.952$). Under water stress, the fungus accumulated a combination of different polyols important in osmoregulation, which depended on the solute used to generate the stress. Arabitol and glycerol were the main polyols accumulated in osmotically modified media, whereas erythritol was the main polyol that was accumulated in media amended with PEG. The results found that *Pochonia chlamydosporia* may use different osmoregulation mechanisms to overcome osmotic and matric stresses.

Keywords: Biological control, water stress; *Pochonia chlamydosporia*; root-knot nematodes; cyst nematodes; endogenous reserves.

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Introduction

The anamorphic and facultative parasitic fungus, *Pochonia chlamydosporia* (Goddard) Zare & Gams has been associated with soils which suppress the multiplication of cyst nematode populations (Kerry, Crump and Mullen, 1982). Atkins et al. (2003a) suggested the fungus has potential as a biological control agent against root-knot (*Meloidogyne* spp.) and cyst nematodes (*Globodera* spp. and *Heterodera* spp.) (Atkins, Clark, Kerry and Sosnowska, 2002, Tobin, Haydock, Hare, Woods and Crump, 2008). In order to provide effective control of nematode multiplication, *P. chlamydosporia* must establish in the soil and rhizosphere and survive, even in the absence of nematode hosts, and should infect, parasitise and consume nematode eggs when they are produced on roots (Kerry and Jaffee, 1997). However, the successful development of *P. chlamydosporia* as a biocontrol agent is not only dependent on the ability of the organism to kill the nematode target pest; other factors including those involved in the production of the fungus are important, as they affect the growth of the fungus in soil after its release (Kerry et al., 1993). The efficacy of some fungal biological control agents in soil has not been consistent because fungi can be markedly influenced by environmental conditions, including the availability of water, which affects survival and performance (Cook and Baker, 1983). Additionally, the shelf-life of a formulated fungal-based biocontrol agent can be much affected by its ability to withstand drying. Some information is available on the water relations of *P. chlamydosporia*, particularly the effects of water stress on hyphal growth and production of chlamydospores (Kerry, Irving and Hornsey, 1986, Bourne and Kerry, 1999, Noreen, Amer-Zareen, Zaki and Shaukat, 2001). However, tolerance to solute and matric stress has been little studied in this fungus. In soil and crop residues, matric potential is the major component of total water potential (Magan and Lynch, 1986) and therefore, matric stress is thought to have greater effect on fungal growth in soil and spore germination than osmotic stress (Brownell and Schneider, 1985).

In order to overcome water stress, microbial cells must be able to take up water when their internal water potential is less than that of the environment. The water potential gradient across the cell membrane results in the movement of water from the side with greater potential until, by the development of cell turgor, water potential inside the cell is in equilibrium with that outside (Cook and Baker, 1983). Osmoregulation is an energy-requiring process and may be accomplished internally by the accumulation of compatible solutes. When grown under low water potentials (water-stress conditions), fungi are able to lower their intracellular water potential accordingly, by synthesis of polyhydroxyalcohols (polyols), organic acids and sugars (Brown, 1978, Luard, 1982, Meikle, Chudek, Reed and Gadd, 1991, Van Eck, Prior and Brandt, 1993, Hallsworth and Magan, 1994a, Hallsworth and Magan, 1994b). These solutes are accumulated in protoplasmic and vacuolar spaces in fungal propagules (Brown and Simpson, 1972), and regulate the intracellular

osmotic potential (Pascual, Melgarejo and Magan, 2000). By altering the conditions of growth, such as reducing the availability of water in the medium, fungi are seen to change the quantity and/or proportion of the endogenous reserves accumulated (Ramos, Magan and Sanchis, 1999), which may improve spore germination rate (Hallsworth and Magan, 1995) and change the virulence of certain species (Chandler, Andersen and Magan, 2005). Therefore, effective physiological manipulation of inocula may improve spore quality and the establishment of the fungus in soil, thus leading to improved efficacy of fungal-based biocontrol agents (Magan, 2001). The objectives of this study were to: (a) quantify the effect of varying levels of solute (ionic, non-ionic water stress) and matric stress on the radial growth of *P. chlamydosporia* and (b) measure the accumulation of endogenous low and high molecular weight polyols (glycerol, erythritol, arabitol and mannitol) and a sugar (glucose) in response to osmotic and matric water stress, in three isolates of the fungus.

Materials and methods

Fungal isolates

The identity of three isolates (10, 280 and 392) of *P. chlamydosporia* was checked using specific diagnostic primers derived from the ß-tubulin gene, and confirmed to be *P. chlamydosporia* using PCR (Hirsch, Mauchline, Mendum and Kerry, 2000). DNA fingerprinting enabled the discrimination between different isolates of *P. chlamydosporia* grown in pure culture (Arora, Hirsch and Kerry, 1996). The isolate 392, originally isolated from Cuba, was identified as *P. chlamydosporia* var. *catenulata*, and could also be distinguished from isolates of *P. chlamydospor*ia var. *chlamydosporia* using specific PCR primers (Atkins et al., 2003b). The three fungal isolates are maintained as freeze-dried samples in a collection at Rothamsted Research, UK.

Media

The medium used in this study was potato dextrose agar (PDA), prepared according to the instructions of the manufacturer (Oxoid). The osmotic potential of the PDA (-0.7 Mpa) was modified to -1.4, -2.8 and -7.1 MPa (Magan, 1997) by the addition of the ionic solute, potassium chloride (KCl) or the non-ionic solute, glycerol (Dallyn and Fox, 1980).

For modification of the matric potential, PDA was omitted and known amounts of PEG 8000 were used, resulting in matric potentials of -1.4, -2.8 and -4.2 MPa. The water potential generated by PEG 8000 is mainly (99%) due to matric forces (Steuter, Mozafar and Goodin, 1981). The medium was enriched with peptone (10 g l^{-1}) and glucose (40 g l^{-1}) and was autoclaved prior to use. Sterile disks of capillary matting (8.5 cm diameter) were placed in sterile 9 cm Petri dishes to which 20 ml of the room temperature cooled medium was added. The matting was overlaid with a

sterile disk of black polyester lining cloth and then a cellophane disk (Ramos et al., 1999), to provide support for fungal growth in the liquid medium.

Inoculation and measurement

Petri dishes were inoculated centrally with a 5 mm diameter agar plug taken from the edge of seven-day-old colonies growing on PDA (Ramos et al., 1999). Using a completely randomised design, five dishes per treatment combination (solute × water potential) were inoculated and incubated at 25°C; those at the same water potential were placed in sealed polyethylene bags (Marín, Sanchis and Magan, 1995). The growing colonies were measured along two diameters at right angles after 5, 7, 10, 12, 14, 18 and 25 days, which was prior to any colony reaching the edge of its dish. Additionally, the numbers of spores (chlamydospores) produced after 25 days of growth in osmotically modified media were recorded in all isolates, at each level of water potential.

Quantification of polyols and glucose

A sample of 100 mg of fresh, aerial mycelia from each of three randomly selected replicate dishes of each treatment combination of the 25 day-old-cultures was mixed with 1 ml of HPLC grade water in a 2 ml microtube (Eppendorf) and sonicated for two minutes using a Soniprep 150 (Fisher Scientific UK), at 28 µm amplitude. After immersion in a boiling water bath for 5.5 minutes, the samples were left to cool (Hallsworth and Magan, 1994a) and 667 µl of acetonitrile were added to maintain a 40:60 acetonitrile:water ratio, the same ratio as in the mobile phase of the HPLC. The tubes were then centrifuged for 10 minutes at 1150× g (Microfuge). The supernatant was filtered through 0.2 µm aperture membrane filters (13 mm diameter, Whatman) and injected into a Gilson HPLC for quantification of solutes. The HPLC was fitted with a Hamilton HC-75 Ca^{2+} column, and a refraction index detector. The mobile phase was 40:60 acetonitrile:water and the flow rate was 1 ml min^{-1}. The solutes of particular interest from this analysis were four polyols (glycerol, erythritol, arabitol and mannitol) and one sugar (glucose). Solute quantification was measured using the Gilson software adapted to the HPLC equipment. Peak areas were integrated by the software and amounts expressed as mg g^{-1} fresh biomass based on the calibration curves and the pure sugar alcohols or glucose.

Statistical analysis

Effects of osmotic and matric potential on the radial growth rate in three isolates of P. chlamydosporia

A two-stage modelling approach was used for analysis of fungal radial growth (Mead, Curnow and Hasted, 2003). Firstly, the radii of the colonies were plotted against time for each replicate dish and

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were found to increase linearly. Linear regression was applied to estimate the radial growth rate (mm/day) for each colony. As the second stage of the analysis, the radial growth rates were modelled on the different water potentials for osmotic and matric stress, in separate analyses for each type of stress.

Modification of osmotic potential

A parallel curve regression and exponential model was used to describe the radial growth rates for osmotic stress. The form of this model was

$$Y = a + b \exp(c\,(MPa + 0.7))$$

where Y represents the estimated radial growth rates, $a + b$ represents the radial growth rate in the control condition (MPa = -0.7) and c is the rate of exponential decay in the radial growth rate with decreasing MPa. The statistical significance of differences between the two types of solute used (KCl or glycerol) and the three isolates were then assessed in terms of whether separate values for the *a, b* or *c* parameters were required in the model for the categories of these different factors.

Modification of the matric potential

A parallel lines regression was applied to describe the radial growth rates for matric stress. A linear model and analysis was used to determine whether separate intercept or slope parameters were required for the three isolates.

The estimated MPa for zero radial growth was also estimated using the fitted regression curves or lines.

The radial growth rates at each level of matric or osmotic potential were compared separately by analysis of variance (ANOVA). The data were checked for Normality and constant variance by plotting histograms of residuals and plotting the residuals against the fitted values. No transformation was required. Following ANOVA, least significant differences (LSD) were used to statistically separate the means, at the 5% level of significance. The Genstat® (2007) statistical system was used for all analyses.

Effects of osmotic and matric potential on the accumulation of endogenous reserves in three isolates of P. chlamydosporia

Modification of osmotic potential

For polyol data from HPLC, ANOVA was applied to assess the significance of differences between isolates, media and variation in the osmotic potential. Due to two of the isolates (isolate 10 and 392) having no observations for erythritol and total polyols for the treatment using glycerol, at -2.8 MPa, the method of Residual Maximum Likelihood (REML) was applied (Patterson and Thompson, 1971) as implemented in GenStat (2007). This method takes account of the unbalance,

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while still providing an assessment of treatment effects via approximate F-tests, standard errors of difference (SED) between means and LSD (5%).

Modification of matric potential

Similar analysis was applied to data from the experiment investigating the effect of matric potential in the accumulation of endogenous reserves. In this case, there were no observations for glycerol, at -2.8 MPa, therefore, REML was used. As the polyol data showed a clear skewed distribution, data were transformed to the natural logarithm scale.

Results

Effects of osmotic and matric potential on the radial growth rates in three isolates of P. chlamydosporia

Modification of osmotic potential

The mean radial growth of colonies for each of the three fungal isolates, in response to osmotic stress was found to be linear over time (Figure 1). At -7.1 MPa, none of the *P. chlamydosporia* isolates tested grew in the medium modified with glycerol, but continued growing at this water potential in the medium modified with KCl (Figure 1). Having applied the linear regression model to each replicate, the average of the estimated rates of radial growth of *P. chlamydosporia,* for each combination of solute, isolate and water potential level was calculated (Figure 2). The exponential model revealed significant differences between the solutes KCl and glycerol ($p<0.001$), but no differences ($p>0.05$) were found in the rates of decrease for the three isolates (Figure 2). The model shows that the exponential rate of decrease in radial growth using KCl (0.115) was approximately half of the rate of decrease using glycerol (0.228). Analysis of Variance of the estimated radial growth rates at different osmotic potentials showed a three-way interaction between solute, isolate and MPa level ($p<0.001$); although the overall main effect of isolate was not significant ($p=0.759$), which supports the result found using the exponential model.

Modification of matric potential

When PEG 8000 was added to the medium for modification of the matric potential, the variation in the radial growth rates with decreasing MPa was linear ($p<0.001$), and separate lines for isolates were fitted (Figure 3). The Analysis of Variance of radial growth rates for matric potential gave a significant interaction between isolate and MPa level ($p<0.001$), with both main effects also being significant ($p<0.006$).

Given the models, the limits of radial growth potential were estimated (Table 1). The fungus was generally more tolerant to the ionic solute KCl than to glycerol. In matrically modified media, the limits for growth differed from -5.16 ± 0.19 to -6.38 ± 0.39 MPa, depending on the

isolate (Table 1). Chlamydospores were produced by all isolates in the unmodified medium but few or none were produced at lower osmotic potentials (Table 2).

Effects of osmotic and matric potential on the accumulation of endogenous reserves in three isolates of P. chlamydosporia

Modification of osmotic potential

The total amounts of polyols accumulated intracellularly were greater and significantly different in osmotically modified media ($p<0.05$, using LSD), when compared with those endogenous reserves accumulated in unmodified media, with the exception of media modified with KCl at -1.4 MPa (Table 3-A). However, no significant differences in the amounts of total polyols were found between isolates in response to modification of the osmotic potential ($p=0.952$).

The proportion of polyols accumulated by the fungus depended on the solute used to generate the water stress (Figure 4). When grown in media osmotically modified with glycerol, the fungus accumulated proportionately greater amounts of glycerol compared to the other polyols and in the control medium (Figure 4). In media osmotically modified with KCl, the accumulation of arabitol at -1.4 and -2.8 MPa was proportionately greater than in unmodified media (Figure 4). In the media modified with glycerol, greater amounts of glucose were detected, compared with the amounts accumulated in colonies growing in media where water was freely available (Table 3-A). However, fungal growth in media modified with KCl did not result in a significant change in the accumulation of glucose ($p>0.05$) (Table 3-A). There was a highly significant interaction ($p<0.001$) between isolates, media and level of water potential in the accumulation of arabitol and mannitol. Comparing specific quantities (on the natural logarithm scale), there was a greater ($p <0.05$) accumulation of arabitol at -2.8 and and -7.1 MPa, in all isolates compared to the control (Table 4-A). However, no significant differences ($p>0.05$) between isolates were found in the accumulation of glycerol and erythritol (Table 4-A).*Modification of matric potential*

The quantity of polyols accumulated by the fungus in media modified with PEG was greater and significantly different ($p<0.05$) compared to that accumulated in unmodified media, in all the isolates tested (Table 3-B). Significant differences in the amounts of total polyols were found between isolates in response to matric stress levels ($p<0.001$). However, the three-way interaction between isolates, matric stress levels and solute was not significant ($p=0.247$). The total amounts of polyols accumulated were greatest when the fungus grew at water potentials of -4.2 MPa, in media amended with PEG (Table 3-B). In matrically modified media, a large quantity of erythritol was accumulated in all the isolates, and this increased substantially with the reduction of the water potential (Figure 4-B). There was a significant interaction ($p<0.001$) between isolates and level of water potential in all types of polyols (Table 4-B).

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Discussion

For the first time, the effects of osmotic and matric potential on radial growth and accumulation of polyols were studied in colonies from different isolates of *P. chlamydosporia*. In this study, the fungus grew faster in a non-modified medium, when water was freely available (-0.7 MPa). Similar results were found by Kerry *et al.*(1986), who studied the effects of pH and water potential on the growth of six isolates of the fungus on a basal medium amended with a mixture of salts or with sucrose. However, matric stress, a component of the water potential relevant to growth and colonisation in soil (Brownell and Schneider, 1985), was not considered in their study. In the present study, radial growth rates decreased substantially with the increase of osmotic or matric stress. Although radial growth rates were lower in PEG-modified media, the fungus was relatively tolerant to matric stress. Interestingly, differences between isolates were found in response to matric stress on the PEG-modified media but isolates responded similarly on the medium modified with the ionic and non ionic solute, used to subject the fungal isolates to osmotic stress. Tolerance to matric stress may have been related to the accumulation of high amounts of erythritol in PEG-modified media, which enables enzyme systems to work effectively in such conditions. Low-molecular weight erythritol is known to regulate intracellular water potential efficiently, and is accumulated by xerophylic fungi in response to water stress (Hallsworth, 1995).

All the isolates tended to be more sensitive to the non-ionic solute glycerol than to the ionic KCl. At -7.1 MPa, the fungus was able to continue growing in a medium amended with the ionic solute, but not in a medium amended with glycerol at this level of MPa. Low concentrations of ionic solutes, such as KCl, may stimulate growth in some fungi (Larsen, 1986), as K ions aid transport across the mycelial cell walls to enable better intracellular osmotic adjustment. Therefore, it is possible that KCl from the medium may have been utilised to provide increased tolerance to osmotic stress.

In media modified with glycerol, *P. chlamydosporia* accumulated large amounts of glycerol, which were much greater than in unmodified media. This accumulation was possibly a response to regulate intracellular osmotic potential. As glycerol was used to modify the medium, it may have been passively accumulated by the fungus which in turn helped it to tolerate the increase in stress. Glycerol appears to be of crucial importance in many fungi, as it protects enzymes from accumulation of sodium and from loss of water, both of which may denature the enzymes (Luard, 1982).

The quantities of the major sugar alcohol accumulated in colonies of *P. chlamydosporia* depended on the solute used to generate the water stress. Whereas arabitol and glycerol were the

main polyols accumulated in osmotically modified media with ionic and non-ionic solutes, respectively, erythritol was the main sugar accumulated in media amended with PEG.. The versatility to accumulate different polyols and perhaps different sugars under different stress conditions might bring advantage of allowing the fungus to survive in soil when environmental conditions are less favourable or highly variable. In some fungi, enhanced accumulation of compatible solutes contributed to an improvement in viability (Hallsworth and Magan, 1995, Mokiou, 2005, Teixidó et al., 2005) and even virulence (Chandler et al., 2005). Therefore, the viability of the spores produced under different stress regimes might have been different, although this aspect was not covered in the study, as few chlamydospores were produced on osmotically modified media, after 25 days of growth (Table 2). As chlamydospores are the preferred type of inoculum to establish the fungus in soil (De Leij and Kerry, 1991) it was considered that any improvement through the incorporation of polyols could be greatly outweighed by the reduction in chlamydospore production. To conclude, the presence of free water available in the agar media favoured the growth of *P. chlamydosporia*, when compared to growth rates in media where water was restricted. Fungal growth was affected by both osmotic and matric stress, although this species can tolerate matric stress levels relevant to those that occur in soil. Under water stress, the fungus accumulated a combination of different sugar alcohols important in osmoregulation, the differential and proportional amounts of which were dependent on the solute used. The research presented in this paper provides some new information suggesting the involvement of different mechanisms of osmoregulation in *P. chlamydosporia* as a response to osmotic and matric stress on mycelial growth and the accumulation of endogenous reserves in spores. Further research is needed to study the effects of such changes on fungal survival and activity in soil.

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Figure 1. Effect of osmotic potential (glycerol and KCl modified media) on the average radial diameter (mm) over time (days) in three isolates of *Pochonia chlamydosporia*; [isolates 10 (A, B), 280 (C, D) and 392 (E, F)], at 25°C; (×, -7.1 MPa; ▲, -2.8 MPa; ■, -1.4 MPa and ♦, -0.7 MPa). Solid lines show the fitted regressions through the means.

Figure 2. Comparison of radial growth rates (mm/day) in three isolates of *Pochonia chlamydosporia* in response to osmotic potential (MPa). [● – glycerol, isolate 10; ■ – glycerol, isolate 280; ▲- glycerol, isolate 392; ○- KCl, isolate 10; □ - KCl, isolate 280; Δ - KCl, isolate 392; — -Fitted curve for glycerol: Y = -0.294 + 1.402 *exp*(0.228 (*MPa* + 0.7)); ---- -Fitted curve for KCl: Y = -0.294 + 1.402 *exp*(0.115 (*MPa* + 0.7)); R^2 for model = 74.4%, s^2 = 0.042, df =99]. The least significant difference (LSD) at the 5% level for comparisons of means is taken from the ANOVA of these data.

Figure 3. Comparison of radial growth rates (mm/day) in three isolates of *Pochonia chlamydosporia* in response to matric potential (MPa). [● - PEG, isolate 10; ■ - PEG, isolate 280; ▲- PEG, isolate 392; — - Fitted line for isolate 10: Y = 1.677+ 0.323 *MPa*; ----- -Fitted line for isolate 280: Y = 1.429 + 0.241 *MPa*; ····· -Fitted line for isolate 392: Y= 1.327 + 0.208 *MPa*; R^2 for model = 92.7%, s^2 = 0.010, df =40]. The least significant difference (LSD) at the 5% level for comparisons of means is taken from the ANOVA of these data.

Figure 4. Relative proportion (%) of polyols [▨ - glycerol, ■ - erythritol, ▥ - arabitol and □ - mannitol] accumulated in biomass of three isolates of *Pochonia chlamydosporia* [A- isolate 10, glycerol; B-isolate 10, KCl; C- isolate 280, glycerol; D- isolate 280, KCl, E- isolate 392, glycerol; F- isolate 392, KCl; G- isolate 10, PEG; H- isolate 280, PEG; I- isolate 392, PEG], after 25 days of growth, at 25 ºC.

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Table 1. Estimated limits of water potential (MPa) required for *Pochonia chlamydosporia* radial growth, in different solutes with standard errors (SE).

Table 2. Mean and standard error of mean (SEM) number of chlamydospores produced per Petri dish, in each treatment, after 25days of growth, at 25ºC.

Table 3. Effect of ionic (KCl) and non ionic (glycerol) osmotic potential (A) and matric potential (B) on the intracellular accumulation of total polyols and glucose in three isolates of *Pochonia chlamydosporia*, at 25ºC. Means in each column for statistical comparison given the ANOVAs are followed by the least significant difference (LSD) at the 5% level of significance. Differences between isolate were not statistically significant ($p>0.05$) for total polyols, so means for comparison in this case (in bold) are taken across isolate.

Table 4. Effect of ionic (KCl) and non ionic (glycerol) osmotic potential (A) and matric potential (B) on the intracellular accumulation of glycerol, erythritol, arabitol and mannitol in three isolates of *Pochonia chlamydosporia*, at 25ºC. Means in each column for statistical comparison given the ANOVAs are followed by the least significant difference (LSD) at the 5% level of significance. Differences between isolate were not statistically significant ($p>0.05$) for glycerol and erythritol, so means for comparison in this case (in bold) are taken across isolate.

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Table 1

Isolate	Solute	Limit of water potential required for growth (MPa)†	SE
All	KCl	-14.27	2.55
All	Glycerol	-7.57	0.89
10	PEG	-5.16	0.19
280		-5.94	0.31
392		-6.38	0.39

†- As estimated from the fitted models (see figures 2 and 3)

Table 2

Water potential (MPa)	Solute	Isolate					
		10	SEM	280	SEM	392	SEM
-0.7	Unmodified	4250	250	9300	500	2200	100
-1.4	Glycerol	100	50	0	*	0	*
-2.8		0	*	0	*	0	*
-7.1		†		†		†	
-1.4	KCl	250	50	1250	350	450	50
-2.8		0	*	0	*	350	150
-7.1		0	*	0	*	0	*

† No growth

* No standard error as 0 chlamydospores were always produced.

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Table 3

(A) Amount (mg/g) mycelial colonies (Log-transformed)

Isolate	Water potential (MPa)	Solute	Total Polyols (df=30)	Glucose (df=34)
10	-0.7	Unmodified	9.26	8.14
	-1.4	Glycerol	10.31	9.86
	-1.4	KCl	9.38	9.22
	-2.8	Glycerol	ND†	10.38
	-2.8	KCl	10.11	8.57
	-7.1	KCl	10.30	8.50
280	-0.7	Unmodified	9.07	7.69
	-1.4	Glycerol	10.88	9.56
	-1.4	KCl	9.19	7.94
	-2.8	Glycerol	ND†	8.91
	-2.8	KCl	9.50	7.98
	-7.1	KCl	10.09	8.58
392	-0.7	Unmodified	8.96	7.70
	-1.4	Glycerol	10.63	9.60
	-1.4	KCl	9.27	7.75
	-2.8	Glycerol	ND†	8.63
	-2.8	KCl	9.75	8.12
	-7.1	KCl	10.85	9.76
LSD (5%)				1.000
	-0.7	Unmodified	**9.10**	
	-1.4	Glycerol	**10.61**	
	-1.4	KCl	**9.28**	
	-2.8	KCl	**9.79**	
	-7.1	KCl	**10.41**	
LSD (5%)			0.361	

†ND- Not determined

(B) Amount (mg/g) mycelial colonies (Log-transformed)

Isolate	Water potential (MPa)	Solute	Total Polyols	Glucose
10	-0.7	Unmodified	9.26	8.14
	-1.4	PEG	10.39	9.77
	-2.8	PEG	11.27	10.81
	-4.2	PEG	12.48	9.49

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280	-0.7	Unmodified	9.07	7.69
	-1.4	PEG	9.89	8.56
	-2.8	PEG	11.09	10.35
	-4.2	PEG	12.27	10.81
392	-0.7	Unmodified	8.96	7.70
	-1.4	PEG	9.45	8.99
	-2.8	PEG	11.30	9.92
	-4.2	PEG	11.62	9.54
LSD (5%) (df=22)			0.422	0.797

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Table 4

(A)

Amount (mg/g) mycelial colonies (Log- transformed)

Isolate	Water potential (MPa)	Solute	Glycerol (df =35)	Erythritol (df =28)	Arabitol (df=36)	Mannitol (df=35)
10	-0.7	Unmodified	7.43	7.68	7.46	8.47
	-1.4	Glycerol	9.73	7.75	7.49	9.06
	-1.4	KCl	5.43	7.87	8.06	8.34
	-2.8	Glycerol	12.8	9.45	8.49	9.73
	-2.8	KCl	7.86	8.09	9.58	8.19
	-7.1	KCl	7.87	10.49	8.63	8.19
280	-0.7	Unmodified	7.75	7.72	7.46	7.76
	-1.4	Glycerol	10.46	8.07	7.78	8.75
	-1.4	KCl	7.91	7.64	7.80	7.77
	-2.8	Glycerol	12.19	8.79	8.20	9.06
	-2.8	KCl	7.59	7.69	8.81	7.79
	-7.1	KCl	7.9	9.39	8.24	8.31
392	-0.7	Unmodified	7.38	7.66	7.49	7.73
	-1.4	Glycerol	10.40	7.95	7.63	8.07
	-1.4	KCl	8.03	7.68	7.83	7.77
	-2.8	Glycerol	12.61	8.73	7.79	8.10
	-2.8	KCl	7.62	7.93	9.21	8.08
	-7.1	KCl	8.60	9.14	10.14	8.98
LSD (5%)					0.676	0.582
	-0.7	Unmodified	**7.52**	**7.69**		
	-1.4	Glycerol	**10.12**	**7.93**		
	-1.4	KCl	**7.92**	**7.73**		
	-2.8	Glycerol	**12.53**	ND[†]		
	-2.8	KCl	**7.69**	**7.90**		
	-7.1	KCl	**8.12**	**9.67**		
LSD (5%)			0.472	0.373		

[†]-Not determined

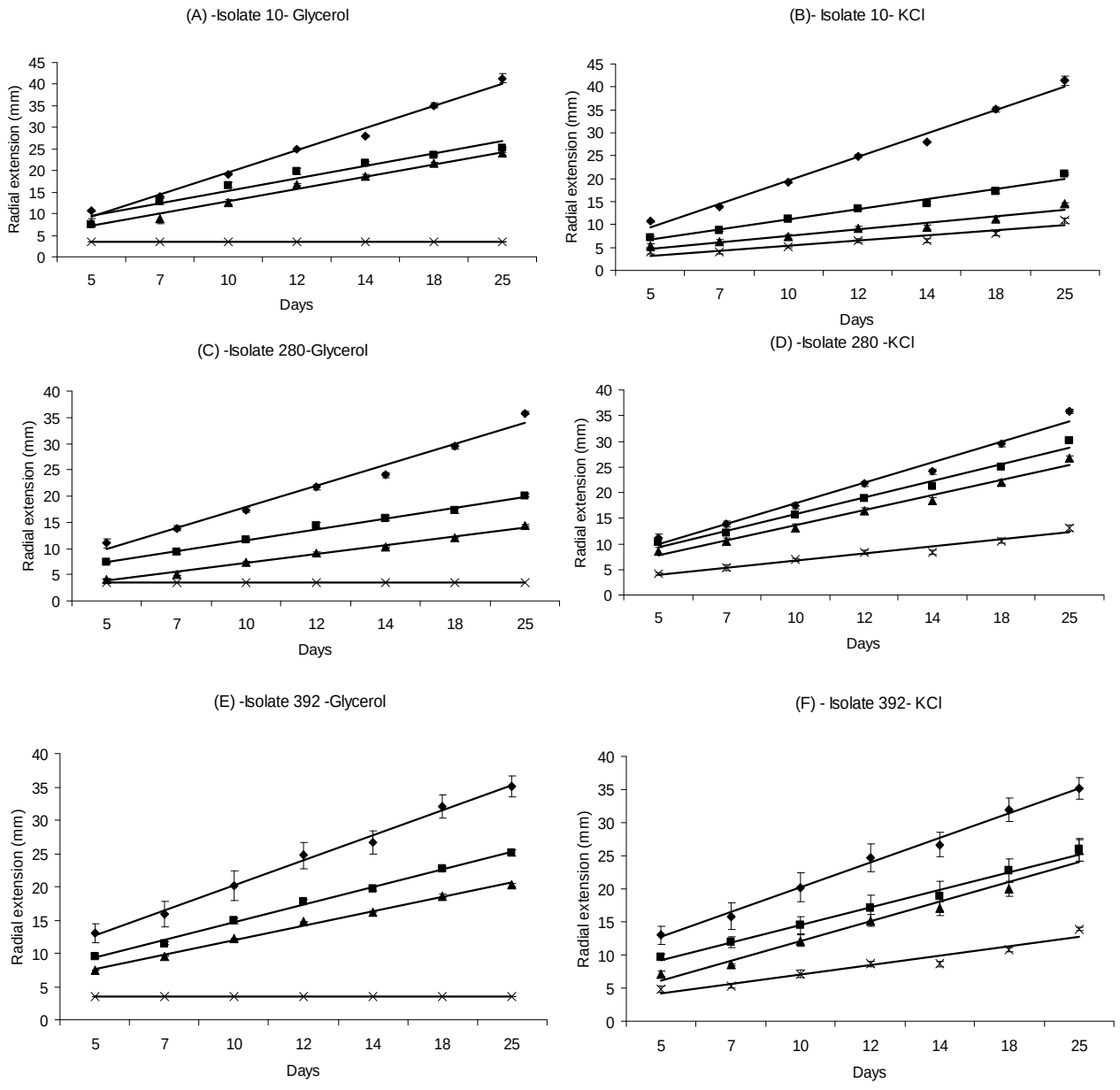
(B)

Isolate	Water potential (MPa)	Solute	Glycerol (df=14)	Erythritol (df=22)	Arabitol (df=22)	Mannitol (df=22)
10	-0.7	Unmodified	7.43	7.68	7.46	8.47
	-1.4	PEG	7.38	10.12	7.97	7.83
	-2.8	PEG	8.36	11.21	7.69	7.74
	-4.2	PEG	8.38	12.43	8.41	8.36
280	-0.7	Unmodified	7.75	7.72	7.46	7.76
	-1.4	PEG	7.47	8.68	7.47	9.20
	-2.8	PEG	ND[†]	10.91	8.17	8.52
	-4.2	PEG	7.69	12.23	7.80	7.96
392	-0.7	Unmodified	7.38	7.66	7.49	7.73
	-1.4	PEG	7.45	7.81	8.26	8.31
	-2.8	PEG	7.51	10.75	10.03	9.02

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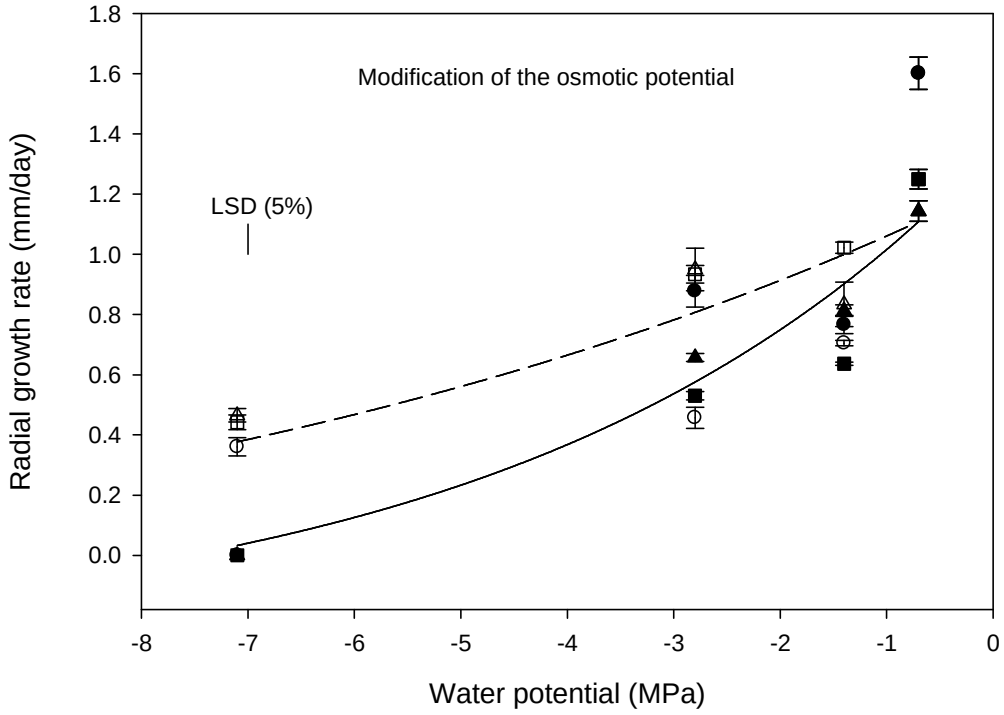
	-4.2	PEG	7.46	10.87	10.48	9.99
LSD (5%)			0.311	0.478	0.645	0.640

Figure 1



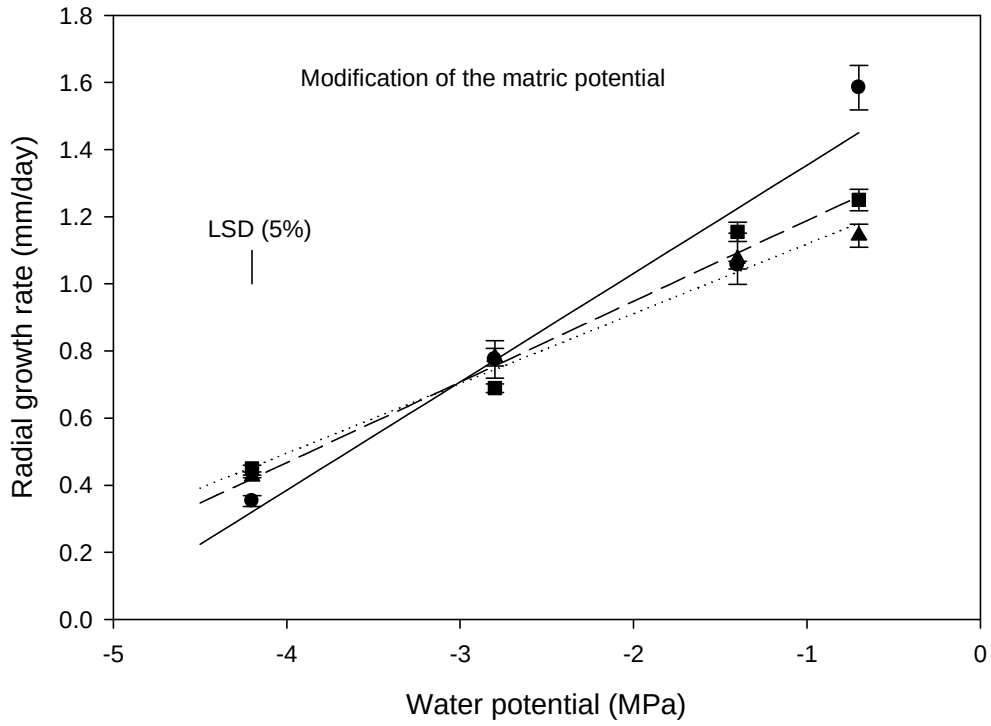
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Figure 2



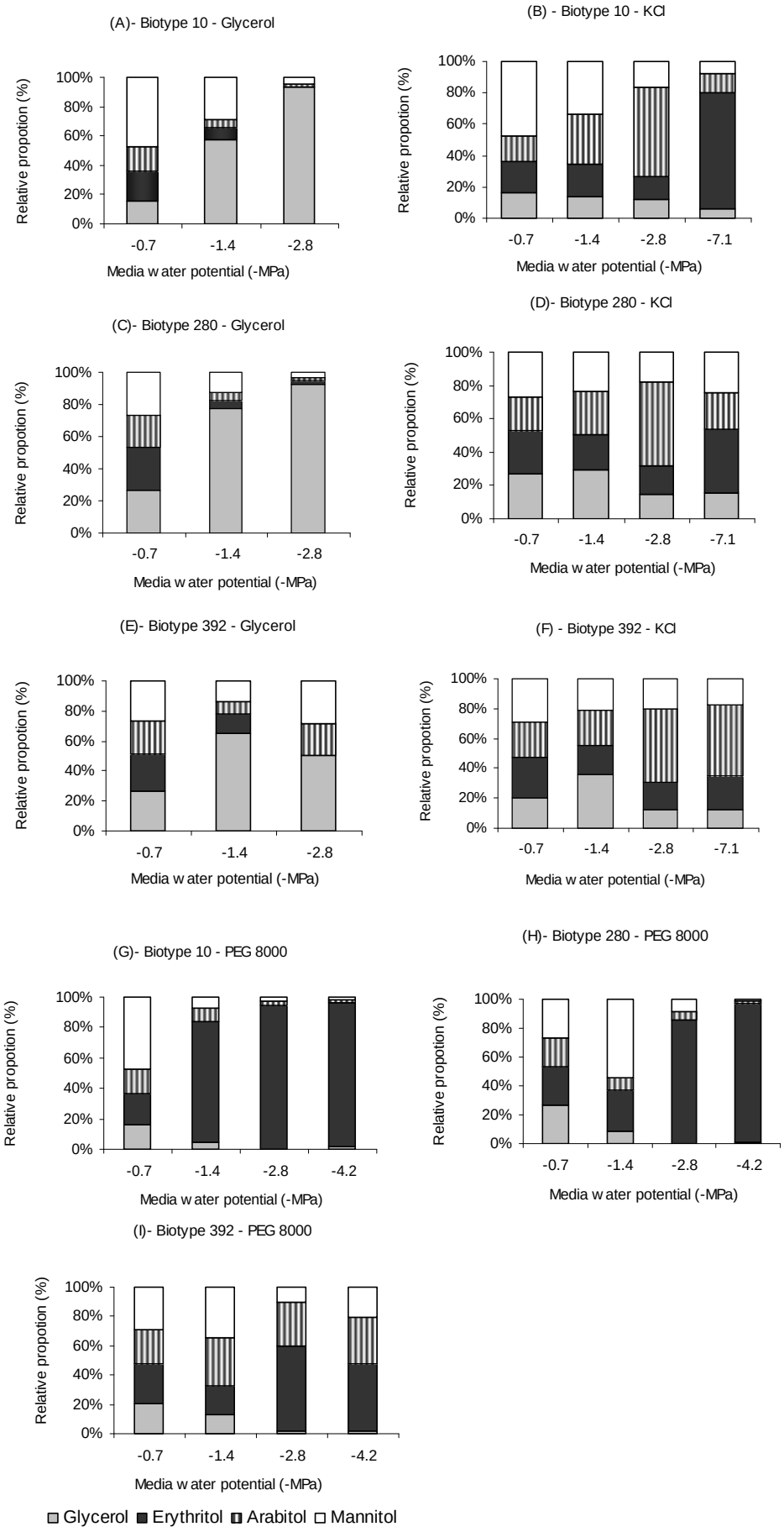
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Figure 3



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