Root responses to soil physical constraints: Quantitative gene expression analysis

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ABSTRACT

Root elongation and exploration can be constrained by soil that is too hard for roots to penetrate through, soil that contains too much water (which results in hypoxyia due to too little oxygen) or because as the soil dries the water potential falls. Studies of the impact on gene expression in relation to soil water, soil strength and porosity are limited, partly because of the difficulty in doing *quantitative* studies on roots that have been *grown in soil*. Many drought gene expression studies use root material grown in hydroponics containing osmotic compounds to simulate drought conditions, while other more recent studies have used material grown in soil amended with sand and peat.

We have harvested seedling root material of Barley (*Hordeum vulgare*) plants grown in a wide range of soil conditions. Soil conditions have been quantified (e.g soil strength, soil macroporosity, water content) using measurements related to the least limiting water range approach. Using quantitative RT-PCR we are analysing the gene expression of DHN4 (a gene previously shown to be up regulated in drought conditions), an aquaporin and other candidate genes. Future work may include candidate genes that are potentially involved in root penetration, such as those involved in the production of mucilage and border cells.

Key words: Barley, Soil strength, Drought tolerance, qRT-PCR, Least limiting water range.

1 INTRODUCTION

Plant roots can experience a range of physical constraints during growth in soil. Since soil strength is a result of both the intrinsic matrix and its water content, soil strength will vary depending on rainfall patterns. To separate plant responses to specifically a lack of water rather than responses to soil strength the underlying physics of the soil needs to be explored. Using the context of the least limiting water range (Leao, 2004), which allows the calculation of matic potential limits to root growth such as soil strength (2MPa), wilting point (-1.5MPa), and oxygen deficiency (10% air filled porosity) we are exploring root molecular responses to soil physical constraints. Figure 1.shows an example result of the least limiting water range calculations using the spreadsheet provided by Leao, 2004. The calculations suggest that for this particular soil there are combinations of bulk density and water content, that would expose roots to water deficiency but not to growth restriction by soil strength, or combinations where roots would be exposed to restriction by soil strength but they would not be exposed to water stress. This paper aims to demonstrate the quantification of the gene expression of a limited group of aquaporins in root samples grown in soil with quantified physical properties.

2 METHODS

2.1 Soil preparation

Sandy loam soil was collected from two separate plots, part of a tillage trial at SCRI Invergowrie, Dundee, UK. Plots were under continual winter barley cultivation and cultivated by ploughing to

a standard depth of 20cm followed by compaction (Compacted) or were direct drilled (No-till). Soil cores were equilibrated to known matric potential using tension tables.

2.2 Plant Growth

Hordeum vulgare cv Pastoral grain were incubated between several layers of damp filter paper at 15^{o}C for 2-3 days until root length had reached between 5 and 10 mm long. Seedlings were stored at 4^{o}C until required. For growth experiments, seedlings were transferred to a small hole in the top of the soil cores, each was sealed in an individual plastic bag to prevent drying. Cores containing seedlings were transferred to a growth chamber at 15^{o}C for 48 hours. Plant roots were extracted from the soil cores, the longest root was measured, and all roots were frozen in liquid N_2 for molecular analysis.

2.3 Soil Physical measurements

Penetrometer resistance was measured using a 1 mm diameter penotrometer probe at a rate of 4 mm/min, for 12 cores/ per treatment (readings averaged 5 mm to 15 mm depth).

2.4 Quantitative analysis

RNA was extracted from frozen root tissue using Promega PolyAtract mRNA extraction kit. cDNA production was performed using superscript II. Quantitative PCR was performed on a Roche 2. LightCycler. The control gene used in this study was GAPDH. Primers were designed against sequence homology between HvPIP2;1 AB009307, HvPIP1;3 AB009308 (Katsuhara, 2002).

3 RESULTS AND DISCUSSION

3.1 Soil physical properties

Figure 2 shows the changes in physical properties for two soils, compaction and No-till across a range of water potentials. Penetrometer resistance was significantly affected by both the matric potential and by the soil tillage treatment (Figure 2A. $P<0.001$, $P<0.001$). The No-till had statistically significantly lower penetrometer resistance, than the compacted soil at all matric potentials except for at -10kPa. The water release characteristics did not differ significantly between the soils ($p=0.820$, Figure 2B). In contrast, the air filled porosity of the soils was significantly greater for the No-till soil ($p=0002$, Figure 2C)

The two soils impeded root growth compared with the maximum root growth achieved at -5kPa for the compacted soil and in the saturated soil for the No-till. Root growth was signifcantly impeded due to both the reduction in water availability (more negative matric potential, $p<0.001$) and between the two soils ($p=0.023$). Despite having a lower overal soil strength, the No-till soil impeded root growth more at the same matric potentials. Although differences in the volumetric water content was limited between the two soils, significant variation in airfilled porosity was found. Both soils had less than 10% airfilled porosity for the saturated treatment.

3.2 Quantitative analysis

It was not possible to accurately quantify the level of expression in the Saturated No-till samples due to low overall RNA recovery. However the relative expression levels of the aquaporin RNA in all the other samples are shown in Figure 3. Overall there was statistically significant increase in expression as soil dried ($p=0.008$). There was no significant difference between the soils ($p=0.1$). Previously Katsuhara, 2002 found that HvPIP2-1 and HvPIP1;3 transcripts increased

over time in barley shoots exposed to salt stress, whereas transcript levels remained constant. However they also showed a reduction in PIP2;1 protein in roots in response to salt stress. Further work is now underway to explore these relatioships in more detail.

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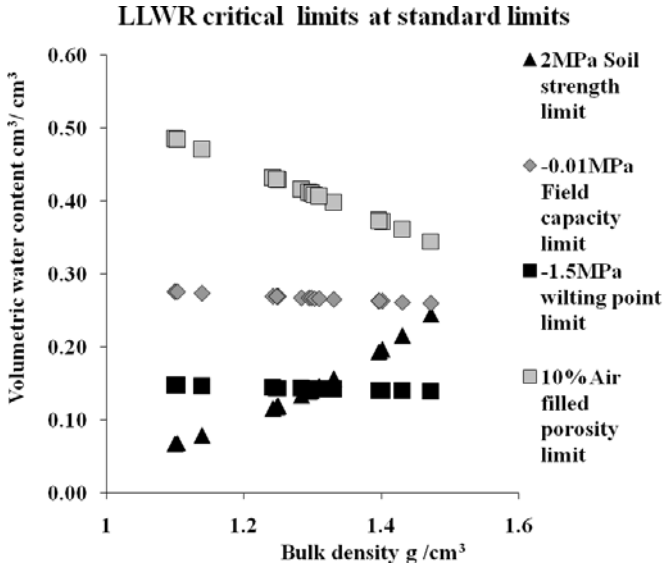

Figure 1. Least limiting water range for repacked cores from plough sandy loam soil

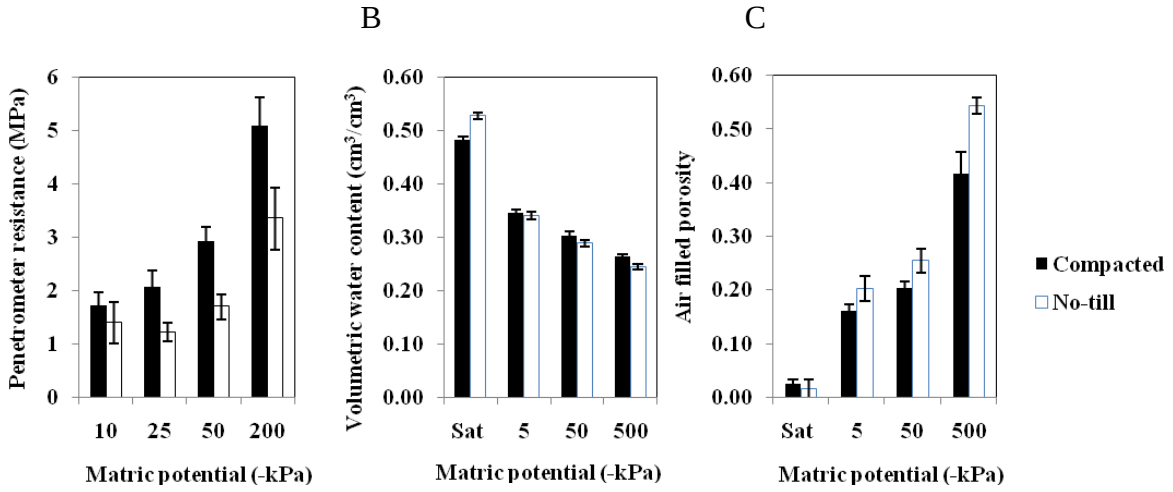

Figure 2. Physical properties of Compaction and No-till soil at a range of soil water potentials. A: Penetrometer resistance, B: Volumetric water content, C: Air filled porosity.

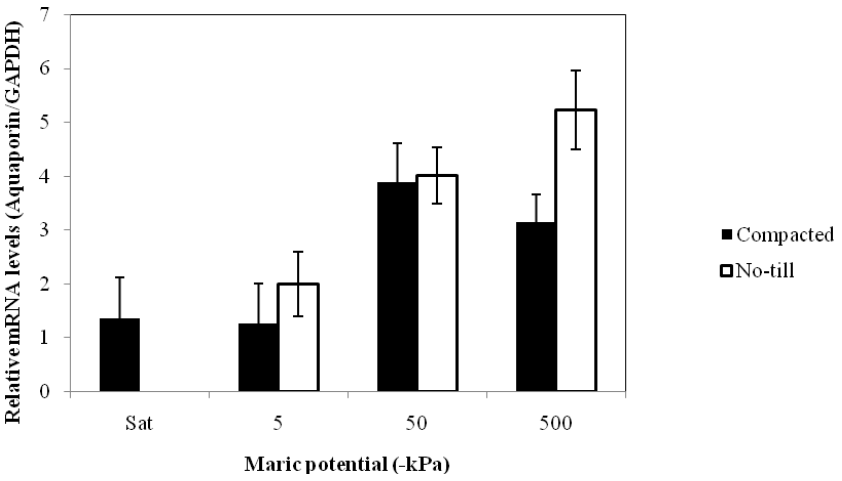

Figure 3. Relative expression of an aquaporin, control GAPDH.

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