## Title

Impact of soil tillage on the robustness of the genetic component of variation in phosphorus (P) use efficiency in barley (Hordeum vulgare L.)



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## [1] Introduction

Phosphorus (P) is an essential plant nutrient that limit agricultural production on a global scale. It is therefore desirable that the P-use efficiency of agricultural plants be improved. Many plant species are adapted to P-deficiency and have developed a range of mechanisms that enhance their ability to acquire P from soil (White et al. 2005; Vance et al., 2003). These include modifications to root structure (Hammond & White, 2008), the formation of symbioses with mycorrhizae (Smith & Read, 1997), and the production of root exudates such as organic anions (Ryan et al., 2001) and phosphatase enzymes (Tadano et al., 1993; George et al., 2002). P-use efficiency is therefore a multi-mechanistic trait whose genetic control is likely to be greatly affected by environment (George et al. 2008) Although genotypic variation in P-use efficiency of cereals is reported (Batten & Khan, 1987; Jones et al., 1989; Jones et al., 1992; Gahoonia & Nielsen, 1996; Manske et al., 2000; Osborne & Rengel, 2002a,b; Wang et al., 2005), few studies have investigated this variation in contrasting environmental conditions and the robustness of the genetic component of variation is rarely tested. In this paper we establish the P-nutrition characteristics of barley associated with sufficiency then describe a study of the P-use efficiency in an association mapping population of both winter and spring barley. This paper investigates the impact of a tillage treatment on the robustness of the genetic component of the variation between barley genotypes.

## [2] Materials and Methods

### *P-response of barley*

Topsoils (0-10 cm depth) were collected from a site near SCRI, Dundee, Scotland, which were typical of arable soil of the region and defined as a Cambisol (FAO-Unesco classification). Soils were air-dried, mixed and passed through a 2 mm sieve to remove coarse material and vegetative matter. The soils were either left un-amended or fertilized with inorganic phosphate ($KH_2PO_4$) at four different rates, 100, 250, 500 and 1000 mg P $kg^{-1}$ soil. Soils were mixed with fertilizer in 20 kg lots in a cement mixer at approximately 75 rpm for 30 minutes. All soils were then watered to and maintained at 80% field capacity as determined by gravimetric water content and incubated at ambient temperature for 28 days in the glasshouse prior to planting. Three replicate pots each containing the equivalent of 0.75 kg of air-dried soil of the five soil P treatments were sown with four surface-sterilised seeds of the local standard barley (*Hordeum vulgare* L.) variety (cv Optic). Uniform-sized seeds were germinated prior to planting until their radicles were between 5 and 10 mm long. The seedlings were subsequently thinned to two uniform plants per pot one week after planting. The soils were maintained at ~80% field capacity during the growth period and all nutrients, except P, were provided weekly by addition of 5 mL $pot^{-1}$ of a nutrient solution (3 mM $NH_4SO_4$, 2 mM $KNO_3$, 1 mM $MgSO_4$, 10 mM $Ca(NO_3)_2$, 80 μM FeEDTA, and micronutrients [B, Cu, Mn, Zn, Mo and Co]). Plants were grown in a randomized design in a glasshouse at 22/14 °C (day/night) with an approximate 16 h day-length. Pots were rotated between glasshouse benches regularly to reduce effects of possible environmental gradients. Plants were harvested after 42 d growth and shoot biomass determined after oven drying for one week at 65 °C.

### *Screening of association mapping population for shoot P concentration under different cultivation treatments*

An association mapping population was grown in the field under two different cultivation treatments of a long-tern field trial. Soil conditions were imposed over a number of years in a field trial located on the SCRI site near Dundee, Scotland (Newton et al, 2008). The soil was a Cambisol with a sandy-loam surface texture. It had a pH of 5.7, was freely drained and underlain

by colluvial sand at 60 cm depth. To reduce in-field variability the entire site was initially ploughed to 20 cm, power harrowed and sown with a single spring barley variety (Optic) in 2003. Five cultivation treatments were established in triplicate in autumn 2003 that imposed different levels of soil disturbance ranging from light to heavy disturbance in the order: (1) zero-tillage, (2) minimum tillage to 7 cm depth, (3) conventional plough to 20 cm depth, (4) plough to 20 cm followed by compaction and (5) deep plough to 40 cm depth. These treatments provide different physical constraints to root impedance and water availability. Differences in root impedance have been demonstrated to have a major impact on penetration resistance (Hallett et al. unpublished). Fifteen blocks measuring 33 x 33m were marked out in an even grid with five blocks in each of three north-south columns representing the three treatment replicates. Blocks were separated from each other by 3m wide strips which were sown with grass seed after the first trial year was sown. Within each block, trial plots measured 1.55m wide by 2.1m long and were sown at 250 seed $m^{-2}$ in early November 2007 or at 360 seed $m^{-2}$ in early April 2008 for winter and spring varieties, respectively. Fifty-six lines of winter barley and 64 lines of spring barley, identified as a population useful for association genetics (Thomas et al., unpublished), were sown to each cultivation treatment. Prior to the year of sampling, the trial was sown in three successive seasons. The data presented in this paper were derived from samples taken from only the plough treatment, which represents the typical agricultural practice for the region, and minimum tillage, which is gaining popularity because of fuel costs and perceived benefits to soil conservation.

***Shoot sampling and P analysis***

Flag leaf samples were taken at growth stage 21 from both barley plants grown in pots and each genotype grown in the field and samples were kept on ice until they could be frozen. Samples were freeze dried before milling to a flour. Phosphorus concentration in shoots was determined on milled samples. One hundred mg of shoot material was digested in 5 mL of 18 M $H_2SO_4$ at 360 °C for 20 mins, after which an excess of 30% hydrogen peroxide was added until digests cleared (Heffernan, 1985). Concentrations of P in diluted digests were determined by reaction with malachite green (Irving & McLauglin, 1990).

***Data calculation and statistical analysis***

All data are presented as the mean of three replicates and bars represent standard errors of the mean. Significant differences were established using ANOVA, and treatment means compared by LSD (P = 0.05) (Genstat v9; Rothamsted Experiment Station, U.K.). Relationships between both shoot biomass and P concentration in the glasshouse experiment were related to the P added using regression. From these regression it was possible to determine the critical P concentration for barley to reach 75% growth in this soil. Mean data from the different cultivation treatments within each population of genotypes were compared using regression and $r^2$ and 95% confidence intervals derived. The critical P concentration to achieve 75% growth was also plotted. All data were tested for normality prior to analysis.

**[3] Results**

The shoot dry weight of spring barley cultivar Optic increased significantly ($p<0.05$) to an asymptote with increasing P addition to the soil (Figure 1A). This growth response was described by an exponential rise to a maximum of the form $y = a + b(1-\exp^{(cx)})$. Using this equation it was possible to calculate the amount of P that needs to be added to this soil to achieve a critical proportion of maximal growth. For the purposes of this experiment 75% of maximal growth was used to define the critical growth of the barley plants, which was achieved by an addition of 175 mg P $kg^{-1}$ soil. The shoot P-concentration of these plants also increased

significantly ($p<0.05$) with the addition of P to soil (Figure 1B), however this did not reach an asymptote with the relationship being described by a linear equation ($y= a + bx$). This allowed the calculation of the critical P concentration in the shoots to achieve 75% growth which, on this soil, was 2.56 µg P $g^{-1}$ DW. These results demonstrate that soils typical of the arable north east of Scotland are responsive to the addition of P when barley is grown and that reasonably large additions of P are required to achieve growth approaching the physiological maximum for the barley.

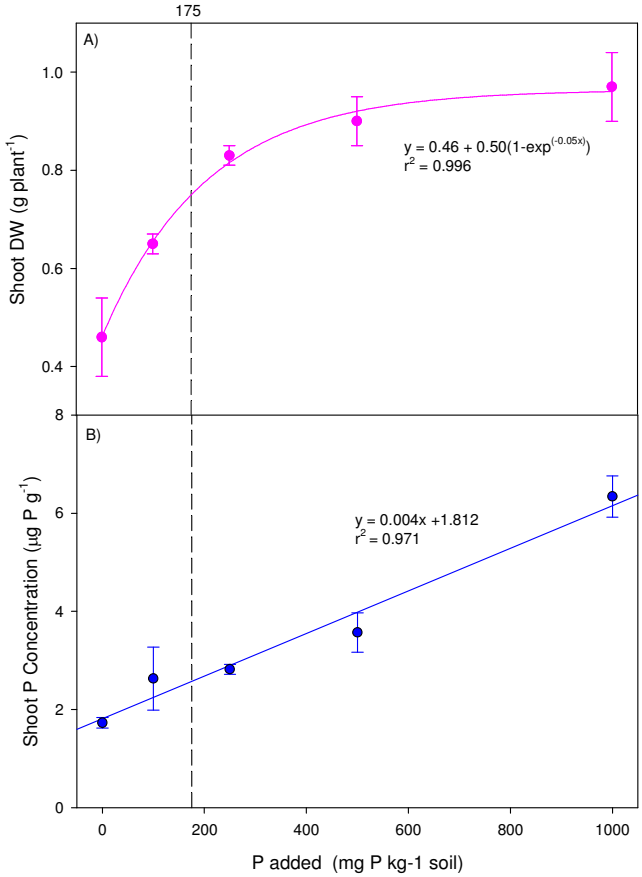


**Figure 1.** Response of (A) shoot biomass and (B) shoot P concentration of young barley plants to increasing amount of P-added to soil in a glasshouse experiment. Values are the mean of three replicates and bars represent standard errors of the mean. The amount of P added to achieve 75% maximal growth on this soil is calculated to be 175 mg P $kg^{-1}$ soil. The critical shoot P concentration to achieve this growth is calculated to be 2.56 µg P $g^{-1}$ DW.

When grown in the field, winter barley genotypes had significantly ($p<0.05$) greater P concentration in flag leaves than spring barley genotypes (Figure 2). This may be an indication of the greater root growth associated with winter germplasm (Hallet et al., unpublished) which would allow greater access to P resources. Within the spring and winter genotypes there was significant ($P<0.05$) variation in shoot P concentration ranging from 1.7 to 3.0 and 1.5 to 2.5 µg P $g^{-1}$ DM, respectively (Figure 2). Of particular interest was that some of the winter barley genotypes (Avenue, Gleam, Estrel, Pict and Magie) were able to achieve the critical shoot P concentration necessary to achieve 75% maximal growth in this soil under conventional cultivation. In contrast none of the spring genotypes were able to achieve this. Moreover, none of either winter or spring varieties passed the critical level in the minimum till cultivation treatment.

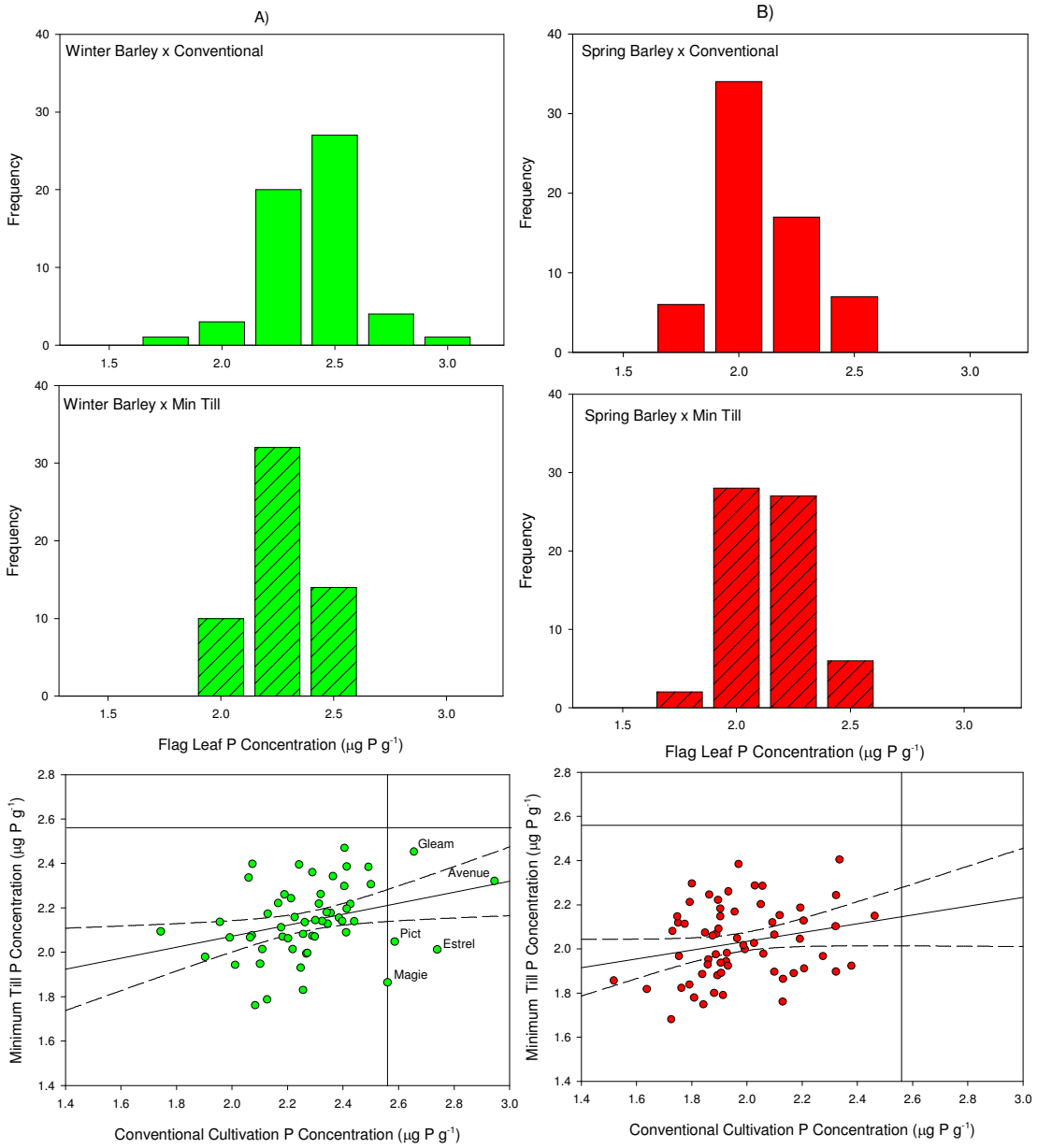


**Figure 2.** Frequency histograms of shoot P concentration (µg P $g^{-1}$ DM) for (A) winter barley genotypes (n = 56) and (B) spring barley genotypes (n = 64) grown under conventional plough and minimum till cultivation in the field. The relationship of the shoot P-concentration for each genotype between each cultivation treatment is also presented. Regression of the data and 95% confidence intervals for that relationship are presented. Also plotted is the critical P-concentration (2.56 µg P $g^{-1}$) derived from figure 1. All data are the mean of three replicates. Only genotypes which show greater P-concentration than the critical to achieve 75% growth on this soil are labeled.

There was also a significant ($p<0.05$) impact of the cultivation treatment on the concentration of P in shoots, however this effect was dependent on which set of genotypes were considered (Figure 2). In the winter germplasm the minimum till cultivation treatment caused a decline in the shoot P concentration while in the spring germplasm there was a slight increase in the average shoot P concentration. The impact of the cultivation treatment on the ability of the various genotypes to acquire P is demonstrated even more strongly when the shoot P concentration of the various genotypes is correlated between the different cultivation treatments

(Figure 2). The lack of correlation demonstrates that there is no significant relationship between the genotypes, either winter or spring germplasm, when grown in the different cultivation treatments. In fact, only 32% of the winter genotypes fall within the 95% confidence intervals of the relationship, while even fewer, only 19%, of the spring genotypes fall within the corresponding confidence interval. This suggests that the vast majority of barley genotypes have a differential response in P-nutrition to cultivation treatments, with approximately equal numbers being more suited to minimum tillage and vice versa. Interestingly, of the five winter genotypes which showed P concentrations greater than the critical for 75% maximal growth in conventional cultivation treatments, only one (Gleam) demonstrated a suitability for growth in minimum till cultivation.

The availability and cycling of P for the conventionally cultivated and minimum till treatments are likely to be quite different due to differences in the biological and physiocochemical conditions of these treatments. For example microbial community structure and size, rooting depth, and water relations are all likely to be different. These differences will go some way to explaining why there is no relationship between genotypes of the association mapping populations between the two treatments. It is likely that the traits which allow some genotypes to successfully acquire P in the conventional plough cultivation treatment will be quite different to those of which benefit P-nutrition in minimum tillage treatment, therefore compromising the use of the genetic variation to predict useful genotypes for the different treatments.

Results here are analogous with results on studies on genotypic variation in P-use efficiency in wheat (Manske et al., 2000, Liao et al., 2008; George et al., 2008) which demonstrate that the ability of the wheat lines to acquire P was greatly dependent on soil type and only a small proportion of the variability in shoot growth and P content was attributable to genotypic differences. It is therefore imperative that screening for P-use efficiency in cereals be performed on soils rather than in hydroponics or agar (Hayes et al., 2004) and ideally on a range of different soil types and under different agronomic treatments as the genetic control of this trait on one soil is likely to be different under a range of conditions.

The development of SNP (single nucleotide polymorphism) based high throughput genome wide assays which use hundreds of SNP's to identify single markers from association mapping populations are a powerful tool to allow genotypic variation identified in studies like this to identify markers for P-use efficiency in barley. However, while there may be significant genotypic variation in these association mapping populations, the genetic component of this variation is not always robust between treatments.

In conclusion, we have shown that genotypic variation in P-use efficiency is present in association mapping populations grown in the field. However, such variation is not related between soil cultivation treatments, where differences in root abiotic stresses will have a large impact on root growth and nutrient acquisition. It is therefore important that when screening for markers for multi-mechanistic traits, such as P-use efficiency, that screening occurs in a number of environments so that the robustness of the genotypic variation can be tested.

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