Prospects for more phosphorus efficient subterranean clover

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Abstract
Subterranean clover has a requirement for relatively high soil P fertility to achieve high yields. However, some other pasture legumes can yield as well as subterranean clover with less applied P as a consequence of having roots that can explore soil more effectively. The aim of this work was: (i) to determine whether there is variation in yield among cultivars of subterranean clover when grown in low-P soil, and (ii) to determine the root morphology traits underpinning this variation. Thirty cultivars of subterranean clover were grown at an intermediate and high level of soil P availability in a pot experiment. The cultivars had similar shoot yields in the high-P soil, but varied two-fold in shoot yield at the intermediate level of P supply. In a further experiment, the root morphology acclimation of five of these cultivars was assessed in response to seven rates of soil P availability. Cultivars that had a combination of high root length density and high specific root length were able to maintain higher soil exploration at lower concentrations of available P. They acquired more P from low-P soil and yielded better than the cultivars with roots poorer at nutrient foraging. The results indicate that there is significant intra-specific variation in P-acquisition efficiency among subterranean clover cultivars and this is potentially useful for improving pasture yields in marginally P-deficient soils.

Keywords
Trifolium subterraneum, critical phosphorus requirement, root hair length, root hair cylinder volume.

Introduction

Subterranean clover (*Trifolium subterraneum*) is the most widely grown legume in temperate pastures in southern Australia, but it has a high critical external requirement for phosphorus (P) (i.e. a high soil test P (STP) concentration is needed to achieve near-maximum yield) relative to the grasses with which it is commonly grown (Haling et al. 2016a). This drives low P-use efficiency because P loss (through erosion, leaching and runoff) and P accumulation (i.e. in high P-sorbing soils) are positively correlated with STP concentration (Simpson et al. 2015). Use of pasture legumes that can yield well at lower STP concentrations could improve the P efficiency of pastures and may reduce the P-input cost of production (Simpson et al. 2014).

Comparative studies of the shoot and root growth of a range of legume species in response to P have identified serradellas (*Ornithopus compressus* and *O. sativus*.) as having substantially lower critical external requirements for P than subterranean clover (Haling et al. 2016a). Furthermore, the serradella species differ in root and rhizosphere traits, but it is their root morphology that enables them to yield well in soil with low plant-available P (Haling et al. 2016a; 2016b). A combination of relatively high root length density (root length per unit soil volume), high specific root length (root length per unit root mass) and long root hairs enables the serradellas to maximise soil exploration at low soil P supply and capture more P than can subterranean clover. Although subterranean clover develops high root length density in response to low soil P supply, its relatively low specific root length and short root hairs constrain the effectiveness of dry matter allocation to roots for soil exploration.

Serradellas are already used in pastures in some areas of southern Australia (e.g. sandy, acid soils). However, the species are not as widely adapted as subterranean clover (Hayes et al. 2015). Consequently, there is a need to investigate both the scope to improve the adaptation range of serradellas, and the potential for improving the P-acquisition efficiency of subterranean clover on soils with little available P. This paper reports experiments intended: (i) to assess the variation in yield in response to low soil-P availability among

30 cultivars of subterranean clover in controlled conditions; and (ii) to investigate the importance of root morphology for P acquisition in subterranean clover using a subset of cultivars.

Methods

Variation in yield in low soil P among 30 cultivars of subterranean clover

The shoot growth of 30 cultivars of subterranean clover was measured in response to an "intermediate" and "high" level of soil P availability. A sandy loam soil (8.3 mg kg^{-1} Colwell-extractable P; 0.5M $NaHCO_3$ pH 8.5) was steam pasteurised, sieved to < 2 mm, and mixed with lime (to raise $pH_{(Ca)}$ to 5.2) and P-free basal nutrients. Pots (200 mm height; 86 mm internal diameter) were filled with a bottom layer of 0.9 kg (oven-dry equivalent) of the low-P soil followed by a top layer of 0.3 kg of the soil mixed with either 40 or 250 mg P/kg. The high-P treatment was sufficient to achieve maximum yield for subterranean clover (Haling et al. 2016a). The two layers of soil were used to mimic the stratification of P that occurs in the field. Plants were grown as microswards with reflective sleeves fitted to the outside of the pot; these were raised daily to equal plant height. Plants were grown in a controlled environment cabinet for five weeks (15-20°C; photon flux density 620 µmol/m^2/s^1; 12 h light/ dark). Three replicates were grown for each combination of cultivar and P.

Root morphology acclimation of 5 cultivars of subterranean clover in response to low soil P supply

Five cultivars of subterranean clover were grown at seven rates of P applied to the topsoil in a pot experiment (0, 10, 20, 40, 60, 80 and 250 mg P kg^{-1}). The cultivars were selected based on a relatively low (Napier and Riverina; ssp. *yanninicum*), intermediate (Seaton Park; ssp. *subterraneum*) and high (Leura and Losa; ssp. *subterraneum*) yield in the 40 mg P/kg treatment in the experiment described above. The experimental setup and growth conditions were as described above. Five replicates were grown for each combination of cultivar and P. At harvest, the P-amended topsoil layer was cut into two quarters and one half segment. Roots were washed from all of the topsoil segments and from the subsoil. Roots from one quarter topsoil segment were scanned and root length and diameter analysed using WinRHIZO software (Regent Instruments Inc., Quebec, Canada). Root mass was determined for roots from both the scanned quarter and from the half segment, and used to estimate the total mass and length of roots in the topsoil (based on specific root length of roots from the scanned segment i.e. root length per unit root mass). Roots from the remaining quarter were stored at 4°C in 50% ethanol. Root hairs on these samples were photographed using a camera-mounted microscope and root hair length was measured using ImageJ (U. S. National Institutes of Health, Maryland, USA). Total plant P uptake was determined after ashing (550°C) shoots and roots from both the topsoil and subsoil, dissolving the ash in 2 M HCl and analysing P concentration colourimetrically. Data from both experiments were analysed using general analysis of variance (Genstat 16^{th} Edition, VSN International, UK) with cultivar and P as factors.

Results

Among the 30 cultivars of subterranean clover there was a two-fold range in shoot yield in the 40 mg P/kg treatment, despite similar maximum yields (i.e. yield at 250 mg P/kg) (Figure 1).

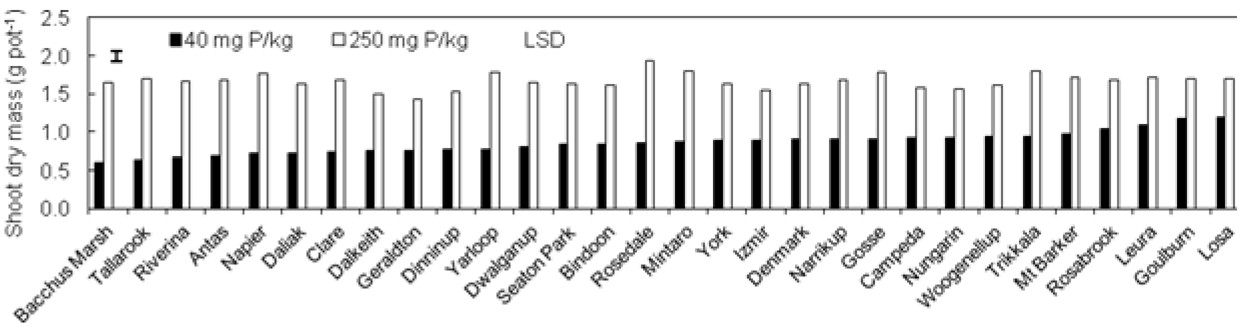

Figure 1. Shoot dry mass of 30 cultivars of subterranean clover grown with 40 and 250 mg P/kg applied in the topsoil layer of the pot. Bar shows LSD (*P*=0.05; n=3) for interaction of cultivar x P supply.

In the second experiment, shoot growth of all of the cultivars increased linearly between 10 and 80 mg P/kg topsoil (Figure 2a). Maximum shoot yields (i.e. shoot dry mass at 250 mg P kg^{-1}) among the five cultivars were generally similar; however, cultivars differed in their critical external requirement for P (i.e. P required to achieve maximum yield).

The root length density (i.e. root length per unit topsoil volume) of the cultivars increased between 1.4- and 2.1-fold in response to decreased P supply (Figure 2b). Seaton Park, Leura and Losa achieved similar maximum root length densities, but differed in ability to maintain a high root length density at low soil P supply. Losa and Leura were able to maintain high root length density at lower rates of applied P than Seaton Park. Riverina and Napier had significantly lower maximum root length densities compared with those of Leura and Losa. Napier only achieved its maximum root length density at a relatively high level of P supply.

Napier and Riverina had the lowest average specific root lengths across all P levels (112 and 114 m g^{-1}, respectively), while Losa had the highest average specific root length (182 m g^{-1}) (data not shown). Average specific root lengths of Leura and Seaton Park were between those of the other cultivars (144 and 145 m g^{-1}, respectively). Specific root length was decreased at the lower P supply rates. Average root hair length of the five cultivars ranged from 0.36 mm (Leura) to 0.40 mm (Losa) (data not shown). Root hairs were marginally shorter in the unamended soil compared with their lengths in soil to which P had been applied.

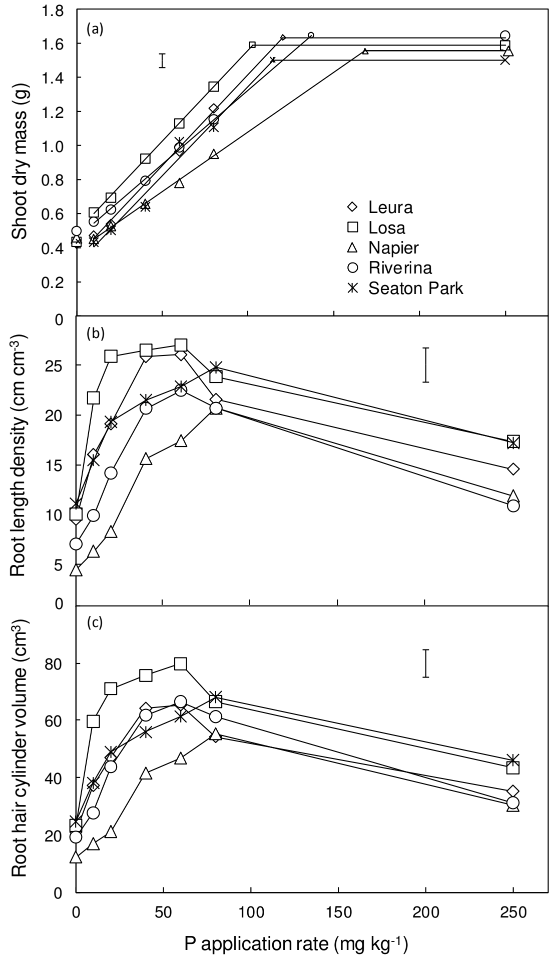

Figure 2. (a) Shoot dry mass (b) root length density in the P-fertilised topsoil layer and (c) root hair cylinder volume in the P-fertilised topsoil layer for five cultivars of subterranean clover. In (a) the critical external requirement for P (i.e. P required to achieve near-maximum yield) is denoted by the smaller symbols and was calculated as the intersection of maximum shoot yield (assumed to occur at 250 mg P kg^{-1}) and the linear response to P application between 10 and 80 mg P/kg^{-1} (NB: growth between 0 and 10 mg P kg^{-1} was lagged for some cultivars). In (b) root length density was calculated as the length of roots per unit volume of the P-fertilised topsoil. In (c) root hair cylinder volume was calculated as the volume of the cylinder encompassing the root and root hair zone. Bars show LSD (*P*=0.05; n=5) for interaction of cultivar x P supply.

The root hair cylinder volume of the five cultivars (i.e. the rhizosphere volume of the root system delineated by the length of root hairs and the average radius of the roots) (Figure 2c) increased in response to lower P supply and followed a similar trend to the response of root length density (Figure 2b). Losa achieved the largest maximum root hair cylinder volume and maintained larger root hair cylinder volumes at lower rates of P supply than all other cultivars. Napier had the lowest maximum root hair cylinder volume and had smaller root hair cylinder volumes than all other cultivars at P rates between 10 and 60 mg P kg^{-1}. Leura, Seaton Park and Riverina demonstrated responses between those of Losa and Napier.

Discussion

This work indicates that there is significant intra-specific variation in the ability of cultivars of subterranean clover to yield at intermediate levels of soil P supply and therefore indicates scope to select among existing cultivars for better performance on soils of lower P fertility. However, further work is required to validate these results under field conditions. Furthermore, it must be considered that even the best performing cultivars of subterranean clover have a significantly higher requirement for P than that of potential alternative legumes (e.g. serradellas; Haling et al. 2016a).

Root morphological traits that influence total soil exploration were found to significantly influence the ability of the subset of five cultivars of subterranean clover to acquire P and yield in low P soil. A major factor influencing soil exploration was the ability to develop and maintain high root length density at low concentrations of available P. High specific root length was an important factor in the achievement of a high root length density; most likely because it enabled more efficient allocation of dry matter for soil exploration. Root hairs were short and there was limited variation in their length among the cultivars. Root hair length is a significant factor conferring P-acquisition efficiency in more P-efficient legume species (Haling et al. 2016b), but the present results suggest that there may only be limited scope to breed for longer root hairs within subterranean clover.

Conclusion

This work demonstrates significant intra-specific variation in the ability of subterranean clover to yield at intermediate levels of soil P supply. Cultivars that had a combination of high root length density and high specific root length were able to maintain higher soil exploration at lower concentrations of available P. This enabled them to acquire more P and yield better in marginally P-deficient soil than cultivars with poorer nutrient foraging attributes.

Acknowledgements

The work was predominantly funded by Meat and Livestock Australia (MLA) and Australian Wool Innovation Limited (AWI) as part of “Phosphorus-efficient legume pasture systems” (B.PUE.0104) with data analysis continuing under “RnD4P-15-02-016 Phosphorus Efficient Pastures” a project supported by funding from the Australian Government Department of Agriculture and Water Resources as part of its Rural R&D for Profit programme and from MLA, Dairy Australia and AWI. The work was funded in part by the Rural & Environment Science & Analytical Services Division of the Scottish Government.

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