Digging up the hidden half of Australian barley

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Abstract
Barley crops are usually exposed to water stress, especially during critical periods of grain set and filling. In addition to direct effects of water stress, there is a drought-induced nitrogen deficit, and shortage of water can also affect phosphorous uptake. The objective of this work was to compare the root system, nitrogen, and phosphorus uptake in a historic collection of Australian barley varieties adapted to winter rainfall environments. We observed modifications in morpho-physiological root traits as well as in the uptake of N and other nutrients per unit of root length of barley plants associated with the year of release of the cultivars. This is one of the first studies indicating the indirect effect of selection for yield on root and nutritional traits in Australian barley.

Keywords
Roots, N uptake, drought adaptation, phenotyping.

Introduction
Crop physiology provides a reference to both breeding and agronomy. Breeding for drought environments can take advantage of the genetic variation in root attributes such as root length, rooting depth, and degree of root–soil contact which are linked to water uptake (Palta et al., 2011; Passioura, 1983). Selection for yield over five decades has reduced the size of the root system and increased nitrogen uptake per unit root length in Australian wheat (Aziz). Maccaferri et al. (2016) have found diversity in the root growing angle for elite durum wheat. Robinson et al. (2018) analysed a panel of 216 breeding lines from the Northern Region Barley Breeding program in Australia and indicated root angle was strongly associated with yield. The objective of the current work was to analyse the changes in the root system, nitrogen and phosphorus uptake in a historic collection of South Australian barley varieties. It is hypothesised that similar to wheat, there was a redundancy in roots that led to smaller root system in newer varieties.

Methods
Experimental design
We grew a historic collection of SA barley cultivars in rhizo-boxes (Figure 1) to map the growth, and distribution of the root system, and measured nitrogen and phosphorus uptake. Given the intensity of sampling and detail, we only used six genotypes that are genetically close. The cultivars were selected to represent barley breeding during the last 120 years: Prior A (released in 1903), Proctor (1952), Clipper (1968), Schooner (1983), Sloop (1997) and Compass (2015). Four plants of each cultivar were grown in each rhizo-box. Rhizo-boxes were glass-walled plastic boxes filled with soil to a depth of 1.0 m (Figure 1). The soil was a sandy loam from Roseworthy (lat -34.51, long 138.68). Sterilised soil was packed to a bulk density of approximately 1.35 g/cm^3 to fill the box. At sowing, on 27 February 2020, the equivalent of 100 kg N/ha was incorporated (as a multiple fertilizer Osmocote) and mixed into the top 0.1 m of soil. Plants were grown at 23/14 ºC day/night temperature with a daylength of 12 h. Water was applied till boxes reached field capacity just before sowing, 800 cm^3 were applied every 10 days after plants reach the second visible node stage.

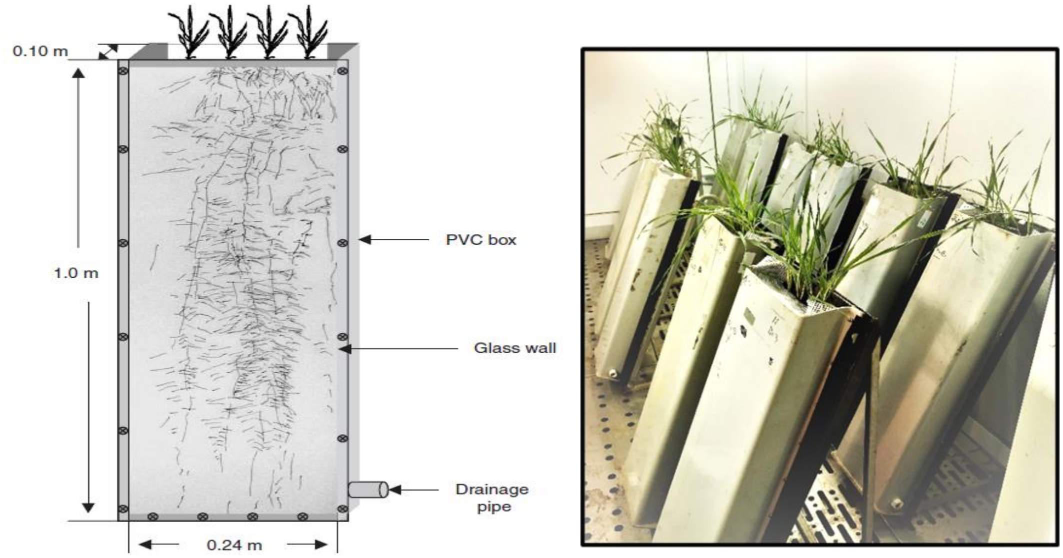

Figure 1: A: Scheme of the rhizo-box used for growing the plants and studying root growth (credit Liao et al. (2006)). B: View of the trial in a controlled environment room at the Plant Research Centre, Waite Campus.

The rhizo-boxes were arranged in a randomized complete block design with four replicates for each cultivar. Root traits were analysed as a function of the year of release of the cultivars. The absolute and relative (% yr^{-1}) rate of genetic change of each trait was calculated as the slope of the least square regression between the trait and year of release. The relative rate was calculated in relation to the newest cultivar (Fischer et al., 2014). We report *p*-value as a continuous quantity, and Shannon information transform [$s = -\log_2(p)$] as a measure of the information against the tested hypothesis (Greenland, 2019).

Results

Morpho-physiological traits

Root length increased at a rate of 7.7 cm y^{-1} or 0.002 % y^{-1} (Figure 2a). A similar trend was observed for root length density, which increased at a 0.00068 cm cm^{-3} y^{1} (Figure 2b) or 0.0027 % $year^{-1}$, and for the extension rate of the roots at 0.16 cm d^{-1} y^{-1} or 0.0032% y^{-1} (Figure 2c). Total root biomass and total root biomass per shoot did not change with year of release (Figure 2d and e). Days to reach 1 m depth were shortened from older to newer cultivars at 1.3 d every 10 years of release (Figure 2f).

Nitrogen and phosphorus uptake

Nitrogen uptake per cm of root decreased with the year of release of the cultivars at a rate of 0.88 mg cm^{-1} $year^{-1}$ or 0.004 % $year^{-1}$ (Figure 3a). There was no change in P uptake per cm of root with year of release (Figure 3b). For both nutrients, there was no association between uptake per unit of biomass of roots and the year of release of the cultivars (Figure 3c and d).

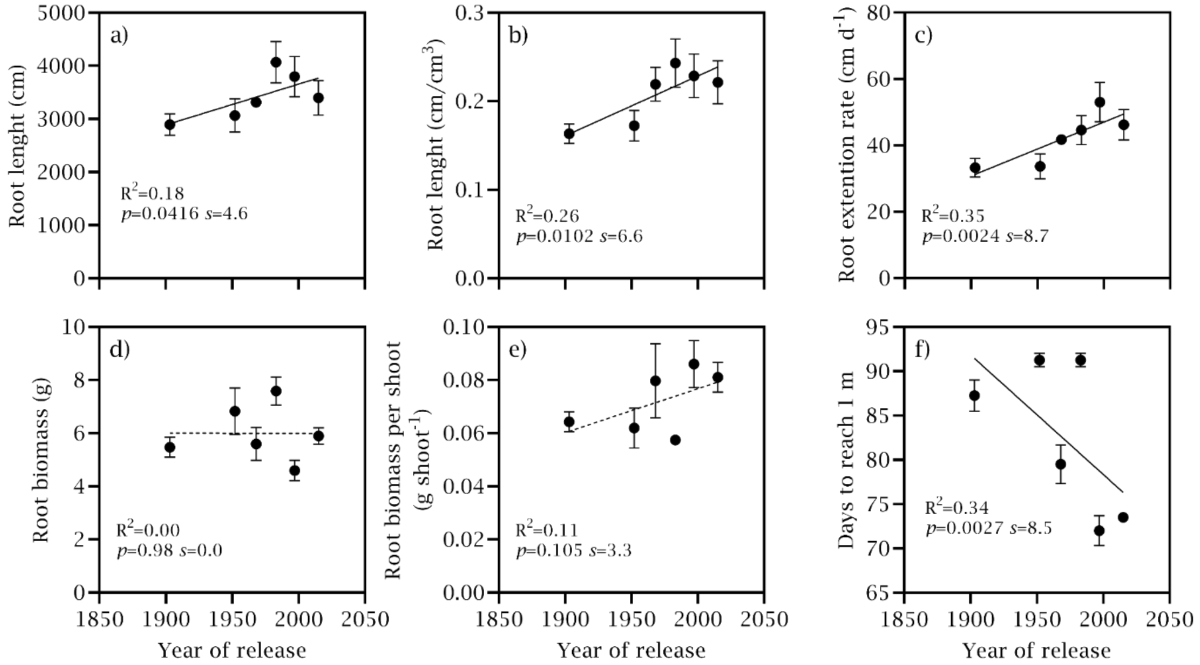

Figure 2. Morphological traits as a function of year of release of historical barley cultivars. Solid lines are least squares regressions for p < 0.05, s > 4.3, and dashed lines for p > 0.05, s < 4.3. Vertical bars indicate two standard errors of the mean.

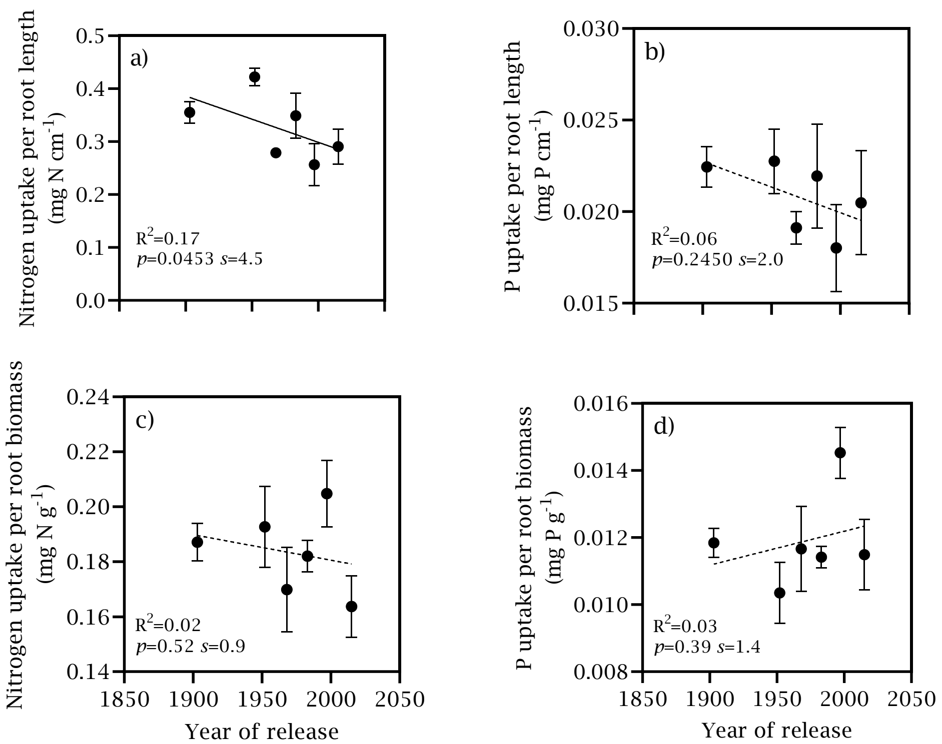

Figure 3. Nitrogen and phosphorus uptake per unit of root length and biomass as a function of the year of release of historical barley cultivars. Solid lines are least squares regressions for p < 0.05, s >4.3, and dashed lines for p > 0.05, s < 4.3. Vertical bars indicate two standard errors of the mean.

3.3 Sulphur, potassium, and micronutrients

The amount of nutrient per cm of root length decreased with year of release for S, K, Ca, Mg, Na, B, and Cu (Table 1). Trends were weaker for Zn and Mn, and not evident for Fe, Al, and Mb (data not shown).

	Nutrient								
	S	K	Ca	Mg	Na	B	Cu	Zn	Mn
Rate	-1.3×10^{-4}	-1.6×10^{-3}	-3.3×10^{-4}	-8.8×10^{-5}	-1.8×10^{-4}	-3.8×10^{-6}	-3.2×10^{-6}	-9.8×10^{-6}	-3.0×10^{-5}
R^2	0.23	0.21	0.41	0.44	0.33	0.22	0.33	0.16	0.14
p	0.018	0.025	0.001	0.000	0.003	0.021	0.003	0.052	0.076
s	5.8	5.3	10.3	11.3	8.2	5.6	8.2	4.3	3.7

Table 1: Rate of genetic change for the nutrient uptake per cm of root per year. Rates are slopes of least-square regression between uptake and year of release.

Conclusion

Breeding for yield and agronomic adaptation in barley has indirectly selected for faster root growth at the expense of nutrient uptake per unit root length. This is the mirror image of wheat, where selection for yield reduced root system size and increased N uptake per unit root length (Aziz et al., 2017). Direct comparisons between wheat and barley are needed to confirm this apparent divergence. Further field work is necessary to confirm our findings in controlled environment.

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