

Article

# Characterization of Root and Shoot Traits in Wheat Cultivars with Putative Differences in Root System Size

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Abstract: Root system size is a key trait for improving water and nitrogen uptake efficiency in wheat (Triticum aestivum L.). This study aimed (i) to characterize the root system and shoot traits of five wheat cultivars with apparent differences in root system size; (ii) to evaluate whether the apparent differences in root system size observed at early vegetative stages in a previous semi-hydroponic phenotyping experiment are reflected at later phenological stages in plants grown in soil using large rhizoboxes. The five wheat cultivars were grown in a glasshouse in rhizoboxes filled to 1.0 m with field soil. Phenology and shoot traits were measured and root growth and proliferation were mapped to quantify root length density (RLD), root length per plant, root biomass and specific root length (SRL). Wheat cultivars with large root systems had greater root length, more root biomass and thicker roots, particularly in the top 40 cm, than those with small root systems. Cultivars that reached anthesis later had larger root system sizes than those that reached anthesis earlier. Later anthesis allowed more time for root growth and proliferation. Cultivars with large root systems had 25% more leaf area and biomass than those with small root systems, which presumably reflects high canopy photosynthesis to supply the demand for carbon assimilates to roots. Wheat cultivars with contrasting root system sizes at the onset of tillering (Z2.1) in a semi-hydroponic phenotyping system maintained their size ranking at booting (Z4.5) when grown in soil. Phenology, particularly time to anthesis, was associated with root system size.

Keywords: root system size; phenology; root biomass; root length; root mapping

# 1. Introduction

There is no consensus on whether root system size in wheat is critical for improving water and nitrogen uptake, despite the common assumption that large root systems capture more water and nitrogen and produce more biomass and greater yields [1,2]. Genotypic variation in wheat root system size exists [3,4], providing the opportunity to improve water and nutrient uptake efficiency in cereal crops [5–7]. Whether the size of the root system is a candidate trait for improving water and nitrogen uptake efficiency needs investigation. However, as a first step, wheat genotypes with putative differences in root system size need to be identified and characterised.

The size of the root system is determined by the total root biomass and cumulative root length [1,8]. It is assumed that a large root system requires more photosynthetic assimilates for its production, proliferation, growth and function. It has been estimated that the amount of photosynthetic assimilates invested to produce one unit of root dry matter can produce double that of shoot dry matter [9].



The extra carbon investment in root biomass and root length, which is mainly used in root respiration before floral initiation [10], is reflected in shoot growth, particularly before booting [2]. While wheat with large biomass and root length and more prolific root systems can improve water [9] and nitrogen uptake in dry environments and deep sandy soils [11–13], wheat root systems with small biomass and root length have been unintentionally selected in modern cultivars [14]. In Australia, wheat varieties released in the last 50 years have progressively had less root biomass, root length and root length density, while nitrogen uptake has increased [15]. This raises the question, to what extent do wheat breeders need to select for root system size and what is the efficiency of water and nitrogen uptake per unit of root length and root biomass?

In this study, we characterized the root system and shoot traits in five wheat cultivars with putative differences in the total root biomass and cumulative root length at the onset of tillering (Z2.1) from a previous semi-hydroponic phenotyping study. We also evaluated whether the apparent differences in root system size at tillering in a preliminary semi-hydroponic phenotyping study were reflected at later phenological stages when wheat was grown in soil using large rhizoboxes. It was hypothesized that genotypic differences in wheat root system size at early vegetative stages (tillering, Z2.1) grown under semi-hydroponic conditions would be reproducible at late phenological stages (booting, Z4.9), when grown in soil with large rhizoboxes. To test this hypothesis, five wheat cultivars were grown in rhizoboxes to map and observe differences in root growth, branching and distribution [15–19].

#### 2. Materials and Methods

# 2.1. Plant Material and Growth Conditions

Five wheat cultivars with apparent differences in total root biomass and cumulative root length at the onset of tillering (Z2.1) from a previous semi-hydroponic phenotyping study were used in this study. The cultivars were Ghurka and Bahatans-87, released in 1916 and 1924, respectively; Hartog, released in 1983; and Tincurrin and Harper, released in 1978 and 2010, respectively (Table 1).

**Table 1.** Five wheat cultivars with the country of origin, year of release and apparent root biomass and cumulative root length at the onset of tillering (Z2.1) identified in a phenotyping study using a semi-hydroponic system. Supplementary data from a previous semi-hydroponic phenotyping study are available in Table S1.

Cultivars	Country of Origin	Year of Release	Total Root Biomass	Cumulative Root Length		
Ghurka	Australia	1916	Large	Large		
Tincurrin	Australia	1978	Small	Small		
Hartog	Australia	1983	Medium	Medium		
Harper	Australia	2010	Small	Small		
Bahatans-87	Algeria	1924	Large	Large		

The five cultivars were grown in 24-L glass-walled rhizoboxes ( $0.24 \text{ m} \log \times 0.10 \text{ m} \text{ wide} \times 1.0 \text{ m}$  deep) filled with soil. Plyvinyl chloride (PVC) rhizoboxes have been previously described in root studies [12,15,16,20]. Briefly, the acrylic glass side was covered with a removable, black PVC sheet to avoid light exposure to the roots. The rhizoboxes were placed on steel stands at a 30° angle to force the roots to grow along the acrylic glass side as described elsewhere [12,16,19–22]. The visibility of the root system does not differ between rhizoboxes angled of 30 or 45° when seeds are sown in contact with the acrylic glass side [17,23]. The 30° inclination facilitates root system mapping when using large rhizoboxes [24].

Top soil (0–15 cm) was collected from a field site at Cunderdin ( $31^{\circ}64'N$ ,  $117^{\circ}24'E$ ), Western Australia, and was classified as a reddish-brown sandy clay loam: Red Calcic Dermosal [25]. The soil consisted of 63.5% sand with a particle size between 0.010–0.020 inches, 8.3% silt and 28.3% clay with a pH, measured in a 1:5 suspension of soil in 0.01 M CaCl<sub>2</sub>, of 6.0. The soil contained 6 µg g<sup>-1</sup>

of nitrate–N, 4  $\mu$ g g<sup>-1</sup> of ammonium–N, 46  $\mu$ g g<sup>-1</sup> of Colwell P and 691  $\mu$ g g<sup>-1</sup> of Colwell K [15]. Air-dried soil was sieved to 2 mm and mixed uniformly with 25% in (vol.) of washed and air-dried river sand to improve drainage [15]. The soil was packed to a bulk density of approximately 1.56 g cm<sup>-3</sup> [15]. Compound fertiliser equivalent to 65 kg ha<sup>-1</sup> N, 79 kg ha<sup>-1</sup> P, 71 kg ha<sup>-1</sup> K and trace amounts of micronutrients (S, Cu, Zn, Mo and Mn), optimal amounts for wheat grown on Cunderdin soils [26,27], was mixed homogeneously into the top 0.1 m of soil in each rhizobox before sowing.

The experiment was conducted in an evaporatively cooled glasshouse at The University of Western Australia, Perth, Australia (31°93′S, 115°83′E) from May to July 2017 with an average air temperature of 16 °C, maximum temperature of 22 °C, minimum temperature of 9 °C, relative humidity of 61% and 10–11 h of natural light. The five cultivars were grown in a completely randomized block design with three replicates.

Four homogenous pre-germinated seeds were sown equidistant from each other at a depth of 2 cm in each rhizobox, ensuring that the seeds were in contact with the acrylic glass wall. This corresponded to a field sowing density of 160 plants  $m^{-2}$  [28]. The plants were hand-watered as required with tap water to maintain the soil water content close to field capacity and to avoid excessive drainage. Plant phenology was monitored regularly using the scale of Zadoks and Chang [29]. The experiment ended at 63 days after sowing (DAS) when some roots had reached the bottom of the rhizoboxes.

A separate, but simultaneous experiment was conducted to monitor, in detail, the phenological development in each cultivar. The experiment was conducted in pots (25 cm diameter, 30 cm height), with eight plants per pot, corresponding to a field sowing density of 160 plants  $m^{-2}$  [28], using the same five cultivars, the same soil and similar a plant density as those used in the rhizobox study and grown in the same glasshouse with similar temperature, light and watering conditions. Time to tillering (Z2.1), time to booting (Z4.9) and time to anthesis (Z 6.1) were recorded [29] (Table 2). Differences in the phenological development between plants grown in pots and rhizoboxes were not expected to be due to the differences in soil volume between the pot experiment (15L) and the rhizoboxes (24L) [30,31], since phenology in wheat is driven by temperature and natural light [32,33].

**Table 2.** Time in days after sowing (DAS) to the onset of tillering (Z2.1) measured in five wheat cultivars grown in rhizoboxes, and to booting (Z4.9) and anthesis (Z6.1) measured in the same cultivars growing in pots in similar conditions. Developmental stages were reached at the same time (DAS) in both the pot and rhizobox experiments.

Cultivars	Time to Tillering (Z2.1)	Time to Booting (Z4.9)	Time to Anthesis (Z6.1)	
	(DAS)	(DAS)	(DAS)	
Ghurka	16	92 a	104 a	
Tincurrin	17	59 c	75 b	
Hartog	15	57 c	67 b	
Harper	18	85 b	104 a	
Bahatans 87	14	95 a	107 a	
LSD $_{p < 0.05}$	ns	4.2	6.9	

For each parameter, data with the same letter indicate no significant difference between cultivars (p < 0.05), ns = not significant.

## 2.2. Root Traits: Non-Destructive Measurements

Root growth was monitored weekly from seven DAS through the glass wall of each rhizobox by removing the black PVC cover sheet. Visible new roots were marked on transparency films using a black waterproof, permanent pen. Immediately afterwards, the visible new roots were also marked on the acrylic glass of the rhizobox so that new root growth could be recognized at the next measurement. The glass wall was covered with the black PVC cover sheet immediately after the glass was marked. The transparent film was scanned at 600 pixels per mm using a portable scanner (Jenkins PS4100: East Bentleigh, Vic, Australia) and root images were analyzed for root length using WinRhizo Pro software (v2009, Regent Instrument, Quebec, QC, Canada).

#### 2.3. Destructive Measurements

When the experiment ended at 63 DAS, destructive above-and below-ground measurements were taken. The plants in each rhizobox were harvested by cutting the shoots from the roots at the crown. Aboveground measurements included shoot biomass, leaf area (LA), leaf biomass, specific leaf area (leaf area per unit leaf weight, SLA), tiller number, and plant height. Leaf area was measured using a portable leaf area meter (LI-3000, Li-COR Biosciences, Lincoln, NE, USA). Shoots, spikes and leaves were separated and oven-dried at 70 °C (Heratherm OMS 100, Thermo Scientific, Langenselbold, Germany) before being weighed on a precision balance (Voyager<sup>®</sup>, Ohaus Corporation, Parsippany, NJ, USA).

Destructive belowground measurements included root length, root biomass, root length density (root length per unit of soil volume; RLD) and specific root length (root length per unit of biomass; SRL). Each rhizobox was opened by removing the glass wall, and the soil profile was sampled in 0.2 m sections from the top by cutting the soil with a carbon steel filling blade. The roots in each section were recovered from the soil by washing through a 1.4-mm sieve to produce a clean sample Palta and Fillery [34]. The recovered roots from each 0.2-m soil section were placed in plastic bags and stored at 4 °C until being scanned at 400 dpi per mm (Epson Perfection V800, Long Beach, CA, USA) to quantify root morphological traits. The root samples were dried and weighed after scanning as per the shoot samples. Root images were analyzed using WinRHIZO Pro software (v2009, Regent Instrument, Quebec, QC, Canada) [35]. The total root length density was calculated as the total root length divided by the soil volume. The distribution of RLD in the soil profile was calculated as the root length in 0.2 m sections from the top to the bottom of each rhizobox divided by the soil volume of the corresponding section (0.0024 m<sup>3</sup>). The specific root length (SRL), an indirect measure of the thickness of the root system, was estimated as the total root length divided by the total root biomass [8,10–12,15,16,19,20].

#### 2.4. Statistical Analysis

The data were checked for normal distribution and equal variance and then analysed with one-way ANOVAs. Multiple comparisons were made using Fisher's least significant difference (LSD) tests (p = 0.05) using R package Agricolae [36]. The cumulative root length data were analysed using the repeated measures model. The covariance structure that was selected as the best, based on the value of deviance and Akaike information criterion (AIC), was unstructured. The model uses days and cultivars and their interactions. The analysis was conducting using Genstat statistical software 18th edition. A Pearson correlation analysis of the mapped root length (total root length of the visible root system measured non-destructively through the glass-wall of the rhizobox), total root length (total length of the root system measured destructively at the end of the experiment), root biomass, specific root length, root:shoot ratio, leaf area, specific leaf area, leaf biomass, shoot biomass, tiller number and plant height was undertaken. All statistical analyses were performed using R software version 3.4.2 [37].

# 3. Results

#### 3.1. Shoot Traits

All five wheat cultivars emerged within 2–3 DAS. The onset of tillering (Z2.1) occurred at 14–18 DAS, with no significant differences between the five cultivars (Table 2).

The leaf area ranged from 402 cm<sup>2</sup> plant<sup>-1</sup> in Hartog to 627 cm<sup>2</sup> plant<sup>-1</sup> in Bahatans-87. The cultivars with large root systems (Ghurka and Bahatans-87) had 24% more leaf area than those with small root systems (Tincurrin and Hartog) (Table 3). Leaf biomass ranged from 1.51 g plant<sup>-1</sup> in Hartog to 2.12 g plant<sup>-1</sup> in Bahatans-87; again, the cultivars with large root systems had more leaf biomass than those with small root systems (Table 3). The specific leaf area (SLA) ranged from 255 cm<sup>2</sup> g<sup>-1</sup> in Harper to 312 cm<sup>2</sup> g<sup>-1</sup> in Tincurrin. The high SLA in Tincurrin indicates that it has the thinnest leaves among the five cultivars (Table 3). The tiller number varied from 4.6 tillers plant<sup>-1</sup> in Hartog to 8.8 tillers plant<sup>-1</sup> in Bahatans-87. The shoot biomass ranged from 3.2 g plant<sup>-1</sup> in Ghurka to

5.1 g plant<sup>-1</sup> in Hartog (p < 0.05). Hartog and Tincurrin had 21% more shoot biomass than Ghurka, Harper and Bahatans-87 (Table 3).

Cultivars	LA SLA		LB Tillers		Shoot biomass	Н
	$(cm^2 plant^{-1})$	$(cm^2 g^{-1})$	(g plant <sup>-1</sup> )	(tillers $plant^{-1}$ )	(g plant <sup>-1</sup> )	(cm)
Ghurka	555 ab	277 с	2.00 ab	5.3 b	3.2 b	44 b
Tincurrin	493 bc	312 a	1.58b c	5.7 b	4.6 ab	65 a
Hartog	402 c	267 cd	1.51 c	4.6 b	5.1 a	70 a
Harper	491 bc	255 d	1.93 abc	4.8 b	3.5 b	43 b
Bahatans-87	627 a	295 b	2.12 a	8.8 a	3.3 b	46 b
LSD $p < 0.05$	113	14	0.42	2.1	1.4	7.1

**Table 3.** Leaf area (LA), specific leaf area (SLA), leaf biomass (LB), tiller number , shoot biomass and plant height (H) of five wheat cultivars at harvest (63 DAS).

For each parameter, data with the same letter indicates no significant difference between cultivars (p < 0.05).

## 3.2. Root Traits

Cumulative visible root length increased up until 42 DAS, and after that, the rate of cumulative root length [growth] started to decline. Differences in cumulative visible root length between the cultivars became apparent from 35 DAS and differed significantly (p < 0.05) from 49 DAS (Figure 1). At 63 DAS (Figure 2), the cumulative visible root length ranged from 9.08 m plant<sup>-1</sup> in Hartog to 12.5 m plant<sup>-1</sup> in Bahatans-87; Bahatans-87 and Ghurka had a 2.54 m plant<sup>-1</sup> (31%) longer root length than Hartog and Tincurrin.

The total root length at harvest ranged from 199 m plant<sup>-1</sup> in Hartog to 278 m plant<sup>-1</sup> in Bahatans-87; Ghurka and Bahatans-87 had 70.5 m plant<sup>-1</sup> greater (26% greater) root length than Hartog and Tincurrin (Table 4). Root biomass at harvest ranged from 1.05 g plant<sup>-1</sup> in Tincurrin to 2.03 g plant<sup>-1</sup> in Bahatans-87; Bahatans-87 and Ghurka had an additional 0.79 g plant<sup>-1</sup> of root biomass than the other three cultivars. The SRL ranged from 139 m g<sup>-1</sup> in Bahatans-87 to 202 m g<sup>-1</sup> in Tincurrin; Tinticurrin presented 61.5 m g<sup>-1</sup> greater SRL than Ghurka and Bahatans-87, indicating that Ghurka and Bahatans-87 had 30% thicker roots than Tincurrin (Table 4). The RLD ranged from 9.3 cm cm<sup>-3</sup> in Hartog to 11.64 cm cm<sup>-3</sup> in Harper; Ghurka, Harper and Bahatans-87 had 1.76 cm cm<sup>-3</sup> greater RLD than Hartog and Tincurrin (16% higher RLD). The root:shoot ratio ranged from 0.22 in Hartog to 0.62 in Ghurka; the root:shoot ratios of Ghurka and Bahatans-87 were 2.6 higher those than in Tincurrin and Hartog, which is likely due to the cultivars with large root systems having 1.6 g plant<sup>-1</sup> less shoot biomass (32% less shoot biomass) than those with small root systems (Table 4).



**Figure 1.** Cumulative visible root length measure non-invasively every 7 days by root mapping through the glass wall of each rhizobox from the time the plants were 7 to 63 days after sowing (DAS). Vertical bars represent LSD at p < 0.05. The time to tillering for the five cultivars is indicated by T and the time to booting for the cultivars Tinticurrin and Hartog is indicated by B. Time to booting for the Harper, Ghurka and Bahatans-87 cultivars is not indicated, as booting in these cultivars occurred at 85, 92 and 95 DAS, respectively. The vertical bars represent LSD at p < 0.05. Supplementary detail data on the cumulative visible root length, measured by root mapping through the glass wall of each rhizobox, is available in Table S2.



**Figure 2.** Example of the root distribution patterns of Ghurka, Tincurrin, Hartog, Harper and Bahatans-87 drawn on the glass panels of the rhizoboxes at 63 DAS.

Cultivars	Root Length	Root Biomass	Specific Root Length	fic Root Length Shoot ength Density Biomas		Root:Shoot Ratio
	(m plant <sup><math>-1</math></sup> )	(g plant $^{-1}$ )	$(m g^{-1})$	$(\mathrm{cm}\ \mathrm{cm}^{-3})$	(g plant $^{-1}$ )	
Ghurka	273 a	1.94 a	142 c	11.37 ab	3.2 b	0.62 a
Tincurrin	211 b	1.05 b	202 a	9.75 bc	4.6 ab	0.23 c
Hartog	199 b	1.10 b	178 b	9.30 c	5.1 a	0.22 c
Harper	230 ab	1.44 b	162 bc	11.64 a	3.5 b	0.41 b
Bahatans-87	278 a	2.03 a	139 c	10.84 abc	3.3 b	0.61 a
$LSD_{n < 0.05}$	56	0.47	35	1.85	1.4	0.12

**Table 4.** Root length, root biomass, specific root length, root length density, shoot biomass and root:shoot ratio of five wheat cultivars at harvest (63 DAS).

For each parameter, data with the same letter indicates no significant difference between cultivars (p < 0.05).

Most of the root biomass (80%) was distributed in the top 0.4 m of the soil profile; however, differences in root biomass distribution occurred among the cultivars (Figure 3a). Bahatans-87 and Ghurka (large root system) had 37% and 46%, respectively, of their total root biomass allocated into the top 0.2 m layer of the soil profile, compared with 46% and 47%, respectively, in Tincurrin and Hartog (small root systems). The percentage of the total root biomass that Bahatans-87 allocated in the top 0.2 m layer differed significantly from the other cultivars (p < 0.01). Bahatans-87 and Ghurka had 30% and 36%, respectively of their total root biomass in the 0.2–0.4 m soil layer, compared with 34% and 39%, respectively, in Tincurrin and Hartog. Despite the differences in the grams allocated by cultivars at this level, there were no significant differences between cultivars in the percentage that the cultivars allocated at a depth of 0.2–0.4. At a depth of 0.4–1.0 m, Bahatans-87 had 34% of its total biomass, compared with ~20% in the other cultivars.

Most of the root length was also distributed in the top 0.4 m of the soil profile in all five cultivars. About 50% of the total root length was in the top 0.2 m of the soil profile, and 30% was in the 0.2–0.4 m layer. The RLD in the 0–0.2 m layer of the soil profile was highest in Bahatans-87, followed by Ghurka and Harper, with Hartog and Tincurrin having the lowest RLD. At a depth of 0.2–0.4, Ghurka and Harper had higher RLD than the other cultivars. Below 0.6 m, Bahatans-87 had the highest RLD value of the five cultivars. The lowest values in the RLD throughout the 1-m soil profile were presented by Hartog, and Tincurrin had similar values (Figure 3b).

SRL decreased in all cultivars down the soil profile (Figure 3c). Tincurrin had the highest SRL in the top 0.6 m of the soil profile. Ghurka had the lowest SRL in the top 0.2 m layer, while Bahatans-87 had the lowest SRL in the 0.2–0.6 m layer.



**Figure 3.** Destructively measured vertical profiles (0.2 m sections from the top to bottom of each rhizobox) of (**a**) root biomass, (**b**) root length density and (**c**) specific root length. The horizontal bars represent LSD at p < 0.05 for comparison between cultivars at each depth increment in the soil profile. Detailed supplementary data on the distribution down the soil profile of root biomass, RLD and SRL are available in Table S3.

# 3.3. Correlation between Traits

There was a strong positive correlation (r = 0.82) between the root length mapped on the glass surface and the scanned total root length at harvest (Table 5). Leaf area was positively correlated with leaf biomass (r = 0.91) and tiller number (r = 0.75) (Table 5).

<b>Table 5.</b> Pearson correlations ( <i>r</i> ) between 12 traits investigated in wheat cultivars. RL = root length;
MRL = mapped root length; RB = root biomass; SRL = specific root length; RLD = root length density;
R: S = root: shoot ratio; LA = leaf area; SLA = specific leaf area; LB = leaf biomass; SB = shoot biomass;
T = tiller number; H = plant height.

Traits	RL	MRL	RB	SRL	RLD	R:S	LA	SLA	LB	SB	Т
MRL	0.82 ***										
RB	0.87 ***	0.80 ***									
SRL	-0.50	-0.51	-0.84 ***								
RLD	0.32	0.08	0.39	-0.48							
R:S	0.72 **	0.68 **	0.92 ***	-0.87 ***	0.61 **						
LA	0.86 ***	0.92 ***	0.77 ***	-0.43	0.12	0.62					
SLA	-0.03	0.13	-0.07	0.21	-0.22	-0.05	0.28				
LB	0.90 ***	0.88 ****	0.83 ***	-0.53	0.23	0.66 **	0.91 ***	-0.15			
SB	-0.19	-0.26	-0.46	0.65 **	-0.74 **	-0.75 **	-0.22	-0.02	-0.22		
Т	0.64	0.61	0.63	-0.41	0.10	0.51	0.75 **	0.39	0.59	-0.09	
H	-0.56	-0.53	-0.67 **	0.70 **	-0.83 ***	-0.83 ***	-0.49	0.24	-0.61	0.81 ***	-0.29

\*\*, \*\*\* significant at p < 0.05 and p < 0.01 probability levels, respectively.

Root length was positively correlated with root biomass (r = 0.87; p < 0.01) and the root: shoot ratio (r = 0.72; p < 0.05). Root biomass was negatively correlated with SRL (r = -0.84; p < 0.01) and positively correlated with the root:shoot ratio (r = 0.92; p < 0.01). SRL was negatively correlated with the root:shoot ratio (r = -0.87; p < 0.01), while RLD was positively correlated (r = 0.61; p < 0.05) (Table 5).

Root length was positively correlated with leaf area (r = 0.86; p < 0.01) and leaf biomass (r = 0.90; p < 0.01). A similar correlation was found between the root biomass and shoot traits. SRL was positively correlated with shoot biomass (r = 0.65; p < 0.05) and plant height (r = 0.70; p < 0.01). RLD was negatively correlated with plant height (r = -0.83; p < 0.01). The root: shoot ratio was positively correlated with leaf biomass (r = 0.66; p < 0.05), and negatively correlated with shoot biomass (r = -0.75; p < 0.05) and plant height (r = -0.83; p < 0.01). The root: shoot ratio was positively correlated with leaf biomass (r = 0.66; p < 0.05), and negatively correlated with shoot biomass (r = -0.75; p < 0.05) and plant height (r = -0.83; p < 0.01) (Table 5). Shoot biomass included leaf biomass, stems and young spikes. The negative correlation between shoot biomass and RLD and the R:S ratio occurred mainly because the Tincurrin and Hartog cultivars, which have small root systems, were already in their reproductive stage (booting stage) at 63 DAS when the experiment was terminated. The Harper and Bahatans-87 cultivars, which have large root systems, were in their vegetative stages (late tillering).

## 4. Discussion

#### 4.1. Characterising the Root Systems of Cultivars with Contrasting Root System Sizes

At 63 DAS, Bahatans-87 and Ghurka, which have putative large root systems, had higher cumulative root length and total root biomass than Tincurrin and Hartog (p < 0.05), which have putative small root systems, when grown in soil up to booting, Z4.5. The differences in root system size were mainly due to cumulative root length and total root biomass due to root branching and proliferation rather than differences in rooting depth. Bahatans-87 and Ghurka had more root branching and proliferation in the top 40 cm of the soil profile than the other cultivars. Vertical growth of the root system in Bahatans-87 was 37% faster than the other cultivars. Apart from Bahatans-87, root extension down the soil profile did not differ between the cultivars, suggesting that the seminal roots in the other four cultivars had similar rates of vertical growth. Specific root length (SRL) is an indirect measure of root thickness [12]. Bahatans-87 and Ghurka had the lowest SRL down the soil profile, indicating thicker roots than Tincurrin and Hartog. In particular, Ghurka had a lower SRL than Bahatans-87 having a lower SRL than Ghurka.

The non-destructive measurement of root length by mapping indicated that all cultivars have similar root lengths until 35 DAS; differences in root length and branching between cultivars became apparent mainly after tillering at the beginning of stem elongation. Differences in root length after

35 DAS were reported for nine wheat cultivars, released across five decades, grown in rhizoboxes [15], reporting similar values of total root length before tillering, and the differences were associated with the onset of stem elongation (Z3.0). When the root growth of vigorous and non-vigorous wheat genotypes was compared in rhizoboxes by Liao, Palta and Fillery [12], visual differences in root length and proliferation from the one-leaf stage (10 DAS) were monitored, but it was not until the onset of stem elongation (30 DAS) that significant differences occurred. These findings not only reflect the plasticity of the wheat root system when different cultivars are grown under different conditions [2,15,38–43], but uncover the perils of conducting early-stage root phenotyping in wheat [44,45]. Despite the high plasticity of the root system, wheat cultivars, when grown in the field or in rhizoboxes, maintained a similar ranking in root biomass and root length density under well-watered and moderate water deficit conditions [1,46].

#### 4.2. Negative Relationship Between Phenology and Root System Size

Time to booting (Z4.5) in Tincurrin and Hartog, which have small root systems, was 36 days earlier than Bahatans-87 and Ghurka, which have large root systems (p < 0.05). A similar study with nine wheat cultivars observed differences of only seven days [15]. The large discrepancy in our study possibly reflects cultivar differences in photoperiod and vernalisation requirements [47–49] or temperature responses [49,50]. However, under field conditions, wheat cultivars with less root biomass reached anthesis up to 26 days earlier than cultivars with more root biomass [40], which may be due to slower phenological development in some cultivars, allowing root systems to grow and proliferate for longer than cultivars with faster phenological development [15]. The allocation of photosynthetic assimilates to the wheat root system and root growth rates abruptly fell from 48% to 17% at floral initiation (double ridge) and subsequently, declined slowly to about 4% at booting (Z4.1) [10,51]. This implies that, the slower phenological development in Bahatans-87 and Ghurka was likely associated with their large root systems. On the other hand, the earlier time to booting (Z4.1) in Tincurrin and Hartog was likely associated with their small root systems, because their roots grew slowly, and they reached booting much earlier than the other cultivars [52].

#### 4.3. The Relationship Between Root System Size and Shoot Traits

Bahatans-87 and Ghurka, which have large root systems, had more leaf area than Tincurrin and Hartog, which have small root systems (p < 0.05). This is consistent with the finding that wheat root biomass is closely correlated with shoot traits [1], particularly leaf area and leaf biomass [53]. It is likely that Bahatans-87 and Ghurka, which have more leaf area and leaf biomass, would have a higher rate of photosynthesis per plant [1] which would result in more C assimilates allocated to roots [51]. The cultivars with large root systems had higher root:shoot ratios, indicating that a higher proportion of the total plant biomass was allocated to the roots [54]. The root:shoot ratio is closely related to wheat cultivar phenology as it decreases with developmental stage [40]. Cultivar differences in the root:shoot ratio may be due to the delayed start to reproductive development in the cultivars with large root systems (Bahatans-87 and Ghurka), extending the period of investment to root biomass [40]. Indeed, by the time the cultivars with small root systems (Tincurrin and Hartog) had reached Z55 (half ear emergence), those with large root systems were only at mid-tillering (Z25).

There is no consensus on whether the size of the root system in wheat is paramount for improving water and nitrogen capture, despite most studies relating root system size with water and nitrogen uptake, suggesting that large root systems can capture more water and more nitrogen and produce more biomass than small root systems [1,2]. Vigorous wheat genotypes with large root systems that proliferate early are the most effective for capturing nitrogen in deep sandy soils [1,11,12], but they can deplete the soil water fast and early, causing premature water deficits [2]. Few non-vigorous wheat genotypes with small root systems can capture as much nitrogen per plant as those with large root systems [55]. Furthermore, the root system of wheat varieties released in Australia in the last five decades has progressively declined in size, while nitrogen uptake has increased, mainly due to an

increased efficiency in capturing nitrogen [15]. This indicates that nitrogen uptake and, likely, water uptake, are not always directly or entirely associated with large root systems. The question remains, to what extent does the size of the root system influence nitrogen and water uptake efficiency.

# 5. Conclusions

Wheat cultivars with large and small root systems at early vegetative stages (Z2.1) of growth in a semi-hydroponic system maintained the size of their root system at later stages (booting stage Z4.5) when grown in soil. Wheat cultivars with large root systems had greater root length, root biomass, root branching and thicker roots, particularly in the top 40 cm of the soil profile than cultivars with small root systems. Cultivars that took longer to reach anthesis had larger root systems than those that reached anthesis earlier, indicating that they had more time for root growth and that the size of the root system is likely related to phenology. The size of the wheat root system is likely to be associated with more photosynthetic assimilates being allocated to roots [1,10,56]. Cultivars with large root systems had 25% more leaf area and leaf biomass, presumably increasing canopy photosynthesis to supply the demand for carbon assimilates to roots. The characterization of the root system size and shoot traits of in five wheat cultivars in this study will assist further investigations on the role of root system size in the efficiency of water and nutrient uptake.

Supplementary Materials: The following are available online at http://www.mdpi.com/2073-4395/8/7/109/s1.

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