Crop Ecology & Physiology

ARTICLE

Characterization of root traits for improvement of spring wheat in the Pacific Northwest

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Funding information

Washington Grain Commission, Grant/Award Number: 3019–7768; U.S. Department of Agriculture; NIFA Hatch project, Grant/Award Number: 1014527

Abstract

Understanding the genetic basis of root traits provides essential information on a largely untapped resource for crop improvement, as roots are instrumental for the uptake of water and nutrients. However, breeding for improved root traits is challenging due to laborious and time-consuming root phenotyping in soil. Our studies sought to uncover spatiotemporal root-growth dynamics of mature plant root systems in five spring wheat (Triticum aestivum L.) cultivars, Louise, Alpowa, Hollis, Drysdale, and Dharwar Dry, and a facultative spring landrace, AUS28451 using the in situ minirhizotron technique. The 2-yr greenhouse study revealed that the root system grows rapidly after early node elongation to gain maximum size during anthesis, after which root growth slows and transitions to senescence. We were able to detect quantifiable differences among wheat cultivars in root traits in both 5-d old seedlings and root systems at anthesis. Furthermore, the positive correlation of the observed root traits with grain yield and the consistency in root traits observed using minirhizotrons and through extraction of young and mature root systems has reinforced the experimental results. A negative correlation was found between root number, area, and length and root diameter. We found that the spring wheat cultivars, AUS28451, Dharwar Dry, and Alpowa, had increased root number, area, and length, but also increased time to heading. The results from this study can be further leveraged to screen breeding lines for root traits of interest, as well as assess the heritability of root traits for dryland farming in the inland Pacific Northwest.

1 | INTRODUCTION

A better physiological understanding of root growth dynamics and architecture is crucial to optimize crop performance through the modification of below-ground root traits adapted to environmental and climatic extremes (Khan, Gemenet, & Villordon, 2016; Postma, Schurr, & Fiorani, 2014; Uga et al.,

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2013). Nonetheless, root systems are not easily accessible for phenotyping specific traits in soil, either in the greenhouse or in natural field settings. However, dynamic properties of the root system can be investigated in nondestructive soil-based studies which maintain the topology and architecture (Johnson, Tingey, Phillips, & Storm, 2001).

Root phenotyping techniques during preanthesis and at anthesis in soil-filled pots and columns have the potential to generate valuable information on adult plant root traits to address the shortfalls of seedling root studies (Manschadi, Hammer, & Christopher, 2008; Watt, Wasson, &

Abbreviations: BVR, biomass to volume ratio; DAP, days after planting; R/S, root/shoot biomass ratio; RER, root elongation rate; RWD, root weight density.

Chochois, 2013a). Minirhizotrons are nondestructive in situ root phenotyping systems suitable for both greenhouse pots and field landscapes that enable the acquisition of highquality data on root growth, demography, and dynamics in a spatiotemporal context (Crocker et al., 2003; Polomski & Kuhn, 2002). Ideally, minirhizotrons are used to trace fine roots (<2-mm diameter), as coarse roots (>2-mm diameter) are not accurately measured since the entire root surface does not come in contact with the minirhizotron imaging tubes (Johnson et al., 2001). The time-intensive process for extracting quantitative data from the scanned images is the most challenging issue in this system. Artifacts such as crossing and overlapping of the root segments in the image window add complexity as the root system grows and develops (Lobet et al., 2017).

Field-based root phenotyping is complicated by heterogeneity of the soil profile, especially if the goal is to screen a large number of plant species or individuals (Wasson et al., 2012; Zhu, Ingram, Benfey, & Elich, 2011). Moreover, fieldbased studies can be confounded by the genotype \times environment interactions which may mask the genetic potential or variance in root growth of individual cultivars (Paez-Garcia et al., 2015; Rich & Watt, 2013). Greenhouse studies allow a large number of plants to be phenotyped with the additional ability to impose different abiotic stresses in a climatically controlled environment (Jeudy et al., 2016; Watt et al., 2013b). Characterizing root traits in soil-filled pots and chambers can mimic natural environments. However, selection of an appropriate pot size is essential to avoid impediments to root growth and ensure reproducible and unbiased experimental results (Poorter, Bühler, Van Dusschoten, Climent, & Postma, 2012).

Breeding for beneficial root traits has gained increased attention in the recent years since plant breeders have already focused considerable effort on enhancing grain yield by modifying aboveground shoot structures (Richards et al., 2010). Previous research has found that root system architecture of specific crop species can be altered to improve desirable agronomic traits such as yield, drought tolerance, and resistance to nutrient deficiencies (Tuberosa et al., 2002; Uga et al., 2013). Furthermore, crop simulation models suggested that narrowand deep-rooting traits increase biomass accumulation and grain yield in wheat (Triticum aestivum L.) and maize (Zea mays; Hammer et al., 2009; Manschadi, Christopher, deVoil, & Hammer, 2006). Higher root surface area is another desirable root trait in crop species since it increases the absorptive area for water and nutrient uptake, thus benefitting the crop, particularly in the soil with a limited supply of essential resources (Narayanan, Mohan, Gill, & Vara Prasad, 2014; Newman, 1966). Herein, we characterize root traits of three commonly grown spring wheat cultivars in the inland Pacific Northwest, Louise, Alpowa, and Hollis, in addition to two drought-tolerant cultivars, Dharwar Dry and Drysdale, and a facultative spring landrace, AUS28451, to determine the

Core Ideas

- Increased root growth occurs after node elongation with maximum growth at anthesis.
- Drysdale, Dharwar Dry, and AUS28451 have potential to enhance root growth in spring wheat.
- Root diameter is negatively correlated with root surface area, root length, and root volume.
- Seminal root growth rate is a good predictor of overall root system architecture.

genetic potential of these cultivars for improving root architectural traits for dryland farming environments.

2 | MATERIALS AND METHODS

2.1 | Growing conditions and planting materials

The experiments were performed in a climate-controlled greenhouse environment at the Wheat Plant Growth Facility at Washington State University in Pullman, WA, for two consecutive years, 2015-2016 and 2016-2017. Five spring wheat cultivars, including three inland Pacific Northwest cultivars, Alpowa (PI 566596), Hollis (PI 632857), and Louise (PI 634865); the Australian cultivar, Drysdale; an Indian landrace, Dharwar Dry; and one facultative spring wheat landrace from Iran, AUS28451 (PI 621458) were replicated four times in a completely randomized design to study root system architecture traits throughout their growth period. These cultivars were considered to serve as a basis for screening root traits in populations developed from the crosses of Louise \times AUS28451, Hollis \times Drysdale, and Louise \times Alpowa to breed for drought and disease tolerance (Martinez et al., 2018; Thompson, Smiley, & Garland-Campbell, 2015). In the first year, the experiment was conducted in 40-L black plastic pots (NSW15TB, Nursery Supplies, Sumner, WA) from November 2015 to February 2016 (except for AUS28451 which was harvested on June 2016). The experiment was repeated from September 2016 to January 2017 (AUS28451 was harvested in April 2017) in the second year to assess the heritability and robustness of the observed root traits. Sungrow SS#6 RSi potting mix without perlite (Sun Gro Horticulture, Agawam, MA) was used in 40-L pots in both years. In addition, a duplicate experiment was conducted at the same time both years in 3-L pots filled with 1:1 fine sand (<1 mm)/regular potting mix, Sungrow SS#1 F1P RSi, for shoot and root biomass extraction. Both 3-L and 40-L pots were kept in the same greenhouse until simultaneous harvesting under identical growth conditions. The average daily temperature maintained in the greenhouse was 20-22°C, the atmospheric CO_2 concentration was 729–741 mg m⁻³, photosynthetically active radiation (PAR) was approximately 170–210 µmole m⁻² s⁻¹, and relative humidity was approximately 37%. The plants were grown in 16:8 h light/dark cycle.

Surface sterilized seeds (0.6% sodium hypochlorite) of individual wheat cultivars were allowed to germinate first in petri dishes containing 2.3 g L^{-1} Murashige and Skoog Minimal (MS) growth media with 3 g L^{-1} Gelzan CM agar (Phytotech Labs, Shawnee Mission, KS) for a week in the growth chamber (22°C; 16:8 h light/dark; ~100 µmol m^{-2} s⁻¹). Two seedlings were then transplanted into the greenhouse pots at 7 d post germination and were thinned to one after a week. Regular irrigation was supplied along with slow-release fertilizer Osmocote (14:14:14 N/ P/K; Scotts Miracle Gro, Marysville, OH) to ensure a non-limiting supply of essential nutrients. Seedling root growth experiments were performed under controlled laboratory conditions in 150 by 15 mm petri dishes (VWR International, Radnor, PA). Seedlings of all cultivars were germinated and seminal growth rate of the first root pair was measured from five individual seedlings per dish over 5-d period. These experiments were repeated up to eight times.

2.2 | Installation of the minirhizotron system, image acquisition, and processing

The CI-600 minirhizotron system is comprised of a transparent tube and a root imaging unit (CID Bio-Science, Camas, WA). A 105-cm-long acrylic tube (6.5-cm inner diameter) was installed at an angle of 45° off the vertical axis in each 40-L pot filled with potting mix as per manufacturer's instructions (CID Bio-Science, 2016) prior to planting (Supplemental Figure S1). The CI-600 in situ root imager was inserted down the tubes to capture color images of live roots that came in contact with the tubes under the soil. The scanning resolution of the image was adjusted to 300 dpi in the first year and was raised to 600 dpi in the second year to ensure quality root images. Technically, the scanner head takes four images (hereafter called a window) at consecutive depths down the tube. However, given the pot height of less than half a meter in this study, part of the wheat root system exposed to only half of the third window and the entire fourth window were combined for image analysis (Supplemental Figure S1c, S2).

The root tubes were sequentially imaged at 3-d intervals beginning 18 d after planting (DAP) when transplanted seedling roots were first visible in all tubes until physiological maturity in the first-year study to gain a close understanding of the root growth dynamics. The time point of 18 DAP was selected as root growth was only observed after 10 DAP and was negligible until 18 DAP. Root images taken up to 48 DAP were considered to capture root growth trends preanthesis as the five spring wheat cultivars were at or approaching the early reproductive stage (Z50), except the facultative landrace AUS28451 (Supplemental Table S4). The final root scan at anthesis of individual cultivars was analyzed because no additional root growth was observed after anthesis at a pot depth of 43 cm (Supplemental Figure S3–S8). Root scanning in the second year was performed at a 6-d interval with all other procedures and sampling intervals kept consistent with the first-year study.

After visual observations of images, root images were analyzed quantitatively at 6-d intervals to capture root growth dynamics during the vegetative stage. The images obtained from the minirhizotron system were processed using Root-Snap! version 1.3.2.25 software to generate quantitative measurements of root traits (CID Bio-Science; Supplemental Figure S2). A number of root traits from the observed imaging windows including number of roots, root length, root surface area, root volume, average root diameter along with an additional parameter, root elongation rate, were measured and subject to statistical analyses.

2.3 | Harvest and measurements

The crop growth stage was recorded at each scanning date based on the Zadoks growth scale (Zadoks, Chang, & Konzak, 1974; Supplemental Figure S3-S8). In addition, days to heading and maturity were also noted for each of the six cultivars (Supplemental Table S4). The data on shoot biomass and grain yield from both 3-L and 40-L pots in the first year and additional data on root biomass from the 3-L pot in the second year were recorded for statistical analysis. Briefly, the entire potting mixture after harvesting the shoot biomass at harvesting maturity stage was placed in a 2-mm sieve to separate bigger debris and organic matter from the roots. Roots were washed gently with a hose using lukewarm water by spreading the soil sample in the sieve plate to get rid of small soil particles and perlite adhering to the root. Organic matter that still clung to the root system was further removed with the help of tweezers. Both shoot and root biomass were stored in a Ziploc bag at -80°C and then freeze-dried for 72 h before recording the total dry plant biomass for data analysis. Further, root weight density (RWD) was also calculated by dividing plant root biomass with the soil volume from 3-L pots.

2.4 | Statistical analysis

Pearson correlation coefficients were calculated for shoot biomass and grain yield from 40-L pots with root traits measured at the heading stage. Correlation coefficients were also calculated between the root and shoot parameters obtained from the 3-L pots with those from the 40-L pots. Since the randomly assigned minirhizotron tubes within individual pots

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for each cultivar were sequentially scanned at a 6-d interval from 18-48 DAP, this study was considered a repeatedmeasures experiment. The analysis of variance was performed using the MIXED procedure of the SAS statistical package (SAS Institute, 1990). Pooled analysis of 2-yr of data with six-time point measurements taken on six wheat cultivars was performed under a completely randomized design (CRD) and analyzed as a three-way factorial of study year, cultivar, and plant age (DAP). Similarly, 2-yr of measurements of root traits during anthesis were treated as independent variables from prior measurements and analyzed separately in each year. The assumption of normality was tested by observing trait histograms and normal probability plots as well as conducting Kolmogorov-Smirnov (P < .05) tests using Proc Univariate in SAS. Response root variables from the repeated measures study violating the normality assumption included root number, root length, root surface area, and root volume so these were log transformed (log_{10}) while average root diameter was inverse power transformed (x^{-1}) . The rest of the data on root and shoot attributes from the 40-L and 3-L pot experiments were analyzed without data transformation.

The best-fit covariance model for the repeated measures data was also assessed based on the information criteria (i.e., smallest Bayesian information criterion, value) as described by Moser (2004). The most parsimonious covariance model was the heterogeneous first-order autoregressive [ARH(1)] which was used for the whole analysis. The least square means were calculated from the mixed model analysis, and the significant differences in the trait mean values were determined using the adjusted Tukey-Kramer procedure at the 5% level of significance (P < .05). The macro PDMIX800 within the SAS Proc Mixed interface was used for simple pairwise comparison of mean components. In addition, polynomial contrast was performed to detect root growth trends over time in the repeated measures data.

For the seedling root growth experiments, each individual seminal root was treated as a single replicate (experimental unit) and analyzed using repeated measures analysis of variance as above. Since 10% of the seminal root growth rate measurements were zero due to stagnant or cessation of root growth until 5 d after plating throughout the study period, the number 1 was added to the whole dataset as a constant value prior to log-transformation to meet the assumptions of normality.

3 | RESULTS

3.1 | Root growth dynamics during the preanthesis growth phase

Prior to heading, from 18–48 d after planting (see Supplemental Figure S3–S8 for Zadoks growth stage), the roots of the different cultivars responded in a similar way over the two study years and the root traits were highly heritable as indicated by the nonsignificant year \times cultivar interaction effect for all parameters. The root growth over time was also consistent across the 2 yr as indicated by the nonsignificant threeway interaction between study year, cultivar, and plant age (DAP; Supplemental Table S1). The orthogonal polynomial contrast revealed a significant quadratic growth trend in the mean number of roots prior to heading (Figure 1a). Root parameters such as root length, root surface area, and root volume changed over time following a significant positive linear, cubic growth trend prior to heading (Figure 1b–1d). However, average root diameter showed a significant negative growth trend suggesting that wheat produces finer roots as crop growth escalates (Figure 1e).

Cultivars did differ for total number of roots prior to heading as indicated by the significant interaction between plant age and cultivar for this trait (Supplemental Table S1). Dharwar Dry $(2.4 \pm 0.06 \text{ and } 2.6 \pm 0.07)$ along with Alpowa $(2.2 \pm 0.06 \text{ and } 2.5 \pm 0.07)$, AUS28451 $(2.1 \pm 0.06 \text{ and } 2.4 \pm 0.07)$, and Drysdale $(2.2 \pm 0.06 \text{ and } 2.3 \pm 0.07)$ showed a significantly higher number of roots at both 42 and 48 DAP, respectively, compared with Hollis $(2.0 \pm 0.06 \text{ and } 2.2 \pm 0.07;$ Supplemental Table S5). However, root length, root surface area, root volume, and average root diameter showed nonsignificant differences among cultivars when analyzed at specific growth stages (Supplemental Table S6–S9).

3.2 | Root system architecture traits significantly differed among spring wheat cultivars at the heading stage

Root traits of all the cultivars were measured at their respective heading stage (Z59) which is defined as 50% of heads emerged from the flag leaf, regardless of the time required for the transition to flowering. AUS28451, a facultative spring landrace, went through a long vegetative phase compared with other cultivars before entering the reproductive stage (Supplemental Table S4). In 2015–2016, the total number of roots was found to be significantly higher in AUS28451 (610 ± 41) , followed by Alpowa (543 ± 83) , and Dharwar Dry (439 \pm 26), with the least number observed in Hollis (171 ± 24) . Similarly, AUS28451 showed the highest number of roots (2226 ± 280) in 2016–2017 (Figure 2a). Root length was significantly longer in AUS28451 (6423 ± 647 and 16717 ± 2744 mm, respectively) in both study years while shorter in Hollis (1611 \pm 223 and 1904 \pm 339 mm, respectively; Figure 2b).

There was discernible variation in root surface area among cultivars with higher values observed in AUS28451 ($8200 \pm 881 \text{ mm}^2$) and Alpowa ($8199 \pm 1090 \text{ mm}^2$) in the first year, and in AUS28451 ($16538 \pm 2760 \text{ mm}^2$), Dharwar Dry ($6494 \pm 1622 \text{ mm}^2$), and Alpowa ($4775 \pm 929 \text{ mm}^2$) in the



FIGURE 1 Increase in the mean number of roots (a) from 18–48 d after planting followed significant quadratic trend (P < .0001) while root length (b), root surface area (c), and root volume (d) followed significant cubic growth trend (P = .0128, P < .0001, and P < .0001, respectively). Average root diameter followed a significant negative quartic trend (P = .0145) (e). Each point within the scatter plot represents the mean value of six cultivars replicated four times over two study years (n = 48)

second year (Figure 2c) with similar results for root volume in 2015–2016 (Figure 2d). However, the average root diameter of individual cultivars exhibited negative correlation with the rest of the root traits measured. In 2016–2017, AUS28451 had the smallest root diameter (0.29 ± 0.01 mm) in contrast with the largest diameter observed in Drysdale (0.36 ± 0.02 mm) and Hollis (0.35 ± 0.02 mm). Moreover, Dharwar Dry and Alpowa exhibited higher values for root number, root length, surface area, and volume, but had smaller root diameters (Figure 2e).

In addition, average root elongation rate (RER) calculated by dividing total root length by the growth stage (in terms of days after planting) of individual cultivars was measured. Since the rooting depth was limited by pot depth in our study, RER could be a promising trait for providing realistic information on root growth metrics and also add robustness to the minirhizotron results. We found similar results with Alpowa, Dharwar Dry, Drysdale, and AUS28451 exhibiting significantly higher RER (ranging 59–85 mm d⁻¹) compared with Hollis (31 mm d⁻¹; Figure 2f).



FIGURE 2 Root traits compared among six spring wheat cultivars grown in 40-L pots during heading stage for two consecutive years, 2015–2016 and 2016–2017. Number of roots (a), root length (b), root surface area (c), root volume (d), average root diameter (e), and root elongation rate (f) were separately analyzed in each individual study year. Different letters on the bar graph within the study year indicates significant difference in root traits among wheat cultivars at P < .05 using the Tukey-Kramer test. NS means nonsignificant at P < .05. The bars indicate standard error of the mean. The top-down order of the cultivars within the legend follows left to right in the bar graph for individual year

2016-2017

3.3 | Root traits were positively correlated with shoot biomass, grain yield, and days to heading

Year

2015-2016

To explore whether there is correlation between root traits and grain yield, analysis was performed between the individual root traits and yield measured from the direct harvesting of plants grown in 40-L pots for 2 yr. AUS28451 was excluded from the correlation analysis as this facultative landrace with increased data values and higher correlation coefficient skewed the data trends and was likely to influence any relationship between grain yield and root traits. The higher values for root traits in the second year compared with the first year in AUS28451 is likely to change the overall relationship (compare Figure 3 and Supplemental Figure S9 for

Year

2016-2017

2015-2016



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FIGURE 3 Correlation of different root system architecture traits (a–e) and shoot biomass (f) with grain yield as observed from simple linear regression analysis. There was a strong positive correlation of shoot biomass (f), moderate positive correlation of root surface area (c), root volume (d), and root elongation rate (e) and slight positive correlation of number of roots (a) and root length (b) with grain yield at P < .05. Data points observed from 2 yr of study for all cultivars except AUS28451 are included

correlation with and without AUS28451). As expected, shoot biomass was positively correlated with grain yield ($r^2 = .94$, P < .0001) whereas, root surface area ($r^2 = .35$, P < .0001), root volume ($r^2 = .40$, P < .0001), and root elongation rate ($r^2 = .30$, P = .0003) showed a moderate correlation to grain yield. While statistically significant (P < .05), root number and root length showed only a slight correlation to grain yield ($r^2 < .3$; Figure 3). In addition, all the root traits, except average root diameter, were positively correlated with each other as well as with shoot biomass, whereas root diameter had a significant negative correlation with the number of roots (Supplemental Table S10).

Since we were interested in examining the possible effects of phenology on root growth and development in spring wheat cultivars further, correlation analysis was performed between days to heading and root traits measured in the 40-L pot experiment. Interestingly, we found that the number of roots, root length, root surface area, root volume, and root elongation rate were moderately correlated with days to heading both in the absence and presence of AUS28451 (Supplemental Figure S10 and S11, respectively).

3.4 | Differential root biomass among wheat cultivars supports the minirhizotron-based study

In addition to the minirhizotron-based study, we measured the total root biomass of spring wheat cultivars grown in 3-L pots at harvesting maturity in 2016–2017 (Figure 4). There was significant variation observed among spring wheat cultivars for total root biomass per plant. The highest root biomass observed in AUS28451 (5.61 ± 0.38 g plant⁻¹) was more than 20-fold higher than the least biomass producing cultivars, Hollis and Louise (0.25 ± 0.11 and 0.26 ± 0.05 g plant⁻¹, respectively; Supplemental Figure S12; Figure 4b). Root/Shoot biomass (R/S) ratio was significantly higher in AUS28451 (0.13 ± 0.01) followed by Drysdale (0.05 ± 0.02 ;



FIGURE 4 Shoot and root phenotypes of six wheat cultivars in the 40-L (top panel; a) and 3-L (bottom panel; b) pots, respectively. The facultative spring landrace 'AUS28451' remained at the tillering stage while the other five cultivars were at the grain filling stage as observed in 40-L pot experiment (a). Differential root biomass of spring wheat cultivars was observed in 3-L pots performed at the same time and in the same environmental conditions (b). The white scale bar at the top of panel b equals 1 cm

Figure 5). Similarly, RWD was also noted to be significantly higher in AUS28451 (1.87 \pm 0.13 mg cm⁻³) while lowest in Hollis and Louise (0.08 \pm 0.04 and 0.09 \pm 0.02 mg cm⁻³, respectively; Figure 5).

3.5 | Effect of pot size on the shoot and root attributes underpins phenotypic plasticity in spring wheat cultivars

The use of compact pots in climate-controlled greenhouse studies is a common practice as they increase the number of replicates while efficiently using limited space. We sought to examine both the difference in shoot attributes across the cultivars as well as variation of traits between 40-L and 3-L pot experiments at maturity for two independent years. Total shoot biomass and grain yield were found to be higher in AUS28451 (217 ± 7 and 76 ± 3 g plant⁻¹, respectively) along with Alpowa (112 ± 20 and 49 ± 7 g plant⁻¹, respectively) and Dharwar Dry (98 ± 16 and 44 ± 7 g plant⁻¹, respectively) when compared with other cultivars in 40-L pots in a pooled analysis (Supplemental Table S11). This result further reinforces the result of correlation analysis discussed above, suggesting that higher root trait values observed in these cultivars at the heading stage might have contributed to the higher shoot biomass along with grain yield. Both shoot biomass and



FIGURE 5 Root weight density (RWD) and root/shoot ratio (R/S) among spring wheat cultivars in the small pot (3 L) study during 2016–2017. Different letters in both lower and uppercase indicate a significant difference in RWD and R/S ratio respectively among cultivars at P < .05. The bars indicate standard error of the mean



FIGURE 6 Correlation of shoot biomass obtained from the pot size study (3 L vs. 40 L) during 2015–2016 and 2016–2017. 'AUS28451' was excluded from the study in both years. Each point is the average of four replicates from an individual year on each of five spring wheat cultivars (n = 4)

grain yield were also noted to be higher in AUS28451 (46 \pm 5 and 13 \pm 1.7 g plant⁻¹, respectively) and Alpowa (20 \pm 0.5 and 9 \pm 0.3 g plant⁻¹, respectively) in the 3-L pot experiment (Supplemental Table S12). However, AUS28451 showed the lowest harvest index (HI) in both the 3-L and 40-L pot experiments (0.27 \pm 0.02 and 0.35 \pm 0.01, respectively) (Supplemental Table S11, S12).

Moreover, we found a strong positive correlation between shoot biomass obtained from 40-L and 3-L pot (Supplemental Figure S13). However, the relationship was moderate when AUS28451 was not included in the analysis, which shows that extreme value of AUS28451 might have substantially influenced the results (Figure 6). As expected, the positive relationship shows that shoot biomass increases with the increase in pot size.

In this context, total plant biomass (both shoot and root) to pot volume ratio (BVR) has is one of the important criteria



FIGURE 7 Total plant biomass/pot volume ratio (BVR) among spring wheat cultivars in the small pot (3 L) study during 2016–2017. Different letters indicate a significant difference at P < .05. The dotted line indicates the threshold BVR of 2 g L⁻¹ as suggested by Poorter et al. (2012). All cultivars except Hollis exceeded the threshold level of BVR. The bars indicate standard error of the mean

both for understanding the effect of pot size and selection of appropriate pot size. Interestingly, it was noted that none of the cultivars, except Hollis, grown in 3-L pot had BVR ≤ 2 g L⁻¹ (Figure 7).

3.6 | Seminal root growth rates follow a similar pattern observed in adult plant root traits

As our greenhouse study did not capture early primary and seminal root growth, we also measured seedling root growth rate in a plate-based assay. Seminal root growth rate was significantly higher in AUS28451, Dharwar Dry, and Drysdale at Day 4 and 5 in the 5-d old seedlings as compared with Alpowa, Louise, and Hollis (Supplemental Table S13; Supplemental Figure S14), which is consistent with our results in the greenhouse studies.

4 | DISCUSSION

The purpose of this study was to characterize root traits of six wheat cultivars that are either commonly grown in the inland Pacific Northwest (Louise, Alpowa, and Hollis) or landraces, that is, drought-tolerant cultivars (Dharwar Dry, Drysdale, and AUS28451) that are being used to enhance drought tolerance or pathogen resistance (Thompson et al., 2015). In addition, we wanted to determine if the drought-tolerant cultivars and landraces (Dharwar Dry, Drysdale, and AUS28451) have a common ideotype that could be bred or selected for secondarily by breeders. Previous studies have proposed increased root length and reduced root diameter for better scavenging of the least mobile nutrients and water while conserving soil moisture during drought stress resembling the steep, cheap, and deep root ideotype proposed by Lynch (2013; Narayanan et al., 2014; Richards & Passioura, 1989; Watt et al., 2013a).

Since the facultative spring wheat AUS28451 showed a prolonged vegetative stage, it is not surprising that it achieved greater overall root growth. AUS28451 had significant variation in root length between the first and second year, although still significantly higher than other cultivars in both years. The longer root length observed in the second year of study is probably due to longer growth duration in the vegetative phase (additional 24 d before heading), thereby increasing the canopy function and carbon supply to the root system in the second year compared with the first year (Edwards, Benham, Marland, & Fitter, 2004; Supplemental Table S4).

Additionally, the spring wheat cultivars Alpowa and Dharwar Dry also showed a later transition to flowering allowing them to accumulate increased root biomass. Alpowa harbors a single weaker vernalization gene, and thus still requires a short period (1–2 wk) of cold treatment (Martinez et al., 2018). The slower early root growth of Alpowa and Dharwar Dry might be beneficial for initial water conservation while helping to escape drought stress during rapid growth at later stages due to photoperiod insensitivity as described for Alpowa (Santra, Santra, Allan, Campbell, & Kidwell, 2009). Above all, the positive correlation between days to heading and root traits observed in minirhizotron study suggest that phenology is likely a key driver for the difference in root traits observed among the six wheat cultivars examined herein.

Root diameter is often negatively correlated with other root traits, particularly under drought and in-field conditions. For example, a greenhouse study on winter wheat at the preflowering stage showed that total root length correlated negatively with root diameter (Awad, Byrne, Reid, Comas, & Haley, 2017). Likewise, a field-based study in maize found that diameter decreased along the root and change in diameter was correlated with length (Wu, Pages, & Wu, 2016). However, the average root diameter reported in this study was consistent with the 0.14-0.30 mm range of fibrous wheat root system reported by Manschadi, Manske, and Vlek (2013). Narayanan et al. (2014) observed a positive correlation between shoot biomass and several root traits such as rooting depth, root dry weight, root length, root surface area, and root volume of spring wheat grown in soil-filled columns in greenhouse conditions. Although shoot biomass and grain yield were measured from individual plants, the positive relationship between grain yield and root traits suggest that root traits have a remarkable influence on grain yield. In fact, higher root number, longer root length, and the higher surface area could contribute toward yield enhancement in wheat. A suite of studies performed in rice (Oryza sativa; Uga et al., 2013), corn (Lynch, 2015), and wheat (Wasson et al., 2012) have already demonstrated that modification of some specific root traits such as root length, root diameter, and branching angle can boost crop yield by optimizing resource acquisition from the soil. In this context, reduced root diameter in AUS28451, Alpowa, and Dharwar Dry coupled with increased root length, as observed in our study, could be a cost-efficient strategy for better exploration of sparse water and nutrients stored in the deeper soil profile.

Root-shoot biomass partitioning is considered an important strategy for efficient use of resources, particularly when plants are exposed to drought and nutrient deficiencies. The results from the R/S ratio in this study were slightly inconsistent with the study conducted by Manschadi et al. (2008), where Dharwar Dry outperformed most of the tested cultivars. However, the difference in the Dharwar Dry R/S ratio observed in the two studies was probably due to the difference in sampling time. The root biomass was sampled at early vegetative stage (33 DAP) in the previous study, while the current study examined the ratio at physiological maturity.

The ratio of root biomass to soil volume is another root parameter indicative of how well roots can explore soil resources (Ma, Wood, & Bransby, 2000). Higher RWD in the topsoil layer can enhance nutrient absorption, while higher root density in deeper soil is essential for reaching stored water (Pandey, 2012). Cultivars capable of increasing root biomass by forming deep, branched root systems are considered ideal for capturing moisture from deeper soil profiles (Manschadi et al., 2008; Watt et al., 2013b). Overall, AUS28451 had significantly higher root biomass, R/S ratio, and RWD in the 3-L pot experiment which was consistent with the higher root traits values observed in the minirhizotron study in 40-L pots. The results strongly suggest that the larger root biomass of AUS28451 is likely to provide better adaptation to environmental extremes, such as moisture stress and poor soil fertility.

Shoot biomass was more than five times and grain yield was more than six times higher in the 40-L pots than in the 3-L pots when averaged across six spring wheat cultivars for 2 yr (Supplemental Table S11, S12), suggesting that plants have substantial phenotypic plasticity to adjust their growth to their surroundings. Previously, it has been proposed that small pots might be a constraint for root growth and proliferation of both above- and below-ground biomass (Poorter et al., 2012). All the cultivars, except Hollis, exceeded the minimum threshold BVR of 2 g L⁻¹. Our findings are not surprising since nearly 65% of the controlled experiments in a mega-study failed to consider pot size (Poorter et al., 2012). Nonetheless, the positive correlation between 3-L and 40-L pots in this study suggests that plants grown in small pots are representative of those grown in big pots.

The results from early growth of seminal roots is important for the implication of seedling root traits for both plant establishment and the relation to dynamic root functions as plants grow and mature (Golan, Hendel, Méndez Espitia, Schwartz, & Peleg, 2018). The seminal roots and their laterals also contribute significantly to form the bulk of the fibrous wheat root system (Osmont, Sibout, & Hardtke, 2007) which have been subjected to selection pressure during crop domestication for promoting seedling recovery against drought stress (Golan et al., 2018). On average, the spring wheat cultivars produced 1.2-cm seminal root growth per day across the 5d study period (data not shown) which was similar to the 1–3 cm of primary (seminal) axile root elongation of wheat seedlings observed in a paper roll experiment (Watt et al., 2013b). Interestingly, the significantly higher seminal root growth rate was identified in AUS28451, Dharwar Dry, and Drysdale, which was indicative of increased root length, volume, and area found in this study.

5 | **CONCLUSIONS**

With advancing developmental stage, initiation and growth of nodal roots along with the branching of different root classes to higher orders is a common phenomenon (Manschadi et al., 2013). The increase in root number via branching is an important root trait as it enhances associated root traits, such as root surface area and root volume, in later stages (Newman, 1966). Thus, Dharwar Dry, AUS28451, and Alpowa produced the highest root number and showed the possibility for higher order root traits in later growth stages. Also, it is interesting to note that these three cultivars were at late-tillering (Z29) to early node elongation stage (Z31) until 48 DAP displaying the initiation of root branching while Drysdale, Hollis, and Louise were already advancing to booting and later stages (Z41-Z55) with well-branched root systems (Supplemental Figure S3–S8). Additionally, the significant increase in root growth observed from 36 DAP onward in most of the cultivars suggests that the wheat root system engages in increased growth after early node elongation stage (Z31) until anthesis.

The study of root traits in controlled conditions is crucial for comparisons with natural environments (Zhu et al., 2011) and helps to maximize heritable phenotypic variance. In the field, root growth can be severely arrested due to edaphic constraints such as extreme drought (Richards et al., 2010), and root systems exhibit a substantial degree of phenotypic plasticity in response to environmental cues (Kano, Inukai, Kitano, & Yamauchi, 2011; Osmont et al., 2007; Palmer, Bush, & Maloof, 2012). In fact, Hollis and Drysdale did not differ significantly in dryland farming conditions in terms of root number, length, and volume in field settings (Ghimire, 2017). Nonetheless, the genetic variability identified in this research, and knowledge gained from these studies can be leveraged to develop drought-resilient wheat cultivars with improved root systems through molecular and quantitative genetic approaches (de Dorlodot et al., 2007; Watt et al., 2013a) targeting dryland farming communities, particularly in the face of global climate change (IPCC, 2014).

ACKNOWLEDGMENTS

We would like to acknowledge generous support from the Washington Grain Commission grant number 3019–7768 to CMS, KGC, and KAS. This article is based on work that is supported by the US Department of Agriculture (USDA) NIFA Hatch project 1014527.

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How to cite this article: Ghimire B, Hulbert SH, Steber CM, Garland-Campbell K, Sanguinet KA. Characterization of root traits for improvement of spring wheat in the Pacific Northwest. *Agronomy Journal*. 2020;112:228–240. https://doi.org/10.1002/agj2.20040