Seedling and field assessment of wheat (*Triticum aestivum* L.) dwarfing genes and their influence on root traits in multiple genetic backgrounds

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Abstract

Deployment of the *Rht-B1b* and *Rht-D1b* dwarfing genes helped facilitate the Green Revolution to increase wheat yields globally. Much is known of the influence of these genes on plant height and agronomic performance, but not of their effects on root architecture. We assessed 29 near-isogenic lines (NILs) representing 11 Green Revolution and alternative dwarfing genes across multiple genetic backgrounds for root architecture characteristics in controlled and field environments. Genetic background did not influence plant height, but had a small and significant (*P* < 0.05) effect on root architecture. All dwarfing gene NILs were significantly (*P* < 0.01) shorter compared with tall controls. The Green Revolution *Rht-B1b* and *Rht-D1b* sometimes had longer seedling roots but were not different from their respective tall controls for root depth in the field. The *Rht8*, *Rht12*, and *Rht18* dwarfing gene NILs produced long seminal roots in seedling pouches, and a greater maximum rooting depth (MRD) and root penetration rate (RPR) in the field. Genotypic increases in MRD and RPR were strongly correlated with increased harvest index and grain yield, particularly in dry environments. Careful root phenotyping highlights the potential of novel dwarfing genes for wheat genetic improvement under water-limited conditions.

Keywords: Alternative dwarfing genes, breeding, drought, maternal, root architecture, seed size.

Introduction

Plant height of wheat was significantly reduced with the introduction of reduced height (*Rht*) genes distributed globally with the Green Revolution. The Green Revolution represented a scientific-led framework to increase global grain production and food security through integration across multidisciplinary research activities. Improved management strategies for...
irrigation and fertilization were coordinated with deployment of high-yielding germplasm with improved resource utilization efficiency supported by greater investment in crop research, and improved infrastructure and policy support (Pingali, 2012). The linking of high-yielding germplasm, photoperiod insensitivity, and major dwarfing genes in varietal development has been estimated to have increased wheat productivity ~1.0% per year (Evenson and Gollin, 2003; Lumpkin, 2015).

The most widely used dwarfing genes in wheat breeding are Rht-B1b (formerly Rht1) and Rht-D1b (formerly Rht2) bred from the Japanese wheat landrace Daruma. Both dwarfing genes reduce plant height through reduced sensitivity to the growth hormone gibberellic acid (GA). This GA insensitivity reduces cell expansion to decrease cell length and leaf and stem size (Keyes et al., 1989; Peng et al., 1999). Another group of dwarfing genes, termed ‘alternative dwarfing genes’, reduce plant height while maintaining GA sensitivity (Rebetzke et al., 2012). One GA-sensitive (GAS) dwarfing gene, Rht8, was derived from the Japanese wheat landrace Akakomugi, and reduces plant height through sensitivity to brassinosteroids (Gasperini et al., 2012). This gene is associated with greater cell size in seedling tissue to increase leaf area and coleoptile length (Botwright et al., 2001, 2005).

The successful adoption of wheat dwarfing genes was evidenced by widespread adoption of Rht-B1b, Rht-D1b, or Rht8 in >70% of wheat varieties grown globally by the 1990s (Evans, 1998). Several studies have evaluated the agronomic and physiological performance of the Green Revolution dwarfing genes (e.g. Flintham et al., 1997; Casebow et al., 2016). Shorter elongating stems reduce competition to increase assimilate partitioning to ears, thereby increasing floret fertility and kernel number, and ultimately harvest index (HI) (Fischer and Stockman, 1986; Flintham et al., 1997; Butler et al., 2005). Both Rht-B1b and Rht-D1b are molecularly and physiologically similar, reducing plant height by 14–25% depending on genetic background and environment (Flintham et al., 1997). In contrast, the alternative Rht8 dwarfing gene, present in many eastern European varieties due to chromosomal linkage with the photoperiod-insensitive Ppd-D1a gene, is more variable in its plant height reduction (8–38%) (Ellis et al., 2004; Rebetzke et al., 2012).

Despite their widespread adoption, several studies have reported a negative influence of the GA-insensitive (GAI) dwarfing genes on agronomic performance particularly in less favourable growing environments. For example, Butler et al. (2005) reported that non-Rht tall lines were equal to, or greater than, GAI Rht-B1b and Rht-D1b siblings in grain yield, test weight, and kernel weight across contrasting irrigation regimes. Similar reductions in biomass and yield have also been reported elsewhere for Rht-B1b and Rht-D1b, and particularly when assessed in droughted environments (e.g. Mathews et al., 2006; Jatayev et al., 2020). The GAI dwarfing genes have been widely reported to reduce coleoptile length to reduce seedling establishment especially when sown deep (Trehowan et al., 2001; Ellis et al., 2004; Rebetzke et al., 2007). Further, Rht-B1b has been reported to reduce photosynthetic rate in the flag leaf and reduce grain protein content (Jobson et al., 2019).

All dwarfing genes have been reported to decrease canopy height, but their influence on root growth and architecture are not well understood. Comparisons between dwarfing genes have largely been restricted to seedling root architecture in controlled environments (e.g. McCaig and Morgan, 1993; Wojciechowski et al., 2009) with some field assessment of rooting depth and root length density (e.g. Siddique et al., 1990; Hodgkinson et al., 2017). Genetic assessment has focused on mapping populations (e.g. Bai et al., 2013), comparisons between historic and modern wheat varieties (e.g. Waines and Ehdaie, 2007; Friedli et al., 2019), and less frequently between dwarfing gene near-isogenic lines (NILs) (e.g. Miralles et al., 1997; Wojciechowski et al., 2009). The wide range of experimental and sampling methods, and inconsistency in findings between studies, has highlighted uncertainty in the influence of wheat dwarfing genes on root growth (e.g. McCaig and Morgan, 1993; Miralles et al., 1997).

Deeper roots are hypothesized to increase late-season access to water and nutrients particularly in rainfed environments. Kirkegaard et al. (2007) demonstrated how uptake of even small amounts (10.5 mm) of water from depth later in the season increased water use efficiency to significantly increase grain yield in rainfed wheat crops. Deeper roots have also been suggested as a key breeding objective for accessing additional nutrient and water resources from greater soil depth to increase wheat yields (Araus et al., 2002; Wasson et al., 2014). However, despite the well-established influence of dwarfing genes on shoot growth (e.g. Botwright et al., 2005; Rebetzke et al., 2014), how dwarfing genes influence root growth in wheat is poorly understood. In this study, we assessed root growth for 11 dwarfing genes representing multiple genetic backgrounds in seedling and adult plants in controlled and field environments.

Materials and methods

Wheat genotypes

Wheat genotypes were contained in four sets representing different wheat genetic backgrounds and containing between 2 and 11 different dwarfing (Rht) genes (Tables 1–3). Set one contained CSIRO-developed, BC2-derived dwarfing gene NILs in the tall, Australian variety ‘Halberd’. A representative tall sister NIL (‘HalberdT’) was selected and used as the tall control for direct comparisons with each of the dwarfing gene NILs containing either Rht-B1b, Rht-D1b, Rht-B1c, Rht-D1c, Rht5, Rht8, Rht9, Rht12, Rht13, or Rht18. Seven lines were randomly sampled for each dwarfing gene to minimize bias through sampling of genetic background. Set two contained BC2- derived NILs for six dwarfing genes (Rht-B1b, Rht-D1b, Rht-B1c, Rht4, Rht5, and Rht8) and the tall parent in the Russian ‘spring’ variety Miranovskaya (hereafter ‘M808S’) (Loskutova, 1998). Set three consisted of four CIMMYT-based, spring maturity pairs near isogenic for Rht-B1b (Galvez 87, Nesser 90, and Seri 82) and Rht-D1b (Pavon 76), and their tall siblings (Trehowan et al., 2001). All NILs were BC3 derived. Set four contained CSIRO-developed, BC3-derived Rht13
Fig. 1. Weather conditions for Yanco 2018 (A), Condobolin 2017 (B), and Condobolin 2018 (C). The red line represents the smoothed weather curve, and actual measurements are the blue line. Irrigation events are shown for pre-anthesis irrigation (filled circles) and pre- and post-anthesis irrigation (filled squares) regimes. Average anthesis date was (A) 13 Oct (B) 7 Oct (C) 8 Oct.

Experiment management

A series of experiments were conducted in 2017 and 2018 at two sites in the southern wheatbelt of New South Wales, Australia: the Department of Primary Industries Research Station at Yanco (–34.6281645, 146.431761E; elevation 136 m) and the Neil Fettell Irrigation Centre at Condobolin (–33.047495S, 147.238776E; elevation 195 m). The Yanco experiments were undertaken at the Yanco Managed Environment Facility (see Rebetzke et al., 2013 for details).

Experiments at both sites were managed according to standard practice with plots sown mid- to late May following a pulse crop rotation and summer fallow. At Yanco, fertilizer was supplied as 100 kg ha$^{-1}$ MAP (Incitec Pivot Fertilisers) with the seed while at Condobolin, fertilizer was supplied as 105 kg ha$^{-1}$ Starter 15 (Incitec Pivot Fertilisers) also with the seed. Nitrogen requirements were monitored and supplemental urea (Incitec Pivot Fertilisers) was supplied as required based on crop growth predictions with the TopCrop® modelling program. Experiments were baseline rainfed, with two supplemental irrigation regimes being applied at each location (Fig. 1). In irrigation regime one (I1), irrigation was supplied up to anthesis (Z55–60) and then stopped (i.e. terminal drought with a final irrigation supplied at Z45–5). Irrigation regime two (I2) was as for I1 but with 1–2 additional irrigations at and after anthesis. Supplemental irrigation was supplied as needed to achieve: (i) the long-term average in-season rainfall with regime I1; and (ii) 25% above average in-season rainfall with regime I2. Timing and amounts of irrigation were determined using soil type and rainfall input data in the StressMaster® modelling program. At Yanco, irrigation was supplied by a travelling irrigator, and in 2018, a pre-sowing irrigation of 158 mm was applied. After sowing and during vegetative growth, a total of 112 mm of irrigation was supplied in five events in I1 and I2, and an additional 93 mm in I2 applied across three events post-anthesis (Fig. 1). At Condobolin, water was supplied with flood irrigation and the amount of water was estimated at 100 mm per irrigation event. The soil profile at Condobolin was full prior to sowing in 2017 so only a single irrigation was applied during vegetative growth (I1 and I2) and one irrigation at anthesis (I2). Irrigation at Condobolin in 2018 was as for 2017 except that an additional flood irrigation was supplied pre-sowing in both I1 and I2. The soil at Yanco is classified as a red chromosol (Merriungue loam to Merriungue sand) and Condobolin as a grey vertosol (Isbell, 1996).

At Yanco, all four sets of lines were grown under the two irrigation regimes (I1 and I2) in a p-rep ($r=1.5$) row–column experimental design with four blocks (two blocks per irrigation regime). Space was limited at Condobolin so only the Halberd NILs were grown in 2017 under I2 and in 2018 under both irrigation regimes (I1 and I2). Genotypes at
Where $m$ is the experiment mean, $L_i$ is the effect of the $i$th location; $Y_j$ is randomized to blocks at Yanco. As whole plot at Condobolin and as a block design with irrigation levels at Condobolin were replicated four times in a row–column experimental design. Irrigation treatments were applied as a split-plot design (irrigation as whole plot) at Condobolin and as a block design with irrigation levels randomized to blocks at Yanco.

In all experiments, 6 m long plots were sown at a target density of 160 plants m$^{-2}$ using seed from irrigated field trials harvested the previous year. Plots at Yanco were seven rows wide and spaced 25 cm apart, and at Condobolin were 10 rows wide and spaced 18 cm apart. At maturity, plots were end-trimmed to 5 m length and border rows were removed, leaving the middle rows for harvest.

Weather data were obtained from on-site weather stations or a nearby weather station provided through the Australian Government Bureau of Meteorology weather service (www.bom.gov.au).

**Field measurements**

Mature plant height and grain yield were recorded for each plot. Plant height was measured in the middle row as the distance from the ground to the top of the head excluding the awns. Grain yield (per unit area) was calculated from the weight of machine-harvested grain and measured plot length and width (as number of rows×spacing). At Yanco, anthesis date (Zadoks et al., 1974), anthesis biomass, and HI were measured, and maturity biomass was calculated. For anthesis biomass, four bordered rows of 30 cm were cut at ground level at anthesis (Z65), dried for 4 d at 72 °C, and weighed. For HI, three 50 cm long bordered rows were cut at maturity (Z99), weighed, and then threshed. The HI was calculated as threshed grain weight–total bundle weight, and maturity biomass was calculated as plot yield–HI.

Maximum root depth was determined for each plot after harvest using a modification of the core-break method (Wasson et al., 2014). Briefly, 4 × 4.2 cm diameter soil cores were inserted in the soil to a depth of 2 m using a tractor-mounted, hydraulic soil corer. Two cores were taken on, and two cores between, sowing rows in the middle of each plot. Intact cores of 30 cm were cut at ground level, with significant reductions for the Rht-D1b leaf as it was not fully elongated (Supplementary Table S1). All dwarfing genes was less evident on the length of the second leaf one in Rht-D1b, Rht-D1c, Rht-D1d, and Rht-D1f (Supplementary Table S1). In the Halberd genetic background, Seedlings were harvested at approximately the 1.6 leaf stage (Table 1). The GAS dwarfing gene NILs were barely present at the 1.6 leaf stage in all varieties except for the Rht-D1c parent. (Supplementary Table S1). The GAS dwarfing gene NILs were reduced or equal in size to the Rht-D1c parent. (Supplementary Table S1). The GAS dwarfing gene NILs were reduced or equal in size to the Rht-D1c parent. (Supplementary Table S1).

**Statistical analysis**

A combined analysis of variance and covariance over sites, years, and irrigation regimes was performed for all characters using the SAS mixed model procedure Proc MIXED (Littell et al., 1996). Dwarfing gene, genetic background, and irrigation regime were assumed fixed effects, whilst blocks, sites, and years were random effects in the linear model containing both main effects and their interactions. The general statistical model was:

$$Y_{ijklr} = m + L_i + Y_j + LY_{ijk} + R/LY_{r(ik)} + I_k + LY_{ik} + LLY_{ijk} + GL_{ijk} + G_{li} + GLY_{ij} + GLY_{ijk} + GY_{ijk} + GLYI_{lik} + GLYI_{ijk} + e_{ijklr}$$

where $m$ is the experiment mean, $L_i$ is the effect of the $i$th location; $Y_j$ is the effect of the $j$th year; $LY_{ijk}$ is the effect of the interaction between location $i$ and year $j$; $R/LY_{r(ik)}$ is the effect of the $r$th block nested in the $i,j$th location and year; $I_k$ is the effect of the $k$th irrigation; $LY_{ik}$ is the effect of the interaction between location $i$ and irrigation $k$; $LY_{ijk}$ is the effect of the interaction between location $i$, year $j$, and irrigation $k$; $GLY_{ij}$ is the effect of the interaction between genotype $l$ and location $i$, year $j$, and irrigation $k$; $GY_{ijk}$ is the effect of the interaction between genotype $l$, location $i$, and irrigation $k$; $GLY_{ijk}$ is the effect of the interaction between genotype $l$, year $j$, and irrigation $k$; and $e_{ijklr}$ is the residual.

Years, locations, and blocks were random effects, and irrigation and genotypes were fixed effects. The model was modified replacing genotype terms with genetic background and NILs nested within genetic backgrounds when comparing specific dwarfing gene effects. Further, appropriate site and year main and interaction effects were removed when analysis was undertaken for specific sites (e.g. Condobolin versus Yanco) and/or years (e.g. Yanco 2018 irrigated and rainfed).

In a separate analysis, genotypes were considered as random effects to obtain variance and covariance estimates in the linear mixed model. For root measurements, individual scorers undertaking root measures and the measured core length were included as fixed linear covariates in the mixed model. Genetic correlations and their SEs were estimated for all characters after Holland (2006). The testing of NIL means was undertaken using a priori pre-planned contrasts which use a 1 degree of freedom test under the null hypothesis of no dwarfing gene effect. In the mixed model, all fixed effects were tested with a Wald’s test. Heritability was estimated on an entry-mean basis after Holland et al. (2003). The effects of individual dwarfing genes on measured characters was determined in statistical comparisons with the relevant near-isogenic tall (Halberd, M808S, and CIMMYT) or Rht-B1b/Rht-D1b (Australian varietal) controls using an orthogonal linear vector fitted in the CONTRAST statement in Proc MIXED (Littell et al., 1996). Unless otherwise indicated, statistical significance is reported at $\alpha=0.05$. Figures were constructed in R version 3.5.2 (R Core Team, 2013) and SigmaPlot version 14.0 (Systat Software, San Jose, CA, USA).

**Results**

**Seedling measurements in controlled environments**

Seedlings were harvested at approximately the 1.6 leaf stage (Supplementary Table S1). In the Halberd genetic background, the length of the first seedling leaf was significantly ($P<0.01$) reduced in lines containing the GAI dwarfing genes (Rht-B1b, Rht-D1b, Rht-B1c, and Rht-D1c) compared with the tall Halberd control (Table 1). The GAS dwarfing gene NILs were not different in length from the tall controls. The effect of dwarfing genes was less evident on the length of the second leaf as it was not fully elongated (Supplementary Table S1). All NILs had decreased length of leaf one in the M808S background, with significant reductions for the Rht-D1b, Rht-B1c, and Rht4 dwarfing gene NILs. In the Australian varietal backgrounds, length of leaf one in Rht-B1b or Rht-D1b parental NILs was commonly reduced or equal in size to the Rht13 and Rht18 NILs. The only exception was in the Yitpi background.
Table 1. Seedling leaf and root means for dwarfing gene near-isogenic lines (NILs) representing multiple genetic backgrounds

| Background/NIL | Length leaf 1 (mm) | No. of seminal roots (n) | Total seedling root length (mm) | Total seminal root length (mm) | Average seminal root length (mm) |
|----------------|--------------------|--------------------------|---------------------------------|-------------------------------|----------------------------------|
| Halberd        |                    |                          |                                 |                               |                                  |
| HalberdT (Tall)| 144.7              | 4.65                     | 1357                            | 1230                          | 266                              |
| Halberd Rht-B1b| 127.8**            | 5.56**                   | 1450                            | 1435*                         | 258                              |
| Halberd Rht-D1b| 125.9**            | 5.04*                    | 1341                            | 1341                          | 266                              |
| Halberd Rht-D1c| 97.9**             | 5.00*                    | 1458                            | 1368                          | 274                              |
| Halberd Rht12  | 135.7              | 4.92                     | 1390                            | 1264                          | 240*                             |
| Halberd Rht13  | 155.6              | 4.89                     | 1457                            | 1351                          | 276                              |
| Halberd Rht18  | 137.9              | 5.11*                    | 1495                            | 1365*                         | 267                              |
| M808S          |                    |                          |                                 |                               |                                  |
| M808S (Tall)   | 157.3              | 4.33                     | 1200                            | 1200                          | 277                              |
| M808S Rht-B1b  | 142.0              | 4.67                     | 1373                            | 1373                          | 294                              |
| M808S Rht-D1b  | 134.4*             | 5.20*                    | 1466*                           | 1466*                         | 282                              |
| M808S Rht-D1c  | 99.7**             | 4.50                     | 1287                            | 1287                          | 286                              |
| M808S Rht4     | 130.8*             | 4.00                     | 1352                            | 1282                          | 321**                            |
| M808S Rht5     | 140.7              | 5.00                     | 1275                            | 1256                          | 251                              |
| M808S Rht8     | 141.7              | 4.33                     | 1183                            | 1133                          | 262                              |
| Australian varietal backgrounds |           |                          |                                 |                               |                                  |
| cv. Espada     | 119.2              | 4.60                     | 1193                            | 1148                          | 250                              |
| Espada Rht13   | 125.8              | 4.67                     | 1257                            | 1257                          | 269                              |
| Espada Rht18   | 118.5              | 3.83*                    | 1040                            | 1040                          | 272*                             |
| cv. Gregory    | 118.0              | 4.83                     | 1327                            | 1288                          | 267                              |
| Gregory Rht13  | 138.5              | 4.83                     | 1217                            | 1191                          | 247                              |
| Gregory Rht18  | 116.8              | 4.60                     | 1257                            | 1257                          | 273                              |
| cv. Mace       | 103.8              | 5.20                     | 1231                            | 1231                          | 237                              |
| Mace Rht18     | 122.9*             | 5.20                     | 1412                            | 1412*                         | 272*                             |
| cv. Magenta    | 112.0              | 5.00                     | 1540                            | 1559                          | 308                              |
| Magenta Rht18  | 140.4**            | 4.80                     | 1332                            | 1332                          | 278                              |
| cv. Scout      | 99.3               | 4.83                     | 1006                            | 1008                          | 209                              |
| Scout Rht18    | 108.6              | 4.40                     | 1213*                           | 1154*                         | 258**                            |
| cv. Yitpi      | 128.0              | 5.40                     | 1518                            | 1495                          | 277                              |
| Yitpi Rht18    | 112.8**            | 5.25                     | 1301**                          | 1288**                        | 245                              |

where the Rht-D1b parent was significantly (P<0.01) greater for length of leaf one (Table 1). Averaged across varietal backgrounds, Rht13 (+11%) and Rht18 (+7%) were significantly greater in length than their Rht-B1b and Rht-D1b parents.

Numbers of seminal roots were equal to, or increased, for all dwarfing gene NILs compared with their respective tall controls, and consistently across both Halberd and M808S genetic backgrounds (Table 1). The GAI dwarfing gene NILs produced significantly greater numbers of seminal roots particularly in the Halberd background. Numbers of seminal roots in Rht-B1b or Rht-D1b parental NILs were generally the same as their corresponding Rht13- or Rht18-carrying NILs in the Australian varietal backgrounds. One exception was Espada where the Rht18 NIL produced significantly fewer roots than the Rht-D1b-containing parent. Total root length (seminal+branch roots) (TRL) and seminal root length (seminal roots only) (SRL) were highly correlated ($r_s=0.93, P<0.01$), and were equal to, or increased, in size, for all dwarfing gene NILs compared with the tall (Halberd and M808S) and most Rht-B1b or Rht-D1b (Australian varietal) controls (Table 1). The Yitpi Rht18-containing NIL was significantly (P<0.01) reduced for TRL and SRL compared with Yitpi.

Dwarfing gene NILs with increased SRL typically had greater numbers of seminal roots (e.g. Halberd containing Rht-B1b and Rht18, and M808S containing Rht-D1b). Numbers of seminal roots were positively correlated with increases in SRL ($r_s=0.63; P<0.01$), and average SRL was consistent for all dwarfing gene NILs except reduced length for Rht-D1c (Halberd).
and greater length for Rht4 (M808S) and Rht18 (Espada, Mace, and Scout). Root angle between seminal roots two and three, and seminal roots four and five, was similar for all dwarfing gene NILs, except Rht5 which had a narrower root angle in the Halberd genetic background (Supplementary Table S1).

**Field environments**

Daily maximum and minimum temperatures, rainfall, and solar radiation are given for all site×year irrigation treatments in Fig. 1. In all experiments, air temperatures were consistent with long-term minimum and maximum temperatures at each site. The Yanco in-season rainfall was 144 mm (long-term average 238 mm) while the total water supplied to the crop including irrigation was 271 mm and 363 mm for Yanco 2018 I1 (pre-anthesis irrigation only) and I2 (pre- and post-anthesis irrigation), respectively. For Condobolin 2017 I2, the in-season rainfall was 229 mm (long-term Condobolin average of 231 mm), while rainfall in Condobolin 2018 was considerably reduced, with 158 mm in the growing season with some larger rainfall events occurring later during grain filling. As irrigation was supplied at Condobolin through flood irrigation, the amount of water supplied to each sowing cannot be estimated exactly.

**Above-ground measurements under field conditions**

**Plant height**

Repeatability for plant height was high ($R^2=0.93 \pm 0.04; P<0.01$), reflecting small dwarfing gene×environment and residual variances from the combined mixed model analysis. All dwarfing gene NILs were significantly ($P<0.01$) reduced in plant height compared with their respective tall control in Halberd, M808S, and CIMMYT genetic backgrounds (Table 2). The GAI Rht-B1c and Rht-D1c, and GAS Rht12 dwarfing genes all produced extreme dwarf phenotypes (reductions of 42–57%), while the remaining dwarfing genes reduced plant heights by 8–39% across both Halberd and M808S backgrounds. Height reductions for the Rht-B1b and Rht-D1b GAI dwarfing genes were 8% and 13%, and 10% and 8%, respectively, in the Halberd and M808S backgrounds, and were consistent with the 10–13% height reduction for Rht-B1b and Rht-D1b genes in the CIMMYT semi-dwarf NILs (Table 2). A similar reduction in height with Rht-B1b and Rht-D1b across the Halberd, M808S, and CIMMYT genetic backgrounds reflected the statistically non-significant dwarfing gene×background interaction across all dwarfing gene NILs (data not shown). In the Australian varietal backgrounds, NILs containing the Rht13 or Rht18 alternative dwarfing genes were equal, or shorter, in plant height than their Rht-B1b or Rht-D1b recurrent parents (Table 2). On average, both the GAS Rht13 and Rht18 dwarfing genes reduced height by 9% compared with their respective Rht-B1b- or Rht-D1b-containing parents.

**Development**

Development as Zadoks score varied between NILs in all genetic backgrounds. Generally, NILs containing alternative dwarfing genes Rht4, Rht5, Rht8, and Rht9 accelerated development toward anthesis, whereas Rht-B1c, Rht-D1c, and Rht12 slowed development when compared with their respective Halberd tall and M808S controls (Table 2). The dwarfing gene×genetic background interaction was statistically significant, with the Rht-B1b gene delaying development relative to the tall control in the M808S and Seri genetic backgrounds ($P<0.01$), but not in the Halberd background. Conversely, the Rht-D1b gene hastened development in the Halberd background ($P<0.01$) but was not statistically different from tall controls in the M808S and CIMMYT genetic backgrounds (Table 2). In the Halberd background, the Rht13 gene hastened, and Rht18 delayed, development. Development was generally accelerated and anthesis date earlier with Rht13 and Rht18 compared with Rht-B1b or Rht-D1b in the Australian varietal backgrounds. Exceptions were the Espada and Gregory backgrounds where Rht13 and Rht18 genes varied in their effect on plant development.

**Crop biomass and grain yield**

Grain yields for the majority of dwarfing gene NILs were similar to, or greater than, those of their respective tall controls (Table 2). The dwarfing gene×genetic background interaction was statistically significant, with some dwarfing gene NILs varying for grain yield across genetic backgrounds. For example, grain yield for the extreme dwarfing gene Rht-B1c NIL was significantly less than for the tall Halberd control but significantly greater than for the M808S tall control. The Rht-D1b-carrying NILs were significantly higher yielding in the Halberd and most CIMMYT backgrounds but not in M808S, while Rht-B1b NILs were lower yielding in the M808S background but not in Halberd or Seri backgrounds (Table 2). Despite the change in ranking for Rht-B1b, Rht-B1c, and Rht-D1b, some genes (e.g. Rht8) were consistent for grain yield across genetic backgrounds. Grain yields for the Rht18 NILs were commonly equal to, or greater than, yields for their tall or Rht-B1b- or Rht-D1b-carrying parents. In contrast, Rht13 was associated with significantly reduced grain yield in the Espada and Gregory backgrounds (Table 2).

Across all genetic backgrounds, increases in HI were positively correlated with changes in grain yield ($r=0.84, P<0.01$). Dwarfing gene×genetic background interaction was non-significant, with HI being commonly greater for dwarfing gene NILs except for Rht-B1c and Rht-B1b in the Halberd and M808S backgrounds, respectively (Table 2). In the Australian varietal backgrounds, Rht13 was associated with significantly reduced HI whereas Rht18 was commonly equal to or greater for HI (Table 2).

Differences among dwarfing gene-carrying NILs were large and statistically significant for anthesis and maturity biomass (Table 2). The dwarfing gene×genetic background interaction

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**Table 2**

| Dwarfing Gene | Genetic Background | Plant Height Reduction (%) | Development | Crop Biomass | Grain Yield |
|---------------|--------------------|-----------------------------|-------------|-------------|-------------|
| Rht-B1b       | Halberd            | 8                          | Accelerated |             |             |
| Rht-D1b       | M808S              | 13                         | Accelerated |             |             |
| Rht13         | CIMMYT             | 8                          | Delayed     |             |             |
| Rht18         | Australian         | 10                         | Delayed     |             |             |

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was statistically significant with Rht-D1b-, Rht5-, and Rht8-containing NILs, significantly smaller for anthesis biomass than the tall control in the Halberd background, but larger than M808S in the M808S background. Extreme dwarf GAI Rht-B1c or Rht-D1c NILs produced among the smallest anthesis biomass in the Halberd background, whereas the GAS Rht12 extreme dwarf NIL was not statistically different from the tall control. A large dwarfing gene×background interaction for maturity biomass was strongly evident with reranking of means for the GAI Rht-B1b- or Rht-D1b-containing NILs, and GAS Rht5 and Rht8 dwarfing gene NILs between the Halberd and M808S backgrounds (Table 2). The Rht13 dwarfing gene NILs were equal to or greater in maturity biomass across all backgrounds, while Rht18 NILs were larger in biomass in the Halberd background and equal to, or smaller than, their Rht-B1b or Rht-D1b parents in the Australian varietal backgrounds.

Phenotypic variation for root depth under field conditions

In the full mixed linear model, dwarfing gene NIL, genetic background, operator (i.e. root counter), and length of the soil core were all statistically significant ($P<0.01$) for MRD and RPR. Repeatability for MRD was intermediate in size ($R^2=0.56 \pm 0.09; P=0.01$), reflecting a small and non-significant dwarfing gene×environment interaction but large residual and sampling variances (data not shown). Repeatability for RPR, was also intermediate in size ($R^2=0.52 \pm 0.10; P=0.01$). As for MRD, the dwarfing gene×environment interaction was small and not significant, while residual and sampling variances were large (data not shown).

Maximum rooting depth

Maximum rooting depth varied significantly ($P<0.01$) across experiments, ranging from 133 cm at Condobolin in 2017 (with post-anthesis irrigation, 12) to 93 cm at Yanco in 2018 (pre-anthesis irrigation only, 11). In 2018, mean MRD for pre- and pre+post-anthesis irrigation was 115 cm and 123 cm (Condobolin), and 93 cm and 103 cm (Yanco), respectively (Fig. 2). The increase in MRD with post-anthesis irrigation was statistically significant ($P<0.01$) at 6.9% and 9.9% for Condobolin and Yanco, respectively, and was consistent across the two sites with their contrasting soil types.

The MRD was generally equal to or greater at maturity for dwarfing gene NILs when compared with their respective tall or Rht-B1b/Rht-D1b controls across genetic backgrounds (Table 3). Background genetic effects were small but significant ($P<0.01$), with the greatest MRD in the Australian varietal (109 mm) and smallest in the CIMMYT (99 mm) genetic backgrounds (data not shown). The background×dwarfing gene interaction was small and not statistically significant, with NILs ranking similarly across backgrounds for MRD. One exception was the extreme GAI Rht-B1c-carrying NIL which was similar in MRD to the Halberd control (+6%) but significantly deeper (+23%) than M808S (Table 3). The MRD of Rht8 NILs was significantly deeper in both Halberd and M808S genetic backgrounds (+5%). The Rht-B1b or Rht-D1b NILs were not statistically different for MRD from the tall controls in the Halberd, M808S, and three of the four CIMMYT genetic backgrounds (Table 3). The Rht18 NIL varied in MRD across Australian varietal backgrounds (+6% to 24%), and were on average significantly ($P<0.01$) deeper rooting (+16%) than their Rht-B1b or Rht-D1b parents (Table 3). To a lesser extent, the Rht13 dwarfing gene averaged +7% greater rooting depth over commercial parents.

Figure 3 summarizes mean MRD for all NILs carrying GAI, GAS, or no (tall) dwarfing genes in the four background sets. Both GAS- and GAI-containing dwarfing gene NILs were significantly deeper rooting than the tall controls in the Halberd and M808S backgrounds, and not different from each other in the M808S background. However, in both the Halberd and Australian varietal backgrounds, GAS NILs were significantly ($P<0.01$) greater than GAI NILs for MRD (Fig. 3).

Root penetration rate

RPR was only measured at Yanco in 2018. The average RPR was significantly ($P<0.01$) greater (+13%) in the post-anthesis irrigation treatment (8.94 mm d$^{-1}$) compared with the pre-anthesis irrigation treatment (8.15 mm d$^{-1}$).

Genetic background differences were large and significant ($P<0.01$) for RPR, ranging from 8.2 mm d$^{-1}$ for NILs in the CIMMYT background, to intermediate 8.4 mm d$^{-1}$ and 8.5 mm d$^{-1}$ in the M808S and Halberd backgrounds, respectively, and a high rate of 8.8 mm d$^{-1}$ for NILs in Australian varietal backgrounds (Fig. 3). The dwarfing gene×background interaction was small but statistically significant. The GAI NILs were, on average, larger than the tall NILs for RPR in the M808S and CIMMYT backgrounds, but this largely reflected the greater RPR for Rht-B1c and Seri Rht-B1b in the M808S and CIMMYT backgrounds, respectively (Table 3). The RPR was significantly ($P<0.01$) greater for GAS than GAI dwarfing gene NILs in the Halberd and Australian varietal backgrounds, and were larger but not significantly different in the M808S background (Fig. 3). The Rht8 dwarfing NIL was significantly ($P<0.01$) larger for RPR in the Halberd and M808S backgrounds, and Rht9 and Rht12 dwarfing NILs were significantly greater in the Halberd background (Table 3). The Rht13-containing NILs were associated with significantly ($P<0.01$) increased RPR in the Halberd background but were not different from Rht-B1b or D1b NILs in the Australian varietal backgrounds (Table 3). In contrast, the Rht18 NILs were equal to or significantly greater ($P<0.01$) for RPR in Australian varietal backgrounds.

Genotypic relationships for root and agronomic phenotypes

Across both irrigation regimes at Yanco in 2018, genotypic increases in MRD were moderately correlated with increases
Table 2. Predicted values for growth and development characteristics for dwarfing gene near-isogenic lines (NILs) across multiple genetic backgrounds measured across multiple field environments

| Background/NIL      | Plant height (cm) | Zadoks score | Anthesis biomass (kg ha⁻¹) | Grain yield (t ha⁻¹) | Harvest index | Maturity biomass (t ha⁻¹) |
|---------------------|------------------|--------------|----------------------------|----------------------|---------------|--------------------------|
| Galvez (Tall)       | 108              | 56.7         | 1036                        | 2.71**               | 0.246         | 11.130*                  |
| cv. Galvez (Rht-B1b)| 97 (–10.2**)     | 58.6         | 1090                        | 3.24                 | 0.260         | 14.107                   |
| Nesser (Tall)       | 107              | 55.6         | 1218                        | 2.84**               | 0.231**       | 12.403                   |
| cv. Nesser (Rht-B1b)| 93 (–13.1**)     | 56.4         | 1135                        | 4.19                 | 0.368         | 11.408                   |
| Pavon (Tall)        | 105              | 57.1         | 967*                        | 2.50                 | 0.184*        | 14.788*                  |
| cv. Pavon (Rht-D1b) | 94 (–10.5**)     | 57.1         | 1199                        | 2.87                 | 0.273         | 10.640                   |
| Seni (Tall)         | 101              | 60.7**       | 847*                        | 3.83                 | 0.320*        | 11.472                   |
| cv. Seni (Rht-B1b)  | 88 (–12.1**)     | 55.3         | 1058                        | 4.41                 | 0.393         | 11.362                   |
| Australian varietal backgrounds                                                                                                           |
| cv. Espada (Rht-D1b)| 84               | 59.5         | 1379                        | 4.602                | 0.343         | 12.506                   |
| Espada Rht13        | 77 (–8.3**)      | 63.8**       | 860**                       | 4.096*               | 0.312**       | 13.981*                  |
| Espada Rht18        | 83 (–1.2)        | 52.2*        | 1061**                      | 4.348                | 0.336         | 12.657                   |
| cv. Gregory (Rht-B1b)| 99              | 49.3         | 1138                        | 4.061                | 0.325         | 12.228                   |
| Gregory Rht13       | 85 (–14.1**)     | 45.8*        | 1058*                       | 3.448**              | 0.244**       | 12.375                   |
| Gregory Rht18       | 88 (–11.1**)     | 53.5**       | 1242*                       | 4.014                | 0.310         | 12.778                   |
| cv. Mace (Rht-D1b)  | 89               | 57.4         | 1155                        | 4.688                | 0.335         | 14.528                   |
| Mace Rht18          | 87 (–2.2)        | 55.6         | 1172                        | 4.769                | 0.366**       | 13.055**                 |
| cv. Magenta (Rht-D1b)| 82             | 49.0         | 1250                        | 4.162                | 0.310         | 12.686                   |
| Magenta Rht18       | 89 (–8.5**)      | 48.3         | 993**                       | 3.541**              | 0.285         | 11.502                   |
| cv. Scout (Rht-D1b) | 87               | 58.7         | 1066                        | 4.175                | 0.307         | 14.629                   |
| Scout Rht18         | 75 (–13.8**)     | 68.3**       | 956**                       | 4.517                | 0.324         | 11.499**                 |
| cv. Yitpi (Rht-D1b) | 90               | 51.8         | 997                         | 4.175                | 0.307         | 12.457                   |
| Yitpi Rht18         | 83 (–7.8**)      | 55.5**       | 1020                        | 4.640*               | 0.363**       | 11.338                   |

Rht, reduced major height gene with Rht-B1b, Rht-B1c, Rht-D1b, and Rht-D1c genes conferring GA insensitivity (GAI), and Rht4, Rht5, Rht8, Rht9, Rht12, Rht13, and Rht18 genes conferring GA sensitivity (GAS). Zadok’s scores were undertaken 115–118 d after sowing. Percentage reduction in plant height relative to control is given in parentheses. Significance levels represent comparisons with the tall or Rht-B1b, Rht-D1b controls, and are designated as: *P<0.05; **P<0.01.

* Measured at Yanco in 2018 only.
in RPR \((g=0.46 \pm 0.06; P<0.01)\). Delayed development (i.e. lower Zadoks scores) were moderately correlated with genotypic reductions in RPR \((g=0.36 \pm 0.09; P<0.05)\) despite genotypic increases in MRD with later flowering \((g=-0.38 \pm 0.08; P<0.05)\) (Table 4).

Genotypic increases in MRD were positively correlated with decreases in average SRL and HI \((P<0.01)\), and were independent of changes in plant height, development, and anthesis under field conditions (Rich et al., 2020). Therefore, a potential effect of narrower root angles on MRD for specific genotypes cannot be dismissed solely based on our seedling studies.

The shorter lengths of leaf one in the controlled-environment study (Table 1) confirmed previously observed reductions in seedling growth with the GAI dwarfing genes Rht-B1b, Rht-D1b, Rht-B1c, and Rht-D1c (e.g. Botwright et al., 2001, 2005; Addisu et al., 2009; Rebetzke et al., 2014). Further, the greater leaf length of GAS Rht5, Rht8, Rht9, Rht12, Rht13, and Rht18 dwarfing genes was consistent with greater leaf size reported for these genes elsewhere (e.g. Addisu et al., 2009; Rebetzke et al., 2012).

The numbers of seminal roots varied together with SRL \((g=0.63, P<0.01)\), highlighting the importance of factors contributing to greater seminal root number in seedling root architecture. Seminal root number is under strong maternal and genetic control (Meyer, 1976, and references therein), and increases in embryo size are linked to increased frequency and size of seminal roots four, five, and six (Rebetzke et al., 2022). The larger number of seminal roots in NILs containing GAI Rht-B1_ and Rht-D1_ alleles contributed to their greater SRL, while average SRL was not different from that of tall controls (except Rht-D1c which produced significantly shorter seminal roots). The reduced average SRL for Rht-D1c seedlings is consistent with other reports of decreased TRL of Rht-D1c-containing NIL seedlings in controlled environments (Wojciechowski et al., 2009; Bai et al., 2013).

Narrow seminal root angles contribute to greater rooting depth in rice (Oyanagi et al., 1993) and wheat (Richard et al., 2015). In the present study, only the single Rht5-carrying Halberd NIL produced a significantly narrower root angle (seminal roots four and five) than the respective tall control (Table 1). Root angle for the Rht5 NIL in the M808S background was not different from that of the tall control M808S. Neither the Halberd nor M808S Rht5 NILs were significantly different in MRD from their respective tall controls in the field (Table 3). Root angle measured in growth cabinet-grown seedlings was unrelated to root angle measured under field conditions (Rich et al., 2020). Therefore, a potential effect of narrower root angles on MRD for specific genotypes cannot be dismissed solely based on our seedling studies.

**Genotypic variation in plant height under field conditions**

All dwarfing gene NILs reduced mature plant height. Height reductions varied from 10% (Rht-B1b) to 55% (Rht-D1c), and were consistent in effect across contrasting genetic backgrounds (Table 2). The NILs carrying the Rht-B1c, Rht-D1c, and Rht12 dwarfing genes exhibited the largest reductions in plant height, and Rht-B1b and Rht-D1b NILs the smallest height reduction. The change in plant height was consistent...
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with ranking for Rht-B1b, Rht-D1b, Rht-B1c, Rht8, Rht-D1c, and Rht12 dwarfing gene NILs in the winter varietal background Mercia (Addisu et al., 2009). However, whereas the Rht8 NIL was similar in height to Rht-B1b and Rht-D1b NILs in the winter Mercia (Addisu et al., 2009) and Paragon (Casebow et al., 2016) backgrounds, height reduction was greater for Rht8 NILs in the spring Halberd and M808S backgrounds.

Table 3. Predicted values for maximum root depth and root penetration rate for dwarfing gene near-isogenic lines (NILs) across multiple genetic backgrounds measured across multiple field environments

| Background/NIL | Maximum rooting depth (cm) | Root penetration rate (mm d⁻¹) |
|---------------|-----------------------------|-------------------------------|
| Halberd       |                             |                               |
| HalberdT (Tall) | 110                        | 8.15                          |
| Halberd Rht-B1b | 113 (+2.2)                | 8.27 (+1.9)                   |
| Halberd Rht-D1b | 110 (+0.1)                | 8.52 (+4.5)                   |
| Halberd Rht-B1c | 112 (+1.8)                | 8.47 (+4.0)                   |
| Halberd Rht-D1c | 102 (–7.9**)              | 7.22 (–11.9**)                |
| Halberd Rht5   | 110 (–0.3)                 | 8.63 (+6.0*)                  |
| Halberd Rht8   | 113 (+2.6*)                | 8.96 (+9.9***)                |
| Halberd Rht9   | 115 (+4.3*)                | 8.97 (+10.1***)               |
| Halberd Rht12  | 116 (+5.2**)               | 8.99 (+10.4**)                |
| Halberd Rht13  | 114 (+3.8*)                | 8.66 (+6.3**)                 |
| Halberd Rht18  | 113 (+2.5*)                | 8.22 (+0.8)                   |
| M808S          |                             |                               |
| M808S (Tall)   | 106                        | 7.62                          |
| M808S Rht-B1b  | 107 (+0.4)                 | 7.63 (+0.1)                   |
| M808S Rht-D1b  | 112 (+5.2)                 | 8.23 (+8.0)                   |
| M808S Rht-B1c  | 133 (+25.2**)              | 9.56 (+25.5***)               |
| M808S Rht4     | 116 (+9.4*)                | 8.91 (16.9*)                  |
| M808S Rht5     | 112 (+4.8)                 | 8.19 (+7.5)                   |
| M808S Rht8     | 119 (+12.5*)               | 9.08 (+19.2**)                |
| CIMMYT         |                             |                               |
| Galvez (Tall)  | 116                        | 8.71                          |
| cv. Galvez (Rht-B1b) | 118 (+1.3)            | 8.71 (+0.0)                   |
| Nesser (Tall)  | 98                         | 7.38                          |
| cv. Nesser (Rht-B1b) | 109 (+9.7)            | 8.10 (+9.9)                   |
| Pavon (Tall)   | 110                        | 8.24                          |
| cv. Pavon (Rht-D1b) | 107 (–2.7)            | 8.08 (–1.9)                   |
| Seri (Tall)    | 98                         | 7.48                          |
| cv. Seri (Rht-B1b) | 118 (+20.2**)         | 8.38 (+12.5)                  |
| Australian varietal backgrounds |                         |                               |
| cv. Espada (Rht-D1b) | 105                   | 7.69                          |
| Espada Rht13   | 110 (+4.6)                 | 8.53 (+10.4)                  |
| Espada Rht18   | 127 (+21.4***)             | 9.59 (+24.6***)               |
| cv. Gregory (Rht-B1b) | 116               | 8.38                          |
| Gregory Rht13  | 122 (+5.9)                 | 8.81 (+5.2)                   |
| Gregory Rht18  | 121 (+4.7)                 | 9.08 (+8.4)                   |
| cv. Mace (Rht-D1b) | 111                 | 8.11                          |
| Mace Rht18     | 122 (+9.7**)               | 9.39 (+15.7*)                 |
| cv. Magenta (Rht-D1b) | 115               | 8.30                          |
| Magenta Rht18  | 129 (+12.2*)               | 9.27 (+11.7*)                 |
| cv. Scout (Rht-D1b) | 111            | 8.41                          |
| Scout Rht18    | 121 (+8.6*)                | 9.66 (+14.8*)                 |
| cv. Yitpi (Rht-D1b) | 111            | 8.08                          |
| Yitpi Rht18    | 130 (+17.6**)              | 10.4 (+28.7***)               |

Rht, reduced major height gene with Rht-B1b, Rht-B1c, Rht-D1b, and Rht-D1c genes conferring GA insensitivity (GAI), and Rht4, Rht5, Rht8, Rht9, Rht12, Rht13, and Rht18 genes conferring GA sensitivity (GAS). Zadok’s scores were undertaken 115–118 d after sowing. Percentage reduction in plant height relative to control is given in parentheses. Significance levels represent comparisons with the tall or Rht-B1b, Rht-D1b controls, and are designated as: *P<0.05; **P<0.01.

* Measured at Yanco in 2018 only.
of height across the different genetic backgrounds (Table 1) and dwarfing genes reported elsewhere (e.g. Addisu et al., 2009), increases confidence in the genetic effects of dwarfing genes on root architecture and other measured traits.

**Root depth varies across dwarfing genes**

Genotypic variation for MRD and RPR was large and repeatable within and across the different genetic backgrounds. Repeatability was not as large as for plant height (cf. 0.92, 0.56, and 0.52 for height, MRD, and RPR, respectively), but was moderately consistent with heritabilities for MRD in Wasson et al. (2017) and larger than heritabilities reported elsewhere (e.g. Guo et al., 2020). Spatial variation within and between field plots together with factors including coring depth and root count can all contribute to the large sampling variation commonly encountered with field root phenotyping (Wasson et al., 2012; Guo et al., 2020). As reported in Guo et al. (2020), genotype (here dwarfing gene x genetic background) variances for MRD and RPR were large relative to genotype x environment interaction variances, suggesting that only a few well-managed environments containing deep and unconstrained soils are needed for assessing genotypic differences in MRD and RPR.

Time of anthesis varied across dwarfing genes and backgrounds (Table 2). Anthesis in *Rht-B1b*, *Rht-D1b*, *Rht13*, and *Rht18*-carrying NILs was delayed in some but not all backgrounds, whereas *Rht-B1c*-containing NILs were consistently delayed and *Rht8* NILs were consistently quicker to reach anthesis. Dwarfing genes have been reported to vary in their influence on plant development (e.g. Addisu et al., 2010; Rebetzke et al., 2012). The *Rht8* gene is associated with earlier flowering through genetic linkage with the photoperiod insensitivity gene *Ppd-D1a* on chromosome 2DS (Worland et al., 1998). Delays in flowering extend the period for root growth

### Table 4. Genetic correlations (±SE) for maximum root depth and root penetration rates with agronomic traits assessed at Yanco in 2018 for both irrigated and rainfed environments

| Character                  | Maximum rooting depth | Root penetration rate |
|----------------------------|-----------------------|-----------------------|
| Zadoks score               | -0.38 ± 0.08**       | 0.36 ± 0.09**        |
| Mature plant height        | -0.16 ± 0.11ns       | -0.03 ± 0.16ns       |
| Anthesis biomass           | 0.09 ± 0.10ns        | 0.03 ± 0.23ns        |
| Grain yield (all)*         | 0.45 ± 0.16**        | 0.53 ± 0.10**        |
| - Irrigated only           | 0.35 ± 0.14*         | 0.33 ± 0.15*         |
| - Droughted only           | 0.57 ± 0.18**        | 0.75 ± 0.10**        |
| Harvest index (all)*       | 0.56 ± 0.14**        | 0.47 ± 0.22*         |
| - Irrigated only           | 0.49 ± 0.16**        | 0.38 ± 0.15*         |
| - Droughted only           | 0.58 ± 0.18**        | 0.76 ± 0.08**        |
| Maturity biomass (all)*    | 0.20 ± 0.24ns        | 0.21 ± 0.14ns        |
| - Irrigated only           | 0.30 ± 0.21ns        | 0.13 ± 0.15ns        |
| - Droughted only           | 0.06 ± 0.24ns        | 0.22 ± 0.18ns        |

Significance levels are designated as: *P < .05; **P < .01; and ns, P > .05.

*a Combined irrigated and droughted environments.

Australian varieties Espada and Gregory were both represented with GAS *Rht13* and *Rht18* dwarfing gene NILs, with *Rht13* reducing height to a greater extent than *Rht18*. A strong height-reducing effect for *Rht13* was also reported by Divashuk et al. (2020) and Rebetzke et al. (2011). Notwithstanding, the extent of height reduction with *Rht13* was contingent on genetic background (cf. Halberd, Espada, and Gregory backgrounds) (Table 2). Similarly, *Rht18* NILs varied in height depending on genetic background (cf. Mace and Scout backgrounds). The potential for differential ranking for plant height across dwarfing genes emphasizes the need for assessment across both genetic backgrounds and environments.

The large number of random lines representing each dwarfing gene in our study, together with the consistency in ranking
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to increase MRD (Kirkegaard and Lilley, 2007). In the present study, the range in development across dwarfing gene NILs was considered small and the genetic correlation between Zadoks score and MRD modest in size. Further, in the controlled-environment study, genotypic differences for root traits were apparent on seedlings assessed at the same early growth and development stage. Together, these results suggest that dwarfing genes influenced root growth independent of development in the NILs studied.

Delayed flowering across genotypes was modestly correlated with genotypic increases in MRD consistent with reports of extended root growth with slower development to flowering (Kirkegaard and Lilley, 2007). Delaying flowering should allow for deeper root growth and access to available water, but later flowering in many environments can delay grain growth into hot and dry conditions to reduce grain yield and quality. Genotypic variation in RPR was large and repeatable, and genetically correlated with MRD, whereas development score was negatively correlated with RPR. Faster rates of root growth may provide a robust and inexpensive surrogate for MRD independent of crop development. Potential differences in RPR could be simply assessed for large numbers of genotypes in tubes containing sieved field or commercial soils in controlled environments. Further studies are needed to validate the potential for high-throughput phenotyping of RPR in controlled and field environments.

Maximum root depth and RPR were significantly greater for most GAS and GAI dwarfing gene-containing NILs when compared with the respective controls (Fig. 3). Differences between the GAS and GAI groups were in themselves smaller but statistically significant, with the GAS dwarfing NILs an average 2.1% and 4.6% greater for MRD and RPR, respectively. Our data highlight the opportunity for use of GAS dwarfing genes to improve rooting depth, and the consistency of GAS dwarfing NILs for MRD and RPR across multiple genetic backgrounds. For example, MRD in Rht13- and Rht18-containing NILs was equal to, or greater than, the respective Rht-B1b- or Rht-D1b-containing parents in the Australian varietal backgrounds. The advantages of increased coleoptile length and early shoot vigour with GAS dwarfing genes is widely accepted (e.g. Rebetzke et al., 2012; Zhao et al., 2022), and Rht18-based, semi-dwarf wheat varieties are now available commercially. However, the influence of GAS dwarfing genes on root growth and architecture remains to be understood. Even small increases in MRD can increase late-season access to water deep in the soil profile to improve the marginal water use efficiency of rainfed crops (Kirkegaard et al., 2007). The value of deep soil water was demonstrated in the experiment at Yanco in 2018 with the strong and positive genetic correlation for grain yield and both MRD and RPR, and particularly in the droughted environment (Table 3). Further, the strong genetic correlation for root depth parameters with HI but not biomass at maturity suggests that genotypic differences in post-anthesis water use may be contributing to increases in grain yield. Greater water use after flowering has previously been shown to be associated with increases in HI and grain yield in droughted environments (Passioura, 1977), while deeper roots have been shown to increase water use and wheat yields across different drought experiments (e.g. Palta et al., 2011; El Hassouni et al., 2018; Li et al., 2019).

Improving inference when phenotyping root growth and architecture

The need for multiple, deep soil cores to account for root plasticity with soil variability has limited most genetic studies to seedling growth in controlled environments or early growth stages in the field (e.g. Wojciechowski et al., 2009). However, poor correlation between assessments representing different stages of crop development, growth cabinet and field, and different soil mimics (e.g. gels and hydroponics) (Wojciechowski et al., 2009) highlight uncertainty when concluding among studies about genetic factors affecting root architecture.

The ranking of genotypes for seedling root architecture correlates poorly with root architecture in field-grown seedlings (Wojciechowski et al., 2009) and adult (Rich et al., 2020) plants. Seed source and size are well known to affect seedling shoot and root growth (Roach and Wulff, 1987), yet few seedling studies acknowledge the importance of controlling maternal factors when comparing among genotypes. Indeed, seed size is rarely reported or standardized, and dwarfing genes are well established to influence seed number and seed size (e.g. Flintham et al., 1997; Butler et al., 2005). Bai et al. (2013) reported strong positive correlations for seed weight and plant height, with a range of seedling root size attributes measured on genotypes in a wheat mapping population. Further, their quantitative trait locus (QTL) analysis identified overlapping genomic regions for seed weight, seedling root growth, and plant height (including the Rht-D1 locus on chromosome 4D). Their results support other research (e.g. Singh et al., 2017) highlighting the need to control seed weight in seedling root and shoot assessment, and particularly for factors known to affect seed weight such as plant height. Covariance analysis has been used to adjust for large mean seed size differences between dwarfing gene NILs (e.g. Wojciechowski et al., 2009). However, as plant height and seed size differences are confounded (i.e. not independent), inference here on the effects of dwarfing genes on seedling root growth should be treated with caution.

Comparisons of root architecture between short and tall height phenotypes have rarely been undertaken on near isolines or siblings to control genetic background and restrict inference to specific dwarfing genes. For example, numerous studies report reductions in sizes of root systems of modern Rht-B1b and Rht-D1b semi-dwarf varieties in comparisons between modern and older, tall or landrace wheat varieties (e.g. Waines and Ehdaie, 2007; Bai et al., 2013; Bektas et al., 2016; Subira et al., 2016; Aziz et al., 2017; Friedli et al., 2019; Fradgley et al., 2020).

Indeed, comparisons of field-grown tall and GAI semi-dwarf
NILs (at flowering) were not different for root length or biomass (McCaig and Morgan, 1993), or semi-dwarf NILs were larger for root biomass and not length (Miralles et al., 1997).

Evidence for post-anthesis root growth?

Roots were measured growing an average 6.9–9.9% deeper for plots supplied with an additional 1–2 irrigations at and/or after anthesis. The increased MRD was consistent across the two sites despite their contrasting in soil type from a light clay-loam to a heavier vertosol. This evidence for post-anthesis root growth is counter to the belief that root growth ceases at anthesis (Siddique et al., 1990), and that assimilates in demand for grain growth are preferentially allocated away from growing roots (Andersson et al., 2005; Kirkegaard and Lilley, 2007). Additional root growth at depth was unlikely to be due to deeper wetting with irrigation to depths below 90 cm (Asseng et al., 1998), suggesting continued partitioning of assimilate to roots. Post-anthesis root growth has been reported for different wheat varieties assessed in root boxes in a controlled environment (Manschadi et al., 2006), and with post-anthesis fungicide application in the field (Ford et al., 2006). In the latter, increases in root length of 45.6% and 11.5% were measured in fungicide-treated plots in two seasons, with the largest increase in the season with the greatest fungal infection. Similarly, Gregory et al. (1978) reported post-anthesis wheat root growth below 80 cm depth despite a reduction in overall root dry weight. They hypothesized that senescence in some parts of the root system enabled continued root growth and exploration at depth. These different findings suggest that root growth may continue at depth if conditions are favourable (i.e. soils contain adequate moisture and are non-toxic), and support the need for root phenotyping to be undertaken at maturity.

Conclusions

This is the first report of field assessment of wheat dwarfing gene NILs for MRD and RPR, and their relationship with grain yield across multiple genetic backgrounds. All dwarfing genes decreased mature plant height and, in most cases, were equal to or greater for MRD and RPR compared with their respective tall or GAS dwarfing gene controls. Deeper roots were genetically associated with greater HI and increased grain yield particularly in droughted environments. The GAS dwarfing genes showed promise in increasing root depth while maintaining reductions in plant height. Our results quantified the effects of the different dwarfing genes on root growth to aid in informing breeders when selecting genotypes with improved water and nutrient uptake for droughted environments.

Supplementary data

The following supplementary data are available at JXB online.

Table S1. Mean values for different root architecture traits measured on near-isogenic wheat genotypes in a root pouch screen in a controlled environment.

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Author contributions

GR and CI: design and coordination; PH: conducting the controlled experiment and collecting data; DS and KB: managing the field experiments and collecting above-ground measurements; PH, DS, KB, GR, and CI: collecting field data on below-ground roots; GR and CI: data analysis; CI and GR: writing, with all authors reviewing and commenting.

Conflict of interest

The authors have no conflicts to declare.

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Data availability

The data supporting the findings of this study are available from the corresponding author, Greg Rebetzke, upon request.

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