# Gibberellin-sensitive dwarfing gene *Ddw1* has no negative effect on the root system of field-grown winter rye

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## ARTICLE INFO

## ABSTRACT

Rye is a small-grain cereal with an extensive and well-branched root system that is particularly suitable for grain production in marginal environments where other grain cereals, especially wheat, cannot thrive well. However, no significant genetic gain could be achieved to improve plant height and lodging resistance in the last 30 years in rye. Thus, the relatively low grain-to-straw ratio currently hinders rye from becoming more integrated into cereal crop rotations. Here, we report first results on the effects of the gibberellin-sensitive dwarfing gene *Ddw1* on the root system and aboveground traits of rye under field conditions. We assessed root traits of semidwarf and tall genotypes in two field environments at the time of anthesis using soil coring, the core-break method and root scans. Semidwarfs revealed no negative but slightly positive effects on rooting depth, total root length, root length density, and cumulative root length distribution. While plant height was significantly reduced, no increase in harvest index and grain yield was observed in semidwarf prototypes. We conclude that rye does not lose the advantages of its deep and widely branched root system with the introgression of *Ddw1*, especially not on marginal sites.

## 1. Introduction

Rye (*Secale cereale* L.) is one of the minor cereal crops with an area under cultivation of 4.3 million hectares worldwide in 2021, representing about 1.5 % of the total cereal cultivation area. Over 80 % of all rye is grown in Europe, mainly in Central and Eastern Europe (FAO, 2022). Rye is particularly suitable for grain production under extreme climatic conditions and marginal soils with low fertility (Kottmann et al., 2016). It has a higher yield potential than wheat on sandy, infertile and poorly drained soils (Hübner et al., 2013), and it is considered as a comparably drought tolerant cereal crop (Schittenhelm et al., 2014). Rye might therefore be a promising crop to secure grain production under more frequent and severe drought conditions in the future. One of the main reasons of rye's advantages under unfavourable

growth conditions is its extensive and well-branched root system (Dittmer, 1937), with a higher root dry weight compared to wheat and triticale (Sheng and Hunt, 1991). This highly developed root system enables for a very efficient uptake of water and nutrients using 20–30 % less water per produced unit of dry matter compared to wheat (Starzycki, 1976). Further positive characteristics of rye, such as a lower carbon footprint compared to wheat (Riedesel et al., 2022) can contribute to a more sustainable and resource-efficient grain production. However, a lack of significant genetic gain to improve plant height and lodging resistance in the last 30 years (Laidig et al., 2021) hinders rye from becoming more integrated into cereal crop rotations. In wheat, the introgression of both gibberellin (GA)-insensitive (e.g. *Rht-B1b*, *Rht-D1b*) and GA-sensitive dwarfing genes (e.g. *Rht8*) made an important contribution to the "green revolution" (Milach and Federizzi, 2001). In rye,

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the dominant dwarfing gene *Ddw1* has been used in breeding of open pollinating cultivars for the genetic improvement of plant height (Torop et al., 2003; Tenhola-Roininen and Tanhuanpää, 2010). However, this approach is limited by increased frequencies of tall plants, as heterozygous semidwarf genotypes carrying the undesired recessive wild-type allele are difficult to detect within the seed multiplication process of open-pollinating populations (McLeod et al., 2000). As a result, winter rye was by far the tallest among five cereal crops in a recent survey of German variety trials on long-term breeding progress for yield and yield-related traits (Laidig et al., 2021). In contrast to random mating rye populations, *Ddw1* has only recently been used in hybrid breeding (Hackauf et al., 2022). Recent progress in harnessing the potential of rye genomic resources has led to the development of closely linked markers for the GA-sensitive dwarfing gene *Ddw1* (Braun et al., 2019), allowing marker-assisted breeding for germplasm improvement and development of semidwarf rye cultivars with previously unattainable precision.

To the best of our knowledge, there is no information available so far on possible changes in the root system of rye due to the introgression of dwarfing genes. In wheat, centuries of breeding altered root system architecture, as phenotyping of the 'hidden half' and selection for desirable root characteristics were limited despite the importance of the root system in improving the acquisition of water and nutrients (Voss-Fels et al., 2017). Fradgley et al. (2020) reported differences in several root traits between historic and modern wheat cultivars; for example, modern wheat cultivars had fewer nodal and seminal roots per plant. McGrail and McNear (2021) studied a diverse panel of winter wheat cultivars released between 1803 and 2002 in a root phenotyping platform and reported a reduction in root system size and growth rates with increased year of release. In addition, root sizes of modern wheat cultivars were found to be smaller compared with that of older landraces in a study from Waines and Ehdaie (2007). They assume that the root system of modern cultivars is sufficient under optimal growth conditions, but insufficient for the optimal uptake of water and nutrients under conditions of abiotic stress like drought. In line with this assumption, Subira et al. (2016) found in greenhouse experiments with 24 semidwarf (*Rht-B1b*) and tall (*Rht-B1a*) durum wheat cultivars grown in PVC tubes that the reduction in root biomass in semidwarf cultivars was most pronounced in the lowest soil layer. This suggests their limited capacity to extract water from deeper soil layers. Zhang et al. (2009) on the other hand, state that recent semidwarf cultivars of wheat are even more efficient regarding water use. They observed a decrease in total root length mainly in the topsoil, leading to a higher proportion of roots in deeper soil layers. Siddique et al. (1990) also reported a higher root length density in the upper soil layer (0–40 cm) in an older variety compared to a more recent variety. Friedli et al. (2019) found in their study with 14 winter wheat genotypes grown in columns in the greenhouse under drought and well-watered conditions, that wild type cultivars rooted deeper than semidwarf cultivars under well-watered conditions. Under drought, however, both cultivars rooted to equal depth, indicating that modern semidwarf cultivars responded with enhanced root allocation to deeper soil layers (Friedli et al., 2019).

As empirical evidence on the effects of dwarfing genes on the root system of rye is not yet available, the specific objectives of our study are to (i) clarify the effect of *Ddw1* on maximum rooting depth, total root length, root length density, and cumulative root length distribution in field-grown winter rye compared to their near-isogenic full-sibs and modern rye cultivars, and to (ii) evaluate the effect of *Ddw1* on selected aboveground traits.

## 2. Materials and methods

### 2.1. Plant material

In the field experiments, we included three groups of winter rye with three genotypes in each group: Three semidwarf P-type experimental hybrids carrying the *Ddw1* dominant allele, three near-isogenic tall full-sibs (without *Ddw1*), as well as three modern rye cultivars. The *Ddw1* gene was introgressed into elite inbred lines of the Petkus gene pool using novel markers generated by *de novo* transcriptome sequencing and comparative mapping (Braun et al., 2019). These homozygous semidwarf lines as well as their near-isogenic tall full-sibs were used to establish homozygous semidwarf and tall male-sterile single-cross testers, which served as seed parents for the development of the experimental hybrids. To ensure maximal fertility restoration in semidwarfs, the pollen parent carried a non-adapted fertility restorer gene, which was introduced by marker-assisted backcrossing (Hackauf et al., 2012; Hackauf et al., 2017). As modern cultivars, 'SU Perspectiv', 'SU Bebop', and 'SU Performer' were included in the experiment. We refer to these three genotype groups as 'semidwarf', 'near-isogenic tall' and 'cultivar', respectively. All plant materials are proprietary to HYBRO Saatzucht GmbH & Co. KG (Kleptow, Germany).

### 2.2. Field experiments

We carried out the field trials in 2021 and 2022 at the Julius Kühn Institute's experimental fields in Groß Lüsewitz (GL) and Braunschweig (BS), respectively. GL is located in Mecklenburg-Western Pomerania near the Baltic Sea (54.07 N, 12.31 E, 45 m elevation); the predominant soil is Haplic Luvisol over loamy sand, with a soil texture of 78.1 % sand, 17.9 % silt, and 4.0 % clay in the upper 30 cm. BS is located in Lower Saxony (52.30 N, 10.44 E, 80 m elevation). Its soil is classified as Haplic Luvisol, with a soil texture of 78.5 % sand, 15.5 % silt, and 6.0 % clay (0–150 cm). Here, the loamy sand topsoil (0–60 cm) is followed by almost pure sand. Both GL and BS are characterised by a temperate oceanic climate (Cfb) with mean temperatures of 9.6 °C (GL) and 10.0 °C (BS) and a mean precipitation of 730 mm (GL) and 755 mm (BS). The two site-year combinations are considered as environments.

The experiment was set up as an incomplete split-plot design with genotype group as subplot and three replications. Cultivars of similar height were sown adjacent to each subplot to minimise boundary effects. The winter rye cultivar 'SU Cossani' was sown adjacent to the tall hybrids and the cultivar subplots, and the winter triticale cultivar 'Tulus' and the winter rye cultivar 'Durinos' were sown adjacent to semidwarf subplots in GL and BS, respectively.

Sowing was done with a Haldrup SB 25 (Haldrup, Ilshofen, Germany) in GL and a Wintersteiger Nursery Master (Wintersteiger, Ried, Austria) in BS, each at a seeding density of 240 seeds m$^{-2}$. The plot sizes were 6 m$^2$ (4.75 × 1.25) in GL and 8.1 m$^2$ (6.5 × 1.25) in BS. Nitrogen was applied as calcium ammonium sulphate with 163 (GL) and 120 kg N ha$^{-1}$ (BS), split into two applications (beginning of vegetation and BBCH 31). Additional fertilisation was applied based on soil analyses to ensure optimal nutrient supply. Pesticides were applied according to local practice to avoid any biotic stress. Growth regulators were used in BS only. Here, tall hybrids and cultivars were treated with growth regulators. Additional data on crop management can be found in Table 1.

**Table 1**

Crop management in the environments Groß Lüsewitz and Braunschweig.

| | Groß Lüsewitz 2021 | Braunschweig 2022 |
|---|---|---|
| Seeding date | 02 October 2020 | 27 October 2021 |
| Harvest date | 11 August 2021 (313 days after seeding) | 02 August 2022 (297 days after seeding) |
| N-fertilisation (calcium ammonium sulphate) | 73 kg N/ha (02 March 2021) 90 kg N/ha (27 April 2021) Total: 163 kg N/ha | 60 kg N/ha (09 March 2022) 60 kg N/ha (19 May 2022) Total: 120 kg N/ha |
| Additional fertilisation | 0.88 kg P$_2$O$_5$ /ha (05 November 2020) 60 kg P$_2$O$_5$/ha, 140 kg K$_2$O/ha (11 May 2021) | 160 kg K$_2$O/ha, 24 kg MgO/ha, 50 kg SO$_3$/ha (28 February 2022) |

## 2.3. Root sampling and analysis

Root sampling was performed at anthesis. Three soil cores (Ø 6 cm, depth 150 cm) were taken from each plot by means of a petrol-powered percussion coring system and a hydraulic pull device (Nordmeyer Geotool, Berlin, Germany). The soil cores were taken in rows directly below single plants, which were cut off at ground level before sampling and their number of tillers was recorded. The soil cores were separated into 10 cm segments, and the core-break method was performed according to Smit et al. (2000) in order to assess the number of roots per $cm^2$ (RN, roots $cm^{-2}$). Additionally, the maximum rooting depth (RD_max, cm) was defined as the maximum depth where roots were found. On a subsample of each 10 soil cores from GL and BS, the individual 10 cm segments were washed with a 'GVP 13100′ hydropneumatic elutriation system (Gillisons's Variety Fabrication, Benzonia, USA) to separate the roots from the soil. Roots were further separated from soil and other organic materials by hand with tweezers and magnifier lamps. The roots were scanned in a water-filled tray with an Epson Expression 10000XL scanner (Seiko Epson Corporation, Suwa, Japan), with the resolution set to 800 dots per inch. The root scans were analyzed with the image analysis software Rhizovision Explorer (Seethepalli et al., 2021) in order to obtain root length. Analysis mode was set to 'broken roots', image thresholding level was '220′, and the maximum size to filter non-root objects was set to 0.1 $mm^2$. Root pruning was enabled with a threshold of '10′.

A linear regression analysis was performed between RN (from the core-break method) and RL (from the root scans) determined from the same soil cores and depth segments on a subsample of each 10 soil cores based on the method of Wasson et al. (2014), in order to estimate root length for all soil cores and depths. Because of different soil textures in GL and BS, site-specific linear regressions were calculated:, in order to compare the results from both sites:

$$\text{Groß Lüsewitz: RL} = 1447 \cdot 6x + 148 \cdot 7 \ (R^2 = 0 \cdot 82, \ n=144) \qquad (1)$$

$$\text{Braunschweig: RL} = 983 \cdot 2x + 34 \cdot 4 \ (R^2 = 0 \cdot 83, \ n=148) \qquad (2)$$

Whereas RL is the calculated root length and x is RN (number of roots $cm^{-2}$).

Root length density (RLD, cm $cm^{-3}$) was calculated by dividing RL (cm) by the soil volume of each depth section (282.7 $cm^3$). Additionally, total root length (TRL, cm $core^{-1}$) was calculated. For further analyses, RLD was calculated for the depth sections 0–30, 30–60, 60–90, 90–120, and 120–150 cm.

Cumulative root length distribution was calculated by the asymptotic nonlinear ß-model developed by Gale and Grigal (1987):

$$Y = 1 - ß^d \qquad (3)$$

whereas Y is the cumulative root length distribution (ranging from 0 to 1) from the soil surface to depth d (in cm). The regression coefficient *ß* is a measure of vertical root distribution which was estimated with the 'nls' function in R (R Core Team, 2022). Higher ß indicates a higher proportion of root length in deeper soil layers.

The phenological growth stages 'beginning of stem elongation' (growth stage 30), 'beginning of heading' (growth stage 51), 'beginning of flowering' (growth stage 61), 'end of flowering' (growth stage 69), and 'fully ripe' (growth stage 89) were recorded in BS according to the BBCH scale for cereals (Lancashire et al., 1991) and expressed as day of year. Each growth stage was reached when 50 % of the plants per plot had reached the respective stage.

## 2.4. Aboveground traits

Plant height (PH, cm) was recorded at anthesis. Harvest was performed at full maturity on August 11, 2021 in GL and on August 2, 2022 in BS. Before harvesting the experimental plots with a plot combine,

whole plants of 0.5 $m^2$ (GL) and 1 $m^2$ (BS) were cut by hand at ground level. The plants were separated into spikes and straw, the spikes were threshed and winnowed, and the chaff was added to the straw. The grain yield of the hand-harvested plants was added to the machine harvested grain yield. Grain yield (GY), straw yield (SY), and total aboveground biomass yield (BIO) are reported as g $m^{-2}$ at 86 % dry matter content. The yield components spikes $m^{-2}$, grains $spike^{-1}$, and thousand grain weight (TGW) were determined, and the harvest index (HI) was calculated as GY / BIO.

## 2.5. Monitoring of soil water content

The soil water content was measured with the portable soil moisture probe Diviner 2000 (Sentek Technologies, Stepney, Australia) in BS only. Six PVC tubes with a diameter of 5 cm and a depth of 150 cm were installed evenly distributed over the entire experiment. Soil moisture readings were taken in 10 cm intervals from 5 to 115 cm twice a week with the Diviner 2000 probe. The dimensionless readings were converted to plant available water content (in %) by means of a site and depth specific calibration.

## 2.6. Meteorological parameters

Air temperature, precipitation, and further meteorological parameters were measured directly in the field in BS using a mobile weather station (Pessl Instruments, Ried, Austria). In GL, the weather data was provided by the adjacent German Weather Service station number 1803 (Groß Lüsewitz).

## 2.7. Statistics

Genotypes were not evaluated individually, but in their respective genotype group (semidwarf, near-isogenic tall, cultivar). Analyses of variance were performed with the 'lm' function of R (R Core Team, 2022), with genotype group and environment as fixed effects. For the analysis of total root length and root length density, the number of tillers of the sampled plant was set as a covariable in the analysis. Means were compared via Tukey post-hoc test ($\alpha = 0.05$) within the 'emmeans' package in R. All graphs were created in R with the 'ggplot2′ package (Wickham, 2016).

## 3. Results

### 3.1. Environmental conditions

The average air temperature in the main growing season between April and July was 13.4 °C and 15.0 °C in GL and BS, respectively (Fig. 1). The heat stress threshold of 27 °C was exceeded in GL and BS on 14 and 34 days, respectively. The amount of precipitation between April and July was comparable at both locations. In GL, however, April and June were low in precipitation with 25.9 and 21.4 mm, while in BS, March was very low in precipitation with 6.4 mm. In BS, however, the plants received an additional irrigation of 30 mm each in April and May. In the seasonal soil moisture course in BS it can clearly be seen that the two irrigation events replenished the plant available water, but it decreased strongly especially after flowering (Fig. 2). Gradually, the water of the deeper soil layers was also withdrawn.

### 3.2. Maximum rooting depth

At both sites, no differences in the maximum rooting depth between the three genotype groups could be detected, whereas semidwarfs revealed a slightly higher – but non-significant – maximum rooting depth in GL (Fig. 3). Again, there were significant differences between the sites: on average, the roots reached a maximum depth of 122 cm in GL, and 98 cm in BS.
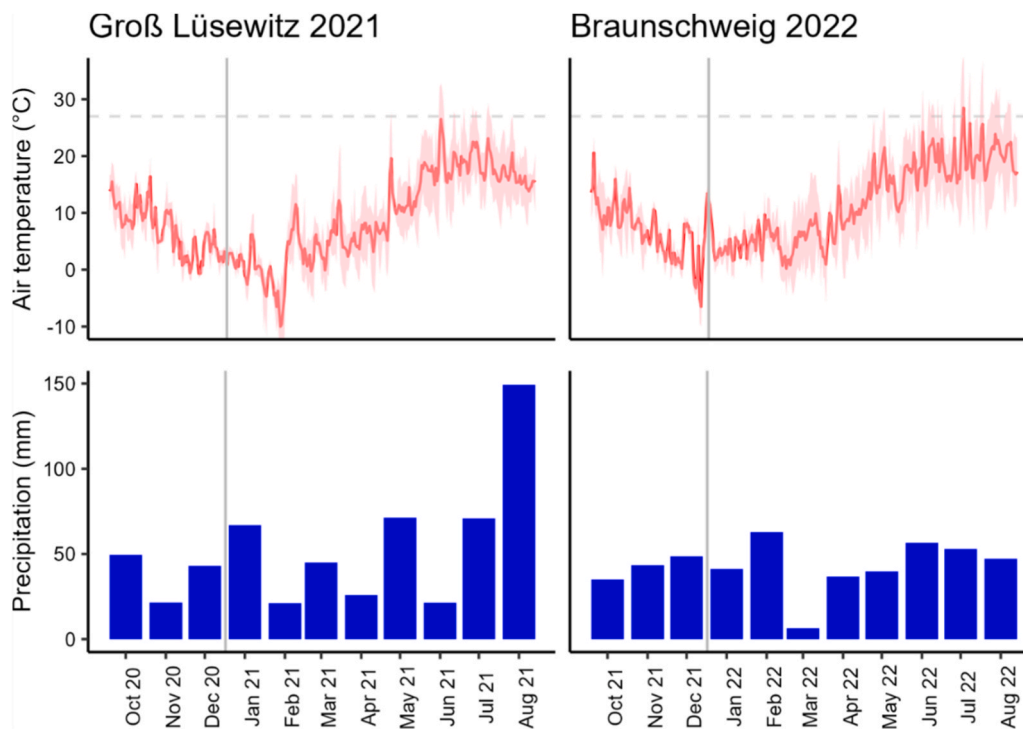
**Fig. 1.** Above: mean daily air temperature (red line) and the range between daily minimum and maximum temperatures (pink area). Below: monthly precipitation from sowing until harvest in GL (left) and BS (right). The vertical grey lines indicate the turn of the year, the horizontal grey dashed lines indicate 27 °C (heat stress threshold for winter wheat according to Rezaei et al., 2015). A heat stress threshold for winter rye is not yet available.
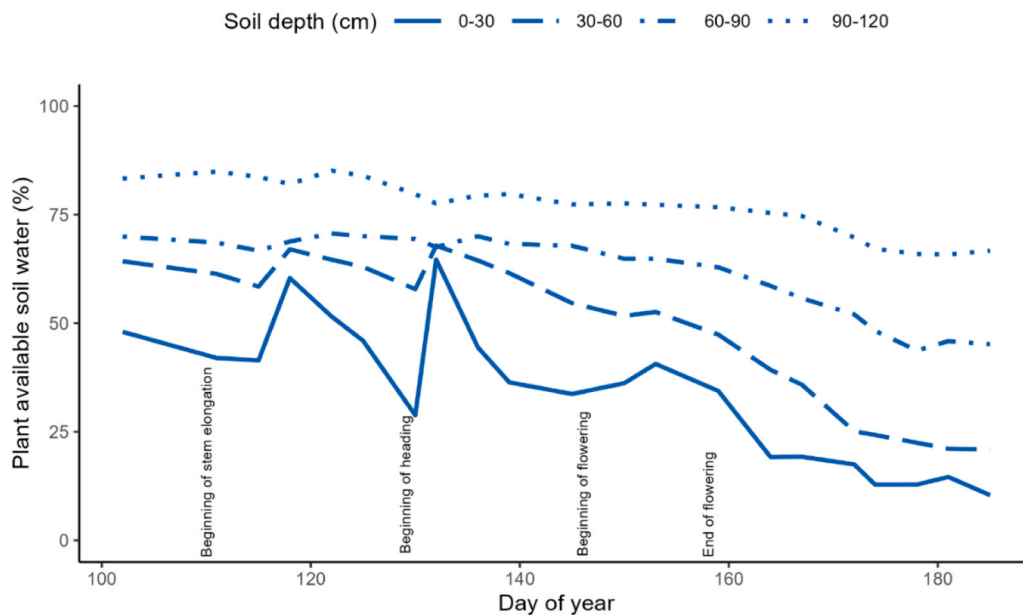


**Fig. 2.** Plant available soil water (in % of the total plant available water capacity) for the soil depth 0–30 cm, 30–60 cm, 60–90 cm, and 90–120 cm from day of year 100–185 in BS. The vertical text indicates the growth stages of semidwarfs. The peaks at days of year 117 and 131 indicate irrigation events with 30 mm each.

### 3.3. Total root length

The highest total root length was found in semidwarfs in GL with a mean of 14051 cm core$^{-1}$ (Fig. 4). However, the differences within the environments were not significant. In contrast, significant differences between environments could be observed: While the mean total root length in GL was 12,222 cm, in BS it was about 60 % less at 5197 cm. Likewise, the variability of the total root length was significantly higher in GL than in BS.

### 3.4. Root length density

Root length density showed significant differences between the sites (Table 2). In GL, the root length density in the topsoil ranged from 7.78 to 9.81 cm cm$^{-3}$, whereas in BS it was significantly lower at 3.32–3.83 cm cm$^{-3}$. A similar pattern was observed in the subsequent soil layers: root length densities were significantly higher in GL than in BS. This was particularly evident in the 120–150 cm soil layer, where only very few roots were found at this soil depth in BS. There was little
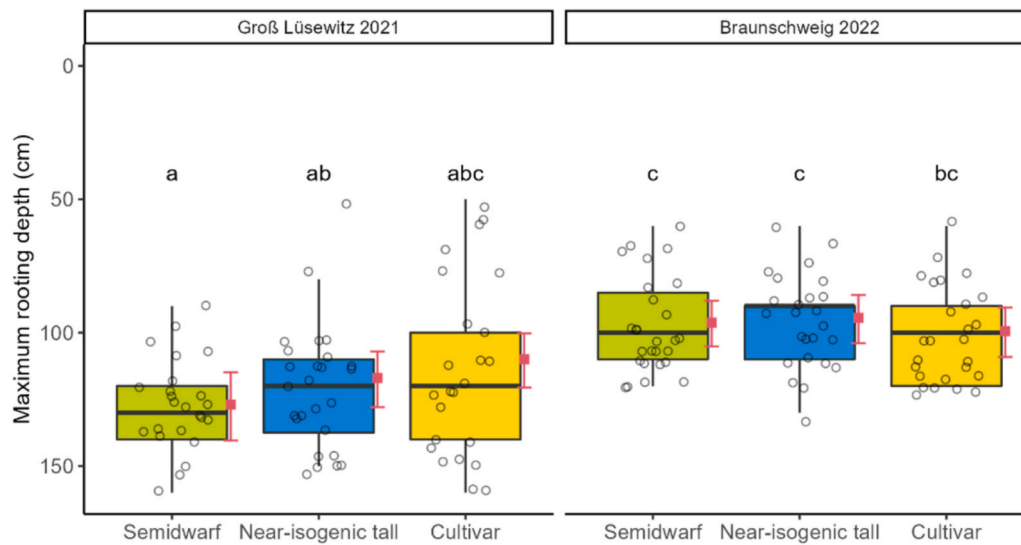
**Fig. 3.** Maximum rooting depth of three genotype groups in Groß Lüsewitz (GL) and Braunschweig (BS). Adjusted mean values (points estimated by the model and the 95% confidence interval (error bar)) are given in red. Groups that do not share a letter are significantly different to each other (α = 0.05).
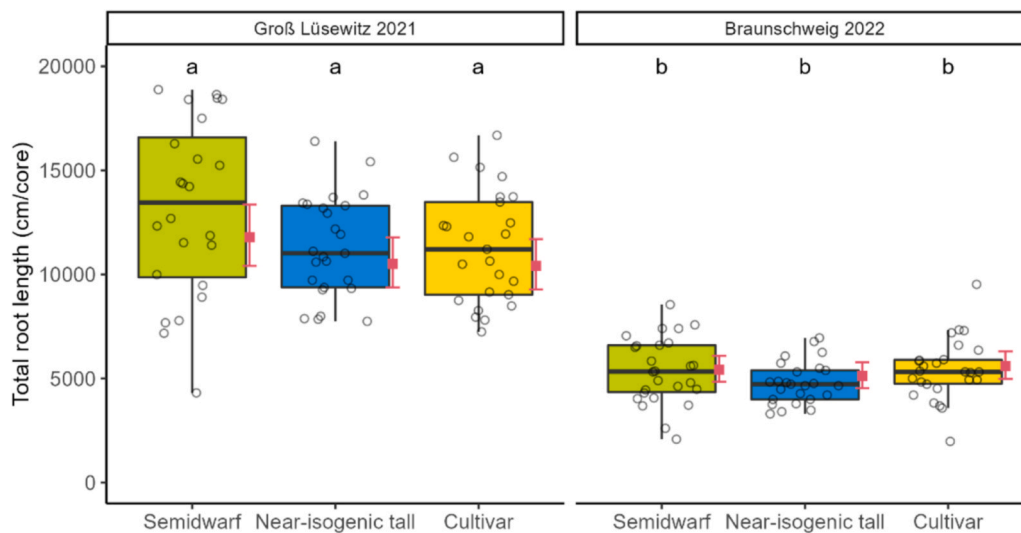


**Fig. 4.** Boxplots for total root length per soil core in Groß Lüsewitz (GL) and Braunschweig (BS) for each genotype group. Adjusted mean values (points estimated by the model and the 95 % confidence interval (error bar)) are given in red. Groups that do not share a letter are significantly different to each other (α = 0.05).

**Table 2**

Root length density (RLD, cm cm$^{-3}$) in different soil depths in Groß Lüsewitz (GL) and Braunschweig (BS) for the genotype groups 'semidwarf', 'near-isogenic tall', and 'cultivar'. Different letters in each column indicate significant differences (α = 0.05).

| | Root length density (cm cm$^{-3}$) | | | | |
| --- | --- | --- | --- | --- | --- |
| | 0–30 cm | 30–60 cm | 60–90 cm | 90–120 cm | 120–150 cm |
| **Groß Lüsewitz (GL)** | | | | | |
| Semidwarf | 9.81[a] | 1.79[a] | 0.99[a] | 0.70[a] | 0.28[a] |
| Near-isogenic tall | 7.97[a] | 1.36[ab] | 0.64[b] | 0.55[ab] | 0.16[ab] |
| Cultivar | 7.78[a] | 1.77[a] | 0.54[b] | 0.42[abc] | 0.15[ab] |
| **Environmental mean** | **8.52** | **1.64** | **0.72** | **0.56** | **0.20** |
| **Braunschweig (BS)** | | | | | |
| Semidwarf | 3.70[b] | 0.80[c] | 0.42[b] | 0.16[c] | 0.00[b] |
| Near-isogenic tall | 3.32[b] | 0.70[bc] | 0.42[b] | 0.10[c] | 0.01[b] |
| Cultivar | 3.83[b] | 0.70[c] | 0.42[b] | 0.27[bc] | 0.01[b] |
| **Environmental mean** | **3.62** | **0.73** | **0.42** | **0.18** | **0.01** |

difference between the genotype groups within the sites. In the 60–90 cm soil layer, however, semidwarfs in GL had a significantly higher root length density than their near-isogenic full-sibs and cultivars. In general, semidwarfs were always ahead in root length density, but the differences were rarely statistically significant.

## 3.5. Cumulative root length distribution

The cumulative root length distribution was similar for all genotype groups in both GL and BS, and no significant differences were found between the sites and genotype groups (Fig. 5). The extinction coefficient ß varied only minimally with values between 0.95 and 0.96. The percentage of roots in the topsoil (0–30 cm) varied between 70.7 % and 74.6 %. All genotype groups had slightly higher proportions of roots in deeper soil layers in GL compared to BS. For example, at the GL site, on average 6.7 % of the total root length was found below 90 cm, whereas at BS it was 3.8 %.

## 3.6. Aboveground traits

Plant height at flowering was significantly reduced by 35% (GL) and 19% (BS) in semidwarfs in comparison to their near-isogenic tall full-sibs. There was no significant difference in plant height between the near-isogenic tall full-sibs and cultivars (Fig. 6). However, the difference between the sites is remarkable: the mean plant height of semidwarfs was 99 cm in GL and 78 cm in BS. Near-isogenic tall hybrids and cultivars reached a height of over 150 cm in GL, while their maximum plant height was 95 cm in BS.

Mean environmental grain yields were similar in GL and BS with 1008 and 1004 g m$^{-2}$, respectively (Table 3). Semidwarfs had a significantly lower grain yield in GL compared to near-isogenic tall full-sibs and cultivars. In BS, no significant difference in grain yield was found between the genotype groups. Straw yield was lower in semidwarfs compared to their near-isogenic full-sibs and cultivars in both GL and BS. The mean harvest index in BS was 0.53, with no differences between the genotype groups. Harvest indices were lower in GL than in BS with a mean of 0.47. Here, semidwarfs and near-isogenic tall full-sibs had a significantly lower harvest index than cultivars. At both locations, semidwarfs had the highest number of spikes m$^{-2}$, the differences were

however not significant at both sites. Semidwarfs and near-isogenic tall full-sibs had significantly more kernels per spike than cultivars in BS. The lowest thousand grain weight was found in semidwarfs at both locations, followed by their near-isogenic tall full-sibs and cultivars..

## 4. Discussion

### 4.1. Root traits of semidwarfs

In general, the environment - especially the soil properties - has by far the greatest influence on root characteristics under field conditions, while the effects of genotypes are comparatively small (Acuna and Wade, 2012). The high variation between plots and soil cores observed in our trials (see Fig. 4) is commonly reported with root phenotyping under field conditions (Wasson et al., 2012). In our experiments, the effect of the environment was highly significant for all root traits, indicated by a deeper rooting, higher total root length, and higher root length density in GL compared to BS. These differences between the environments is most likely due to the different soil properties, but may also be caused by the different precipitation pattern - and thus different soil moisture content. In contrast to the significant differences in root parameters between the two environments, we have found only minor differences in root parameters between genotype groups (see Table 3).

The similar maximum rooting depth of semidwarf and tall genotypes is comparable to results from Ingvordsen et al. (2022), where rooting depth of wheat semidwarfs (both GA-sensitive and GA-insensitive) was generally equal to or slightly higher than their tall counterparts. Reduced rooting depth due to dwarfing genes would be particularly critical in rye, since it is especially grown in environments with low rainfall and soils with low water holding capacity. Reduced rooting depth may impede the access to subsoil water, which is especially important on marginal sites (Kirkegaard et al., 2007; Ober et al., 2014).

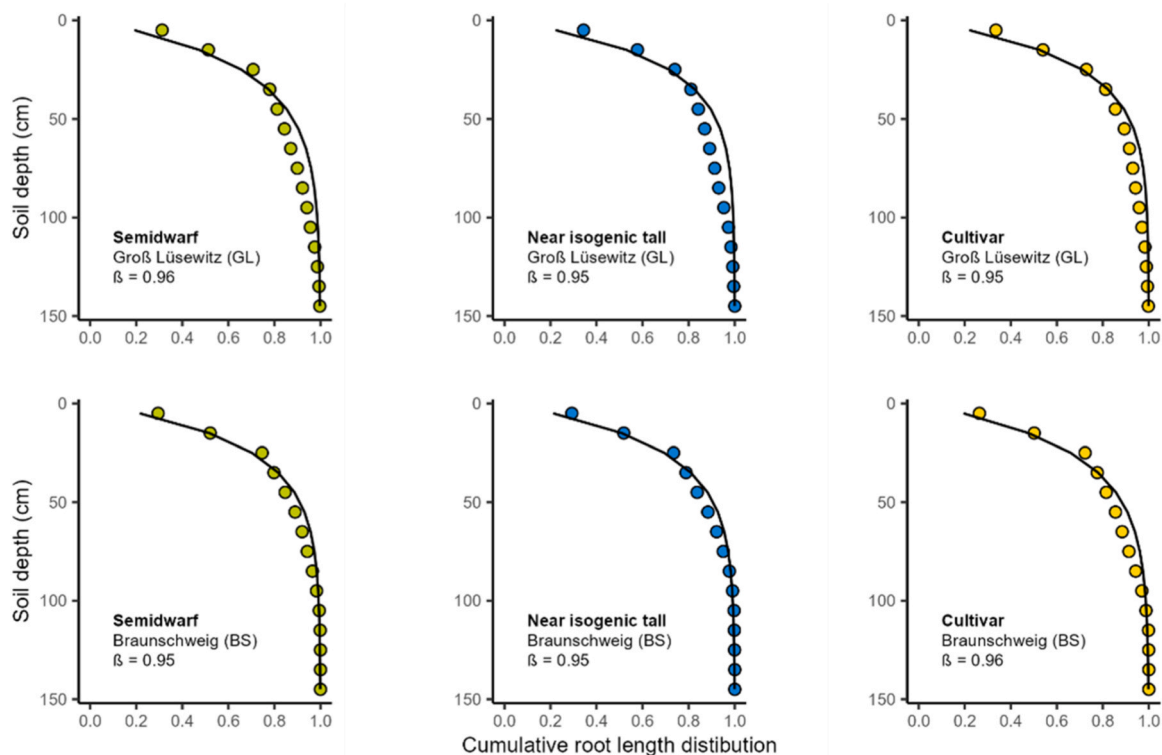Both total root length and root length density at different depths



**Fig. 5.** Cumulative root length distribution (0 − 1) for three genotype groups in Groß Lüsewitz (above) and Braunschweig (below). Dots denote the measured values and the lines denote the curve fitting. The extinction coefficient ß indicates the vertical root distribution, whereas high ß indicates a higher proportion of roots in deeper soil layers.
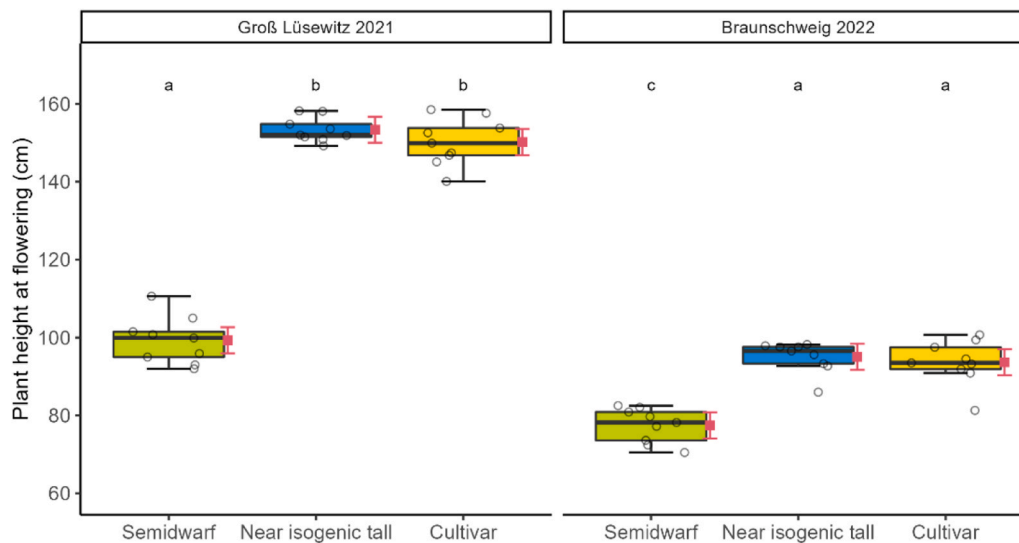
**Fig. 6.** Plant height at flowering of genotype groups 'semidwarf', 'near-isogenic tall', and 'cultivar' in GL and BS. Adjusted mean values (points estimated by the model and the 95 % confidence interval (error bar)) are given in red. Groups that do not share a letter are significantly different to each other (α = 0.05).

**Table 3**
Grain yield (GY), straw yield (SY), total aboveground biomass yield (BIO), harvest index (HI), spikes m$^{-2}$, grains spike$^{-1}$, and thousand grain weight (TGW) in GL and BS for the genotype groups 'semidwarf', 'near-isogenic tall', and 'cultivar'. Different letters in each column indicate significant differences (α = 0.05).

| | GY (g/m$^2$) | SY (g/m$^2$) | BIO (g/m$^2$) | HI (-) | Spikes m$^{-2}$ (#) | Kernels spike$^{-1}$ (#) | TGW (g) |
|---|---|---|---|---|---|---|---|
| **Groß Lüsewitz (GL)** | | | | | | | |
| Semidwarf | 852[a] | 996[b] | 1848[a] | 0.46[a] | 563[b] | 65.9[c] | 21.9[a] |
| Near-isogenic tall | 1061[bc] | 1246[c] | 2306[b] | 0.46[a] | 510[ab] | 68.6[c] | 28.9[b] |
| Cultivar | 1112[c] | 1150[c] | 2262[b] | 0.49[b] | 481[ab] | 67.2[c] | 34.3[c] |
| **Environment mean** | **1008** | **1131** | **2139** | **0.47** | **518** | **67.2** | **28.4** |
| **Braunschweig (BS)** | | | | | | | |
| Semidwarf | 957[ab] | 835[a] | 1792[a] | 0.53[c] | 493[ab] | 58.0[b] | 33.5[c] |
| Near-isogenic tall | 1014[bc] | 912[ab] | 1926[a] | 0.53[c] | 461[a] | 56.0[b] | 39.4[d] |
| Cultivar | 1040[bc] | 925[ab] | 1966[a] | 0.53[c] | 492[ab] | 50.1[a] | 42.4[e] |
| **Environment mean** | **1004** | **891** | **1895** | **0.53** | **482** | **54.7** | **38.4** |

revealed only minor differences between genotype groups. Semidwarfs had a slightly higher total root length and a higher root length density in the topsoil compared to tall genotypes, the differences could however not be statistically confirmed. The higher values compare well with the higher tillering of semidwarfs. Although the number of spikes m$^{-2}$ (recorded at maturity) did not differ significantly between the genotype groups, we recorded a higher number of tillers in the semidwarf plants under which the soil cores were taken (data not shown). This is in contrast to the general theory that reduced tillering triggers increased total root length as more carbon can be allocated from above- to belowground organs (Richards et al., 2007). The higher root length density of semidwarfs in deeper soil layers in GL point in the direction of the considerations of Zhang et al. (2009), who suggest that semidwarfs are more efficient with regard to water use because of a higher proportion of roots in deeper soil layers. Higher root length density in deeper soil layers could not be observed in BS, despite comparable yields between semidwarfs and their tall full-sibs. However, this may also have been due to the generally less favourable soil conditions: Below 90 centimetres, the root development in BS is hindered by almost pure sand.

Cumulative root length distribution was similar between the genotype groups, indicated by the comparable regression coefficients (Fig. 6). A large part of the roots was found in the topsoil, which is comparable to values reported by White et al. (2015). They found between 61 % and 96 % of wheat roots in the upper 50 cm at different locations, whereas 81–85 % of the root length was found in the upper 50 cm in our study. The root length in the subsoil is therefore only a small proportion of the total root length, but nevertheless important: Manschadi et al. (2006) demonstrated that an increased root length in deeper soil layers supports more water extraction during grain filling with positive yield effects. As we could not detect differences between semidwarf and tall genotypes in terms of a higher proportion of roots in the subsoil, we conclude that *Ddw1* has no negative effect on the cumulative root length distribution in semidwarfs.

Anthesis was chosen as sampling date in the current study, as root size of winter cereals is assumed to reach its maximum at this time and declines thereafter, when grains become the major sink for assimilates (Gregory et al., 1978; Siddique et al., 1990). Friedli et al. (2019) however reported post-anthesis root growth in some modern wheat cultivars in reaction to drought stress during grain filling. They conclude that the ability to adjust root growth during grain filling might be especially important in modern (semidwarf) genotypes, which, under optimal growth conditions, do not root as deeply as older cultivars. Further sampling at full maturity or continuous monitoring of root growth by minirhizotrons (Yang et al., 2021) could provide additional insights in this regard.

As root phenotyping under field conditions is very time consuming and labour intensive, we only examined three genotypes per group here. An alternative approach is high-throughput phenotyping of root system architecture under controlled conditions. This approach has advantages over field phenotyping in terms of speed, number of genotypes, and accuracy (Atkinson et al., 2019). However, a major disadvantage is that the results of high-throughput phenotyping are not easily transferable to

**Table 4**
F values and Pr(>F) from analysis of variances for maximum rooting depth (RD_max), total root length (TRL), and root length density in different depths (RLD 0–30 cm, RLD 30–60 cm, RLD 60–90 cm, RLD 90–120 cm, and RLD 120–150 cm), grain yield (GY), straw yield (SY), total aboveground biomass yield (BIO), harvest index (HI), spikes m$^{-2}$, grains spike$^{-1}$, thousand grain weight (TKW), plant height at anthesis (PH), Degrees of freedom: genotype group: 2; environment: 1, GxE: 2. * ** , * *, and * indicate significance at $p < 0.001$, $p < 0.01$, and $p < 0.05$.

| Trait | Genotype group | | Environment | | G x E | |
|---|---|---|---|---|---|---|
| | F | Pr (>F) | F | Pr (>F) | F | Pr (>F) |
| *Belowground* | | | | | | |
| Max. rooting depth | 1.09 | | 34.29 | * ** | 2.10 | |
| Total root length | 3.37 | * | 357.90 | * ** | 1.03 | |
| Root length density (0–30 cm) | 3.38 | * | 352.39 | * ** | 2.12 | |
| Root length density (30–60 cm) | 0.55 | | 106.55 | * ** | 0.32 | |
| Root length density (60–90 cm) | 5.63 | * * | 28.90 | * ** | 0.01 | * |
| Root length density (90–120 cm) | 1.47 | | 49.62 | * ** | 4.54 | * |
| Root length density (120–150 cm) | 1.41 | | 32.33 | * ** | 1.74 | |
| *Aboveground* | | | | | | |
| Grain yield | 13.61 | * ** | 0.04 | | 3.81 | * |
| Straw yield | 19.41 | * ** | 117.32 | * ** | 5.16 | * * |
| Total aboveground biomass | 19.41 | * ** | 26.28 | * ** | 4.07 | * |
| Harvest index | 6.72 | * * | 232.70 | * ** | 8.40 | * ** |
| Spikes m$^{-2}$ | 2.31 | | 3.70 | | 1.69 | |
| Kernels spike$^{-1}$ | 4.30 | | 123.91 | * ** | 5.52 | * * |
| Thousand grain weight | 129.77 | * ** | 343.98 | * ** | 3.51 | * |
| Plant height | 290.11 | * ** | 1122.40 | * ** | 76.20 | * ** |

field conditions (Wasson et al., 2014; Clarke et al., 2017). In parallel to the field evaluation presented in the present study, we also analysed root system architecture of 48 semidwarfs and their 48 near-isogenic tall full-sibs at early growth stages in a high-throughput phenotyping experiment based on Hund's germination pouch system (Hund et al., 2009). The recorded root system architecture traits in early development stages under several environmental conditions support the results described in the present study (Kucherova et al., in preparation).

### 4.2. Aboveground traits of semidwarfs

The most obvious morphological effect was - as expected - the reduced plant height of semidwarfs by 35% (GL) and 19% (BS) compared to their near-isogenic tall full-sibs. Since near-isogenic tall full-sibs in BS were treated with chemical growth regulators, the 35 % height reduction in GL corresponds to the genetic effect of *Ddw1* on plant height. Similar reductions in plant height were reported in both GA-sensitive (Kantarek et al., 2018; Chernook et al., 2019) and GA-insensitive dwarfing genes (Subira et al., 2016) in other cereals. Rebetzke et al. (2012) for example reported plant height reductions by GA-sensitive dwarfing genes in wheat ranging from 7 % (*Rht8*) to 55 % (*Rht5*). In rye, which is the only small-grain cereal that did not experience significant breeding progress regarding plant height over the last decades (Laidig et al., 2021), *Ddw1* might provide a breeder's option to improve lodging resistance, which is closely related to plant height and the major cause of yield losses in rye (Geiger and Miedaner, 2009).

As a consequence of a reduced plant height, Miralles and Slafer (1995) reported a higher harvest index as a higher proportion of dry matter was allocated to the grains. Rebetzke and Richards (2000) and Rebetzke et al. (2012) reported a genetic correlation in wheat between reduced plant height and dry matter partitioning to grains, also resulting in higher harvest indices and grain yields. In contrast, we did not observe an improved harvest index or higher grain yields in semidwarfs carrying Ddw1 compared to their near-isogenic tall full-sibs and cultivars. In wheat, a higher harvest index resulting in a yield advantage was observed for semidwarf cultivars, which varies with spring or winter habit, genetic background, and environmental conditions (Butler et al., 2005). However, wheat grain yield does not depend on the presence of dwarfing genes per se, but on an optimal height for a given environment (Richards, 1992; Miralles and Slafer, 1995; Flintham et al., 1997). Considering the importance of the stem as storage organ for assimilates (Wojcieska et al., 1974) as well as its share in photosynthetic activity (Nalborczyk et al., 1981) in rye, the plant height of semidwarfs reported here seemed to be below the optimum. Likewise, the flag leaves of semidwarfs may not yet have compensated the reduced plant height with increased photosynthetic rates, that would result in a biomass similar to that of tall rye, as has been reported for semidwarf wheat (Lecain et al., 1989; Morgan et al., 1990; Flintham et al., 1997). This assumption is supported by the significantly lower thousand grain weight of the semidwarfs compared to near-isogenic tall genotypes and cultivars.

The similar environmental mean grain yields in BS and GL – despite more favourable environmental conditions in GL - are most likely caused by the use of growth regulators on near-isogenic tall genotypes and cultivars in BS. The total aboveground biomass was consistently higher in GL, but due to lower harvest indices, the grain yield was similar to BS. Notably, semidwarfs in BS showed a similar yield level compared to the near-isogenic tall full-sibs. This could be due to the smaller difference in plant height between semidwarf and near-isogenic tall full-sibs in BS (17.6 cm) compared to GL (54.1 cm) caused by the application of growth regulators in the near-isogenic tall full-sibs in BS, which is the current standard in conventional rye cultivation to prevent lodging and assure harvestability of modern cultivars. Thus, the advantage of a longer stem (= higher photosynthetic performance) of the near-isogenic tall full-sibs was much lower in BS. Additionally, the weather conditions in GL seemed to be more favourable for plant growth: For example, the average temperature in GL was lower with fewer days exceeding the heat stress threshold of 27 °C. The course of the soil water in BS indicates a water deficit during grain filling (see Fig. 2). It can therefore be assumed that the plants at BS had to cope with more drought and heat stress than in GL. The same yield level of semidwarf and tall genotypes in BS could therefore cautiously be interpreted as increased drought tolerance. Plaza-Wüthrich et al. (2016) reported a possible link between GA inhibitors with both lodging and drought tolerance in the small grain cereals tef (*Eragrostis tef)* and finger millet (*Eleusine coracana* L.). Torop et al. (2003) reported a higher drought tolerance in Russian population rye cultivars carrying the *Ddw1* dwarfing gene. In line with their results, the altered GA content by *Ddw1* in the present study might have led to a higher drought tolerance of semidwarfs. In wheat, however, it is often reported that semidwarf cultivars suffer from drought more than tall cultivars and display lower grain yields (see opinion from Jatayev et al., 2020). It needs to be considered, however, that *Ddw1* belongs to the class of GA-sensitive dwarfing genes, while the *Rht* genes in wheat are GA-insensitive (Braun et al., 2019). Therefore, the different results for rye and wheat do not necessarily contradict each other. However, as grain yield is a complex inherited trait in rye (Siekmann et al., 2021), no general conclusions can be drawn from our results regarding the yield enhancing potential of the *Ddw1* introgression in rye. Further research under diverse environmental conditions including drought is needed.

### 5. Conclusion

This is the first report on a field evaluation of the effect of the GA-sensitive dwarfing gene *Ddw1* on root system and aboveground traits in winter rye. The effects of *Ddw1* on maximum rooting depth, root length, root length density and cumulative root length distribution were marginal, and negative effects were not observed. As expected, *Ddw1* reduced the plant height in the experimental semidwarf hybrids.

Comparable yield levels to their near-isogenic tall full-sibs under more unfavourable site conditions in BS provide certain indication on potentially increased stress tolerance. We conclude from our results that *Ddw1* has no negative impact on the root system of rye as compared to modern cultivars, and that rye does not lose the advantages of its deep and widely branched root system to *Ddw1*. However, a further evaluation of semidwarf rye under diverse environmental conditions is necessary to strengthen this conclusion.

## Funding

## CRediT authorship contribution statement

**Lorenz Kottmann:** Methodology, Investigation, Formal analysis, Supervision, Project administration, Conceptualization, Visualization, Writing - original draft. **Wanda Burzik:** Investigation, Formal analysis, Writing – review & editing. **Til Feike:** Conceptualisation, Writing – review & editing. **Dörthe Siekmann:** Resources, Writing – review & editing. **Franz Joachim Fromme:** Writing – review & editing, Resources. **Veronika Kucherova:** Writing – review & editing. **Bernd Hackauf:** Funding acquisition, Conceptualisation, Investigation, Writing – review & editing.

## Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

## Data availability

Data will be made available on request.

## Acknowledgements

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