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A wheat/rye polymorphism affects seminal root length and yield across different irrigation regimes

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HIGHLIGHT

A wheat/rye polymorphism in chromosome 1B affects seminal root length and lateral root proliferation and is associated with differences in drought and waterlogging tolerance in the field.

ABSTRACT

The introgression of a small segment of wheat (*Triticum aestivum* L.) chromosome arm 1BS in the distal region of rye (*Secale cereale* L.) 1RS.1BL arm translocation in wheat (henceforth $1RS^{RW}$) was previously associated with reduced grain yield, carbon isotope discrimination and stomatal conductance, suggesting reduced access to soil moisture. Here we show that lines with the normal 1RS arm have longer roots than lines with the $1RS^{RW}$ arm in both field and hydroponic experiments. In the $1RS^{RW}$ lines, differences in seminal root length were associated with a developmentally regulated arrest of the root apical meristem (RAM). Approximately 10 days after germination, the seminal roots of the $1RS^{RW}$ plants showed a gradual reduction in elongation rate, and stopped growing a week later. Seventeen days after germination, the roots of the $1RS^{RW}$ plants showed altered gradients of reactive oxygen species and emergence of lateral roots close to the RAM, suggesting changes in the root meristem. The $1RS^{RW}$ lines also showed reduced biomass (estimated by Normalized Differences Vegetation Index) and grain yield relative to the 1RS lines, with larger differences under reduced or excessive irrigation than under normal irrigation. These results suggest that this genetic variation could be useful to modulate root architecture.

KEY WORDS: wheat, rye, roots, lateral roots, reactive oxygen species (ROS), waterlogging, drought tolerance.

INTRODUCTION

Approximately 750 million tons of wheat (*Triticum aestivum* L.) are produced worldwide every year (FAO, 2018), but further increases are required to feed a growing human population. One understudied area that can contribute to these yield increases is the role of different root architectures on wheat adaptation to different soils. Although some progress has been made in the understanding of root development and architecture in Arabidopsis (Liu *et al.*, 2017; Yu and Luan, 2016), this knowledge is lacking in grass species (Voss-Fels *et al.*, 2018). There have been some examples of phenotypic selection of root architecture in breeding programs (Richards *et al.*, 2010; Wasson *et al.*, 2012), but those methods are laborious and can be accelerated by a better understanding of the genes controlling wheat root architecture.

Rye (*Secale cereal* L.), a close relative of wheat, is more tolerant to water shortages than wheat, and has been reported to have a more robust root system. The translocation of the short arm of rye chromosome one (1RS) to wheat chromosome 1B (1RS.1BL) contributes to above ground biomass (Shearman *et al.*, 2005) and better performance under drought stress (Ehdaie *et al.*, 2012; Ehdaie *et al.*, 2003; Hoffmann, 2008; Moreno-Sevilla *et al.*, 1995; Zarco-Hernandez *et al.*, 2005). To address breadmaking quality problems associated with the 1RS.1BL translocation (Fenn *et al.*, 1994), a recombinant 1RS chromosome including two wheat 1BS chromosome segment introgressions (henceforth $1RS^{WW}$) was developed to eliminate the two rye regions associated with the bread-making quality problems (Lukaszewski, 2000).

We introgressed the newly engineered chromosome into the spring wheat variety 'Hahn' and generated 1RS/$1RS^{WW}$ near isogenic lines (NILs). Previous field trials showed that the Hahn 1RS lines had significantly higher yield and better canopy water status than the $1RS^{WW}$ NILs in both well-watered and water-stressed environments, although the differences were larger in the latter (Howell *et al.*, 2014). From a cross between Hahn-$1RS^{WW}$ and Hahn-1RS, we generated two additional NILs, one carrying the distal ($1RS^{RW}$) and the other the proximal wheat segment ($1RS^{WR}$). The two NILs carrying the distal rye region (1RS and $1RS^{WR}$, henceforth $1RS^{xR}$) showed significant improvements in grain yield and canopy water status compared to NILs carrying the distal wheat segment ($1RS^{WW}$ and $1RS^{RW}$, henceforth $1RS^{xW}$). The $1RS^{xR}$ NILs also showed higher carbon isotope discrimination and increased stomatal conductance, suggesting improved access to soil moisture relative to the $1RS^{xW}$ NILs (Howell *et al.*, 2014).

In the winter of 2013, heavy rains waterlogged a UC Davis experimental field that affected the four 1RS NILs at the early tillering stage. Although the affected areas were irregular, the $1RS^{xR}$ were less affected than the $1RS^{xW}$ NILs. Based on this observation and previous results, we hypothesized that the $1RS^{xR}$ lines might have a more extensive root system than the $1RS^{xW}$ lines, which helped them tolerate both waterlogging in this experiment and water shortages in the previously published experiments (Howell *et al.*, 2014).

The first objective of this study was to characterize the effect of the wheat-rye polymorphism in the distal region of the 1RS.1BL translocation on root architecture in the field, and on plant biomass and grain yield under normal, excessive or reduced irrigation. After we observed that the lines with the distal wheat segment had shorter seminal roots than the lines with the distal rye segment in hydroponic conditions, we also decided to study the effect of these genotypes on seminal root growth rates, distribution of reactive oxygen species, and distribution of lateral roots. The implications of the observed differences in root development and architecture are discussed.

MATERIALS AND METHODS

Plant materials

In this study, we used four near isogenic lines (NILs) that showed differences in grain yield in previous work (Howell *et al.*, 2014). The recurrent common wheat parent of these NILs is the spring wheat cultivar 'Hahn' developed by the International Maize and Wheat Improvement Center (CIMMYT). The Hahn cultivar carries the complete 1RS translocation from rye, and the three NILs differed from Hahn either in the presence of a distal interstitial segment of wheat chromatin ($1RS^{RW}$), a proximal interstitial segment of wheat chromatin ($1RS^{WR}$), or both ($1RS^{WW}$) (Howell *et al.*, 2014). The interstitial wheat segments were introgressed from the common wheat cultivar 'Pavon 76' (also developed at CIMMYT) to eliminate the *Sec-1* locus from 1RS and to incorporate the *Glu-B3/Gli-B1* locus from 1BS into the 1RS chromosome to improve bread-making quality (Lukaszewski, 2000). The source of this 1RS arm was the rye cultivar 'Petkus', and the resulting 1RS.1BL translocation became widely distributed in wheat breeding programs around the world (Landjeva *et al.*, 2006; Rabinovich, 1998; Schlegel and Korzun, 1997; Zhang *et al.*, 2011).

All field experiments were conducted at the University of California field station in Davis, CA (38°32′ N, 121°46′ W), which has deep Yolo loam soils (fine-silty, mixed, superactive, nonacid, thermic Mollic Xerofluvent). Experiments were sown in plots at a density of 300 grains per m^2 (3 million grains per hectare).

Waterlogging experiments

Controlled waterlogging experiments were conducted during the 2013-2014 and 2015-2016 growing seasons. An additional experiment was performed in 2014-2015 but it was not analyzed due to severe weed problems. The experiments were planted in November and harvested in June (experiments are designated by their harvest year).

The two waterlogging experiments were organized in a split-plot randomized complete block design (RCBD) with four blocks in 2014 and three blocks in 2016. Within each block, the main factor was irrigation treatment, and within each irrigation treatment – block combination, the Hahn 1RS, $1RS^{WW}$, $1RS^{RW}$, and $1RS^{WR}$ genotypes were used as sub-plots. The average trait values of the $1RS^{xR}$ and $1RS^{xW}$ NILs were compared to determine the effect of the distal rye and wheat chromosome segments.

In the 2014 field experiment, each block included two different irrigation regimes as main plots. The first treatment was based on plant needs and normal practices in California's Sacramento Valley and is designated hereafter as normal irrigation. The second treatment, referred hereafter as waterlogging, consisted of artificial flooding twice a week starting in late January and ending in late March during the tillering stage, followed by normal irrigation. Water was applied via flood irrigation, and the soil profile remained saturated. While plants were not kept fully or partially submerged, there were persistent pools of water on the soil surface indicating a waterlogged environment. Each genotype was planted in three adjacent 1 m rows (experimental unit) with 30.5 cm spacing between rows at a rate of 30 grains per row. Genotypes were separated by an empty row (61 cm spacing), and treatments were separated by a minimum of a border row, an irrigation levee, and another border row, leaving in excess of three meters between experimental units of different treatments. Experimental units were replicated six times within each of the four blocks in an RCBD pattern and were used as sub-samples. At the end of the season, each set of three rows was harvested and grain yield was recorded. The average of

the six subsamples was used as a single data point in the statistical analysis. Canopy Spectral Reflectance (CSR) measurements were taken for all subsamples on two days (April 17th and April 30th, both post-anthesis). Subsamples were averaged within days, and day averages were used as repeated measures.

Canopy spectral reflectance measurements were taken with the "ASD HandHeld 2 Pro" spectrometer from Malvern Panalytical. Measurements were taken using a "scanning" method in which 50 measurements were taken on a single plot and averaged to give a single reflectance spectrum. From these measurements, differences in biomass between genotypes were estimated using the Normalized Difference Vegetation Index (NDVI), which was calculated using the formula (R900 nm-R680 nm)/(R900 nm+R680 nm), where R = reflectance at the specified wavelength.

In the 2016 field experiment, each block included three irrigation treatments. The first treatment was grown under normal irrigation as described above. The waterlogging treatment included flood irrigations three times a week, from the beginning of February (later than in 2014 due to a wet winter) to the end of February, followed by normal irrigation. The terminal drought treatment was grown under normal irrigation conditions until late March (before booting stage), and no additional irrigations after that point. Within each block–treatment combination, each genotype was machine sown in 2.23 m^2 plots (1.83 x 1.22 m), which were combine-harvested at maturity. In 2016, CSR measurements were taken as described above on March 24th (booting), April 6th (anthesis), April 13th and April 28th (both post-anthesis). Days were used as repeated measurements and were analyzed as sub-sub-plots in an RCBD split-split-plot design using conservative degrees of freedom (df) for days and all their interactions (this was not necessary in 2014 because there were only 2 days and 1 df). After the CSR measurements were completed, an irrigation pipe ruptured flooding several sections of the experiment on April 29th, resulting in increased variability in the final yield measurements. Flooding was irregular and inconsistent across blocks, with major effects on replications two and three of the drought treatment and replication two of the waterlogging treatment.

Root depth experiments

The field experiment to estimate root length was conducted after a maize crop harvested in the summer of 2016. The field was organized in an RCBD with six blocks and four genotypes per block. Plots were machine sown in 4.5 m^2 plots in November 2016 and were grown under normal irrigation conditions. To obtain soil core samples at specific depths and avoid differential soil compaction, we excavated ~2 m deep trenches cutting perpendicular across the middle of plots including complete blocks one (March 3rd, 2017, North side), three (March 20th, 2017, North side) and six (March 9th, 2017, South side) to expose the root system. We took horizontal soil core samples from the center of each block at 20 cm intervals using a thin-walled copper pipe (5.08 cm diameter x 35 cm long= 709.4 cm^3). Core samples were taken from 20 to 140 cm in the first block and from 20 to 180 cm in blocks three and six (Supplementary Fig. S1) after we discovered the presence of roots at 140 cm in block 1. Plants were at the tillering stage at the time of the root sampling.

Determination of root parameters in soil samples

Soil core samples were washed using a hydro-pneumatic elutriation system from Gillison's Variety Fabrications, Inc. (Smucker, McBurney, and Srivastava 1982). After washing and sorting white turgid roots from other organic matter and decayed roots of the previous maize crop (easily distinguished by color, texture and diameter), we suspended the roots in water and scanned them using an EPSON Expression 11000XL flatbed scanner. Scanned root images were analyzed using the WinRhizo software package. Measurements of dry root biomass are not reported because they were too variable due to small biomass, stray soil contaminants, and changes in ambient moisture. The 20 cm sampling point was not used because the large amount of root biomass and organic matter present in these samples made them difficult to clean and measure.

Since all root measurements were performed using soil cores of identical volume (709.4 cm^3) we refer to these measures as densities (except for average root diameter). Differences in total root length, surface and volume density, average root diameter, and root tips and fork densities were analyzed using a split-plot design with genotypes ($1RS^{xR}$ and $1RS^{xW}$) as main plots and depth as subplot. This is a conservative statistical analysis because it reduces the df for genotype from 3 to 1. Therefore, we also compared the two same pairs of genotypes using statistical contrasts in an ANOVA including all four genotypes. To account for the inability to randomize depths, we used a conservative estimate of the df for subplots and for the interaction between subplot and

main plot. Conservative df were calculated by dividing their df by the number of subplots. This strategy is similar to that used for repeated measures in time and does not affect comparisons among main plots (genotypes), which are the main objective of this study. Homogeneity of variance and normality of the residuals was confirmed for all the individual ANOVAs performed at each depth for all parameters. When necessary, data was transformed using power transformations to satisfy ANOVA assumptions.

Hydroponics experiments

Hydroponic experiments were performed in growth chambers at 22-23 °C with a photoperiod of 16 h light / 8 h dark provided by fluorescent lights supplemented with incandescent lighting. In all experiments, grains were imbibed at 4°C for four days, after which they were placed at room temperature. Once most of the grains had germinated and the coleoptiles had emerged, healthy seedlings were transferred to a mesh suspended on water (UCD) or $CaCl_2$ (0.5 mM, Chascomús). Four days later, healthy seedlings were transferred to tanks with growth solutions (KH_2PO_4 0.2 mM, $MgSO_4.7H_2O$ 1.0 mM, $CaCl_2$1.5 mM, KCl 1.5 mM, H_3BO_3 1 µM, $(NH_4)_6Mo_7O_{24}.4H_2O$ 0.05 µM, $CuSO_4.5H_2O$ 0.5 µM, $ZnSO_4.7H_2O$ 1 µM, $MnSO_4.H_2O$ 1 µM, FeEDTA 0.1 mM, $Ca(NO_3)_2$ 1.0 mM, $(NH_4)_2SO_4$ 1.0 mM). After removing the grain, seedlings were wrapped at the crown with foam and inserted in holes pre-cut in a foam core board placed on top of the solution. Nutrient solution was changed two to three times a week for the duration of the experiment. Particular details of the methods used in the two laboratories are described below:

Davis, CA, USA: The experiments were performed in 13 L hydroponic tanks containing the nutrient solution. Twenty-four seedlings were placed in each tank in a six by four pattern. All genotypes were included in one tank and, if necessary, multiple tanks were used as replications.

In the experiments to study the effect of different nitrogen sources (nitrate and ammonium) and concentrations (0.2 and 2 mM), seedlings were grown in normal growth solution for seven days (from 5 to 12 days after germination (DAG)) and then transferred to four separate tanks with each of the four nitrogen sources for 10 days (12 to 22 DAG). Roots were measured at 22 DAG. Each tank included 6 replications of each of the four genotypes organized in a completely

randomized design. The results were analyzed in a 2 x 2 factorial ANOVA with distal and proximal 1RS regions as factors and wheat or rye chromosome segments as levels.

For the analysis of distances between the first lateral roots and the RAM, the four genotypes were grown in a tank with normal nitrogen conditions (2 mM nitrate) in a completely randomized design (n=12). Lines carrying distal rye ($1RS^{xR}$) or wheat ($1RS^{xW}$) segments were compared using a t-test. *Chascomús, Buenos Aires Argentina*: The $CaCl_2$ (0.5 mM) from the germination tank was replaced by nutrient solution on the 4^{th} day. On the 5^{th} day, plants were transferred to 350 mL pots containing nutrient solution, with each pot (plant) being a replicate. Pots were rotated every two days to ensure that they occupied different positions within the growth chamber. For the root elongation time course, the length of the second longest seminal root was measured daily four hours after the start of the light period, starting 6 DAG. Within each experiment, data was analyzed as repeated measures (split-plot in time with conservative df). A combined ANOVA was performed using experiments as blocks.

Nitro blue tetrazolium (NBT) and 2',7'–dichlorofluorescin diacetate (DCF-DA) staining

The NBT staining experiments were performed using 5 cm root sections excised from the second longest root of 1RS and $1RS^{RW}$ plants 17 DAG. This root segment was placed for 90 min in a 0.1 mg mL^{-1} NBT solution dissolved in 200 mM potassium phosphate buffer, pH 7.6, in darkness. For the DCF-DA staining similar root segments were placed in the same buffer supplemented with 10 µM DCF-DA for 60 min, in darkness. Roots segments stained with NBT or DCF-DA were washed in the same buffer for 30 min and placed on a slide. Roots were observed using a Zeiss Discovery.V20 (Carl Zeiss MicroImaging, Germany) stereomicroscope equipped with a coaxial fluorescence mechanism. Pictures were obtained with an Axiocam 512 color (Carl Zeiss MicroImaging, Germany). The images were processed with the ImageJ software obtaining a longitudinal profile of color and fluorescence intensities. These experiments were performed in Chascomús.

RESULTS

$1RS^{xW}$ lines showed less tolerance to waterlogging than $1RS^{xR}$ lines in the field

Controlled waterlogging experiments performed in 2014 and 2016 using the four Hahn NILs showed that the NDVI and yield responses of the two lines carrying the distal rye chromosome segment ($1RS^{xR}$) were similar to each other and different from the two lines carrying the distal wheat chromosome segment ($1RS^{xW}$), which were also similar to each other (Supplementary Fig. S2). These results were consistent across years and irrigation treatments, so for simplicity we grouped the genotypes with the same distal segment for the following statistical analyses and figures.

***2014 field experiment*:** The repeated measures ANOVA analysis (two days) of the RCBD split-plot experiment harvested in 2014 showed significant differences between control and waterlogging conditions (main treatment) for NDVI (P = 0.0025, Supplementary Table S1), but the effect of the treatment on yield was not significant (P = 0.1556, Supplementary Table S3). For both traits we detected highly significant differences between genotypes (sub-plots, P <0.0001), with the $1RS^{xR}$ lines showing higher NDVI and grain yield than the $1RS^{xW}$ lines (Fig. 1). The NDVI differences between genotypes under waterlogging conditions were larger than under normal irrigation. This was reflected in a significant interaction between waterlogging and genotype (P = 0.0034, Supplementary Table S1) that can be visualized in the interaction graph as lack of parallelism between lines (Fig. 1). The same trend was observed for yield (Fig. 1), but the interaction was not significant (P =0.1101, Supplementary Table S3), likely due to the conservative statistical analyses used for these tests (see Material and Methods) and the higher variability of the yield data.

Since the genotype x day interaction for NDVI was significant, we also performed separate statistical analyses for each of the two days. Each of the two days show similar results, with significant differences between treatments, genotypes and genotype x treatment interactions (Supplementary Table S2).

***2016 field experiment*:** This RCBD split-split-plot experiment included three irrigation treatments (control, waterlogging, and drought). To facilitate the comparisons with the 2104 experiment and to simplify the statistical analysis of the interactions, we present the ANOVA tables for waterlogging (Supplementary Tables S4-6) and drought separately (Supplementary Tables S7-9), although they share the same control treatment.

In the waterlogging experiment, we observed significant differences between treatments for NDVI (P = 0.0010, Supplementary Table S4) but not for yield (P = 0.9772, Supplementary Table S6). The differences between genotypes were highly significant for both NDVI (P <0.0001, Supplementary Table S4) and grain yield (P =0.0054, Supplementary Table S6), despite the rupture of an irrigation pipeline that affected the treatments and blocks in irregular patterns leading to increased variability of the grain yield results (all NDVI measurements were obtained before the incident). For both parameters the $1RS^{xR}$ lines showed higher values than the $1RS^{xW}$ lines (Fig. 1). We observed a consistent trend for larger NDVI differences between genotypes in the waterlogging treatment than in the control (Fig. 1), but the waterlogging by genotype interaction was marginally non-significant in the overall NDVI analysis (P = 0.0783, Supplementary Table S4) and not significant for yield (P = 0.2888, Supplementary Table S6). For NDVI, the individual ANOVAs by day showed marginally non-significant interactions for the measurements taken in the middle of the waterlogging experiment (April 6 P = 0.06 and April 16 P = 0.07), but not for those closer to the beginning and end of the measurement window (March 24 P = 0.91 and April 28 P = 0.16, Supplementary Table S5). The differences between genotypes were highly significant for all four individual ANOVAs (Supplementary Table S5).

No significant differences were detected between the drought and control treatments for NDVI (P = 0.1130) and grain yield (P = 0.4587). By contrast, highly significant differences were detected between genotypes for both parameters (NDVI P = 0.0002, Supplementary Table S7, grain yield P = 0.0088, Supplementary Table S9). We also detected a significant treatment by genotype interaction for NDVI (P = 0.0308, Supplementary Table S7) but not for yield (P = 0.8561, Supplementary Table S9), likely due to the additional variability generated by the irrigation pipe incident. In the four individual ANOVAs by day, the $1RS^{xR}$ lines showed higher NDVI and grain yield than the $1RS^{xW}$ lines (Fig. 1, Supplementary Fig. S2, Supplementary Table S8).

Taken together, these results confirmed the better performance of the $1RS^{xR}$ lines compared to $1RS^{xW}$ lines, with larger differences observed under excessive or insufficient irrigation than under control irrigation (Fig. 1).

$1RS^{xW}$ lines have shorter roots than $1RS^{xR}$ lines in the field

Total root length density: The graph for total root length density (total root length in a 709.4 cm^3 soil volume) at different soil depths showed consistent differences between genotypes grouped by the distal rye or wheat segments (Fig. 2A). The total root length densities were consistently higher in the $1RS^{xR}$ than in the $1RS^{xW}$ NILs through the soil profile, with the largest absolute differences detected at 40 cm (Fig. 2A).

The overall ANOVA for total root length density showed significant differences between the $1RS^{xR}$ and $1RS^{xW}$ genotypes (P =0.0263, Supplementary Table S10). The limited df resulting from the averaged genotypes make this analysis very conservative. To increase the power of the analysis, we performed a statistical contrast between the two lines with the distal rye segment and the two lines with the distal wheat segment in a separate ANOVA including all four genotypes. In this analysis with a higher number of df, the difference between the $1RS^{xR}$ and $1RS^{xW}$ genotypes was highly significant (P = 0.004, Supplementary Table S11).

The overall ANOVA showed a marginally significant interaction between genotype and depth (P = 0.0497), but it become non-significant when adjusted using conservative df (P = 0.190, Supplementary Table S10). The total root length density decreases with depth, which minimizes the absolute value of the differences between genotypes at more extreme depths. To address this limitation, we explored the differences between genotypes expressed as a percent of the $1RS^{xR}$ values ((($1RS^{xR}$ - $1RS^{xW}$) / $1RS^{xR}$) *100). This analysis showed that the relative difference in total root length between $1RS^{xW}$ and $1RS^{xR}$ plants was larger at the deepest sampling points (Supplementary Fig. S3A, regression between depth and percent difference P = 0.002). No roots were detected for $1RS^{xW}$ at 180 cm.

Finally, we performed individual ANOVAs by depth and detected significant differences between genotypes at three depths and marginally non-significant differences at two (Fig. 2A, Supplementary Table S12). In summary, Fig. 2A and the statistical analyses in Supplementary Tables S10 to S12 indicated that the $1RS^{xR}$ lines have more extended and deeper root systems than the $1RS^{xW}$ lines.

Average root diameter and combined root traits: In the overall split-plot ANOVA for average root diameter, the effect of depth was significant, even when using conservative df (P= 0.02, Supplementary Table S13). The difference between the average $1RS^{xR}$ and $1RS^{xW}$ genotypes was marginally non-significant in the conservative analysis (P = 0.0649, Supplementary Table S13),

but was highly significant in the statistical contrast between the pairs of genotypes in the ANOVA including the four genotypes (P = 0.006, Supplementary Table S14). In the analyses by individual depths, we observed similar average root diameters for the $1RS^{xR}$ and $1RS^{xW}$ genotypes between 40 and 100 cm, but those values started to differentiate at 120 cm (P= 0.07) and were significant at 140, 160 and 180 cm. At all these depths, the average root diameter was larger in the $1RS^{xR}$ than in the $1RS^{xW}$ lines (Fig. 2B, Supplementary Table S15).

A similar result was observed also when the differences in root diameter were calculated as the decrease in $1RS^{xW}$ relative to $1RS^{xR}$. In this graph, a sharp increase in the effect of genotype was observed below the 100 cm depth (Supplementary Fig. S3B, regression between depth and relative root diameter P = 0.002). Taken together, these results suggest that the main seminal and/or adventitious roots from the $1RS^{xR}$ lines reach deeper in the soil than the$1RS^{xW}$ lines, for which only thinner roots are found deeper in the soil profile.

The other root traits measured with WinRhizo showed a profile similar to the one observed for total root length density (Supplementary Fig. S4). This is not surprising because root surface and volume density are a function of root length and diameter, and the number of root tips and forks is mainly affected by the abundance of roots. The contrasts between $1RS^{xW}$ and $1RS^{xR}$ in the ANOVAs using the four genotypes were significant for root surface (P =0.005), root volume (P =0.008), number of tips (P =0.02) and number of forks densities (P =0.003, Supplementary Fig. S4). Significant differences were also detected in three to four of the ANOVAs for individual depths in each of the traits (Supplementary Fig. S4).

$1RS^{xW}$ lines have shorter primary roots than $1RS^{xR}$ in hydroponic cultures

The changes in total root length density and diameter with soil depth include both seminal and adventitious roots. To see if the differences observed between the $1RS^{xW}$ and $1RS^{xR}$ lines in the field could be also detected at early growth stages, when the root system is dominated by seminal roots, we performed hydroponics experiments.

Differences in root length: In the hydroponic experiments, the $1RS^{xR}$ lines showed significantly longer seminal roots than the $1RS^{xW}$ lines (Fig. 3A). Since root length was not significantly different between lines with the same distal segment, those lines were averaged for the statistical

analyses (Fig. 3B). Highly significant differences in seminal root length were detected between the $1RS^{xW}$ and $1RS^{xR}$ plants in all four nitrogen treatments (Fig. 3B), which indicates that these differences are robust across different ionic environments.

Root elongation time course: The previous results indicated that the differences in primary root length between the $1RS^{xR}$ and $1RS^{xW}$ plants start at an early stage of root development. These differences were consistent between the lines with the same distal chromosome segment, so root growth experiments were carried out using only the 1RS and $1RS^{RW}$ lines (Supplementary Tables S16-S19). In the overall ANOVAs for accumulated root length, highly significant differences were detected between genotypes over time ($P < 0.0001$, Supplementary Table S16, Fig.4A) and elongation rate (Supplementary Table S18, Fig. 4B). The differences between days and the genotype by day interactions were also highly significant for both parameters ($P < 0.001$, Supplementary Tables S16 and S18). In separate ANOVAs performed for individual days, we detected significant differences in accumulated root length starting at 11 DAG, although differences were already close to significant at 10 DAG ($P = 0.053$, Supplementary Table S17, Fig. 4A). In the same experiments, the differences in the rate of elongation were significant from day 8.5 ($P = 0.0328$, Supplementary Table S19, Fig. 4B).

$1RS^{xR}$ and $1RS^{xW}$ lines differ in the distribution of lateral roots and reactive oxygen species (ROS)

In seminal roots of the $1RS^{xR}$ plants imaged at 22 DAG, lateral roots started to appear 10.42 ± 0.47 cm from the root tip (Fig. 5 and Supplementary Fig. S5). By contrast, the seminal roots of the $1RS^{xW}$ plants showed lateral roots appearing significantly closer to the RAM (2.43 ± 0.44 cm, $P < 0.0001$, Supplementary Fig. S5). No significant differences were detected between lines differing in the proximal wheat and rye segments (Fig. S5).

Since ROS gradients affect root elongation (Tyburski *et al.*, 2009), we estimated their distribution in seminal roots 17 DAG by measuring the amount of formazan produced from the reduction of NBT and fluorescence in DCF-DA staining. Formazan intensity, associated with superoxide anions, was similar between genotypes near the root tips (distal region) but was significantly lower in the $1RS^{RW}$ than in the 1RS roots starting at 650 μm from the root tip ($P < 0.05$), and becoming even more pronounced after 860 μm ($P < 0.001$, Fig. 6A). Fluorescence

intensity from the DCF-DA staining, associated mainly with hydrogen peroxide, peroxynitrite and hydroxyl radicals, showed a very different pattern, with significantly higher intensities in $1RS^{RW}$ relative to the 1RS roots between 250 and 950 μm, and even higher differences between 350 and 640 μm measured from the root tip ($P < 0.001$, Fig. 6B).

Finally, we compared the distribution of formazan during lateral root development in 1RS and $1RS^{RW}$ (Supplementary Fig. S6). No differences between genotypes were detected at 6 DAG, but at 9 DAG NBT staining revealed lateral root primordia close to the RAM in the $1RS^{RW}$ but not in the 1RS line. By 15 DAG large lateral roots were formed close to the RAM in $1RS^{RW}$, but no lateral root primordia were observed in the same region in the 1RS line. These results confirmed the differences in the distribution of lateral roots between the two genotypes.

DISCUSSION

$1RS^{xR}$ genotypes show deeper roots in the field and increased tolerance to waterlogging and terminal drought

Previous studies have shown an association between the introgression of the rye 1RS arm in wheat and improved resistance to water stress (Ehdaie *et al.*, 2012; Ehdaie *et al.*, 2003; Hoffmann, 2008; Moreno-Sevilla *et al.*, 1995; Zarco-Hernandez *et al.*, 2005). In three of these studies, the 1RS.1BL lines showed increased root biomass compared to the non-1RS control lines in large pot or sand-tube experiments. However, these differences were not validated in the field. In this study we showed that differences in grain yield and biomass between plants carrying a complete 1RS translocation and NILs with an introgressed distal wheat chromosome segment (1BS) are associated with differences in total root length density and average root diameter in the field. Field excavations of the four different 1RS NILs provided an opportunity to visualize the differences in their root systems and to quantify these differences using horizontal soil cores at consistent depths.

This experiment confirmed the hypothesis that the $1RS^{xR}$ lines have a higher root density throughout the soil profile, with roots that reach deeper in the soil than the $1RS^{xW}$ lines (Fig. 2A). The more extensive root system of the $1RS^{xR}$ lines relative to the $1RS^{xW}$ lines may have

contributed to their better tolerance to drought and waterlogging conditions in the experiments presented in this study (Fig. 1), and to the higher carbon isotope discrimination and increased stomatal conductance values detected in a previous study (Howell *et al.*, 2014). Through their deeper root system, the $1RS^{xR}$ plants can access more stored soil moisture and nutrients, keep their stomata open longer, and generate additional photosynthetic products and biomass than the $1RS^{xW}$ plants. However, we cannot rule out the possibility that the genes in the distal 1BS introgression may have a more direct effect on aerial biomass or on other anatomical and/or physiological root differences known to impact tolerance to waterlogging and drought (Bailey-Serres and Voesenek, 2008; Berger *et al.*, 2016).

$1RS^{xW}$ shows an earlier arrest of seminal root growth than $1RS^{xR}$ in hydroponic culture

The differences in root depth observed between the Hahn $1RS^{xR}$ and $1RS^{xW}$ NILs in the field were paralleled by drastic changes in seminal root length in hydroponic cultures (Fig. 3A). These differences were robust across experiments and were detected with different nitrogen sources and concentrations (Fig. 3B). We hypothesize that these early differences in seminal root length may have contributed to the observed differences in total root length density observed in the deepest soil core samples in the field (Fig. 2, Fig. 4B).

The early and consistent differences in root growth under controlled conditions provided the opportunity to study the process in detail. During the first week of development, root growth occurred at the same rate for both genotypes, suggesting that the differences were not primarily associated with embryonically determined differences in root elongation. Instead, differences in root growth consistently manifested during the second week across multiple experiments. The growth rate of the seminal roots of the $1RS^{xW}$ plants gradually decreased during the second and third week, to come close to zero by the end of the third week, whereas growth continued in the $1RS^{xR}$ plants (Fig. 4A). The consistent timing of these events suggests that these changes are developmentally regulated.

The growth arrest of the seminal roots in the $1RS^{xW}$ plants was accompanied by the proliferation of lateral roots in close proximity to the RAM, suggesting important changes in the RAM. The RAM consists of a quiescent center (QC) surrounded by stem cells that generate new daughter cells, which undergo additional divisions in the proximal region of the meristem and differentiate

in the transition zone (Heyman *et al.*, 2014). At a cellular level, a balance between cell proliferation and cell elongation/differentiation determines root growth rate (Pacifici *et al.*, 2015). The arrest of the growth of seminal roots in $1RS^{xW}$ plants suggests a modification in cell proliferation and/or cell elongation/differentiation. Additional studies will be required to determine if this arrest involves changes in the QC and/or modifications in the root regions adjacent to the meristem. In any case, the dramatic reduction in seminal root growth and increased lateral root proliferation close to the RAM argues for an early developmental program switch in the regulation of the RAM in the $1RS^{xW}$ plants.

The distribution of ROS along seminal roots differs between $1RS^{xW}$ and $1RS^{xR}$ lines

The transition from cell proliferation to cell elongation and differentiation and the subsequent development of lateral roots depends on the distribution of ROS along the root axis, specifically on the opposing gradients of superoxide and hydrogen peroxide. Superoxide is predominant in dividing cells in the meristematic zone, while hydrogen peroxide is predominant in elongated cells in the differentiation zone (Dunand *et al.*, 2007; Tsukagoshi *et al.*, 2010; Voothuluru and Sharp, 2013; Yamada *et al.*, 2018). The balance between these ROS modulates the transition between root proliferation and differentiation zones.

Seventeen days after germination, the apical region of 1RS seminal roots showed opposing gradients of superoxide and hydrogen peroxide characteristic of elongating roots (Fig. 6). A different ROS distribution was detected in the arrested $1RS^{xW}$ roots, where superoxide was restricted to the distal ~700 μm and increased levels of DCF-DA fluorescence were detected between 250-950 μm in the cell proliferation zone (Fig. 6). The contrasting patterns of ROS distribution reflect the major developmental changes that differentiate the seminal roots of the 1RS and $1RS^{xW}$ genotypes.

Studies in Arabidopsis have shown that changes in ROS distribution can be triggered by the altered expression of major genes that control the size of the meristematic zone. These genes include *UPBEAT1* (*UPB1*), a basic helix-loop-helix (bHLH) transcription factor that regulates the meristematic zone size by restricting H_2O_2 distribution in the elongation zone (Tsukagoshi *et al.*, 2010). In addition, *ROOT MERISTEM GROWTH FACTOR 1* (*RGF1*) and the transcription

factor *RGF1 INDUCIBLE TRANSCRIPTION FACTOR 1* (*RITF1*) that mediates *RGF1* signaling can modulate the distribution of ROS along the root developmental zones leading to enhanced stability of PLETHORA2 (PLT2)(Yamada *et al.*, 2018). Reduced expression of *PLETHORA* in the root apical region (Chen *et al.*, 2011) or changes in its distribution (Ercoli *et al.*, 2018) have been associated with impaired root growth. To test if these Arabidopsis results are applicable to wheat, we are initiating expression studies of these genes in the $1RS^{xR}$ and $1RS^{xW}$ lines.

It remains unknown if the differential pattern of ROS distribution in the roots of the $1RS^{xW}$ plants is the result of changes in the wheat homologs of these central developmental genes or a more direct effect on genes affecting the redox balance in different developmental root zones. The differences in superoxide and hydrogen peroxide distribution between the seminal roots of the $1RS^{RW}$ and 1RS plants were measured after the arrest in root growth (Fig. 6). Therefore, we currently do not know if the changes in ROS distribution are a cause or consequence of the changes observed in root growth and lateral root proliferation close to the RAM.

Conclusions and future directions

Results presented here indicate that the differences in grain yield between the $1RS^{xW}$ and $1RS^{xR}$ lines were preceded by significant differences in aerial biomass. These differences were generally larger under restricted or excessive irrigation than under normal irrigation, suggesting that the introgression of the distal wheat segment in the $1RS^{xW}$ lines resulted in a reduced tolerance to these stresses.

The hydroponic studies showed that the introgression of the distal wheat chromosome segment in $1RS^{xW}$ resulted in a developmentally regulated arrest of seminal root growth associated with drastic changes in ROS gradients along the roots and the distribution of lateral roots close to the RAM. Since the $1RS^{xW}$ and $1RS^{xR}$ lines are highly isogenic (Howell *et al.*, 2014), they provide a useful tool to identify the genes that regulate this major developmental change in the wheat RAM. The identification of these genes can not only contribute to our basic understanding of the regulation of root development in grasses, but also provide tools to modulate wheat root architecture.

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Supplementary information

Supplementary Figures

Supplementary Tables

Supplementary Table S14. Average root diameter contrast.

Supplementary Table S15. Average root diameter contrast by depth.

Supplementary Table S16. Repeated measures for root length

Supplementary Table S17. Root length (mm) by day.

Supplementary Table S18. Repeated measures for root elongation rate.

Supplementary Table S19. Root elongation rate by day.

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FIGURE LEGENDS

Fig. 1. NDVI (top row) and yield (bottom row) under normal irrigation (control), waterlogging and drought conditions. Lines with the distal rye segment ($1RS^{xR}$) showed significantly higher NDVI and yield values ($P<0.0001$) than the lines with the distal wheat segment ($1RS^{xW}$) in all experiments. Differences between genotypes were generally larger under water stress conditions. Error bars are SE of the means across blocks.

Fig. 2. Change in total root length density and average root diameter in $1RS^{xW}$ and $1RS^{xR}$. **A**) Total root length density. Relative changes are presented in Supplementary Fig. S3A and S3B. **B**) Average root diameter. *P* values represent differences between $1RS^{xW}$ and $1RS^{xR}$ at individual depth (Tables S12 and S15). Error bars represent SE of the means across blocks. Asterisks or numbers above error bars are *P*-values for the difference between genotypes at individual depths. * = P <0.05, ** = P <0.01, *** = P <0.001.

Fig. 3. Differences in primary root length between plants carrying the distal rye (1RS and $1RS^{WR}$) and wheat chromosome segments ($1RS^{RW}$ and $1RS^{WW}$) in hydroponic culture. **A**) Representative plants in normal culture medium. **B**) Seminal root length in hydroponic media using nitrate or ammonium at two different concentrations (2.0 mM and 0.2 mM). ** = P <0.01, *** = P <0.001.

Fig. 4. Time course of primary root growth of plants carrying the distal rye (1RS) or wheat chromosome segments ($1RS^{RW}$) in hydroponics culture. LS means and standard errors are from the ANOVA of the three combined experiments. **A**) Cumulative length of the second longest seminal root (mm, Tables S16 and S17). **B**) Elongation rate of the second longest seminal root (mm/ h, Tables S18 and S19).* = P <0.05, ** = P <0.01, *** = P <0.001.

Fig. 5. Development of laterals on the seminal roots of plants carrying the distal rye or wheat segments. Plants were grown for 22 days in hydroponics under low nitrogen conditions (0.2 mM nitrate). Arrows indicate the root apical meristem (RAM) of the seminal roots. The inset shows a detailed view of the seminal root tips for the four genotypes. $1RS^{xW}$ lines show lateral roots close to the RAM.

Fig. 6. Distribution of ROS along the main roots of plants carrying the distal rye (1RS) or wheat chromosome segments ($1RS^{RW}$) 17 days after germination. **A**) Formazan signal intensity along

the roots as determined with NBT (mainly detects superoxide anions). **B**) Fluorescence signal intensity along the root as determined by DCF-DA (mainly detects hydrogen peroxide). Zero indicates the most distal point of the root (root tip). Representative NBT and DCF-DA stained roots from the corresponding experiment (number indicates growth rate in mm h^{-1} of the individual roots at the last sampling time). Gray circles connected with a line indicate the interval over which the indicated significance threshold is met, an arrow indicates the interval is not bounded. * = $P < 0.05$, *** = $P < 0.001$, n=5.

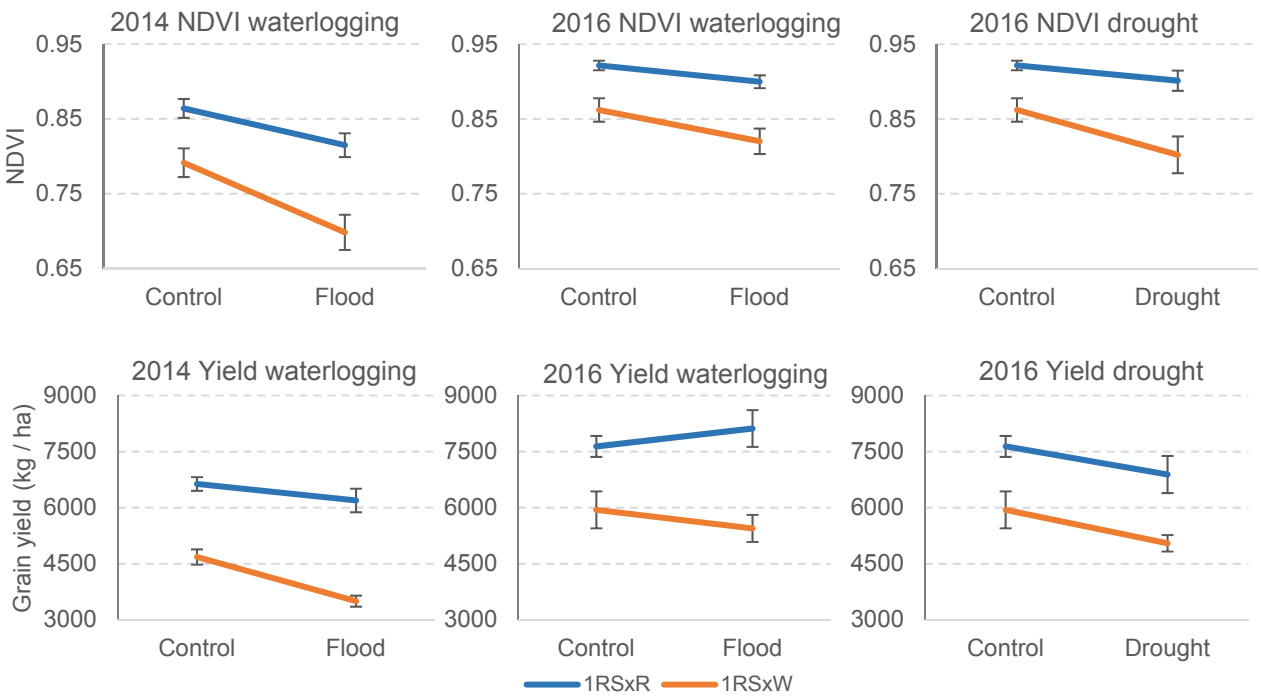

Fig. 1

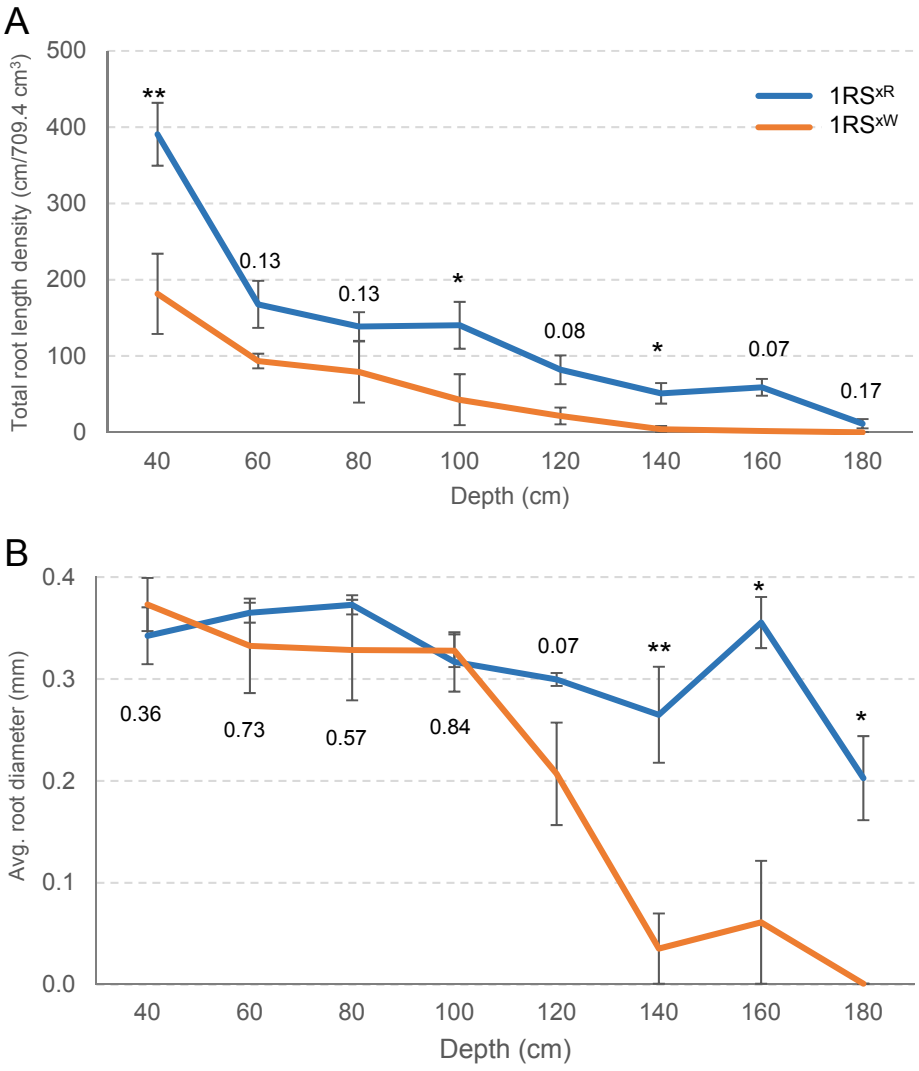

Fig. 2

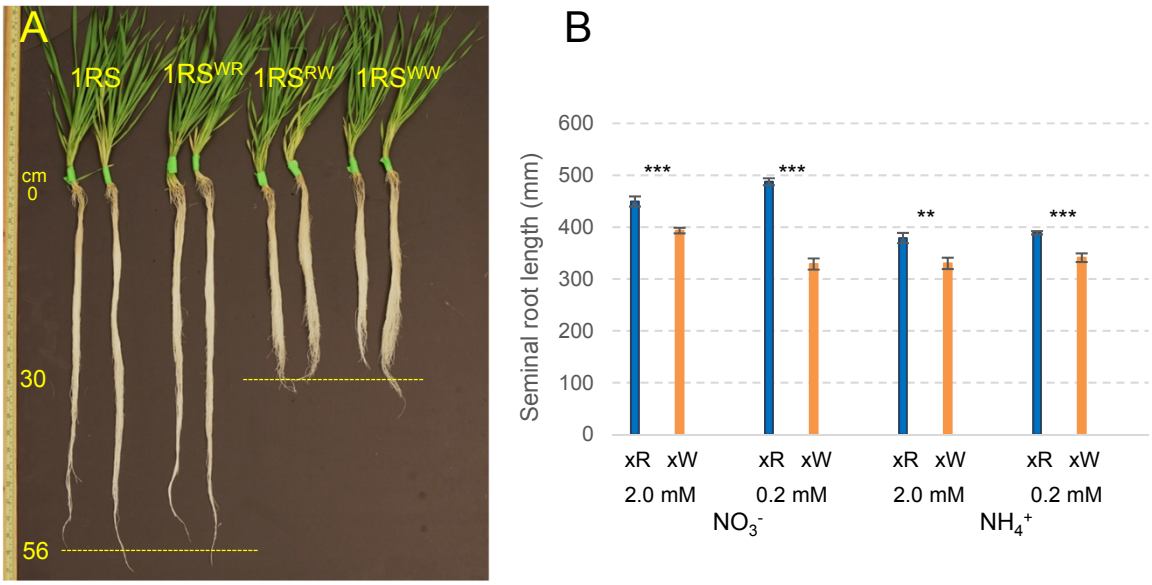

Fig. 3

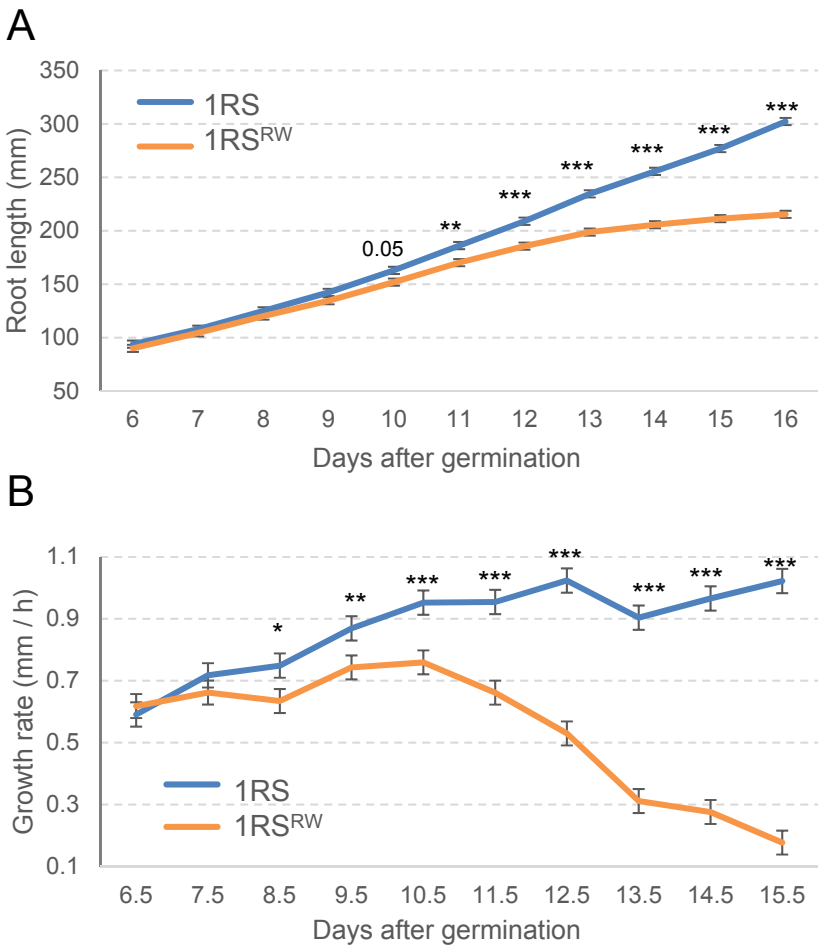

Fig. 4

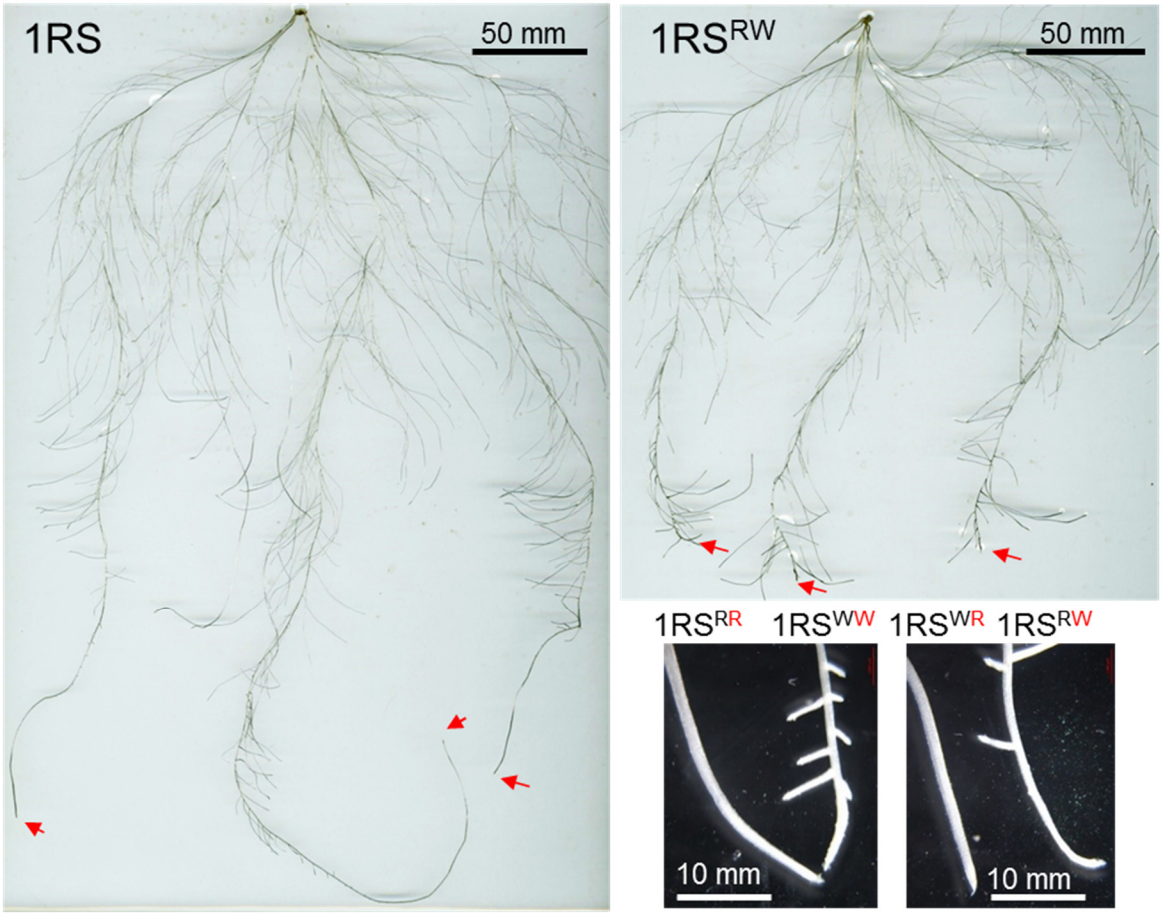

Fig. 5

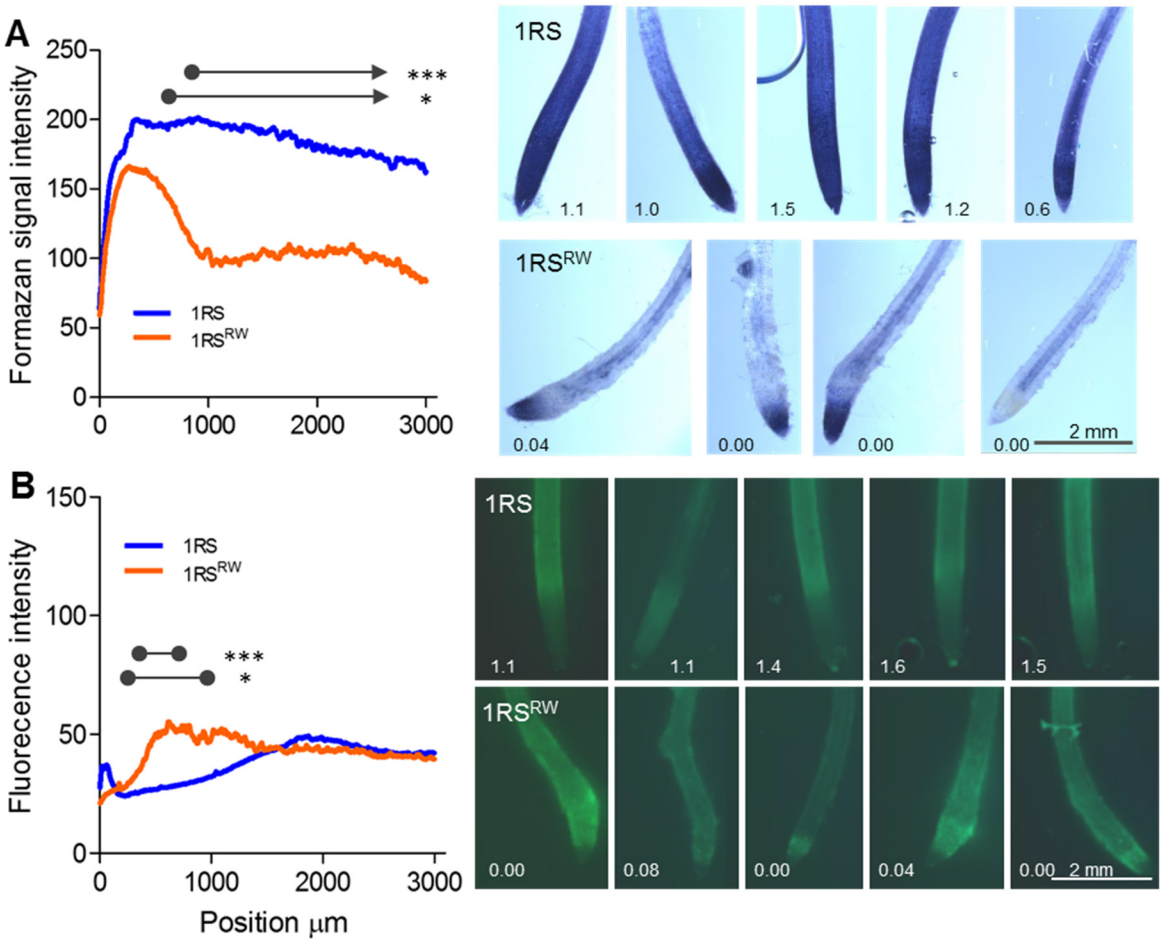

Fig. 6