A wheat/rye polymorphism affects seminal root length and is associated with drought and waterlogging tolerance

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Running title: A wheat/rye polymorphism affects root length

Date of submission: November 5, 2018

Tables: 0
Figures: 6
Words: 6462
Supplementary Tables: 16
Supplementary Figures: 6

HIGHLIGHT

A wheat/rye polymorphism in chromosome one affects seminal root length and apical dominance and is associated with differences in drought and waterlogging tolerance in the field.

ABSTRACT

The introgression of a small segment of wheat chromosome 1BS in the distal region of the rye 1RS 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. In this study, we show that the $1RS/1RS^{RW}$ polymorphism causes differences in root length in field and hydroponic experiments. In the latter, 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 a loss of apical dominance. The $1RS/1RS^{RW}$ isogenic lines also differed in plant biomass and grain yield under normal, terminal drought, and waterlogging field conditions. The differences were larger in fields with reduced or excessive irrigation. These results suggest that this polymorphism may be useful to modulate root architecture and mitigate the negative impacts of excess or reduced water in wheat production.

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

63 **INTRODUCTION**

64 Approximately 750 million tons of wheat are produced worldwide every year (FAO, 2018), but
65 further increases are required to feed a growing human population. Minimizing yield losses from
66 water stress and waterlogging can contribute to these increases (Mittler and Blumwald, 2010;
67 Tester and Langridge, 2010). One strategy to minimize these losses is the development of crops
68 with root architectures better adapted to different soil types and growth cycles (Manschadi *et al.*,
69 2006; Voss-Fels *et al.*, 2018). Although some progress has been made in the understanding of
70 root development and architecture in Arabidopsis (Liu *et al.*, 2017; Yu and Luan, 2016), this
71 knowledge is insufficient to engineer useful solutions for specific stress conditions in distantly
72 related grass species (Voss-Fels *et al.*, 2018). One of the traits that contributes to determine plant
73 performance under water limitation is the early capacity of roots to explore the soil at depth
74 (Manschadi *et al.*, 2006); an attribute that could be affected in waterlogged soils (Rich and Watt,
75 2013).

76 Rye, a close relative of wheat, is more tolerant to water shortages than wheat, and several studies
77 have pointed to a more robust root system. The translocation of the short arm of rye chromosome
78 one (1RS) to wheat chromosome 1B (1RS.1BL) contributes to above ground biomass (Shearman
79 *et al.*, 2005) and better performance under drought stress (Ehdaie *et al.*, 2012; Ehdaie *et al.*,
80 2003; Hoffmann, 2008; Moreno-Sevilla *et al.*, 1995; Zarco-Hernandez *et al.*, 2005).
81 Unfortunately, the 1RS.1BL translocation also brings with it dough stickiness and reduced gluten
82 strength, which negatively affect breadmaking quality limiting its utilization in wheat breeding
83 programs focused on this trait (Fenn *et al.*, 1994). To address this problem a recombinant 1RS
84 chromosome including two wheat 1BS chromosome segment introgressions (henceforth $1RS^{ww}$)
85 was developed to eliminate the two rye regions generating the problems in bread-making quality
86 (Lukaszewski, 2000).

87 We introgressed the newly engineered chromosome into the variety 'Hahn' using six
88 backcrosses, generating $1RS/1RS^{ww}$ near isogenic lines (NILs). Previous field trials showed that
89 the Hahn 1RS lines had significantly higher yield and better canopy water status than the $1RS^{WW}$
90 NILs in both well-watered and water-stressed environments, although the differences were larger
91 in the latter (Howell *et al.*, 2014). We previously intercrossed the 1RS and $1RS^{WW}$ lines and
92 generated two additional NILs, one carrying the distal ($1RS^{RW}$) and the other carrying the

93 proximal ($1RS^{WR}$) wheat segment. The two NILs carrying the distal rye region (1RS and $1RS^{WR}$,
94 henceforth $1RS^{xR}$) showed significant improvements in grain yield and canopy water status
95 compared to NILs carrying the distal wheat segment ($1RS^{WW}$ and $1RS^{RW}$, henceforth $1RS^{xW}$).
96 This indicates that the distal wheat region was the one responsible for the differences. NILs with
97 the distal rye segment also showed higher carbon isotope discrimination and increased stomatal
98 conductance, suggesting that these plants had improved access to the water present in the soil
99 (Howell *et al.*, 2014).

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

106 In this study, we showed that the wheat-rye polymorphism in the distal region of the 1RS.1BL
107 translocation is responsible for differences in root length in the field. These genotypes also
108 displayed differences in plant biomass (as determined by canopy spectral reflectance) that were
109 generally larger in fields with excessive or reduced irrigation than in fields under normal
110 irrigation. Using hydroponic experiments, we showed that the lines with the distal wheat
111 segment had shorter seminal roots than the lines with the distal rye segment. The shorter roots
112 were associated with an earlier arrest of the root apical meristem (RAM) growth, development of
113 lateral roots closer to the RAM and changes in the distribution of reactive oxygen species.

114

115 **MATERIALS AND METHODS**

116 **Plant materials**

117 In this study, we used four near isogenic lines (NILs) that showed differences in grain yield
118 under water stress in a previous work (Howell *et al.*, 2014). The recurrent common wheat parent
119 of these NILs is the cultivar 'Hahn' developed by the International Maize and Wheat
120 Improvement Center (CIMMYT). The Hahn cultivar carries the complete 1RS translocation, and
121 the three NILs differed from Hahn either in the presence of a distal interstitial segment of wheat

122 chromatin (henceforth $1RS^{RW}$), a proximal interstitial segment of wheat chromatin (henceforth
123 $1RS^{WR}$), or both (henceforth $1RS^{WW}$) (Howell *et al.*, 2014). The interstitial wheat segments were
124 introgressed from the common wheat cultivar 'Pavon 76' to eliminate the *Sec-1* locus from 1RS
125 and to incorporate the *Glu-B3/Gli-B1* locus from 1BS into the 1RS chromosome to improve
126 bread-making quality (Lukaszewski, 2000).

127

128 **Waterlogging experiments**

129 Controlled waterlogging experiments were conducted at the University of California field station
130 in Davis, CA (38°32′ N, 121°46′ W) during the 2013-2014 and 2015-2016 growing seasons. An
131 additional experiment was performed in 2014-2015 but it was not analyzed due to severe weed
132 problems. The experiments were planted in November and harvested in June (the harvest year
133 will be used hereafter to designate the experiments).

134 The two waterlogging experiments were organized in a split-plot randomized complete block
135 (RCBD) design with four blocks in 2014 and three blocks in 2016. Within each block, the main
136 factor was irrigation treatment, and within each irrigation treatment – block combination, the
137 Hahn1RS, $1RS^{WW}$, $1RS^{RW}$, and $1RS^{WR}$ genotypes were used as sub-plots. The average of 1RS
138 and $1RS^{WR}$ was compared with the average of $1RS^{WW}$ and $1RS^{RW}$ to determine the effect of the
139 distal rye and wheat chromosome segments.

140 In the 2014 field experiment, each block included two different irrigation regimes as main plots.
141 The first one was based on plant needs and normal practices in California Sacramento Valley and
142 the second one consisted of artificial flooding twice a week starting in late January and ending in
143 late March 2014 during the tillering stage. Water was applied via flood irrigation, and the
144 schedule was set so that the soil profile remained saturated. While plants were not kept fully or
145 partially submerged, there were persistent pools of water on the soil surface indicating a
146 waterlogged environment. Each genotype was planted in three adjacent 1 m rows (experimental
147 unit) at a rate of 30 grains per row. These experimental units were replicated six times within
148 each of the four blocks in an RCBD pattern and were used as sub-samples. At the end of the
149 season, each set of three rows was harvested and grain yield was recorded in grams. The average
150 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 (4/17and 4/30). Subsamples were averaged within days, and day averages were used as repeated measures.

Canopy spectral reflectance (CSR) 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 together to give a single representative 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).

In the 2016 field experiment, each block included three irrigation treatments. The first one was a normal irrigation with water provided as needed. The waterlogging treatment included flood irrigations three times a week that were applied as described above. Irrigations were initiated at the beginning of February (later than in 2014 due to a wet winter) and ended at the end of February. In the drought treatment, irrigation ceased in late March before the booting stage. Within each block – treatment combination, each genotype was machine sown at a density of 3 million grains per hectare 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 four different days (3/24, 4/6, 4/13, and 4/28). 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 for days and all their interactions (this was not necessary in 2014 because there were only 2 days and a single degree of freedom). After the CSR measurements were completed, an irrigation pipe ruptured flooding several sections of the experiment in an irregular pattern (4/29) and increased variability in the final yield measurements.

Root depth experiments

The field experiment to estimate root length was conducted at the University of California field station in Davis, CA, which has deep Yolo loam soils (fine-silty, mixed, superactive, nonacid, thermic Mollic Xerofluvent). The field was organized as a randomized complete block design with six blocks and four genotypes per block. Plots were machine sown at a density of 3 million grains per hectare in 4.5 m^2 plots in November 2016.

To obtain soil samples at specific depths and avoid differential soil compaction, we excavated ~2 m deep trenches cutting perpendicular across the middle of plots and including complete blocks one (3/3/2017, North side), three (3/20/2017, North side) and six (3/9/2017, South side) to expose the root system. We then 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 roots from other organic matter, 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. We also attempted to measure root biomass, but the small biomass of the dried roots combined with small stray soil contaminants and changes in ambient moisture made it difficult to quantify root biomass, so only the WinRhizo measurements are reported. 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 accurately.

Since all root measurements were performed using soil cores of identical volume (709.4 cm^3) we will refer to these measures as densities. 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 (average of 1RS and $1RS^{WR}$ vs. average of $1RS^{WW}$ and $1RS^{RW}$) as main plots and depth as subplot. This is a very 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 statistically randomize depths, we used a conservative estimate of the degrees of freedom (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 the one use for repeated measures in time and does not affect comparisons among main plots (genotypes), which are the

210 main objective of this study. Homogeneity of variance and normality of the residuals was
211 confirmed for all the individual ANOVAs performed at each depth for all parameters. When
212 necessary, data was transformed using power transformations to satisfy ANOVA assumptions.

213

214 **Hydroponics experiments**

215 The hydroponic experiments were performed in growth chambers set at 22-23 °C with a
216 photoperiod of 16 h light / 8 h dark provided by a fluorescent light source supplemented with
217 incandescent lighting. In all experiments, grains were imbibed at 4°C for four days, after which
218 they were placed at room temperature. Once the majority of the grains had germinated and the
219 coleoptiles had emerged, healthy germinated seedlings were transferred to a mesh suspended on
220 water (UCD) or $CaCl_2$ (0.5 mM, Argentina). Four days later, healthy seedlings were transferred
221 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
222 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,
223 $MnSO_4.H_2O$ 1 μM, FeEDTA 0.1 mM, $Ca(NO_3)_2$ 1.0 mM, $(NH_4)_2SO_4$ 1.0 mM). After removing
224 the remaining grain, seedlings were wrapped at the crown with foam and inserted in holes pre-
225 cut in a foam core board placed on top of the solution. Nutrient solution was changed two to
226 three times a week for the duration of the experiment. Particular details of the methods used in
227 the two laboratories are described below:

228 *Davis, CA, USA*: The experiments were performed in 13 L hydroponic tanks (ULINE part
229 number S-19507) containing the nutrient solution. Twenty-four seedlings were placed in each
230 hydroponic tank in a six by four pattern. All genotypes were included in one tank, and if
231 necessary, multiple tanks were used as replications.

232 In the experiments aimed to study the effect of different sources of nitrogen and of their external
233 concentrations, seedlings were grown in normal growth solution for seven days (from 5 to 12
234 DAG) and then transferred to low nitrogen solution for another 10 days (12 to 22 DAG). Roots
235 were measured and imaged with a flatbed scanner at 22 DAG.

236 *Chascomús, Buenos Aires Argentina*: The $CaCl_2$ (0.5 mM) from the tank where the grains were
237 germinated was replaced by nutrient solution on the 4th day. On the 5th day, plants were
238 transferred to 0.350 L pots containing aerated nutrient solution, with each pot (plant) being a

239 replicate. Pots were rotated every two days to ensure that they occupied different positions
240 within the growth chamber. For the root time course, the length of the second longest seminal
241 root was measured four hours after the start of the light period, starting 6 DAG.

242

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

244 Five centimeter root sections were carefully excised from the second longest root of 1RS and
245 $1RS^{RW}$ plants 17 DAG. For the NBT staining this segment was placed for 90 min in a 0.1 mg/mL
246 NBT solution dissolved in 200 mM potassium phosphate buffer, pH 7.6, in darkness. For the
247 DCF-DA staining root segments were placed in the same buffer supplemented with 10 µM DCF-
248 DA for 60 min, in darkness. Roots segments stained with NBT (detecting superoxide anions) or
249 DCF-DA (detecting hydrogen peroxide, peroxynitrite and hydroxyl radicals) were washed in the
250 same buffer for 30 min and placed on a slide. Roots were observed using a Zeiss Discovery.V20
251 (Carl Zeiss MicroImaging, Germany) stereomicroscope equipped with a coaxial fluorescence
252 mechanism. Pictures were obtained with an Axiocam 512 color (Carl Zeiss MicroImaging,
253 Germany). The images were processed with the ImageJ software obtaining a longitudinal profile
254 of color and fluorescence intensities.

255

256 **RESULTS**

257 **$1RS^{xW}$ lines showed less tolerance to waterlogging than $1RS^{xR}$ lines in the field**

258 To test if the $1RS^{xR}$ lines have better tolerance to waterlogging than the $1RS^{xW}$ lines we
259 performed controlled waterlogging experiments in 2014 and 2016 using the four Hahn NILs. The
260 analysis of the data using the four different genotypes showed that the NDVI and yield responses
261 of the lines carrying the distal rye chromosome segment (1RS + $1RS^{WR}$ =$1RS^{xR}$) were similar to
262 each other and different from the lines carrying the distal wheat chromosome segment ($1RS^{WW}$ +
263 $1RS^{RW}$ =$1RS^{xW}$), which were also similar to each other (Supplementary Fig. S2). These results
264 were consistent across years and irrigation treatments, so for simplicity we grouped the
265 genotypes with the same distal segment for the following statistical analyses and figures.

266 ***2014 field experiment*:** The repeated measures ANOVA analysis (two days) of the RCBD split
267 plot experiment harvested in 2014 showed significant differences between control and

268 waterlogging conditions (main treatment) for NDVI (P = 0.0025, Supplementary Table S1), but
269 the effect of the treatment on yield was not significant (P = 0.1556, Supplementary Table S3).
270 For both traits we detected highly significant differences between genotypes (sub-plots, P
271 <0.0001), with the $1RS^{xR}$ lines showing higher NDVI and grain yield than the $1RS^{xW}$ lines (Fig.
272 1). The NDVI differences between genotypes under waterlogging conditions were larger than
273 under normal irrigation. This was reflected in a significant interaction between waterlogging and
274 genotype (P = 0.0034, Supplementary Table S1) that can be visualized in the interaction graph
275 as lack of parallelism between lines (Fig. 1). The same trend was observed for yield (Fig. 1), but
276 the interaction was not significant (P =0.1101, Supplementary Table S3), likely due to the
277 conservative statistical analyses used for these tests (see Material and Methods) and the higher
278 variability of the yield data.

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

283 ***2016 field experiment*:** This RCBD split-split-plot experiment included three irrigation
284 treatments (control, waterlogging, and drought). To facilitate the comparisons with the 2104
285 experiment and to simplify the statistical analysis of the interactions, we present the ANOVA
286 tables for waterlogging (Supplementary Tables S4-6) and drought separately (Supplementary
287 Tables S7-9), although they share the same control treatment.

288 In the waterlogging experiment, we observed significant differences between treatments for
289 NDVI (P = 0.0010, Supplementary Table S4) but not for yield (P = 0.9772, Supplementary
290 Table S6). The unanticipated rupture of an irrigation pipeline (after all NDVI measures were
291 completed) affected the treatments and blocks in irregular patterns leading to increased
292 variability of the grain yield results. This increased variability might have contributed to the lack
293 of significant differences in grain yield between treatments. In spite of this incident, the
294 differences between genotypes were highly significant for both NDVI (P <0.0001,
295 Supplementary Table S4) and grain yield (P =0.0054, Supplementary Table S6). Similar to the
296 2014 experiment, the $1RS^{xR}$ lines showed higher NDVI and grain yield than the $1RS^{xW}$ lines
297 (Fig. 1). We observed a consistent trend for larger NDVI differences between genotypes in the

298 waterlogging treatment than in the control (Fig. 1), but the interaction between waterlogging and
299 genotype was marginally non-significant in the overall NDVI analysis (P = 0.0783,
300 Supplementary Table S4) and not significant for yield (P = 0.2888, Supplementary Table S6).
301 For NDVI, the individual ANOVAs by day showed marginally non-significant interactions for
302 the measurements taken in the middle of the waterlogging experiment (April 6 P = 0.06 and
303 April 16 P = 0.07), but not for those closer to the beginning and end of the measurement window
304 (March 24 P = 0.91 and April 28 P = 0.16, Supplementary Table S5). The differences between
305 genotypes were highly significant for all four individual ANOVAs (Supplementary Table S5).

306 No significant differences were detected between the drought and control treatments for NDVI
307 (P = 0.1130) and grain yield (P = 0.4587). By contrast, highly significant differences were
308 detected between genotypes for both parameters (NDVI P = 0.0002, Supplementary Table S7,
309 grain yield P = 0.0088, Supplementary Table S9). We also detected a significant treatment by
310 genotype interaction for NDVI (P = 0.0308, Supplementary Table S7) but not for yield (P =
311 0.8561, Supplementary Table S9), likely due to the additional variability generated by the
312 irrigation pipe incident. In the four individual ANOVAs by day, the $1RS^{xR}$ lines showed higher
313 NDVI and grain yield than the $1RS^{xW}$ lines (Fig. 1, Supplementary Fig. S2, Supplementary Table
314 S8).

315 Taken together, these results confirmed the better performance of the $1RS^{xR}$ lines compared to
316 $1RS^{xW}$ lines, under control, waterlogging and water deficit conditions. The differences between
317 genotypes tended to be larger under excessive or insufficient irrigation than under control
318 irrigation (Fig. 1), which suggests that the presence of the distal 1RS segment helped to mitigate
319 the negative impacts of waterlogging and drought on biomass accumulation and grain yield.

320

321 **$1RS^{xW}$ lines have shorter roots than $1RS^{xR}$ lines in the field**

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

327 The overall ANOVA for total root length density showed significant differences between the
328 averages of the $1RS^{xR}$ and $1RS^{xW}$ genotypes (*P* =0.0263, Supplementary Table S10). The limited
329 degrees of freedom resulting from the averaged genotypes make this analysis very conservative.
330 To increase the power of the analysis, we performed a statistical contrast between the two lines
331 with the distal rye segment and the two lines with the distal wheat segment in a separate
332 ANOVA including all four genotypes. In this analysis with a higher number of degrees of
333 freedom, the difference between the $1RS^{xR}$ and $1RS^{xW}$ genotypes was highly significant (*P* =
334 0.004, Supplementary Table S11).

335 The overall ANOVA showed a marginally significant interaction between genotype and depth (*P*
336 = 0.0497), but it become not significant when adjusted using conservative degrees of freedom (*P*
337 = 0.190, Supplementary Table S10). The total root length density decreases with depth, which
338 minimizes the absolute value of the differences between genotypes at more extreme depths. To
339 address this limitation, we explored the differences between genotypes expressed as a percent of
340 the $1RS^{xR}$ values (($(1RS^{xR} - 1RS^{xW}) / 1RS^{xR}) *100$). This analysis showed that the relative
341 difference in total root length between $1RS^{xW}$ and $1RS^{xR}$ plants was larger at the deepest
342 sampling points (Supplementary Fig. S3A, regression between depth and percent difference *P* =
343 0.002). No roots were detected for $1RS^{xW}$ at 180 cm.

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

349 ***Average root diameter and combined root traits***: In the overall split-plot ANOVA for average
350 root diameter, the effect of depth was significant, even when using conservative degrees of
351 freedom (*P*= 0.02, Supplementary Table S13). The difference between the average $1RS^{xR}$ and
352 $1RS^{xW}$ genotypes was marginally non-significant in the conservative analysis (*P* = 0.0649,
353 Supplementary Table S13), but was highly significant in the statistical contrast between the pairs
354 of genotypes in the ANOVA including the four genotypes (*P* = 0.006, Supplementary Table
355 S14). In the analyses by individual depths, we observed similar average root diameters for the
356 $1RS^{xR}$ and $1RS^{xW}$ genotypes between 40 and 100 cm, but those values started to differentiate at

357 120 cm (P= 0.07) and were significant at 140, 160 and 180 cm. At all these depths, the average
358 root diameter was larger in the $1RS^{xR}$ than in the $1RS^{xW}$ lines (Fig. 2B, Supplementary Table
359 S15).

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

366 The other root traits measured with WinRhizo showed a profile similar to the one observed for
367 total root length density (Supplementary Fig. S4). This is not surprising because root surface and
368 volume density are a function of root length and diameter, and the number of root tips and forks
369 is mainly affected by the abundance of roots. The contrasts between $1RS^{xW}$ and $1RS^{xR}$ in the
370 ANOVAs using the four genotypes were significant for root surface (P =0.005), root volume (P
371 =0.008), number of tips (P =0.02) and number of forks densities (P =0.003, Supplementary Fig.
372 S4). Significant differences were also detected in three to four of the ANOVAs for individual
373 depths in each of the traits (Supplementary Fig. S4).

374

375 **$1RS^{xW}$ lines have shorter primary roots than $1RS^{xR}$ in hydroponic cultures**

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

380 ***Differences in root length***: In the hydroponic experiments, the 1RS and $1RS^{WR}$ lines showed
381 significantly longer seminal roots than the $1RS^{RW}$ and $1RS^{WW}$ lines (Fig. 3A). Since root length
382 was not significantly different between lines with the same distal segment, those lines were
383 averaged for the statistical analyses (Fig. 3B). The differences in seminal root length between the
384 $1RS^{xW}$ and $1RS^{xR}$ plants were detected in experiments using two sources of nitrogen (nitrate and

385 ammonium) at two different concentrations (2.0 and 0.2 mM, Fig. 3B), which indicates that these
386 differences are robust across different ionic environments.

387 ***Root elongation time course***: The previous results indicated that the differences in primary root
388 length between the $1RS^{xR}$ and $1RS^{xW}$ plants start at an early stage of root development. These
389 differences were consistent between the lines with the same distal chromosome segment, so the
390 following experiments were carried out using only the 1RS and $1RS^{RW}$ lines. To characterize the
391 growth response in more detail, we performed daily measurements of roots and calculated
392 elongation rates. Within each experiment, data was analyzed as repeated measures (split plot in
393 time with conservative degrees of freedom). The experiment was repeated three times and
394 overall ANOVAs were performed using experiments as a blocking factor, with replications
395 nested within experiments (Supplementary Tables S16-S19).

396 In the overall ANOVAs, the two genotypes showed highly significant differences over time ($P <$
397 0.0001) in accumulated root length (Supplementary Table S16, Fig.4A) and elongation rate
398 (Supplementary Table S18, Fig. 4B). The differences between days and the interaction genotype
399 by day interactions were also highly significant for both parameters ($P < 0.001$, Supplementary
400 Tables S16 and S18). In separate ANOVAs performed for individual days, we detected
401 significant differences in accumulated root length starting at 11 DAG, although differences were
402 already close to significant at 10 DAG ($P = 0.053$, Supplementary Table S17, Fig. 4A). In the
403 same experiments, the differences in the rate of elongation were significant from day 8.5 ($P =$
404 0.0328, Supplementary Table S19, Fig. 4B).
405

406 **$1RS^{xR}$ and $1RS^{xW}$ lines differ in root apical dominance and in the distribution of reactive oxygen**
407 **species (ROS)**

408 To test if the arrest in seminal root growth in the $1RS^{RW}$ plants was associated with a loss of
409 seminal root apical dominance, we characterized the distribution of lateral roots. Figure 5
410 showed clear differences between the seminal roots of 1RS and $1RS^{RW}$ plants imaged at 22
411 DAG. In the seminal roots of the $1RS^{xR}$ plants, lateral roots started to appear after the first 90.1 ±
412 2.7 mm from the root tip suggesting active apical dominance (Fig. 5 and Supplementary Fig. S5).
413 By contrast, the seminal roots of the $1RS^{xW}$ plants showed lateral roots appearing on average

414 14.7 ± 2.5 mm from the root apex. The differences between genotypes were highly significant (P
415 < 0.0001, Supplementary Fig. S5).

416 Since ROS gradients affect root elongation (Tyburski *et al.*, 2009), we estimated their
417 distribution in seminal roots 17 DAG by measuring the amount of formazan produced from the
418 reduction of nitro blue tetrazolium (NBT) and fluorescence in DCF-DA staining. Formazan
419 intensity, associated with superoxide anions, was similar in the distal region of the roots but was
420 significantly lower in the $1RS^{RW}$ than in the 1RS roots proximal to 650 μm ($P < 0.05$), and even
421 lower after 860 μm ($P < 0.001$, Fig. 6A). Fluorescence intensity from the DCF-DA staining,
422 associated mainly with hydrogen peroxide, peroxynitrite and hydroxyl radicals, showed a very
423 different pattern, with significantly higher intensities in $1RS^{RW}$ relative to the 1RS roots between
424 250 and 950 μm, and even higher differences between 350 and 640 μm ($P < 0.001$, Fig. 6B).

425 Finally, we compared the distribution of formazan during lateral root development in the same
426 two genotypes (Supplementary Fig. S6). No differences between genotypes were detected at 6
427 DAG, but at 9 DAG NBT staining revealed lateral root primordia close to the RAM in the
428 $1RS^{RW}$ but not in the 1RS line. By 15 DAG large lateral roots were formed close to the RAM in
429 $1RS^{RW}$, but no lateral root primordia were observed in the same region in the 1RS line. These
430 results confirmed the differences in apical dominance between the two genotypes.

431

432 **DISCUSSION**

433 **$1RS^{xR}$ genotypes show deeper roots in the field and increased tolerance to waterlogging and**
434 **terminal drought**

435 Previous studies have shown an association between the introgression of the rye 1RS arm in
436 wheat and improved resistance to water stress (Ehdaie *et al.*, 2012; Ehdaie *et al.*, 2003;
437 Hoffmann, 2008; Moreno-Sevilla *et al.*, 1995; Zarco-Hernandez *et al.*, 2005). In three of these
438 studies, the 1RS.1BL lines showed increased root biomass compared to the non-1RS control
439 lines in large pot or sand-tube experiments. However, these differences were not validated in the
440 field. In this study we showed that differences in grain yield and biomass between plants
441 carrying a complete 1RS translocation and NILs with an introgressed distal wheat chromosome
442 segment (1BS) are associated with differences in total root length density and average root

443 diameter in the field. Field excavations across three blocks of a field experiment including the
444 four different 1RS NILs provided an opportunity to visualize the differences in their root systems
445 and to quantify these differences using horizontal soil cores at consistent depths.

446 This experiment confirmed the hypothesis that the $1RS^{xR}$ lines have a higher root density
447 throughout the soil profile, with roots that reach deeper in the soil than the $1RS^{xW}$ lines (Fig.
448 2A). The more extensive root system provides a simple explanation for the higher carbon isotope
449 discrimination and increased stomatal conductance values observed in the $1RS^{xR}$ plants in
450 previous field experiments (Howell *et al.*, 2014). Through their deeper root system, the $1RS^{xR}$
451 plants can access more stored soil moisture, keep their stomata open longer, generate additional
452 photosynthetic products and biomass than the $1RS^{xW}$ plants. This was reflected in this study in
453 significantly higher NDVI values during the vegetative phase and increased grain yield values in
454 the$1RS^{xR}$ plants relative to the $1RS^{xW}$ plants, particularly under limiting irrigation (Fig. 1).

455 A previous study using eleven triticales showed that lines with better drought tolerance displayed
456 root traits less affected by drought and waterlogging than the susceptible lines (Grzesiak *et al.*,
457 2002). In our study we observed that the $1RS^{xR}$ lines performed better than the $1RS^{xW}$ lines
458 under both drought and waterlogging conditions (Fig. 1). The better performance of the $1RS^{xR}$
459 lines under waterlogging may be associated with their more extensive and deeper root system,
460 but we cannot rule out the possibility that anatomical and/or physiological differences known to
461 impact tolerance to waterlogging (Bailey-Serres and Voesenek, 2008) also affected the
462 differences observed in this study. In wheat, short-term periods of waterlogging are known to
463 affect the functionality of the root system (Malik *et al.*, 2001). Interestingly, an early study
464 comparing one triticales and two wheat accessions after two cycles of waterlogging and drainage
465 (seven days long each) found that the seminal roots of the triticale were twice as long as the
466 seminal roots of the wheat cultivars (Thompson *et al.*, 1992). In a later study preformed in a
467 stagnant medium that resulted in the death of the complete seminal root system, it was shown
468 that the same triticale accession had a higher ratio of nodal roots/shoot fresh weight and a larger
469 proportion of aerenchyma in root cross-section (18% vs. 14 and 12% in the two wheat cultivars).
470 This trait was likely associated with improved O_2 diffusion from shoots (Watkin *et al.*, 1998).
471 Although the number of accessions included in the previous studies was too small to make any
472 generalization, it indicates that additional studies will be required to determine if the $1RS^{xR}$ and

473 $1RS^{xW}$ lines differ in anatomical or physiological traits that can affect their differential tolerance
474 to waterlogging.

475

476 **$1RS^{xW}$ shows an earlier arrest of seminal root growth than $1RS^{xR}$ in hydroponic culture**

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

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

492 The growth arrest of the seminal roots in the $1RS^{xW}$ plants was accompanied by the proliferation
493 of lateral roots in close proximity to the RAM, suggesting a loss of apical dominance. The RAM
494 consists of a quiescent center (QC) surrounded by stem cells that generate new daughter cells,
495 which undergo additional divisions in the proximal region of the meristem and differentiate in
496 the transition zone (Heyman *et al.*, 2014). At a cellular level, a balance between cell proliferation
497 and cell elongation/differentiation determines root growth rate (Pacifici *et al.*, 2015). The arrest
498 of the growth of seminal roots in $1RS^{xW}$ plants suggests a modification cell proliferation and/or
499 cell elongation/differentiation. Additional studies will be required to determine if this arrest
500 involves changes in the QC and/or modifications in the root regions adjacent to the meristem. In
501 any case, the dramatic reduction in growth and apical dominance argues for an early
502 developmental program switch in the regulation of the RAM in the $1RS^{xW}$ plants.

503

504 **The distribution of ROS along seminal roots differed between 1RS^{xW} and 1RS^{xR} lines**

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

512 Seventeen days after germination, the apical region of the seminal roots of the 1RS lines showed
513 opposing gradients of superoxide and hydrogen peroxide characteristic of elongating roots (Fig.
514 6). A different ROS distribution was detected in the arrested 1RS^{xW} roots, where superoxide was
515 restricted to the distal ~700 μm and increased levels of DCF-DA fluorescence were detected
516 between 250-950 μm in the cell proliferation zone (Fig. 6). The contrasting patterns of ROS
517 distribution reflect the major developmental changes that differentiate the seminal roots of the
518 1RS and 1RS^{xW} genotypes.

519 Changes in ROS distribution can be triggered by the altered expression of major genes that
520 control the size of the meristematic zone (De Tullio *et al.*, 2010). These genes include *UPBEAT1*
521 (*UPB1*), a basic helix-loop-helix (bHLH) transcription factor that regulates the meristematic
522 zone size by restricting H_2O_2 distribution in the elongation zone (Tsukagoshi *et al.*, 2010). In
523 addition, *ROOT MERISTEM GROWTH FACTOR 1* (*RGF1*) and the transcription factor *RGF1*
524 *INDUCIBLE TRANSCRIPTION FACTOR 1* (*RITF1*) that mediates *RGF1* signaling can modulate
525 the distribution of ROS along the root developmental zones leading to enhanced stability of
526 PLETHORA2 (PLT2)(Yamada *et al.*, 2018). Reduced expression of *PLETHORA* in the root
527 apical region (Chen *et al.*, 2011) or changes in its distribution (Ercoli *et al.*, 2018) have been
528 associated with impaired root growth.

529 It remains unknown if the differential pattern of ROS distribution in the roots of the 1RS^{xW}
530 plants is the result of changes in the wheat homologs of these central developmental genes or a
531 more direct effect on genes affecting the redox balance in different developmental root zones.
532 The differences in superoxide and hydrogen peroxide distribution between the seminal roots of

533 the $1RS^{RW}$ and 1RS plants were measured after the arrest in root growth (Fig. 6). Therefore, we
534 currently do not know if the changes in ROS distribution are a cause or consequence of the
535 changes observed in root growth and apical dominance.

536

537 **Conclusions and future directions**

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

543 The hydroponic studies showed that the introgression of the distal wheat chromosome segment
544 in $1RS^{xW}$ resulted in drastic changes in apical dominance, location of lateral roots and ROS
545 distribution along the roots. Since the $1RS^{xW}$ and $1RS^{xR}$ lines are highly isogenic (Howell *et al.*,
546 2014), they provide a useful tool to identify the genes that regulate this major developmental
547 change in the wheat root apical meristem. The identification of these genes can not only
548 contribute to our basic understanding of the regulation of root development in grasses, but also
549 provide tools to modulate wheat root architecture and mitigate negative impacts of water stress in
550 wheat production.

551

552 **ACKNOWLEDGEMENTS**

553 This project was supported in the US by the Agriculture and Food Research Initiative
554 Competitive Grant 2017-67007-25939 (WheatCAP) from the USDA National Institute of Food
555 and Agriculture, by the International Wheat Yield Partnership (IWYP), and the Howard Hughes
556 Medical Institute; and in Argentina by the CONICET and the ANPCYT (PICT 2014-1887).

557

558

Supplementary information

Supplementary Figures

Supplementary Fig. S1. Excavation of the first block including the four genotypes.

Supplementary Fig. S2. Plant biomass and grain yield.

Supplementary Fig. S3. Percent decrease in total root length density and diameter.

Supplementary Fig. S4. Differences between $1RS^{xR}$ and $1RS^{xW}$ in different root parameters.

Supplementary Fig. S5. Distance between the primary root apex and the first lateral root

.Supplementary Fig. S6. Distribution of nitro blue tetrazolium (NBT) staining during root development.

Supplementary Tables

Supplementary Table S1. NDVI waterlogging 2013-2014.

Supplementary Table S2. NDVI waterlogging 2013-2014 by day.

Supplementary Table S3. Yield waterlogging 2013-2014.

Supplementary Table S4. NDVI waterlogging 2015-2016.

Supplementary Table S5. NDVI waterlogging 2015-2016 by day.

Supplementary Table S6. Yield waterlogging 2015-2016.

Supplementary Table S7. NDVI drought 2015-2016.

Supplementary Table S8. NDVI drought 2015-2016 by day.

Supplementary Table S9. Yield drought 2015-2016.

Supplementary Table S10. Total root length density.

Supplementary Table S11. Total root length density contrast.

Supplementary Table S12. Total root length density contrast by depth.

Supplementary Table S13. Average root diameter.

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. Plant biomass estimated by 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. Decrease 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. * = 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) with n= 12 plants per genotype in each treatment ($1RS^{xR}$ *vs.* $1RS^{xW}$).

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 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). 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). *= $P < 0.05$, ***= $P < 0.001$, n=5.

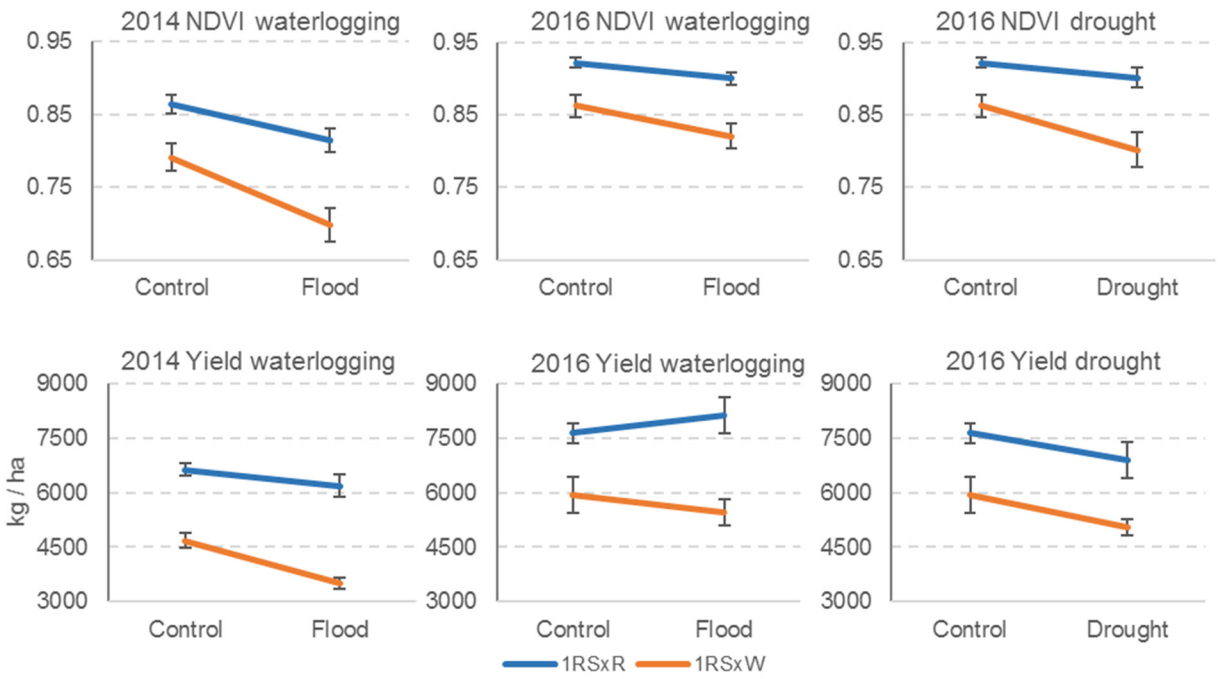
2014 NDVI waterlogging
2016 NDVI waterlogging
2016 NDVI drought
0.95
0.85
0.75
0.65
Control
Flood
Drought
2014 Yield waterlogging
2016 Yield waterlogging
2016 Yield drought
kg / ha
9000
7500
6000
4500
3000
1RSxR
1RSxW

Fig. 1

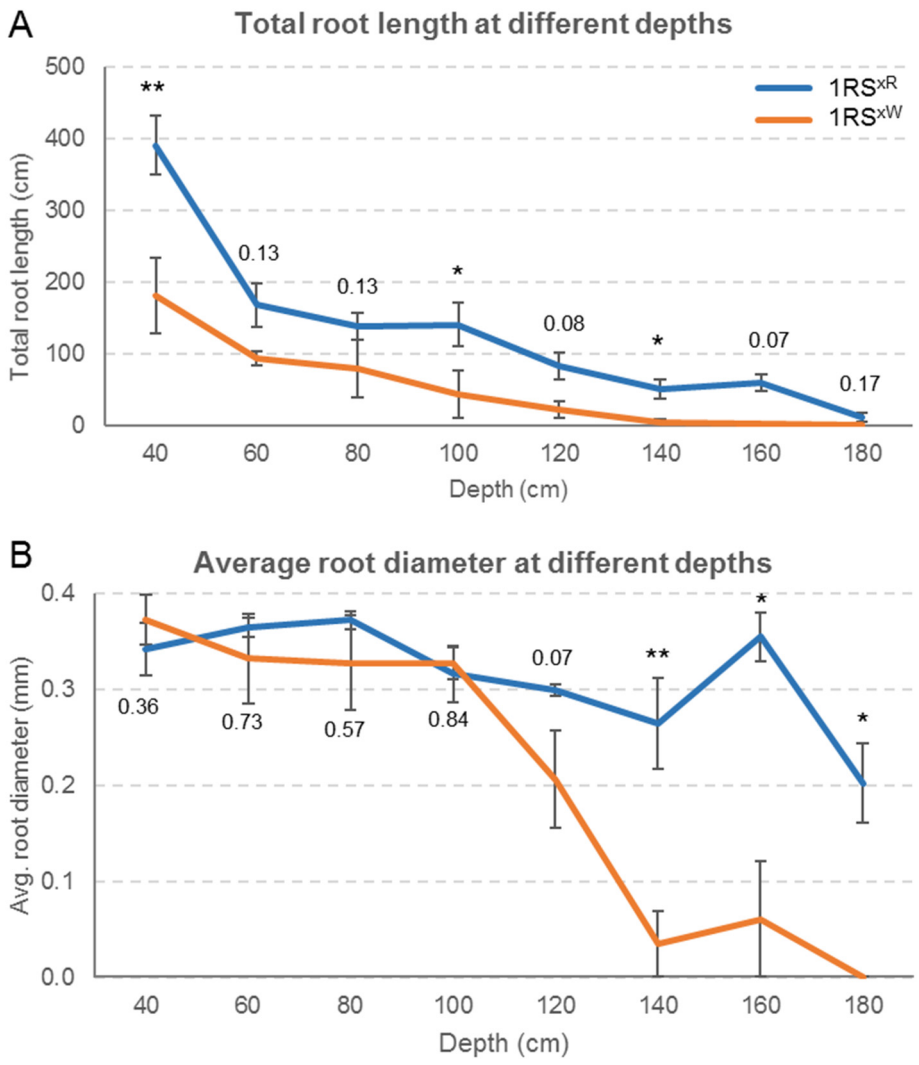
A
Total root length at different depths
Total root length (cm)
500
400
300
200
100
0
**
0.13
0.13
*
0.08
*
0.07
0.17
1RS^xR
1RS^xW
40
60
80
100
120
140
160
180
Depth (cm)
B
Average root diameter at different depths
Avg. root diameter (mm)
0.4
0.3
0.2
0.1
0.0
0.36
0.73
0.57
0.84
0.07
**
*
*
40
60
80
100
120
140
160
180
Depth (cm)

Fig. 2

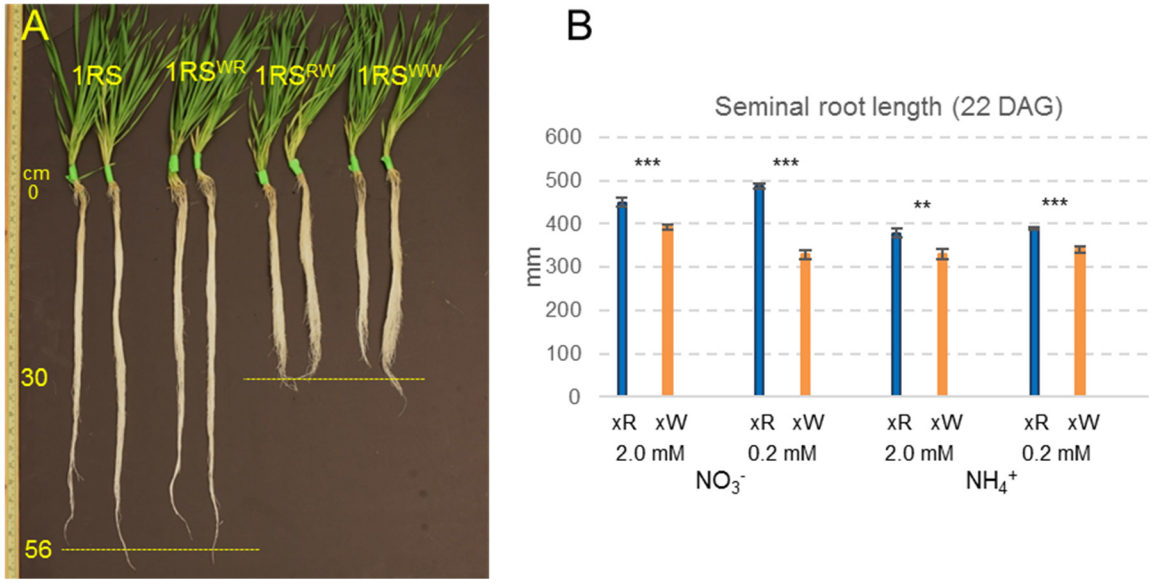
A
1RS
1RSWR
1RSRW
1RSWW
cm
0
30
56
B
Seminal root length (22 DAG)
600
500
400
300
200
100
0
mm
***
***
**
***
xR xW
2.0 mM
xR xW
0.2 mM
NO3-
xR xW
2.0 mM
xR xW
0.2 mM
NH4+

Fig. 3

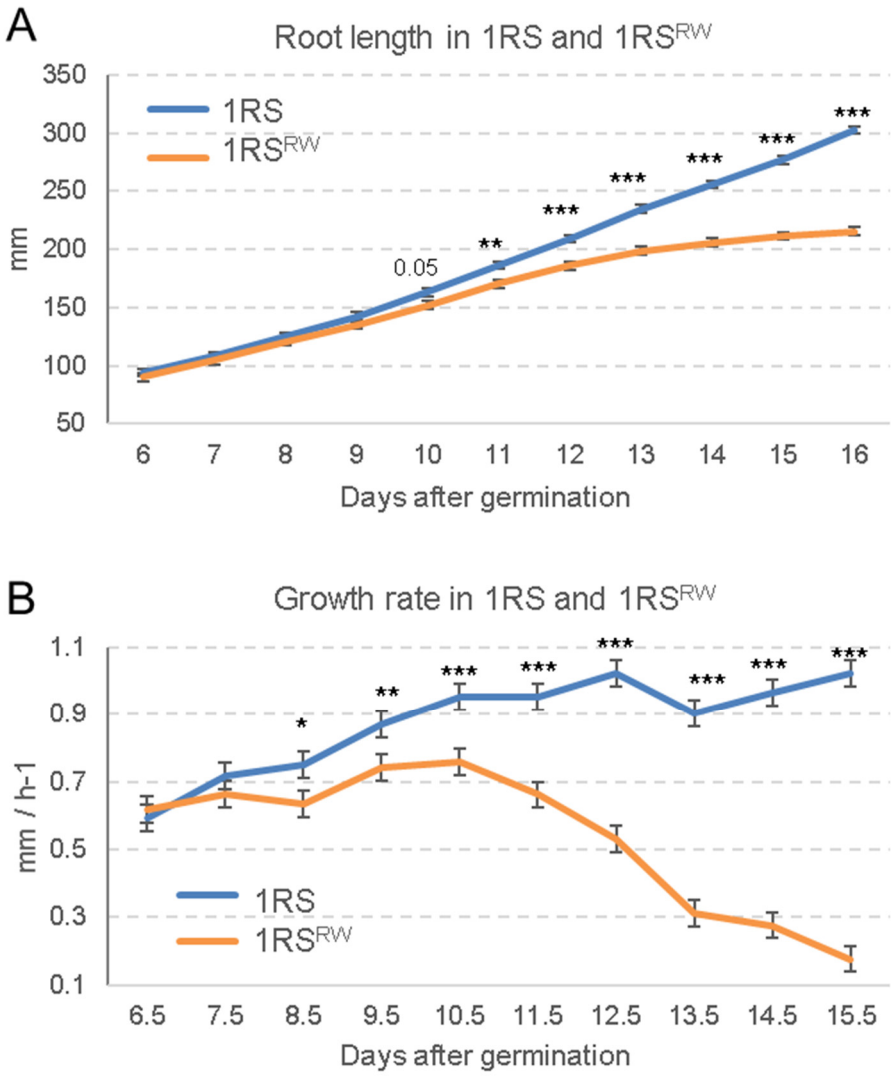
A
Root length in 1RS and 1RSRW
350
300
250
200
150
100
50
mm
1RS
1RSRW
0.05
**
***
6
7
8
9
10
11
12
13
14
15
16
Days after germination
B
Growth rate in 1RS and 1RSRW
1.1
0.9
0.7
0.5
0.3
0.1
mm / h-1
1RS
1RSRW
*
**
***
6.5
7.5
8.5
9.5
10.5
11.5
12.5
13.5
14.5
15.5
Days after germination

Fig. 4

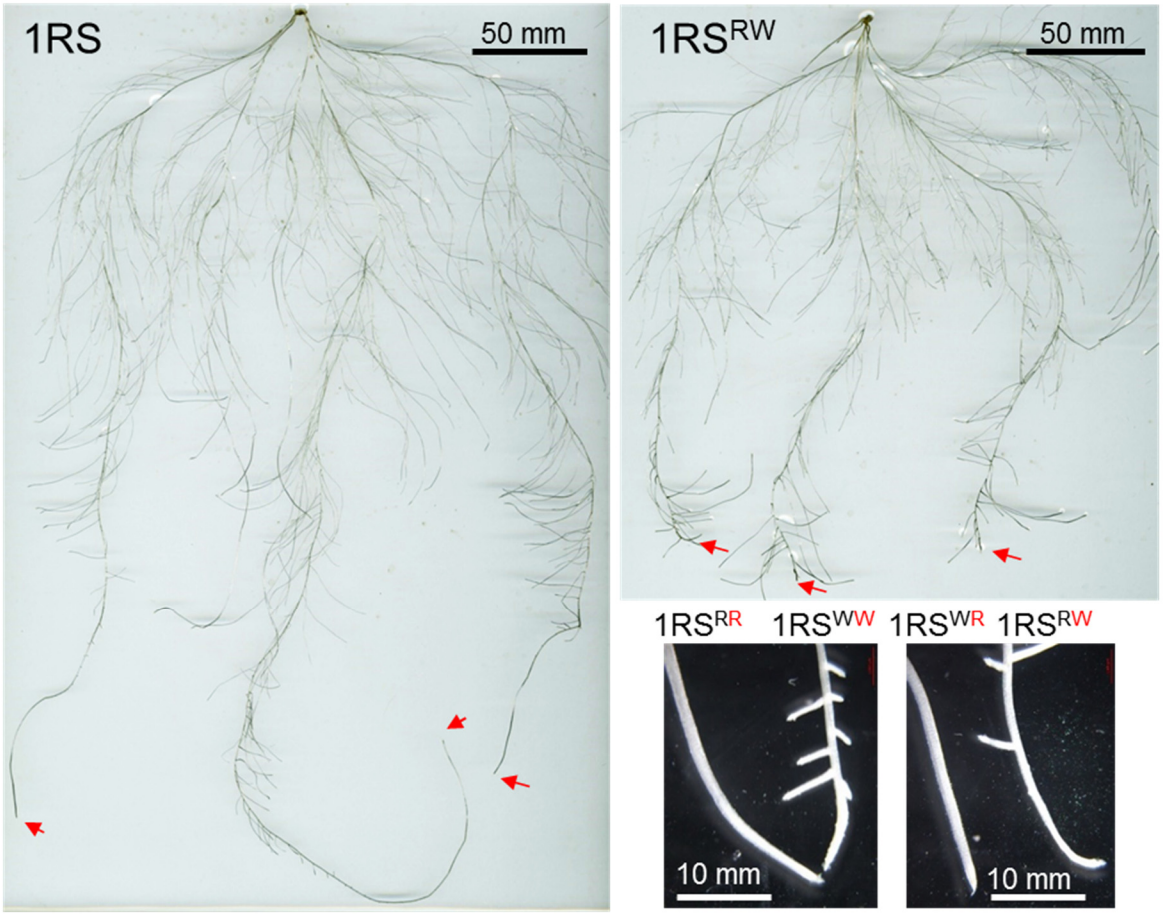

Fig. 5

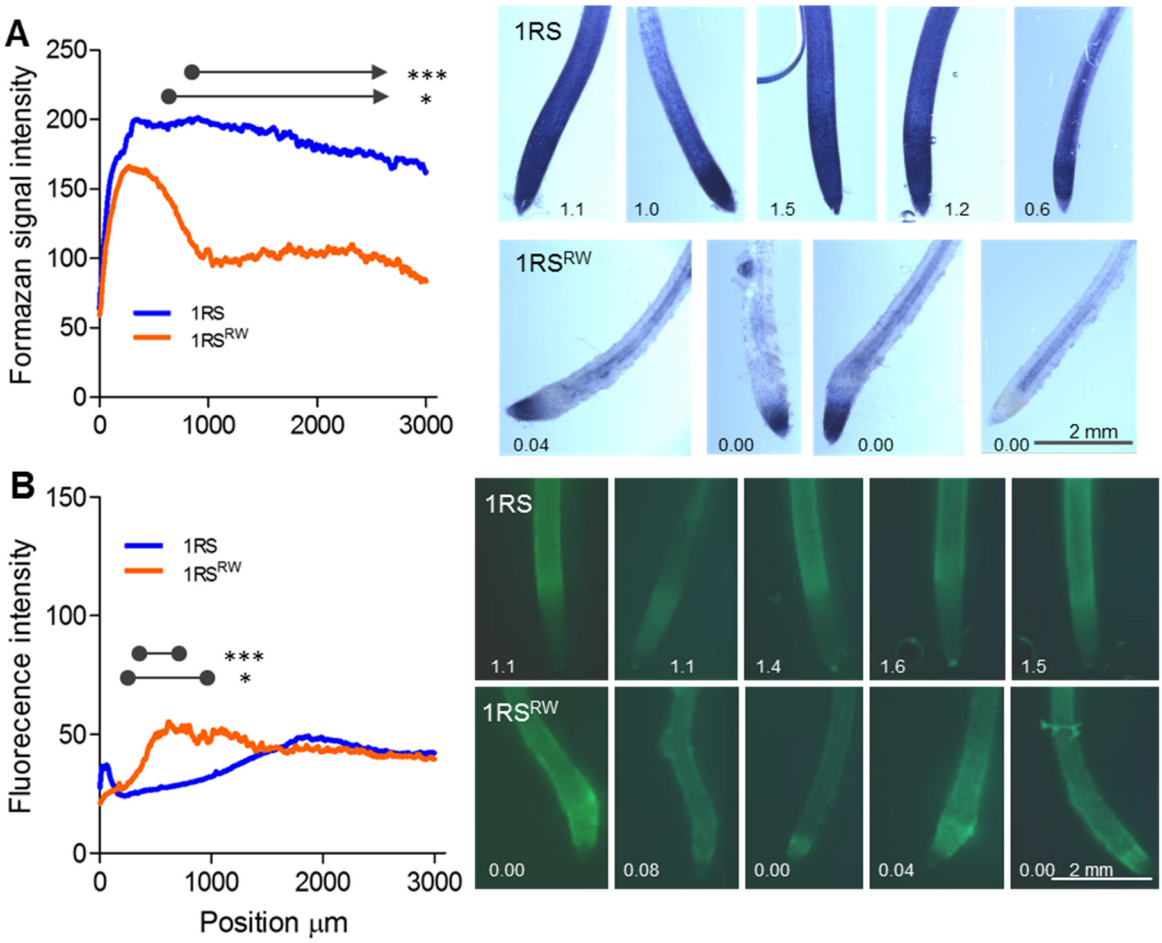
A
Formazan signal intensity
250
200
150
100
50
0
0
1000
2000
3000
***
*
1RS
1RS^RW
1RS
1.1
1.0
1.5
1.2
0.6
1RS^RW
0.04
0.00
0.00
0.00
2 mm
B
Fluorescence intensity
150
100
50
0
0
1000
2000
3000
Position µm
1RS
1RS^RW
***
*
1RS
1.1
1.1
1.4
1.6
1.5
1RS^RW
0.00
0.08
0.00
0.04
0.00
2 mm

Fig. 6