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Does Morphological and Anatomical Plasticity during the Vegetative Stage Make Wheat More Tolerant of Water Deficit Stress Than Rice?[1][OPEN]

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Water scarcity and the increasing severity of water deficit stress are major challenges to sustaining irrigated rice (*Oryza sativa*) production. Despite the technologies developed to reduce the water requirement, rice growth is seriously constrained under water deficit stress compared with other dryland cereals such as wheat (*Triticum aestivum*). We exposed rice cultivars with contrasting responses to water deficit stress and wheat cultivars well adapted to water-limited conditions to the same moisture stress during vegetative growth to unravel the whole-plant (shoot and root morphology) and organ/tissue (root anatomy) responses. Wheat cultivars followed a water-conserving strategy by reducing specific leaf area and developing thicker roots and moderate tillering. In contrast, rice 'IR64' and 'Apo' adopted a rapid water acquisition strategy through thinner roots under water deficit stress. Root diameter, stele and xylem diameter, and xylem number were more responsive and varied with different positions along the nodal root under water deficit stress in wheat, whereas they were relatively conserved in rice cultivars. Increased metaxylem diameter and lower metaxylem number near the root tips and exactly the opposite phenomena at the root-shoot junction facilitated the efficient use of available soil moisture in wheat. Tolerant rice 'Nagina 22' had an advantage in root morphological and anatomical attributes over cultivars IR64 and Apo but lacked plasticity, unlike wheat cultivars exposed to water deficit stress. The key traits determining the adaptation of wheat to dryland conditions have been summarized and discussed.

Among cereals, rice (*Oryza sativa*) and wheat (*Triticum aestivum*) are the most important staple food crops, and they belong to the family Poaceae. These two cereals share a common ancestor and diverged about 65 million years ago (Sorrells et al., 2003). Rice eventually developed strong adaptation potential for fully flooded conditions across tropical to temperate environments, while wheat became well adapted to aerobic conditions mostly restricted to temperate environments. Rice, with a semiaquatic behavior, consumes about 30% of the total fresh water available for agricultural crops worldwide, which equates to a 2- to 3-fold higher consumption than other cereals such as wheat and maize (*Zea mays*; Peng et al., 2006). Despite a significantly lower water requirement, the potential yield of wheat in a favorable environment (9 tons ha^{-1}) is comparable with the yield of fully flooded rice (9 tons ha^{-1}) in the dry season at the International Rice Research Institute (IRRI; Fischer and Edmeades, 2010). Hence, rice records very low water productivity compared with wheat and other dryland cereals. Because of growing concerns about water scarcity and the increased frequency and magnitude of water deficit stress events under current and future climates, increasing or even sustaining rice yield under fully flooded conditions is highly challenging. To minimize the total water requirement for cultivating rice, several water-saving technologies have been developed, such as direct-seeded aerobic rice cultivation (Bindraban et al., 2006). These water-saving technologies increased water productivity substantially compared with flooded conditions but were invariably associated with a yield penalty. A major challenge that water-saving technologies including aerobic rice currently face is the lack of mechanistic understanding for further genetic improvement.

By virtue of its wider adaptation to a range of edaphic conditions, rice is considered to possess the diversity to adapt to upland or aerobic scenarios extending into water deficit conditions (Khush, 1997). Genetic differences in rice root biomass and rooting depth and variation in root morphology with water deficit stress exposure are well documented (Kato et al., 2006, 2007; Henry et al., 2011; Kano et al., 2011). But the underlying mechanisms differing across diverse germplasm that influence water uptake under water deficit stress are not fully understood (Gowda et al., 2011). A recent report

[1] This work was supported by the Wageningen University Fund (Ph.D. stipend and field work at the International Rice Research Institute to N.K.); by the Federal Ministry for Economic Cooperation and Development, Germany; and by the Cereal Systems Initiative for South Asia (U.S. Agency for International Development-Bill & Melinda Gates Foundation).

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has documented water deficit-tolerant genotypes recording a lower bleeding rate and narrow xylem diameter under stress (Henry et al., 2012). Contrastingly, a higher root hydraulic conductivity helped to maintain a higher photosynthetic rate (Adachi et al., 2010), with tolerant cultivars maintaining greater root hydraulic conductivity than susceptible cultivars (Matsuo et al., 2009). Furthermore, upland rice cultivars with deeper roots outperformed lowland cultivars possessing a shallow root system when encountered with water deficit stress (Uga et al., 2013). Additionally, major-effect grain yield metaquantitative trait loci under water deficit stress identified in rice were found to colocalize on the genomes of other dryland cereals such as wheat, maize, and pearl millet (*Pennisetum glaucum*; Swamy et al., 2011), indicating a possible common evolutionary pathway for water deficit adaptation across cereals. Despite these achievements and the relatedness among cereals, rice does not respond in a way similar to other dryland cereals to water deficit stress conditions. To bring in a revolutionary change in future breeding strategies for upland/aerobic rice and for water deficit tolerance in rice, there is a need for a fundamental understanding and identification of the key traits that determine the water deficit stress response in well-adapted dryland cereals. Hence, comparing whole-plant responses (shoot and root) of rice with those of other dryland cereals such as wheat is essential.

A comparative study between two C_3 cereals (rice and wheat) will help identify the core adaptive mechanisms and/or a suite of traits that render wheat to grow with less water and more tolerance of water deficit stress. Such comparative analysis should target key morphological, physiological, anatomical, and agronomic traits throughout the crop growth cycle, as water deficit stress occurs at both early (vegetative stage) and late (reproductive stage) seasons in rice (Pandey et al., 2007). Extensive research efforts are currently ongoing to reduce the impact of water deficit stress during the reproductive stage in rice (Venuprasad et al., 2008; Verulkar et al., 2010; Vikram et al., 2011; Kumar et al., 2014) and in wheat (Olivares-Villegas et al., 2007; Lopes and Reynolds, 2010; Pinto et al., 2010). Therefore, our study focused on stress during the vegetative stage to identify key checkpoints that determine whole-plant responses of representative rice cultivars adapted to lowland, upland/aerobic, or water deficit conditions and of wheat cultivars with moderate to high water deficit tolerance. Cultivars from both species were exposed to moisture levels that resemble aerobic conditions and water deficit stress during the vegetative stage. Our study follows a previous report that has successfully demonstrated the approach to expose rice and wheat to the same moisture stress conditions (Praba et al., 2009) and is designed to address the following specific objectives: (1) to quantify the adaptive plasticity in shoot and root morphology and biomass partitioning among different plant parts (leaves, stem, and root); (2) to estimate the key supportive physiological mechanisms such as whole-plant water use efficiency (WUE) and leaf-level carbon isotope discrimination ($\Delta^{13}C$); and (3) to dissect root anatomical plasticity across different key zones in both rice and wheat roots exposed to water deficit stress.

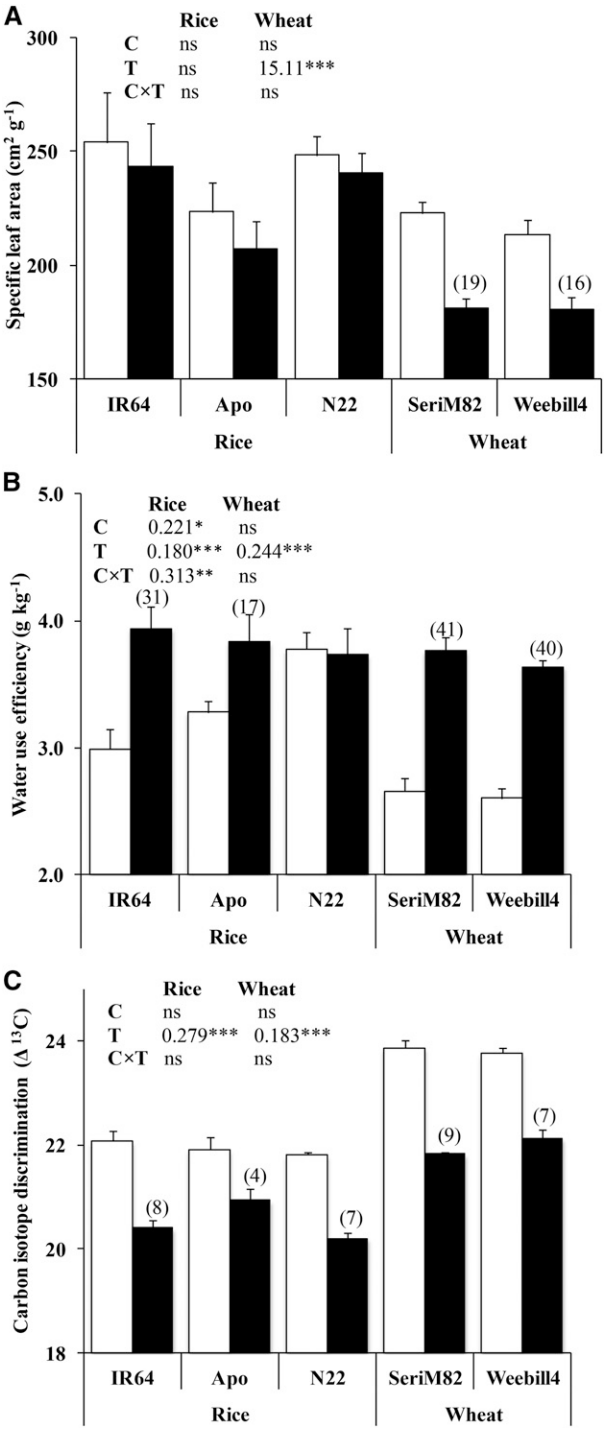

Figure 1. Specific leaf area (A), whole-plant WUE (B), and $\Delta^{13}C$ (C) of rice and wheat (mean ± SE). White columns represent control, and black columns represent water deficit stress. Values in parentheses represent the significant percentage change (increase or decrease) over the control. The ANOVA results with LSD values are given for cultivar (C), treatment (T), and cultivar-treatment interaction (C×T). Significance levels are as follows: $^{*}P < 0.05$, $^{**}P < 0.01$, and $^{***}P < 0.001$.

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Finally, novel traits that benefit dryland adaptation in wheat compared with high water-requiring rice cultivars are highlighted.

RESULTS

Shoot Morphology and Whole-Plant and Leaf-Level WUE

Significant reductions in total leaf area, total biomass, and cumulative water transpiration were recorded under water deficit stress in rice cultivars, with stronger reductions in the tolerant cv Nagina 22 (N22; $P < 0.001$) and in both wheat cultivars ($P < 0.01$ to $P < 0.001$; Supplemental Table S1). Specific leaf area and tiller number decreased under water deficit stress only in wheat cultivars ($P < 0.001$; Fig. 1A; Supplemental Table S1). Whole-plant WUE increased in response to water deficit stress in two out of three rice cultivars (rice 'IR64,' 31%; rice 'Apo,' 17%) and in both wheat cultivars (approximately 40%). The tolerant rice 'N22' recorded higher WUE than the other two cultivars in the absence of stress and was not altered by water deficit stress; hence, significant cultivar and treatment interaction ($P < 0.01$; Fig. 1B) was observed. $\Delta^{13}C$ of leaf is often used as a proxy to measure WUE (lower $\Delta^{13}C$ means higher WUE; Impa et al., 2005). In both species, water deficit stress had a strong effect on $\Delta^{13}C$ ($P < 0.001$), but there were no cultivar differences ($P > 0.05$). On average, $\Delta^{13}C$ decreased by 6% in rice cultivars and by 8% in wheat cultivars. The absolute value of $\Delta^{13}C$ was higher in wheat cultivars than in rice cultivars (Fig. 1C).

Biomass Partitioning among Leaf, Stem, and Root

In general, both species recorded higher biomass partitioning to leaf and stem than to root, with a higher proportion of biomass partitioned to roots in wheat than in rice (Supplemental Table S1). Leaf weight ratio (LWR) and stem weight ratio (SWR) varied only among rice cultivars ($P < 0.01$), with a significant effect of water deficit stress ($P < 0.05$ to $P < 0.01$). The susceptible rice 'IR64' had 16% lower LWR and 24% higher SWR with water deficit stress. The tolerant rice 'N22' had lower LWR and higher SWR than other cultivars and was not altered by water deficit stress. In both species, root weight ratio did not differ significantly among cultivars and treatments ($P > 0.05$), but an increasing trend was observed with the tolerant rice 'N22' and both wheat cultivars in water deficit stress.

Root Morphology

Root morphological traits such as maximum root length, total root length, and root length density did not differ with either cultivars or stress treatments in rice ($P > 0.05$), but root volume and root biomass differed with both cultivars and treatments ($P < 0.05$ to $P < 0.01$; Supplemental Table S2). Conversely, in both wheat cultivars, the water deficit stress treatment effect was highly significant for all the above-mentioned traits ($P < 0.05$ to $P < 0.001$), but there were no cultivar differences. The maximum root length of the two wheat cultivars was increased in response to water deficit

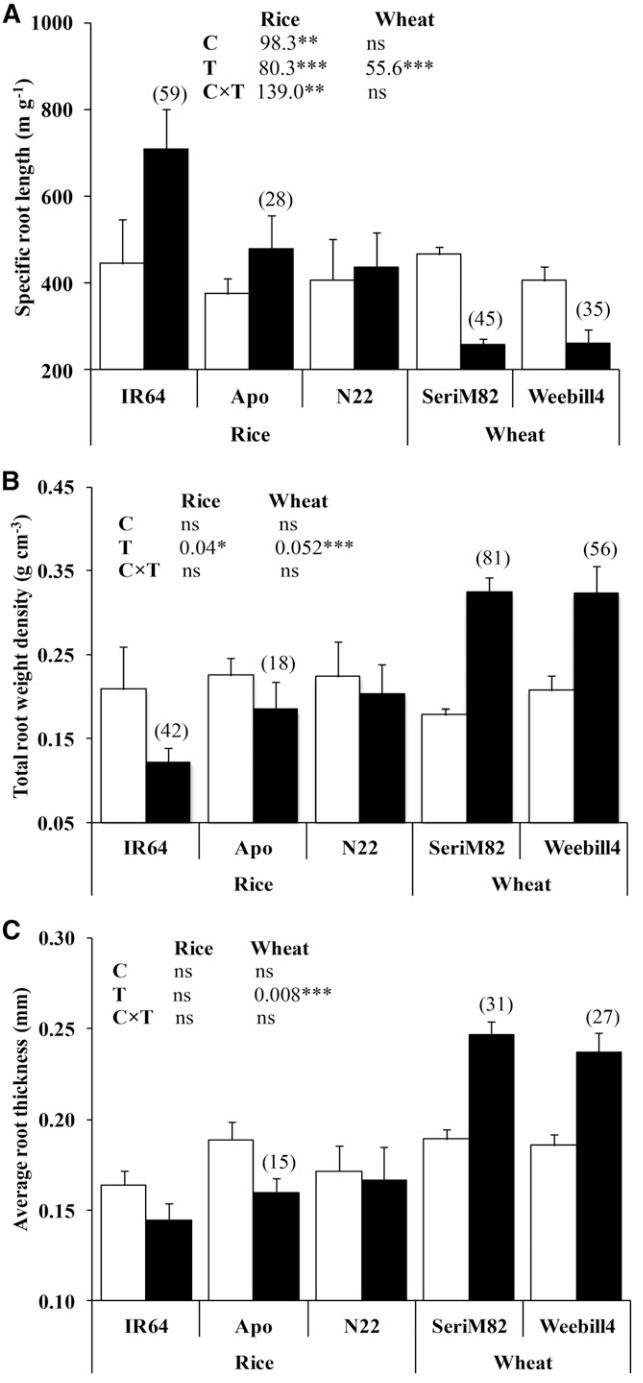

Figure 2. Specific root length (A), total root weight density (B), and average root thickness (C) of rice and wheat (mean ± SE). White columns represent control, and black columns represent water deficit stress. Values in parentheses represent the significant percentage change (increase or decrease) over the control. The ANOVA results with LSD values are given for cultivar (C), treatment (T), and cultivar-treatment interaction (C×T). Significance levels are as follows: $*P < 0.05$, $**P < 0.01$, and $***P < 0.001$.

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stress compared with control conditions (Supplemental Table S2).

Specific Root Length, Average Root Thickness, and Total Root Weight Density

Specific root length (SRL), expressed as the ratio of root length to root biomass, is a key indicator of root thickness. In response to water deficit stress, SRL increased significantly in two of the three rice cultivars (rice 'IR64,' 59%; rice 'Apo,' 28%) but decreased in both wheat cultivars by approximately 40% (Fig. 2A). The SRL is independently controlled by two other components, root thickness and root weight density (Ostonen et al., 2007). Our results support this, with an increased SRL in rice 'IR64' determined mainly by

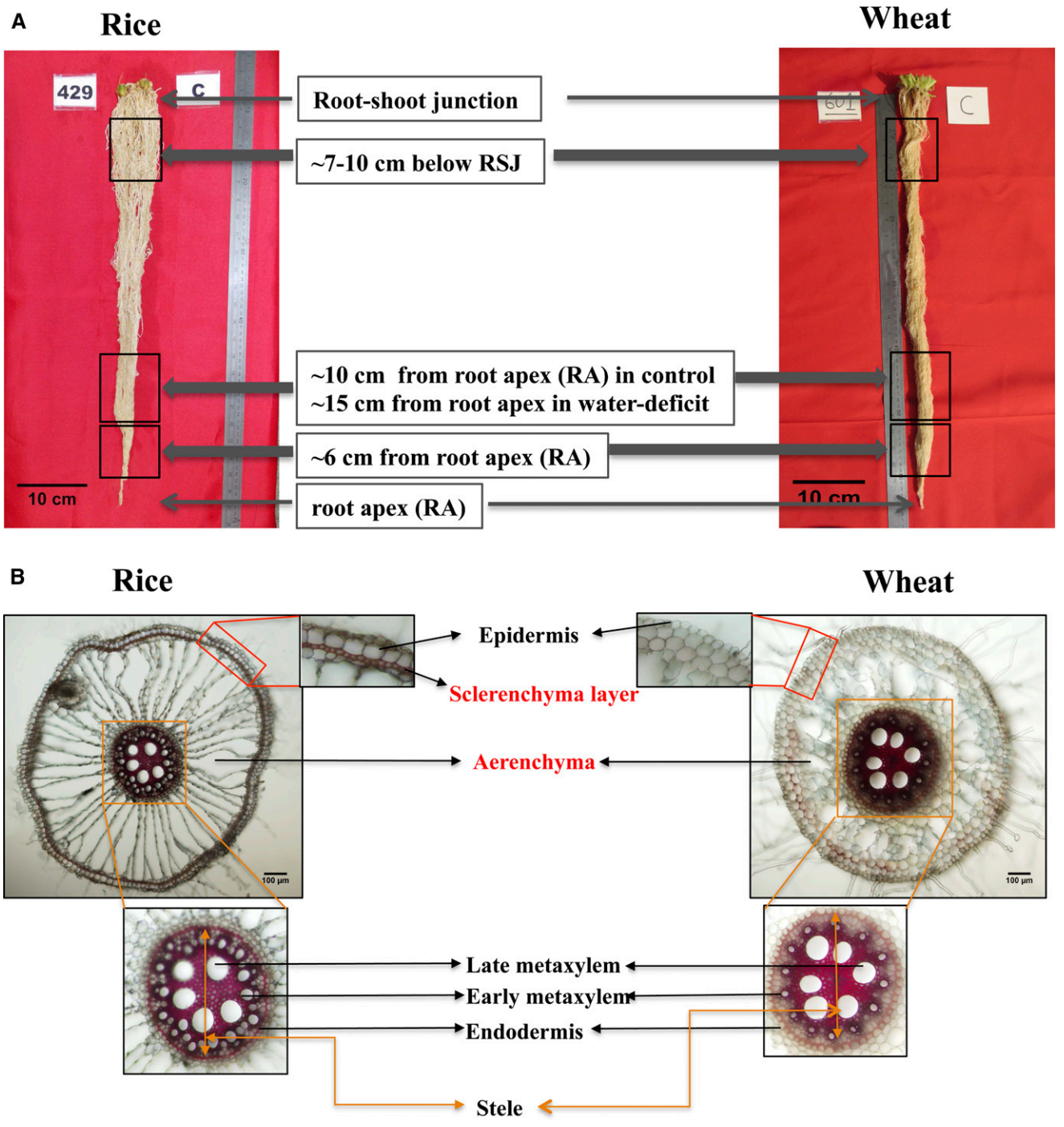

Figure 3. A, Root samples were collected in three different zones on nodal roots for anatomy study. B, Radial root cross sections showing anatomical variation in rice and wheat. Scale bars on root images = 10 cm (A) and 100 μm (B).

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reduced total root weight density (42%), while in cv Apo this was due to a reduction in both average root thickness (15%) and total root weight density (18%). On average, the lower SRL in the wheat cultivars was due to a greater increase in total root weight density (69%) than in average root thickness (29%; Fig. 2, B and C).

Radial Root Anatomy

To further confirm the observed variation in root morphology, we investigated root anatomical variables at three different locations along the root length (Fig. 3). Note that root cross sections were stained with phloroglucinol to assess the secondary cell wall thickening and lignin deposition under water deficit stress. While it appears that there were changes in the staining pattern (e.g. cv Weebill4 under water deficit treatment; Fig. 4), the results were not consistent across replications in both species; thus, we will not discuss such changes.

Root Diameter

Variation in root diameter is due to change in the number and size/width of cortical cells and in stele diameter. In both species, root diameter varied significantly with cultivar ($P < 0.05$ to $P < 0.001$) and position along the root ($P < 0.001$). A significant effect of water deficit stress on root diameter was documented with rice cultivars ($P < 0.01$; Supplemental Table S3). Root diameter at the root-shoot junction (RSJ; Fig. 3A) decreased with stress exposure in rice 'IR64' (25%), with no change in the aerobic cv Apo and the tolerant cv N22 (Fig. 4A). A clear pattern was not observed at 10 to 15 cm from the root apex (RA; Fig. 4B). However, an opposite response was observed at 6 cm from the RA, where the tolerant cultivars showed lower root diameter (cv N22, 19%; cv Apo, 20%), with no change in cv IR64 (Fig. 4C). Unlike in rice cultivars, root diameter at the RSJ increased significantly in both wheat cultivars (wheat 'SeriM82,' 42%; wheat 'Weebill4,' 30%), but at two other positions a decreasing trend was observed (i.e. 10–15 cm from the RA and 6 cm from the RA; Fig. 4).

Stele Diameter and Stele Diameter in Proportion to Root Diameter

Stele is the central part of the root system that contains vascular tissue (i.e. xylem and phloem; Fig. 3B). Both cereals recorded strong cultivar and spatial (different positions along the root) variations ($P < 0.001$) for stele diameter (Supplemental Table S3). Stele diameter at the RSJ did not differ in any of the rice cultivars (Fig. 5A), but the tolerant cv N22 maintained a higher stele diameter at 10 to 15 cm from the RA and at 6 cm from the RA (Fig. 5, B and C). Additionally, stele

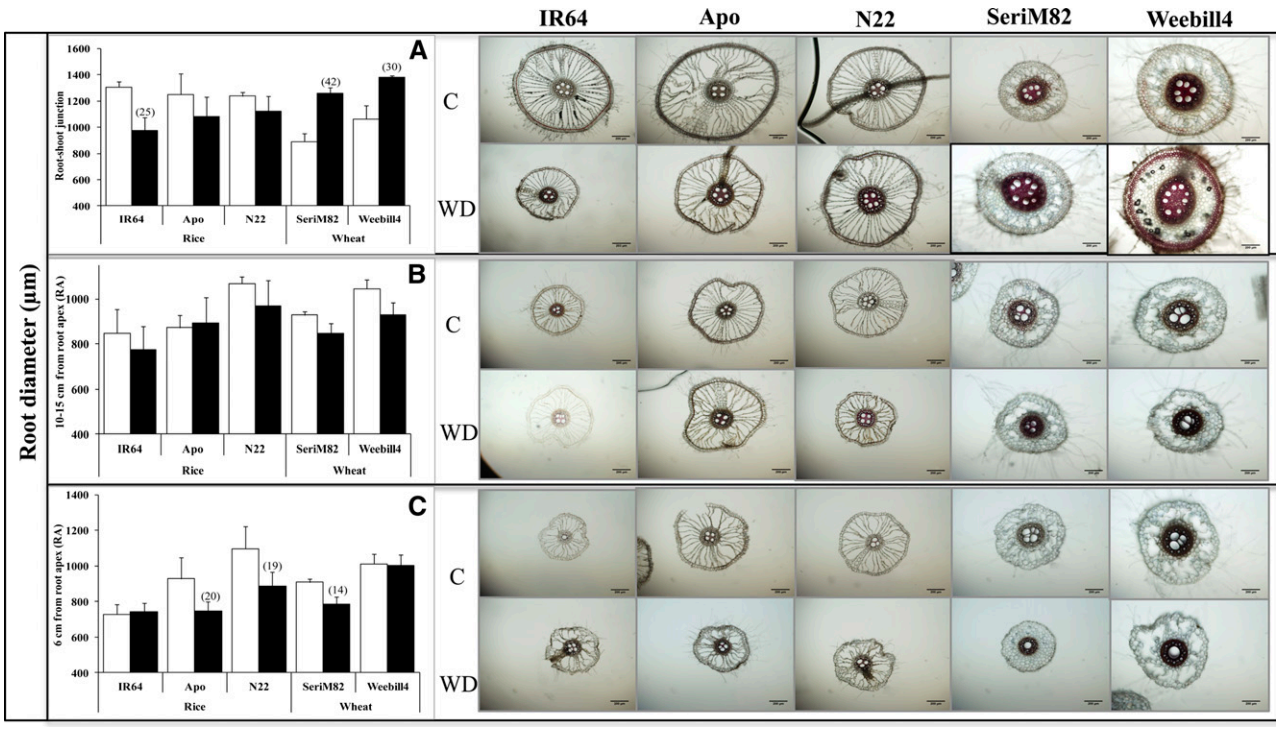

Figure 4. Root diameter at the RSJ (A), 10 to 15 cm from the RA (B), and 6 cm from the RA (C) on nodal roots of rice and wheat cultivars (mean ± SE). White columns represent control (C), and black columns represent water deficit stress (WD). Values in parentheses represent the significant percentage change (increase or decrease) over the control value. Scale bars on root images = 200 μm.

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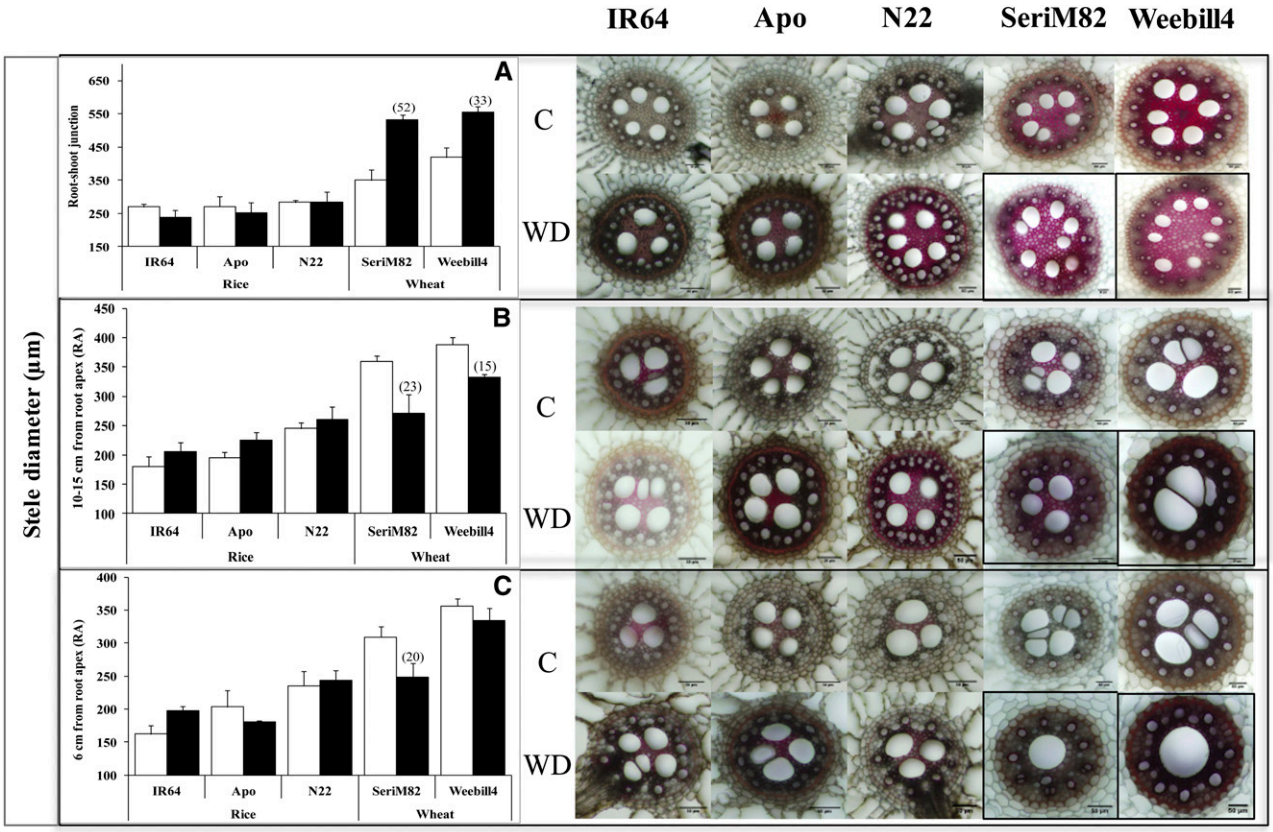

Figure 5. Stele diameter at the RSJ (A), 10 to 15 cm from the RA (B), and 6 cm from the RA (C) on nodal roots of rice and wheat (mean ± SE). White columns represent control (C), and black columns represent water deficit stress (WD). Values in parentheses represent the significant percentage change (increase or decrease) over the control value. Scale bars on root images = 50 μm.

diameter was more stable and was not affected by water deficit stress in rice ($P > 0.05$). Unlike in rice, stele diameter increased significantly under water deficit stress at the RSJ in wheat cultivars, with wheat 'SeriM82' (52%) showing a greater increase than wheat 'Weebill4' (33%; Fig. 5A). Stele diameter responded in an opposite manner, with a strong reduction at two other positions on roots (10–15 and 6 cm from the RA) in cv SeriM82 and at 10 to 15 cm from the RA only for cv Weebill4 (Fig. 5, B and C).

Stele diameter in proportion to root diameter (SD:RD) was strongly affected by water deficit stress in rice ($P < 0.001$) and lacked cultivar and tissue position variation on nodal roots. By contrast, wheat cultivars documented a significant variation along the root tissue position ($P < 0.001$) and its interaction with treatment ($P < 0.05$; Supplemental Table S3). Wheat cultivars maintained higher SD:RD (approximately 35%–40%) than rice cultivars (approximately 20%–25%) across three root positions sectioned (Fig. 6). An increasing trend with SD:RD was observed in response to water deficit stress in all three rice cultivars (Fig. 6), but this was least affected by water deficit stress in wheat, except for a noticeable reduction at 10 to 15 cm from the RA for cv SeriM82 (Fig. 6B).

Late Metaxylem Diameter and Number

Late metaxylem diameter (LMXD) remained relatively constant in rice cultivars across different root tissue positions under both control and water deficit stress, with a narrow variation ($P < 0.05$) recorded among cultivars (Supplemental Table S3). The effect of water deficit stress on LMXD was not significant, but a decreasing trend was observed across all three rice cultivars near the RSJ (Fig. 7A). Late metaxylem number (LMXN) varied significantly with root tissue position ($P < 0.01$), cultivar, and treatment ($P < 0.05$) in rice. Among rice cultivars, lowland-adapted rice 'IR64' had lower LMXN at 6 cm from the RA in non-stress conditions, but upon exposure to stress, LMXN increased significantly and was similar to that of other cultivars (Fig. 7F). Unlike in rice, LMXD varied with cultivar and tissue position and their interaction in wheat ($P < 0.001$; Supplemental Table S3). In both control and water deficit stress, wheat cultivars maintained higher LMXD at 10 to 15 cm from the RA and at 6 cm from the RA compared with RSJ, except for cv SeriM82 recording a 28% lower LMXD at 10 to 15 cm from the RA under water deficit stress (Fig. 7B). LMXD increased greatly in both wheat cultivars at

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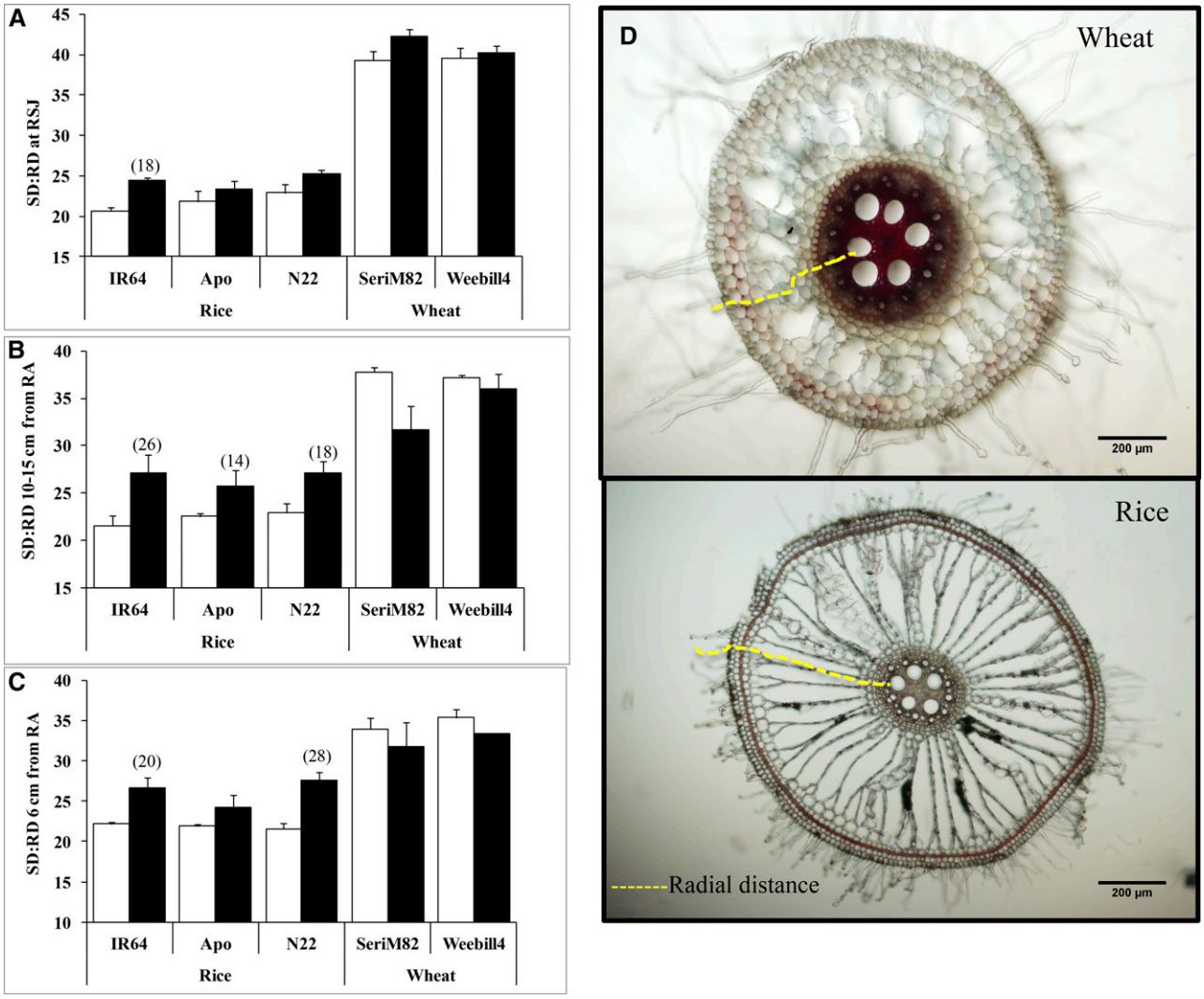

Figure 6. SD:RD (%) at the RSJ (A), 10 to 15 cm from the RA (B), and 6 cm from the RA (C) on nodal roots of rice and wheat (mean ± SE). White columns represent control, and black columns represent water deficit stress. A pictorial representation of radial distance in wheat and rice is shown in D. Values in parentheses represent the significant percentage change (increase or decrease) over the control value. Scale bars on root images = 200 μm.

6 cm from the RA under water deficit stress exposure, with the increase being higher in wheat 'Weebill4' (51%) than in wheat 'SeriM82' (30%; Fig. 7C). Additionally, LMXN displayed strong interaction between treatment and tissue position ($P < 0.001$). Exposure to water deficit stress resulted in an increase in LMXN at the RSJ in wheat cultivars (Fig. 7D), but this decreased at two other positions, with a highly significant reduction observed at 6 cm from the RA (Fig. 7, E and F). According to Hagen-Poisseuille's law, the flow of water in any given conduit is the fourth power of the radius of the conduit. Theoretically calculated axial conductance by modified Hagen-Poisseuille's law also followed a pattern similar to that of LMXD across three different positions on nodal roots in both species (Fig. 8).

DISCUSSION

We compared rice and wheat for their adaptive responses in root morphology and anatomy to water deficit stress. The findings from our study are discussed below.

Reduced Specific Leaf Area Is a Determining Factor for Increased WUE under Stress in Wheat

Reducing specific leaf area in response to water deficit stress to conserve water has been documented across crop species (Rao and Wright, 1994; Araus et al., 1997; Craufurd et al., 1999; Nautiyal et al., 2002), and the same was observed with wheat cultivars (Fig. 1A). Increased WUE under water deficit conditions is well known (Blum, 2009). Our results also documented

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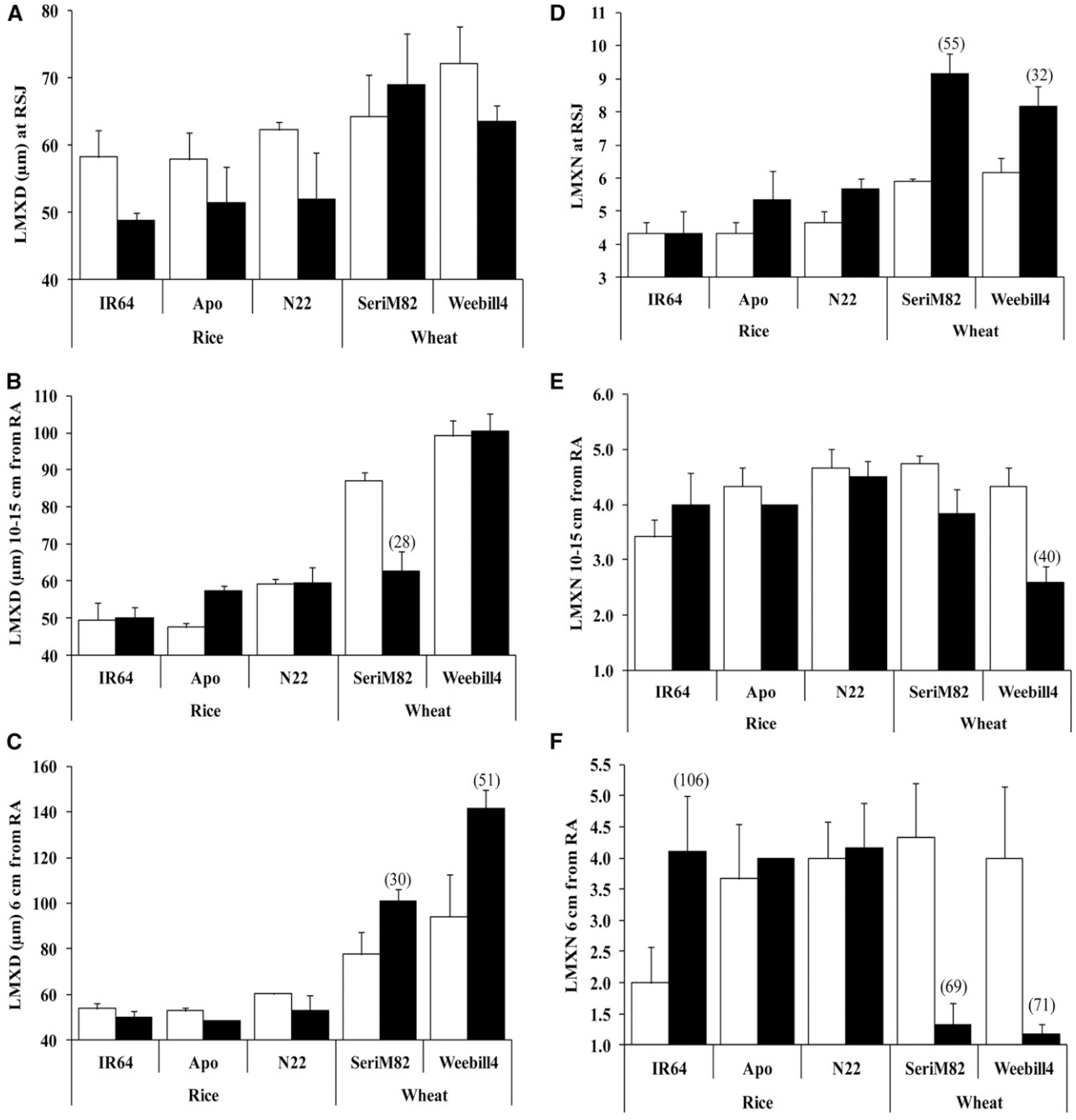

Figure 7. LMXD and LMXN at the RSJ (A and D), 10 to 15 cm from the RA (B and E), and 6 cm from the RA (C and F) on nodal roots of rice and wheat (mean ± SE). White columns represent control, and black columns represent water deficit stress. Values in parentheses represent the significant percentage change (increase or decrease) over the control value.

increased WUE with cultivars of both species except for rice 'N22' under water deficit stress; in wheat, this increased WUE could be due to reduced specific leaf area. Variation in $\Delta^{13}C$ is determined by the balance between stomatal conductance and carboxylation efficiency (Farquhar et al., 1989). Both species had comparable reductions in $\Delta^{13}C$ in water deficit stress (Fig. 1C), but possibly for different reasons. Lower specific leaf area leads to higher carboxylation rate, but under water deficit conditions, limitation of stomatal conductance also reduces photosynthesis. Therefore, reduced $\Delta^{13}C$ under water deficit stress in wheat could be due to both lower stomatal conductance and higher carboxylation rate (Condon et al., 1990), but in rice it could be due to lower stomatal conductance only.

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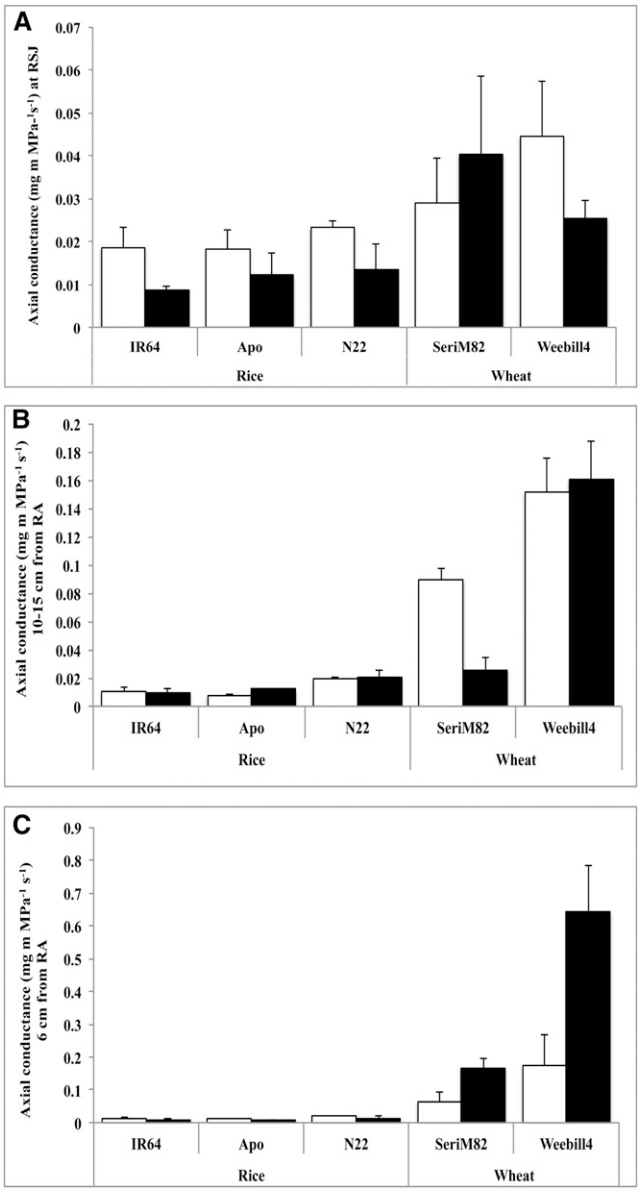

Figure 8. Theoretically calculated axial hydraulic conductance at the RSJ (A), 10 to 15 cm from the RA (B), and 6 cm from the RA (C) on nodal roots of rice and wheat cultivars (mean ± SE). White columns represents control, and black columns represent water deficit stress.

SRL Displays Opposing Responses among Rice and Wheat Cultivars under Water Deficit Stress

SRL captures the overall effect of both root thickness and root weight density (Fitter, 2002). In our study, SRL increased under water deficit stress in rice 'IR64' and 'Apo' because of reduced average root thickness, while lower SRL in wheat cultivars resulted from increased average root thickness and total root weight density (Fig. 2). Our results suggest that rice cultivars (except for rice 'N22') aimed for a rapid water-acquisition strategy, since thinner roots (higher SRL) increase overall root hydraulic conductance by exploring more soil volume for water and enabling rapid uptake of water (Reich et al., 1998; Eissenstat and Achor, 1999; Solari et al., 2006; Hernández et al., 2010). This strategy could lead to higher susceptibility to water deficit stress due to quicker water depletion (Ryser, 2006). On the other hand, the two wheat cultivars employed a conservative strategy by developing thicker roots and exploring less soil volume for water by reducing root length density. Thicker roots enhance soil-penetrating ability to access deeper layers in drying soil conditions (Davies and Bacon, 2003). This can be substantiated by our results on maximum root length. Although the full potential to express maximum root length of wheat could be influenced by limited pot size in our study, it increased in response to water deficit stress in wheat, while this was not the case with rice (Supplemental Table S2; Supplemental Fig. S1). Among the rice cultivars, tolerant rice 'N22' followed a conservative strategy by reducing root length density (Supplemental Table S2), but, unlike wheat, it lacked plasticity in SRL, average root thickness, and total root weight density (Fig. 2).

Stele Diameter Was More Responsive to Water Deficit Stress in Wheat Than in Rice

The SD:RD provides an indirect measure of cortex tissue area/width. The stele size and SD:RD are lower in wetland than in dryland plants (McDonald et al., 2002), and our result confirms this difference between rice and wheat (Figs. 5 and 6). This anatomical feature in wetland species aims to optimize the consumption of oxygen under waterlogging (Armstrong and Beckett, 1987; Armstrong et al., 1991; Aguilar, 1998). A distinct sclerenchyma layer as an apoplastic barrier to impede radial oxygen loss was observed in rice, even in the absence of waterlogging (Supplemental Fig. S2). These root anatomical features in rice have an advantage under flooded conditions but could affect root water uptake. An inverse relationship between overall radial hydraulic conductance and cortex width has been documented (Rieger and Litvin, 1999). Radial hydraulic conductance is lower in rice than in other cereals (Miyamoto et al., 2001), possibly because of larger aerenchyma in the cortex (Ranathunge et al., 2003).

There was a significant position effect on the stele tissue, with higher stele diameter at the RSJ than at the other two positions (10–15 and 6 cm from the RA) in both species but more conspicuously with wheat (Figs. 5 and 6). Although the exact ecophysiological significance of such a gradient in stele tissue is unclear, it could play an important role in maintaining water uptake by improving the internal aeration of roots, particularly near the root tip. The availability of oxygen is known to decrease with increasing depth of the soil, and smaller stele tissue tends to prevent oxygen deficiency to support uninterrupted xylem transport (Gibbs et al., 1998). Furthermore, water uptake by a region close to the root tip appears to be a predominant

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feature of all cereal roots (Greacen et al., 1976). An increased stele diameter near the RSJ in wheat may play a supportive role in water transport rather than in direct water uptake, but smaller stele diameter at the other two positions near the RA (Fig. 5) could help in maintaining water uptake under stress. Contrary to this, stele diameter did not differ significantly under water deficit in rice, but increasing trends of SD:RD were documented across all three positions along the root (Fig. 6). A similar increase in SD:RD under water deficit stress was identified previously in rice (Henry et al., 2012). The above response demonstrates the attempt of rice to reduce radial distance under water deficit stress by decreasing cortex width (i.e. an increase in SD:RD without changing stele diameter) to improve radial hydraulic conductance (for a pictorial representation of radial distance in rice and wheat, see Fig. 6D). Together, the observed variation in root diameter under water deficit in wheat was mostly due to a change in stele diameter, but in rice it was due to variation in cortex width (Fig. 4). Both wheat cultivars and the tolerant rice 'N22' maintained higher stele diameter, substantiating its role in water deficit stress adaptation.

Xylem Developmental Plasticity Was More Responsive to Water Deficit Stress in Wheat Than in Rice

The development of LMXD and LMXN varied strongly along the root length in wheat, with lower xylem diameter but a higher number near the RSJ and higher diameter but a lower number at 10 to 15 cm and 6 cm from the RA. Unlike in wheat, xylem diameter and number were least affected by either water deficit stress or along the three different positions in rice roots (Fig. 7). Bulk flow of water or axial conductance is known to be closely related to the cross-sectional area or diameter of xylem vessels (Niklas, 1985). Hence, wheat cultivars would have higher water uptake than rice cultivars because of higher xylem diameter (Fig. 7), which was confirmed with calculated axial conductance (Fig. 8). Recently, it has been hypothesized that combining low axial conductance (narrow xylem diameter) at the base of the root system (i.e. closer to the RSJ) with higher axial conductivity (higher xylem diameter) near the root tips in deeper soil facilitates effective water use until flowering and grain development (Wasson et al., 2012). This is a pilot report showing such a developmental gradient in xylem diameter along the root length in both wheat cultivars, and it was confirmed with calculated axial hydraulic conductance. A large proportion of the lateral roots generally are developed toward the RSJ part compared with the root tip (Bramley et al., 2009). Therefore, under water deficit increase in LMXN near the RSJ can help increase the uptake of water by lateral roots from the top soil layers but strongly decrease it at the root tip (6 cm from the RA) to conserve moisture in lower soil profiles. In summary, the response of xylem diameter and number to water deficit stress in wheat was a novel finding, and this provides additional mechanistic understanding of wheat root plasticity toward adapting to water deficit stress.

CONCLUSION

A comprehensive analysis of two diverged species, one adapted to flooded conditions and the other to

Table I. *Summary of adaptive changes in key morphological, physiological, and root anatomical traits in response to water deficit stress of tolerant rice and wheat cultivars*

Symbols are as follows: =, no change; +, increase; −, reduction in water deficit (+/−, less than 10%; ++/−−, more than 10% to less than 20%; +++/−−−, more than 20% to less than 35%; and ++++/−−−−, more than 35%–50%).

Parameter	Water Deficit Adaptive Traits	Root Tissue Position	Rice		Wheat	
			'Apo'	'N22'	'SeriM82'	'Weebill4'
Morphology						
Shoot	Tiller no.		=	=	−−−	−−−
	Specific leaf area		=	=	−−	−−
Root	Root length density		=	−−−−	−−−−	−−−−
	SRL		+++	=	−−−−	−−−−
	Average root thickness		−−	=	+++	++++
	Total root weight density		−−	=	++++	++++
Physiology	Whole-plant WUE		++	=	++++	++++
	$\Delta^{13}C$		−	−	−	−
Nodal root anatomy	Stele diameter	RSJ	=	=	++++	+++
		10 to 15 cm from the RA	=	=	−−−	−−
		6 cm from the RA	=	=	−−	=
	LMXD	RSJ	=	=	=	=
		10 to 15 cm from the RA	=	=	−−−	=
		6 cm from the RA	=	=	+++	++++
	LMXN	RSJ	+++	+++	++++	+++
		10 to 15 cm from the RA	=	=	−−	−−−−
		6 cm from the RA	=	=	−−−−	−−−−

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aerobic conditions, allowed us to demonstrate the functional role of organ/tissue plasticity for adapting to water deficit stress. Both wheat cultivars had thicker leaves and roots and moderate tillering that help conserve soil moisture during vegetative-stage water-deficit stress (Table I). Plasticity in stele and xylem diameter and xylem number along the root length in wheat cultivars facilitates the efficient use of available moisture under water deficit stress. Therefore, future studies should aim toward establishing the relationship between root morphology, anatomy with yield, and yield components under water deficit conditions.

MATERIALS AND METHODS

Plant Materials and Growth Conditions

Greenhouse and controlled-environment experiments were conducted to compare the vegetative-stage water deficit stress response of rice (*Oryza sativa*) and wheat (*Triticum aestivum*) with emphasis on root morphological and anatomical plasticity. Three rice cultivars, rice 'IR64' (susceptible to water deficit stress), rice 'Apo' (aerobic/water deficit tolerant), and rice 'N22' (water deficit and high-temperature tolerant), were chosen for our study based on previous reports (Liu et al., 2006; Jagadish et al., 2011; Rang et al., 2011; Venuprasad et al., 2012). The two wheat cultivars selected were wheat 'SeriM82,' which is moderately susceptible (Pfeiffer, 1988) to tolerant of water-limited conditions (Villareal et al., 1995), and wheat 'Weebill4,' a highly drought-tolerant check cultivar (Reynolds et al., 2007; Praba et al., 2009). Dormancy of rice seeds was broken after exposure to 50°C for 3 d, and pregerminated seeds were sown in white-painted pots (55 cm long and 15-cm diameter) as recommended by Poorter et al. (2012) to minimize the confounding effects of increasing temperature of pot surface and soil. The pots were filled with 11 kg of clay loam soil and maintained under natural greenhouse conditions at the IRRI during the 2012 wet season (i.e. during the season when temperature in the greenhouse and pot can be controlled best). Each pot was drilled with holes on either side at the bottom for imposing controlled water deficit stress and lined with polythene covers to facilitate easier separation of roots from soil at the end of the treatment. Simultaneously, wheat seeds were directly sown in pots with the same dimensions and maintained in controlled-environment large walk-in chambers (10.6-m^2 area), built as an extension to the greenhouse where the rice plants were maintained. The chambers were maintained at day/night temperatures of 21°C/18°C, 60% to 70% relative humidity, 16-h/8-h light/dark cycle, and light at 650 μmol m^{-2} s^{-1}, following Praba et al. (2009). Across both cereals and the treatments imposed, three replications were maintained and placed in a completely randomized design.

Water Deficit Stress Imposition, Cumulative Water Transpiration, and Whole-Plant WUE

Both rice and wheat plants were maintained at two moisture regimes: control at 100% field capacity that is the maximum soil moisture content after drainage of excess water, resembling an aerobic condition; and water deficit stress at 55% to 60% field capacity. Water deficit stress was imposed after seedling establishment (i.e. 15 d after seedling emergence), before which all the pots were maintained uniformly at 100% field capacity. Pots with the control treatment were maintained at 100% field capacity throughout the experiment, while water deficit stress was imposed by unplugging the stoppers at the bottom of the pots. A standardized gravimetric approach of daily pot weighing (Raju et al., 2014) was followed to gradually attain 55% to 60% field capacity and thereafter maintained at the same level until the end of the experiment (for details, see Supplemental Fig. S3). Once the target stress level was reached, daily consumed water due to transpiration was replenished by adding an exact amount of water to bring back the moisture content to the desired target in each pot. The soil surface was covered with a circular polythene sheet to protect from direct evaporative loss of water, and a slit across the radius of the polythene sheet prevented heat buildup on the soil surface. In addition, a set of filled pots without a plant was also maintained to correct for evaporative loss of water from the opening created by the slit in the circle-shaped polythene sheet. Daily pot weights recorded for 30 consecutive days of the stress period were used to calculate the daily evapotranspiration. After correcting for evaporative loss from empty pots, actual transpiration was calculated. Finally, daily actual transpiration was summed for the 30-d period to calculate cumulative water transpired. Whole-plant WUE (g kg^{-1}) was calculated as the ratio of total biomass (root and shoot) to cumulative water transpired.

Shoot Morphology

Following 30 d of stress, plants were harvested 45 d after sowing, tiller numbers were counted, and total leaf area was estimated by a leaf area meter (LI-3000; LI-COR). Leaves and stems were separately oven dried at 70°C for 72 h to compute specific leaf area and shoot biomass.

Leaf $\Delta^{13}C$

Top-most fully expanded leaves from four to five tillers per plant were collected from control and water deficit-stressed pots immediately before relieving stress separately for three replications, then oven dried and ground to fine powder. Samples were analyzed for carbon isotope composition by a stable isotope ratio mass spectrometer facility available in the analytical service laboratory of the IRRI (http://asl.irri.org/lims/). The analytical precision of the samples was within 0.1%. Furthermore, the $\Delta^{13}C$ value was calculated relative to the atmospheric carbon isotope composition as follows (Farquhar et al., 1989):

$$\Delta^{13}C = \frac{\delta^{13}C_a - \delta^{13}C_p}{1+\delta^{13}C_p/1000}$$

where $\delta^{13}C_a$ and $\delta^{13}C_p$ denote the carbon isotope compositions of atmosphere (−8‰) and leaf sample, respectively.

Root Sample Processing

The entire column of soil along with the roots was placed on a 1-mm sieve and meticulously washed using a gentle stream of water to minimize the loss of small roots and root hairs. The rice root system is mainly composed of nodal roots and only one radicle or seminal root (primary root), with the latter growing to a maximum length of 15 cm and being viable until the seven-leaf stage. On the contrary, wheat develops and maintains several seminal roots until maturity (Yoshida and Hasegawa, 1982). To make a meaningful comparison between rice and wheat, the nodal root was investigated in our study. Across both rice and wheat cultivars, three replicate root sections (2–3 cm) were collected from three different positions along the nodal root for root anatomy study: (1) near the RSJ; (2) approximately 15 cm from the RA from water deficit-stressed samples and approximately 10 cm from the RA on control samples, following Henry et al. (2012); and (3) at 6 cm from the RA in both treatments (Fig. 3A). Collected samples were stored in 40% (v/v) alcohol to study root anatomy. The remaining whole-plant root samples were placed in 20% (v/v) alcohol and stored at 4°C for root scanning and image analysis.

Root Image Acquisition and Root Morphology Traits

Root samples stored in 20% (v/v) alcohol were cut meticulously to fit the scanner tray and aligned vertically on plates to avoid overlapping. An 8-bit grayscale image was acquired by scanning with an Epson Perfection 7000 scanner at a resolution of 600 dots per inch next to a ruler. After capturing the image, root samples were oven dried at 70°C for 72 h to record the total root biomass. Morphological attributes such as total root length, average root thickness, and root volume were computed by analyzing images with WinRHIZO Reg 2012b software (http://www.regent.qc.ca/assets/winrhizo_software.html). To avoid underestimation of fine root lengths during image processing, the threshold pixel was adjusted to automatic mode (Kato et al., 2010; Kato and Okami, 2011).

Derived Shoot and Root Growth Parameters

LWR, SWR, and root weight ratio were calculated as ratios of leaf, stem, and root weight to total biomass. Average specific leaf area was calculated as the ratio of total leaf area to leaf dry weight. Root length density was calculated as the ratio of total root length to the volume of soil in the pot, and total root weight density was calculated as the ratio of root length density to root

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biomass. Specific root length was calculated as the ratio of total root length to root biomass.

Root Anatomy

To investigate root anatomical features, samples stored in 40% (v/v) ethanol obtained from three different positions along the root (Fig. 3A) were hand sectioned with a razor blade using a dissection microscope. Root sections were stained with 0.5% (w/v) phloroglucinol in water followed by 20% (v/v) HCl (Jensen, 1962) for lignin staining. Images of the root sections were acquired with a Zeiss Axioplan 2 compound microscope with 50× and 100× magnification. At least three to five root images per replicate and tissue position were considered for measuring anatomical traits such as root cross section diameter, stele diameter, LMXD, and sclerenchyma with ImageJ software (for details, see Abramoff et al., 2004). A schematic sketch of the different root anatomical traits measured using ImageJ is provided in Figure 3B.

Theoretically Calculated Axial Conductance

If the number of xylem vessels is n, their overall theoretical axial conductance (K_h; mg m MPa^{-1} s^{-1}) was calculated with the modified Hagen-Poisseuille's law described by Tyree and Ewers (1991) and Tombesi et al. (2010):

$$K_h = \frac{\pi\rho}{128\eta}\sum_{i=1}^{n} d_i^4$$

where d_i is the radius of the ith vessel in meters, ρ is the fluid density (assumed to be 1×10^9 mg m^{-3}), and η is the viscosity (assumed to be 1×10^{-9} MPs·s).

Statistical Analysis

The shoot and root morphological data were analyzed to check the significance level through ANOVA in GenStat Release13 (https://www.vsni.co.uk/genstat), with cultivar and treatment as main factors. However, for root anatomy data, root tissue position was included in the analysis as a factor along with cultivar and treatment.

Supplemental Data

The following supplemental materials are available.

Supplemental Figure S1. Maximum root length of rice and wheat cultivars.

Supplemental Figure S2. Variation in the sclerenchyma layer of rice and wheat cultivars.

Supplemental Figure S3. Rate of water depletion in percentage field capacity.

Supplemental Table S1. ANOVA for shoot morphological-, physiological-, and biomass-partitioning traits.

Supplemental Table S2. ANOVA for root morphological attributes.

Supplemental Table S3. ANOVA for root anatomical traits.

Received November 8, 2014; accepted January 16, 2015; published January 22, 2015.

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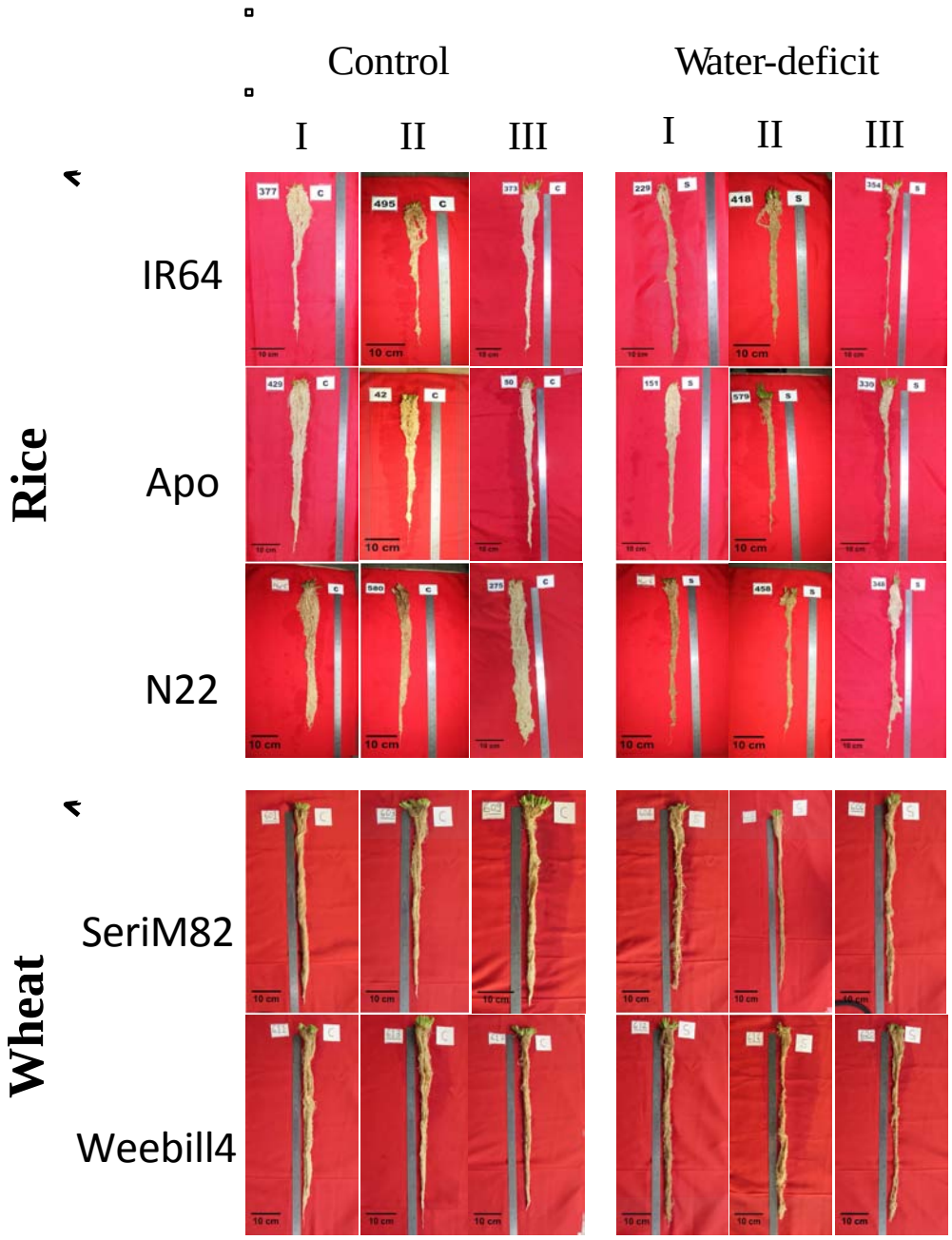
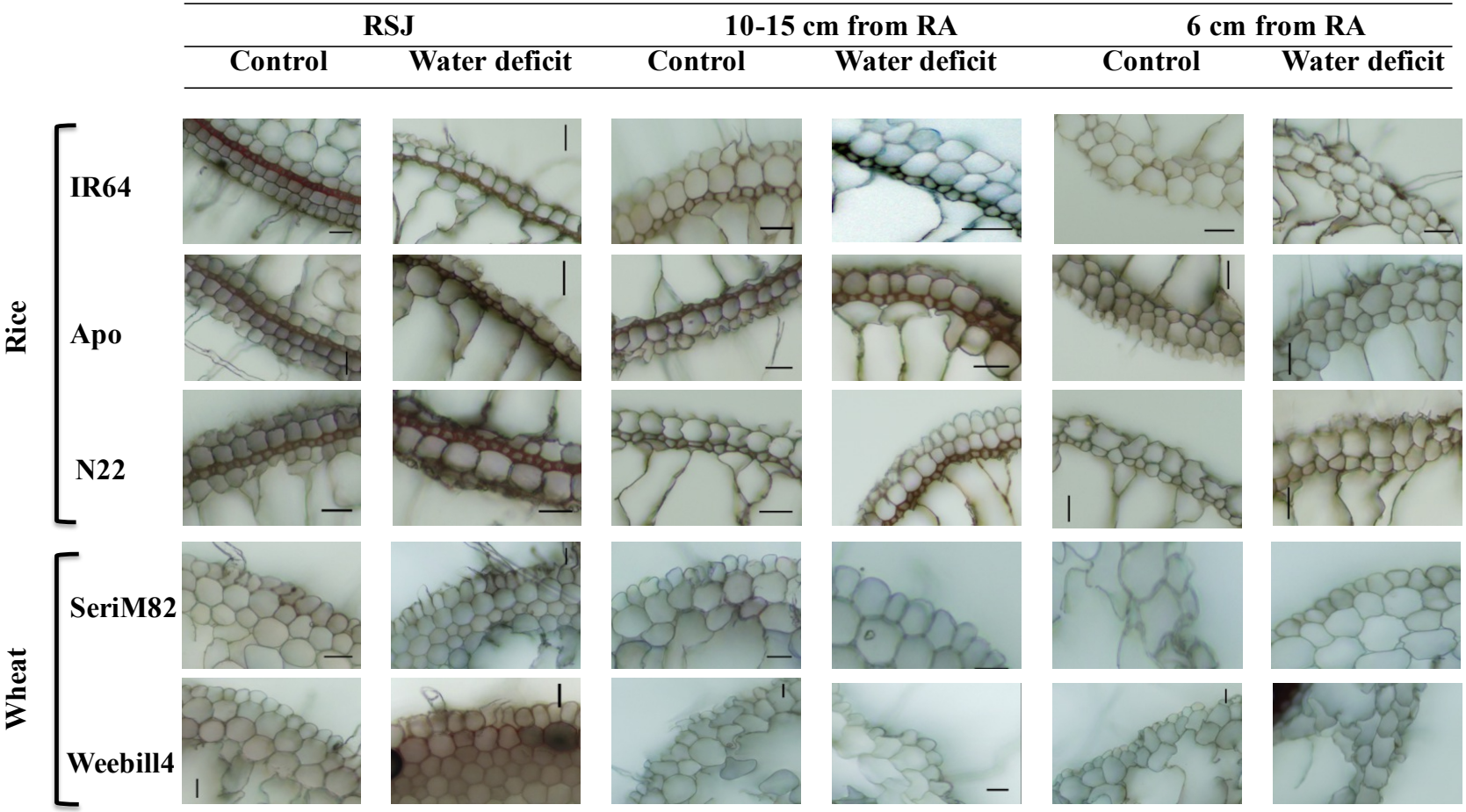
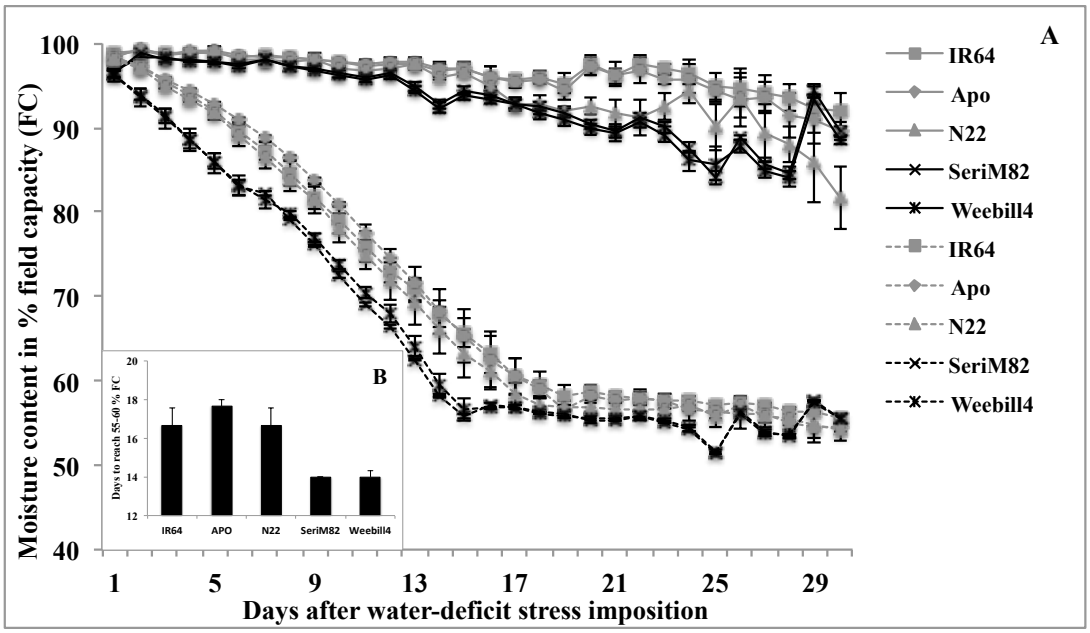


Supplementary Figure S1: Maximum root length (MRL) of rice (IR64, Apo, and N22) and wheat (SeriM82 and Weebill4) cultivars under control and water-deficit stress. Figure consists of replicated images (n=3). Scale bar on each image represents=10 cm.



Supplementary Figure S2: Variation in sclerenchyma layer in different root zones of rice and wheat in control and water-deficit conditions. Scale bar on each image represents=30μm.



Supplementary Figure S3: rate of water depletion calculated based on pot weighing data for both rice (IR64, Apo, and N22) and wheat (SeriM82 and Weebill4) cultivars and expressed as moisture content in % field capacity **(A)**. Number of days taken after stress imposition to reach the desired water-deficit stress expressed in % field capacity (FC) for both rice and wheat cultivars **(B)**. Compared to rice, wheat cultivars reached the target-water-deficit stress (55-60 % FC) 3 days earlier but no cultivar differences were observed in either species.

Supplementary Table S1: Tiller number, total biomass, cumulative water transpiration, and biomass partitioning to various organs (leaf, stem, and root) of rice and wheat cultivars in control and water-deficit conditions. Values in table represent mean±SE and analysis of variance result with least significant difference (LSD) for each trait.

Species	Cultivar	Treatment	TLA	TN	TB	CWT	LWR	SWR	RWR
Rice	**IR64**	Control	1282±178	19.67±4.48	10.50±1.98	3.49±0.60	0.495±0.008	0.408±0.015	0.097±0.010
	IR64	Water-deficit	855±149	18.67±1.45	8.36±1.06	2.15±0.35	0.416±0.012	0.505±0.010	0.079±0.002
	N22	Control	2544±347	21.00±2.52	23.78±2.83	6.35±0.92	0.431±0.027	0.473±0.023	0.096±0.012
	N22	Water-deficit	1120±238	16.67±1.86	10.94±1.90	2.89±0.39	0.423±0.027	0.465±0.011	0.111±0.016
	APO	Control	1451±106	14.33±1.86	13.24±2.00	4.02±0.55	0.502±0.016	0.401±0.009	0.098±0.008
	APO	Water-deficit	1007±188	13.33±1.20	10.02±1.44	2.65±0.47	0.481±0.017	0.433±0.012	0.085±0.007
Wheat	**SeriM82**	Control	2005±254	34.67±2.19	18.45±1.41	6.94±0.36	0.488±0.048	0.364±0.033	0.147±0.016
	SeriM82	Water-deficit	1077±68	23.67±2.33	12.50±0.01	3.32±0.10	0.475±0.016	0.365±0.013	0.160±0.004
	Weebill4	Control	2261±26	29.67±1.20	19.55±0.84	7.55±0.55	0.543±0.016	0.313±0.006	0.143±0.010
	Weebill4	Water-deficit	1085±121	18.33±2.40	12.03±1.04	3.30±0.26	0.499±0.008	0.347±0.006	0.155±0.014
LSD ($P < 0.05$)									
Rice		**Cultivar (C)**	297.0^{***}	4.417^{*}	2.721^{***}	0.838^{**}	0.031^{**}	0.028^{**}	0.018^{ns}
		Treatment (T)	242.5^{***}	3.606^{ns}	2.222^{***}	0.684^{***}	0.026^{*}	0.023^{**}	0.015^{ns}
		C×T	420.0^{**}	6.246^{ns}	3.849^{**}	1.185^{*}	0.045^{ns}	0.040^{**}	0.026^{ns}
Wheat		**Cultivar (C)**	380.1^{ns}	5.840^{ns}	1.681^{ns}	0.690^{ns}	0.067^{ns}	0.047^{ns}	0.026^{ns}
		Treatment (T)	380.1^{**}	5.840^{**}	1.681^{***}	0.690^{***}	0.067^{ns}	0.047^{ns}	0.026^{ns}
		C×T	537.5^{ns}	8.260^{ns}	2.377^{ns}	0.976^{ns}	0.095^{ns}	0.067^{ns}	0.036^{ns}

TLA=total leaf area (cm^2 $plant^{-1}$), **TN**=tiller number per plant, **TB**=total biomass (g $plant^{-1}$), **CWT**=cumulative water transpiration (kg $plant^{-1}$), Δ^{13}**C**=carbon isotope discrimination, **LWR**=leaf weight ratio [g (leaf) g^{-1} (plant)], **SWR**=stem weight ratio [g (stem) g^{-1} (plant)], **RWR**=root weight ratio [g (root) g^{-1} (plant)]. **Significance level:** **P*<0.05, ***P*<0.01, ****P*<0.001, **ns**=non-significant, **SE**=standard error.

Supplementary Table S2: Root morphological attributes of rice and wheat in control and water-deficit conditions. Values in table represent mean±SE and analysis of variance result with least significant difference (LSD) for each trait.

Species	Cultivar	Treatment	MRL	TRL	RLD	RV	RB
Rice	**IR64**	Control	46.83±6.93	0.49±0.19	5.85±2.27	10.46±4.07	1.00±0.19
	IR64	Water-deficit	51.72±6.83	0.46±0.06	5.48±0.69	7.51±0.38	0.66±0.10
	N22	Control	53.3±1.21	0.97±0.31	11.7±3.78	22.94±5.37	2.35±0.53
	N22	Water-deficit	55.03±3.49	0.57±0.22	6.83±2.70	11.98±3.01	1.24±0.31
	APO	Control	52.6±5.83	0.49±0.10	5.95±1.20	15.47±2.48	1.31±0.23
	APO	Water-deficit	51.73±2.61	0.42±0.11	5.12±1.36	8.64±1.88	0.85±0.14
Wheat	**SeriM82**	Control	55.67±3.18	1.26±0.14	15.11±1.63	37.41±4.37	2.72±0.38
	SeriM82	Water-deficit	64.33±0.88	0.52±0.03	06.21±0.35	26.15±2.75	2.00±0.04
	Weebill4	Control	61±2.08	1.12±0.05	13.52±0.62	33.35±2.55	2.81±0.32
	Weebill4	Water-deficit	64±2.65	0.48±0.04	05.78±0.46	22.27±2.11	1.86±0.24
LSD (*P*<0.05)							
Rice		**Cultivar (C)**	6.60^{ns}	0.286^{ns}	3.440^{ns}	5.052^{*}	0.486^{**}
		Treatment (T)	5.39^{ns}	0.233^{ns}	2.809^{ns}	4.125^{**}	0.397^{**}
		C×T	9.34^{ns}	0.404^{ns}	4.865^{ns}	7.144^{ns}	0.687^{ns}
Wheat		**Cultivar (C)**	5.12^{ns}	0.172^{ns}	2.065^{ns}	5.550^{ns}	0.472^{ns}
		Treatment (T)	5.12^{*}	0.172^{***}	2.065^{***}	5.550^{**}	0.472^{**}
		C×T	7.24^{ns}	0.243^{ns}	2.920^{ns}	7.840^{ns}	0.667^{ns}

MRL=maximum root length (cm)**, TRL**=total root length (km plant^{-1}), **RLD**=root length density (cm cm^{3}), **RV**=root volume (cm^{3}), **RB**=root biomass (g plant^{-1})**. Significance level:** **P*<0.05, ***P*<0.01, ****P*<0.001, **ns**=non-significant, **SE**=standard error.

Supplementary Table S3: Analysis of variance results for nodal root anatomical traits in rice and wheat. Values in table represent least significant difference (LSD) for each trait.

Rice	RD	SD	LMXD	LMXN	SD:RD	AC
Cultivar	108.3^{*}	21.11^{***}	4.239^{*}	0.647^{*}	1.262^{ns}	0.0040^{**}
Treatment	088.5^{**}	17.24^{ns}	3.461^{ns}	0.528^{*}	1.030^{***}	0.0033^{*}
Tissue position	108.3^{***}	21.11^{***}	4.239^{ns}	0.647^{**}	1.262^{ns}	0.0040
Cultivar×Treatment	153.2^{ns}	29.86^{ns}	5.995^{ns}	0.914^{ns}	1.785^{ns}	0.0057
Cultivar×Tissue position	187.6^{ns}	36.57^{ns}	7.342^{ns}	1.120^{ns}	2.186^{ns}	0.007
Treatment×Tissue position	153.2^{ns}	29.86^{ns}	5.995^{ns}	0.914^{ns}	1.785^{ns}	0.0057^{*}
Cultivar×Treatment×Tissue position	265.4^{ns}	51.72^{ns}	10.383^{ns}	1.584^{ns}	3.091^{ns}	0.0099
Wheat						
Cultivar	066.5^{***}	22.20^{***}	8.92^{***}	0.664^{ns}	2.122^{ns}	0.062^{***}
Treatment	066.5^{ns}	22.20^{ns}	8.92^{ns}	0.664^{ns}	2.122^{ns}	0.062^{**}
Tissue position	081.4^{***}	27.18^{***}	10.93^{***}	0.814^{***}	2.599^{***}	0.0759^{***}
Cultivar×Treatment	094.0^{ns}	31.39^{ns}	12.62^{ns}	0.940^{ns}	3.001^{ns}	0.0877^{*}
Cultivar×Tissue position	115.1^{ns}	38.44^{ns}	15.45^{*}	1.151^{ns}	3.675^{ns}	0.0074^{**}
Treatment×Tissue position	115.1^{***}	38.44^{***}	15.45^{***}	1.151^{***}	3.675^{*}	0.107^{***}
Cultivar×Treatment×Tissue position	162.0^{ns}	54.37^{ns}	21.85^{ns}	1.627^{ns}	5.197^{ns}	0.152^{*}

RD=root diameter (µm), **SD**=stele diameter (µm), **LMXD**=late metaxylem diameter (µm), **LMXN**=late metaxylem number, **SD:RD**=stele diameter in proportion to root diameter (%), **AC**=axial conductance (mg m MPa^{-1} s^{-1}) . **Significance level:** **P*<0.05, ***P*<0.01, ****P*<0.001, **ns**= non-significant.

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