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Abstract : The role of root signals in water deficit responses of rice (*Oryza sativa* L.) is important in the alternate flooding and drying conditions encountered in the rainfed lowlands, where the abundant roots in shallow soil layers may generate signals when droughted, with consequent reduction in stomatal conductance (g_s) and growth, despite the likelihood of additional water in deeper soil layers. This study was conducted to confirm the presence of root signals, explore their nature and plant responses, consider the suitability of the methods, and discuss implications for adaptation under rainfed lowland drought. A split-root technique was used in greenhouse studies, whereby roots were divided into two sections: flooded and droughted. The decrease in g_s and transpiration rate (Tr) due to drying of a portion of the roots, and their apparent recovery upon severing of this root portion, were consistent with the role for signals. The field study confirmed the evidence for root signals during progressive soil drying, whereby g_s and Tr decreased before leaf water potential (Ψ_L) started to decline. The increase in leaf ABA concentration under field drought, and its strong association with soil moisture tension and g_s, suggested its involvement in mediating stomatal responses during early drought in rice. The recovery in Ψ_L after severing of droughted roots in the greenhouse could be attributed to increased hydraulic conductance. These responses imply a role for both chemical and hydraulic signals in rice, which have important implications for adaptation and crop performance in contrasting rice ecosystems.

Key words : Drought, Rainfed lowland, Rice, Root signals, Stomatal conductance, Water deficit.

Rice (*Oryza sativa* L.) is grown on about 46 million hectares in the rainfed lowlands of South and Southeast Asia (MacLean et al., 2002), where the fields are bunded and with little or no irrigation water. Grain yields in this ecosystem average only 2.3 t ha^{-1}. The root system of the rice crop generally remains shallow (Pantuwan et al., 1997; Samson et al., 2002) and is exposed to both anaerobic flooded and aerobic droughted conditions due to alternate flooding and drying of the soil. This is in contrast with the uplands, where the soil is not flooded and remains mostly aerobic. Additional traits may therefore be needed to cope with the anaerobic-aerobic transitions during drought in the rainfed lowlands (Fukai et al.,1999a; Wade et al., 1999a). During drying periods, as water is rapidly lost from soil surface layers, plant growth and productivity are restricted through reduced availability of water and nutrients (Otoo et al., 1989; Fukai et al., 1995, 1999b; Wade et al., 1998). In such situations, root signals may be important for regulating stomatal closure and water loss, and may even be stronger than in other ecosystems due to the existence of a large number of root tips in shallow layers of surface soil.

During water stress, stomata respond to hydraulic as well as chemical signals (Pospíšilová, 2003). Accumulated evidence suggests that both chemical and hydraulic signals are operative and integrated in the regulation of leaf growth and stomatal conductance when plants are grown under drought stress (Davies et al., 1994; Comstock, 2002). Their relative importance is likely to depend on plant size and growth conditions (Tardieu and Davies, 1993). The hydraulic signals involve changes in pressure potential in the xylem as well as changes in water content of the guard cells and other epidermal cells. On the other hand, abscisic acid (ABA) synthesized in roots under water stress and transported to leaves may act as a root-to-shoot chemical signal of water stress conditions and, together

with ABA synthesized in the leaves themselves, induce stomatal closure (Liang et al.,1997). ABA not only alters stomatal opening but also increases the root hydraulic conductivity (Hose et al., 2002). A common view of the functional mode of the two signal types in controlling leaf expansion growth and stomatal behavior has been proposed: at mild soil water deficits, chemical signals are produced in roots and transported via xylem to shoots where they reduce leaf growth and stomatal conductance (Ali et al., 1998). When soil water deficit becomes more severe, hydraulic signals from the change in hydrostatic pressure play the major role (Liu et al., 2003).

Root tips have been reported to communicate with shoots to regulate stomatal closure as soil dries, even when plant water status is high; this observation is termed root signals (Sharp and Davies, 1979; Davies and Zhang, 1991; Davies et al., 1994). Root tips appear to respond to soil drying by increasing the biosynthesis of ABA (Zhang and Davies, 1989; Hartung et al., 2002; Hose et al., 2002). Root signals were reviewed by Comstock (2002), who concluded that ABA, transported in the xylem from root to shoot and perceived at the guard cell, is an essential regulating factor for stomatal closure under drought stress. ABA plays an important role in the plant's response to water deficit (Pospíšilová, 2003), as established in recent reviews on changes in ABA content during water deficit, as well as the response of stomata to ABA signalling (Assmann and Armstrong, 1999; Blatt, 2000; Rock, 2000; Ng et al., 2001; Schroeder et al., 2001; Wilkinson and Davies, 2002; Zhang et al., 2006). This signalling mechanism provides the plant with a stomatal response system in which water potential is perceived in the root as a primary indication of soil water status. ABA is thought to have an important role in root-to-shoot communication (Zhang and Davies, 1990), although questions still arise as to whether ABA in the xylem stream is sufficient to affect stomatal behavior in drying soils. Hartung et al. (2002) observed that ABA is derived to a significant extent from synthesis in the root tissues and that the greatest accumulation is often observed in the root tips as a result of vacuolization of cells with a high percentage of cytosol, a compartment where ABA is formed. ABA is then released into the xylem, and thus, transported to the leaves via the transpiration stream. Consequently, ABA xylem-transported from root tips in drying soil is widely considered to be the dominant factor in regulating stomatal closure under early drought (Sharp et al., 1990; Davies and Zhang, 1991; Sharp, 2002).

Field experiments on wheat subjected to intermittent drought showed that soil surface drying increased both xylem and bulk-leaf abscisic acid content even before a measurable change in leaf water potential (Ψ_L) could be detected in droughted plants (Ali et al.,

1999). More severe drought reduced the leaf water content and transpiration rate (Tr) and increased ABA accumulation in leaves, together with gradual osmotic adjustment and senescence of older leaves. The osmotic adjustment sustained leaf turgor pressure during soil drying. These authors concluded that (1) non-hydraulic root signals resulted in early drought adaptation under mild water stress conditions by reducing leaf growth and stomatal conductance (g_s); and (2) osmotic adjustment sustained turgor during moderate and severe water stress. Although changes in xylem ABA have been considered to act as a chemical signal during the early stages of soil drying (Davies and Zhang, 1991), hydraulic signals from decreases in hydrostatic pressure in xylem tissue, become more significant as soil water deficit increases (Liu et al., 2003). The interaction between g_s and hydraulic conductance regulates leaf water potential, with the decrease in both hydraulic conductance and g_s resulting in cavitation during water stress in rice (Sperry, 2000). These authors observed that much of the stomatal closure during drought was a result of the amplifying effects of declining hydraulic conductance. Most of this decline in hydraulic conductance occurred in the xylem of the root system rather than in the shoots, because roots are more susceptible to cavitation. Rice, for example, becomes more than 50% cavitated, even under well-watered conditions, and much of the drop in its leaf-specific hydraulic conductance during drought can be attributed to xylem cavitation (Stiller et al., 2003). Thus, in well-watered rice, cavitation was found to be occurring on a diurnal basis by exhibiting root pressure and guttation (Sperry et al., 2003). These osmotic pressures have been implicated in the refilling of cavitated vessels in rice (Stiller et al., 2003), and their observations suggest a vital role of root pressure in the maintenance of hydraulic conductance and gas exchange. Nevertheless, as has been argued by Comstock (2002), the pattern of interaction between the two signal types (i.e., chemical and hydraulic) in a wide range of plant species, as well as the course of the responses with time, are still poorly understood.

In rice, Fukai and Cooper (1995) speculated that root signals may be important in the fluctuating water conditions of the rainfed lowlands,, where the abundant roots in the shallow soil layers may send signals when droughted, despite the likelihood of sufficient moisture in deeper soil layers. Some initial evidence in support of this proposal is available from Dingkuhn et al. (1999) who worked with two rice species, *Oryza glaberrima* and *Oryza sativa*, in hydromorphic soils in the uplands. Moreover, in a soil-drying greenhouse experiment conducted on rice, Bano et al. (1993) observed that when the soil surrounding some part of the root system dried out while other roots remained in a moist soil

environment, stomatal conductance decreased but leaf water potential remained unchanged. Consequently, Bano et al. (1993) concluded that the decrease in stomatal conductance could not be attributed to drought stress in the leaf, but was possibly caused by a root-sourced signal transmitted through the xylem. Likewise, Kobata et al. (1994) surmised that sterility in drought-stressed lowland rice "Nipponbare" stressed at the reproductive stage was associated with a chemical transported from roots in drying soils. Kobata and Hara (1994) identified ABA as the chemical exuded from roots which affected stomatal aperture. These authors concluded that stomatal conductance was controlled by a soil-moisture-dependent root signal, that was deemed to be the probable link between soil matric potential and g_s. One aspect that has been largely overlooked is the hydraulic conductance of rice and its drought-induced reduction due to xylem cavitation (Stiller et al., 2003). Exposure to the anaerobic-aerobic transitions in rainfed lowland drought may assist elucidation of the nature of root signals.

In this study, rice cultivars were grown in greenhouse experiments using a split-root system, where some roots were kept well-watered while others were exposed to drying soil. Severing of roots in drying soil was also used to determine if root signals generated from these roots in response to drought were interrupted. A field experiment was also conducted to assess the presence of root signals in actual rainfed lowland field conditions characterized by cycles of flooding and soil drying. Physiological parameters such as leaf water potential, stomatal conductance and transpiration rate were monitored daily in progressively drying soil with gradually increasing soil matric tension. Consequently, this paper aims to (1) provide evidence confirming the presence of root signals during rainfed lowland drought, (2) explore the nature of these root signals and the responses in stomatal conductance, transpiration rate and leaf water potential, (3) consider the suitability of the methods adopted for examining these responses, and (4) discuss the implications for adaptation to rainfed lowland drought. A second paper examines variation between cultivars in these responses.

Materials and Methods

1. Greenhouse experiment I
(1) Cultural details
A split-root experiment was conducted between February 20 and April 9, 2001 in a greenhouse at the International Rice Research Institute, Los Baños, Philippines (14° 11' N, 121° 15' E, 23 m altitude). A polyvinyl chloride (PVC) pot with a 20-cm inner diameter and 55-cm height was used as an outer container, inside which a PVC pipe with a 15-cm inner diameter and the same height was placed. Sandy
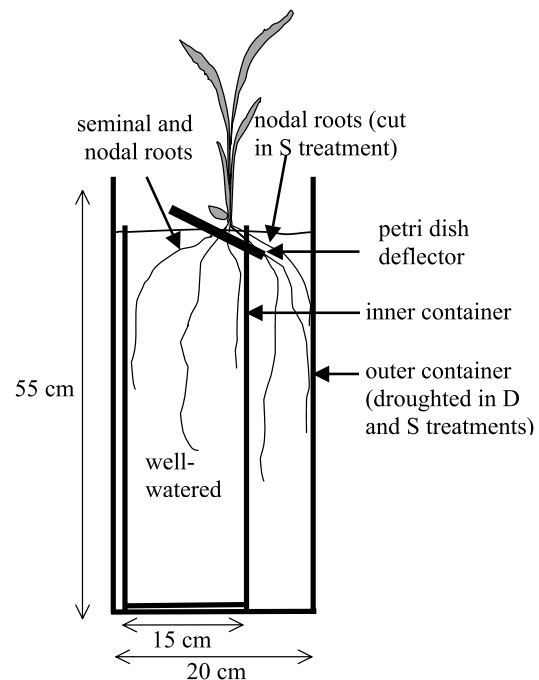

Fig. 1. Cross section of the pot setup used in the first greenhouse split-root experiment at IRRI in 2001. The setup allowed roots to grow either into the inner well-watered container or into the outer container which was subjected to drought in the Droughted (D) and Severed (S) treatments. The Petri dish was used to deflect some of the nodal roots to the outer container.

loam soil (35% clay, pH 5.7) was added to both inner and outer pots to within 5 cm from the top of both containers. The volume of soil in the inner container was one-third greater than that in the outer container. A 150-mm petri dish with a 3-mm diameter hole in the center was placed at a 45° angle at the mouth of the inner pipe to hold a single 8-d-old seedling and to serve as a deflector of nodal roots (Fig.1).

The following treatments were imposed in a randomized complete block design with 5 replicates:
- W, well-watered; watered daily and kept flooded to about 2.5 cm above the soil surface,
- D, droughted; same as W until 19 d after transplanting, after which the outer container was drained and water was withheld thereafter, so that roots in the outer container would be subjected to progressive water deficit, and
- S, severed; the same as D for water regime, but as defined below, nodal roots growing toward the outer container were severed at the base of the tillers during the progressive water deficit.

Roots in treatment S were to be severed after water was withheld, at a time when the W and D treatments did not differ significantly in Ψ_L, but g_s and Tr were significantly lower in treatment D. The D treatment was designed to test whether a response to drought could be observed in Ψ_L, g_s, and Tr, from withholding

water from a portion of the root system, with the hypothesis that any change would result from a positive signal from root tips in drying soil. The S treatment then tested whether severing the portion of roots subjected to drought in the outer container would result in any recovery in Ψ_L, g_s, and/or Tr, with the hypothesis that severing of roots in drying soil would remove the source of the positive signal from root tips.

The lowland *indica* rice line, IR62266-42-6-2 (IR62266), was used in this experiment, as it performs well when subjected to drought in the fluctuating water (flood-drought) environment of the rainfed lowlands (Wade et al., 1999b), and is noted for its more gradual water extraction from depth and its greater ability for osmotic adjustment (Kamoshita et al., 2000, 2004). The seminal root of each pre-germinated seed was carefully inserted through the hole in the petri dish of each pot, allowing the root system to be split, with the seminal and some nodal roots growing through the hole and into the inner pot, and the remaining nodal roots being deflected by the petri dish to the outer container (Fig. 1). The D and S treatments were imposed on the outer container, while the inner container in D and S, and both containers in W, were kept flooded throughout the experiment. No disease or insect damage was observed during the experiment and the distance between neighboring pots was kept at 40 cm to minimize mutual shading. NPK was applied respectively, as, 2.73 g of urea, 1.84 g of solophos, and 1.04 g of muriate of potash, to each pot.

(2)   Meteorological data

Daily minimum and maximum air temperatures were recorded using a hygrothermograph and evaporation was measured with 7 pan evaporimeters each 20-cm in diameter, randomly placed inside the greenhouse. The average daily minimum and maximum air temperatures during the experiment were 27.9°C and 36.3°C, respectively, and average pan evaporation was 4.9 mm d^{-1}.

(3)   Physiological measurements and plant sampling

Leaf stomatal conductance, instantaneous Tr and Ψ_L were monitored daily from the day water was withheld in the drought treatment. The g_s and Tr of the second-youngest fully-expanded leaf were measured at 1000 h under similar light and humidity conditions on both the abaxial and adaxial sides of the leaf using a Li-Cor 1600 steady state porometer (Li-Cor Corporation, Lincoln, NE, USA). The same leaf used for g_s and Tr measurements was detached and used to measure Ψ_L using a Scholander pressure chamber (Plant water status console, Soil Moisture Equipment Corporation, Santa Barbara, CA, USA). Shoots were sampled at the start of withholding water (19 DAS), and then at 32 DAS and 39 DAS. The latter two sampling dates marked the time of severing roots (13 DAWW, days after withholding water) and harvest (20 DAWW). At each sampling, shoots were harvested at the ground
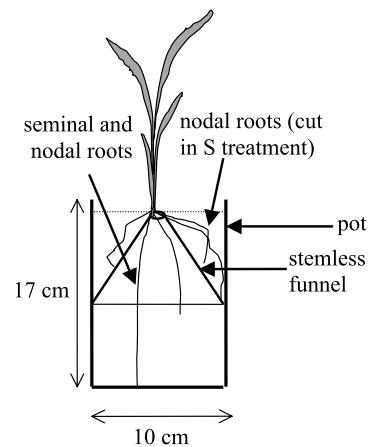

Fig. 2.   Cross section of pot setup used in the second greenhouse split-root experiment at Nagoya, Japan in 2004. The funnel is introduced to split the roots to grow either through the funnel to the submerged lower portion of the container or become confined to the portion of the pot above the funnel which is subjected to drought in the Droughted (D) and Severed (S) treatments.

level, oven-dried at 70°C for 48 h and weighed.

Tr reported here is a relative measure of instantaneous transpiration rate, which is influenced by the chamber size in the steady state porometer, the light intensity, and the leaf-air vapour pressure difference. While this instantaneous Tr is not the same as the actual transpiration rate of the leaf outside of the chamber, instantaneous Tr has often been used as a basis for comparison of cultivars exposed to similar water deficit conditions (Yambao et al., 1992; Dingkuhn et al., 1999; Jia and Zhang, 1999; Angelocci et al., 2004). Instantaneous Tr was a more sensitive measure of differences between cultivars than g_s in the second paper (Siopongco et al., 2008).

2.   Greenhouse experiment II
(1)   Cultural details

A second split-root experiment was conducted from June 21 to July 29, 2004 in a greenhouse at the Graduate School of Bioagricultural Sciences, Nagoya University, Chikusa, Nagoya, Japan (35° 09' N, 136° 56' E). Plastic pots, 9.5 cm in diameter and 16.5 cm in height, were used. Inverted funnels with stems removed were placed inside these containers (Fig. 2), and river sand (pH 6.0) was added to each pot, both below and above the funnel. Single 4-d-old pregerminated IR62266 seeds were placed on the top of the funnel with the seminal root inserted in the funnel hole. The seminal root and some nodal roots were allowed to grow through the hole and into the lower core of sand while the remaining nodal roots were deflected by the funnel to grow in the upper part of the pot (Fig. 2).

A completely randomized design with four

Table 1. Mean daily weather data and number of non-rainy days during the two drought periods in the field experiment.

Drought duration (DAS)	Maximum temperature (°C)	Minimum temperature (°C)	Evaporation (mm d⁻¹)	Irradiance (MJ m⁻² d⁻¹)	Rainfall (mm d⁻¹)	Non-rainy days (number)
41 to 68	30.8	24.0	2.9	14.2	3.7	10 (0.4)*
69 to 97	30.9	23.4	3.3	15.4	2.5	14 (0.5)

*, Numbers in parentheses indicate ratio of non-rainy to rainy days.

replications was used with the following treatments imposed: well-watered (W), watered daily with full-strength Hoagland solution so the sand surface was kept continuously saturated; drought (D), the same as W until 29 d after transplanting, after which the solution level was lowered to just below the level of the funnel, thereby withholding water from the roots above the funnel; and severed (S), the same as D in water regime, but the nodal roots growing above the funnel were severed at the base of the tillers 2 d after withholding water. Nodal roots were again severed when W and D treatments did not differ significantly in Ψ_L, but g_s and Tr were significantly lower in treatment D.

(2) Meteorological data

The minimum and maximum daily air temperatures and relative humidity were recorded with a hygrothermograph. The average daily minimum and maximum air temperatures during the experiment were 25.1°C and 39.1°C, respectively, and the average relative humidity was 62%.

(3) Physiological measurements and plant sampling

The changes in g_s, Tr and Ψ_L were monitored daily from the day water was withheld. The g_s and Tr of the second-youngest fully-expanded leaf were measured at 1000 h using a Li-Cor 6200 portable photosynthesis system. Leaf water potential was measured on the same leaf used for g_s and Tr measurements using a leaf cutter psychrometer (J. R. D. Merrill Specialty Equipment, Logan, Utah, USA), and the output was read using a microvoltmeter (model HR-33T, Wescor Inc. Logan, Utah, USA). Shoots were sampled at the start of withholding water (28 DAS), and at 30 DAS and 32 DAS. The latter two sampling dates represented the time of severing roots (2 DAWW) and harvest (4 DAWW).

3. Field experiment

(1) Cultural details

This experiment was conducted at the International Rice Research Institute, Los Baños, Laguna, Philippines, during the wet season of 2002. The land was prepared by multiple plowing, rotovation and harrowing. Bunds of 15 cm height and 25 cm width were constructed around each 5 m by 5 m plot. Twenty-five-cm deep and 40-cm wide canals were dug between neighboring bunds to prevent water from moving between plots. Molluscicide (Bayluscide a.i.

ethanolamine salt of niclosamide) and pre-emergence herbicide (Machete a.i. butachlor) were applied before sowing. A split-split plot design with 4 replications was used, with two drought timings as main plots, (41 to 68 DAS and 69 to 97 DAS); two water regimes as subplots, (well-watered, with water maintained at 5 cm depth, and droughted with irrigation and rainfall excluded in the specified periods); and two entries as sub-subplots, (the upland *japonica* line CT9993-5-10-1-M [CT9993] and IR62266). CT9993 is reported to have a deeper root system (Azhiri-Sigari et al., 2000; Wade et al., 2000), and is specifically adapted to particular drought situations in the rainfed lowlands (Wade et al., 1999b). Three-day-old pregerminated seeds of both entries were sown on 25 September 2002, at a 25 cm by 25 cm spacing. Removable rainout shelters were installed over the plots in which water was to be withheld, as described by Regmi (1995).

(2) Meteorological and field hydrological data

Rainfall, solar radiation and maximum and minimum temperatures were monitored in a nearby weather station located at about 600 m from the experimental plots (Table 1). The second drought-stress period was during relatively hotter and drier conditions than the first drought-stress period. Free water table was monitored daily with piezometers inserted to a depth of 80 cm within each plot, and daily soil moisture tension was monitored by mercury tensiometers placed at 10 and 40 cm depths in each plot. The piezometers and tensiometers were fabricated, installed and monitored using the protocol described by O'Toole and Maguling (1981).

(3) Physiological measurements

Daily g_s and Tr of the second-youngest fully-expanded leaf were measured using a Li-Cor 1600 porometer starting at 1000 h. The same leaf was used to measure Ψ_L using a Scholander pressure chamber as described previously. For ABA analysis, leaves were sampled at 1000 h, placed in aluminum foil, then immediately dipped in liquid N and stored at −80°C until analyzed. Extraction and purification were performed following the protocol of Dunlap and Guinn (1989). Samples were then analyzed using an HPLC consisting of an LC 10AT HPLC pump, SIL-10AD auto-injector, a CTO-10Avp column oven and an SPD-10 Avp detector (Shimadzu Corporation, Kyoto, Japan).
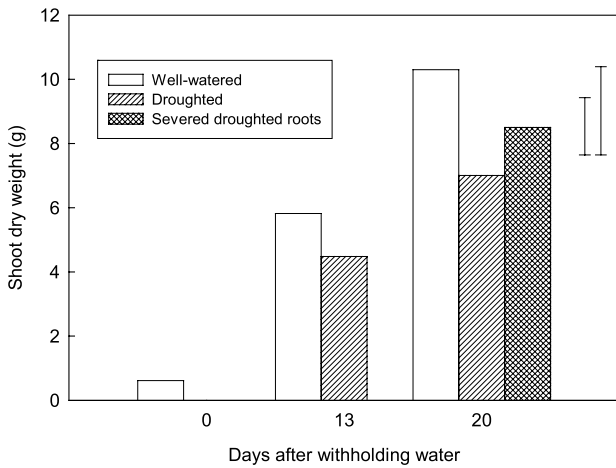
Fig. 3. Shoot dry weight (g plant[-1]) of IR62266 measured at of 0, 13 and 20 d after withholding water in greenhouse experiment I at IRRI in 2001. Short and long vertical bars indicate pooled $LSD_{0.05}$ and pooled $LSD_{0.01}$, respectively.

4. Statistical analysis

Analysis of variance was conducted using the designs specified above for each experiment using Systat 7.0 (SPSS, 1997), and $LSD_{0.05}$ and $LSD_{0.01}$ were calculated, for comparing treatments and their interactions at each sampling date, using the Tukey method. Pooled $LSD_{0.05}$ and pooled $LSD_{0.01}$ were presented in each figure as short and long vertical bars, respectively, which allowed more rigorous comparisons across treatments and their interactions at each sampling date, because they took account of the magnitude of the variation from one sampling date to another. Pooled LSD was calculated as the square root of the sum of squares of the individual LSDs divided by the number of cases: Pooled LSD = SQRT [SUM (LSD^2) /N] Mean values were graphed using SigmaPlot 2002 (SPSS, 2001).

Results

1. Greenhouse experiments

(1) Effects of water stress on biomass production

In the first greenhouse experiment using rice cultivar IR62266, the shoot dry weight in the D treatment was lower than that in the well-watered treatment by 13 DAWW, but the difference was not significant (Fig. 3). At 20 DAWW, shoot dry weight in D was significantly lower than that in W. Severing of roots in the S treatment resulted in a dry weight intermediate between the values in D and W at 20 DAWW. No significant differences in shoot fresh weight, tiller number or plant height were observed in the second greenhouse experiment (data not shown), which was terminated just 2 d after root excision and 4 d after withholding water.

(2) Effects of drought on g_s, Tr and Ψ_L

The Ψ_L in the drought treatment was significantly lower than in W throughout the first greenhouse
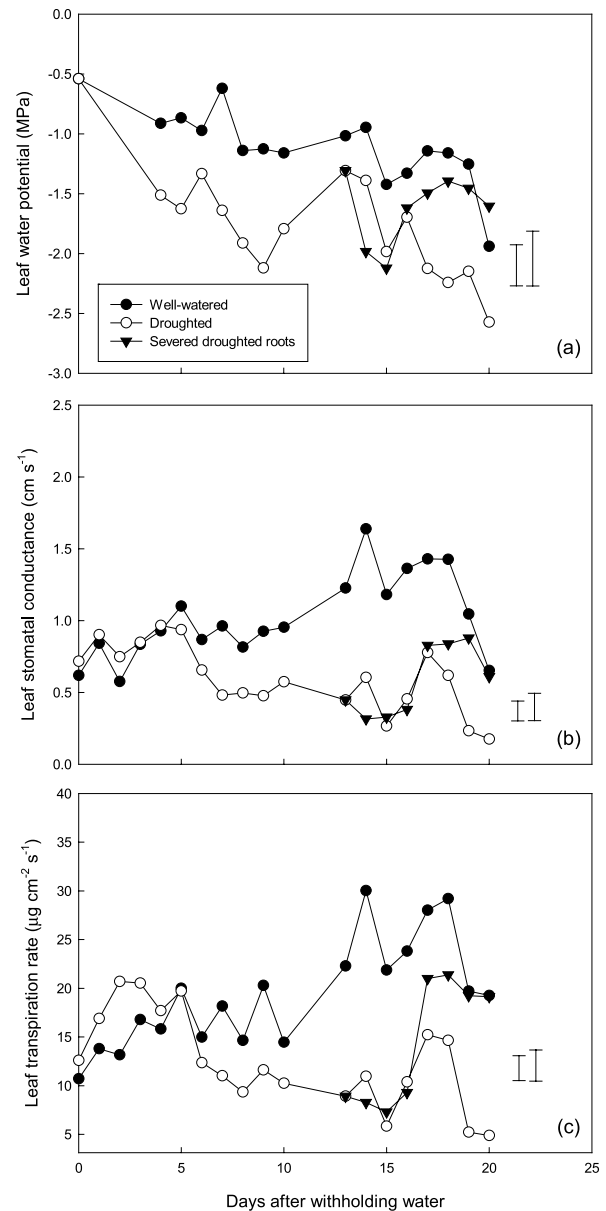

Fig. 4. (a) Leaf water potential, (b) stomatal conductance and (c) transpiration rate of IR62266 in response to drought imposed in outer container at 20 DAS in greenhouse experiment I at IRRI in 2001. Short and long vertical bars indicate pooled $LSD_{0.05}$ and pooled $LSD_{0.01}$, respectively.

experiment, except at day 13, where it was only 0.4 MPa less than in W (Fig. 4a). By about 5 to 7 DAWW, both g_s and Tr decreased significantly in D, and with the difference progressively widening with time as drought progressed (Fig. 4b,c). Following drainage, Tr was initially slightly greater in D, and significantly so at about 2 d after withholding water (Fig. 4c). Nodal roots were severed in the outer container in treatment S on day 13, when the difference in Ψ_L between W and D was small and not statistically significant. With severing, Ψ_L and g_s dropped significantly the next day (day 14) in S, before both of them and Tr recovered to
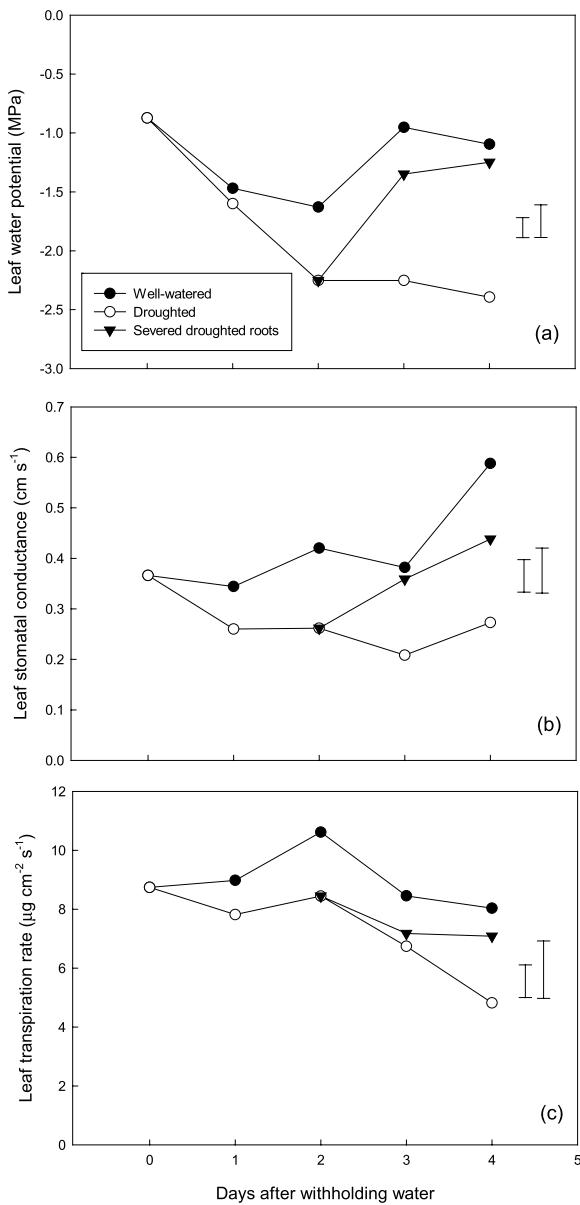
Fig. 5.　(a) Leaf water potential (b) stomatal conductance and (c) transpiration rate of IR62266 in response to drought imposed on roots growing above the funnel at 29 DAS in greenhouse experiment II at Nagoya, Japan in 2004. Short and long vertical bars indicate pooled $LSD_{0.05}$ and pooled $LSD_{0.01}$, respectively.

the same level as of the well-watered treatment at day 20. In contrast, all three parameters in D continued to decline significantly until day 20. In the well-watered treatment, however, values of Ψ_L, g_s, and Tr were also lower at days 19 and 20, when the maximum temperature exceeded 40°C, and evaporative demand was high (data not shown).

In the second greenhouse experiment, nodal roots of plants subjected to drought above the funnel were severed on the second day after withholding water, when the differences in Ψ_L between W and D were small (about 0.2 MPa) and not significantly different on day 1 (Fig. 5). After severing roots, both Ψ_L and g_s of plants in S increased progressively with time until the experiment was terminated 2 d later. However, in D, the parameters either did not change (Ψ_L, g_s) or continued to decrease (Tr) through harvest. In this experiment, a significant reduction in g_s was observed on day 1, one day before a significant reduction in Ψ_L was apparent.

2.　Field Experiment
(1)　Weather conditions

Rainfall was sporadic during both periods of water stress (41 to 68 DAS and 69 to 97 DAS; Fig. 6), yet the droughted plots were protected by rainout shelters. Rainy days were generally lower in solar radiation. Soil moisture tension (SMT) rose steadily under the rainout shelters, with the rise being more rapid in the top 10 cm. The odd values of SMT from days 22–24 at 40 cm depth cannot be explained. Comparably, water table depth decreased progressively to reach about 80 cm from the soil surface by the end of the experiment in both drought periods.

(2)　Drought stress between 41 and 68 DAS (1st cycle)

As values of Ψ_L, g_s, and Tr only rarely differed significantly between lines within each treatment, mean values are presented (Fig. 7). After water was withheld in D, Ψ_L was slightly lower than in W, but the difference was not statistically significant until day 25 after withholding water. In W and D, g_s and Tr, values oscillated until day 14, when values became significantly lower in D, but recovered again for a few days following rain, when solar radiation was lower (Fig. 6a). After day 19, however, values of both g_s and Tr in D dropped significantly below the values obtained from plants subjected to W through the end of this treatment cycle. In the W, the decline in Tr on days 20 and 24 to 27 was associated with lower minimum temperature and lower vapor pressure deficit (data not shown).

(3)　Drought stress between 69 and 97 DAS (2nd cycle)

The two cultivars rarely differed significantly in Ψ_L, g_s, or Tr within treatments, and so mean values are presented as in the first cycle (Fig. 8). The Ψ_L was significantly lower in D starting at day 18, and continued to decline progressively with time until harvest at day 28. However, reduction in g_s and Tr started earlier, at days 7–10, and then became consistently and progressively greater with time after day 13–14, through day 28. The smaller difference between values in W and D observed on days 11 and 12 was associated with 32 mm of rain on day 11 (Fig. 6d) and the associated lower vapor pressure deficit.
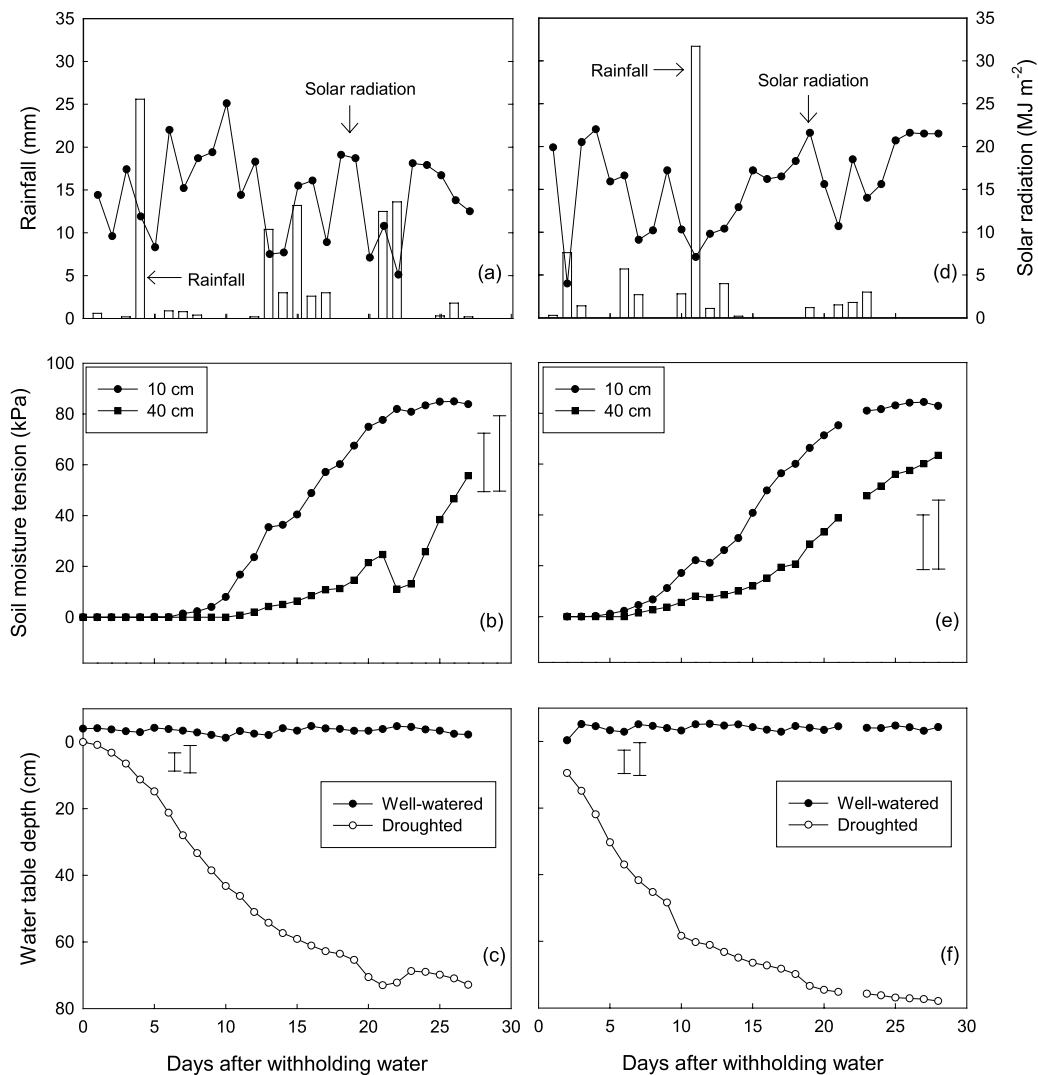
Fig. 6. (a, d) Rainfall and solar radiation, (b, e) soil moisture tension data means of the two rice lines, CT9993 and IR62266, of droughted plots, and (c, f) depth to free water table in droughted plots from (a, b, c) 41 to 68 DAS and (d, e, f) 69 to 97 DAS in the field experiment at IRRI in 2002. Rainfall was excluded from droughted plots using portable rainout shelters. Short and long vertical bars indicate pooled $LSD_{0.05}$ and pooled $LSD_{0.01}$, respectively.

(4) Leaf ABA Concentration in drought stress between 69 and 97 DAS (2nd cycle)

ABA concentration was measured using leaf samples with similar age to those used for leaf water potential measurements. As leaf ABA concentration rarely differed significantly between lines, mean values were again presented (Fig. 9). ABA concentration in leaves in D was low, ranging from undetectable to below 100 ng g^{-1} fresh weight. In contrast, ABA concentration in leaves in W increased progressively from 5 DAWW and reached a maximum of about 300 ng g^{-1} fresh weight, at about 13 to16 DAWW. ABA levels then decreased thereafter, and reached values similar to those obtained in W after about day 20.

Discussion

These results demonstrate that rice is highly sensitive to water deficit under the alternate anaerobic flooded and aerobic droughted conditions commonly experienced in the rainfed lowland ecosystem. Symptoms of water deficit including reduced growth were observed, even when water was available just below the soil surface layer. We demonstrate that this extreme sensitivity in rainfed lowland conditions is likely to be associated with root-to-shoot communication, associated with roots in the drying soil surface layer. The effect of these signals is more apparent under natural field conditions and particularly when soil drying occurs more gradually, as observed during the second drying cycle of the field experiment. This is further substantiated by greenhouse studies, where removal of the source (roots growing in drying soil) resulted in recovery of leaf water potential and stomatal conductance and
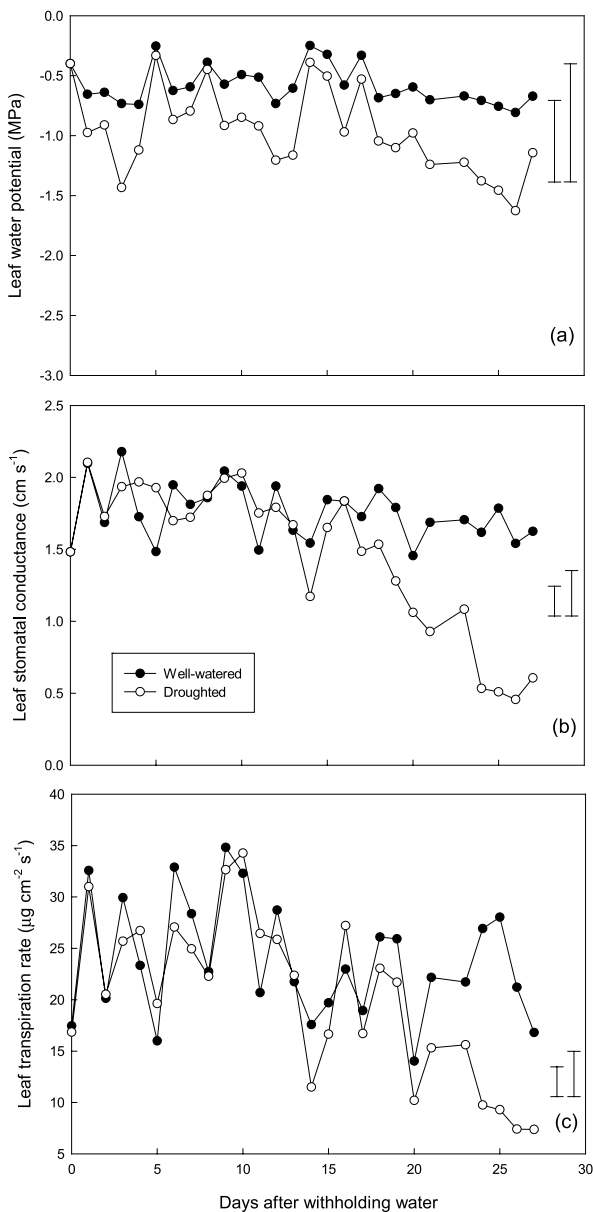
Fig. 7.   (a) Leaf water potential, (b) stomatal conductance and (c) transpiration rate measured in the field experiment in response to withholding water from 41 to 68 DAS in the field at IRRI in 2002. Data are means of the two rice lines, CT9993 and IR62266, and short and long vertical bars indicate pooled $LSD_{0.05}$ and pooled $LSD_{0.01}$, respectively.
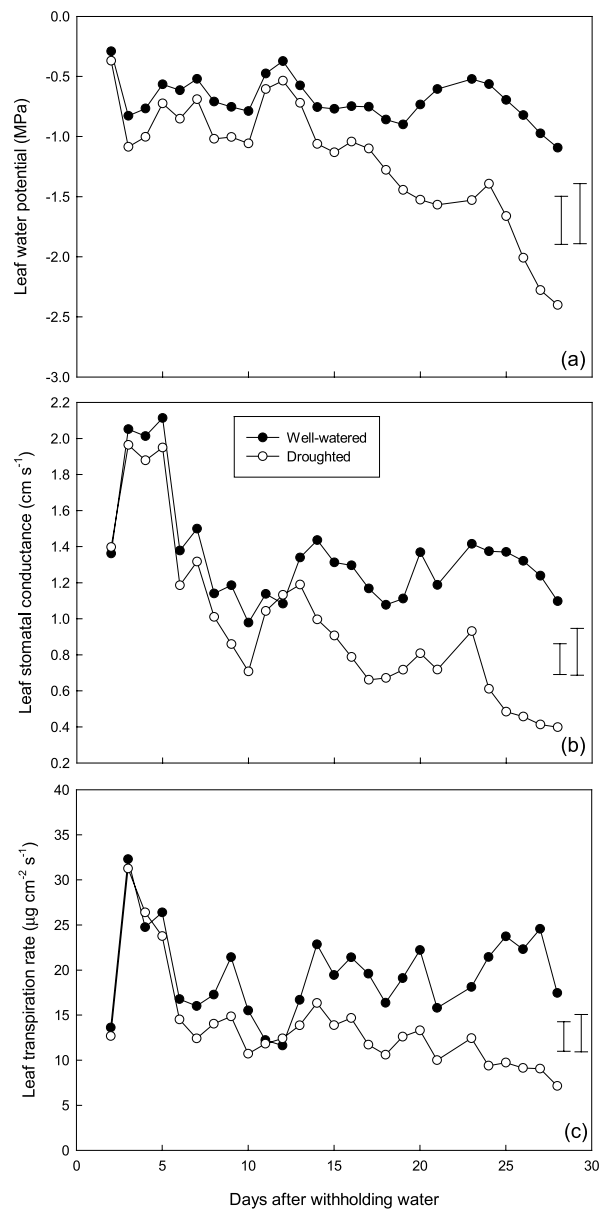
Fig. 8.   (a) Leaf water potential (b) stomatal conductance and (c) transpiration rate in response to drought imposed between 69 and 97 DAS in the field experiment at IRRI in 2002. Data are means of the two rice lines, CT9993 and IR62266, and short and long vertical bars indicate pooled $LSD_{0.05}$ and pooled $LSD_{0.01}$, respectively.

resumption of growth.

1.   Evidence for root-to-shoot communication in rice

In the first greenhouse experiment, the initial decrease and recovery in Ψ_L under drought from 4–13 DAWW, and the initial increase in Tr with drainage at 2 DAWW are discussed in sections 3 and 5, respectively.

In the first greenhouse experiment, while g_s decreased under drought from day 6 and Tr from day 7 after withholding water, Ψ_L under drought dropped without recovery after g_s and Tr, from day 14 onwards (Fig. 4). This response was much clearer

in the second greenhouse experiment, where the decrease in g_s under drought occurred one day before Ψ_L (Fig. 5). When roots in dry soil were severed in both experiments, the observed recovery in Tr and g_s suggested the interruption of a positive signal, "an inducible signal added to the transpiration stream under drought", perceived from roots in the drying soil (Comstock, 2002).

In the field experiment, when water was withheld from 41 to 68 DAS, both g_s and Tr decreased significantly from day 18 onwards, yet significant reduction in Ψ_L was apparent a few days later,
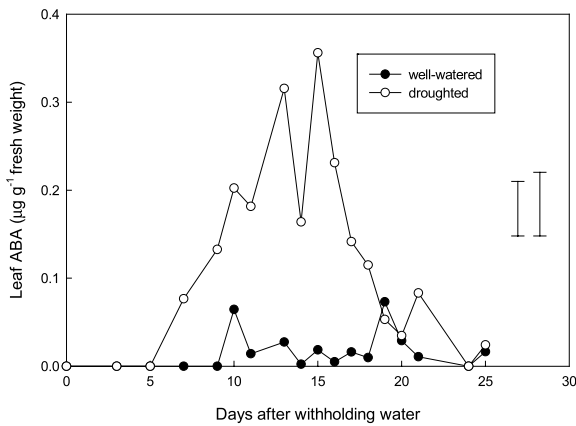
Fig. 9. Leaf ABA concentration in response to drought imposed between 69 and 97 DAS in the field at IRRI in 2002. Data are means of the two rice lines, CT9993 and IR62266, and short and long vertical bars indicate pooled $LSD_{0.05}$ and pooled $LSD_{0.01}$, respectively.

starting at 25 DAWW (Fig. 7). Likewise, when water was withheld from 69 to 97 DAS, both g_s and Tr in D, decreased significantly during days 7 to 10 after withholding water, and again from day 13 onwards, yet, significant reduction in Ψ_L was not observed until about 18 DAWW (Fig. 8). The fact that g_s and Tr began to decline a few days before a significant reduction in Ψ_L in both field experiments provided strong evidence for the existence of root-sourced signals in rice when surface soil started to dry. This is supported by the stronger negative correlation of soil moisture tension with g_s than with Ψ_L (R = −0.71[*] and −0.30[ns], respectively). These findings are consistent with the previous studies of Bano et al. (1993), Davies et al. (1994), Dingkuhn et al. (1999), and Liu et al. (2003), all of them suggested a primary role for root signals in regulating g_s and leaf growth in rice and other crops under water deficit. Our data provide strong evidence for root-to-shoot communication in rice, whereby the portion of roots in drying soil wielded a regulatory function on leaves, but this function was gradually removed upon excision of the droughted roots. This is discussed further below.

2. Nature of the root signal

During the second cycle of drought in the field experiment, the concentration of ABA in leaves of water-stressed plants reached a maximum at 13 to 16 d after water was withheld (Fig. 9), which coincided with the period when g_s and Tr dropped significantly and progressively thereafter, in the droughted plots (Fig. 8). This increase in ABA concentration in conjunction with decreasing g_s and Tr provides evidence for ABA as a potential candidate for root signaling early in the water deficit. The rise in leaf ABA coincided almost perfectly with the commencement of the drop in gs in mild water deficit. After 15 DAWW, leaf ABA started

to decline, presumably due to decreased delivery from roots due to slower water movement, and possibly the breakdown of ABA in the leaf. Under severe water deficit, however, ABA is no longer important, because leaves lose their turgor due to reduced leaf water potential; guard cells subsequently lose turgor, and stomata close. A hydraulic signal (increasing hydraulic resistance in xylem vessel due to cavitation) may help to explain this lowered leaf water potential, especially as water deficit intensifies (Ali et al., 1998; Liu et al., 2003).

Sauter et al. (2001) indicated that roots were able to 'measure' decreasing soil water availability during a period of drought (perhaps as changes in root water status), which resulted in an increased release of ABA from the stellar tissues of roots to the xylem vessels. After xylem transport to the shoot, guard cells responded rapidly and sensitively to increased ABA concentrations resulting in reduced transpirational water loss. With regards to the source of leaf ABA, strong evidence was established in previous studies that ABA is synthesized in increasing quantities in roots in drying soils (Neales et al., 1989; Zhang and Davies, 1991; Bano et al., 1993; Zhang et al., 2006) and then translocated via the xylem stream to the leaf to induce stomatal response. The decrease in leaf ABA concentration as drought progressed (Fig. 9) seems to coincide with the strong reduction in Ψ_L after day 24 (Fig. 8), suggesting that ABA ceased to accumulate in leaves when Ψ_L became sufficiently low. This could also be explained by a consequent reduction in transpiration rate and thus, the reduced delivery of root-sourced ABA through the transpiration stream, as g_s and Tr declined after day 13 (Fig. 8), and possibly by the breakdown of ABA in the leaf. This is similar to the response to ABA accumulation observed by Jia and Zhang (1999) where declining transpiration rate followed closely the decreasing trend of g_s.

Substantial evidence was established, which suggests ABA as the primary candidate for mediating stomatal response to soil drying (Sharp, 2002), with ABA being produced in water-stressed roots and transported through the xylem to be perceived at the guard cells in the leaf where g_s is regulated. For example, Henson et al. (1989a,b) and Jensen et al. (1989) reported that stomata opening was controlled by root-borne stress signals transported by the transpiration stream, and only to a lesser extent by the stress condition experienced by leaf tissues.

Ideally, root-sourced ABA in the transpiration stream should be measured rather than leaf ABA, but the importance of xylem ABA is widely recognized, with more ABA coming from roots in drying soils (Bano et al., 1993; Kobata and Hara, 1994; Kobata et al., 1994). Jackson (2002) implied that transpiration-borne root signals can be demonstrated convincingly only if there is a change in the flux of the solute out of the

root and into the shoot. A change in concentration in the transpiration stream can indicate a genuine signal, but only if the extent of change exceeds that of any alteration in transpiration rate or in stomatal conductance. Furthermore, stomata were found to respond mainly to prevailing ABA concentration in the xylem stream rather than to the accumulated amount of xylem-derived ABA in the leaves (Jia and Zhang, 1999). To establish a definitive role for ABA as the chemical signal involved in the regulation of stomatal behavior in the early response of rice to surface soil drying would require further measurements.

The nature of root signals has been the subject of study in various plant species in the past decade. Several studies (Tardieu et al., 1992; Salah and Tardieu, 1997; Comstock, 2002) suggested that stomatal response to drought was the result of an integration of hydraulic and chemical root-generated signals. These authors outlined the great diversity of stomatal behavior, such that a single mechanism even if well documented in all its aspects, cannot be generalized to explain the physiology of stomata under all water-stress conditions (Salleo et al., 2000).

Further evidence has established a secondary response of stomata to some component of leaf water potential or a "hydraulic signal" (Comstock, 2002). Boyer (1989) argued that drying of the soil may result in the collapse of the water potential gradient between xylem and actively-dividing cells. When roots were severed, Salleo et al. (2000, 2004) showed that plants closed their stomata to reduce the occurrence of cavitation and to prevent the decrease in Ψ_L. The apparent recovery in Ψ_L after root severing observed here (Fig. 4), which is discussed further below, may indicate the contribution of an hydraulic signal affecting stomatal conductance.

3.   Initial changes in leaf water potential

In the first greenhouse experiment, Ψ_L dropped significantly in D after the pots were drained, before apparently recovering at 13 DAWW (Fig. 4). This apparent increase in Ψ_L may be partially explained by the findings of Stiller et al. (2003), who showed that even at -2 MPa, rice roots were vulnerable to cavitation, with xylem conductivity in leaves dropping by as much as 90%. Nevertheless, the plants were able to refill cavitated xylem conduits and restore lost xylem hydraulic conductance. Likewise, Vysotskaya et al. (2003) found variation in root hydraulic conductance which allows plants to compensate for limited water supply and maintain water balance. In our data (Fig. 4), there was even some recovery in Ψ_L. We propose that cavitation in larger xylem vessels and roots, accompanied by a decrease in g_s, may have allowed Ψ_L to drop by 4 DAWW then gradually to improve by 9 DAWW, as a result of continued but gradual water supply via smaller xylem vessels and roots which did

not cavitate or which were able to refill. This remains to be experimentally tested.

4.   Subsequent changes in leaf water potential after root severing

In the first greenhouse experiment, severing of roots in treatment S on day 13 resulted in a significant drop in Ψ_L and g_s on day 14 (Fig. 4). This may be attributed to the plant's response to wounding of roots providing only a temporary check, and the plants were able to recover quickly within one day. In contrast with the first greenhouse experiment, the second experiment was short in duration due to a smaller pot size that mediated a more rapid response, with less opportunity to express the compensations with wounding. Nevertheless, any wounding response in experiment 1 was quickly overridden by the hypothesized response to root signals. More importantly, despite differences in the duration of both greenhouse experiments, as well as differences in the lag time, leaf water potential started to recover after the severing of roots subjected to water stress (Figs. 4 and 5). A similar response was observed by Shane and McCully (1999), who found that leaf water potential increased in maize when all roots, but one, were severed, when compared with plants with intact roots. They concluded that a plant with excised roots could supply the shoot with sufficient water for it to survive by way of its remaining xylem vessels embolizing less frequently or refilling more quickly, so that the remaining root was more efficient. Stiller et al. (2003) also observed this ability in rice to refill cavitated xylem conduits and restore lost xylem hydraulic conductance. They also noted that reduction in hydraulic conductance could have come from cavitation in roots and stems and soil drying. Similarly, in an excised root experiment in wheat, Vysotskaya et al. (2003) showed evidence that variation in root hydraulic conductance provided a powerful mechanism to compensate for limitations in water supply, allowing plants to maintain an adequate water balance which complemented stomatal control of water loss. Consequently, we propose that cavitation and hydraulic signals may be important in rice in rainfed lowland drought, especially as the water deficit becomes severe. The putatively greater role for hydraulic signals during severe water deficit is explored in a companion paper, together with evidence for differences between cultivars (Siopongco et al., 2008).

5.   The influence of weather

Responses in greenhouse and field were subject to variation in weather conditions, despite the use of pots in the greenhouse and rainout shelters in the field. For example, g_s and Tr were lower in W at days 19 and 20 in greenhouse experiment 1 (Fig. 4), where maximum temperature exceeded 40°C and evaporative demand was high (data not shown). Tr dropped in W in the

field in the first drought period (41 to 68 DAS), when minimum temperature and vapor pressure deficit were lower during days 26 and 27 (data not shown). Likewise, in the second drought period (69 to 97 DAS) in the field, differences between treatment W and D narrowed on days 11 and 12, following 32 mm of rain on day 11, when minimum temperatures and vapour pressure deficit again were lower, reducing evaporative demand. A smaller but similar event was also observed around days 15 and 16 in the first drought period in the field. Such weather events buffered the responses discussed elsewhere in the paper, without altering the conclusions.

Conclusions

The use of the split-root protocol in the greenhouse experiments made it possible to demonstrate the presence of root signals in water-stressed rainfed lowland adapted rice lines. This was shown by the reduction in stomatal conductance when only a portion of the root system was subjected to drought, and the apparent recovery when the portion of roots in drying soils was severed, which was consistent with a positive root-sourced signal. Further support was provided from both drying cycles in the field experiment, in which g_s and Tr declined earlier in response to drying of the surface root zone, before Ψ_L started to decline. The stronger correlation of soil moisture tension with stomatal conductance than with leaf water potential was also consistent with this view. Our field data implicated ABA as a potential candidate for chemical root signaling in early drought in rice, since its increased concentration in leaves coincided with critical changes in stomatal behavior as well as in soil moisture tension. Further studies are needed, however, to confirm ABA's involvement in root signaling in rainfed lowland rice, by establishing its root origin and delivery through the xylem sap at a rate and duration sufficient to induce stomatal closure.

We found three interesting phenomena: rice responded sharply to drying of a portion of its root system by closing stomata, and the stomata reopened when roots in the drying soil were severed. Furthermore, leaf water potential recovered after severing roots in drying soils. These responses, which were revealed by the split-root/root-severing in the drying soil technique, imply an important role for hydraulic signals in rice, especially in the fluctuating water (anaerobic flood-aerobic drought) environments of the rainfed lowlands. The role of hydraulic signals, such as of decreased hydraulic conductance through cavitation, in stomatal response to drought requires further study, to clarify the mechanisms and likely implications in contrasting growing conditions. We propose that these phenomena may have great impact in rainfed lowland drought, where water availability is heterogeneous in time and space. We conclude that

further research is needed on hydraulic signals in rice, and their implications for crop performance.

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