Stomatal Responses in Rainfed Lowland Rice to Partial Soil Drying; Comparison of Two Lines

Joel DLC. Siopongco[1], Kazumi Sekiya[2], Akira Yamauchi[2], James Egdane[1], Abdelbagi M. Ismail[1] and Len J. Wade[3]

(*[1]International Rice Research Institute, DAPO Box 7777, Metro Manila Philippines;*
[2]Graduate School of Bioagricultural Sciences, Nagoya University, Chikusa, Nagoya 464-8601, Japan;
[3]Charles Sturt University, E.H. Graham Centre for Agricultural Innovation, Wagga Wagga NSW 2678, Australia)

Abstract : Previously, we demonstrated that root tips in drying soil communicate with shoots for stomatal closure in rainfed lowland rice, despite further water being available at depth. This study examines variation between two lines in root signals. Rice lines CT9993 and IR62266 were grown in the field, and in the greenhouse with the split-root root-sever wax-layer system, to investigate their responses to mild and severe water deficit by monitoring stomatal conductance (g_s), leaf water potential and leaf ABA concentration. In the greenhouse, root systems were divided, withholding water from one portion, and in some cases, severing the droughted portion of roots to remove the signal. Wax layers differing in strength were placed at hardpan depth. Roots of CT9993 were better able to penetrate the wax layers. IR62266 exhibited stronger responses than CT9993, with IR62266's stomatal conductance dropping sharply under water deficit, and recovering at slower rates but less completely, when roots subjected to drying soil were severed. The greater stomatal response in IR62266 was associated with a higher leaf ABA concentration during early water deficit, which in turn was associated with its greater number of roots in drying soil. In the field, a second reduction in g_s was observed under severe water deficit, with stronger signals in IR62266 associated with more conservative water use as soil drying intensified. To better exploit subsoil water in mild or transient water deficit, selection for reduced root signals might be warranted.

Key words : Hardpan, Leaf water potential, Mechanical impedance, Rainfed lowland, Rice, Root signals, Stomatal conductance, Water deficit.

Rice is grown on 132 million hectares in diverse environments, a third of which is rainfed lowland (MacLean et al., 2002; O'Toole, 2004). Drought-prone rainfed lowlands are commonly subjected to cycles of flooded, anaerobic and dry, aerobic soil conditions (Wade et al., 2000), the occurrence and duration of which are unpredictable. Rice crops in these ecosystems are at risk of exposure to drought stress leading to variable low yields averaging about 2.3 t ha^{-1} (Wade et al., 1999a). The root system is usually shallow (Wade et al., 1998), partly because of the restrictive effect of anaerobic soil conditions on root growth. Confounding the effect of drought on the root system is the presence of mechanical soil impedance or hardpans at 20 to 30 cm from the soil surface, which are usually created by farm practices such as plowing and puddling (O'Toole and De Datta, 1986; Siopongco et al., 1994; Hoque and Kobata, 1998; Samson and Wade, 1998).

Plant water status during the transition period from anaerobic and flooded to aerobic and water-deficit conditions is crucial to drought resistance and recovery. Enhanced capacity of the root system to penetrate the hardpan is considered essential for the development of deeper roots under rainfed lowland conditions (Fukai and Cooper, 1995) and is a key factor for drought adaptation in areas where water supply is limited (O'Toole, 1982; Yu et al., 1995). Capacity to adapt to cycles of wet and dry conditions can be linked with the ability of the roots to proliferate quickly in the subsurface layers of the soil prior to and during the early stages of drought (Bañoc et al., 2000a, b), to enable continued water extraction and maintenance of leaf water potential.

In response to hardpans or compacted soils, Hartung et al. (1994), Mulholland et al. (1996, 1999), Hussain et al. (1999) and Roberts et al. (2002) showed that increased ABA export from roots encountering compacted soil was responsible for the concurrent reductions in stomatal conductance in various crops. However, Ismail et al. (1994) found no evidence for the involvement of ABA as a root signal in mediating responses to restricted root growth in cowpea. In rice, little is known regarding the effect of mechanical

Abbreviations : ABA, abscisic acid; DAS, days after sowing; DAWW, days after withholding water; Ψ_L, leaf water potential; SMT, soil moisture tension; g_s, stomatal conductance; Tr, transpiration rate.

impedance on root-shoot signaling.

To study the effects of hardpans in the laboratory or in the greenhouse, Yu et al. (1995) and Clark et al. (2000) devised screening methods using wax layers of varying hardness for examining penetration ability of rice root systems. While Yu et al. (1995) identified lines that were divergent in their capacity to penetrate the wax barrier in aerobic soils, Clark et al. (2002) successfully correlated laboratory findings with data obtained from field experiments (Samson et al., 2002). The importance of roots capable of penetrating a hardpan in the rainfed lowlands was examined by Samson et al. (2002) in a drought-prone environment where the clayey subsoils were heavily compacted. In this study, lines were found to adapt to changing soil conditions in different ways as drought progressed. Consistent with the results of Azhiri-Sigari et al. (2000) and Kamoshita et al. (2000) in the greenhouse, IR58821 and CT9993 were more able to develop roots at depth, while IR62266 was slower in extracting water from depth but favored osmotic adjustment. Similar results were obtained in controlled conditions by Babu et al. (2001).

The study of partially-stressed root systems is important, especially under rainfed lowland conditions, because of the unique hydrological characteristics, not only the alternating wet and dry cycles but also the continuing relatively-wet conditions below the hardpan. There is a need for plants to respond to abiotic stresses, such as drought and/or hardpans, be it momentary or for survival, when drought persists for longer duration. One possible mechanism for this adaptation is via root-to-shoot signaling to control stomatal conductance when soil drying is perceived or mechanical impedance is encountered by the roots. Within the root system, the tips may be the main source of signaling activity, especially when they are growing in water-limited conditions or under high mechanical impedance, by sending inhibitory signals to the leaves (Passioura, 2002).

Overall, a complex root-shoot signaling system comprising two signal types has been proposed for the control of leaf stomatal behavior. At mild soil water deficits, chemical signals are produced in roots and transported via xylem to the 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 perform a major role (Liu et al., 2003). In rice, for instance, ABA has been reported to be the hormone involved in chemical root signaling (Bano et al., 1993), while cavitation has been shown to occur on a diurnal basis via root pressure and guttation (Sperry et al., 2003), with osmotic pressure building in roots implicated in the refilling of cavitated vessels (Stiller et al., 2003). Their observations suggested a vital role

for root pressure in the maintenance of hydraulic conductance.

The previous study in this series, (Siopongco et al., 2008), demonstrated the existence of root signals in rainfed lowland rice. The decrease in g_s and transpiration rate (Tr) before any decrease in leaf water potential (Ψ_L), which was associated with increased soil moisture tension (SMT) and a rise in leaf ABA, was consistent with the influence of a chemical signal under mild drought. Under severe water deficit, however, ABA was no longer important, because leaves lose their turgor due to reduced Ψ_L, guard cells subsequently lose turgor, and stomata close. A hydraulic signal (increasing hydraulic resistance in xylem vessels due to cavitation) may explain this lowered Ψ_L especially as water deficit intensified (Ali et al., 1998; Liu et al., 2003). This manuscript builds upon this framework by exploring evidence for differences between lines in stomatal responses and root signals under mild and severe water deficit in the greenhouse and the field.

Consequently, in this study, greenhouse experiments were conducted using the split-root, root-severe in drying soil system. The root-to-drying soil and root-to-wet soil contact represented root-soil conditions found in rainfed lowland, where roots were subjected to fluctuating soil moisture conditions, i.e., from submerged and anaerobic to dry and aerobic. Wax layers were placed at 15 or 20 cm depths, to simulate hardpans that may be encountered by rice roots in the rainfed lowland fields. The entries included were the upland *japonica* line CT9993-5-10-1-M (CT9993) and the lowland *indica* line IR62266-42-6-2 (IR62266). CT9993 is reported to have a deeper root system (Kamoshita et al., 2000; 2004), and is specifically adapted to particular drought situations in the rainfed lowlands (Wade et al., 1999b). IR62266 performs well when subjected to drought in the fluctuating water (flood-drought) environments of the rainfed lowlands (Wade et al., 1999b; Siopongco et al., 2005, 2006), and is noted for its more gradual water extraction from depth and its greater ability for osmotic adjustment (Kamoshita et al., 2000, 2004).

The objectives were to (1) examine differences between the two contrasting lines in responses of stomatal conductance, transpiration rate and leaf water potential to water deficit and soil mechanical impedance, and (2) consider any differences between the lines in mild and severe water deficit. The implications for rice improvement in fluctuating water environments are discussed.

Materials and Methods

1. Experiment 1
(1) Cultural details
This experiment was conducted between 16 January to 7 March 2002, in a greenhouse at the International

Rice Research Institute (IRRI), Los Baños, Philippines (14°11'N, 121°15'E, 23 m altitude). A split-root protocol with an inner and an outer container, based on the protocol of Siopongco et al. (2008), was used in this experiment. A split-split-plot design with 7 replications was used, with three water-root treatments as main plots, two artificial hardpan treatments as subplots, and two lines as sub-subplots. The water-root treatments were similar to Siopongco et al. (2008) : well-watered in both containers (W), water deficit in the outer container but without severing roots (D), and with roots in the drought-stressed outer container severed (S). The hardpan treatments were : (1) control or soft wax, 3% paraffin wax with 97% petroleum jelly; and (2) hard wax, 60% paraffin wax with 40% petroleum jelly. IR62266 and CT9993 were used to assess genotypic differences with lines similar in phenology but differing in their strategies of drought response, favoring either drought tolerance or drought avoidance, respectively. Extra pots were prepared to accommodate destructive sampling for daily leaf water potential.

Wax layers were prepared by melting together paraffin wax and petroleum jelly following the procedure described by Clark et al. (2000). The mixture was poured into molds of 20 cm in diameter and 3 mm in depth before it hardened. A 10-cm diameter pipe was then punched through the middle leaving a 20-cm ring of wax with a 10-cm diameter hole. The artificial hardpans were designed to provide a contrast in mechanical impedance, from almost nil (3 : 97 wax : petroleum jelly) to significant (60 : 40). The low impedance hardpan was included as a quantitative check on root penetration below the layer, as described by Clark et al. (2000).

A cylindrical pot made from polyvinyl chloride (PVC), with 20 cm in internal diameter and 55 cm in height with an inner plastic sleeve, was filled with air-dried Maahas clay soil (28% clay, 44% silt, and 28% sand; pH 5.2; Wopereis, 1993) to a height of 30 cm (Fig. 1). The 3-mm thick wax layer was then placed flat on the soil, and a PVC pipe 10 cm in internal diameter and 25 cm in height was placed centrally over the hole in the middle of the hardpan. A petri dish with a 3-mm hole drilled in the middle was placed at a 45-degree angle just below the lip of the inner pot to serve as a deflector of roots. On the lower side of the petri dish, a 2-mm slit was made in the inner pot through which the roots could pass. The inner and outer pipes were then filled with soil to 5 cm below the top of both pots, so that the petri dish was just covered with soil, and forming a surface layer above the hardpan of 20 cm depth (Fig. 1). A hole was drilled and plugged in the outer container just above the bottom seal, and another one just above the hardpan in the outer container. The plugs were removed from both positions at the start of withholding water in the
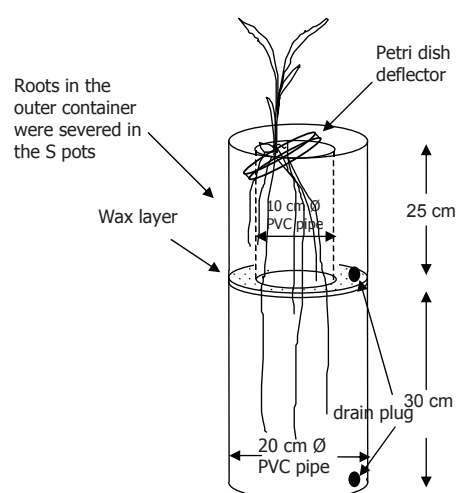

Fig. 1. Pot setup with variable hardpans used in Experiment 1. The setup allowed roots to grow either into inner well-watered container or into the outer container which was subjected to water deficit in the Droughted (D) and Severed (S) treatments. An artificial hardpan was placed at the base of the outer container to provide impedance to root growth. A petri dish was used to deflect some of the nodal roots to the outer container.

D and S treatments. This protocol was intended to simulate rainfed lowland soil hydrology, where roots in the surface layer (outer container) are subjected to faster soil drying conditions above the hardpan, while roots that penetrate to depth (inner container) would have access to a greater volume of wet soil for a longer duration (Samson et al., 2002).

One 8-d old seedling was planted in each pot with five or more nodal roots allowed to pass through the 3-mm diameter hole in the petri dish. The rest of the nodal roots were deflected by the petri dish to the outer container. The pots were watered daily and a flooding depth of 2 cm was maintained throughout in W, or until drainage in D and S treatments. The exterior of the pot was covered with aluminum foil to minimize any rise in soil temperature that might affect root growth. 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. Preventive measures were taken to preclude damage from diseases and insects. The distance between neighboring pots was more than 40 cm to minimize mutual shading. At 20 d after sowing (DAS), the plugs were removed and water was withheld for 18 d in D and S treatments. Roots were severed 11 d after withholding water (DAWW) in the S treatment. The plants were harvested on 7 March 2002, at 50 DAS. A hygrothermograph was installed to monitor minimum and maximum daily temperatures, and evaporation was measured with 5 pan evaporimeters randomly distributed in the greenhouse.

(2) Physiological measurements and plant sampling
Leaf water potential (Ψ_L) was measured daily with a

Scholander pressure bomb (Soil Moisture Equipment Corporation, Santa Barbara, CA, USA). Stomatal conductance (g_s) and transpiration rate (Tr) on both abaxial and adaxial sides of the leaf were measured daily between 1000−1200 h with a steady state Li-Cor 1600 porometer (Li-Cor Corporation, Lincoln, NE, USA). Tr as 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 lines exposed to similar water deficit conditions (Yambao et al., 1992; Dingkuhn et al., 1999; Jia and Zhang, 1999; Angelocci et al., 2004).

Leaves were sampled for ABA analysis following measurements of Ψ_L, g_s and Tr at 7, 13 and 18 DAWW. The leaf blades were placed in aluminum foil then immediately immersed in liquid N and stored at -80 °C until analysis. Extraction and purification were done following the protocol of Dunlap and Guinn (1989). Samples were then fed into an HPLC system consisting of an LC 10AT HPLC pump, SIL–10AD auto injector, a CTO-10AVP column oven and an SPD 10 AVP (Shimadzu Corporation, Kyoto, Japan). Readings for ABA were based on a retention time of 11.531 min.

At harvest, the plastic sleeve containing the roots and soil was carefully removed and root axes were counted as those which penetrated the wax layer, did not penetrate the wax layer and those which escaped by passing in between the wall of the outer container and the wax layer.

2. Experiment 2
(1) Cultural details
Details of this experiment, conducted in the field at IRRI, Philippines during the 2002 wet season, were reported by Siopongco et al. (2008), but additional data on genotypic variation are provided here. Briefly, 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 lines as sub-subplots (CT9993 and IR62266). Three-day old pregerminated seeds of both entries were sown on 25 September 2002, at a 25 cm by 25 cm spacing. Movable 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. The second drought-stress period was hotter and drier than the first drought-stress
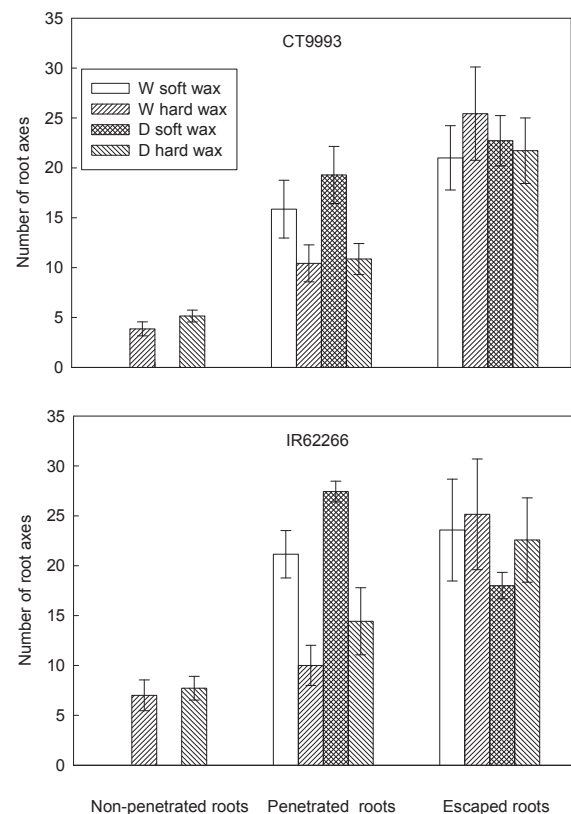

Fig. 2. Number of root axes that either remained above the hardpan, penetrated the hardpan, or escaped between the hardpan and the wall of the outer and inner cylinders in response to drought and wax layer at 50 DAS in Experiment 1. W - well-watered in both containers; D - drought in the outer container. Vertical bars indicate ± SE.

period. In each plot, free water table was monitored daily with piezometers inserted to a depth of 80 cm, and daily soil moisture tension was monitored by mercury tensiometers placed at 10 and 40 cm depths. The piezometers and tensiometers were fabricated, installed and monitored using the protocol described by O'Toole and Maguling (1981). Daily Ψ_L, g_s, Tr and ABA were measured as described above.
(3) 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). Comparisons between parameters were achieved using regression analysis.
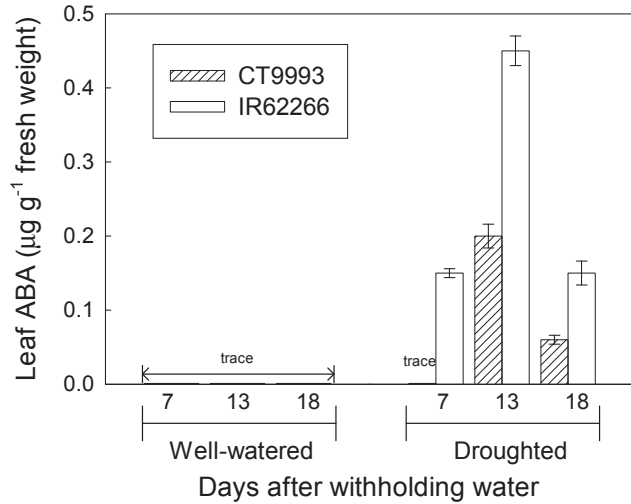

Fig. 3. Leaf ABA concentration measured at 7, 13, and 18 DAWW in CT9993 and IR62266 in well-watered and water-deficit treatments in Experiment 1. Vertical bars indicate ± SE.

Results

1. Experiment 1
(1) Timing of events, weather conditions and sampling

Water was withheld from drought treatments from 20 DAS, roots were severed 11 days later, with harvest after further 7 days following root severing. Daily solar radiation, minimum and maximum temperature, and pan evaporation averaged 9.9 MJ m^{-2} d^{-1}, 21.7°C, 27.7°C and 4.4 mm d^{-1}, respectively. Solar radiation was 57% of actual values from the IRRI wetland meteorological station 500 m away, as defined by Kamoshita et al. (2002).

(2) Root penetration of wax layer

A greater number of roots penetrated the soft wax than the hard wax in both lines and water regimes (Fig. 2). Generally, IR62266 had a higher number of root axes that penetrated the wax layers, especially the soft wax in D treatment. While no roots failed to penetrate the soft wax layer, about 4.5 and 7.5 roots failed to penetrate the hard wax in CT9993 and IR62266, respectively. The percentage of root axes
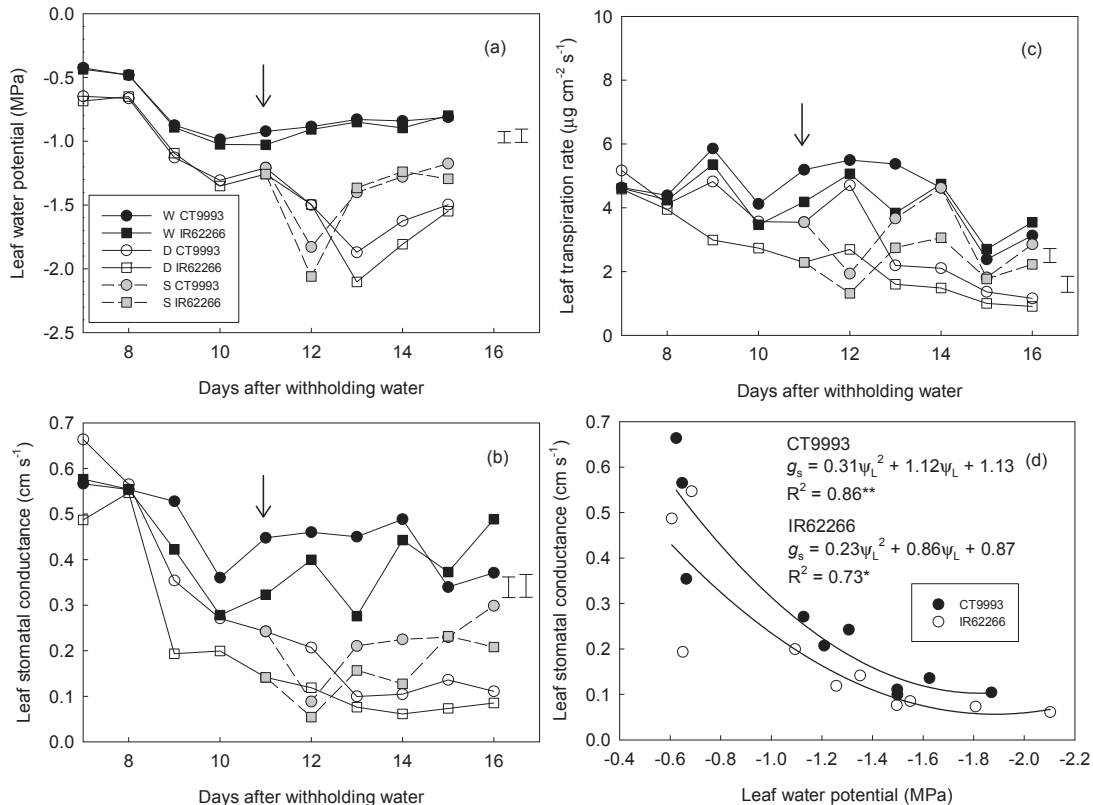

Fig. 4. Time course of (a) leaf water potential, (b) stomatal conductance, (c) transpiration rate, and (d) stomatal conductance in relation to leaf water potential of droughted CT9993 and IR62266, averaged across wax layer treatments in response to drought and mechanical impedance in Experiment 1. W, well-watered in both containers; D, drought in the outer container but without severing roots; S, roots in the water-stressed outer container severed. Vertical arrows indicate the time when roots in the outer container were severed in treatment S. Short and long vertical bars indicate pooled LSD$_{0.05}$ and pooled LSD$_{0.01}$, respectively. *P<0.05 and **P<0.01.

that penetrated the hard wax under well-watered conditions was 66% in CT9993 and 53% in IR62266. Under drought treatments, roots penetrating the hard wax were 56% in CT9993 and 47% in IR62266. The number of escaped roots did not differ significantly between treatments and lines.

(3)　Leaf ABA concentration

ABA was monitored in leaves of plants grown under the no-hardpan treatment at 7, 13 and 18 DAWW (Fig. 3). ABA concentration in the well-watered treatment remained below the detection limit but was detected under drought. At day 7, a low level was detected in leaves of IR62266 only. By day 13, higher concentrations of ABA were detected in leaves of both lines, with considerably higher concentration in leaves of IR62266. Leaf ABA was 0.45 μg g^{-1} FW in IR62266 at 13 DAWW, more than double that of CT9993, which had no detectable leaf ABA at 7 DAWW. By day 18, the level of ABA had dropped substantially in leaves of both lines, though IR62266 still maintained a significantly higher leaf ABA concentration.

(4)　Leaf water potential, stomatal conductance and transpiration rate

Fig. 4a, b and c show Ψ_L, g_s, and Tr, respectively, of both lines averaged across wax layers. Significant differences were observed between W, D and S treatments (Fig. 4), but not between impedance treatments (not shown). Before examining differences between lines, we summarize the average responses in W, D and S over wax layers. On average, Ψ_L of plants subjected to drought in the outer pot dropped significantly below those of the well-watered plants, especially after day 9 (Fig. 4a). Roots in the droughted outer container were severed at 11 DAWW, while the Ψ_L of droughted plants remained within −1.2 MPa. The Ψ_L dropped significantly one day after root severing (12 DAWW), but gradually recovered over the next three days to values intermediate between W and D treatments. Values of Ψ_L under D treatment declined progressively until 13 DAWW and remained significantly below that of S to the end of the experiment. Stomatal conductance of D declined significantly from 9 DAWW, and continued to decline progressively with time in the continuously-droughted treatment (D) through to the end of the experiment (Fig. 4b). In the S treatment, where roots were severed on day 11, g_s declined on day 12, returned to values prior to severing on about days 13 and 14, then increased further by day 16, to reach an intermediate level between W and D. Values of Tr declined steadily and significantly under drought (Fig. 4c). Following severing on day 11, Tr dropped on day 12, and recovered thereafter, to values approaching those before severing.

In comparing the two lines, no differences in Ψ_L were observed (Fig. 4a). However g_s under W was often significantly higher in CT9993 than IR62266 (e.g., 0.43
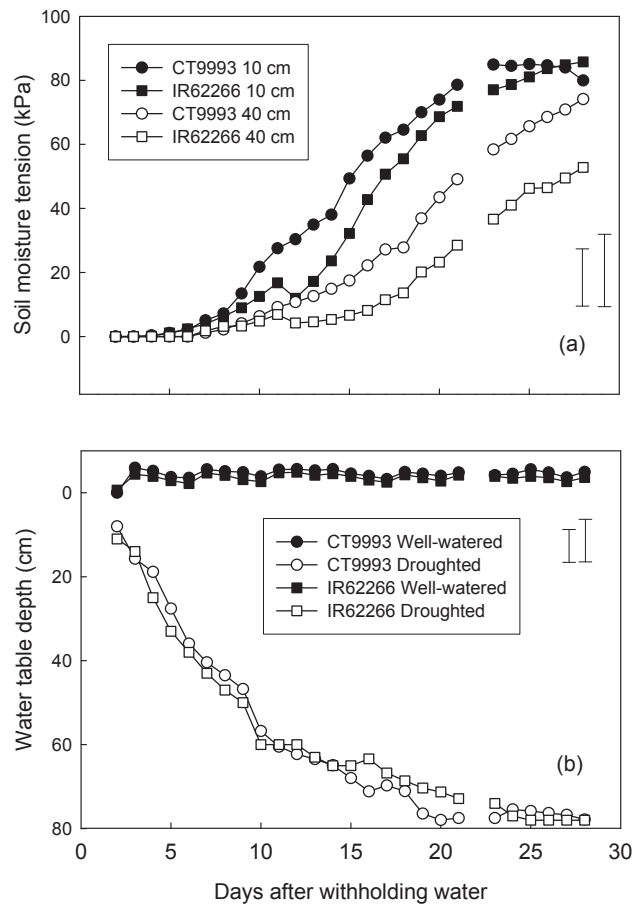

Fig. 5.　(a) Soil moisture tension data of CT9993 and IR62266, in droughted plots, and (b) depth to free water table of CT9993 and IR62266 at 69 to 97 DAS in the field. Short and long vertical bars indicate pooled LSD$_{0.05}$ and pooled LSD$_{0.01}$, respectively.

and 0.38 for 9-16 DAWW, respectively, P<0.05; Fig. 4b). Cultivar differences were especially important as water deficit intensified from 9–12 DAWW, with g_s being more stable in CT9993 but dropping substantially and significantly in IR62266 (0.27 and 0.16, respectively, P<0.05) in D. Thereafter, g_s was low in both lines in D from 13–16 DAWW (0.11 and 0.07, respectively). Upon severing of droughted roots at day 11, g_s in S decreased at 12 DAWW in the two lines, but there was a greater increase from 13–16 DAWW in g_s of CT9993 than in that of IR62266 (0.24 and 0.18, respectively, P<0.05). Relative to W, Tr was maintained in CT9993 but dropped significantly in D in IR62266 from 9–12 DAWW (4.16 and 2.68, respectively, P<0.05; Fig. 4c). After day 13, Tr was low in both lines in D and continued to decline from 13–16 DAWW (1.70 and 1.25, respectively). With severing, Tr dropped for one day at 12 DAWW in both lines, but increased thereafter in IR62266 to values approaching those before severing in D. In contrast, for CT9993, Tr increased from days 13 to 16 to values that did not differ significantly from the W treatment. Consequently, Tr
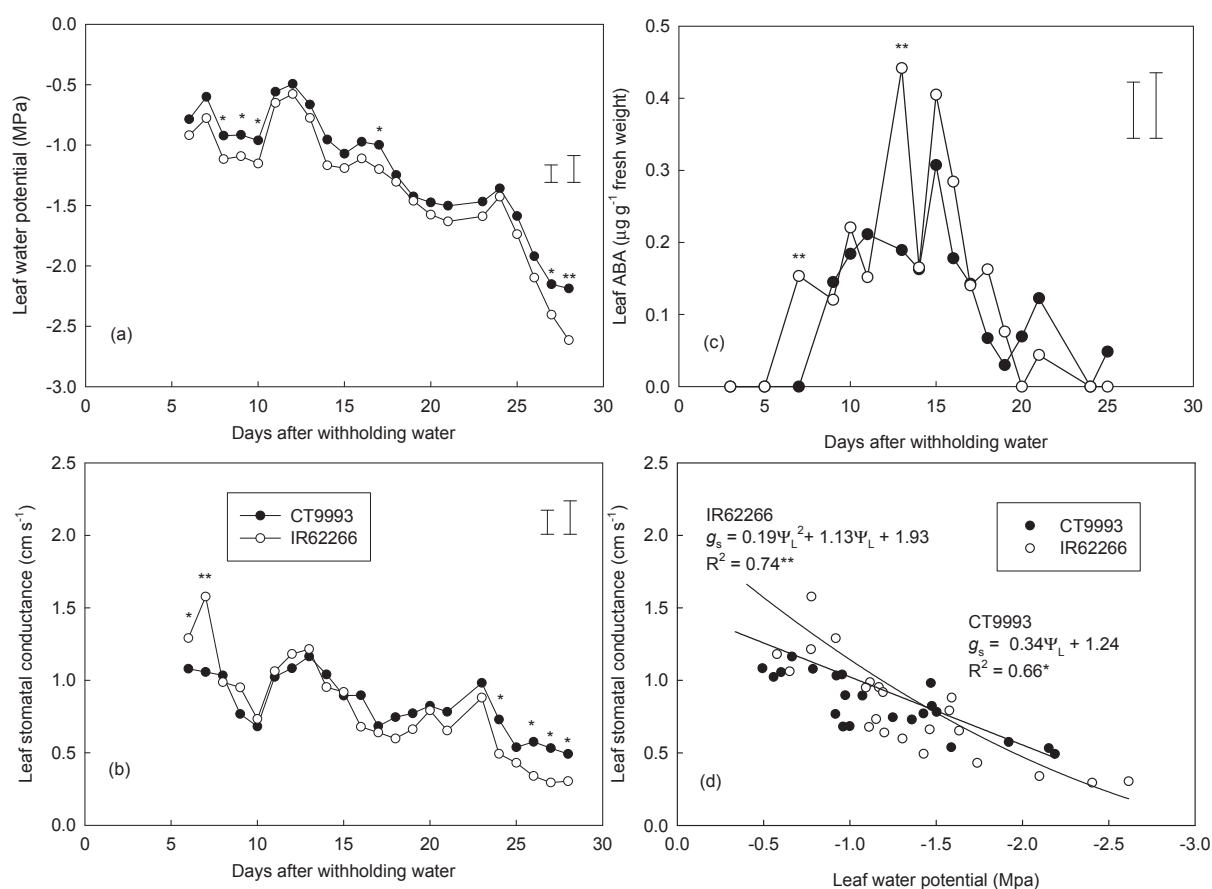
Fig. 6. Time course of (a) leaf water potential, (b) stomatal conductance, (c) leaf ABA concentration, and (d) stomatal conductance in relation to leaf water potential of droughted CT9993 and IR62266 during the drying cycle 69 to 97 DAS in the field. Short and long vertical bars indicate pooled $LSD_{0.05}$ and pooled $LSD_{0.01}$, respectively. $*P<0.05$ and $**P<0.01$.

under S treatment in CT9993 was significantly higher than in IR62266 from 13–16 DAWW (3.24 and 2.45, respectively, $P<0.05$). The relationship between leaf stomatal conductance and leaf water potential for both lines is shown in Fig. 4d. As Ψ_L decreased, g_s decreased significantly ($P<0.05$ and $P<0.01$) in a curvilinear fashion in both lines, with higher values of g_s in CT9993 throughout the range in Ψ_L.

2. Experiment 2
(1) Weather conditions, soil moisture tension (SMT) and water table depth (WTD)

Rainfall was sporadic during both periods of water deficit (41 to 68 DAS and 69 to 97 DAS; data not shown), yet the droughted plots were protected by rainout shelters. Rainy days were generally lower in solar radiation and in vapor pressure deficit. Responses in SMT and WTD were consistent for both periods of water deficit, but are only shown for the second cycle from 69–97 DAS (Fig. 5a, b). SMT rose steadily under the rainout shelters, with the rise being more rapid in the top 10 cm, and with SMT increasing more rapidly in CT9993 than in IR62266 at both 10-cm and 40-

cm depths (Fig. 5a). WTD decreased progressively to reach about 80 cm from the soil surface by the end of the experiment (Fig. 5b), but the lines did not differ significantly in WTD.

(2) Leaf water potential, stomatal conductance and transpiration rate

In the field, lines separated in Ψ_L and g_s at 8–10 and 6–7 DAWW, respectively, with significantly lower values in IR62266 for Ψ_L and CT9993 for g_s (Fig. 6a, b). The lines did not differ significantly in these parameters again until 17, 27 and 28 DAWW for Ψ_L and after 24 DAWW for g_s, when Ψ_L and g_s showed greater and significant decline in IR62266 as water deficit intensified. Leaf ABA concentration was significantly higher in IR62266 at 7 DAWW and again at 13 DAWW, but declined in both lines by 24 DAWW (Fig. 6c).

The relationship between g_s and Ψ_L for both lines is shown in Fig. 6d. As Ψ_L decreased, g_s decreased significantly ($P<0.05$ and $P<0.01$) in both lines, with a more rapid decrease to lower g_s values at high Ψ_L in IR62266, where the curvilinear regression was statistically significant.

(3) Leaf ABA concentration

Leaf ABA concentration was plotted against SMT at 10 cm depth measured from 3 to 13 DAWW (Fig. 7a), when SMT started to increase during early water deficit treatment in the field experiment (Fig. 5a). Leaf ABA concentration increased faster in IR62266 than in CT9993 per unit increase in SMT, attaining double the concentration in IR62266 (P<0.05). Relative leaf water potential (%Ψ_L) and relative stomatal conductance (%g_s) were plotted against leaf ABA concentration from 3 to 16 DAWW; the relative values were calculated from the ratio between daily values and the reference value at 3 DAWW. One data point for %g_s was excluded from the regression for CT9993 at 7 DAWW. While no relationship was found between leaf ABA concentration and %Ψ_L (Fig. 7b), %g_s decreased exponentially with leaf ABA concentration, before starting to rise again at higher leaf ABA concentrations, later during water deficit (Fig. 7c). The lines differed significantly in this relationship, with a greater decline in %g_s in IR62266 as leaf ABA concentration increased.

Discussion

In rainfed lowlands, rice fields are subjected to alternate flooding and drying, based on rainfall and soil characteristics, with the consequent anaerobic and aerobic conditions in the rooting zone (Sharma et al., 1995) providing complexity for root growth and nutrient availability. Compacted soils are also very common in rainfed lowland rice fields and occur in an array of soil textures (Wade, 1996). Pronounced hardpans are usually present immediately beneath the plow layer and they can decrease root growth below them by as much as 60–80% (Kundu et al., 1996). In studying hardpans in pot experiments, Clark et al. (2002) considered wax layers to be analogous to hardpans because they allowed mechanical impedance to be varied independently of water status and aeration. Thus, the use of the split-root systems setup (Fig. 1), with artificial hardpans and two contrasting rice lines, allowed the interactive effects of drought, mechanical impedance and genotype on g_s, Tr and Ψ_L to be examined in the greenhouse, with the field experiment providing an important contrast.

1. Root signals in response to mechanical impedance in the greenhouse

In all water regime and wax layer treatment combinations, all roots were able to penetrate the 3% wax indicating its effectiveness as a control or negligible barrier. Although CT9993 had fewer roots than IR62266, these few roots were able to penetrate the wax layers better than IR62266 (Fig. 2). In this study, CT9993 showed higher hardpan penetration ability than IR62266 regardless of water regime. The percentage of root axes that penetrated the artificial
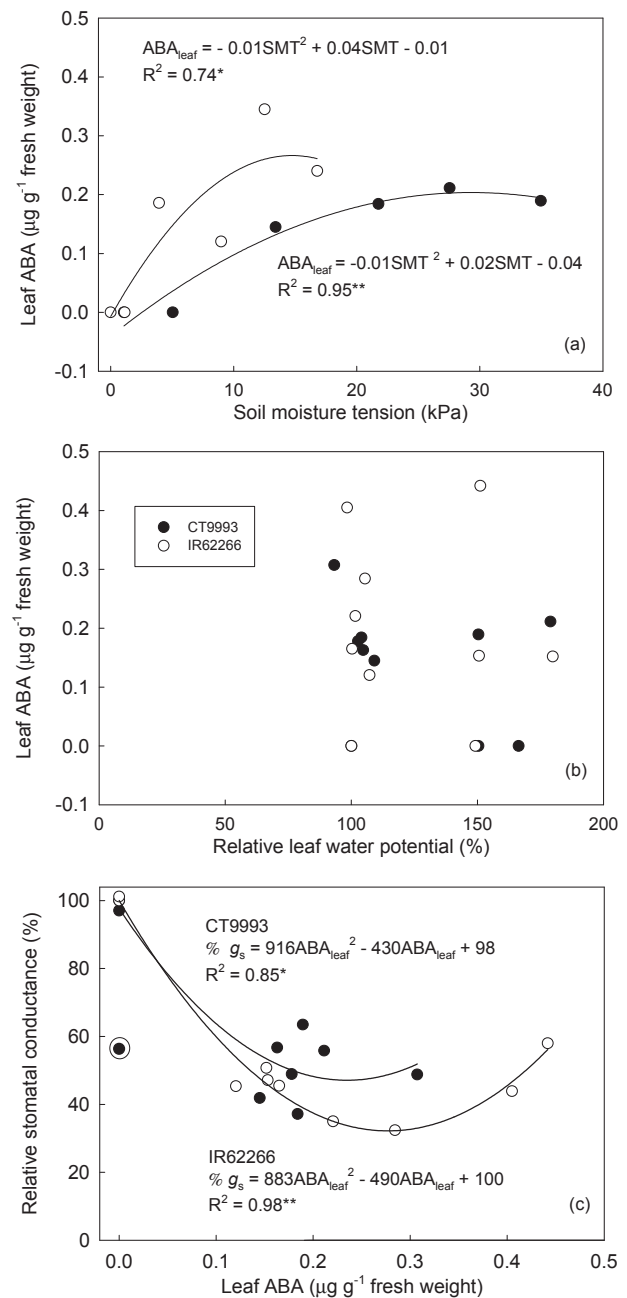

Fig. 7.   (a) Leaf ABA concentration as affected by changes in soil moisture tension at 10 cm depth; (b) leaf ABA concentration in relation to relative leaf water potential and (c) relative stomatal conductance in relation leaf ABA concentration of droughted CT9993 and IR62266 during the drying cycle 69 to 97 DAS in the field. The circled point was excluded from the regression for CT9993 in (c). *P< 0.05 and **P<0.01.

hardpan was greater in CT9993 than in IR62266 under both well-watered (66% and 53%, respectively) and drought treatments (56% and 47%), respectively. The greater inherent ability of CT9993 to elongate roots at depth was also reported by Azhiri-Sigari et al. (2000), and was consistent with field studies (Samson et al., 2002), and other wax-layer experiments (Babu et al.,

2001).

The similar Ψ_L and g_s of the plants grown under both soft and hard wax layers under all water regimes with or without root severing implied a negligible effect of impedance on root signals in this study (Fig. 4a, b, c), which differs from other reports (Mulholland et al., 1996; 1999; Hussain et al., 1999; Roberts et al., 2002). The reason for this discrepancy could be attributed to the numbers of roots escaping between the wax layer and the wall of the cylinder. In future studies, wax layers needed to be sealed to the pot wall, so that roots are not allowed to escape past the wax layer in controlled conditions. Presumably, an insufficient number of roots were impeded relative to those escaping around the wax layer. Consequently, no conclusive evidence on root signaling in response to mechanical impedance is available from this study.

2. Root signals in response to water deficit in the greenhouse

Relative to W, stomatal conductance in D started to decline at around 9 DAWW, before Ψ_L dropped substantially at 11 DAWW (Fig. 4a). These responses were in accord with the results of Siopongco et al. (2008), which implicated leaf ABA as a chemical signal from roots to reduce stomatal conductance under mild drought. Upon severing roots subjected to drought, reductions in both Ψ_L and g_s were observed for one day, which were probably due to wounding, but both parameters resurged strongly and thereafter, continued to increase progressively with time. This resurgence was better observed in g_s of CT9993 than in that of IR62266 (Fig. 4b). The decline in both g_s and Ψ_L with prolonged drought contrasted with their subsequent recovery after severing of roots subjected to water deficit. These responses were in accord with the conclusions of Siopongco et al. (2008), which implicated hydraulic signals in this recovery of Ψ_L and g_s with severing. These results suggest that, in the absence of root signals from shallow roots in drying soils after severing, water uptake from roots in deeper wetter layers could become sufficient to maintain leaf water potential. Whether such putative access of penetrating roots to water in deeper layers is of agronomic benefit would depend upon the magnitude of water storage in deeper soil layers, how long the reserve would last and when the store would be replenished. To do so, roots would need to proliferate quickly in subsoil layers under mild drought (Bañoc et al., 2000 a, b), in order to maintain water extraction and leaf water potential as water deficit increases. For rice to better exploit subsoil water in mild or transient water deficit, selection for reduced root signals may be warranted.

3. Stomatal conductance, leaf ABA concentration and SMT in mild drought

Leaf ABA concentration was directly proportional to SMT, especially during the initial soil drying phase (Fig. 7a). ABA concentration started to increase in leaves of IR62266 at much lower soil moisture tension than in CT9993 (Fig. 7a), attaining consistently higher leaf ABA concentration in IR62266 than in CT9993 in both the greenhouse (Fig. 3) and the field (Fig. 6c). This is further illustrated with relative stomatal conductance (Fig. 7c), which declined more sharply and for longer duration in IR62266, as indicated by the greater magnitude of its regression parameters. As noted previously, stomata respond to root-sourced ABA delivered through the transpiration stream, so leaf ABA concentration is a less sensitive measure of the chemical signal under mild drought. Accordingly, the one data point excluded from the regression for CT9993 at 7 DAWW may represent an early response to xylem-delivered ABA, before a change in leaf ABA could be detected at 9 DAWW (Fig. 6c). Stomatal sensitivity to leaf ABA concentration also seemed to decline as the water deficit progressed (Fig. 7c). Consequently, IR62266 was more sensitive to soil drying, accumulated leaf ABA more rapidly, tended to close its stomata at a much lower soil moisture tension, closed its stomata faster and for longer in mild water deficit than the upland line CT9993.

4. Leaf ABA concentration, transpiration rate and root number in mild drought

In the greenhouse, transpiration rate (Fig. 4c) and stomatal conductance (Fig. 4b) followed similar patterns of response and recovery from root severing. However, the recovery upon severing roots in drying soil was greater in Tr than in g_s, specifically at 13 to 16 DAWW (Fig. 4c), where Tr in severed roots recovered to values of the well-watered treatment in CT9993, but less so in IR62266. With the greater number of roots in IR62266 (Fig. 2), Tr dropped substantially under drought, and did not recover fully to its previous level after severing roots in drying soil. In contrast, the reduction in Tr under drought was still statistically significant in CT9993, but was less severe, and when roots in drying soil were severed, the recovery in Tr to well-watered levels was complete. The magnitude of these responses seemed to be related to root number (Fig. 2) and leaf ABA concentration (Fig. 3). Fewer roots in CT9993 were associated with lower leaf ABA concentration, less reduction in g_s and Tr as water deficit progressed, and greater recovery on severing roots in drying soil. Further study is recommended to identify the number of root tips in drying soil needed to send sufficient signal that elicits stomatal response under mild drought, the critical concentrations of ABA and other factors needed to trigger this response,

and their impact on water use, biomass and crop performance.

5. Root signals in response to severe water deficit in the field

It is important to note, however, that soil volume is limited in pots, and plants in the field may have access to a greater soil volume, potentially revealing additional evidence related to prolonged drought. Consequently, it is vital to compare the greenhouse data with field data, where the duration of effective water deficit was considerably greater (16 d in the greenhouse compared with more than 28 d in the field to drop to $1.0\,\mu g\,cm^{-2}\,s^{-1}$, respectively).

In the field, stomatal conductance declined sharply with the initial increase in leaf ABA concentration (Fig. 6b, c). This decline preceded the observed rise in leaf ABA at 7 DAWW, which was consistent with a stomatal response to root-sourced ABA delivered directly via the xylem, as has been proposed by Ali et al. (1998). We attempted to measure xylem ABA, but were not able to extract sufficient xylem sap, which is difficult to obtain in rice under water deficit (Yano et al., 2006). Values for leaf ABA fluctuated between sampling times, and any initial variation between lines in g_s as leaf ABA first increased at 6–7 DAWW was short-lived, and thereafter, the lines did not differ in g_s until about 24 DAWW, which was after leaf ABA had declined to near zero. A reduced rate of delivery of root-sourced ABA with reduction in hydraulic conductance and Tr, as well as accelerated breakdown when water deficit intensified, may account for low leaf ABA late during water deficit. This was confirmed in Fig. 6d, where the relationship between Ψ_L and g_s only became statistically significant at lower Ψ_L when drought became severe. Further data are needed to fully explore the relationship between critical ABA and other signals and stomatal response, and how they may vary between lines.

In contrast to the greenhouse study, the initial decrease in g_s was rapid in the field trial. Presumably, as roots could access further water from deeper soil profile, the plants were able to continue to maintain a reduced rate of water loss with partially closed stomates for an extended period, and it was only when water supply became more restricted that further reduction in g_s was observed.

From about 24 DAWW, g_s declined further in both lines, with the decline being more severe in IR62266. By this time, soil moisture tension had increased to 80 kPa at 10 cm and more than 50 kPa at 40 cm depth in both lines (Fig. 5a), but with a greater soil moisture tension in CT9993 at 40 cm depth. CT9993 has been reported to have a greater root penetration and proliferation at depth (Babu et al., 2001), which may have assisted access to more soil water from deeper soil layers, thus maintaining its g_s for longer duration (Fig. 6b). As this second decline in g_s occurred after

leaf ABA had already declined to near zero, it is likely that hydraulic signals from the change in hydrostatic pressure played the major role here, as proposed by Liu et al. (2003). Our results suggest a vital role for the maintenance of some hydraulic conductance and gas exchange, and the maintenance of Ψ_L as water deficit progresses, which was more evident in the field with the longer duration of severe water deficit. Continuing functionality would permit recovery on relief from water deficit, but the challenge remains to better match the rate of water loss with the timing of any expected replenishment of water supply.

Furthermore, variation between lines was more significant in the field for the second reduction in g_s (24–28 DAWW), which we have associated with hydraulic conductance as soil drying intensifies. Interestingly, IR62266, the line that was more responsive to ABA in the first cycle (early water deficit) in the greenhouse, was also more responsive to changes in hydraulic conductance in the second cycle (severe water deficit) in the field

Conclusions

These studies have established that as water deficit progressed, stomatal conductance declined more rapidly and to a greater extent in the lowland line, IR62266 than in the upland line, CT9993. Likewise, when roots in drying soil were severed, there was a greater recovery in g_s, and especially in Tr in CT9993 than in IR62266. These responses were in accord with a greater increase in leaf ABA concentration during early water deficit in IR62266, which in turn, seemed to be associated (Fig. 2) with a greater number of roots in drying soil in this lowland *indica* cultivar, which tillers more freely (Azhiri-Sigari et al., 2000; Babu et al., 2001; Samson et al., 2002; Siopongco et al., 2005, 2006). Likewise, as water deficit intensified in the field, there was a greater second decline in g_s and Tr in IR62266, perhaps related to a greater decline in its hydraulic conductance. These responses were consistent with the greater increase in SMT under CT9993 (Fig. 5a), which implied that CT9993 used water faster than IR62266 as drought progressed, but without considerable reduction in g_s, which was in accord with previous reports (Kamoshita et al., 2000, 2004; Wade et al., 1999b). This paper now adds evidence for a genotypic difference in root signals, that may more fundamentally account for the differential adaptive behavior of these contrasting lines under water deficit. Stronger root signals in IR62266 seem to be associated with more conservative water use (Kamoshita et al., 2000; 2004) and broader adaptation across rainfed lowland environments (Wade et al., 1999b). This is consistent with the high sensitivity of lowland rice even to partial soil drying, such as transitory disappearance of surface soil water very early in a period of water deficit (Fukai and Cooper, 1995; Fukai et al., 1995;

Jearakongman et al., 1995). Given a need for rice plants in various environments to exploit soil water at depth (e.g., rainfed lowland) or to ignore transient mild water deficit (e.g., aerobic rice), selection for reduced root signals may be effective. Weaker root signals would be expected to provide a greater advantage in target environments in which it is unlikely that water deficit will be severe, or where water deficit is regularly replenished. These recommendations require further testing and further research is warranted on the implications of root signals for adaptation of rice to contrasting environments.

References

Ali, M., Jensen, C.R., Mogensen, V.O., Andersen, M.N. and Henson, I.E. 1998. Early signals in field-grown wheat in response to shallow soil drying. Aust. J. Plant Physiol. 25 : 871-882.

Angelocci, L.R., Marin, F.R., Oliveira, R.F. De and Righi, E. Z. 2004. Transpiration, leaf diffusive conductance, and atmospheric water demand relationship in an irrigated acid lime orchard. Braz. J. Plant Physiol. 16 : 53-64.

Azhiri-Sigari, T., Yamauchi, A., Kamoshita, A. and Wade, L.J. 2000. Genotypic variation in response of rainfed lowland rice to drought and rewatering. II. Root growth. Plant Prod. Sci. 3 : 180-188.

Babu, R.C., Shashidhar, H.E., Lilley, J.M., Thanh, N.D., Ray, J.D, .Sadasivam, S., Sarkarung, S., O'Toole, J.C. and Nguyen, H.T. 2001. Variation in root penetration ability, osmotic adjustment and dehydration tolerance among accessions of rice adapted to rainfed lowland and upland ecosystems. Plant Breed. 120 : 233-238.

Bano, A., Dörffling, K., Bettin, D. and Hahn, H. 1993. Abscisic acid and cytokinins as possible root-to-shoot signals in xylem sap of rice plants in drying soil. Aust. J. Plant Physiol. 20 : 109-115.

Bañoc, D.M., Yamauchi, A., Kamoshita, A., Wade, L.J. and Pardales Jr., J.R. 2000a. Dry matter production and root system development of rice cultivars under fluctuating soil moisture. Plant Prod. Sci. 3 : 197-207.

Bañoc, D.M., Yamauchi, A., Kamoshita, A., Wade, L.J. and Pardales Jr., J.R. 2000b. Genotypic variations in response of lateral root development to fluctuating soil moisture in rice. Plant Prod. Sci. 3 : 335-343.

Clark, L.J., Aphale, S.L. and Barraclough, P.B. 2000. Screening the ability of rice roots to overcome the mechanical impedance of wax layers : importance of test conditions and measurement criteria. Plant Soil 219 : 187-196.

Clark, L.J., Cope, R.E., Whalley, W.R., Barraclough, P.B. and Wade, L.J. 2002. Root penetration of strong soil in rainfed lowland rice : comparison of laboratory screens with field

performance. Field Crops Res. 76 : 188-198.

Dingkuhn, M., Audebert, A.Y., Jones, M.P., Etienne, K. and Sow, A. 1999. Control of stomatal conductance and leaf rolling in O. sativa and O. glaberrima upland rice. Field Crops Res. 61 : 223-236.

Dunlap, J.R. and Guinn, G. 1989. A simple purification of indole-3-acetic acid and abscisic acid for GC-SIM-MS analysis by microfiltration of aqueous sample through Nylon. Plant Physiol. 90 : 197-201.

Fukai, S. and Cooper, M. 1995. Development of drought-resistant cultivars using physiomorphological traits in rice. Field Crops Res. 40 : 67-86.

Fukai, S., Rajatasereekul, S., Boonjung, H. and Skulkhu, E. 1995. Simulation modelling to quantify the effect of drought for rainfed lowland rice in Northeast Thailand. In : IRRI : Fragile Lives in Fragile Ecosystems. Proceedings of International Rice Research Conference, IRRI, Los Baños, Philippines. 657-674.

Hartung, W., Zhang, J. and Davies, W.J. 1994. Does abscisic acid play a stress physiological role in maize plants growing in heavily compacted soil? J. Exp. Bot. 45 : 221-226.

Hoque, M.M. and Kobata, T. 1998. Growth responses of drought resistant rice cultivars to soil compaction under irrigated and succeeding non-irrigated conditions during the vegetative stage. Plant Prod. Sci. 1 : 183-190.

Hussain, A., Black, C.R., Taylor, L.B. and Roberts, J.A. 1999. Soil compaction. A role for ethylene in regulating leaf expansion and shoot growth in tomato? Plant Physiol. 121 : 1227-1237.

Ismail A.M., Hall A.E. and Bray E.A. 1994. Drought and pot size effects on transpiration efficiency and carbon isotope discrimination of cowpea accessions and hybrids. Aust. J. Plant Physiol. 21 : 23-35.

Jearakongman, S., Rajatasereekul, S., Naklang, K., Romyen, P., Fukai, S., Skulkhu, E., Jumpaket, B. and Nathabutr, K. 1995. Growth and grain yield of contrasting rice cultivars grown under different conditions of water availability. Field Crops Res. 44 : 139-150.

Jia, W. and Zhang, J. 1999. Stomatal closure is induced rather by prevailing xylem abscisic acid than by accumulated amount of xylem-derived abscisic acid. Physiol. Plant. 51 : 501-531.

Kamoshita, A., Wade, L.J. and Yamauchi, A. 2000. Genotypic variation in response of rainfed lowland rice to drought and rewatering. III. Water extraction during drought period. Plant Prod. Sci. 3 : 189-196.

Kamoshita, A., Wade, L.J., Ali, M.L., Pathan, M.S., Zhang, J., Sarkarung, S. and Nguyen, H.T. 2002. Mapping QTLs for root morphology of a rice population adapted to rainfed lowland conditions. Theor. Appl. Genet. 104 : 880-893.

Kamoshita, A., Rodriguez, R., Yamauchi, A. and Wade, L.J. 2004. Genotypic variation in response of rainfed-lowland rice to prolonged drought and rewatering. Plant Prod. Sci. 7 : 406-420.

Kundu, D.K., Ladha, J.K. and Lapitan de Guzman, E. 1996. Tillage depth influence on soil nitrogen distribution and availability in a rice lowland. Soil Sci. Soc. Am. J. 60 : 1153-1159.

Liu, F., Jensen, C.R. and Andersen, M.N. 2003. Hydraulic and chemical signals in the control of leaf expansion and stomatal conductance in soybean exposed to drought stress. Funct. Plant Biol. 30 : 65-73.

MacLean, J.L., Dawe, D.C., Hardy, B. and Hettel, G.P. (Eds.).

2002. Rice Almanac : Sourcebook for the Most Important Economic Activity on Earth, third ed. CABI Publishing, Wallingford, England, Published in association with : International Rice Research Institute, West Africa Rice Development Association, International Center for Tropical Agriculture, and Food and Agriculture Organization of the United Nations. 1-253.

Mulholland, B.J., Black, C.R., Taylor, I.B., Roberts, J.A. and Lenton, J.R., 1996. Effect of soil compaction on barley (*Hordeum vulgare* L.) growth. I. Possible role for ABA as root-sourced chemical signal. J. Exp. Bot. 47 : 539-549.

Mulholland, B.J., Hussain, A., Black, C.R., Taylor, I.B. and Roberts, J.A. 1999. Does root-sourced ABA have a role in mediating growth and stomatal responses to soil compaction in tomato (*Lycopersicon esculentum*)? Physiol. Plant. 107 : 267-277.

O'Toole, J.C. and Maguling, M.A. 1981. Greenhouse selection for drought resistance in rice. Crop Sci. 21 : 325-327.

O'Toole, J.C. 1982. Adaptation of rice to drought-prone environment. In : Drought Resistance in Crops with Emphasis on Rice. IRRI. 195-213.

O'Toole, J.C. and De Datta, S.K. 1986. Drought resistance in rainfed lowland rice. In : Progress in rainfed lowland rice. IRRI. 145-158.

O'Toole, J.C. 2004. Rice and Water : The Final Frontier. The First International Conference on Rice for the Future. Bangkok, Thailand. 26.

Passioura, J.B. 2002. Soil conditions and plant growth. Plant Cell Environ. 25 : 311-318.

Regmi, K.R. 1995. Influence of water deficit and nitrogen limitation on the phenotypic plasticity and root-shoot partitioning in rice cultivars. Ph.D. thesis. Univ. of the Philippines, Los Baños, The Philippines.

Roberts, J.A., Hussain, A., Taylor, I.B. and Black, C.R. 2002. Use of mutants to study long-distance signalling in response to compacted soil. J. Exp. Bot. 53 : 45-50.

Samson, B.K. and Wade, L.J. 1998. Soil physical constraints affecting root growth, water extraction, and nutrient uptake in rainfed lowland rice. In J.K. Ladha, L.J. Wade, A. Dobermann, W. Reichardt, G.J.D. Kirk and C. Piggin eds., Rainfed Lowland Rice : Advances in Nutrient Management Research. IRRI. 231-244.

Samson, B.K., Hasan, M. and Wade, L.J. 2002. Penetration of hardpans by rice lines in the rainfed lowlands. Field Crops Res. 76 : 175-188.

Sharma, P.K., Ingram, K.T. and Harnpichitvitaya, D. 1995. Subsoil compaction to improve water use efficiency and yields of rainfed lowland rice in coarse-textured soils. Soil Tillage Res. 36 : 33-44.

Siopongco, J.DLC., Ingram, K.T., Pablico, P.P. and Moody, K. 1994. Crop and soil responses to post-emergence tillage and weed control in lowland rice. Expl Agric. 30 : 95-103.

Siopongco, J.DLC., Yamauchi, A., Salekdeh, H., Bennett, J. and Wade, L.J. 2005. Root growth and water extraction response

of doubled-haploid rice lines to drought and rewatering during the vegetative stage. Plant Prod. Sci. 8 : 497-508.

Siopongco, J.DLC., Yamauchi, A., Salekdeh, H., Bennett, J. and Wade, L.J. 2006. Growth and water use response of doubled-haploid rice lines to drought and rewatering during the vegetative stage. Plant Prod. Sci. 9 : 141-151.

Siopongco, J.DLC., Sekiya, K., Yamauchi, A., Egdane, J., Ismail, A.M. and Wade, L.J. 2008. Stomatal responses in rainfed lowland rice to partial soil drying; Evidence for Root Signals. Plant Prod. Sci. 11 : 28-41.

Sperry, J.S., Stiller, V. and Hacke, U.G. 2003. Xylem hydraulics and the soil-plant-atmosphere continuum : opportunities and unresolved issues. Agron. J. 95 : 1362-1370.

SPSS Inc. 1997. SYSTAT 7.0 : New Statistics. Chicago, USA. 303.

SPSS Inc. 2001. SPSS for Windows : Release 12. Chicago, USA. 443.

Stiller, V., Lafitte, H.R. and Sperry, J.S. 2003. Hydraulic properties of rice (*Oryza sativa* L.) and the response of gas exchange to water stress. Plant Physiol. 132 : 1698-1706.

Wade, L.J. 1996. Soil compaction - make it or break it for rainfed lowland ecosystem. In Management of Clay Soils in the Rainfed Lowland Rice-based Cropping System. Proceedings Number 70, ACIAR, Canberra. 243-247.

Wade, L.J., George, T., Ladha, J.K., Singh U., Bhuiyan, S.I. and Pandey, S. 1998. Opportunities to manipulate nutrient by water interactions in rainfed lowland rice systems. Field Crops Res. 56 : 93-112.

Wade, L.J., Fukai, S., Samson, B.K., Ali, A. and Mazid, M.A. 1999a. Rainfed lowland rice : Physical environment and cultivar requirements. Field Crops Res. 64 : 3-12.

Wade, L.J., McLaren, C.G., Quintana, L., Harnpichitvitaya, D., Rajatasereekul, S., Sarawgi, A.K., Kumar, A., Ahmed, H.U., Sarwoto, Singh, A.K., Rodriguez, R., Siopongco, J. and Sarkarung, S. 1999b. Genotype by environment interaction across diverse rainfed lowland rice environments. Field Crops Res. 64 : 35-50.

Wade, L.J., Kamoshita, A., Yamauchi, A. and Azhiri-Sigari, T. 2000. Genotypic variation in response of rainfed lowland rice to drought and rewatering. I. Growth and water use. Plant Prod. Sci. 3 : 173-179.

Wopereis, M.C.S. 1993. Quantifying the impact of soil and climate variability on rainfed rice production. PhD thesis. Wageningen (Netherlands) : Wageningen Agricultural University. 188.

Yambao, E.B., Ingram, K.T. and Real, J.G. 1992. Root xylem influence on the water relations and drought resistance of rice. J. Exp. Bot. 43 : 925-932.

Yano, K., Sekiya, N., Samson, B.K., Mazid, M.A., Yamauchi, A., Kono, Y. and Wade, L.J. 2006. Hydrogen isotope composition of soil water above and below the hardpan in a rainfed lowland rice field. Field Crops Res. 96 : 477-480.

Yu, L.X., Ray, J.D., O'Toole, J.C. and Nguyen, H.T. 1995. Use of wax-petrolatum layers for screening rice root penetration. Crop Sci. 35 : 684-687.