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Response of Leaf Water Potential, Stomatal Resistance, and Leaf Rolling to Water Stress

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ABSTRACT

Numerous studies have associated increased stomatal resistance with response to water deficit in cereals. However, consideration of change in leaf form seems to have been neglected. The response of adaxial and abaxial stomatal resistance and leaf rolling in rice to decreasing leaf water potential was investigated. Two rice cultivars were subjected to control and water stress treatments in a deep (1-meter) aerobic soil. Concurrent measurements of leaf water potential, stomatal resistance, and degree of leaf rolling were made through a 29-day period after cessation of irrigation. Kinandang Patong, an upland adapted cultivar, maintained higher dawn and midday leaf water potential than IR28, a hybrid selected in irrigated conditions. This was not explained by differences in leaf diffusive resistance or leaf rolling, and is assumed to result from a difference in root system extent.

Stomatal resistance increased more on the abaxial than the adaxial leaf surface in both cultivars. This was associated with a change in leaf form or rolling inward of the upper leaf surface. Both responses, increased stomatal resistance and leaf rolling, were initiated in a similar leaf water potential range (−8 to −12 bars). Leaves of IR28 became fully rolled at leaf water potential of about −22 bars; however, total leaf diffusive resistance was only about 4 to 5 seconds per centimeter (conductance 0.25 to 0.2 centimeter per second) at that stage. Leaf diffusive resistance and degree of leaf rolling were linearly related to leaf water potential. Thus, leaf rolling in rice may be used as an estimate of the other two less obvious effects of water deficit.

Increased stomatal resistance as a plant response to water stress, has recently been reviewed (1, 3, 22). Investigations of stomatal response to water stress for upland crop species have shown differing adaxial and abaxial stomatal behavior and critical leaf water potential for stomatal response for several species. The variability associated with the critical leaf water potential within and among species has been accounted for in some cases by the influence of leaf age (5, 7), position in the canopy (5, 21), growth stage observed (19), growth conditions (controlled *versus* field environment (8, 24), and pretreatment water stress history (20) on stomatal response.

Relatively little attention has been paid to changes in leaf form or shape, as plant tissues may begin to "wilt" in similar leaf water potential ranges as those associated with stomatal response. In the case of cereals (grasses) which exhibit leaf rolling, the interaction with stomatal behavior and effect on plant water balance may be significant. Although often alluded to, no reports exist which relate leaf rolling in grasses to leaf water potential and stomatal resistance.

The purpose of the current study was to investigate the response of adaxial and abaxial stomata of rice (*Oryza sativa* L.) to decreasing leaf water potential resulting from slow soil moisture depletion. We also wished to evaluate the effect of leaf rolling on stomatal behavior as this overt visual symptom is widely used as an indication of internal plant water deficit.

MATERIALS AND METHODS

Plant Material and Growing Conditions. Two cultivars of rice (*Oryza sativa* L.), Kinandang Patong (KP) and IR28 which represent upland adapted traditional and lowland adapted modern cultivars respectively, were grown in a greenhouse at the International Rice Research Institute (IRRI), Los Baños, Laguna, Philippines. At this latitude (14° North) and with special modifying equipment, intake, ventilation and exhaust fans, the greenhouse environment was near ambient outside air temperature and water vapor pressure deficit thus closely simulating field conditions.

Control plants were grown in large drums (75-cm diameter and 100 cm deep) which had adequate drainage at the base and were kept well watered through the experiment. Four control drums were used for each variety. Two replications were sampled from the control plants at each sampling.

Stress treatment plants were grown in a large well drained concrete tank (6.8 × 3.5 × 1.35 m) which simulated a deep (100-cm) aerobic upland clay-loam soil with 35 cm of subsoil gravel to enhance drainage. Each cultivar was planted to an area of 11.9 m^2, which was subdivided into four sampling plots. In both treatments, rice was directly sown in dry soils in rows 25 cm apart at a rate of 100 kg/ha (10 g/m^2). The crop was established by administering a 50 mm irrigation each time the soil matric potential at 15 cm depth dried to −0.3 bar during the first 42 days after seeding. Two weeks after seeding, plants were thinned to a uniform stand of 95 plants/m of row. After crop establishment and development of full canopy cover, the soil in the stress treatment tank was allowed to dry gradually over a 29-day period in the vegetative growth stage.

Stomatal Resistance. Measurement of stomatal resistance of the upper (adaxial, r_{ad}) and lower (abaxial, r_{ab}) leaf surfaces was made on the second fully developed leaf from the top of the main culm (the same leaf subsequently sampled for leaf water potential) observed to be healthy and representative of the plant canopy. To minimize disturbance, resistances of the upper and lower leaf surfaces were measured in adjacent positions along the mid portion of the leaf with a Lambda diffusive resistance autoporometer model LI-65, with an LI-20S horizontal sensor having an aperture of 3.5 × 20.0 mm.

The total leaf diffusive resistance to water vapor (R_L) was estimated assuming the two leaf surfaces acted as parallel resistors:

$$R_L = \frac{r_{ad} \times r_{ab}}{r_{ad} + r_{ab}}$$

Leaf Water Potential. A portable pressure chamber (16) was used to estimate leaf water potential. Second fully developed leaves from the top of the main culm were placed in an aluminum foil leaf holder, lined with moist cheese cloth, and then excised at the leaf collar. Leaves were continuously protected by the leaf holder until the measurement was complete. The pressure chamber used N_2 gas at a pressure increase rate of 22 kg cm^{-2} min^{-1}. The equilibrium pressure required to bring water to the cut leaf collar cross-section was recorded as the leaf water potential.

Leaf Rolling Score. Prior to stomatal resistance and leaf water potential measurements, a visual score of the degree of leaf rolling or folding was made on the sample leaf using a 1 to 5 scale with 1 being the first evidence of rolling and 5 being a closed cylinder.

Concurrent measurements of upper and lower stomatal resistance, leaf water potential, and scoring for leaf rolling were made on each sample leaf. Sample leaves were taken from two replications (average of two observations per drum or plot) of the control and four replications of the stress treatment for each measurement period. Measurements were made at 0500 (dawn) and 1300 (midday) h on 12 days of the 29-day stress period.

RESULTS

The 29-day stress period resulted in a slow drying of soil and plants. Figure 1 illustrates the progression of leaf water potential through the drying period. The leaf water potentials of IR28 measured at dawn began to deviate from controls about 12–14 days after stress treatment initiation. Midday values were lower than controls as early as 8–10 days after treatment initiation for both cultivars. Dawn and midday leaf water potentials were lower for IR28 than KP after day 10. Both cultivars exhibited a trend of decreased leaf water potential in response to the stress. Leaf water potential response between the cultivars continued to diverge as stress progressed. When the experiment ended, leaf water potential differed between the cultivars by about 5 bars at dawn and 12 bars at midday.

Figure 2 illustrates the change in midday upper and lower leaf surface stomatal resistance during the treatment period. Again, the divergence in stress and control treatments appears about 10–12 days after treatment. This point is more obvious in IR28 as was the case for decrease in midday leaf water potential (Fig. 1). At that time, 12 days after water was withheld, midday leaf water potential of IR28 was about −12 to −14 bars and KP −9 to −11 bars. No distinction for "critical" leaf water potential was evident between cultivars. Thus, it appears that upper and lower stomata of rice leaves began to respond at midday leaf water potentials of −10 to −13 bars.

The trend of leaf rolling score (Fig. 3) also shows a response at about 10 days after withholding irrigation water. The controls remained unrolled while the degree of leaf rolling in the two cultivars became progressively more pronounced. The difference in response of the two cultivars is similar in timing and degree to that of leaf water potential and stomatal resistance.

DISCUSSION

The two rice cultivars were exposed to the same initial soil and atmospheric condition, however they differed markedly in their

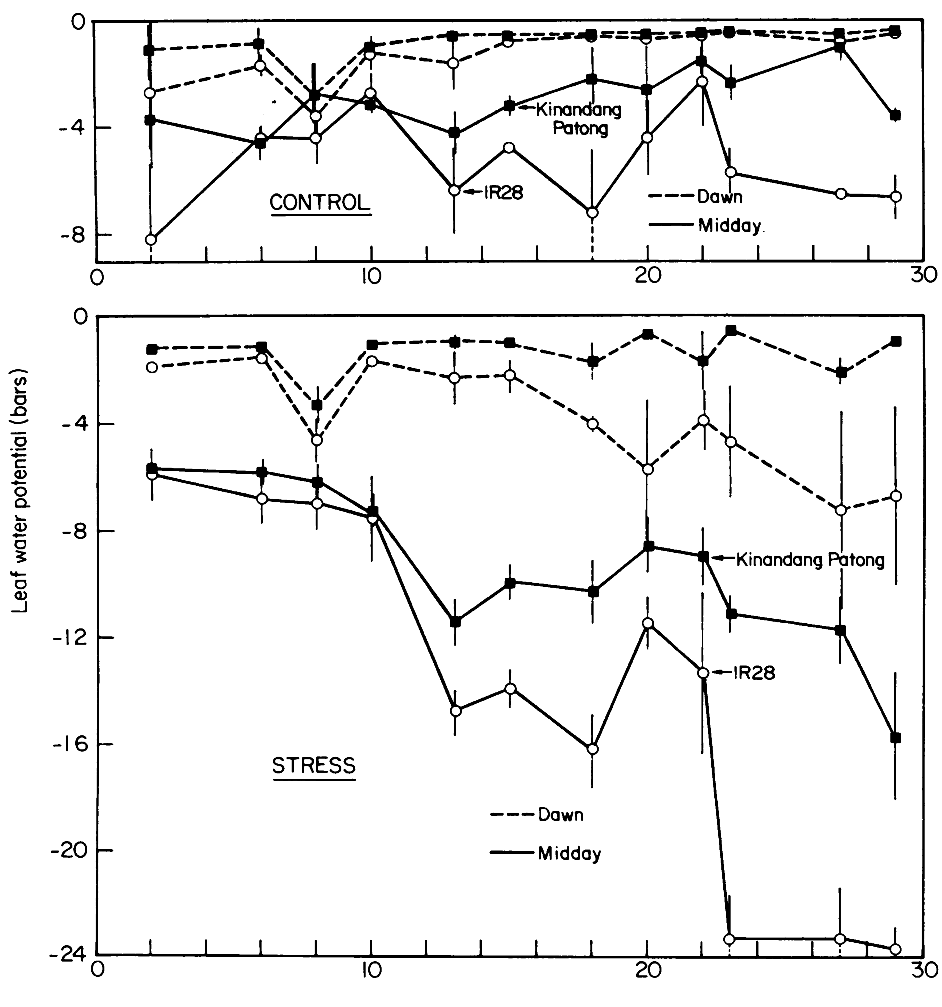

FIG. 1. Dawn (0500 h) and midday (1300 h) estimates of leaf water potential of two rice cultivars (IR28 and Kinandang Patong) subjected to well watered (control) and water stress treatments during a 29-day period. (All data points ± SE.)

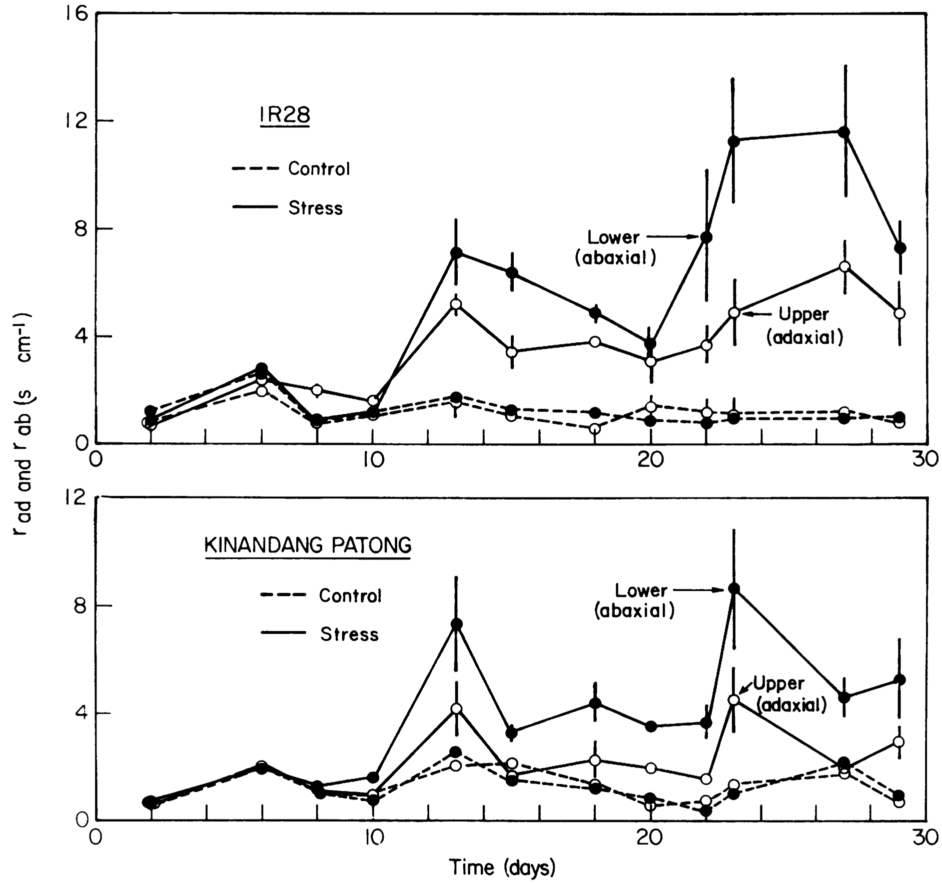

FIG. 2. Diffusive resistance of upper (r_{ad}) and lower (r_{ab}) leaf surfaces of two rice cultivars (IR28 and Kinandang Patong) subjected to well watered (control) and water stress treatments during 29-day period. (All data points ± SE.)

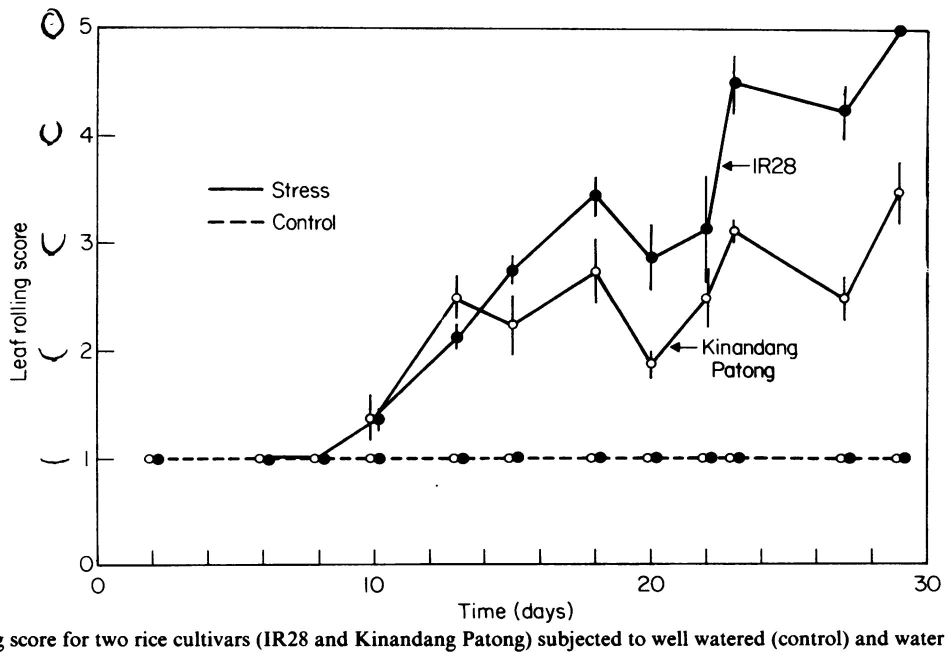

FIG. 3. Leaf rolling score for two rice cultivars (IR28 and Kinandang Patong) subjected to well watered (control) and water stress treatments during 29-day period. (All data points ± SE.)

ability to maintain relatively high leaf water potential, both in terms of rehydration overnight (dawn measurement) and capability to maintain leaf water potential during midday periods of peak evaporative demand (Fig. 1). This was also reflected in the response of other leaf water potential dependent parameters measured: stomatal resistance and leaf rolling. The dawn leaf water potential was thought to be a good estimate of soil water potential with which the cultivar had come into equilibrium during the dark period. Although KP showed a decreasing trend in midday leaf water potential as did IR28, KP rehydrated to higher dawn leaf water potentials throughout the drying period. Examination of the degree of stomatal resistance and leaf rolling response between the cultivars does not explain higher dawn and midday leaf water potentials of KP by greater capability to decrease transpirational water loss. Thus, KP, an upland adapted rice cultivar, probably possesses a superior root system for water absorption allowing better rehydration during night hours and relatively higher water potential during periods of peak evaporative demand. O'Toole and Moya (13) illustrated genotypic variability for the maintenance of leaf water potential among rice cultivars and showed that it was highly correlated to visual drought scoring in rice. Reports of this type of intraspecies difference in maintenance of leaf water potential are few. Blum (2) illustrated differences in maintenance of leaf water potential and stomatal resistance of sorghum cultivars grown in declining soil moisture in Israel.

The stomatal resistance of the upper and lower leaf surfaces differed in response to water stress. As the midday leaf water potential values became more negative the corresponding resistance of the lower leaf surface increased more than the upper surface (Fig. 2). This response is contrary to several other reports on crop species; cotton (7, 17), dry bean (9), and soybean (19). Few reports, however, are available for grasses or cereals. Shimshi (18) reported that upper leaf surface stomata of sorghum closed "earlier" than those of the lower surface. He noted that upper stomatal closure occurred before any visible sign of water stress, such as transient wilting, was evident. However, no estimate of leaf water status was made.

In rice the difference in upper and lower leaf surface stomatal resistance may be an interaction with leaf rolling. In Figures 2 and 3, trends of leaf rolling and stomatal resistance at midday are closely related to leaf water potential. The upper leaf surface resistance is not as responsive to stress as leaf rolling increases. When the upper leaf surface is rolled inside as the leaf's bulliform tissues lose turgor, the upper surface stomata may be responding to a modified microclimate with less incident solar radiation, lower evaporative demand, and possibly lower CO_2 concentration. Figures 2 and 3 also illustrate the effect of stomatal resistance and leaf rolling on the fall of midday water potential seen in Figure 1. The stabilizing effect occurring between days 13 and 22 coincides with the observation of increases in both parameters.

Leaf rolling as a concomitant response of increased stomatal resistance to decreasing leaf water potential has received little attention in physiological studies of grass or cereal species. Parker (14) stated that leaf rolling reduced transpiration in *Stipa tenacissima* and that some Mediterranean grasses reduced transpiration as much as 46 to 63% by rolling. Parker also mentioned that in many species leaf rolling does not occur until the water content has been reduced to lethal levels. The two rice cultivars used in the current study began to roll at relatively high water potentials. Figure 4 illustrates the relationship between leaf rolling and leaf water potential. Both cultivars show a leaf rolling response at leaf water potentials as high as -8 to -10 bars. Full leaf rolling occurred at -20 to -25 bars leaf water potential in IR28 while KP did not reach full rolling. Assuming that decreased exposure of transpirational leaf surface area affects water loss (6, 15), the half-rolling of leaves (leaf rolling score 3) at water potentials of -12 to -15 bars may be a significant additional means of inhibiting water loss from grass species and avoiding severe tissue water deficits (12). The fact that leaf water potential, stomatal resistance, and leaf rolling change together causes difficulty in evaluating the role of leaf rolling in maintenance of leaf water potential.

In rice, leaf rolling begins at relatively high leaf water potentials and progresses across a wide water potential range. The initiation of leaf rolling in rice should not be associated with loss of bulk tissue turgor pressure but only that of the specialized bulliform cell tissues associated with the lateral extensibility of the leaf. Net photosynthesis and translocation of assimilates need to be investigated in relation to this overt symptom of water stress in cereal species.

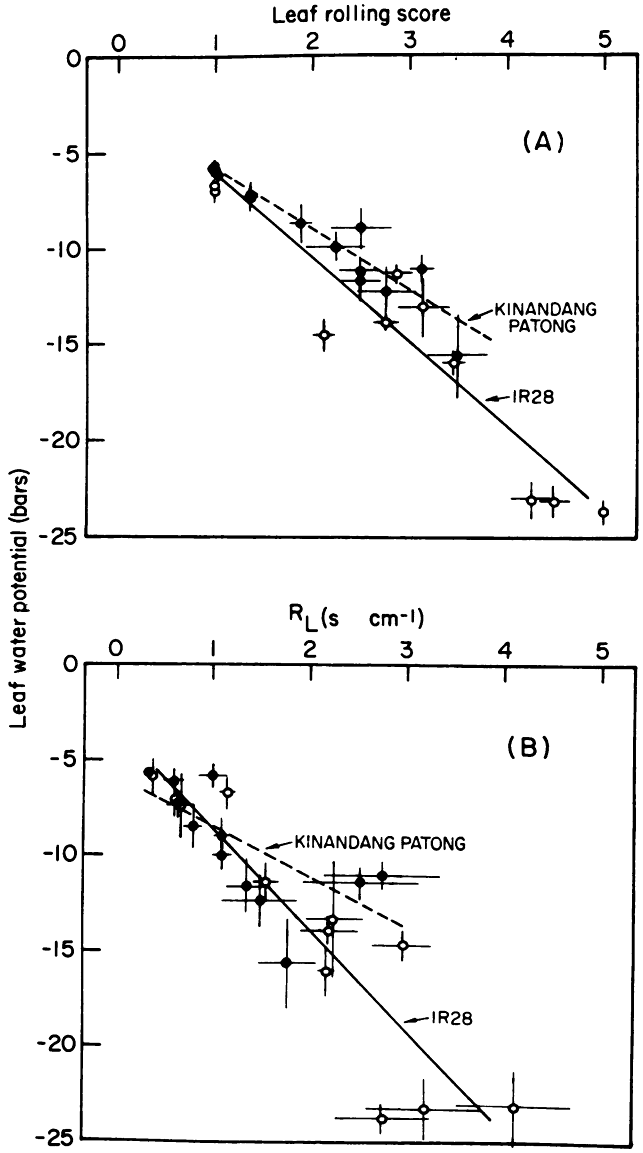

FIG. 4. Relationship between leaf water potential and leaf rolling score (A) and leaf water potential and total leaf diffusive resistance (B) for two rice cultivars subjected to a 29-day drying period. Only midday measurements (N = 12) from stress treatment are plotted. All data ± SE. (A) x = leaf rolling score: IR28$\hat{y} = -1.82-4.47\ (x)$, $r = 0.95^{**}$, KP$\hat{y} = -2.65-3.18\ (x)$, $r = 0.93^{**}$; (B) $x = R_L$: IR28$\hat{y} = -3.29-5.27\ (x)$, $r = 0.81^{**}$, KP$\hat{y} = -5.89-2.69\ (x)$, $r = 0.48$.

Leaf rolling may be a useful indicator of leaf water potential in rice (Fig. 4) and is currently used by breeders selecting for avoidance of water stress in rice (4, 10, 11, 13). Inasmuch as this character is related to the osmotic and turgor pressure components of water potential in specialized bulliform tissues, its use as an indicator of leaf water potential may be jeopardized by the same factors giving rise to variability or shift in water potential thresholds for stomatal closure: leaf age, position, and water stress history.

Generally, there is a critical leaf water potential below which stomata close rapidly over a relatively narrow water potential range (1, 3, 23). In the current study, a critical leaf water potential for stomatal response was not identifiable although leaf diffusion resistance deviated from control levels at about -10 to -12 bars midday leaf water potential 10 to 12 days after water was withheld. The second feature, rapid stomatal closure over a relatively narrow range, was not observed. Attempts were made on five dates throughout the 29-day period to document the stomatal resistance-leaf water potential relationship by intensive diurnal sampling. When these observations were added to Figure 4, they fell on the linear trend although their addition increased the deviation from regression. Figure 4 illustrates the broad range of midday water potential over which total leaf diffusive resistance continued to increase. The gradual increase in stomatal resistance seen in Figure 4, as opposed to rapid closure over a narrow range of leaf water potential, may be a function of adjustment or adaption to the slow progression of soil and plant water deficit imposed in this experimental procedure.

The two rice cultivars appear to differ slightly with respect to the slope relating leaf rolling and leaf resistance to leaf water potential (Fig. 4). In both cases the slope for IR28 is steeper, however only in the leaf diffusive resistance-leaf water potential relationship do the slopes of the cultivars differ significantly (0.05 level). These differences may indicate a greater sensitivity to leaf water potential in KP, the upland adapted cultivar.

Stomatal "closure" is an ambiguous term which refers to initiation of increased resistance in response to decreasing leaf water potential. Midday rice leaf diffusive resistance did not actually reach high levels typical of "closed" stomata of upland crop species. That the stomata were not actually closed may be verified by the fact that predawn total resistances were about 9 to 15 s cm^{-1}. In Figure 4, midday total leaf resistance was only about 4 s cm^{-1} (conductance of 0.25 cm/s) while values of 12 to 20 s cm^{-1} (conductance of 0.08–0.05 cm/s) are common for other crop species at comparable water potentials (20, 21). Such general statements must be made cautiously, however, in light of the variability in the leaf water potential-leaf diffusive resistance relationship attributable to stress prehistory.

Alternatively, a semiaquatic species, such as rice, may not respond in the same manner as a well adapted upland species. In this case, Figure 4 may represent rice's lack of stomatal responsiveness to decreasing leaf water potential. Comparative literature on the stomatal resistance-leaf water potential relationship of rice or other semiaquatic species is unknown to the authors.

The two rice cultivars showed different time courses for the development of plant water deficits. This difference was attributed chiefly to a better water absorption system in the upland adapted cultivar as demonstrated by its maintenance of leaf water potential, especially when observed at dawn. Contrary to reports of many crop species, with decreasing leaf water potential, the upper leaf surface had less diffusive resistance than the lower leaf surface. Rolling inward of the upper leaf surface at the same time in the drying period and same leaf water potential values as increased stomatal resistance illustrates a strong interaction between stomatal resistance and decreased exposure of transpirational leaf surface area as water stress induced adaptive mechanisms in this grass species. From the current results, the relative value of these two responses as adaptive mechanisms to impede water loss during decreasing leaf water potential cannot be evaluated. Total leaf diffusive resistance remained relatively low at leaf water potentials of about −22 bars. This may be alternately interpreted as a result of adjustment or shift in the stomatal resistance-leaf water potential relationship over the slow drying period or as an indication that rice stomata are less sensitive than some other crop species to decreasing leaf water potential.

LITERATURE CITED

1. Begg JE, NC Turner 1976 Crop water deficits. Adv Agron 28: 161–217
2. Blum A 1974 Genotypic responses in sorghum to drought stress. I. Response to soil moisture stress. Crop Sci 14: 361–364
3. Burrows FJ, FL Milthorpe 1976 Stomatal conductance in the control of gas exchange. *In* TT Kozlowski, ed, Water Deficits and Plant Growth, Vol IV. Academic Press, New York, pp 103–152
4. Chang TT, GC Loresto, O Tagumpay 1972 Agronomic and growth characteristics of upland and lowland rice varieties. *In* Rice Breeding, International Rice Research Institute, Los Baños, Philippines
5. Frank AB, JF Power, WO Willis 1973 Effect of temperature and plant water stress on photosynthesis, diffusion resistance and leaf water potential in spring wheat. Agron J 65: 777–780
6. Gates DM 1968 Transpiration and leaf temperature. Annu Rev Plant Physiol 19: 211–238
7. Jordan WR, KW Brown, JC Thomas 1975 Leaf age as a determinant in stomatal control of water loss from cotton during water stress. Plant Physiol 56: 595–599
8. Jordan WR, JT Ritchie 1971 Influence of soil water stress on evaporation, root absorption and internal water status of cotton. Plant Physiol 48: 783–788
9. Kanemasu ET, CB Tanner 1969 Stomatal diffusion resistance in snap beans. I. Influence of leaf water potential. Plant Physiol 44: 1547–1552
10. Loresto GC, TT Chang, O Tagumpay 1976 Field evaluation and breeding for drought resistance. Philippine J Crop Sci 1: 36–39
11. O'Toole JC, TT Chang 1979 Drought resistance in cereals: rice, a case study. *In* H Mussell and RC Staples, eds, Stress Physiology of Crop Plants. Wiley-Interscience, New York, pp 373–405
12. O'Toole JC, RT Cruz, TN Singh 1979 Leaf rolling and transpiration. Plant Sci Letters 16: 111–114
13. O'Toole JC, TB Moya 1978 Genotypic variation in maintenance of leaf water potential in rice. Crop Sci 18: 873–876
14. Parker J 1968 Drought-resistance mechanisms. *In* TT Kozlowski, ed, Water Deficits and Plant Growth, Vol I. Academic Press, New York
15. Ritchie JT, E Burnett 1971 Dryland evaporative flux in a subhumid climate. II. Plant influences. Agron J 63: 56–62
16. Scholander PF, HT Hammer, ED Bradstreet, EA Hemmingsen 1965 Sap pressure in vascular plants. Science 148: 339–346
17. Sharpe PJH 1973 Adaxial and abaxial stomatal resistance of cotton in the field. Agron J 65: 570–574
18. Shimshi D 1964 The use of a field porometer for the study of water stress in plants. Israel J Agric Res 14: 137–143
19. Sionit H, PJ Kramer 1976 Water potential and stomatal resistance of sunflower and soybean subjected to water stress during various growth stages. Plant Physiol 58: 537–540
20. Thomas JC, KW Brown, WR Jordan 1976 Stomatal response to leaf water potential as affected by preconditioning water stress in the field. Agron J 68: 706–708
21. Turner NC 1974 Stomatal behavior and water status of maize, sorghum and tobacco under field conditions. II. At low soil water potential. Plant Physiol 53: 360–365
22. Turner NC 1974 Stomatal response to light and water under field conditions. *In* RL Bieleski, AR Ferguson, MM Cresswell, eds, Mechanisms of Regulation of Plant Growth. Bull 12, R Soc New Zealand, pp 423–432
23. Turner NC 1979 Drought resistance and adaptation to water deficits in crop plants. *In* H Mussell, RC Staples, eds, Stress Physiology of Crop Plants. Wiley-Interscience, New York, pp 343–372
24. Turner NC, JE Begg 1973 Stomatal behavior and water status of maize, sorghum and tobacco under field conditions. I. At high soil water potential. Plant Physiol 51: 31–36