

# Influence of Osmotic Adjustment on Leaf Rolling and Tissue Death in Rice (*Oryza sativa* L.)

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## ABSTRACT

Osmotic adjustment, measured by the lowering of the osmotic potential at full turgor, and its influence on leaf rolling and leaf death was assessed in the lowland rice (*Oryza sativa* L.) cultivar IR36 in both the greenhouse and field. The degree of osmotic adjustment varied with the degree and duration of stress, but was usually 0.5 to 0.6 megapascal (maximally 0.8 to 0.9 megapascal) under severe stress conditions. In leaves in which osmotic adjustment was 0.5 to 0.6 megapascal, leaf rolling and leaf death occurred at lower leaf water potentials in adjusted than in nonadjusted leaves. We conclude that osmotic adjustment aids in the drought resistance of rice by delaying leaf rolling, thereby maintaining gas exchange, and by delaying leaf death.

Several plant processes are affected by loss of turgor during drought (3, 9). Osmotic adjustment, the lowering of osmotic potential by the net increase of intracellular solutes, is now recognized as an adaptive mechanism of many crops to water stress (14, 27, 28). It allows the maintenance of turgor at lower water status, which in turn enables plants to maintain processes such as cell enlargement and stomatal opening (10, 28). Osmotic adjustment has now been observed in several crop species (28). Greenhouse studies on four cultivars of upland rice (*Oryza sativa* L.) have shown that osmotic adjustment of 0.5 to 0.8 MPa occurs in this species (5, 6) and that osmotic adjustment caused leaf elongation to decrease and stop at lower predawn leaf water potentials than in nonosmotically adjusted plants (7).

Leaf rolling and leaf or tiller death also occur as a result of water deficits. Both symptoms are used as criteria in visual scoring of rice varieties being screened for drought resistance (11, 16). Visual drought resistance scores, in particular leaf rolling, are correlated with leaf water potential (20), but since leaf rolling depends on the turgor potential of the leaf bulliform cells, a shift in the osmotic component of the leaf water potential should influence this overt symptom of water stress. Solute accumulation, particularly the increase in sugars, has also been implicated as a factor in increased protoplasmic tolerance for water deficits (15), which may be manifest in an altered relationship between tissue death and tissue water relations.

This paper reports on the degree of osmotic adjustment in a lowland rice cultivar subjected to water deficits and examines the role of osmotic adjustment on leaf rolling and leaf tissue death.

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## MATERIALS AND METHODS

Rice (*Oryza sativa* L. cv IR36) was studied in four experiments, two in the field and two in a greenhouse, at the International Rice Research Institute, Los Baños, Philippines.

In the first experiment, the plants were grown in 18 containers, 0.75 m in diameter, 0.325 m deep, containing 100 L of a well fertilized Maahas clay loam soil. On September 10, seed was sown in 12 locations in each container and grown under well-watered soil conditions for 45 d, after which water was withheld from half of the containers. The remaining half were maintained under well-watered conditions throughout the course of the experiment. Predawn and midday leaf water potentials and turgid osmotic potentials were measured in both the irrigated and unirrigated plants for 41 d after water was withheld from the unirrigated treatment.

In the second greenhouse experiment, pregerminated rice was sown in 10 containers, 0.75 m in diameter and 1.0 m deep, containing a well-fertilized Maahas clay loam soil. On 19 June, 90 seedlings/m of row were planted in 3 rows, 0.2 m apart. Water was withheld from half of the containers 27 d after planting, whereas the remaining containers were watered daily. Predawn and midday leaf water potentials and turgid osmotic potentials were measured 27, 28, and 29 d after the stress treatment was imposed. Samples were also obtained to establish the relationship between leaf death and leaf water potential.

In the first field experiment, the experimental plots of Puckridge and O'Toole (23) were utilized. The plants were sown on December 19 in plots 12 m long and 3 m wide. The soil at the site, a Maahas clay loam, was fertilized at planting and again 54 d from planting (23). The plots were watered uniformly for the first 23 d after planting; thereafter, a line source sprinkler system (8) maintained a continuously decreasing water application rate over the 12-m plot length (4). Midday leaf water potentials and turgid osmotic potentials were measured 57 and 64 d after the differential irrigation treatment was imposed in plots located 0 to 1.5 m, 4.5 to 6.0 m, 7.5 to 9 m, and 10.5 to 12 m from the point of highest water application.

In the second field experiment, the experimental plots of Angus *et al.* (1) were used. The plants were sown on November 30 in 10 × 14-m plots on a site adjacent to that used in the first field study. One plot was irrigated regularly by sprinkler irrigation while the second plot was not. Predawn and midday leaf water potentials were measured in both the irrigated and unirrigated plots on four occasions between 43 and 69 d from sowing. Seventy-one d after sowing, the plants in the irrigated and unirrigated plots were sampled for turgid osmotic potentials and to establish the relationship between leaf rolling and leaf water potential.

Main tillers were cut below water at 0700 h and kept under a polyethylene sheet in distilled H<sub>2</sub>O at low light for 2 to 3 h until rehydrated. To establish the relationship between leaf rolling and leaf water potential, the rehydrated tillers were then air-dried in

the laboratory to achieve different leaf rolling scores from 1 (fully open) to 7 (tightly rolled), and the leaf water potential was measured on each leaf scored for leaf rolling. To establish the relationship between leaf death and leaf water potential, the rehydrated tillers were desiccated in the laboratory until the leaves showed different degrees of irreversible dehydration. During desiccation on the laboratory bench, rice leaf tissue dried from the leaf tip to the basal portion. A distinct visual and tactile change was apparent in the leaf segment that had irreversibly dehydrated which allowed a simple and accurate estimate of per cent leaf death. Leaf length irreversibly dehydrated as a percentage of the total leaf length was used as the measure of leaf death. The water potential was then measured on each leaf scored for leaf death.

Predawn and midday water potentials on the second youngest, fully developed leaf from a main tiller were measured with a Soilmoisture (Soilmoisture Equipment Corp., Santa Barbara, CA) model 3000 pressure chamber. The leaf was excised about 25 mm below the collar and immediately wrapped in a moist cheesecloth to prevent water loss during measurement (21). Procedures were similar for the leaf water potentials of leaves sampled for leaf rolling and leaf death. All samples for the measurement of turgid osmotic potentials were collected at 0700 h. The main tillers were cut below the nodes under water to avoid cavitation and then kept in distilled H<sub>2</sub>O for 2 to 3 h. The second youngest, fully rehydrated leaf was excised and about 7 cm of it was rolled on a pencil and sealed in a small polyethylene bag. The bag was immersed in liquid N<sub>2</sub> for 30 s and then the tissue was allowed to thaw for 5 to 10 min. A 1.7 × 0.5-cm piece of filter paper was then placed on top of the tissue, the bag was resealed, and the filter paper was saturated with sap expressed by a Campbell (Campbell Scientific, Inc., Logan, UT) model J-14 press pressurized to 3.5 MPa for 1 min. The filter paper was inserted into a Merrill (J. R. D. Merrill Specialty Equipment, Logan, UT) model 75-1 psychrometer cup connected to a Wescor (Wescor Inc., Logan, UT) HR33T dewpoint microvoltmeter operating in the psychrometric mode and read after equilibration for 1 to 3 h. The psychrometers were calibrated with NaCl solutions of known potential.

## RESULTS

**Osmotic Adjustment.** In the greenhouse study, water deficits developed slowly in the unirrigated plants with little change in

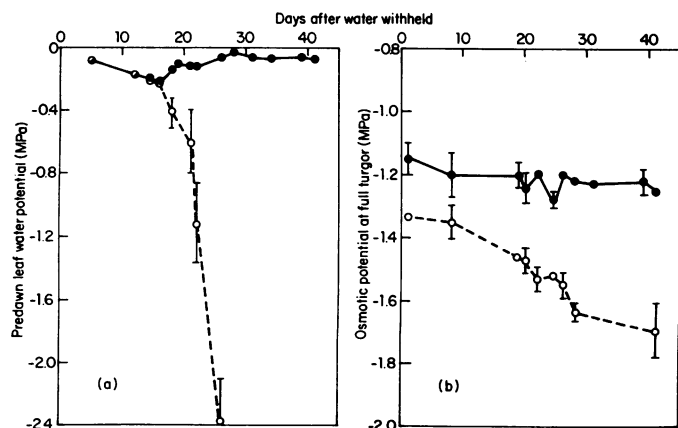


FIG. 1. Change with time in (a) the predawn leaf water potential and (b) the osmotic potential at full turgor in irrigated (●) and unirrigated (○) rice (IR36) in a greenhouse. Values are means  $\pm$  1 SE of the mean of five observations; standard errors of predawn leaf water potentials and osmotic potentials less than 0.05 and 0.025 MPa, respectively, are omitted for clarity.

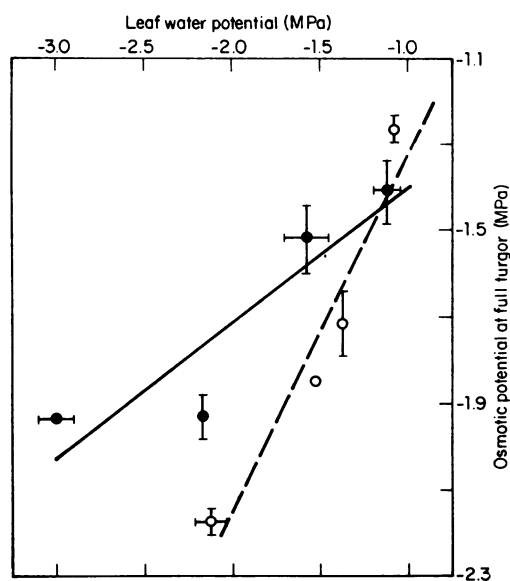


FIG. 2. Relationship between the osmotic potential at full turgor ( $\Psi_{\pi f}$ ) and midday leaf water potential ( $\Psi$ ) in rice (IR36) subjected to four levels of irrigation in the field by a line source sprinkler system. Data obtained 57 (●) and 64 (○) days after establishment of the differential irrigation are presented. The lines give the fitted linear regressions:

- (a), for day 57,  $\Psi_{\pi f} = 0.31 \Psi - 1.09$  ( $r = 0.91$ );  
 (b), for day 64,  $\Psi_{\pi f} = 0.81 \Psi - 0.50$  ( $r = 0.95^*$ ).

Bars give  $\pm$  1 SE of the mean of three observations; standard errors of leaf water potential and osmotic potential less than 0.05 and 0.025 MPa, respectively.

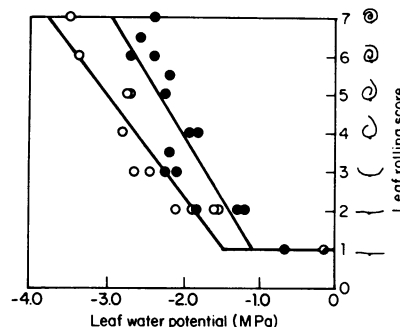


FIG. 3. Relationship between leaf rolling score ( $R$ ) and leaf water potential ( $\Psi$ ) in irrigated (●) and unirrigated (○) rice measured 71 d after planting. The lines give the fitted linear regressions to the data over a leaf rolling score of 1:

- (a), irrigated,  $R = -3.2 \Psi - 2.38$  ( $r = 0.80^{**}$ );  
 (b), unirrigated,  $R = -2.5 \Psi - 2.67$  ( $r = 0.92^{**}$ ).

predawn leaf water potential for the first 16 d and thereafter, steadily decreased to  $-2.4$  MPa 26 d after water was withheld (Fig. 1a). The osmotic potentials at full turgor in the unirrigated plants decreased significantly below the irrigated controls 20 d after water was withheld and continued to decrease with increasing water deficits until 41 d after water was withheld, when the osmotic adjustment, *i.e.* the difference in osmotic potential at full turgor between the control and stressed plants, was about 0.5 MPa (Fig. 1b).

In the field study, the total amount of water received by the plots across the line source decreased with distance from the line, such that the rice plants suffered a greater degree of stress the farther away they were from the line source sprinkler (4, 23). Thus, the plants 0 to 1.5 m from the line were well watered, those 4.5 to 7.5 m from the line were mildly stressed, and those

Table I. Predawn and Midday Leaf Water Potentials and the Leaf Osmotic Potentials at Full Turgor in Unirrigated and Irrigated Rice (cv IR36) Grown in the Greenhouse on Three Days after Water was Withheld from the Unirrigated Plants

Values are means  $\pm$  1 SE of the mean of five observations.

	Days after Water Withheld					
	27		28		29	
	Unirrigated	Irrigated	Unirrigated	Irrigated	Unirrigated	Irrigated
Predawn leaf water potential (MPa)	$-0.57 \pm 0.13$	$-0.12 \pm 0.02$	$-0.46 \pm 0.13$	$-0.12 \pm 0.02$		
Midday leaf water potential (MPa)	$-2.13 \pm 0.16$	$-1.42 \pm 0.04$	$-1.99 \pm 0.09$	$-1.29 \pm 0.09$	$-2.03 \pm 0.10$	$-1.30 \pm 0.10$
Turgid osmotic potential (MPa)	$-1.59 \pm 0.04$	$-1.13 \pm 0.03$	$-1.62 \pm 0.06$	$-1.10 \pm 0.05$	$-1.71 \pm 0.04$	$-1.16 \pm 0.04$

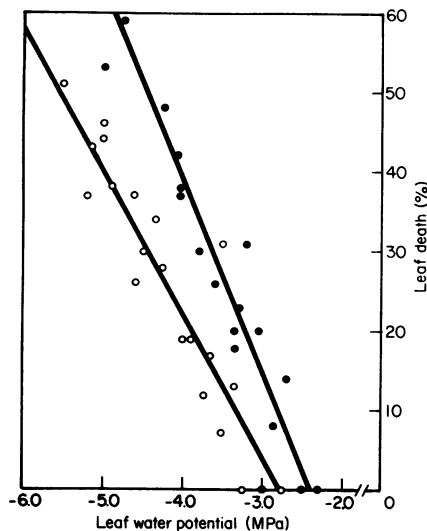


FIG. 4. Relationship between percentage leaf death ( $D$ ) and leaf water potential ( $\Psi$ ) of irrigated (●) and unirrigated (○) rice (IR36) grown in a greenhouse. The lines give the fitted linear regressions:

(a), irrigated,  $D = -24.1 \Psi - 58$  ( $r = 0.95^{**}$ );

(b), unirrigated,  $D = -17.7 \Psi - 49$  ( $r = 0.90^{**}$ ).

10.5 to 12 m from the line were severely stressed (23). The relationship between the osmotic potential at full turgor and midday leaf water potential of the rice plants on two different dates is shown in Figure 2. On both days the leaf osmotic potential at full turgor decreased linearly with midday leaf water potential, indicating an increasing degree of adjustment with increasing water deficits. Between d 57 and d 63, after the differential irrigation treatment was imposed, the water deficits increased but were partially relieved by a rain shower in the early morning of d 64 (23). Thus, the midday water potentials were higher, but the osmotic potentials at full turgor were lower on d 64 than on d 57, leading to the differences in slope on the 2 d. The results indicate that on d 57 from imposition of the treatments, the osmotic adjustment in the most severely stressed plants was 0.5 MPa, whereas on d 64 the osmotic adjustment in the same plants was 0.8 to 0.9 MPa.

**Effect of Osmotic Adjustment on Leaf Rolling.** Angus *et al.* (1) showed that the midday water potential in the unirrigated rice was  $-1.6$  to  $-1.7$  MPa 43 d after sowing. The unirrigated plots decreased to  $-2.6$  MPa 57 d after sowing, and remained at this level for the next 12 d, while the equivalent values in the irrigated plot were  $-1.1$  MPa. At the time of sampling, the leaves in the unirrigated plot were tightly rolled. Leaf osmotic potentials at full turgor of the unirrigated IR36 were  $-2.0$  and irrigated

$-1.4$  MPa, respectively. The osmotic adjustment of 0.6 MPa in the stressed leaves affected the relationship between leaf rolling and leaf water potential (Fig. 3). As shown previously (19, 20), the degree of leaf rolling increased linearly with decrease in leaf water potential below a threshold value. Osmotic adjustment decreased the leaf water potential threshold at which leaf rolling commenced, but the change in leaf rolling with change in leaf water potential was similar in slope to that in the unstressed plants. This indicates that the osmotically adjusted plants maintained turgor not only of the bulk leaf, but also of the bulliform cells controlling leaf rolling. When the leaf water potential in the unirrigated plants was  $-2.6$  MPa, the leaf water potential in the irrigated plants with the same leaf rolling score was  $-2.0$  MPa, indicating that at 0.6 MPa, the osmotic adjustment in the bulliform cells was similar to that in the bulk leaf tissue.

**Effect of Osmotic Adjustment on Leaf Death.** Twenty-seven to 29 d after watering was discontinued, the predawn leaf water potentials in the unirrigated and irrigated rice were  $-0.52 \pm 0.13$  and  $-0.12 \pm 0.02$  MPa, respectively, and the midday leaf water potentials were  $-2.05 \pm 0.12$  and  $-1.34 \pm 0.08$  MPa, respectively (Table I). The leaf osmotic potentials at full turgor on the 3 sampling days are also summarized in Table I. The unirrigated plants adjusted osmotically by about 0.5 MPa. This lowering of the osmotic potential at full turgor affected the degree of leaf death. Figure 4 shows that the degree of leaf death increased linearly as the leaf water potential decreased. However, the degree of leaf death was always less in the unirrigated, osmotically adjusted rice than in the irrigated rice at a given leaf water potential.

## DISCUSSION

This study has shown that the leaves of the lowland rice cultivar IR36 adjust osmotically in response to water deficits. The degree of osmotic adjustment was generally 0.5 to 0.6 MPa, but in one case was as great as 0.8 to 0.9 MPa. Both the degree of stress and the time for development of the stress influenced the degree of osmotic adjustment. Thus, this lowland-improved rice cultivar behaved similarly to, and adjusted to the same degree as, four upland rice cultivars (26). Considering that IR36 was selected for lowland-irrigated conditions, it may appear surprising that its degree of osmotic adjustment is the same as that of the traditional rainfed upland cultivars.

Several morphological and physiological processes have been shown to be maintained, or maintained at a higher rate, as a result of osmotic adjustment (28). For example, when tissue water status progressively decreases in response to a decreasing soil water availability, osmotic adjustment has been shown to maintain stomatal conductances and rates of photosynthesis at relatively higher values (13, 17), to maintain leaf, hypocotyl, and root growth at higher rates (7, 18, 25), and to prevent desiccation

of the apex (2) in osmotically as opposed to nonosmotically adjusted plants. The present study demonstrates that osmotic adjustment also influences the water potential at which leaf rolling and leaf death occur.

Leaf rolling greatly aids grasses, including rice, in minimizing transpirational water loss during water deficits (22). However, when leaves roll, the effective leaf area for light interception is reduced and the diffusive resistance to CO<sub>2</sub> is increased, both of which will reduce photosynthesis. Thus, osmotic adjustment that delays leaf rolling to lower leaf water potentials may be one of the important responses which collectively enable the productive processes of grasses to be maintained at greater leaf water deficits than in nonadjusted plants (5, 6, 10, 26, 28).

Leaf rolling is also used as a visual score for selecting for drought resistance in rice (16). A smaller degree of leaf rolling is taken as indicative of a greater degree of dehydration avoidance by the development of deep roots. Jones (12) showed that the degree of leaf rolling at a particular leaf water potential was dependent on the cultivar and, thus, care must be exercised when using leaf rolling as an index of the degree of water stress or dehydration avoidance. The present study indicates that the degree of leaf rolling will also depend on the ability of the cultivar to adjust osmotically, *i.e.* to tolerate low leaf water potentials, and not simply on its ability to avoid dehydration through a deep root system. This implies that screening for drought resistance on the basis of leaf rolling will select for a range of drought resistance mechanisms.

The present study also shows that the degree of leaf death at a particular degree of water deficit is affected by the degree of osmotic adjustment. However, it is unknown how osmotic adjustment *per se* acts to protect leaf tissue from death at low water potentials. Complete loss of turgor in rice leaf mesophyll tissue may lead to tissue death: osmotic adjustment will lower the water potential at which this occurs. Alternatively, the solutes involved in osmotic adjustment such as sugars and proline may protect the enzymes and thereby maintain tissue viability (15, 24). Finally, with osmotic adjustments, the stomata may not close as early in a stress cycle, thereby preventing leaf damage by maintaining leaf temperatures lower than in nonadjusted leaves. Whatever the mechanisms, the lower leaf death observed in this study in the osmotically adjusted leaves should lead to a better ability to recover functional green leaf area once stress is relieved.

Osmotic adjustment has been identified as a physiologically important drought resistance characteristic (27). The present study indicates that osmotic adjustment aids the productivity of grasses under water deficits by delaying until lower leaf water potentials the leaf rolling response which, although it reduces transpiration and light interception, may be expected to concomitantly reduce photosynthesis. Osmotic adjustment also delays until lower leaf water potential leaf senescence and tissue death which is undoubtedly important in recovery of canopy photosynthetic activity and crop productivity after water stress is relieved.

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