Vulnerability to Hydraulic Dysfunction as Affected by Sowing Date in Rice Leaves

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Abstract: Hydraulic properties of leaf blade were studied to provide basic knowledge to examine relationships between leaf vulnerability to hydraulic dysfunction due to low water potential (Ψ) and plant adaptation to water stress in rice. The objectives were to find the appropriate portion along a leaf blade for measurements of hydraulic dysfunction due to low Ψ and to examine whether leaf vulnerability to hydraulic dysfunction changed with the growing season. The early cultivar Bouzu (to supply materials earlier) and Koshihikari (a leading cultivar of Japan) were grown in pots. Hydraulic profile along a leaf blade was determined under well-watered and drought conditions in the two cultivars. The vulnerability to leaf hydraulic dysfunction was compared between plants sown at different dates only in Koshihikari. In both cultivars, leaf hydraulic conductivity (K) was constant in the basal half of a leaf blade, whereas it became smaller toward the tip and this pattern was maintained under drought conditions, indicating that the basal half was an appropriate portion for estimation of leaf hydraulic dysfunction. In Koshihikari, K decreased as relative water content (RWC) decreased and RWC at which K was reduced to 50% of the maximum (RWC_{50}) was determined for each sowing date. Then leaf Ψ corresponding to RWC_{50} (Ψ_{50}) was estimated from the relationship between Ψ and RWC. As a sowing date was delayed from May to August, Ψ_{50} increased from −1.44 MPa to −1.13 MPa. It was concluded that rice leaves became vulnerable to hydraulic dysfunction due to low Ψ as the growing season was delayed.

Key words: Growing season, Hydraulic conductivity, Leaf, Rice, Vulnerability to hydraulic dysfunction.

Water flow through plant xylem is essential for plants to survive and grow. Recent researches documented that the water flow is disturbed under water stress conditions mainly by cavitation and that xylem vulnerability to hydraulic dysfunction is responsible to plant adaptation to environments (e.g., Tyree and Sperry, 1989; Tyree et al., 1994). Species adapted to dry environments require a more negative water potential (Ψ) for hydraulic dysfunction, indicating that they are less vulnerable than those to humid environments. In these studies it is essential to determine the vulnerability to hydraulic dysfunction and the vulnerability has often been determined on plant segments in laboratories.

Studies on the vulnerability to hydraulic dysfunction have been conducted mainly using trees and shrubs (Tyree et al., 1994). Although hydraulic dysfunction under water stress conditions does occur in crop plants such as maize (Tyree et al., 1986), sugarcane (Neufeld et al., 1992) and rice (Hirasawa et al., 1996; Tsuda et al., 1998), few studies on the vulnerability are available. Genotypic variability in leaf xylem vulnerability to cavitation was found in sugarcane (Neufeld et al., 1992). In rice, variation of the vulnerability to hydraulic dysfunction in panicle neck has been detected between cultivars (Hirasawa et al., 1996). No data, however, is available for leaf vulnerability to hydraulic dysfunction in rice. Therefore, leaf vulnerability to hydraulic dysfunction was examined in this study.

Vulnerability to hydraulic dysfunction due to low Ψ changed with the growing season in silver maple (Acer saccharinum L.) (Tsuda and Tyree, 1997). Leaf vulnerability of rice may also change with the growing season. Regarding morphology, rice leaf is parallel-veined, and the veins are connected with small transverse veins and become thinner toward the leaf tip (Yamazaki, 1961; Chonan et al., 1974; Kono et al., 1982). Leaf hydraulic conductivity may change along a leaf blade, suggesting that the segment portion along a leaf blade should be selected for the measurements on dysfunction.

The objectives of the present study were to clarify the profile of hydraulic conductance along a leaf blade to find appropriate portion for the measurements on dysfunction and to examine whether leaf vulnerability to hydraulic dysfunction due to low Ψ changes with the growing season in rice.

Materials and Methods

1. Plant material

Rice (Oryza sativa L.) plants were grown in soil from seeds in the experimental fields at the Faculty of Bioresources, Mie University, and at the Faculty of Agriculture, Okayama University, through 1996 – 1998. Plants were grown basically by the same method throughout the experimental period. Sixteen seeds were sown in a

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pot having a top diameter of 18 cm and filled with the soil from the experimental fields. Chemical compound fertilizer was incorporated into the soil at a rate of 0.96 g pot⁻¹ each of N, P₂O₅, and K₂O as a basal fertilizer at sowing. Tillers were removed and only the main shoots were grown. Plants were top-dressed at a rate of 0.36 - 0.72 g pot⁻¹ of the same fertilizer depending on the growth.

To examine the profile of hydraulic conductance along a leaf blade, lowland rice cultivar Bouzu plants were grown by the same cultivation procedure as described above in 1996 and 1998 and lowland rice cultivar Koshikari in 1997. Bouzu plants were subjected to various degrees of soil water stress by withholding irrigation up to seven days in 1998. Bouzu is an early cultivar and was used for supplying leaf materials earlier. Koshikari was used because it is a leading cultivar in Japan. The number of pots per plot ranged between 24 and 36.

The effect of sowing date on leaf vulnerability to hydraulic dysfunction due to low Ψ was examined only in Koshikari. Experiments were conducted with five sets of plants designated as May, June, July, August and September plants according to the sowing date in 1997, which was the first day of the month from May to August and on 31 August, for the September plants.

2. Profile of hydraulic conductance along a leaf blade

Plants were grown until the second or third leaf blades from the flag leaf had emerged fully. Shoots were excised at a few centimeters above the soil surface and brought to the laboratory by keeping the shoot base in water and by covering the leaves with aluminum foil to avoid dehydration. The second or third leaf blade was cut at a few centimeters from the ligule under water and the cut end was connected to tubing with a handmade fitting. Water flow was induced through the leaf blade by applying a gravitational pressure of about 0.006 MPa through the tubing after a few centimeters of leaf tip portion was removed. Water efflux from the other end was collected with a 1.5 ml centrifugal tube containing a small piece of cotton for 300 s. Hydraulic resistance (R (MPa s kg⁻¹)) was defined as the applied pressure (MPa) divided by the mass flow rate of the water through the segment (kg s⁻¹).

Following the first measurement of water flow, 3 or 5 cm of the tip portion was cut back, and then water flow was determined again. The successive cut back and determination of water flow were conducted until the length of the leaf blade was reduced to only 5 cm. As a consequence, the leaf blades were divided to several segments. Assuming that water flow through a leaf blade is a series of resistance elements, R of a particular segment removed after the i th measurement was estimated as the difference between R of the i th and the i + 1 th measurements and divided by the length of the removed segment to obtain R per unit length. Conductivity (K), which was defined as conductance per unit length of segment in this study, was given as a reciprocal of the R per unit length. Independently of these measurements, leaf segments of 5 cm were excised at the basal cut end and at 30 cm up to the leaf tip and their K was determined by the same procedure described above only in 1997.

3. Vulnerability to hydraulic dysfunction due to low Ψ

The effect of sowing date on leaf vulnerability to hydraulic dysfunction was assessed only in 1997. When the fourth leaf blade from the flag leaf fully emerged, 30 to 40 shoots were covered with black plastic bags overnight to hydrate the shoots as much as possible. A large amount of guttation was observed at 0700 when shoots were collected. The shoots were cut at a few centimeters above the soil surface and brought to the laboratory by keeping the shoot base in water and by covering the leaves with aluminum foil to avoid dehydration. The fourth leaf blades were cut at the ligule, enclosed in a sheet of aluminum foil of known weight and the aluminum foil including a leaf blade was weighed to obtain the hydrated leaf weight (Wᵢ). After unfolding the aluminum foil, leaf blades were kept on the laboratory bench top for various periods up to six hours to dehydrate them to various degrees. Then the leaf blade was wrapped with aluminum foil to obtain the weight of the dehydrated leaf (Wᵢ). Leaving the leaf wrapped in aluminum foil at least 20 min to stabilize water status, a leaf segment of 5 cm was excised at 5 - 10 cm from ligule under water. Water flow rate through the segment was determined with the procedure described in the previous section and K was calculated as flow rate divided by applied pressure gradient per unit length. At the end of the experiment, the dry weight of the leaf (W₀) was determined and relative water content (RWC) was calculated as follows; RWC = (Wᵢ - W₀) / (Wᵢ - W₀) × 100 (%). Measurement of K in relation to RWC was conducted on the third and second leaves and the flag leaf on different days when each leaf blade emerged fully for each set of plants.

4. Relation between RWC and Ψ

Leaf blades were dehydrated to various degrees on the bench top by the same method as described in the previous section and their Ψ were determined with the pressure chamber method in 1997. RWC was also calculated as described above.

5. Xylem vessel size

Thin transverse sections of leaf blade at about 7 cm from the ligule were manually prepared, stained with safranine dye solution on microscope slides and observed under a light microscope at a magnification of 40× 10. Since individual vessels had elliptical openings, the vessel hydraulic diameters (d) were estimated for each vessel
following Lewis’ method (Lewis, 1992). Assuming laminar flow in ideal capillary tubes, Hagen–Poiseuille law gives that the water flow rate of a vessel under the same pressure gradient is proportional to \( d^4 \) (e.g., Zimmermann, 1971; Milburn, 1979; Tyree et al., 1994). Since there were many vessels in a section, so the mean value of hydraulically weighed diameter (\( \bar{d}_h \)) was calculated as \( \bar{d}_h = \Sigma d^4 / \Sigma d^4 \) (e.g. Davis et al., 1999). Four leaves were measured for each leaf position.

6. Meteorological measurement

Meteorological conditions were measured in 1997 when the effects of sowing date on leaf vulnerability to hydraulic dysfunction were examined. Air temperature and photosynthetically active radiation (PAR) were measured with an air temperature probe (LI-1000–16, Li-Cor, Inc., Lincoln, NE) and a quantum sensor (LI-190SB, Li-Cor, Inc., Lincoln, NE) connected to a data logger (LI-1000, Li-Cor, Inc., Lincoln, NE). A small evaporative pan of 20 cm in diameter was used for determination of daily evaporation (E).

Results and Discussion

1. Hydraulic profile along a leaf blade

The number of leaves examined for hydraulic profile along a leaf blade reached 104 during the three years under both well-watered and water-stress conditions with virtually the same results. The hydraulic profile of a leaf blade is shown as \( R \) as a function of the length of a leaf segment in Fig. 1. \( R \) increased in proportion to the segment length in shorter segments (near ligule), but exponentially in the segments longer than a certain length. The proportional increase in \( R \) indicated that \( R \) per unit length was constant near the leaf base.

In successive cut back measurements, if high resistance exists at a certain point along a leaf blade, it will be a “bottle neck” for water flow and the measured \( K \) value will not be accurate for regions more distal from that point. To verify the hydraulic profile determined by the successive cut back, we compared \( K \) with that of segments from a comparative portion along a leaf blade about 40 cm in length (Fig. 2). Although \( K \) determined by the cut back experiments decreased gradually toward the tip, no difference in \( K \) was detected up to 20 cm from the basal cut end. \( K \) independently determined on the
leaf segments was comparable to that from cut back experiments (compare open triangles with closed circles in Fig. 2). These results coincided with the previous observation that \( R \) increased in proportion to the segment length at the basal half of the leaf blade, and conformed that small \( K \) at the distal half was not due to a "bottle neck".

The proportional increase in \( R \) was detected in almost all samples under well-watered and soil water deficit conditions. Regression coefficient was smaller in the plants under well-watered conditions than in those under soil water stress conditions, indicating \( R \) of given length increased under soil water stress conditions (Fig. 1). Eight samples out of 104 did not show clear proportional increase in \( R \) and were subjected to water stress where leaf \( \Psi \) was less than \(-2 \) MPa. The range of the proportional increase varied from 11 cm to 28 cm depending on the whole leaf length, which ranged between 21 cm and 53 cm. Virtually \( R \) per unit length and \( K \) were constant at the basal half of the leaf blade.

What was responsible for the differences along a leaf blade in \( K \)? Vessel size is sometimes related to \( K \) (Zimmermann, 1971; Miburn, 1979). Assuming Hagen–Poiseuille law for the vessel water flow, \( \Sigma d^4 \) should be related to \( K \). \( \Sigma d^4 \) of the basal segment was \( 972000 \) \( \mu \)m\(^4\) and that of the distal \( 624000 \) \( \mu \)m\(^4\) or 67% of the basal. Since distal \( K \) was 46% of basal \( K \), vessel size could explain 64% of differences in \( K \). One third of differences remained to be explained. Possible explanations might lie in physical character of vessel wall, embolisms etc. and will be subjects of future studies.

The hydraulic profile found here suggested appropriate portion of a leaf blade for the measurements on hydraulic dysfunction although the physiological basis was still unclear. The basal half of a leaf blade was more conductive than the distal half, indicating it is convenient to measure water flow without large errors. Since \( K \) is constant in the basal half, it should not be so critical for the leaf portion. Therefore, the basal half of a leaf blade was used for the measurement on hydraulic dysfunction in the next section.

2. Effect of sowing date on the vulnerability

The total number of leaves emerged on the main shoot was 16 in the May plants. In other words, the flag leaf position on the main shoot was 16. The position decreased as the sowing date was delayed one month, consequently the flag leaf position was 12 in the September plants. Leaf length ranged between 25 cm and 65 cm, depending on the leaf position and sowing date.

\( K \) determined for 5 cm leaf segments at about 10 cm from the ligule decreased with the decrease in \( RWC \) in the May plants (Fig. 3). Sigmoid curve was fit by the least squares method to relation between \( K \) and \( RWC \). The curves were virtually well fit \((P<0.01)\). At saturated status \((RWC=100\%)\), \( K \) had maximum value and ranged from \( 8.7-13.0 \times 10^{-7} \) kg m MPa\(^{-1}\) s\(^{-1}\), and then decreased to zero until \( RWC \) decreased to about 50%. \( RWC \) at which \( K \) decreased to 50% of the maximum value \((RWC_{50})\) was estimated from the fitted curve and ranged from 76.0% - 81.6%. The relation between \( RWC \) and \( \Psi \) was analyzed for each leaf position (Fig. 4). A cubic curve was applied to the relation by the least squares method where \( RWC \) was between 70% - 100% \((P<0.01)\). \( \Psi \) corresponding to \( RWC_{50} \) \((\Psi_{50})\) was ranged from \(-1.20 \) MPa to \(-1.61 \) MPa for each leaf position.

With the same procedure applied to the May plants, \( \Psi_{50} \) was determined for other sowings (Fig. 5). However, the data for the September plants are not shown because the relation between \( K \) and \( RWC \) was not significant. The value for each leaf position did not have a standard error since it was estimated from two regressions, and the average \( \Psi_{50} \) for the uppermost four leaves is shown with the standard error. \( \Psi_{50} \) increased from \(-1.44 \) MPa to \(-1.13 \) MPa \((P<0.05)\), suggesting that rice leaves became more vulnerable as the sowing date was delayed.

The vulnerability to hydraulic dysfunction might be related to environmental conditions, e.g., plants grown under a low irradiance or wet conditions were more vulnerable (Tyree et al., 1994; Cochard et al., 1999). Thus, the meteorological conditions for each sowing date
were examined in relation to the vulnerability. The meteorological conditions during the emergence of four leaves from the flag leaf varied with the sowing date (Table 1). The average of air temperature was highest in the July plants and lowest in the August and September plants. PAR and $E$ were also the highest in the July plants, indicating that the leaves of the July plants grew under highest evaporative demand conditions. This trend in meteorological conditions was not consistent with the changes of vulnerability.

Xylem vessel diameter could be related to the vulnerability when it reflects rigidity of vessel wall (Sperry, 1998). In this study, xylem vessel diameter ranged from 30 $\mu$m to 45 $\mu$m and differed significantly with the sowing date (Fig. 6). Sowing in August and September gave smaller vessels than that in May, June or July. Vessel size could not explain the vulnerability in this study. The reasons for the changes in vulnerability with sowing date require further investigation although loss of cell wall rigidity may be one possible cause.

We found that leaf vulnerability to hydraulic dysfunction changed with the sowing date in rice using leaves dehydrated on the bench top. Adopting this method, we are going to study the relationship between the leaf vulnerability to hydraulic dysfunction and plant adaptation to drought in rice as well as the physiological basis of the hydraulic dysfunction. Our goal is to examine the contribution of the vulnerability to leaf death or stomatal closure in relation to drought resistance in rice.

References


*In Japanese with English summary.

**In Japanese.