Leaf Death and Alteration of Internal Water Relations in Rice (*Oryza sativa* L.) in Response to Water Deficits in the Seedling Stage

Sayeed AHMAD*, Tohru KOBATA** and Sinichi TAKAMI**

(Faculty of Agriculture, Kyoto University, Kitasirakawa,
Sakyo-ku, Kyoto 606 Japan)

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Significant variation in the capacity of rice seedlings to survive under severe water stress exists in the rice germplasm. O'TOOLE et al.⁽⁸⁾ showed that rices from lowland and deep-water areas of Thailand and Bangladesh survived better than rices from upland origin. Similar results were found among Japonica rice⁽⁷⁾: wetland (lowland) cultivars showed better survival than dryland (upland) ones under severe drought.

Adaptation to water stress involves the reduction of cell dehydration either by avoidance or by tolerance of stress⁽⁶⁾. Avoidance in this study is defined as the ability of plants to maintain "extracellular" water potential, while tolerance refers to "intercellular" regulatory functions of plants to maintain physiological processes in face with changing extracellular water potential. In the above cited study⁽⁸⁾, avoidance *per se* was assumed not important, because plant water deficits in terms of leaf water potential developed similarly in the cultivars tested and also because leaf water potential reached quite low values during the drying period. Instead, wetland cultivars were considered to perform better under severe drought as their tolerance was superior to dryland cultivars. However, the factors responsible for such differential tolerance across cultivars are not known.

Tolerance of water stress usually involves the development of low osmotic potentials⁽¹¹⁾ which characterize many plant species found in more arid environment⁽⁶⁾. Thus, O'TOOLE⁽⁹⁾

suggested the difference in basal osmotic potential (osmotic potential measured at full turgor) found in diverse rice ecotypes may represent a long-term constitutive adaptive component. In addition to inherent difference in the basal osmotic potential, differences in osmotic potential can arise from solute accumulation in response to water stress⁽³⁾. Such difference of osmotic potential of stressed and unstressed plants measured at full turgor is termed osmotic adjustment and differential capacity for osmotic adjustment has been observed among species⁽²⁾ and among genotypes of wheat⁽⁵⁾. Since osmotic adjustment enables plants to maintain turgor and turgor-dependent processes to a significantly lower water potential, it is a possible candidate of a factor responsible for differential tolerance of rice seedlings, provided that there are inherent differences among rice cultivars for this capacity. Although occurrence of osmotic adjustment was demonstrated for rice, there was no difference in the degree of osmotic adjustment among the cultivars studied⁽¹⁾. Since they studied only four dryland cultivars, a much broader range of cultivars must be studied in order to find out the differences among cultivars.

This study was undertaken to further examine if there are cultivar differences for rice seedlings to survive under severe drought by comparing cultivars with different hydrological background. Furthermore, we tested the hypothesis that osmotic adjustment is responsible for cultivar differences in seedling survival. Survival ability of plants are evaluated in terms of leaf firing, as the progressive tissue death ultimately results in the death of individual plants. The plants were grown in a very small pot to facilitate the

* Central Extension Resources Development Institute, Joydebpur, Gazipur, Bangladesh.

** Faculty of Agriculture, Shimane University, Nishikawazu-cho, Matsue, 690 Japan.

*** Hokuriku National Agricultural Experiment Station, Inada, Jouetsu, 943-01 Japan.

Fig. 1. Amounts of applied water in different water treatments as function of days after the start of differential irrigation treatment in 2 pairs of rice cultivars. Bars shown on the last day of each irrigation plot denote one standard error of the mean ($n=5$). W and D_1 to D_4 denote irrigation gradients where W is wet and D_4 is driest treatment. Irrigation from D_1 to D_4 treatments was suspended until they reached respective predetermined pot weight. Pot weight was maintained by adding required amount of water daily.

imposition of the similar level of stress, i.e., to exclude the effects of avoidance *per se*.

Materials and Methods

Four rice (*Oryza sativa* L.) cultivars, Nipponbare, IR30, Tachiminori and IRAT13,

Fig. 2. Predawn turgid osmotic potential at different water treatments after 18 days of differential irrigation in 4 rice cultivars. Values are means $\pm$ one standard error of 3 observations. F denotes flooded treatment. See Fig. 1 for other treatments.

Table 1. Predawn leaf water potential (MPa) of four rice cultivars at different levels of irrigation. Values are means $\pm$ one standard error of 3 observations.

Days after treatment	Treatment	Cultivar			
		Nipponbare	Tachiminori	IR 30	IRAT 13
0	—	-0.16 ± 0.05	-0.14 ± 0.05	-0.20 ± 0.03	-0.20 ± 0.04
18	F	-0.15 ± 0.01	-0.15 ± 0.02	-0.15 ± 0.04	-0.15 ± 0.02
	W	-0.13 ± 0.02	-0.20 ± 0.02	-0.13 ± 0.02	-0.22 ± 0.03
	D_1	-1.14 ± 0.14	-1.47 ± 0.12	-0.66 ± 0.01	-0.33 ± 0.04
	D_2	-1.31 ± 0.16	-1.46 ± 0.08	-0.68 ± 0.13	-0.47 ± 0.09
	D_3	-2.09 ± 0.35	-1.81 ± 0.20	-1.13 ± 0.17	-0.82 ± 0.11
	D_4	-2.38 ± 0.46	-1.68 ± 0.10	-0.91 ± 0.07	-1.11 ± 0.23

Note : F and W denote flood and wet soil conditions, respectively, and D_1 - D_4 denote decreasing applied water with increasing number.

Fig. 3. Relationship between predawn leaf water potentials measured in the second leaf from the top and percentages of leaf death measured on whole plant bases, after 18 days of differential irrigation in 4 rice cultivars subjected to 6 levels of irrigation: (1) flooded, (2) wet and (3) to (6) denote D_1 to D_4 as described in Fig. 1. Values are means $\pm$ one standard error of 3 (leaf water potential) or 5 (percentage of leaf death) observations. Standard errors less than 0.03 MPa and 1.0% are omitted for clarity, respectively. Lines are fitted by eye.

were used in this experiment. Nipponbare is an intermediate cultivar and IR30 an semidwarf cultivar, usually grown only under flooded wetland soil conditions, whereas Tachiminori and IRAT13 are intermediate cultivars for cultivation under rainfed dryland soil conditions. All cultivars except IR30, which is from the Indica ecogeographic type, are from the Japonica type.

The experiment was conducted in a naturally lit glasshouse of Kyoto University (HITRON-P-7901, Hitachi Plant Engineering & Construction Co., Ltd) at day/night

Fig. 4. Relationship between predawn leaf water potentials and percentages of leaf death both measured in the second leaf from the top, after 18 days of differential irrigation in 4 rice cultivars subjected to 6 levels of irrigation: (1) flooded, (2) wet, and (3) to (6) denote D_1 to D_4 as in Fig. 1. Values are means $\pm$ one standard error of 3 (leaf water potential) or 5 (percentage of leaf death) observations; standard errors less than 0.03 MPa and 1.0% are omitted for clarity, respectively. Lines are fitted by eye.

temperature of 25/20°C, dew point temperature of 20°C and wind speed of 1 m/s. Plants were grown in a small plastic pot containing 690 g air-dried sandy silt soil. Ammonium sulfate ($(\text{NH}_4)_2\text{SO}_4$), superphosphate (CaP_2O_5) and potassium chloride (KCl) were applied to the soil prior to sowing at the rate of 4.8 g, 2.3 g and 0.7 g per pot. Pregerminated seeds of Nipponbare and Tachiminori were sown on 8 August 1983 (pair 1) and of IR30 and IRAT13 on 13 August 1983 (pair 2). Twenty pots per each irrigation level of four cultivars were used and each pot contained four plants.

Irrigation was suspended 15 days (d) after

Fig. 5. Relationship between dry-matter increase for 18 days of differential irrigation treatment and predawn leaf water potential measured on the last day of treatment. Lines are fitted by eye.

sowing in the pots assigned for dry treatments until a predetermined pot weight was achieved through soil water depletion. Levels of dry treatment ranging from moderate dry (D_1) to severe dry (D_4) were thereafter maintained by adding required amount of water daily so as to keep the respective pot weight constant. Wet treatment (W) received such an amount of water daily as to keep the soil at near field capacity. Thus, a pot of wet treatment received water of 800 to 1,000 ml, whereas D_4 received less than 200 ml of water for 18 day treatment (Fig. 1). Another set of plants was assigned to flood treatment (F) by keeping stagnant water of 3 to 4 cm in depth. Since two pairs of cultivars were sown on different dates and thus grown under different atmospheric demand, each pair received different amount of water during treatment (Fig. 1-a vs. b).

Osmotic adjustment was measured as the difference between osmotic potential at full

Fig. 6. Relationship between predawn leaf water potential and predawn osmotic potential at full turgor in 4 rice cultivars measured at the end of 18 days of differential irrigation treatment. See Fig. 3 for the symbols.

turgor (OP) for the stressed and unstressed plants. We used rehydration technique to determine OP³⁾ as this avoids the dilution effect on the measurements. Predawn leaf water potentials (WP) of recently matured leaves (second from the top) were measured with a pressure chamber at the beginning of differential irrigation treatment and 18 d after treatment, taking the precaution as described by KOBATA and TAKAMI⁴⁾. Predawn leaf water potential herewith refers to the water potentials measured on leaf-blades just before dawn (for simplicity we use leaf water potential instead of leaf blade water potential hereafter). Leaf turgid osmotic potential (OP) was measured on the sap expressed from the excised leaves with a vapor pressure osmometer (Model 5100, Wescor Inc., Logan, Utah, U.S.A.) which was calibrated with known standard solution of sodium chloride (NaCl) at 25°C prior to the measurement. Before measurements, the plants were excavated and rehydrated for four hours at low light flux densities in a humid plastic bag containing shallow distilled water³⁾. The measurements were taken on plants collected before dawn. After 18 d of treatment, leaf length from ligule to tip and its dead portion was measured for leaves fully emerged. For leaves in expansion, length was measured from the base of the plant, i.e., the soil surface. Degree of leaf death

was expressed either on a whole plant basis or on a single leaf basis: the former is the ratio of dead portion of all leaves to total leaf length of a whole plant, whereas the latter is the ratio of dead portion to the whole length of the second leaf from the top.

Results

Gradients of decreasing predawn leaf WP were established in all the cultivars in response to decreasing irrigation (Table 1). Minimum predawn leaf WP developed at D_3 or D_4 in all cultivars. Stress development was more severe in Nipponbare and Tachiminori than in IR30 and IRAT13 at all levels of irrigation.

Leaf OP of all the four cultivars decreased with decreasing irrigation (Fig. 2). Cultivars differed considerably in leaf OP particularly in the driest treatment (D_4): Nipponbare had the lowest leaf OP of all the cultivars and was significantly different from IR30 and IRAT13. Reduction in Tachiminori ranked the second, although it differed significantly only from IRAT13. IRAT13 had the highest leaf OP of all the cultivars in all levels of irrigation and was substantially different from other cultivars in the driest treatment.

Leaf death on a whole plant basis increased with the decrease of WP of the second leaf from the top (Fig. 3). Distinct difference in this response was found between Nipponbare and Tachiminori: wetland Nipponbare was more tolerant to water deficits than dryland Tachiminori (Fig. 3-a). On the other hand, both IR30 and IRAT13 responded more or less similarly (Fig. 3-b).

When leaf death measured on the second leaf was plotted against the leaf WP, response of IR30 became similar to Nipponbare (Fig. 4). That is, the leaf tissue of the wetland cultivars was highly tolerant to leaf water deficits, whereas leaf death rapidly increased with decreasing predawn WP in the dryland cultivars. Dry-matter production decreased with increasing plant water deficits (Fig. 5). In other words, all the cultivars produced less dry-matter when received less amount of water. The decrease of dry-matter production per unit decrease of predawn leaf WP was greater in Tachiminori than in Nipponbare (Fig. 5-a). Similarly, dry-matter production of dryland IRAT13 was more sensitive to decreasing predawn leaf WP although the

trend appeared to be reversed when plant were desiccated more severely (Fig. 5-b).

Discussion

It is well-known that the predawn leaf WP is a good measure of the maximum soil water potential encountered by the roots¹¹⁾. This is also the case with the present study, as suggested by the close correlation between the amount of water applied (Fig. 1) and the predawn leaf WP (Table 1) measured 18 days after treatment. In other words our differential irrigation treatment was well reflected in the gradients of predawn leaf WP established during 18 day treatment.

Predawn leaf WP in each treatment differed across the cultivars, although we tried to impose the same level of plant water stress by using a very small soil container (Table 1). The difference between pair 1 (Nipponbare and Tachiminori) and pair 2 (IR30 and IRAT13) was particularly distinct, as these two group experienced different atmospheric demand of evaporation. Small differences within each group may be due to the differences in leaf area per pot and thus soil water content of pot between each pair of cultivars.

This study has shown that there are cultivar differences in leaf death of rice due to water deficits (Fig. 4). The leaf tissue of Tachiminori and IRAT13 were susceptible, while that of Nipponbare was highly tolerant to water deficits. In IR30, leaf death on a whole plant basis was as high as in IRAT13 (Fig. 3), but that was the result of severe leaf death in lower leaves. Expanding leaves in IR30 during stress development were as in Nipponbare, highly tolerant to water deficits (Fig. 4). Susceptibility to dehydration, as measured in terms of dry-matter production, was also generally higher in dryland than the two wetland cultivars, although the distinction between IR30 and IRAT13 was not clear below WP of -0.1 MPa (Fig 5). Such lower susceptibility of wetland cultivars, both in terms of leaf injury and dry-matter production, suggests that wetland cultivars are likely to survive better than dryland ones in the seedling stage under severe drought, provided that both types of cultivars experience the same degree of plant water deficits. Thus, our results are in accord with earlier studies^{7,8)}, although we did not

measure plant survival directly.

All the four rice cultivars used in this study showed osmotic adjustment in response to water deficits. Leaf turgid osmotic potential decreased in all the cultivars (Fig. 2) as a result of decrease in leaf WP (Table 1). Wetland Nipponbare adjusted to greater extent than dryland Tachiminori. Leaf OP in Nipponbare decreased from -1.0 MPa in flooded treatment to -2.9 MPa in treatment D_4 , i.e., the maximum extent of osmotic adjustment was 1.9 MPa. Corresponding osmotic adjustment was 1.5 MPa in Taciminori. On the other hand, similar extent of osmotic adjustment, 1.2 MPa, was found in IR30 and IRAT13. Extents of osmotic adjustment as observed were therefore different across the cultivars studied. However, the degree of osmotic adjustment was highly correlated to predawn leaf WP and there were no inherent differences in this relationship among four cultivars studied (Fig. 6). Thus, Nipponbare, for instance, adjusted to the most extent simply because it experienced greater stress.

As in the previous studies with largely *Indica* rices^{10,11}, inherent cultivar differences in the capacity of osmotic adjustment in rice were not detected in this study. Below leaf WP of -0.3 MPa, osmotic adjustment increased similarly for all the cultivars tested (as expressed by the line drawn in Fig. 6), although degree of leaf death markedly differed both between Nipponbare and Tachiminori (Fig. 4 -a) and between IR30 and IRAT13 (Fig. 4 -b). Thus, the capacity of osmotic adjustment can not be considered as a candidate to explain the differential capability to withstand lower water potential among diverse cultivars. What causes the differential tolerance of leaf tissue to dehydration among rice cultivars could not be determined from the study. Further studies are needed to look into other possibilities such as the differences in the properties of protoplasm and protoplasmic membranes.

Summary

Leaf injury and reduction in dry-matter production due to severe drought were compared to examine cultivar differences of seedling survival in four rice cultivars of diverse origins and hydrological adaptation. Differential capacity for osmotic adjustment

was also sought among these cultivars. The cultivars studied are two dryland cultivars, Tachiminori and IRAT13, and two wetland cultivars, Nipponbare and IR30. Six irrigation gradients were established during 18 day treatment which was initiated 15 days after sowing under glasshouse condition. Differential plant water deficits in terms of predawn leaf water potentials were developed in response to irrigation treatment in all the cultivars. No consistent difference in predawn leaf water potential were found among cultivars. However, cultivars differed substantially in leaf death and dry-matter production: dryland cultivars were highly susceptible to water deficits compared with wetland cultivars. Cultivars also differed in the extent of osmotic adjustment. Such differences simply arised from the differences in degree of internal water stress developed among the cultivars, because degree of osmotic adjustment was highly correlated to predawn leaf water potential and this relationship was similar in all the cultivars studied. That is, there were no inherent cultivar differences in the capacity of osmotic adjustment and these differences did not correlate with the tolerance of leaf tissue to severe dehydration. Thus, osmotic adjustment is unlikely to be the factor involved in the differential ability for rice seedling to survive under severe drought found between wetland and dryland rice cultivars.

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References

1. CUTLER, J.M., K.W. SHAHAN and P.L. STEPONKUS 1980. Alteration of internal water relations of rice in response to drought hardening. *Crop Sci.* **20**: 307-310.
2. JOHNSON, D.A. 1978. Environmental effects on turgor pressure response in range grasses. *Crop Sci.* **18**: 945-948.
3. JONES, M.M. and N.C. TURNER 1978. Osmotic adjustment in leaves of sorghum in response to water deficits. *Plant Physiol.* **61**: 122-126.
4. KOBATA, T. and S. TAKAMI 1984. Estimation of the leaf water potential in rice by pressure chamber technique. *Japan Jour. Crop Sci.* **53**: 290-298*.

5. MORGAN, J.M. 1977. Differences in osmoregulation between wheat genotypes. *Nature* **270**: 234—235.
6. ——— 1984. Osmoregulation and water stress in higher plants. *Ann. Rev. Plant Physiol.* **35**: 299—319.
7. OKA, H. and E. RO 1957. Drought resistance in rice cultivars. *Agr. & Hort.* **6**: 851—855**.
8. O'TOOLE, J.C., R.S. AQUINO and K. ALLURI 1978. Seedling stage drought response in rice. *Agron. J.* **70**: 1101—1103.
9. ——— 1982. Adaptation of rice to drought prone environments. In *Drought Resistance in Crops with Emphasis on Rice*. (Ed.) IRRI, Los Baños Philippines. 195—213.
10. STEPONKUS, P.L. K.W. SHAHAN and J.M. CUTLER 1982. Osmotic adjustment in rice. In *Drought Resistance in Crop with Emphasis on Rice*. (Ed.) IRRI, Los Baños, Philippines. 181—184.
11. TURNER, N.C. 1979. Drought resistance and adaptation to water deficits in crop plants. In *Stress Physiology in Crop Plants* (Ed.) H. Mussell and R.C. Staples, Willey Interscience, New York. 343—372.
12. ———, J.C. O'TOOLE, R.T. CRUZ, E.B. YAMBAO, Ahmad SAYEED., O.S. NAMUGO, and M. DINGKUN 1986. Responses of seven rice cultivars to water deficits. 2. Osmotic adjustment, leaf elasticity, leaf extension, leaf death, stomatal conductance and photosynthesis. *Field Crops Res.* **13**: 273—286.

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〔和 文 摘 要〕

イネ幼植物の干ばつ下における葉身の枯死および水分生理的变化

Sayeed AHMAD*・小葉田 亨**・高見 晋一***

(*Central Extension Resources Development Institute, バングラデッシュ,

島根大学農学部, *農林水産省北陸農業試験場環境部)

異なった来歴をもつ水稻 (*Oryza sativa* L., 日本晴, IR30) と陸稲 (同, タチミノリ, IRAT13), 各2品種ずつについて幼苗期における乾燥下生存能力を比較した。そして、これら品種間に生存能力の差があるのか、また生存能力と浸透調節能力とは関係があるのかどうかを明らかにしようとした。播種後15日目から18日間、植物体を自然光人工気象室で様々な段階に土壤水分状態を設定したポットで育てた。その結果、土壤への給水量が少ない(第1図)ほど処理18日目の夜明け前の葉身水ポテンシャルは低かった(第1表)。そして、この時の葉身を膨潤させて求めた浸透ポテンシャル値も、ほぼ土壤乾燥の程度に応じて低下した(第2図)。さらにこの低下の程度を湿润区との差から浸透調節量として比べると、陸稲に比べ水稻のほうが大きかった。一方、葉身の枯死率はこの夜明け前の葉身水ポテンシャルと密接な関係があり、水ポテンシャルが低下するにつれて枯死率は増加した(第3図)。そして、葉身水ポテンシャルの低下にともなう枯死率の増大程度は水稻よりも陸稲で著しく(第4図)、乾物生産速度の低下も水稻に比べ陸稲の方がやや大きいという同様の傾向があった(第5図)。しかし、夜明け前の葉身の水ポテンシャルと浸透ポテンシャルとの関係を見ると、この関係には水稻と陸稲間で明確な差は認められなかった(第6図)。すなわち、水稻品種が陸稲品種よりも浸透調節が大きかったのは、水稻品種が単に低い水ポテンシャルにさらされたためであり、本質的な浸透調節能力の差にもとづくものではなかった。

以上のことから、葉身の枯死率と乾物生産からみた乾燥下における幼植物の生存能力は、陸稲品種に比べ水稻品種の方が高いとみなされるにもかかわらず、浸透調節能力には違いが認められないことから、浸透調節は幼苗期のイネの干ばつ下生存能力の差には関与していないものと判断される。