

Interspecific Differences in the Mechanism of Drought Tolerance among Four Cereal Crops*

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Abstract : Interspecific differences in drought tolerance were studied by two solution- and one field-culture experiments in a glasshouse using Japanese millet, sorghum, pearl millet and maize. Water stress treatment for the solution culture was achieved by adding polyethylene glycol 6000 to the solution at a final concentration of 200 g/1000 g H₂O, producing a -0.93 MPa osmotic potential, while that for the field experiment was by suspension of watering. The treatment was made for about two weeks in the vegetative growth stage. Growth parameters and leaf water status were determined at the beginning, during and after the treatment.

Dry weight of plant was generally decreased less by stress in pearl millet and sorghum, while the recovery after removal of stress was faster in sorghum and Japanese millet than in the other species. Osmotic potential (π) as against water potential (Ψ) in stressed leaves decreased more than in the control, except for maize. The decreasing order of relative water content with a decrease of either Ψ or π was as follows : maize > Japanese millet > sorghum > pearl millet. Osmotic adjustment (OA) increased in the following order : sorghum > Japanese millet > pearl millet > maize. From these results, we concluded that sorghum and Japanese millet, especially sorghum, possess a higher dehydration tolerance due to a higher OA and pearl millet due to a larger water retention ability, whereas maize easily wilts because of a smaller OA and water retention ability.

Key words : Drought tolerance, Interspecific difference, Japanese millet, Maize, Osmotic adjustment, Pearl millet, Sorghum, Water stress.

イネ科作物4種における耐乾性機構の種間差異 : 稲田勝美・松浦朝奈・山根昌勝 (鳥取大学乾燥地研究センター)

要 旨 : イネ科作物における耐乾性の種間差異を明らかにするため、ヒエ、モロコシ、パールミレットおよびトウモロコシを用いて水耕および圃場実験を行った。水ストレス処理は栄養成長期に12—14日間行い、水耕実験ではポリエチレングリコール6000を水耕液に加えて最終濃度を200 g/1000 g (浸透ポテンシャル、 -0.93 MPa) とする方法、および圃場実験では灌水を停止する方法によった。生育および葉身の水分状態の測定は、処理開始時、処理中および処理解除後にそれぞれ行った。

処理による乾物重の低下はパールミレットとモロコシが比較的小さく、ストレス解除後の回復ではモロコシとヒエが他よりも速かった。処理区では、水ポテンシャル (Ψ) の低下に対する浸透ポテンシャル (π) の低下が大きかったが、トウモロコシでは対照区との間に差がみられなかった。 Ψ および π の低下に伴う相対含水率の低下する順序はトウモロコシ > ヒエ > モロコシ > パールミレットであった。また、浸透調整 (OA) の大きさは、モロコシ > ヒエ > パールミレット > トウモロコシの順であった。

以上の結果から、モロコシとヒエ、特にモロコシは高いOA能力によって、パールミレットは大きい水分保持能力によってそれぞれ乾燥に耐えるが、トウモロコシはOA能力および水分保持力が小さいためしおれ易いと結論された。

キーワード : 種間差異, 浸透調整, 耐乾性, トウモロコシ, パールミレット, ヒエ, 水ストレス, モロコシ。

Utilizations of drought tolerant species and cultivars are primarily important for crop production in semi-arid regions without irrigation. Drought tolerance can be classified into two groups, dehydration postponement and dehydration tolerance, with the latter involving osmotic adjustment as a real example of acclimation¹²⁾. Osmotic adjustment refers to a

decrease in osmotic potential greater than can be explained by an increase in solute concentration during dehydration¹²⁾.

A number of studies have been reported on varietal differences in osmotic adjustment for sorghum^{3,10,19,22)}, wheat^{9,13,18)}, barley⁶⁾ and soybean²⁰⁾, and on interspecific differences between maize and sorghum^{1,16,17)}, between cotton and sorghum²⁾, among maize, sorghum and tobacco²¹⁾, and among cultivated grasses⁴⁾, respectively. Osmotic adjustment is not detectable in all kinds of plants or in all

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cultivars of a species¹²⁾. It is known that the cause of a decrease in osmotic potential consists of the concentration effect due to water loss from cells and the net accumulation effect of solutes into cells. The latter causes osmotic adjustment²²⁾.

The present study was conducted to find out the interspecific differences in drought tolerance, with a special focus on osmotic adjustment, using four cereal crops which are usually cultivated in rainfed fields.

Materials and Methods

1. Plant materials

Japanese millet (*Echinochloa frumentacea* Link. cv. Hidashirobie), grain sorghum (*Sorghum bicolor* Moench. cv. Feterita Pergamino 1430), pearl millet (*Pennisetum typhoideum* Rich.) and maize (*Zea mays* L. cv. P 3352) were used. Three experiments were successively conducted in a glasshouse in 1990. The first (Exp. 1) and second (Exp. 2) experiments were carried out by means of a solution culture from June 16 to July 12, and July 20 to August 17, respectively. The third experiment (Exp. 3) was conducted in a sandy soil field from August 22 to September 22.

2. Solution culture experiments

Seeds were disinfected and germinated at 30°C. The germinated seeds were placed in plastic baskets [22.6×32.5×7 (H) cm in the inside dimensions and 2×2 mm in the mesh dimensions] and covered with wet vermiculite at about 1 cm depth. The 12 baskets were placed in each solution tank [90×180×15 (H) cm] and the bottom of each basket was dipped into the culture solution to a depth of 0.5–1.0 cm. Nutrient solution was prepared according to Hoagland and Arnon⁸⁾. Concentrations of the solution used were 1/4 of the standard for two weeks after planting and 1/2 for the period thereafter. The pH of the solution was adjusted to about 5.0 with 1 N-H₂SO₄ everyday. The solution was aerated sufficiently with an air compressor. Seedlings were thinned out for spacing and unifying as they grew up.

Water stress treatment was started 5–8 days after planting for half of the plants in each species by adding polyethylene glycol 6000 (PEG) to the culture solution at the rate of 100 g/1000 g H₂O, producing a –0.22 MPa osmotic potential. After one week, PEG

concentration was raised to 200 g/1000 g H₂O, producing a –0.93 MPa. Two weeks after the start of treatment, the plants were transferred to the pure nutrient solution and released from the stress. The other half of the plants were grown in the nutrient solution without PEG giving an osmotic potential of –0.03 MPa as the control. The osmotic potential of the solutions was measured with an Osmometer (Vogel, OM 801).

3. Field culture experiment

Seeds were directly sown in sandy soil and supplied adequately with water. A compound fertilizer (N : P₂O₅ : K₂O = 15 : 15 : 12 in %), dolomite and a magnesium sulfate fertilizer (soluble Mg = 14%) were applied basally at the rates of 60, 100 and 10 g m⁻², respectively, and the same amounts, except for dolomite, were topdressed every 10 days. Stress treatment was given 10–12 days after seeding by stopping irrigation and was terminated 12 days after the start of treatment by rewatering.

4. Measurements of plant growth

Leaf area and dry weights of the top and roots (except for Exp. 3) were measured at the start of the treatment, two times during the treatment, and 4–7 days after the end of treatment. Leaf area per plant was determined with an automatic area meter (Hayashi Denko Ltd., AMM-7). Harvested plants were killed at 115°C for 30 min, then dried at 75°C for 2 days in a ventilating oven. The dry matter was weighed. Ten plants were used for each measurement.

5. Measurements of leaf water status

Water potential (Ψ), osmotic potential (π), turgor potential (P) and relative water content (RWC) were determined at 3 or 4 days, then at 7–11 days after the start of the stress treatment and yet again 4 or 5 days after the end of the treatment through measurements at predawn and near midday (1300–1500 h) using the second whole leaf from the top expanded leaf. Each determination was done with six replications on different leaves. Predawn conditions in Exps. 1 and 2 were simulated by the use of a growth chamber controlled at a temperature of 25°C, RH of 60% and darkness. Intact plants with culture solution were transferred into the growth chamber on the previous evening and kept one night. The Ψ and RWC were measured at about 0900 h. In Exp. 3 the Ψ and RWC

measurements at predawn were carried out at just before sunrise in the field. Water potential was measured with a pressure chamber (PMS Inc., 1002). Immediately after measurement of Ψ , leaf samples were tightly wrapped in aluminum foil, frozen by liquid nitrogen and stored at -40°C . Samples were thawed later and pressed to obtain cell sap. Osmotic potential of the sap was measured with the Osmometer. P was obtained by $\Psi - \pi$. RWC was measured according to Kobata's description¹¹⁾.

6. Calculations of factors affecting osmotic adjustment

The concentration effect ($\Delta\pi_{\text{con}}$) and solute accumulation effect ($\Delta\pi_{\text{acc}}$) were cal-

culated, respectively, according to Wright et al.²²⁾ as follows:

$$\Delta\pi_{\text{con}} = \pi_0 \times \text{RWC}_0 / \text{RWC} - \pi_0$$

$$\Delta\pi_{\text{acc}} = \pi - \pi_0 \times \text{RWC}_0 / \text{RWC}$$

Osmotic adjustment (OA) was calculated by the following equation:

$$\text{OA} = \pi_0 \times \text{RWC}_0 / \text{RWC} - \pi$$

where π_0 and RWC_0 are osmotic potential and relative water content, respectively, in control plants at the first measurement after the start of treatment, and π and RWC are osmotic potential and relative water content respectively, in control and stressed plants at the measuring times thereafter.

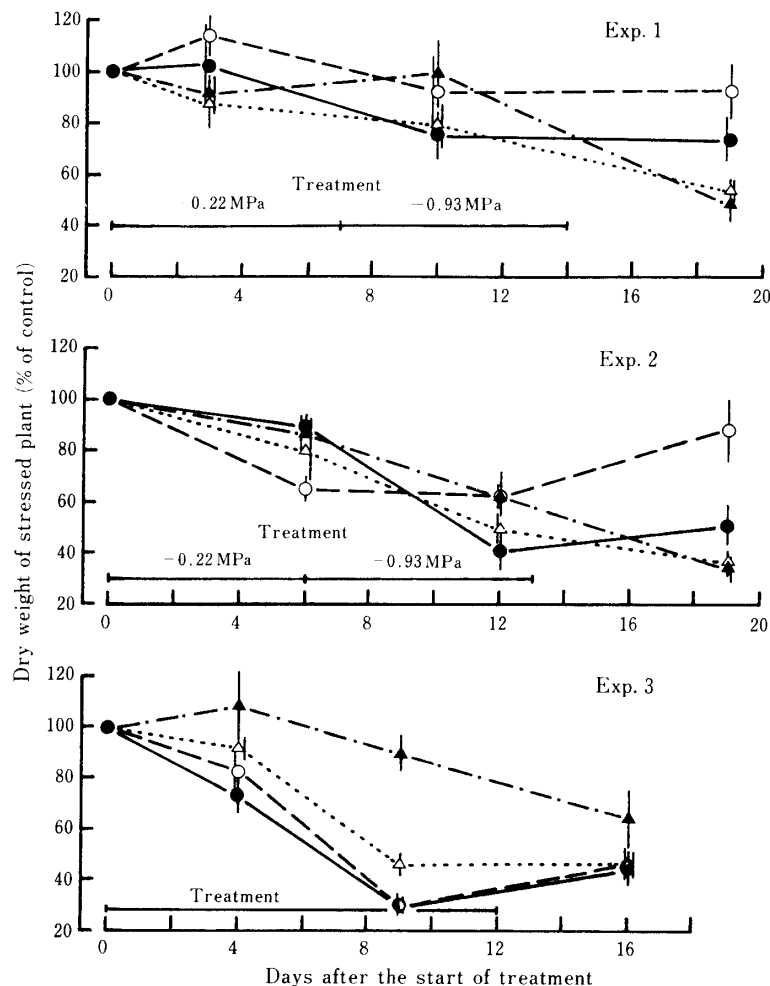


Fig. 1. Effects of water stress on dry weight per plant for four cereal crops after the start of stress treatment.

● : Japanese millet, ○ : Sorghum, ▲ : Pearl millet, △ : Maize
Total (top plus roots) weights were shown in Exps. 1 and 2, but top weights only in Exp. 3. Each value shows the mean of 10 plants. Vertical lines indicate standard deviations.

Results

During the experimental period, environmental conditions varied greatly due to changes in the weather. Within the period of

stress treatment, the average daily solar radiation measured outside of the glasshouse was 14.1, 20.3 and 13.6 MJ m⁻² d⁻¹, and the average daily mean temperature in the glasshouse was 23.3, 27.7 and 25.3°C for Exps. 1, 2

Table 1. Regression coefficients and Ψ at $P=0$ (intercept) as derived from linear regression analysis between P and Ψ in four cereal crops.

		Correlation coefficient between P and Ψ^*		$\Delta P/\Delta \Psi$		Ψ at $P=0$ (MPa)	
		Control	Treatment	Control	Treatment	Control	Treatment
Exp. 1	Japanese millet	0.953	0.950	0.83	0.64	-0.88	-1.09
	Sorghum	0.958	0.835	0.75	0.63	-0.95	-1.22
	Pearl millet	0.908	0.877	0.76	0.65	-1.03	-1.29
	Maize	0.875	0.885	0.59	0.51	-0.98	-1.08
Exp. 2	Japanese millet	0.988	0.933	0.84	0.61	-0.95	-1.30
	Sorghum	0.989	0.908	0.77	0.73	-0.94	-1.21
	Pearl millet	0.973	0.869	0.85	0.84	-1.01	-1.11
	Maize	0.972	0.965	0.73	0.69	-0.84	-0.91
Exp. 3	Japanese millet	0.963	0.952	0.61	0.51	-1.02	-1.16
	Sorghum	0.994	0.984	0.75	0.61	-0.92	-1.26
	Pearl millet	0.936	0.984	0.73	0.82	-0.88	-0.96
	Maize	0.966	0.984	0.65	0.72	-0.89	-0.94

* Significant at 1% level.

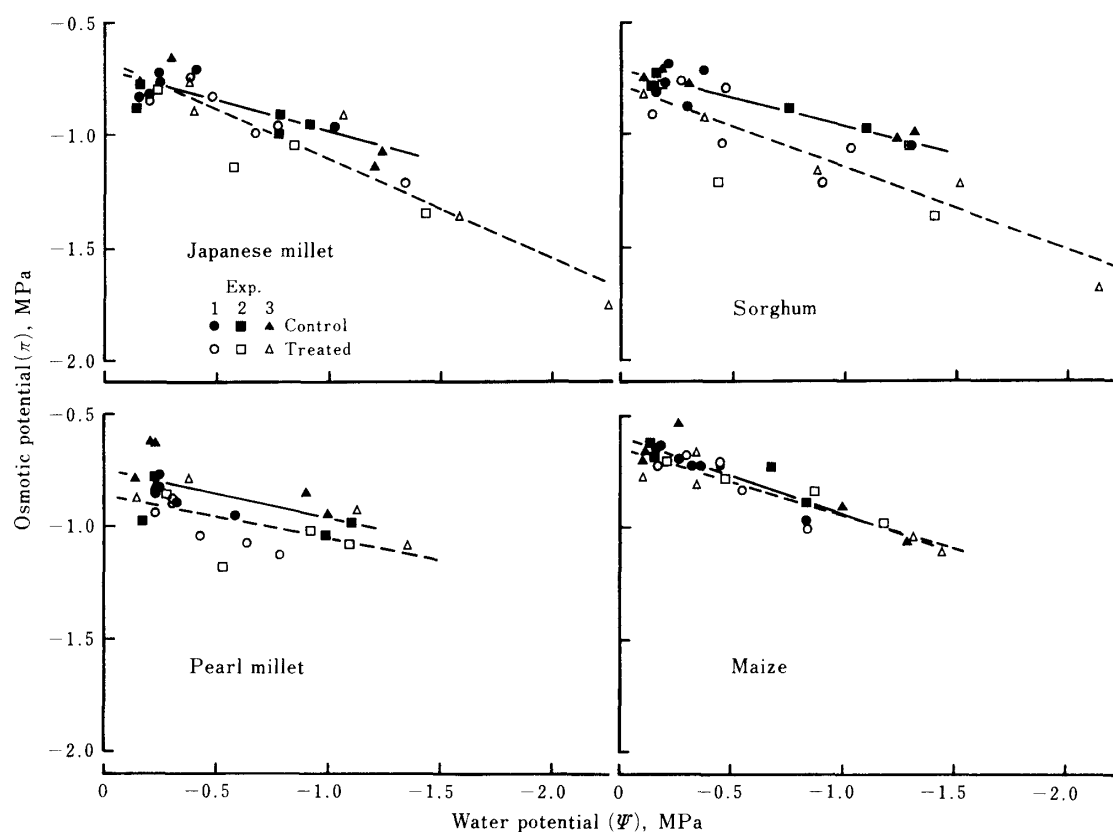


Fig. 2. Relationships between osmotic potential (π) and water potential (Ψ) of leaves for four cereal crops, including all the values determined.

and 3, respectively. Average relative humidity in the glasshouse, however, did not differ greatly among the three experiments; they were 72.6, 75.0 and 73.1%, respectively.

Growth parameters expressed as leaf area and dry weight per plant were generally reduced by water stress in Exps. 2 and 3, but little growth reduction occurred in Exp. 1. Among the species, relative dry weights of stressed plants to control ones at each measuring time were generally reduced less in pearl millet and sorghum than in the other species during stress development. Indications of recovery after removal of the stress were found in sorghum and Japanese millet but were negligible or nonexistent in pearl millet and maize (Fig. 1). Similar results were also obtained for leaf area.

From the relationship between P and Ψ , it was found that P decreased linearly with decreasing Ψ in any species used. The slope of P versus Ψ ($\Delta P/\Delta \Psi$) and the value of Ψ at zero turgor ($P=0$) were calculated from their relationships. These are given in Table 1. The $\Delta P/\Delta \Psi$ in the stressed plants was generally

lower than that in the control. The Ψ at zero turgor in maize was higher than that in the other crops.

The changes in π with the decrease in Ψ during the progress of water stress and recovery for the three experiments are shown in Fig. 2. The values of π relative to values of Ψ in the stressed plants were lower than those in the nonstressed plants, except for maize which showed no clear difference between the two.

The relationship between RWC and Ψ differed greatly among the species (Fig. 3). The sharpest depression in RWC with the decrease of Ψ was observed in maize, followed by Japanese millet, sorghum and pearl millet in the decreasing order.

Differences in osmotic adjustment among the species are shown in Table 2. In all three experiments, a great osmotic adjustment occurred in sorghum under stress. This was less in Japanese millet and pearl millet, and negligible or nonexistent in maize. After removal of the stress, osmotic adjustment was reduced in all species.

To determine the mechanism for the inter-

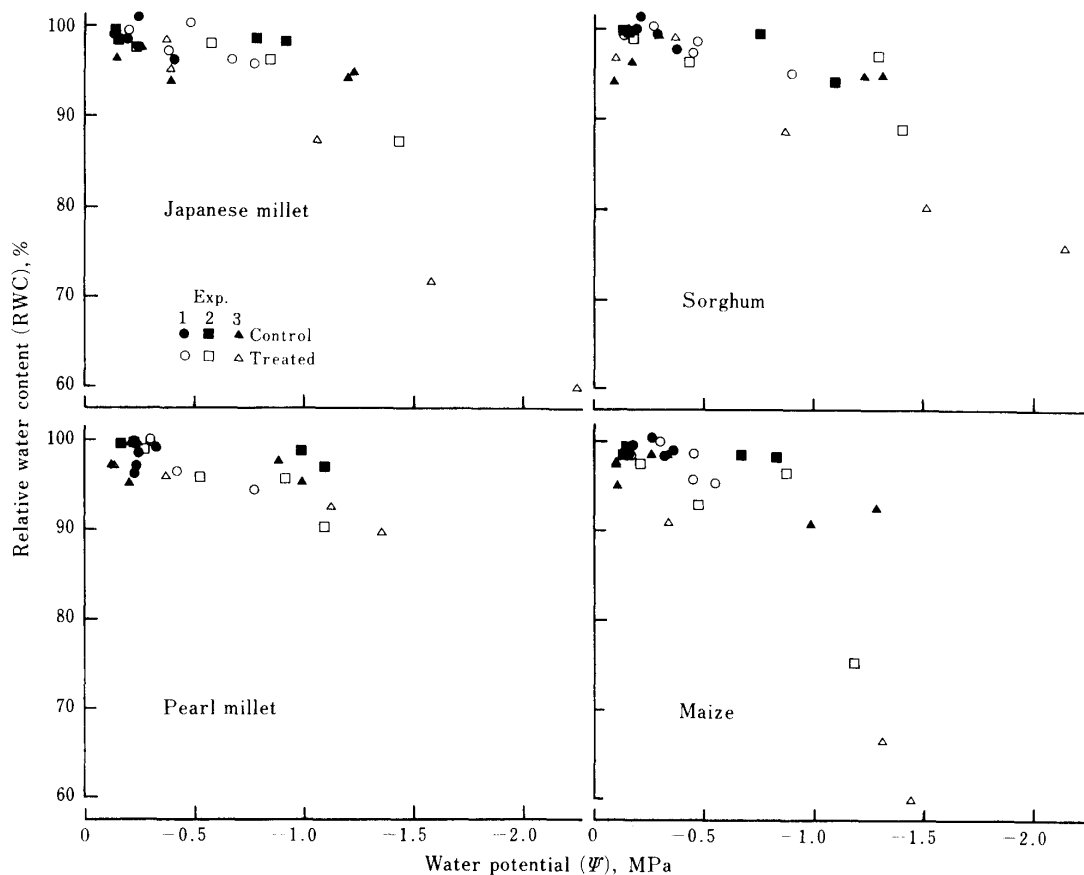


Fig. 3. Relationships between relative water content (RWC) and water potential (Ψ) of leaves for four cereal crops, including all the values determined.

Table 2. Interspecific differences in osmotic adjustment (MPa) among four cereal crops under water stress and after removal of stress.

		Under stress (A)		After stress removal (B)	
		Control	Treatment	Control	Treatment
Exp. 1	Japanese millet	0.08	0.24	0.10	0.11
	Sorghum	0.18	0.48	0.12	0.21
	Pearl millet	0.13	0.33	0.08	0.18
	Maize	0.01	0.10	0.06	0.02
Exp. 2	Japanese millet	0.18	0.47		
	Sorghum	0.16	0.56		
	Pearl Millet	0.25	0.21		
	Maize	0.03	0.13		
Exp. 3	Japanese millet	0.30	0.51	-0.04	0.01
	Sorghum	0.25	0.75	0.06	0.22
	Pearl millet	0.07	0.23	-0.14	0.06
	Maize	0.15	-0.03	-0.17	-0.03

Figures in control and treatment were calculated from values of π and RWC in control plants determined 3 or 4 days after the start of treatment and those in control and stressed plants, respectively, determined 7 to 11 days after the start of treatment (A) and 4 days after termination of treatment (B).

specific differences in π decrease, $\Delta\pi_{\text{con}}$ and $\Delta\pi_{\text{acc}}$ were examined. Remarkable differences for both effects were found between stressed and nonstressed plants in each species and among the species as shown in Fig. 4. $\Delta\pi_{\text{acc}}$ in stressed plants was greatest in sorghum, lower in Japanese millet and least in maize. On the other hand, $\Delta\pi_{\text{con}}$ in pearl millet was lower than in the others. Maize was clearly different from the other species, because $\Delta\pi_{\text{con}}$ was relatively greater than $\Delta\pi_{\text{acc}}$.

Discussion

The present study demonstrates that the effect of water stress on plant growth in dry weight and its restoration after removal of stress were strikingly different among the species studied. The growth response to the stress appeared more clearly when irradiance and temperature were higher (Exp. 2) than when they were lower (Exp. 1), suggesting the large effect of weather on the stress damage of plants.

It is a well known fact that π decreases with a decrease in Ψ in stressed leaves. As a result, P can be kept at a certain high level¹⁰⁾. The value of Ψ at zero turgor seems to be a physiologically important index, because stomatal closure occurs in sorghum and pearl

millet under this condition^{3,7,21,22)}. It was found that the value of either $\Delta\pi/\Delta\Psi$ or $\Delta P/\Delta\Psi$ in stressed plants differs among the species in Exps. 2 and 3, indicating the existence of certain interspecific differences in osmotic adjustment.

In sorghum, it was confirmed that osmotic adjustment due to $\Delta\pi_{\text{acc}}$ certainly occurred in stressed plants. Owing to the osmotic adjustment under stress and its rapid reversion to its original state after removal of stress, it seems that sorghum possesses a strong dehydration tolerance.

A distinct osmotic adjustment occurred also in Japanese millet, although this adjustment was smaller than in sorghum, indicating that Japanese millet has a high dehydration tolerance. In sorghum and Japanese millet, leaf growth and dry matter production were considerably decreased under stress, whereas their recovery took place after removal of the stress. This may be attributable to stored or compensatory growth, because it is suggested that the metabolites, accumulating during the period of suppression of cell enlargement through lack of turgor, are available for cell wall synthesis and other processes associated with growth after turgor is restored¹²⁾.

In maize, the fact that the value of Ψ at zero turgor under stress was highest, suggests

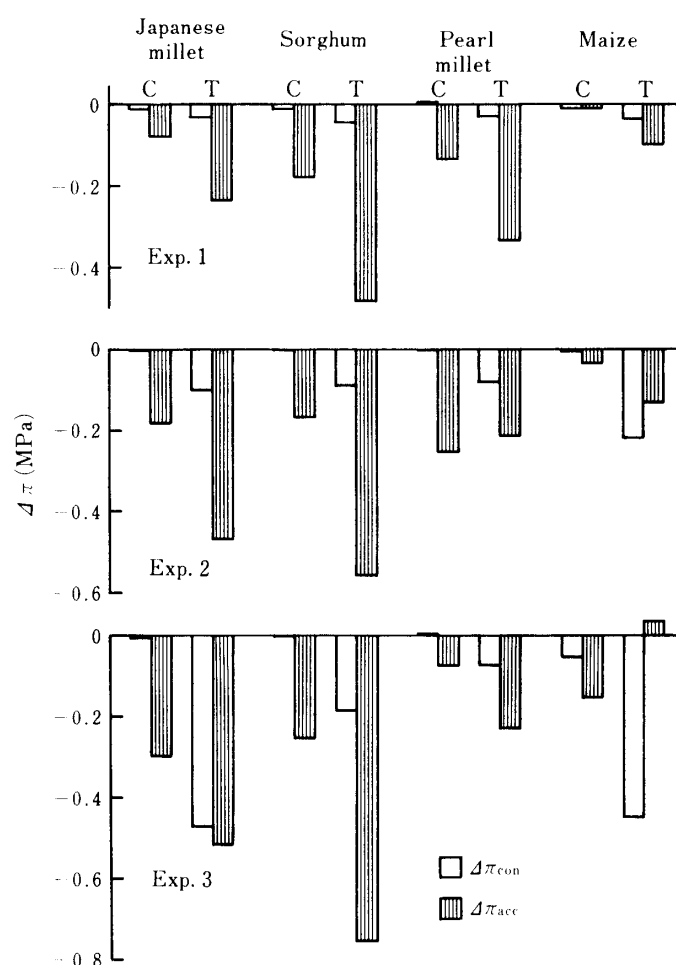


Fig. 4. Contributions of concentration effect ($\Delta\pi_{con}$) and solute accumulation effect ($\Delta\pi_{acc}$) to the decrease of osmotic potential of leaves in control and stressed plants for four cereal crops.

C : control plants, T : stressed plants.

The determination time and calculation method are the same as (A) in Table 2.

that the leaves would wilt more quickly under stress than leaves of other species, as reported by Turner²¹⁾, who compared maize with sorghum and tobacco. The fact that the relative value of $\Delta\pi_{con}$ was higher than $\Delta\pi_{acc}$ in maize under stress, unlike the other species, suggests that loss of water from the cells occurs most easily among the species used^{22,23)}.

Maize also showed a greater decrease in RWC compared with Ψ under stress. This fact suggests that the leaves lose more water under stress and require more water to recover from the stress than leaves of the other species. The present study confirmed the result indicated by Sanchez-Diaz and Kramer¹⁶⁾ that in maize RWC against Ψ widely varies and a large amount of water is lost. Slower recovery

of growth in maize after removal of stress may be caused by a low water retention ability and a small amount of metabolites accumulated in leaf cells.

Pearl millet leaves kept an RWC as high as 90 percent even at daytime under stress. This is supported by a minimum $\Delta\pi_{con}$ observed over the three experiments. There is a small possibility that osmotic adjustment took place in the stressed plants, because the $\Delta\pi_{acc}$ was small. From the fact that $\Delta\pi_{con}$ due to water loss was small, it can be assumed that pearl millet probably has solid cell walls, so that water loss is diminished. However, if water stress becomes greater, a great depression in P will occur^{14,18)}. The slow restoration of growth in pearl millet after removal of stress may be

due to retardation in leaf growth by shortage of metabolites to be used for compensatory growth, and by solid cell walls¹²⁾.

In this study, it was suggested that water retention in plant cells is mainly controlled by osmotic adjustment for sorghum and Japanese millet and by solid cell walls for pearl millet. Plants with a high ability in osmotic adjustment will be more advantageous than solid cell walls because of their quick recovery in leaf growth and dry matter production after removal of stress. The present study shows that the interspecific differences in $\Delta\pi_{acc}$ are consistently detected regardless of solution culture or field culture.

In the field culture (Exp. 3) which root growth was not limited, however, the relative dry weight of shoots in stressed pearl millet plants was much higher than that in the other species. This fact suggests that greater ability of dehydration postponement, separately from dehydration tolerance, may be involved in the mechanisms of drought tolerance in pearl millet, by means of the development of root system and/or the increase in water-absorbing activity of roots under soil water-deficient conditions.

Various indices have already been reported concerning the classification of drought tolerance^{5,6,15,16,18,19)}. This study indicates that $\Delta\pi_{acc}$ may be an effective index to estimate dehydration tolerance in the vegetative stage of plant is concerned. As for the analysis of metabolic substances involved in osmotic adjustment, further investigations will be necessary.

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