

Mechanisms of Different Responses of Leaf Photosynthesis in African Rice (*Oryza glaberrima* Steud.) and Rice (*Oryza sativa* L.) to Low Leaf Water Potential*

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Abstract : This research was conducted to elucidate the mechanisms in water stress tolerance of *Oryza glaberrima* Steud. with special reference to stomatal conductance. Leaf photosynthesis (LPS) of *O. glaberrima* Steud. with developing soil water deficiency, was still maintained above zero when the leaf water potential decreased to as low as -2.0 MPa, while in *O. sativa* L. LPS became zero at -1.6 MPa. Stomatal conductances also changed in parallel with LPS in both species, suggesting that the different response of LPS to reduced leaf water potential is due to the different stomatal response between two species. In the relationship between the stomatal conductance and abscisic acid (ABA) content in the leaves, Saka, a line of *O. glaberrima* Steud., showed a slower decrease of stomatal conductance with increase of ABA than Nipponbare, a cultivar of *O. sativa* L. This attenuated response of stomatal conductance to leaf ABA content in *O. glaberrima* Steud. was supposed to be the cause of maintenance of higher LPS than in *O. sativa* L. under low leaf water potential conditions. The Rubisco content showed no significant difference in the response to developing low leaf water potential between the two species. **Key words** : Abscisic acid, Drought tolerance, *Oryza glaberrima* Steud., *Oryza sativa* L., Photosynthesis, Rice, Stomatal conductance, Water stress.

アフリカイネ (*Oryza glaberrima* Steud.) とイネ (*O. sativa* L.) における葉の水ポテンシャル低下に対する光合成の反応 : 古谷 篤・伊藤亮一・石井龍一 (東京大学農学部)

要 旨 : アフリカイネ (*Oryza glaberrima* Steud.) はイネ (*O. sativa* L.) よりも不良環境に対する耐性が強いと言われている。本報告では、葉の水ポテンシャル低下に対する個葉光合成速度と気孔開度の反応を、*O. glaberrima* Steud. の2系統と*O. sativa* L. の2品種とで比較し、*O. glaberrima* Steud. の有する耐干性機構に関する情報を得ようとした。*O. glaberrima* Steud. は、*O. sativa* L. に比べて、より低い葉の水ポテンシャル条件下でも気孔を開き、光合成を維持していた。これは、乾燥条件下での水分維持という観点からは不利であるが、乾燥条件下でも光合成、ひいては乾物生産を行えるという点では有利な特徴といえる。次に、葉の水ポテンシャル低下にともなう葉中のアブシジン酸 (ABA) 含量の変化を調べた。その結果、ABA 含量は、両種において、葉の水ポテンシャルの低下とともに増加していたが、ABA 含量の増加に対する気孔伝導度の減少程度は、*O. sativa* L. のほうが*O. glaberrima* Steud. よりもはるかに大きいことが判った。このことが、*O. glaberrima* Steud. では、より低い葉の水ポテンシャル条件下でも気孔開度が大きく維持されている原因の一つと考えられた。なお、葉の水ポテンシャルの低下にともなう炭酸固定酵素、リブローズ-1, 5-ビスホスフェートカルボキシラーゼ含量の変化を調べたが、この点については、両種の間に明瞭な差は認められなかった。

キーワード : アブシジン酸, イネ, *Oryza glaberrima* Steud., *Oryza sativa* L., 気孔伝導度, 光合成, 耐干性, 水ストレス。

Two species in the genus of *Oryza*, *Oryza sativa* L. and *O. glaberrima* Steud., are culti-

vated in the world, although cultivation of *O. glaberrima* Steud. is gradually decreasing due to its low potential yield. However, *O. glaberrima* Steud. is supposed to be more tolerant to the unfavorable environments, such as deep water or drought¹⁾, which are occurring widely in West Africa where *O. glaberrima* Steud. is cultivated. In this paper, the mechanisms in water stress tolerance of *O. glaberrima* Steud. were examined with special reference to leaf photosynthesis (LPS) under low leaf water

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potential.

The rate of LPS is limited mainly by two steps, i.e. the step of CO₂ passage through the stomata, and that of CO₂ fixation by the enzyme of ribulose-1, 5-bisphosphate carboxylase/oxygenase (Rubisco). It was reported that when the leaves were detached, accumulation of abscisic acid (ABA) took place in the guard cells, leading to stomatal closure⁵⁾. If the stomata are sensitive to ABA accumulation, they will be rapidly closed with development of water stress, and hence LPS will be reduced even under mild water stress conditions. Thus, high stomatal sensitivity to leaf ABA content might be unsuitable for growth or yield performance of the crop plants in mild water stress conditions. In this paper, stomatal sensitivity to ABA accumulation was examined for *O. glaberrima* Steud. and *O. sativa* L. to elucidate the different mechanisms of water stress tolerance.

It was reported that CO₂ fixation by Rubisco was depressed under water stress conditions^{9,13,14)}. The extent of depression in Rubisco activity with water stress treatment should be examined as a limiting factor of LPS under water stress condition. In this paper, the response of Rubisco content to decrease of leaf water potential was also examined in *O. glaberrima* Steud. and *O. sativa* L.

Materials and Methods

1. Cultivation of plant materials

The seeds of two cultivars of *O. sativa* L., Nipponbare and Reimei, and two lines of *O. glaberrima* Steud., Kamo and Saka from Ghana, were sown in the plastic containers filled with soil in June, 1991. The plants at the 5 leaves stage were transplanted to 5 liter plastic pots with 2 seedlings per pot. Compound fertilizer was applied at the rate of 0.6, 0.9, and 0.8g per pot for N, P₂O₅ and K₂O, respectively. The plants were grown outdoors in the flooded condition.

2. Control and determination of soil water content

When the 10th leaves were fully expanded in August, the flooded water was removed out of the pots, and then the pots were transferred into the artificial lighting room to avoid disturbance of soil water content by the rain. The plants were illuminated by the lamps with 400 $\mu\text{mol m}^{-2}\text{s}^{-1}$ of photosynthetically active radi-

ation (PAR) for 13 hours in a day. When the pots were transferred to the room, the soil water content was about 55% of the wet soil weight. A small amount of water was supplied to the pots every evening for 10 days to avoid a steep decrease of pot water content. The amount of water which should be supplied was determined by weighing the pot before watering. After 10 days, the soil water content was reduced to as low as about 30% of the wet soil weight. Significant difference was not observed between the pots in the final soil water content.

3. Determination of gas exchange rates

The rates of leaf photosynthesis and transpiration were measured every morning on the uppermost fully expanded leaf. The measurement was made for 5 or 6 leaves of each cultivar or line with a semi-closed measuring system (Koito KMC-1000), under the conditions of 350 $\mu\text{L/L}$ of CO₂ concentration, 20°C of dew point, and 27°C of air temperature. The light intensity during the measurement was kept at 1500 $\mu\text{mol m}^{-2}\text{s}^{-1}$ of PAR with metal halide lamps (Toshiba Z39S-A). A water bath was put between the lamps and the leaf chamber to absorb heat from the lamps.

4. Determination of leaf water potential

Leaf water potential was determined with psychrometer (Wescor Inc. HR-33-T) on 3 leaves of each cultivar or line which were used for the gas exchange measurement. Leaf discs of 7 mm diameter were mounted in the psychrometer chambers (Wescor Inc. C-51) which were set in a polystyrene box to keep the constant temperature. The rest parts of the leaves were served for the determination of the contents of ABA and Rubisco.

5. Determination of ABA content

The content of ABA in the leaves was determined only for Nipponbare of *O. sativa* L. and Saka of *O. glaberrima* Steud. The ABA was extracted according to the method by Sakurai et al.¹²⁾, and ABA content was determined by enzyme immunoassay with Phytodetek-ABA kits (Idetek Inc.).

6. Determination of Rubisco content

The Rubisco was extracted according to the method by Makino et al.⁸⁾, and the content was determined with single radial immuno diffusion method¹⁰⁾ with purified Rubisco (Sigma) as the standard protein. The anti-

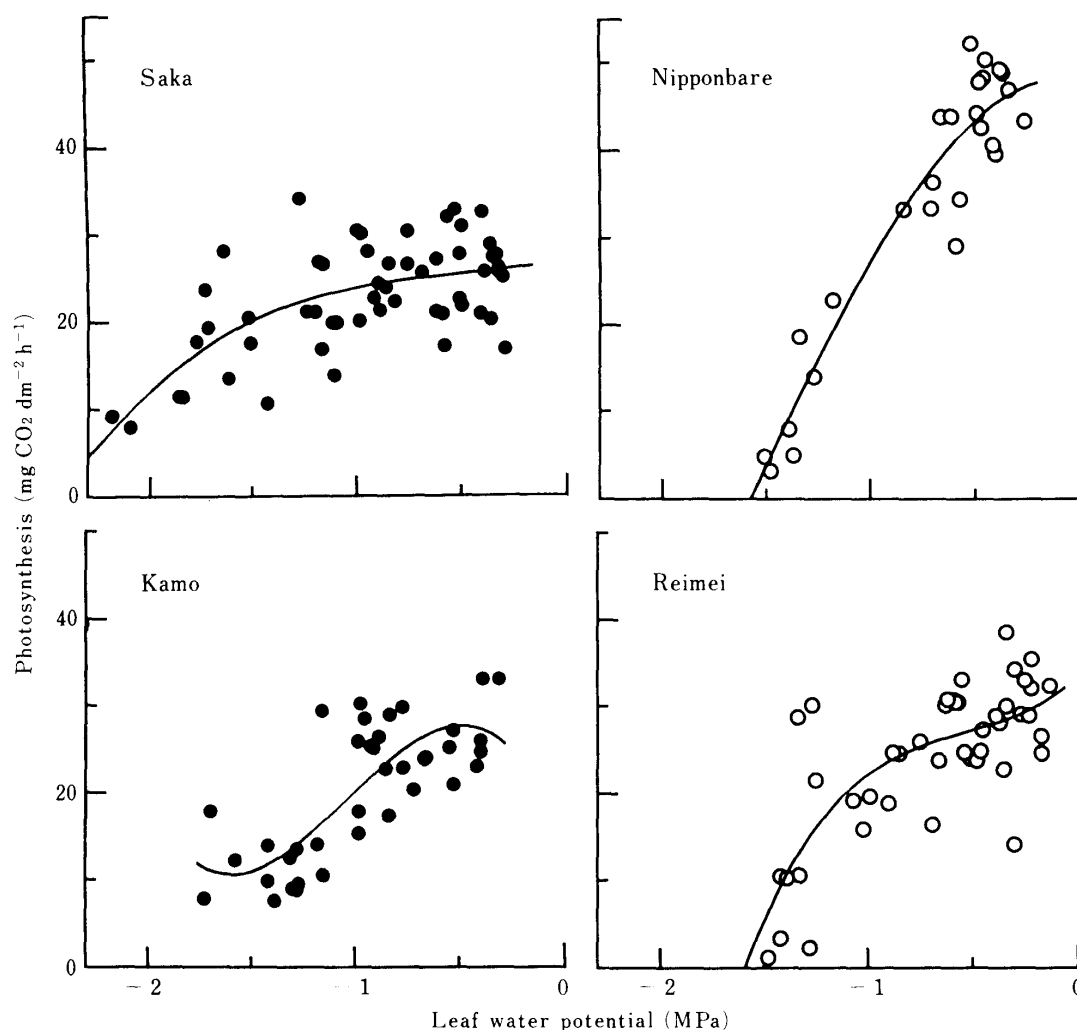


Fig. 1. Relationship of leaf water potential to leaf photosynthesis rate in two lines of *Oryza glaberrima* Steud., Saka and Kamo, and two cultivars of *O. sativa* L., Nipponbare and Reimei.

body of Rubisco was obtained from the rabbit treated with purified Rubisco protein from the leaves of *O. sativa* L.

7. Data processing

The data were plotted in a figure, and they were smoothed with a line by the least squares program of the computer.

Results

Figure 1 shows the change of leaf photosynthesis (LPS) with decrease of leaf water potential due to the soil water depletion. The LPS in Nipponbare and Reimei of *O. sativa* L. showed a rapid decrease with decrease of leaf water potential, while those in Saka and Kamo of *O. glaberrima* Steud. showed a slow decrease with decrease of leaf water potential. The critical leaf water potential where LPS became zero, was about -1.6 MPa in both of Nipponbare

and Reimei, while in Saka and Kamo it was lower than -2.0 MPa. Moreover, the leaves showed a rolling symptom in two cultivars of *O. sativa* L. at about -1.5 MPa of leaf water potential.

The response of stomatal conductance to decreasing leaf water potential was shown in Fig. 2. The stomatal response was almost the same as that of LPS. Therefore, difference in the response of LPS to the decrease of leaf water potential would be attributed mainly to that in the stomatal response.

Figure 3 shows the change of leaf ABA contents in Nipponbare of *O. sativa* L. and Saka of *O. glaberrima* Steud. with decrease of leaf water potential. In both species, ABA increased with decrease of leaf water potential. The ABA content in Saka was much higher than that in Nipponbare even in the condition

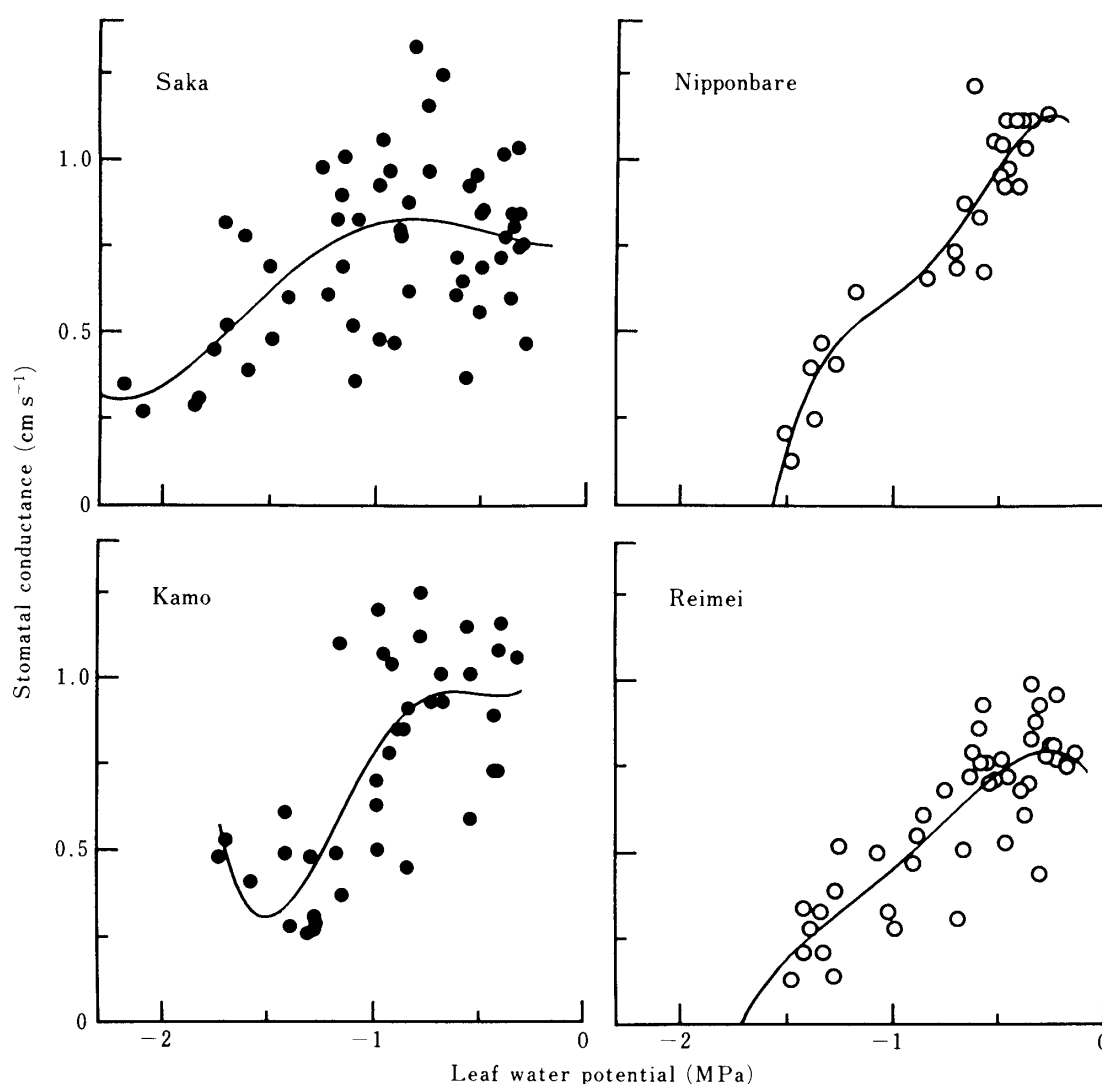


Fig. 2. Relationship of leaf water potential to stomatal conductance in two lines of *Oryza glaberrima* Steud., Saka and Kamo, and two cultivars of *O. sativa* L., Nipponbare and Reimei.

of high leaf water potential. If the stomatal conductance is plotted against ABA contents, Nipponbare showed a steep decrease with increment of leaf ABA content, while Saka showed a slow decrease (Fig. 4).

Rubisco content in the leaves decreased with decrease of leaf water potential (Fig. 5). However, significant difference was not observed between lines or cultivars in decreasing patterns of leaf Rubisco content.

Discussion

1. Response of LPS and stomatal conductance to decreasing leaf water potential

It was shown in the present paper that *O. glaberrima* Steud. was more tolerant to water

stress than *O. sativa* L. on the level of LPS. The water stress tolerance of LPS in *O. glaberrima* Steud. was considered to be due to the stomatal characteristics. As seen in Fig. 2, the plants of *O. glaberrima* Steud. were supposed to be able to keep the stomata open at such low leaf water potentials as -2.0 MPa, while the stomata in *O. sativa* L. were completely closed at such a low leaf water potential. This stomatal characteristics of *O. glaberrima* Steud. are suitable for the growing performance under water stress conditions, although they are not convenient as the survival strategies in severe water stress conditions. Morishima et al.¹¹⁾ found that the plants of *O. glaberrima* Steud. showed a low survival rate and severe damage under the drought conditions,

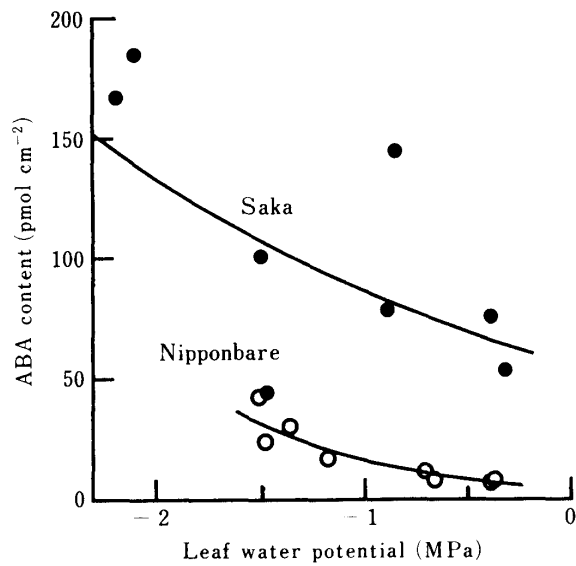


Fig. 3. Relationship of leaf water potential to ABA content in leaf blades in a line of *Oryza glaberrima* Steud., Saka, and a cultivar of *O. sativa* L., Nipponbare.

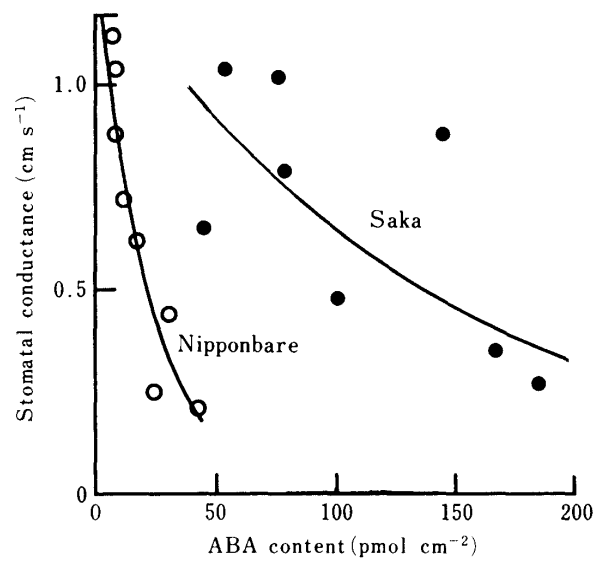


Fig. 4. Relationship of ABA content in leaf blades to stomatal conductance in a line of *Oryza glaberrima* Steud., Saka, and a cultivar of *O. sativa* L., Nipponbare.

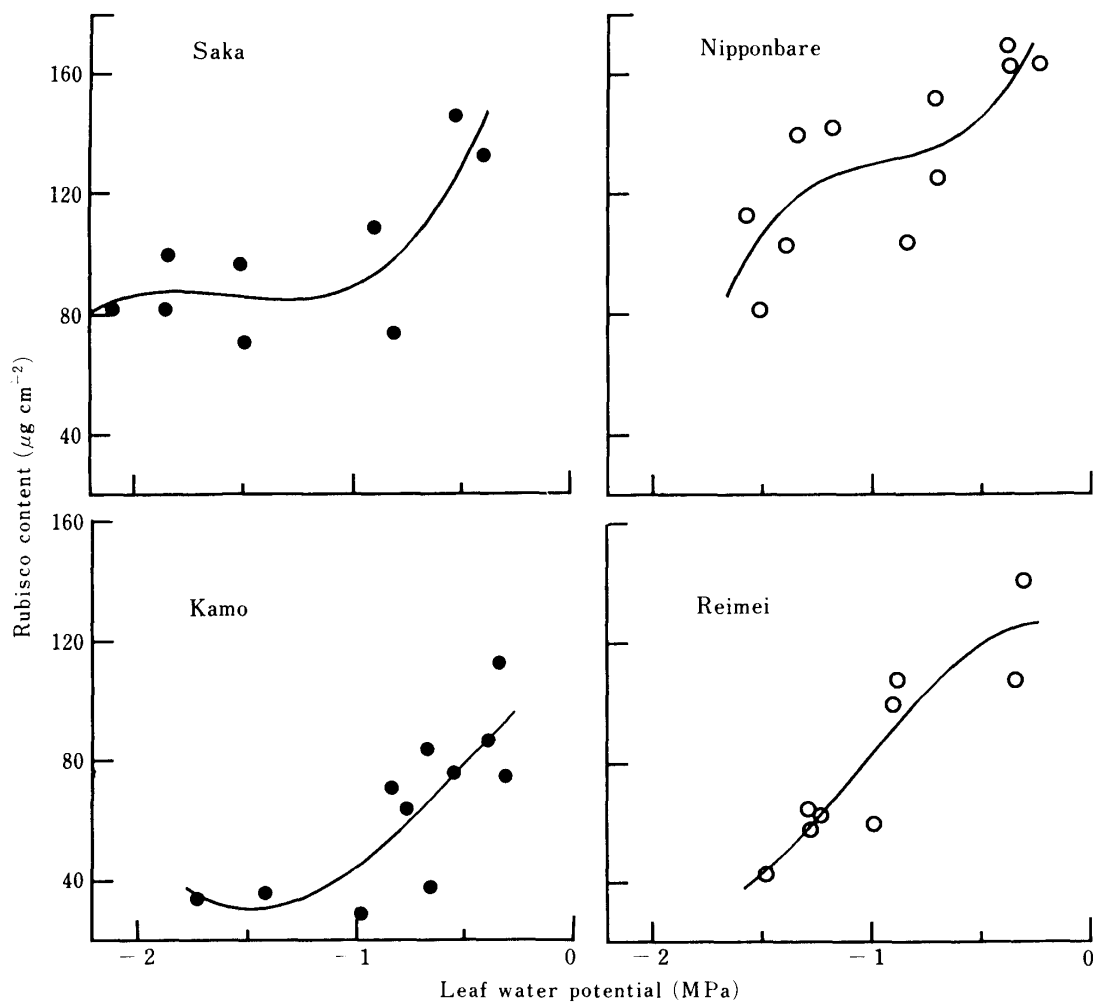


Fig. 5. Relationship of leaf water potential to Rubisco content in leaf blades in two lines of *Oryza glaberrima* Steud., Saka and Kamo, and two cultivars of *O. sativa* L., Nipponbare and Reimei.

compared with other *Oryza* species. From this, they regarded *O. glaberrima* Steud. as an intolerant species to severe drought conditions.

2. Water stress development and ABA accumulation

The ABA content was compared between in Saka, a line of *O. glaberrima* Steud. and in Nipponbare, a cultivar of *O. sativa* L. (Fig. 3). Although Saka showed a large variation of ABA content probably due to the genetic heterogeneity of this line, the present paper showed that the level of ABA in the leaf was higher in *O. glaberrima* Steud. than in *O. sativa* L. through the whole range of leaf water potential determined. Innes et al.⁷⁾ reported with spring wheat that selection for high ABA accumulation under water stress condition led to the cultivars of high yielding and high water use efficiency under water stress conditions. Larque-Saaverda and Wain³⁾ also reported that in maize and sorghum, the leaves of drought tolerant lines or cultivars showed higher ABA accumulation in water stress conditions than intolerant ones. In our data, Saka, a line of *O. glaberrima* Steud., which had been suggested to be drought tolerant, showed higher ABA content than Nipponbare, a cultivar of *O. sativa* L. even in high leaf water potential. In addition to the difference in the level of ABA, the response of stomatal conductance to ABA content was also different between *O. glaberrima* Steud. and *O. sativa* L. Nipponbare showed a steep decrease of stomatal conductance with increase of ABA content, while Saka showed a slow decrease, indicating that the stomata of Nipponbare is more sensitive to ABA content in the leaves as suggested by Henson⁶⁾. It is reported that drought resistance is strengthened by the reduction of sensitivity to ABA content⁴⁾. The stomatal insensitivity of Saka as observed in Fig. 4, might contribute to the maintenance of LPS performance in soil water deficit conditions.

Recently, the research on the mechanism of stomatal closure in relation to ABA accumulation in the leaves has been developed. It has been reported⁵⁾ that stomatal closure takes place before bulk amount of leaf ABA increases. This suggests that enough quantity of ABA for the stomatal closure is released to the apoplast, even if no change of bulk ABA is detected. For stomatal closure, the level of

apoplastic ABA is more important than the level of bulk ABA in a leaf as suggested by Zeevaart¹⁵⁾. The stomatal insensitivity to the bulk ABA in a line of *O. glaberrima* Steud., which was observed in this paper, might be due to low apoplastic ABA level in spite of high ABA contents on the bulk leaf level, or due to low stomatal sensitivity to the apoplastic ABA level.

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