Rice can adapt to excessive soil water regimes and hence is able to grow on flooded soils, which are harmful for the growth of most other crops (Yoshida, 1981; Kramer and Boyer, 1995). However, soil reduction, which occurs in anaerobic soils containing large amounts of organic matter, seriously inhibits the growth and reduces grain yield in rice (Harada, 1950; Baba, 1995). Stomatal closure is a typical symptom of the effects of submergence and soil reduction (Hirasawa et al., 1992; Zhang and Zhang, 1994; Baba, 1995; Kramer and Boyer, 1995). Decrease in leaf diffusive conductance (C_s) by stomatal closure profoundly inhibits gas exchange, thereby decreasing photosynthesis (Kramer and Boyer, 1995). The decrease in C_s may result from low leaf water potential (LWP) through decreased hydraulic conductance in the plant (Hirasawa et al., 1992). Soil reduction in paddy soils results in the production of toxins such as organic acids and hydrogen sulfide (Baba, 1995), which inhibit water absorption by roots and decrease hydraulic conductance in the plant, resulting in a low LWP (Hirasawa et al., 1992). On the other hand, C_s is controlled by non-hydraulic factors such as abscisic acid (ABA), which is supplied from the root system when plants suffer from drought (Davies and Zhang, 1991; Bano et al., 1993; Zhang et al. 2006). ABA from the root has been suggested to be responsible for stomatal closure in flooded pea plants (Zhang and Davies, 1987). Therefore, ABA seems to be the dominant signal controlling C_s in diverse plant species under flood conditions as well as drought conditions (Kramer and Boyer, 1995; Marschner, 1995). However, the effect of non-hydraulic signals on stomatal conductance in rice under anaerobic soils has not been clarified. Our objective was to clarify whether the C_s of rice growing on anaerobic reduced soils is affected by non-hydraulic factors, such as ABA.

Materials and Methods

1. The effect of soil reduction on C_s and LWP

Twenty five germinated seeds of cultivar Nipponbare were sown in a plastic pot with an inside diameter/height of 25/30 cm filled with a mixture of Andosols for rice seedlings (Green Soil, Izumo Green Co., Izumo, Japan) and a sandy soil in a volumetric ratio of 1:1 in 1997 (Pot experiment). The tillers which appeared were cut to keep the main stems uniform following a modified version of the method described by Satake (1972). Before sowing, 0.84 g N pot⁻¹ [(NH₄)₂SO₄], 1.75 g P (as superphosphate of lime) and 1.80 g K (as KCl) were mixed into the soils. At the flower initiation stage 0.42 g N pot⁻¹ was top-dressed. The pots were irrigated and starch (Starch Soluble, Kanto Chemical Co., Tokyo) dissolved in water (50 g per pot) was applied to the soil surface on 18 August. Application of organic materials such as starch to paddy fields markedly accelerates soil reduction through increase in microorganisms and sometime produces hydrogen sulfate (Takai, 1978; Baba, 1995). Eh near the soil surface was measured with pH conductivity meter (D-24, HORIBA Co., Tokyo). This amount of starch reduced the Eh of the soil surface from near 0 to −730 mV within 3 days.

The rice cultivar Nipponbare was grown in silty clay loam in a paddy field in Matsue, Shimane, Japan (Field experiment). The fields where soil condition and fertility have been carefully equalized and managed were used for the experiment. On 23 April 1997, seedlings at the 4-leaf stage which had been grown in seed beds were transplanted in rows 0.30 m apart at a spacing of 0.15 m. Four g N m⁻² [as (NH₄)₂SO₄], 12 g P (as superphosphate of lime) and 12 g K (as KCl) were applied at transplantation, and an additional 2, 4 and 4 g N m⁻² as [(NH₄)₂SO₄] were applied at the early tillering stage, at 3 weeks before heading, and at the full heading date, respectively. The experimental site was a 8.5 × 8.0 m plot. The plants were grown under submerged conditions during the entire growing season. Starch powder (0.5 kg on 16 August and 0.75 kg on 22 August 1997) was applied to the soil surface of a half of the paddy field. Plastic sheets were inserted to a depth of 10 cm from soil surface to separate the
two plots. These amounts of starch were expected to decrease Eh under $-700$ mV within 1 week.

2. Measurement of $C_s$, LWP and other components

The $C_s$ on the abaxial side of the top of the full expanded leaf was measured with a diffusion porometer ($\Delta$elta-T AP4, $\Delta$elta-T Devices, Cambridge, UK) at 10:00 am on sunny days with a photosynthetically active radiation over $1378 \pm 140 \mu$mol m$^{-2}$ s$^{-1}$. LWP was monitored with a pressure chamber (PWSC Model 3005, Soil Moisture Equipment Co., Santa Barbara, CA, USA) following the methods described by Kobata and Takami (1984) during the measurement of $C_s$. After LWP measurement, the samples were immediately placed in air-tight plastic bags, housed in an icebox and stored at $-20^\circ$C. Plant samples were thawed at room temperature and pressurized with a plastic syringe to obtain sap. The osmotic potential (OP) of the sap was measured using a psychrometer chamber with a microvolt meter (C-52-SF and HR-33Tm, Wescor, Inc., Logan, UT, USA). Turgor potential (TP) was calculated from the difference between OP and LWP.

Fig. 1. Leaf diffusive conductance ($C_s$), leaf water potential (LWP), osmotic potential (OP) and turgor potential (TP) of rice after starch application in pot and field experiments. Each value is mean $\pm$ S.E. of three or four observations. The vertical bar indicates a least significant difference at $P<0.05$ by Tukey’s test.
3. Leaf feeding of xylem sap collected from the plants under reduced soil conditions

To examine whether root sap contained any substance that might reduce $C_s$, we fed the exudate collected from the cut end of the shoots of the plants grown in well irrigated and anaerobic reduced soils, to other well-irrigated plants (Field experiment). On the evening of 27 August (12 days after the starch application) shoots were cut with a sharp razor at a height of 10 cm from the soil surface and plastic vials of 1.5 cm$^3$ containing cotton pads were attached to the cut surface, and they were covered and sealed with plastic tape to protect against water loss. In the following morning, the vials were brought to the laboratory and the sap in the cotton pads was gathered by compression with a syringe and stored at $-80^\circ$C. Sap samples of 0.3 cm$^3$ each were placed in 1.5 cm$^3$ vials, which were protected from radiation by aluminum sheets, and the vials were attached to sticks set in the field (Kobata and Hara, 1994). A leaf tip of 1 cm length was cut off in distilled water with scissors and the cut end of the leaf was immersed into sap in the vial, which was closed with a rubber stopper. The plants transplanted on 3 July 1997 and grown under well-irrigated paddy field conditions were fed with the sap at 11:00 on 2 September and 11 September 1997. After the leaf tip was immersed in the sap, the $C_s$ near the leaf tip was measured with a porometer for several hours. The amounts of the sap absorbed by the leaf was determined by weighing the vials before and after feeding. Feeding with xylem sap of the control plants changed $C_s$ less than 10% compared with the feeding with distilled water. The effect of soil reduction on $C_s$ was indicated by relative value to the control.

4. ABA analysis of the plants

The above ground parts of the plants were harvested and cut into small pieces 3 days and 9 days after the starch application in the pot experiment and in the field experiment, respectively, when the $C_s$ significantly lower than that in the control. The 10 g leaf pieces were stored in 60 cm$^3$ of 80% ethanol at $-80^\circ$C. After the solution was fractionated by Cosmosil column (Type 5C18-PABA, Nakarai Chemical Co., Tokyo), the ABA concentration of the fraction was analyzed by high performance liquid chromatography (HPLC) (655 Liquid Chromatograph, L-3000 Photo Diode Array Detector, D-6000 Data Station, Hitachi Co., Tokyo). Concentration of ABA was shown on a fresh weight basis.

Results and Discussion

1. $C_s$ and plant water status under reduced soil conditions

In the pot experiment, $C_s$ started to significantly decrease 2 days after the starch application and
decreased to less than 50% of the control (Fig. 1A). LWP was decreased slightly by starch application at 3 days after the application, but this decrease was not significant (Fig. 1B). OP and TP were not significantly changed by starch application (Fig. 1C, D).

In the field experiment, C_s was decreased significantly by starch application at 8 days after the application (Fig. 1E). Significant decreases in LWP and TP by starch treatment were observed several days after the starch application, although the differences were small (Fig. 1F, H). No significant decrease in OP was observed (Fig. 1G). The results of both experiments showed that C_s under reduced soil conditions significantly reduced before WP and TP started to decrease. Under drought or anaerobic soil conditions, the decrease in C_s started to decline before plant water status changed significantly. When rice leaves were fed with xylem sap gathered from plants under soil reduction, C_s was significantly decreased. Both of these results strongly suggest that non-hydraulic substances contained in xylem sap suppress C_s in leaves with high LWP. We conclude that the non-hydraulic factor plays an important role in stomatal closure under reduced soil conditions in rice.

The higher total-ABA concentration in the shoots under anaerobic reduced soils is accompanied with lower C_s, and hence ABA is suggested to be a chemical signal to control C_s. However, when stomata of young pea leaves closed under flood conditions, ABA in the leaves seemed to come not from roots but from old wilted leaves (Zhang and Zhang, 1994). The possibility of ABA production in response to flood may differ between rice and other crops, because rice roots can survive under stronger anaerobic soil conditions but the roots of other species collapse rapidly and die within a few days under flooding (Zhang and Zhang, 1994). Contributions of ABA to stomatal control in rice under reduced soil conditions should be investigated further.

2. The effect of xylem-sap feeding on C_s

Xylem sap was collected from the cut end of the stem base in the field experiment and fed to well-irrigated plants for four hours at two days. The amount of xylem sap collected in the plot treated with starch was 77% of that in the control (0.34 g tiller$^{-1}$) and 0.12 and 0.16 g tiller$^{-1}$ of xylem sap were absorbed from the vials in the first and second feeding during four hours, respectively. When the xylem sap collected from the plants grown in reduced soil was fed, the C_s was reduced by 16% compared with the control after a 4-hour feeding (Fig. 2).

3. ABA concentrations and C_s

Under starch treatment, trans- and total-ABA concentrations increased to 140% and 113% of the control, respectively, while cis-ABA decreased to 43% (Fig. 3). Thus, starch application increased total-ABA through increases of trans-ABA in the shoots. C_s had a close negative correlation with the trans- and total-ABA concentration in plants suffering from reduction of soil, but not with the cis-ABA concentration. It should be noted that total-ABA plays an important regulatory role in stomatal conductance of rice leaves under reduction soil conditions through the accumulation of trans-ABA.

**Conclusion**

In the plants suffering from anaerobic soil reduction, C_s started to decline before plant water status changed significantly. When rice leaves were fed with xylem sap gathered from plants under soil reduction, C_s was significantly decreased. Both of these results strongly suggest that non-hydraulic substances contained in xylem sap suppress C_s in leaves with a high LWP. We conclude that the non-hydraulic factor plays an important role in stomatal closure under reduced soil conditions in rice.

References

Baba, I. 1995. In T. Matsuo et al. eds., Science of the Rice Plant, Vol. 2 Physiology. Food and Agriculture Policy Research Center, Tokyo, 889-938.

Bano, A. et al. 1993. Aust. J. Plant Physiol. 20 : 109-115.

Davies, W. J. and Zhang, J. 1991. Ann. Rev. Plant Physiol. and Plant Mol. Biol. 42 : 55-76.

Harada, T. 1950. Agric. Hort. 25 : 43-48*.

Hirasawa, T. et al. 1992. Jpn. J. Crop Sci. 61 : 145-152**.

Kobata, T. and Takami, S. 1984. Jpn. J. Crop Sci. 53 : 299-298**.

Kobata, T. and Hara, S. 1994. Jpn. J. Crop Sci. 63 : 638-642.

Kramer, P. J. and Boyer, J. S. 1995. Water Relations of Plants and Soils. Academic Press, San Diego, USA. 193-195.

Marschner, H. 1995. Mineral Nutrition of Higher Plants. Acd. Press, London, UK. 633-635.

Satake, T. 1972. Proc. Crop Sci. Soc. Jpn. 41 : 361-362*.

Takai, Y. 1978. Soil responses to flooded conditions. In K. Kawaguchi ed., Soil Science of Paddy Field. Kodanshya, Tokyo. 23-53*.

Yoshida, S. 1981. Fundamentals of Rice Crop Science. IRRI, Los Banos, Philippines. 65-110.

Zhang, J. and Davies, W. J. 1987. J. Exp. Bot. 38 : 649-659.

Zhang, J. and Zhang, X. 1994. J. Exp. Bot. 45 : 1335-1342.

Zhang, J. et al. 2006. Field Crop Res. 97 : 111-119.

* In Japanese.

** In Japanese with English abstract.