Diurnal Course of Stomatal Aperture of Leaf Blades in Rice Plants

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The plant-water relationship in rice plants has aroused little interest because they are usually grown in submerged paddy fields with enough water supply. However, incipient wilting symptom of leaf blades in rice plants has occasionally been observed on fine days in a hot summer due to the unbalance of water economy. From the facts[9] that water condition of leaf blades has much bearing on stomatal movements it is generally assumed that the stomatal aperture becomes narrower on fine days even when plants are grown in submerged paddy fields.

Generally speaking, the photosynthetic rate of plants is much affected by the stomatal aperture which controls the diffusion rate of carbon dioxide from the atmosphere into the air cavity system inside the leaf when carbon dioxide supply is limiting the photosynthetic rate[8]. Though the prominent effects of stomatal aperture on photosynthetic rate have been emphasized ever since Boysen-Jensen[2], very few researches[10] have been done on the behavior of stomata and on the relations between stomatal aperture and photosynthetic rate in rice plants.

Therefore, the diurnal courses of change in stomatal aperture of leaf blades on the main stem at various growth stages of rice plants (cultivar Manryo) under various weather conditions were studied by using the infiltration method for measuring the stomatal aperture.

Method for measurement of the stomatal aperture

The infiltration solutions used here were a series of solutions made up of a mixture of iso-butanol and ethylene glycol in the proportion of 9 : 1, 8 : 2, 7 : 3 and so on[3]. Stomatal aperture was expressed in terms of composition of the solution penetrating the leaf blade through the stomata and this was referred to as the infiltration score. Scores and composition of mixture in relation to penetration and stomatal aperture are shown in Table 1. When iso-butanol and ethylene glycol mixture at the proportion of 8 : 2 clearly penetrated a leaf blade, but the mixture at the proportion of

Table 1. Scores and composition of mixture (iso-butanol : ethylene glycol) in relation to penetration and stomatal aperture

Composition of mixture: Iso-butanol : Ethylene glycol	Penetration: Stomatal aperture Narrow← →Wide, Scores 1	2	3	4	5
9 : 1	+	+	+	+	+
8 : 2		+	+	+	+
7 : 3			+	+	+
6 : 4				+	+
5 : 5					+

Symbols + indicate that the mixture solution penetrated into leaf blades.

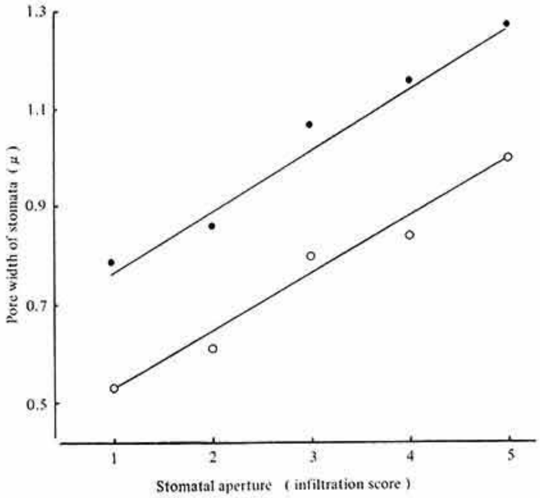

Fig. 1. Comparison between stomatal aperture (infiltration score) and the average of pore width of all stomata (open circles) as well as that of the widest 10 percent stomata (closed circles) measured in a leaf blade.

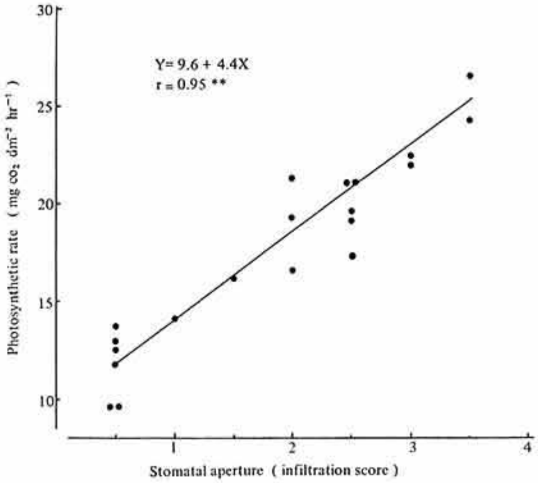

Fig. 2. Relationship between stomatal aperture and photosynthetic rate of leaf blades.

7 : 3 penetrated it very little, the stomatal aperture in this leaf blade was represented by a score of 2.5, the middle value between the scores of 2 and 3. Penetration was indicated as small dark dots of almost the same size just after the infiltration solution was dropped on the abaxial surface of leaf blades. Small dots were so clear that it could be easily distinguished if the solution penetrated leaf blades through the stomata or not, because the size of the stomata was not so different from each other in the middle part of leaf blades and because the penetrated solution was difficult to move in the very narrow intercellular spaces in mesophyll of rice plants.

The stomatal aperture and the pore width of the stomata were measured in the same leaf blade using the infiltration and the microrelief method, respectively[3]. A linear relationship was obtained between the stomatal aperture and the average pore width of all stomata as well as that of the widest 10 percent of stomata measured in a leaf blade (Fig. 1). Furthermore, just after the photosynthetic rate of an intact leaf was measured by aerating method using assimilation chamber, the stomatal aperture of the identical leaf blade was measured by using the infiltration method[6]. High correlation was found between stomatal aperture and photosynthetic rate, the wider the former, the higher the latter (Fig. 2). This relation was not affected by the difference at the growth stage of rice plants or leaf age. It is concluded from these results that the infiltration method using mixed solutions of iso-butanol and ethylene glycol is quite suitable for measuring stomatal aperture as well as for quantitatively estimating the ability for gas diffusion through the stomata of leaf blades for rice plants standing in a paddy field.

Diurnal course of stomatal aperture

Fig. 3 shows the diurnal course of change in stomatal aperture of leaf blades on a cloudy day and a fine day at the booting stage of rice plants growing on the submerged paddy field of the University Farm[4]. On the cloudy and humid day (Aug. 21) the aperture increased slowly in the morning to reach the maximum at about noon and was still kept widely open for some time in the afternoon, then decreased with a decrease in solar radiation toward the evening. While on the fine

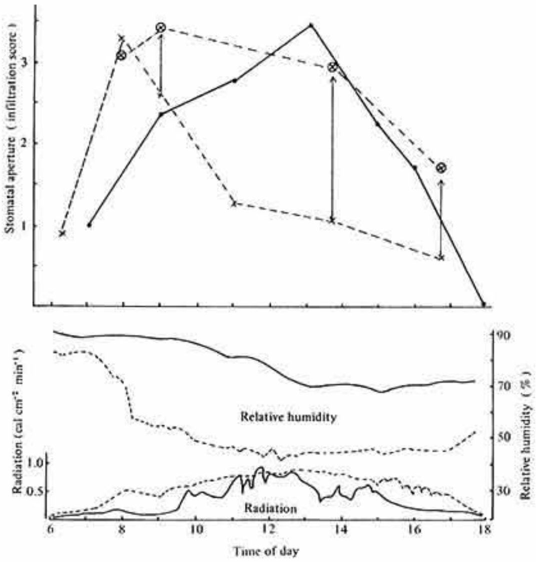

Fig. 3. Diurnal course of the average stomatal aperture of fully-expanded upper four leaves in rice plants at booting stage, with solar radiation and relative humidity on a cloudy day indicated by solid lines (Aug. 21) and on a fine day, by broken lines (Aug. 22).
Symbols surrounded by circles indicate the aperture in plants covered with polyethylene bag (Aug. 22)
Aug. 21 : Rad. 260 cal cm^{-2} day^{-1}, Max. temp. 30.5°C, Min. humid. 67%
Aug. 22 : Rad. 489 cal cm^{-2} day^{-1}, Max. temp. 31.0°C, Min. humid. 42%

and comparatively dry day (Aug. 22) the aperture increased rapidly with an increase in solar radiation early in the morning to reach the maximum at about 8:00 a.m., then decreased very quickly to only 1/2 or less of the maximum in the afternoon. The midday decrease of leaf water content on an areal basis was more remarkable on the fine day, even though the difference of diurnal variations between the cloudy and the fine day was smaller as was expected[7]. After the rice plants with narrow stomatal aperture in the afternoon were kept under highly humid condition by covering them with polyethylene bags for 20–30 min, then the stomata opened again up to near the maximum aperture of the day when solar radiation was enough for their opening. Almost the same results were obtained at different growth stages of rice plants. These results clearly show that the stomata are closed to a considerable extent due to water stress even in leaf blades of those rice plants growing in submerged paddy fields.

The diurnal course of change in stomatal aperture was quite different depending on weather conditions from day to day, but the diurnal maximum of the average aperture in fully-expanded upper four leaves was almost the same irrespective of weather conditions

Table 2. Diurnal maximum stomatal aperture at various growth stages

Growth stage	Leaf position on the stem: Upper← I *	II	III	IV	→Lower V	Average**
Maximum tillering	3.0	3.2	3.0	1.2	1.0	2.6
Panicle initiation	(2.5)***	4.2	4.2	2.8	1.5	3.2
Panicle formation	3.5	3.8	3.8	2.0	0.3	3.3
Booting	3.2****	4.3	3.8	2.5	1.7	3.4
Heading	3.7****	4.0	2.8	2.0	1.7	3.1
Early ripening	3.2	3.2	2.2	1.0	—	2.4
Middle ripening	3.3	3.0	1.5	0.5	—	2.1
Late ripening	2.0	1.3	0.6	0.5	—	1.1

* I Indicates 12th, 14th, 15th and 16th (flag) leaf at maximum tillering, at panicle initiation, at panicle formation and after booting stage, respectively.
** Average stomatal aperture of fully-expanded upper four leaves.
*** An expanding leaf with its elongating sheath.
**** An expanded leaf with its elongating internode.

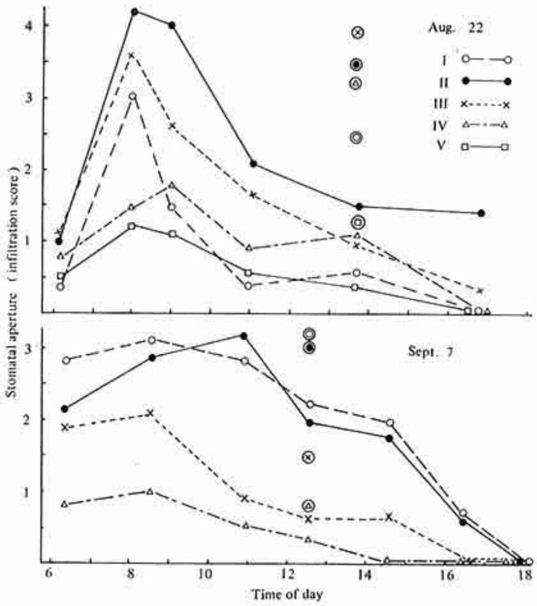

Fig. 4. Diurnal course of change in the stomatal aperture of leaf blades at different positions on the stem in rice plants at the booting (Aug. 22) and ripening (Sept. 7) stages.

I, II···V indicate 16th (flag), 15th··· 12th leaf, respectively, 16th leaf at the booting stage connected with its elongating internode. Symbols surrounded by circles indicate the aperture in plants covered with polyethylene bag.
Sept. 7 : Rad. 380 cal cm^{-2} day^{-1},
Max. temp. 27.5°C, Min. humid. 55%
Aug. 22 : Weather condition is shown in Fig. 3.

except for cloudy days with very low light intensity (Fig. 3)[4]. However, the maximum aperture was different depending on the growth stage, becoming less with advance of ripening after heading, though it was practically the same from tillering to heading stage (Table 2). Furthermore, the aperture reached the maximum earlier in the morning and then decreased more quickly, possibly due to severer water stress compared with that before heading stage when fine weather ensued before and after heading stage[4].

The stomatal aperture of leaf blades was different from each other depending on the leaf position on the stem[5]. As to fully-expanded leaves, the higher their position on the stem, the wider was the diurnal maximum of their stomatal aperture (Table 2). The difference in the aperture due to leaf position was also kept in the diurnal course of stomatal movement (Fig. 4). Moreover, the aperture of lower leaves was closed to a more considerable extent than that of upper ones in the afternoon on fine days, especially at ripening stage. After the stomatal closure in the afternoon the stomata of leaves at every position on the stem opened again up to nearly the diurnal maximum when the plants were covered by polyethylene bags for 20–30 min. As the difference in stomatal aperture due to leaf position was found not only in rice plants grown at the south border of paddy field, but also in those grown in pots where they were cultivated isolatedly, these phenomena probably did not depend on the light deficiency of lower leaves due to mutual shading in the stand, but on the difference in age or physiological characters between upper and lower leaves[5].

The stomatal aperture of an expanding leaf with its elongating sheath was narrower compared with that of the adjacent fully-expanded leaf (Table 2). Also the aperture of the highest, fully-expanded leaf (flag leaf) with its elongating internode of the stem was not so wide as that in the adjacent lower leaf at the booting and heading stage (Table 2). The stomata of the leaves with their elongating sheaths or internodes closed more quickly after reaching their maximum aperture compared with those of leaves at other positions on the stem (Fig. 4). The stomatal closure of these leaves may occure due to severer water stress because the resistance of water transport through vessels is much larger in the differentiating vascular bundles of the elongating sheaths or internodes compared with that of matured ones[5].

Discussion and conclusion

It was made clear from these results that rice plants suffer water stress due to unbalance in water economy, so that the stomata are closed to a considerable extent in the afternoon on fine days accompanied by intense transpiration even when the plants grow in submerged paddy field with negligible influence of water potential surrounding their roots. And the stomatal aperture of fully-expanded leaves at higher positions on the stem was found to be wider all day than that of leaves at lower positions due to the difference in physiological characters.

Considering the stomatal behavior and the close relationship between stomatal aperture and photosynthetic rate[6], stomatal closure owing to water stress, especially in the afternoon on fine days with high light intensity, must have much bearing on the photosynthetic rate in rice plants, that is, the photosynthetic rate will not only be limited by light deficiency due to mutual shading[10,11], but also by the diffusion rate of carbon dioxide which is under the control of stomatal aperture in paddy fields on fine days. Therefore, rice plants can not use solar radiation energy efficiently due to the unbalance of water economy between water absorption through roots and transpiration. A possible suggestion is that the efficiency of light energy utilization in rice plants will be increased by improving the ability of water absorption through roots on fine days, especially that after the heading stage. Furthermore, the low photosynthetic capacity in old leaves with low nitrogen content[1,10] may be related to the narrow stomatal aperture of leaves at lower positions on the stem. At present it would be difficult to come to a firm conclusion before sufficient knowledges is accumulated as to what kinds of physiological characters in rice plants have bearing on the stomatal aperture and the diurnal course of its change.

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