Genotypic Variation of Stomatal Conductance in Relation to Stomatal Density and Length in Rice (Oryza sativa L.)

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Abstract: Stomatal conductance (gₛ) is an important trait responsible for the genotypic difference in gas diffusion for photosynthesis and transpiration in rice (Oryza sativa L.). We measured gₛ, stomatal density and stomatal length (guard-cell length) at two weeks before heading for 64 accessions from a rice diversity research set of germplasm (RDRS) and for three high-yielding cultivars (HYC) under field conditions. Considerable variations in gₛ, and stomatal length were observed among varieties in RDRS, and it was considered that RDRS covers the species diversity of the stomatal characteristics in rice. When it was compared among the varieties with similar plant earliness, gₛ was higher in HYC than in most varieties of RDRS. Stomatal density did not correlate with gₛ, and there was a negative correlation between stomatal density and stomatal length. However, noticeable variance existed in the latter relation, where HYC exhibited a higher stomatal density and slightly shorter stomatal length than RDRS. High gₛ in HYC is attributable to their high stomatal density and moderate specific stomatal conductance (gₛ / stomatal density) while the high-gₛ varieties in RDRS tended to have a lower stomatal density and higher specific stomatal conductance. Stomatal length is related to specific stomatal conductance, but there are remarkable differences between these traits. Specific stomatal conductance in HYC has not reached the upper limit for their stomatal size, which raises a possibility of further improvement of HYC in gₛ.

Key words: Rice (Oryza sativa L.), Rice diverse research set of germplasm (RDRS), Specific stomatal conductance, Stomatal conductance, Stomatal density, Stomatal length.
in rice. Assuming that RDRS almost covers genotypic variation in physiological and morphological traits of rice, a comparison of $g_s$ and its associated traits between RDRS and high-yielding cultivars released recently enables to examine the traits of high-yielding cultivars in rice diversity. This would give a good direction for further improvement in $g_s$.

We measured $g_s$, stomatal density and length at two weeks before heading in RDRS and three high-yielding cultivars under field conditions. The objectives of this study were (1) to evaluate the genotypic variations of $g_s$, stomatal density and stomatal size in rice, using RDRS, (2) to rank $g_s$ and its associated traits of high-yielding cultivars in rice diversity, and (3) to identify the relationships among $g_s$ and its associated traits.

**Materials and Methods**

1. Plant materials and growth conditions

RDRS was used in this study. The names, subspecies, and other information on each variety are listed in the paper by Kojima et al. (2005). Since five accessions (WRC54, WRC97, WRC98, WRC99, and WRC100) were unavailable when our study started, only 64 varieties out of total 69 accessions of RDRS were used. Varieties of RDRS are classified into three genotype groups based on cluster analysis of RFLP data: ‘japonica’ (J) group, consisting of *temperate* and *tropical japonica*, ‘indica 1 (I1) group’ mainly consisting of *aus* varieties, originating mainly from India, Bangladesh, Bhutan, Nepal and Sri Lanka, and ‘indica 2 (I2) group’ consists of *indica* observed in a wide area extending from Madagascar to China. Seven varieties in RDRS are defined as modern varieties, and the others as landraces by Kojima et al. (2005). Three varieties out of the seven modern varieties belong to J group, a variety to I1 group, and three varieties to I2 group. We also used three improved cultivars (HYCs); IR72 released in 1988, Takanari released in 1990 and hybrid-rice, Liang You Pei Jiu (LYPJ) released in 1999. These three cultivars are commonly known as high-yielding rice under irrigated conditions. Modern varieties in RDRS and HYCs are called ‘improved cultivars’ in the present study.

A total of 67 varieties were sown on 4 May, and were transplanted into the paddy field at Kyoto, Japan (35°29′N, 135°47′E, 65m altitude) on 26 May in 2004. The soil was alluvial loam soil classified into Haplaquept. Each plot consisted of 3 rows with 6 hills and hill spacing was 0.3×0.3 m. Each plot was fertilized with 1 g m$^{-2}$ of N, 135º47'E, 65m altitude) on 26 May in 2004. The soil transpiration measurements (LI-6400, LI-COR, Lincoln, NE, USA). The measurements were made at two weeks before heading, but for the cultivars that did not differentiate panicles under natural conditions at Kyoto, $g_s$ was measured at 100 days after transplanting. After panicle initiation was examined under a stereomicroscope (SZ, OLYMPUS, Tokyo, Japan), two weeks before heading was estimated assuming that 32 days are required from panicle initiation to heading. Thus, the leaves for the measurements were the first or second leaves below flag leaves. The measurements were conducted from 0900 to 1200 on fine days at PPFD of 1500 μmol m$^{-2}$ s$^{-1}$ with a LI-COR LI-6400-02B light source. Leaf temperatures, CO$_2$ concentrations surrounding leaf and vapour pressure deficits were 31.6±0.7°C, 348±9 μmol mol$^{-1}$ and 1.3±0.2 kPa, respectively, over measurements. Measurement duration averaged about 60s. The leaf areas enclosed by a chamber were 3-6 cm$^2$.

2. Measurements of stomatal conductance ($g_s$)

We measured $g_s$ in the youngest fully-expanded leaves of each cultivar with 3-5 replicates with a portable-type apparatus for photosynthesis and transpiration measurements (LI-6400, LI-COR, Lincoln, NE, USA). The measurements were made at two weeks before heading, but for the cultivars that did not differentiate panicles under natural conditions at Kyoto, $g_s$ was measured at 100 days after transplanting. After panicle initiation was examined under a stereomicroscope (SZ, OLYMPUS, Tokyo, Japan), two weeks before heading was estimated assuming that 32 days are required from panicle initiation to heading. Thus, the leaves for the measurements were the first or second leaves below flag leaves. The measurements were conducted from 0900 to 1200 on fine days at PPFD of 1500 μmol m$^{-2}$ s$^{-1}$ with a LI-COR LI-6400-02B light source. Leaf temperatures, CO$_2$ concentrations surrounding leaf and vapour pressure deficits were 31.6±0.7°C, 348±9 μmol mol$^{-1}$ and 1.3±0.2 kPa, respectively, over measurements. Measurement duration averaged about 60s. The leaf areas enclosed by a chamber were 3-6 cm$^2$.

3. Measurement of stomatal density and stomatal length

After gas-exchange measurements, the replicas of adaxial and abaxial surfaces of the three measured leaves were made by attaching a SUMP disc (plate B with solution 2, SUMP, Tokyo, Japan) to the middle part of leaf blade. These replicas were observed at 400-fold (10×40) magnification with a microscope (BHS-323, OLYMPUS, Tokyo, Japan). The density of...
stomata was counted in a field of 0.20 mm² with five replicates for each replica. The length of guard cells was measured for 20 stomata selected randomly in a replica. Stomatal density and length for each leaf were the sum and average of those of both surfaces, respectively.

**Results**

A considerable difference was observed in $g_s$ in RDRS, which ranged from 0.21 mol m⁻² s⁻¹ (Rambhog, I2, landrace) to 0.92 mol m⁻² s⁻¹ (Keiboba, I2, landrace) (Fig. 1). The average $g_s$ value of I1 group, 0.55 mol m⁻² s⁻¹, was significantly higher than that of I2 group, 0.41 mol m⁻² s⁻¹, but these groups showed no significant difference from J group (0.44 mol m⁻² s⁻¹ on the average, Table 1). The $g_s$ values of Takanari, IR72 and LYPJ were 0.63, 0.54 and 0.51 mol m⁻² s⁻¹, respectively, and the average $g_s$ value of the improved cultivars (0.55 mol m⁻² s⁻¹) was higher than that of landraces in RDRS (0.46 mol m⁻² s⁻¹).

Stomatal density ranged from 691 no. mm⁻² (Local Basmati, I1, landrace) to 1427 no. mm⁻² (Radin Goi Sesat, I2, landrace, Fig. 2A), and stomatal length ranged from 18.7 µm (Radin Goi Sesat) to 25.2 µm (Tupa729, J, landrace, Fig. 2B). Significant differences were found in both stomatal density and length between I1 and I2 groups (Table 1). Stomatal density of HYC averaged 1341 no. mm⁻², which is comparable with those of the varieties with the highest stomatal density in RDRS. Stomatal length of HYC averaged 21.1 µm, and was similar to the average of RDRS (21.6 µm). Stomatal density was larger in the improved cultivars than in the landraces, and no difference exists in stomatal length. The values of $g_s$ and stomatal length in RDRS showed near normal distributions, while the peak of the distribution of stomatal density appeared at lower stomatal density (Figs. 1 and 2).

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**Table 1.** Mean values of stomatal conductance ($g_s$), stomatal density, stomatal length, and specific stomatal conductance ($g_s$/stomatal density) for three genotype groups in a rice diverse research set of germplasm (RDRS) and for recent high-yielding cultivars (HYC). Means of landraces and improved cultivars are also compared.

| group   | variety | $g_s$  | stomatal density | stomatal length | specific stomatal density |
|---------|---------|--------|------------------|-----------------|--------------------------|
|         |         | mol CO₂ m⁻² s⁻¹ | no. mm⁻² | µm              | nmol CO₂ no.⁻¹ s⁻¹     |
| RDRS    |         |        |                  |                 |                          |
| J       | 14      | 0.44 ab | 962 ab           | 21.5 ab         | 0.46 ab                  |
| I1      | 23      | 0.55 a  | 936 a            | 22.3 a          | 0.60 a                   |
| I2      | 27      | 0.41 b  | 1052 b           | 21.1 b          | 0.40 b                   |
| whole RDRS | 64 | 0.47   | 991 a            | 21.6            | 0.49                      |
| HYC     |         |        |                  |                 |                          |
| landrace | 57      | 0.46 a  | 981 a            | 21.6            | 0.48                      |
| improved* | 10     | 0.55 b  | 1151 b           | 21.5 n.s.       | 0.49 n.s.                 |

* include modern varieties in RDRS and HYC.

Values are expressed as mean of each group.

Figures followed by a different letter and n.s. denote significant difference at 5% level, and non-significance, respectively.
We calculated specific stomatal conductance, where g\textsubscript{s} was divided by stomatal density, to evaluate contribution of a single stomata to g\textsubscript{s}. There was a difference in specific stomatal conductance between I\textsubscript{1} and I\textsubscript{2} groups (Table 1). I\textsubscript{1} group had higher specific stomatal conductance, and I\textsubscript{2} group did lower ones in RDRS. On the other hand, J group exhibited specific stomatal conductance intermediate between I\textsubscript{1} and I\textsubscript{2} groups. Specific stomatal conductance was slightly lower in HYC than in RDRS, but that of the improved cultivars was comparable with that in the landraces.

Table 2 shows the correlation coefficient (r) among stomatal associated traits and the number of days after transplanting to the measurements (DAT) which represents plant earliness. There was a negative correlation between g\textsubscript{s} and DAT, and g\textsubscript{s} in HYCs was higher than that in the varieties having similar plant earliness (r = −0.61, p < 0.01, Fig. 3). A negative correlation was also found between stomatal density and stomatal length (r = −0.59, p < 0.01, Fig. 4), although HYC had the highest stomatal density and medium stomatal length in RDRS.

The value of g\textsubscript{s} in the rice varieties was not significantly related to that of stomatal density (Table 2). Fig. 5 shows the relationship between stomatal density and specific stomatal conductance together with the curves for different g\textsubscript{s} values. The varieties with high g\textsubscript{s} in RDRS tended to have lower stomatal density and higher specific conductance, while HYC had the highest stomatal density and moderate specific stomatal conductance in RDRS. Stomatal length correlated with g\textsubscript{s} positively (r = 0.47, p < 0.01, Table 2). Stomatal length was also related to specific stomatal conductance significantly (r = 0.63, p < 0.01, Fig. 6). Therefore, it appeared that stomatal length affects g\textsubscript{s} through specific stomatal conductance. The broken line in Fig. 6 represents the presumable upper-limit of specific stomatal conductance at a given stomatal length, and substantial differences were observed between specific stomatal conductance of HYCs and the upper limits.

**Discussion**

RDRS exhibited considerable variations in g\textsubscript{s}, stomatal density and stomatal length (Figs. 1 and 2). Maruyama and Tajima (1990) compared some genotype groups, and reported that the abaxial leaf conductance was between the minimum of 0.17 ± 0.02 mol m\textsuperscript{−2} s\textsuperscript{−1} from ten japonica landraces and the maximum of 0.33 ± 0.02 mol m\textsuperscript{−2} s\textsuperscript{−1} in eight indica varieties. These values can be regarded as a half g\textsubscript{s}, in our study, because transpiration from adaxial and
abaxial surfaces are similar in rice leaves. Stomatal density and length ranged from 606 to 1489 no. mm\(^{-2}\) and from 17.1 to 25.6 \(\mu\)m, respectively, in the youngest leaves at tillering and flag leaves of 60 rice varieties (Kawamitsu et al., 1996). Comparison of these results with our measured values suggested that RDRS almost covers the species diversity of the stomatal characteristics in rice although the measurement conditions and the measured leaf positions differed from our study.

The I1 group exhibited a significantly larger \(g_s\), larger stomatal length, and lower stomatal density than the I2 group (Table 1). The I1 and I2 groups include different ecotypes: The I1 group mainly consists of the aus ecotype and I2 group includes various ecotypes other than aus. Tsunoda (1987) mentioned that the aus ecotype with early maturity adapts to upland, and is expected to have resistance property to drought. On the other hand, the aman ecotype, which is confined within the I2 group, adapts to lowland condition where water is abundant and shows high stomatal density and \(g_s\). Therefore, Tsunoda (1987) suggested that adaptation to different water environments caused the differentiation into aus and aman ecotypes. In our study, however, the difference in the stomatal characteristics between the I1 and I2 groups seemed to be related to the difference in plant earliness rather than adaptation to the water environments. This is because there was no consistent difference between I1 and I2 groups in \(g_s\), stomatal density and length among the varieties with similar DAT (Fig. 3). How plant earliness affects stomatal characteristics remains to be resolved.

Some japonica varieties had a considerably high \(g_s\) among the varieties in RDRS. This is interesting since \(g_s\) and/or leaf photosynthetic rate are lower in temperate and tropical japonica than in indica varieties (Weng and Chen, 1987; Maruyama and Tajima, 1990; Peng et al., 1998). This may imply that \(g_s\) of most japonica genotypes can be improved to the level similar to that in indica genotypes.

HYC exhibited higher \(g_s\) compared with the varieties with similar DAT in RDRS (Fig. 5), which associates with cooler canopy and higher leaf photosynthetic rate (Lu et al., 1994; Fischer et al., 1998; Kanemura et al., 2005; Horie et al., 2006). Stomatal density of HYC was also the highest in RDRS, and the average stomatal density of improved cultivars was also greater than that of landraces. The improved rice cultivars released after ‘Green Revolution’ show semidwarf plant statures commonly. LeCain et al. (1989) and Morgan et al. (1990) showed that semidwarf winter wheat lines exhibited smaller cell size and thus higher stomatal density and \(g_s\), compared with their nearly isogenic tall lines. This suggests that cell morphological traits relating to plant height account for the highest stomatal density of HYC. Their high stomatal densities
are partly because the three high-yielding cultivars used here would belong to the I2 group; Takanari and IR72 are the relatives of Milyang23 and IR58, respectively, which belongs to the I2 group (Kojima et al., 2005), and the parents of LYPJ are hsien ecotypes involved in the I2 group (Glaszmann, 1987).

When stomatal density increases independently of stomatal size, improvement of g is expected (Schlüter et al., 2003), but there are a number of experimental reports showing that increased stomatal density does not enhance g. (Jones, 1977; Kawamitsu et al., 1996). This is because stomatal density negatively correlates with stomatal size. A negative correlation was also observed between stomatal density and stomatal size in our study (Fig. 4). However, there is noticeable variance in the quantitative relationship between stomatal density and stomatal size, where HYC with the highest stomatal density exhibited moderate stomatal size of 21.1 µm among rice diversity (average in RDRS: 21.6 µm). This indicates that stomatal size does not vary with the change of stomatal density, when diverse rice genotypes are studied. This agrees with the previous reports for barley (Yoshida, 1978) and Arabidopsis (Schlüter et al., 2003). Our study showed that genotypic difference of stomatal density was not related to that of g, but suggested two different ways to achieve high g. One is to have high specific stomatal conductance, as observed in the varieties in RDRS showing high g, despite with low stomatal density (Fig. 5). The other is to have high stomatal density. Specific stomatal conductance of HYC is equal or lower than that of RDRS and clearly smaller than the varieties with high g, and similar plant earliness, which proved that g of HYC are increased owing to their high stomatal density.

The high g of HYC is partly attributable to their average stomatal length in rice through a positive relation between stomatal size and specific stomatal conductance (Fig. 6). There are remarked differences in specific stomatal conductance at a given stomatal length. These differences would be due to the strong influences of stomatal aperture and pore depth on specific stomatal conductance, as reported for 51 diverse varieties of rice by Maruyama and Tajima (1990). In view of Fig. 6, stomatal length restricts the upper limit of specific stomatal conductance represented by the dashed line in the figure, which could be exceeded with a given length of stomata. The specific stomatal conductance of HYC is considerably lower than the potential curve, suggesting that there is still a possibility to improve g, of HYC through higher specific conductance. The present study showed that, although there is a general trade-off between stomatal density and length, HYC has not only the highest stomatal density but also average stomatal length in rice, and thereby improved g. Information on the variation of the factors determining specific stomatal conductance such as stomatal aperture and pore depth may help further improvement of g, in HYC.

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