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Comparative Study of Stomatal Density and Gas Diffusion Resistance in Leaves of Various Types of Rice

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벼 品種類型間 잎 氣孔密度와 氣體擴散抵抗 比較

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ABSTRACT : Studies were made on differences among types and varieties of rice in stomatal density and gas diffusion resistance, and on the relationship between these traits and photosynthetic rate. Significant differences among types and varieties were found stomatal density and gas diffusion resistance. Generally, stomatal density was higher in indica varieties than in Japonica varieties, gas diffusion resistance was lower in the former than in the later, in varieties developed through indica-japonica hybridization it was intermadiate. The stomatal density was closely positively correlated with the gas conductivity and the net photosynthetic rate, was not correlated with single leaf area, and had significant negative correlation with specific leaf weight. Higher photosynthetic rate of indica varieties mainly results from its high stomatal density and low gas diffusion resistance. The result also suggested that high photosynthetic rate might be obtained if the high stomatal density and low gas diffusion resistance in indica could be combined with the larger specific leaf weight in japonica through crossing between two.

Key words : Stomatal density, Gas diffusion resistance, Net photosynthetic rate, Indica, Japonica

Stomata in the leaves of plants are the principal passage through which carbon dioxide from the surrounding atmosphere enters the leaf and water evaporates from the plant body. Stomata are tied to such physiological prosses as photosynthesis and transpiration, and therefore are a crucial factor affection photosynthetic rate, transpriation intensity, and biomass production capability. Over the past half century, researchers have conducted extensive studies(Yoshida, 1976; Ishihara etal, 1979; Ishii, 1977), from different perspective, on such aspects as the size, density, opening, and gas diffusion conductivity of stomata in leaves, particularly, since the 1970s have further noticed the effect that stomatal density and gas diffusion resistance have on net photosynthetic rate and transpiration intensity(Yoshida, 1976; 1978; Ishihara, 1979; Tsunoda, 1984).

Still there are other people who believe that stomatal density is not necessarily linked to photosynthesis, but has a material effect on transpiration intensity(Tu, 1979). The findings of Tsunoda and others[6] show that stomatal density in the leaf of rice is remmarkably higher than

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in the leaf of other cereal crops such as corn, wheat, and barley, and they think that the high photosynthetic rate in the leaf of indica rice is associated with its high stomatal density and low gas diffusion resistance(Tsunoda, 1984; 1986). Ishihara[4] and others[6] have also detected the dramatic effect that rice stomatal opening has on photosynthesis. In recent years, with the rise of physiological breeding for high photosynthetic efficiency, further probe into the interrelationships between stomatal density, gas diffusion resistance and net photosynthetic rate has become an important aspect of theoretical exploration of rice physiologycal breeding. For this purpose, based on the work of forerunners, this paper made a preliminary investigation into the difference in stomatal density between various types and varieties of rice and its relationship with such properties as gas diffusion resistance and net photosynthetic rate.

Materials and Methods

The first experiment was conducted in the plots at the Rice Laboratory of Shenyang Agriculture University. Test materials included 6 typical indica varieties, 12 typical japonica varieties, 5 indicaclinal varieties developed through crossing indica with japonica, and japonicaclinal varieties bred in a similar way. The plot adopted was 1.2m^2 in size, each consisting of 4 rows, 1m long; intervals between rows and plants were 30cm and 10cm, respectivly. Cultivation and management practice followed those in use for production fields.

During the heading stage, stomatal density on the underside of the flag leaf and its distribution among varieties were obserbed with a microscope, using the collodion. Method taken at random from each variety and 3-5 flag leaves from each plant. For each leaf, counts were 3-5 plants were made in three fields of vision. In the meantime, single leaf area and specific leaf weight were measured. Besides, gas diffusion resistance was determined in vitro with a Model AP-3Non-constant Stomatal Meter in clear windless weather under 30.6 and 58% relative humidity.

The second experiment was conducted in the pots at the same place. Potted plants covering 28 varieties from the above mentioned four types were used as test materials. Net photosynthetic rate of the flag leaf was determined during the heading stage with a Model LHB Infrared CO_2 Analyser, and stomatal density on the underside of the leaf was observed. The measurement was made at between 9 and 11 am summer time under 479 $\mu mol \cdot m^{-2} \cdot s^{-1}$ photon flux density, 325 ppm CO_2 concentration and air flow capacity 1.5$L \cdot min^{-1}$, while the temperature was controlled at about 30℃ by the circulation water cooling mothod.

Results

1. Distribution of stomatal density among varieties

From Table 1, it was clear that, regardless of the type of rice, whether it be indica or japonica, stomatal density on the leaf surface varied with leaf level and with the position in the leaf. Among the top three leaves, the highest stomatal density occurred in the flag leaf, followed by the second leaf, with the third leaf registering the lowest density. On the other hand, for the same level and the same position, stomatal density was higher on the underside of the leaf than on the upside, while for the same level but different position, stomatal density was highest in the midportion of the leaf, second in the upper portion and lowest atthe base. In addition, the difference between the upper and middle portions was relatively slight. Statistical verification also indicated that there existed a marked unevenness in the distribution of stomatal density in leaves among varieties(Table 2). Therefore, in comparing the stomatal densities of different rice types and varieties, it is advisable to take the middle or a little upper portion of the underside of leaves on the same level as denom-

Table 2. Distribution of stomata on leaf surface of rice(no · mm^{-2})

Varieties		Midle part of underside surface of differnt leaf positions			Different part of underside surface of flag leaf			Midle part of upside surface of flag leaf
		Flag leaf	2nd leaf[1]	3rd leaf[1]	Top part	Middle part	Basal part	Stomatal density
Guang-LuAi4	Average	706	606	387	680	706	493	480
(indica)	sd	9.1	13.2	9.9	14.2	9.1	13.3	14.4
Qing-Xi96	Average	600	442	380	581	600	409	440
(japonica)	sd	12.9	15.5	9.5	12.6	12.9	14.3	12.5

Note: [1] counted from the leaf downward

Table 2. Comparisons of differences on stomatal density among leaf positions and parts

Varieties		Flag leaf and 2nd leaf[1]	Flag leaf and 3rd leaf[1]	2nd leaf and 3rd leaf[1]	Middle part and top part	Middle part and basol part	Top part and vasal part	Underside suface and upside surface
Guang-LuAi4	Difference	100	310	219	26	213	187	226
(indica)	t. value	17.6	67.5	16.0	4.3	43.9	30.6	37.4
Qing-Xi96	Difference	158	220	62	19	191	172	160
(japonica)	t. value	22.5	39.1	18.1	2.9	28.1	25.6	25.3

Note : $t_{0.05(7)}=2.365$; $t_{0.01(7)}=3.499$; [1] : Counted from the flag leaf downward

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2. Difference in stomatal density between rice types and varieties

The measurements in the plot experiment manifested that the average stomatal density(at the mid portion of the underside of flag leaves, as was with the case following) of 29 varieties was 596 stomata · mm^{-2}, with a strandard deviation of 57.6. The number for 28 varieties in pot expriment was 624 stomata · mm^{-2}, with standard deviation at 60.7. The two experiments results coincided with those reported by Yoshida and Suzuki[9]. From the distribution shart(Fig. 1), it can be seen that the stomatal density for the majority of varieties was 500-650 stomata · mm^{-2}.

To find out the difference in stomatal densities between rice types, in the plot experiment, the varieties under test were divided into two

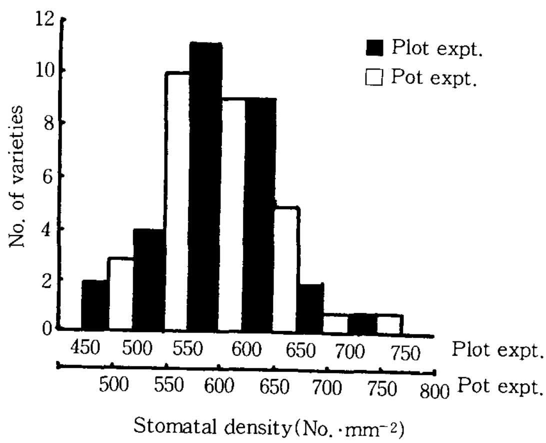

Fig. 1. Distribution of stomatal density.

catagories. It was found that the average stomatal density was 657 stamata · mm^{-2} for the indica varities and 559 stomata · mm^{-2} for the japonica, meaning the density in indica was 17.5% higher than in japonica. The correspond-

Table 3. Comparison of stomatal density among different types of rice(no · mm^{-2})

Types	No.of varieties		Average		Sd		C.V.	
	plot expt	pot expt						
Typical indica varieties	6	5	657	687	33.1	37.1	5.04	5.40
Typical japonica varieties	12	5	553	552	36.9	25.4	6.67	4.60
Indica varieties from indica Ⅱ japonica	5	9	653	671	25.7	38.4	3.95	5.72
Japonica varieties from indica Ⅱ japonica	6	9	568	586	37.7	38.6	6.64	6.58

ing figures for the pot experiment were 678 stomata · mm^{-2} for indica and 570 stomata · mm^{-1} for japonica, the former being 18.9% higher than in indica. It was also clear that within each type the degree of variation in stomatal density was by and large close between varieties.

Next, the indica were further divided into the typical indica type and the indica×japonica indicaclinous type, and the japonoca rices into the typical japonica type and the indica×japonica japonicaclinous type for variance analysis. Results demonstrated that no matter whether it be among the various types or among the diverse varieties within a type there appeared a very significant difference in stomatal density. For the four types followed an order of typical indica〉indicaclinous〉japonicaclinous〉typical japonica(table 3).

Worth mentioning is that of the two types of japonica varieties under test, the japonicalclinal varieties tended to have a higher stomatal density than the typical japonica varieties. For instance, in the plot experiment, out of the six japonicaclinal varieties the stomatal density in four surpassed the total mean value of japonica varieties. In the pot experiment, among the nine varieties, of the same group, the same thing happened in seven of them. For example, on of such varieties. Dian Yu 1, which had the highest stomatal density, recorded 643 stomata per square millimeter of leaf surface, a figure that could almost match the total mean value of japonica varieties.

3. Relationship between stomatal density and net photosynthetic rate

The net photosynthetic rates of flag leaves in 28 varieties were measured under 479μmol · m^{-2} · s^{-1} photon flux density, 325 ppm CO_2 concentration and 30℃, and their stomatal densities obserbed. The results revealed that the average net photosynthetic rate of fourteen japonica varieties was 14.09mg CO_2 dm^{-2} · hr^{-1}, with a stanard deviation of 2.34, variation coefficient of 16.61%. The corresponing figures for fourteen indica varieties were 20.16mg · CO_2 · dm^{-2} · hr^{-1}, 3.62, and 17.94%, respectively. Except for a few varieties, the overall results showed that the net photosynthetic rate was higher in indica rice than in japonica rice. It was also found that all varieties with high stomatal density tended to have a higher net photosynthetic rate. Furthermore, among the japonica varieties tested, an increased net photosynthetic rate was witnessed in the japonicaclinal varieties, compared with the typical japonica varieties. For instance, the stomatal density in such varieties as Dian Yu 1, Liao Jing 5 and Nan Jing 35 was 12.8%, 9.3%, and 8.6% higher than mean value of the japonica varieties respectively. Their net photosynthetic rates were lakewise 17.5%, 21.5%, and 13.9% higher than the mean value of the latter respectively. The results of correlation analysis further proved that a significant positive correlation existed between stomatal density and net photosynthetic rate(Fig. 2).

4. Relationship between stomatal density

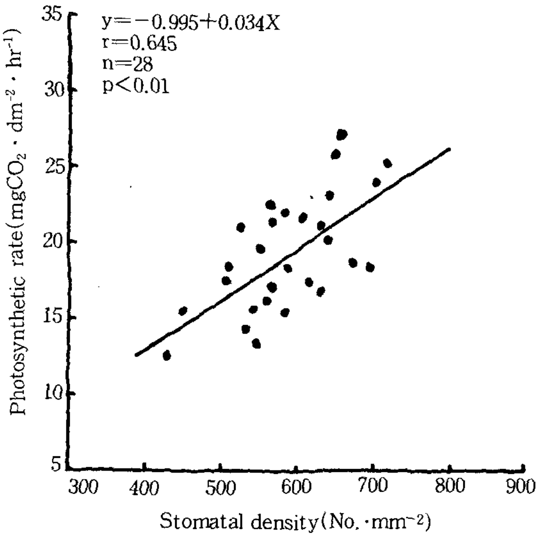

Fig. 2. Relationship between stomatal density and photosynthethic rate.

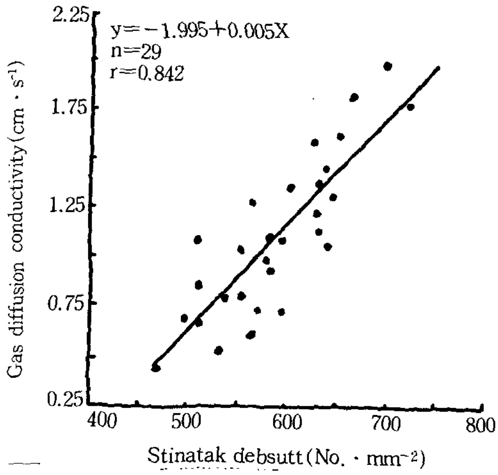

Fig. 3. Relationship between stomatal density and conductivity of gas diffusion.

and gas diffusion resistance

(1) Difference in gas diffusion resistance between rice types and between varieties

Known research results indicate that gas diffusion conductivity(the reciprocal of gas diffusion resistance) in rice leaf is linked to net photosynthetic rate, and that in those varieties that react to carbolic acid(that is, the indica rice) the leaf has a higher gas diffusion conductivity, while in those that do not react to carbolic acid(japonica rice) the leaf has a lower conductivity. In this study, the gas diffusion resistance per unit area of flag leaf in 29 varieties from the above mentioned four rice types was measured, and it was found that the average gas diffusion resistance of the typical indica varieties as well as of the indica × japonica indicaclial varieties was markedly lower than that of the typical japonica varieties and of the *indica × japonica japonicaclinal varieties*(Table 4). Variance analysis also proved that there was a significant difference in gas diffusion resistance among rice types and among varieties, and that among the four rice types the average gas diffusion resistance of the indica × japonica indicaclinal varieties tended to be higher than that of the typical indica varieties, whereas the average gas diffusion resistance of the japonicaclinal varieties was significantly lower than that of the typical japonica varieties. The gas diffusion resistance in the four rice types followed an order that was opposite to the order of stomatal density, that is, typical indica 〈 indicalclinous 〈 japonicaclinous 〈 typical japonica.

(2) Relationship between stomatal density and gas diffusion resistance

To facilitate understanding, first, gas diffusion resistance was converted to gas diffusion conductivity, before making correlation analysis. Results showed that stomatal density was very significantly positively correlated with gas diffusion conductivity(Fig. 3), in other words, the greater the number of stomata in a unit area of leaf, the slighter the gas diffusion resistance, hence the greater the gas diffusion conductivity.

5. Relationship between stomatal density, specific leaf weight and single leaf area

From the results of correlation analysis, it was apparent that stomatal density was inversely correlated to specific leaf weight(Fig. 4), but insignificantly correlated to single leaf area

Table 4. Comparison of gas diffusion resistance of flag leaf among different types of rice(s · cm^{-2})

Types	No. of varaieties	Meximum	Minimum	Average	Sd	C.V.
Typical *indica* varieties	6	0.83	0.53	0.69	0.13	18.84
Typical japonica varieties	12	2.25	1.11	1.43	0.42	29.60
Indica varieties from indica Ⅱ japonica	5	0.93	0.76	0.82	0.07	8.54
Japonica varieties from indica II japonica	6	1.73	0.87	1.16	0.32	27.54

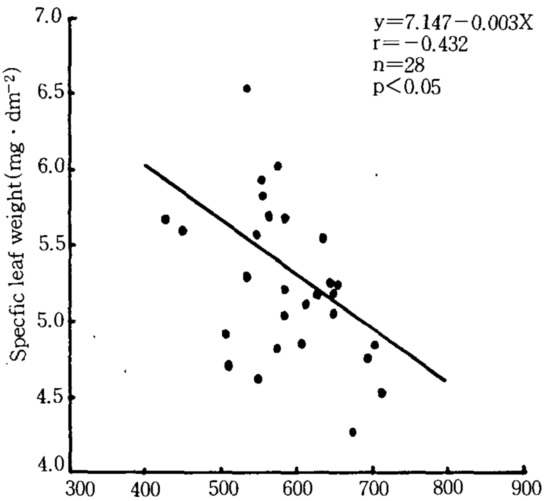

Fig. 4. Relationship between stomatal density and specific leaf weight.

(r=0.3181, p>0.05). Tsunoda reported that most of the varieties with high stomatal density are characterized by relatively thin leaves, fewer and loosely arranged mesophyllous cells, more developed intercellular space and lower mesopyllous resistance, all ravorable to reducing gas diffusion resistance per unit leaf area(Tsunoda, 1986). However, the low RuBPC content and poor carboxylation capability of thin leaves would lead to reduced difference in CO_2 concentration level inside and outside the leaf, which in turn would impede CO_2 absorption. Nevertheless, should the stomatal close, indipendent of the effect of such factors as low temperature and water strees, then stomatal density would remain the determining factor affecting gas diffusion conductivity, especially in semihydrophytic environment, where high stomatal density could ensure easy transport of CO_2 from surrounding atmosphere to photosynthetic cells. If this feature could be properly combined with the trait of thick leaf, that would be favorable to the enhancement of photosynthetic efficiency.

Discussion

Tsunoda and Takahashi[6] pointed out that the stomatal density of the indica rice leaf in the tropical region is significantly higher than that of other cereal crops, such as corn, wheat, and barley, and also significantly higher than that of the japonica rice. And they believe that the low gas diffusion resistance and high photosynthetic rate in indica leaf are probably associated with its high stomatal density(Tsunoda, 1984; 1986). In this study, not only has the fact been proved that both the stomatal density and net photosynthetic rate of the indica rice were higher than that of the japonica, but it was found that the japonicaclinal varieties developed through crossing indica with japonica tended to have a higher stomatal density and not photosynthetic rate than the typical japonica varieties, many of the former had already caught up with or were approaching the indica rice in this respect. This not only demonstrated the relationship exiting between stomatal density, net photosynthetic rate and biomass production, capability, but suggested that crossing indica with japonica could be an effective way to improve the stomatal performance and net photosynthetic rate in japonica rice.

although rice lacks a C_4 photosynthetic system, it exhibits a high net photosynthetic rate per unit leaf area and a maximum crop growth rate that almost rivals that of the C_4 crops(Ishihara, 1979; Yoshida, 1978). According to Tsunoda's view[6] and the results of the present study, the high photosynthetic rate rice leaf, particularly, indica rice leaf is undoubtedly linked to its low gas diffusion resistance, and it is believed that such a low gas diffusion resistance stems mainly from its high stomatal density. While in this study no direct observation was made on the relationship between gas diffusion resistance and net photosynthetic rate, it stands to reason to envision that if we can transfer, through cross breeding, such desirable traits as high stomatal density, hence, low gas diffusion resistance from indica to japonica, that would help enhance the net photosynthetic rate and biomass production capability in japonica rice.

It should be noted that our study revealed that stomatal density was inversely correlated with specific leaf weight, but insignificantly correlated with single leaf area. From the viewpoint of evolution, the change is stomata and in the CO_2 diffusion conductivity of mesophyll is the direct outcome of change in leaf morphology (China, 1975). Recently, Tsunoda[5] suggested that if the property of good aeration in indica rice can be incorporated appropriately with the thick leaf as wild wheat, the result would be beneficial to the enhancement of photosynthesis in the leaf of the rice plant. The problem is that stomatal density and gas diffusion conductivity were inversely correlated to specific leaf weight ($r=-0.4324$, $P<0.05$; $r=-0.5384$, $P<0.01$), but to what extent is that inverse correlation genetically caused, and whether it is possible to incorporate properly, through crossing, the high stomatal density and low gas diffusion resistance of the indica rice with the enormous specific leaf weight of the japonica, all these require further penetrating study.

摘 要

類型이 다른 品種들간에 氣孔密度와 氣體擴散抵抗의 차이와 光合成 速度에 미치는 영향 등을 비교 검토한 바 일반적으로 Indica 品種의 氣孔密度가 Japonica 品種보다 컸으나 氣體擴散은 작았다.

Indica 品種과 Japonica 品種간의 交雜을 통하여 育成한 品種들의 氣孔密度와 氣孔擴散 抵抗은 中間價를 나타냈다.

氣孔密度와 氣體擴散抵抗 光合成 速度 간에는 현저한 正의 相關을 보였지만 특수葉重과는 현저한 負의 相關을 보였고, 單葉面積과의 相關關係는 현저하지 않았다.

Indica 品種의 光合成 速度가 높은 原因은 氣孔密度가 크고 氣體擴散이 낮았기 때문이었다.

Indica 品種과 Japonica品種간의 交雜을 통하여 Indica 品種의 氣孔密度가 크고 氣體擴散抵抗이 낮은 特性과 Japonica 品種의 특수 葉重이 큰 특성을 결합할 수 있다면 光合成 速度를 더욱 높일 수 있으리라 추측된다.

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