Relationship between Stomatal Frequency and Photosynthesis in Barley

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To develop higher yielding varieties, it is necessary to improve not only morphological traits of crops but also physiological traits especially photosynthetic rate (abbreviated Pa, hereafter) of a single leaf. Even though the relationship between Pa of a single leaf and crop yields is not a simple one, Pa of a single leaf may be one of the most important traits for the selection of higher yielding plants. The diffusion of CO_2 into a leaf encounters stomatal resistance (r_s) and mesophyll resistance (r_m). It can be said that the former studies about varietal difference of Pa had been concentrated mainly on r_m differences. Many workers proposed several traits which can be used conveniently as indirect selection criteria, instead of measuring Pa itself, in the selection for high Pa plants. The traits which were reported to correlate with Pa were specific leaf weight, leaf thickness, fresh leaf weight, leaf vein frequency, total chloroplast area, nitrogen content, etc. All of these traits are related to mesophyll structure and function. Selection for these traits means improving the r_m or transport system for products of photosynthetic activity. On the other hand, the effect of changes in r_s on Pa was studied by examining the effect of the degree of stomatal opening on Pa. A difference in r_s caused by the different stomatal openings exerts a big effect on Pa. However, r_s can be controlled not only by the degree of stomatal opening but also by stomatal frequency (stomatal number per unit leaf area, abbreviated Sf, hereafter) in a leaf. The increase of Sf is expected to decrease r_s, thus increasing Pa of the leaf. Therefore, it is expected that Sf can be used in selecting plants of high photosynthetic capacity.

Early investigations on Sf aimed at knowing effects of Sf on transpiration and drought resistance, and it was recognized that high Sf leaf transpires more than low Sf leaf[4]. Research on the relationship of Sf to Pa is rather new. There are only a few reports on it and the relationship between Sf and Pa seems uncertain. In the present report, the relationship between Sf and Pa (apparent Pa of a single leaf) in barley cultivars and closely related iso-genic lines differing in Sf, the varietal difference and heritability of Sf, and the possibility of selecting plants with high photosynthetic capacity by means of Sf are discussed.

Effect of stomatal frequency on photosynthesis

Although CO_2 in air can penetrate into a leaf through cuticle, almost all CO_2 is taken up through stomates. Therefore, it is expected that the number of stomata in a leaf can effect Pa when soil moisture is adequate and transpiration is not inhibited by limited water supply to plants.

Seven spring barley cultivars differing in Sf of the flag leaf were planted in a green house[7]. Three plants from each cultivar were grown with one plant per pot. Pa was measured about two weeks after heading on the three plants of each cultivar. After enclosing the flag leaf in a transparent plastic bag under light for one hour to open the stomata fully, the attached flag leaf was inserted into an air sealed assimilation chamber. After the gas exchange measurements were completed, the leaf was excited and Sf was determined

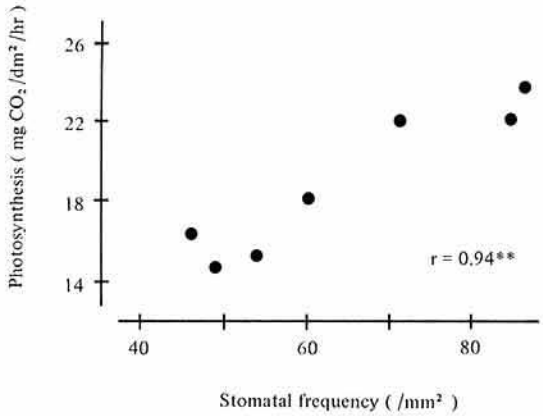

Fig. 1. Relationship between stomatal frequency[1] and photosynthesis[2] of 7 barley cultivars. Each dot is the mean of 3 plants. 1) : lower surface of the flag leaf. 2) : apparent photosynthesis of the flag leaf.

at the widest part of the lower surface of the flag leaf used for the gas exchange measurements using silicon rubber imprint. The relationship between Sf and Pa is shown in Fig. 1. Correlation coefficient between Sf and Pa was 0.94**. Cultivars having high Sf showed high Pa.

Closely related iso-genic line populations were developed by backcrossings with the purpose of obtaining lines having either high or low Sf that would be genetically similar for other characters[6]. The populations were Dickson^3 × CI 4176 (BC_2F_4) (Designated as D population) and Primus II^4 × CI 5064 (BC_3F_4) (designated as P population). Mean Sf and Pa values of high and low Sf lines are

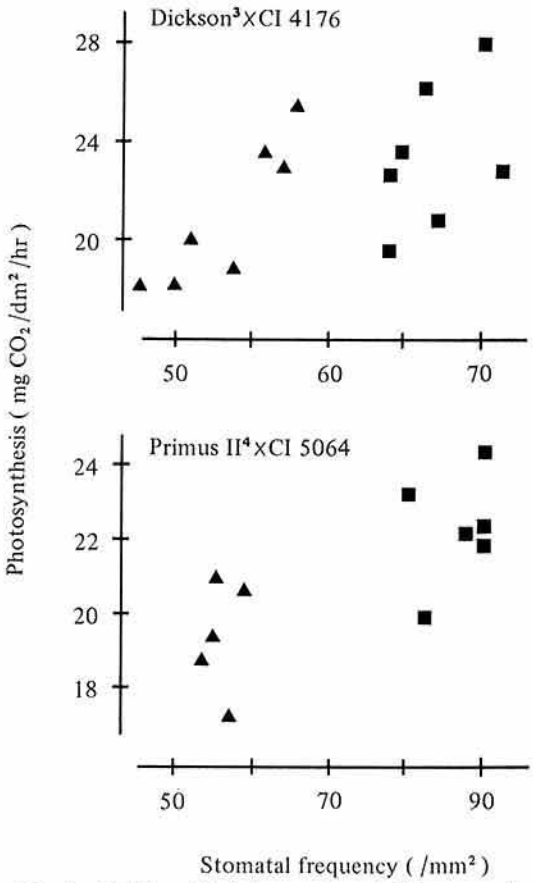

Fig. 2. Relationship between stomatal frequency[1] and photosynthesis[2] of close iso-genic lines of barley. Each dot is the mean of 4 plants. ■ : high, ▲ : low stomatal frequency lines. 1), 2) : refer to Fig. 1.

shown in Table 1. The high and low lines differed significantly in Sf and D and P populations. Mean Pa values were higher in the

Table 1. Mean values for stomatal frequency and photosynthesis in close iso-genic lines of barley

Population[1]	Group[2]	Stomatal frequency[3] (/mm²)	Photosynthesis[4] (mg CO_2/dm²/hr)	Number of lines
D	H	67	23.5	7
	L	53	21.1	7
P	H	87	22.4	6
	L	56	19.4	5

1) : D : Dickson^3×CI 4176, P : Primus II^4×CI 5064 populatiion. 2) : H : hgh, L : low stomatal frequency lines. Each line consisted of four plants. 3) : lower surface of the flag leaf. 4) : apparent photosynthesis of the flag leaf.

high Sf lines than in the low Sf lines in the D population (23.5 vs. 21.1 mg $CO_2/dm^2/hr$) and in the P population (22.4 vs. 19.4). The difference was significant in the P population. In Fig. 2, relationships between Sf and Pa of the two iso-genic lines are shown. It shows clearly that high Sf lines had high Pa.

Effect of stomatal resistance on photosynthesis

Transpiration of leaves used for Pa measurement was determined by an electric hygrometer at the same time of CO_2 measurement to estimate r_s by the Gaastra's method[1]. Fig. 3 shows that high Sf cultivars and isogenic lines had low r_s, and that the cultivars and lines with low r_s had high Pa. It is said that the difference in r_s caused by varying Sf gives a little effect on Pa because the difference in r_m is much larger than r_s values[2,4]. But it is also said that r_m values are overestimated by the Gaastra's method, so that r_m values are probably not so large as compared with r_s values[9], and therefore the change in r_s can effect Pa significantly. It is said that transpiration is controlled only when stomatal opening is narrow. In the present study, gas exchange rates were measured under the condition of wide stomatal opening. Results show that Pa could be controlled through the change in Sf or r_s even if stomata were widely open, which is in contrast to the earlier findings.

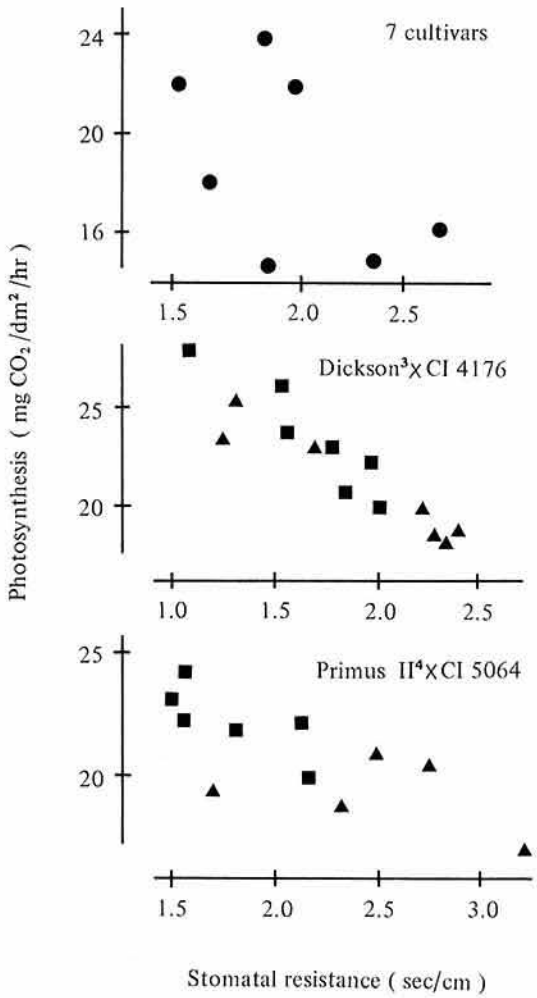

Fig. 3. Relationship between stomatal resistance (r_s) and photosynthesis of three barley populations. 1) : estimated by Gaastra's method. Other notes: refer to Fig. 1 and 2.

Inheritance and variation of stomatal frequency

As discussed previously, a high Sf leaf had high Pa. Thus, high photosynthetic plants can be selected by counting stomata in a leaf if the selection for high Sf plants is possible. To determine the possibility of the selection for high Sf plants, intra-plant variation, varietal differences and heritability of Sf were estimated[8].

Nine winter barley cultivars were planted in field and three imprints were taken from each of distal, middle and basal part of each leaf and sheath for counting stomata. Table 2 shows that Sf on distal part was higher than that on basal part of a leaf and a sheath but the differences were not so large. In lower surface of a flag leaf, the counts were practically the same. Therefore, Sf can be estimated with a relatively high precision. The leaf and sheath Sf was higher in upper positions than in lower positions of a shoot. Sf in upper and lower leaf surfaces did not differ so much.

Correlation coefficients between Sf on lower

Table 2. Stomatal frequencies[1] at different positions of leaves and sheaths of 9 winter barley cultivars

		Basal	Middle	Distal	Mean
L_1[2]	upper	59	61	65	62
	lower	59	60	60	60
L_2	upper	45	48	50	48
	lower	45	46	49	47
L_3	upper	38	41	43	41
	lower	37	41	43	40
L_1	sheath	35	39	42	39
L_2	sheath	27	30	32	30

Each value is the mean of 9 cultivars.
1) : (/mm^2). 2) : L_1 means a flag leaf.

Table 3. Correlation coefficients of stomatal frequency in flag leaf, other leaves and sheaths of 9 winter barley cultivars

L_1 (lower) vs.	Correlation coefficient
L_1 (upper)	0.90**
L_2 (upper)	0.86**
(lower)	0.85**
L_3 (upper)	0.73*
(lower)	0.69*
L_1 sheath	0.68*
L_2 sheath	0.60

Values were calculated using the mean values of each cultivar. L_1 expresses a flag leaf.

surface of a flag leaf and that in other leaves and sheaths were generally high (Table 3). It shows that the selection by means of Sf in a flag leaf results in selecting plants having high Sf in other leaves and sheaths.

Genetical variation of Sf was estimated from a sample collection containing native and improved varieties from Japan and entries from the world collection of barley. Sf of the lower surface of flag leaf ranged from 43 to 87 stomata per mm^2 (6-rowed, hulled), from 46 to 95 (6-rowed, naked) and from 50 to 90 (2-rowed, hulled). The ranges and mean values of Sf did not differ significantly among 6-rowed and 2-rowed or hulled and naked cultivars. Sf of barley flag leaf ranged from 43 to 95 stomates per mm^2, showing a considerably large genetic variation.

To estimate heritability of Sf, cultivars differing in Sf in a leaf were crossed and genetic gain was estimated. Populations used for estimating heritability value were F_2 and F_3 generations of the two iso-genic line populations used for Pa measurements. Sf on the lower surface of the flag leaf of each F_2 plants was counted. High and low Sf plants were selected. Three plants per selected F_3 families were grown and heritability (h^2) was estimated as follows,

$$h^2 = \frac{\text{Genetic gain}}{\text{Selection differential}}$$

Heritability values were 0.46 in Dickson[3] × CI 4176 population and 0.38 in Primus II[4] × CI 5064 population. As heritability values of yield estimated by F_2 plants were generally low, it can be said that the heritability value of Sf was relatively high.

Discussion

Several studies have been done to examine the relationship between Sf and Pa. Some studies were made by comparisons between upper and lower leaf surfaces or comparisons among different species. However, it seems that these comparisons were not proper and exact enough to find the real effect of Sf on Pa. In the present work, the relationship between Sf and Pa was made clear using cultivars and iso-genic lines differing in Sf. A leaf with high Sf had high Pa in all populations examined. Therefore, it can be said that the close association of Sf and Pa was revealed by using the proper and systematic experimental materials. Diffusion of CO_2 into a leaf encounters r_s and r_m. Results here show that the increase of Sf reduced r_s and increased Pa. Although many experiments dealing with varietal differences of Pa concentrated on the part of r_m, the present study proved that r_s also had a close association with Pa. In conclusion, besides mesophyll structure, chlorophyll activity and transport system, Sf in a leaf is one of the most important traits for improving Pa of a leaf because of its

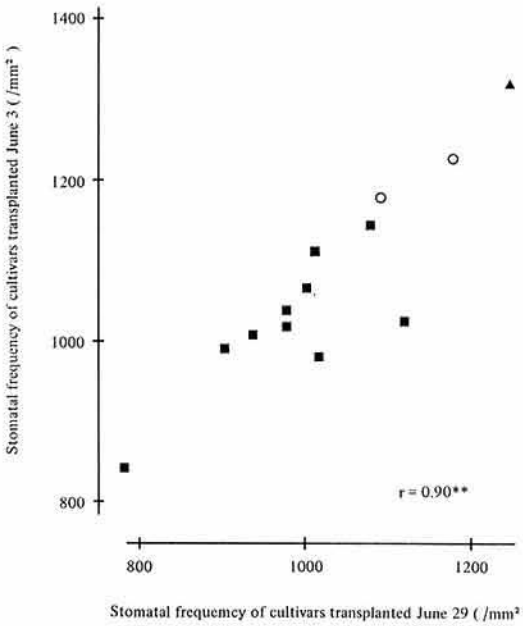

Fig. 4. Relationship between stomatal frequencies[1] of a flag leaf of rice transplanted at different dates. 1) : upper plus lower leaf surface. ■ : Japonica type. ○ : indica type. ▲ : Tongil.

association with Pa, relatively high heritability value and the easiness of the method to estimate it.

In case of other crops except barley, the effect of the change in Sf on Pa is not clear yet. Fig. 4 shows[10] that Sf of rice leaf was much higher than that of barley, Sf of plants transplanted at different dates correlated well, and indica type cultivars examined here and Tongil (indica × japonica type cultivar) had high Sf. Leaf Pa of rice[3] and corn[5] was reported to be influenced by stomatal aperture or stomatal resistance. Further researches will be necessary on the relationship between Sf and Pa or between Pa and stomatal resistance using various crops including rice.

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