Photosynthetic Capacity and Inheritance of V-type Leaf in Rice (*Oryza sativa* L.)

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The rice lines with leaves forming an angle at the midrib toward the adaxial surface (V-type leaves) were selected from the descendants of a cross between Milyang 23 and an offspring of a cross between a *Javanica* cultivar, Arborio and a *Japonica* mutant, Daikoku 1. The photosynthetic rates of the V-type leaves on a projected leaf area basis were significantly higher than those of the ancestor and parent in the irradiance range over 160 Wm^{-2} , but the photosynthetic rates of the V-type leaves on an unfolded leaf area basis were almost the same as those of the ancestor and parent at all the irradiance levels. Nitrogen content of the V-type leaves on an unfolded leaf area basis and leaf thickness did not differ appreciably from the corresponding values in the ancestor and parent although there were significant differences in some cases. The V-type leaf character was found to be regulated by a single recessive gene.

KEY WORDS: *Oryza sativa*, rice, photosynthesis, nitrogen content, leaf thickness, V-type leaf, light interception.

Introduction

As an evolutionary trend, the cultivated plants have larger leaves but a lower photosynthetic ability than their ancestors or wild types (EVANS and DUNSTONE, 1970; BHAGSARI and BROWN, 1986). The lower photosynthetic rates in the cultivars have been ascribed to a "dilution effect" (HESKETH, *et al.* 1981), which implies that the photosynthetic components per unit leaf area become sparse due to leaf expansion. Although the leaf expansion is associated with a lower photosynthetic rate per unit leaf area, it contributes to the increase of the crop yield through the increase of light interception per unit land area (EVANS and DUNSTONE, 1970). However, it would be important to further modify or improve the leaf characters, i.e. thick, small and erect leaves, to raise the current levels of crop yield. The present materials consisting of lines with leaves forming an angle at the midrib of the leaf blade, were bred to examine these problems. First, the formation of an angle at the midrib of the leaf blade results in an erect leaf blade even if it is long. Secondly, the formation of an angle decreases the effective light intercepting area with preservation of the large leaf area of single leaves. In contrast with these beneficial characteristics, however, it is of importance to examine how the photosynthetic ability is influenced by the change of the leaf morphology.

The present experiments were carried out to examine the breeding behavior of the V-type leaf character, as well as the morphological and photosynthetic characteristics of the leaves forming an angle at the midrib.

Materials and Methods

1). *Origin of the materials*

The origin of the rice lines with the leaves forming an angle at the midrib toward the

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Fig. 1. Diagrammatic representation of V-type and flat (normal) leaves inserted into the assimilation chamber with a water jacket.

WJ: Water jacket

AC: Assimilation chamber

PLA: Projected leaf area

ULA: Unfolded leaf area of the V-type leaf

Shadow area: Leaf Blade

Closed circle: Veins

adaxial surface in varying degrees and named V-type leaf lines (Fig. 1), is shown in Table 1. Since one ancestor, Daikoku 1 (a *Japonica* Mutant) which exhibited high rates of photosynthesis (TAKANO and TSUNODA, 1971) is a dwarf (below 60 cm) line, it is not a commercially acceptable type. Another ancestor, Arborio (a *Javanica* cultivar), is a long culm (above 100 cm) line with large grains. In 1974, their F₁ seeds were treated with a mutagen (ethylmethane sulfonate). In 1978, with a view to breeding a rice line enabling to increase the planting density with decreased mutual shading, DAR-12 with deep green

Table 1. Origin of V-type leaf rice lines

Year	Cross and selection					
1973	Daikoku . 1* × Arborio*					
1974	F ₁ * treated with Ethylmethane sulfonate					
1978	F ₅ DAR-12 × Milyang 23*					
1982	F ₄ DARM-7*					
1983	F ₅	DARM-7-1*	DARM-7-2	DARM-7-6	DARM-7-10	DARM-7-11
1984	F ₆	DARM-7-2-8	DARM-7-2-9			
1985		F ₇	F ₇	F ₆	F ₆	F ₆

* Lines with the flat leaf, other lines had the V-type leaves.

and V-type leaves was selected from the descendants of the above-mentioned cross, but it had slightly twisted (droopy) leaves. To improve this leaf character, DAR-12 was crossed to Milyang 23 which is a short culm and high-yielding Korean cultivar with deep green, wide and erect leaves. Their descendants except for one line, DARM-7-1, had deep green and erect V-type leaves and were named DARM-7 family lines. In 1985, the segregation analysis of the V-type leaf character was carried out using the F_2 generation between a F_5 line, DARM-7-2 harbouring the V-type character and a Japanese cultivar, Koshihikari with normal leaves.

2). *Cultivation of plants*

Seeds were sown in polyvinyl-chloride pots (8 cm in diameter and 20 cm deep) filled with normal paddy soil containing 2 g of a commercial compound fertilizer ($N:P_2O_5:K_2O=13\%$ each). The plants grown in a glasshouse were periodically supplied with 1 g of the same fertilizer.

3). *Measurement of photosynthesis*

An open system equipment with an infrared gas analyzer (Hitachi-Horiba, ASSA-1610) was used for the measurement of photosynthesis according to the method of SINGH and SASAHARA (1981). The photosynthetic rates were measured using the latest fully expanded penultimate leaves in three replicates in 1983, and using third to fourth leaves down from the flag leaf in six replicates in 1985. An assimilation chamber made of acrylic resin 0.7 cm in interior thickness and 2.0 cm in interior width was used for the flat leaves. An assimilation chamber consisting of a glass tube 1 cm in interior diameter was used for the V-type leaves (Fig. 1). Both assimilation chambers were enclosed in a water jacket to maintain constant the leaf temperature (25°C) (Fig. 1). Different irradiances were provided by changing the distance of a movable light source with six tungsten lamps (Toshiba, 500W) from the assimilation chamber. Especially, the low irradiances below 100 Wm^{-2} were provided by inserting cheese cloths between the assimilation chamber and the light source. The irradiances were measured with a solarimeter (Noushi-denshi type No. 598) for the same position of the leaf surface in the chamber. Air flow rate was fixed at 6.0 and 6.5 l/min for the flat and V-type leaves, respectively, to obtain the maximum photosynthetic rate, while the relative humidity was maintained at 80%.

4). *Measurement of leaf area and thickness and nitrogen content*

Leaf area was measured with an area meter (Hayashi Denkou, AAM-7 type). As for the V-type leaves, the projected area was measured by putting the leaf margins of the V-type leaves on the under-carrier belt of the area meter like a greek capital lambda (Λ) without pressure from the upper-carrier belt (Fig. 1). Then after the V-type leaf blade was cut along the midrib, the area of each half of the leaf was measured to estimate the area, assuming that the V-type leaf was unfolded. Nitrogen content was determined by the micro-Kjeldahl method. Leaf thickness was estimated by determining the leaf width and area on the photographs of cross-sectional specimens of the leaf pieces which were sampled from the mid-

dle position of the long axis of the leaves according to the following equation.

$$\text{Average thickness} = \text{cross-sectional area} / \text{width}$$

Results

Experiments in 1983

The leaves of DARM-7-1 were significantly thinner than those of DAR-12, DARM-7-2 and Milyang 23. Nitrogen content on an unfolded leaf area basis was significantly higher in

Table 2. Leaf thickness and nitrogen content

Line or Cultivar	Leaf thickness (μm)		Nitrogen content (mg/dm^2)	
	Mean	SE	Mean	SE
DAR-12	113.9 $\pm$ 7.7		14.28 $\pm$ 1.34	
	113.2 $\pm$ 3.8		12.84 $\pm$ 2.30	
DARM-7-1	97.8 $\pm$ 3.5		13.96 $\pm$ 0.43	
DARM-7-2	113.4 $\pm$ 2.1		15.53 $\pm$ 0.62	
DARM-7-2-8,9	100.1 $\pm$ 3.2		12.33 $\pm$ 1.01	
DARM-7-6-1,3	100.5 $\pm$ 5.3		13.10 $\pm$ 0.54	
DARM-7-10-1,2	96.1 $\pm$ 4.3		12.72 $\pm$ 0.28	
DARM-7-11-1,3	111.1 $\pm$ 3.9		12.38 $\pm$ 0.81	
Arborio	121.3 $\pm$ 4.9		9.42 $\pm$ 0.41	
Milyang 23	109.4 $\pm$ 4.7		13.51 $\pm$ 0.32	
	98.9 $\pm$ 1.0		11.61 $\pm$ 0.27	

Note: The thick numerals indicate the results obtained in 1983.

Table 3. Leaf area and angle from the horizon of V-type leaves

Line		Leaf area (cm^2)		Estimated angle (Degree)
		Mean	SE	
DAR-12	Projected	34.60 $\pm$ 0.70		35
	Unfolded	42.02 $\pm$ 1.95		
DARM-7-2*	Projected	20.54 $\pm$ 0.99		50
	Unfolded	31.97 $\pm$ 0.88		
DARM-7-2-8,9	Projected	25.29 $\pm$ 1.53		52
	Unfolded	40.71 $\pm$ 2.41		
DARM-7-6-1,3	Projected	30.11 $\pm$ 1.51		45
	Unfolded	42.67 $\pm$ 2.22		
DARM-7-10-1,2	Projected	27.53 $\pm$ 1.39		49
	Unfolded	41.84 $\pm$ 2.74		
DARM-7-11-1,3	Projected	28.31 $\pm$ 0.27		51
	Unfolded	44.74 $\pm$ 3.00		

Note: The angles were estimated using the following formula:
Degree = $\text{Arccosin}(\text{Projected leaf area} / \text{unfolded leaf area})$

* Results obtained in 1983.

DARM-7-2 than in Milyang 23 and DARM-7-1, but there was no significant difference between DAR-12 and DARM-7-2 (Table 2). The projected and unfolded leaf areas of DARM-7-2 are shown in Table 3 with the estimated angle of the half leaf blade from the horizon.

The photosynthetic rates of DARM-7-2 on a projected leaf area basis were significantly higher than those of the other lines at the irradiance range above 160 Wm^{-2} and seemed to require higher irradiances than 660 Wm^{-2} to reach a plateau. However, the photosynthetic rates on an unfolded leaf area basis were similar to those of DAR-12, DARM-7-1 and Milyang 23 at all the irradiances (Fig. 2).

Experiments in 1985

The differences in the leaf thickness were not significant among the cultivars and lines. However, the nitrogen content of the DAR-12 and DARM-7 family lines was significantly higher than that of Arborio, but not significantly higher than that of Milyang 23 (Table 2). The projected and unfolded leaf areas and the estimated angles of the half leaf blade from the horizon are shown in Table 3.

The photosynthetic rates of the DARM-7 family lines on a projected leaf area basis were significantly higher than those of the cultivars at the 660 Wm^{-2} irradiance. The photosynthetic rates of the DARM-7 family lines on an unfolded leaf area basis were the same as those of the cultivars and DAR-12 at the 660 Wm^{-2} irradiance. Differences among all the photosynthetic rates at the 64 Wm^{-2} were statistically not significant (Fig. 3). The chi-square test of the V-type leaf segregation ratio, shown in Table 4, confirmed that the V-type leaf character was controlled by a single recessive gene. However, the samples were heterogeneous, and the heterogeneity test was not significant.

Fig. 2. Leaf photosynthesis - irradiance curve of three lines and one cultivar.

Discussion

Many studies have been carried out to identify lines or cultivars which show high photosynthetic rates and to analyze the morphological leaf traits relating to photosynthesis (EVANS and DUNSTONE, 1970; KHAN and TSUNODA, 1970 a, 1971; TAKANO and TSUNODA, 1971;

Fig. 3. Leaf photosynthesis at different irradiances.

DORNHOF and SHIBLES, 1976; ZILINSKAS, 1985 and BHAGSARI and BROWN, 1986). In these studies, it has been indicated that the photosynthetic rates are positively correlated with the leaf nitrogen content, but negatively with the area of single leaves as in the results of EVANS and DUNSTONE (1970). Furthermore, it is of interest that the activity of the key enzyme of photosynthesis, i.e. the kinetic constants of ribulose-1,5-bisphosphate carboxylase, showed few differences among rice cultivars belonging to different ecospecies (MAKINO *et al.* 1987). Thus,

Table 4. Heterogeneity chi-square analysis for the V-type leaf at F₂ generation of crosses between F₅ line with the V-type leaf and a Japanese cultivar, Koshihikari

F ₂ family	Number of plants with			Chi-square from 3:1	DF
	Flat leaf	V-type	Total		
1	1501	553	2054	4.05128	1
2	570	177	747	0.67872	1
3	518	173	691	0.00048	1
4	1075	352	1427	0.08433	1
Total of chi-squares				4.81481	4
				0.3 < p < 0.4	
Polled chi-square	3664	1255	4919	0.69127	1
				0.4 < p < 0.5	
Heterogeneity chi-square				4.12354	3
				0.2 < p < 0.3	

it seems important to develop an alternative strategy to increase the plant canopy productivity.

In the present experiments, first, an important basic trait was that in the V-type leaves the nitrogen content on an unfolded leaf area basis and the leaf thickness were identical with the corresponding values of the ancestor and parent. Secondly, the photosynthetic rates on an unfolded leaf area basis were identical with those of the ancestor and parent at all the levels of irradiance and even at the low levels of scattered irradiances. On the other hand, the apparently high photosynthetic rates on a projected leaf area basis were ascribed to the reduction of a projected leaf area due to the formation of an angle at the midrib. That is, by the formation of an angle, the photosynthetic rates on an unfolded leaf area basis appeared to be maintained. Thus, in the plants having the V-type leaf character a rice cano-

py with reduced mutual shading under high planting density and/or high fertilizer level could be obtained. Semi-dwarf strains with a leaf area ranging between 40 to 50% as compared with the reference cultivars, Sasanishiki and Reimei, have been reported (YAMAGUCHI *et al.* 1976). The semi-dwarf strains exhibited photosynthetic rates about 70% higher than those of the reference cultivars. However, the semi-dwarf cultivars are not practical for cultivation even with a high planting density because of their extremely small leaf area. In contrast, the V-type leaves show about the same area on an unfolded basis as that of the normal cultivars.

In the V-type leaves gas exchanges seem to be hampered by the formation of a deeper unstirred air layer on the adaxial surface. However, the apparently high photosynthetic rates of the DARM-7 family lines on a projected leaf area basis indicated that the V-type leaf morphology had not interfered with carbon dioxide exchange, and/or that the abaxial surface of the V-type leaves had compensated for the decrease of gas exchange on the adaxial surface.

Based on the air flow rate and the cross-sectional area of the assimilation chamber, the wind velocity in the assimilation chamber was estimated at 8.3 m/min for the flat leaves and 4.3 m/min for the V-type leaves. However, HESKETH and MUSGRAVE (1962) reported that photosynthesis in corn leaves was hardly influenced by the wind velocity in the range of 4 to 12 m/min. KHAN and TSUNODA (1970 b) reported that the photosynthesis of wheat leaves was not appreciably affected by the wind velocity in the narrow range of 0.6 to 2.5 m/min. Therefore, the difference in the apparent photosynthetic rates between the V-type and flat leaves in the present experiment may have not been associated with the difference in the wind velocity in the two types of assimilation chamber.

It remains to be determined whether the induction of the V-type leaves is related to the effect of the mutagen. However, the characteristics of photosynthesis and leaf morphology were retained throughout different years, indicating that these characters were heritable.

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水稻のV字型葉の光合成特性とその遺伝

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アルポリオと大黒1号の交雑後代系統に密陽23号を交配した後代から, 葉身が中肋を中心として内側に向かってV字型を形成する系統が選抜された. このV字型葉身は, 内側から照射した場合, その投影面積当たりの光合成速度が祖先品種(アルポリオ, 密陽23号)より有意に高い単位投影葉面積当たり光合成速度を示した. ただし, V字型葉身を水平葉面積に変換した場合の単位葉面積当たり光合成速度は祖先品種よりやや高い傾向を示すのみであった. このことは, V字型葉身の状態で水平葉面積に変換した場合の単位葉面積当たり光合成速度を維持していることを示しており, V字型にすることによる葉面積の減少によって相互遮蔽を減少させつつ高い光合成速度を示す群落を構成し得る可能性を示している. ただし, 水平葉と比較してV字型葉は直達光を受ける場合余弦曲線に比例して受光面が減少する不利な面を有している.

水平葉面積に変換した場合の単位葉面積当りV字型葉身の窒素含量及びV字型葉身の厚さ(=平均断面積/葉幅)は祖先品種とほぼ同じ値を示した. ただし, 両形質ともいくつかの場合で有意の差異が見られた.

V字型葉身の特性を有する系統(群)はなお出穂期, 脱粒性などいくつかの農業実用形質に関して若干の分離を示すが, V字型葉身の特性そのものは劣勢の単因子によって支配されていると推定された.