

## Role of Mesophyll Morphology in Determination of Leaf Photosynthesis in Field Grown Soybeans

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### 圃場生育大豆의 葉光合成과정에서 葉肉細胞 形態의 역할

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**ABSTRACT** : Photosynthetic variation in field grown soybean [*Glycine max* (L.) Merr. cv Hodgson78] was studied in relation to leaf anatomical variation. Variations in mesophyll morphology were accentuated by manipulating source and sink size. At R3 stage, two treatments were started : one was thinning and continuous debranching (6.5 plants rather than 26 plants per m of row and remaining plants were debranched weekly), and the other was continuous partial depodding (allowing only one pod to develop at each mainstem node). Gas exchange characteristics, mesophyll cell volume and surface area per unit leaf surface, and microclimatic parameters were measured on the intact terminal leaflet at the 10th node. Observations were made 5 times with 3 to 4 day intervals starting R4 stage. Two models were used to compute leaf photosynthetic rates : one considered no effect of mesophyll morphology on photosynthesis, and the other considered potential effects of variations in mesophyll cell volume and surface area on diffusion and biochemical processes. Seventy nine percent of total photosynthetic variations observed in the experiment was explained by the latter, while 69% of the same variations was explained by the former model. By incorporating the mesophyll morphology concept, the predictability was improved by 14.6% in the field condition.

Additional Index Words : photosynthesis model, leaf anatomy, *Glycine max* (L.) Merr., mesophyll surface area, mesophyll cell volume.

Predicting the supply of photosynthate is a vital part of crop or plant models. Leaf photosynthetic capacity has usually been expressed on a unit leaf area basis in crop models. But positive correlations between leaf morphological traits and leaf photosynthetic capacity (for example, between specific leaf weight and apparent photosynthesis), induced a special interest in modeling the effects of leaf thickness related traits on photosynthesis (Taylor, 1971; Sinclair et al., 1977; Charles-Edwards, 1981; Nobel, 1983). But little effort has been made to test this relationship using field grown species. In some of field crop improvement studies,

specific leaf weight has been widely used as one of selection criteria for high photosynthetic capacity (Pearce et al., 1969; Topark-Ngarm et al., 1977; Lugg and Sinclair, 1979).

The rationale seems to be based on a simple correlation procedure. But some other workers found no such correlation (Watanabe and Tabuchi, 1973; Dunestone et al., 1973; Pallas and Samish, 1974). Also genotypic variations in photosynthetic capacity based on unit leaf weight were reported in a grass species (Wilhelm and Nelson, 1985). This ambiguity might be caused partially by seasonal changes in specific leaf weight of individual leaves (Cole, 1975 ;

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< '91. 11. 30 接受 >

Lugg and Sinclair, 1980) and variations among the leaves at different node positions (Dornhoff and Shibles, 1976). But more importantly, specific leaf weight has less direct cause-effect relationship with photosynthetic capacity than other biological traits such as chlorophyll content, photosynthetic enzymes, electron carriers, and size of CO<sub>2</sub> diffusion site. Specific leaf weight may have some correlations with these direct factors, resulting in positive correlation with photosynthetic capacity. Unfortunately most of these direct factors are difficult, if not impractical, to be directly measured. Furthermore, no single trait has been reported to be solely responsible for observed photosynthetic variations (Shibles et al., 1987). Even though any simulation model could be developed by incorporating all the physiological processes responsible for photosynthesis, it would be impractical to be used for agricultural purposes because all the necessary information will never be acquired.

Photosynthetic capacity expressed on the unit leaf area changes with growing season for individual leaves (Sinclair, 1980; Lauer and Shibles, 1987) and among different leaf positions (Dornhoff and Shibles, 1970), as well as among leaves from different genotypes. Most leaf photosynthesis models for ecological or agricultural purposes, however, have paid little attention to these potential sources of photosynthetic variation. If the same model is used to predict the leaf photosynthetic CO<sub>2</sub> uptake rates at different growth stages, the result might be significantly different from the reality. In order to accommodate photosynthetic variations due to seasonal or positional change, consideration of surface area and cell volume of the mesophyll was suggested in a photosynthesis model and the model was successfully used in the experiment using chamber-grown soybean (*Glycine max* (L.) Merr.) plants treated with different levels of PAR (Yun and Taylor, 1988). The morphological parameters of mesophyll cells, such as the surface and volume, can be relatively easily estimated with sufficient accuracy by a stereological method (Parkhurst, 1982) or a computer-aided tracing method (Longstreth et al., 1982). In the previous experiment (Yun and Taylor, 1988), variation in leaf thickness was induced by an environmental

factor (levels of PAR) within a growth chamber. Under field conditions, there are numerous potential sources for variations.

This study, therefore, was initiated to test the validity of the mesophyll morphology concept in modeling leaf photosynthesis under field conditions. This paper reports the results from observations of the gas exchange and leaf anatomy of terminal leaflets on soybean cultivars manipulated to change the source/sink ratio during seed filling period, and the model computations using the biological and microclimatic data obtained from the same experiment.

## MATERIALS AND METHODS

### Plant Culture

'Hodgson 78' soybeans were grown on a Spilville silt loam soil, which is a fine Mesic Cumulic Hapludoll at the ISU Hinds Research Center near Ames, Iowa. Maize (*Zea mays* L.) was the previous crop. Fertilizer was fall applied at rates of 30 kg P ha<sup>-1</sup> and 75 kg K ha<sup>-1</sup>. Herbicide (Alachlor [2-chloro-2', 6'-diethyl-N-(methoxymethyl)acetanilide] was incorporated before planting to control weeds. Remaining weeds were removed by hand. The plots were sprayed as needed with Carbaryl (1-naphthyl N-methylcarbamate), to control leaf-feeding insects and Benomyl [methyl 1-(butylcarbamoyl)-2-benzimidazolecarbamate], to control powdery mildew (*Erysiphe* spp.). The site was irrigated to maintain adequate water availability.

The soybeans were sown on May 17 in rows 7.3m long and 1.05m wide. A randomized complete block design with three treatment and four replications was used. Two-meter row sections were staked for the three treatments, which were sampled across 5 dates. The plants emerged on May 23 and were thinned to a uniform population of 245,000 plants ha<sup>-1</sup> (26 plants per m of row) on June 5 at the V1 stage (Fehr et al., 1971). On July 11, five plants in each plot were selected on the basis of uniformity. These plants were tagged so they could be easily located throughout the season. Treatments designed to enhance source supply or restrict sink demand were imposed on July 13 at the R3 (V9) stage.

The three treatments consisted of thinning the

stand to 6.5 plants per m of row, continuous partial depodding and control. Thinning a stand to low plant density increases pod set and reproductive sink demand. But it also induces considerable axillary branching, so we also debranched plants weekly in this treatment to prevent development of alternative, competing sinks which might reduce pod set. Partial depodding allowed only one pod to develop at each mainstem node to reduce sink demand.

Measurements were destructive necessitating new plots for each measurement day. Treatments were randomly applied to a sufficient number of plots in each of four blocks to provide adequate material for the experiment.

### Measurements

Apparent photosynthesis (AP), transpiration rate, stomatal resistance, leaf and chamber air temperature, relative humidity, and PAR were determined for the intact terminal leaflet of node 10 of the five tagged plants in each plot using an LI-6000 portable photosynthesis system (LiCor Inc., Lincoln, NE). Five observations were made with 3 or 4 day interval as weather permitted, beginning July 23 until August 6. The beginning date was coincided with full leaf expansion in control plants. The leaves senesced after August 6. Leaves were mostly sunlit throughout the summer, but where they were shaded, we exposed them to sunlight for at least 60 seconds prior to measurement. All observations were made when the PPF was in excess of  $1200 \mu\text{mol m}^{-2} \text{s}^{-1}$ , and between 0930 and 1400 h CDT. Immediately after AP was determined, the leaflet was harvested and its area was measured with an LI-3000 leaf area meter (LiCor Inc., Lincoln, NE). Four  $0.14\text{cm}^2$  leaflet discs were taken from each of the five measured leaflets in each plot (avoiding major vein). These discs were immediately stored in FAA for anatomical observations.

The leaflet discs were dehydrated and embedded with paraffin following NBA-Paraffin procedure (Berlyn and Miksche, 1976). Leaf cross sections were photographed at 200x magnification on a high contrast black and white film (Kodak Technical Pan 2415) with the aid of an automatic photomicrographic system (BH-2 microscope with PM10AD auto-

matic camera, Olympus Optical Co., Tokyo, Japan). Photographic transparencies were mounted as 35mm slides and projected onto a screen ( $15 \times 15 \text{ cm}$ ). Mesophyll cell volume per unit leaf surface (volume ratio, VR) and the cell surface area per unit leaf surface (area ratio, AR) were estimated with the aid of a stereological method suggested by Parkhurst (1982). Leaf thickness (H) was measured on the projected image of the cross section by a ruler in cm and converted to the actual length in  $\mu\text{m}$ .

### Model Computations

The model consists of energy balance and photosynthesis elements which are sequentially related. The formula used for the leaf energy balance equation came from Gates (1980) and used environmental and leaf data (air temperature, TA; absorbed radiation, Qabs; wind velocity, V; relative humidity, RH; leaf resistance to the diffusion of water vapor, Rs; leaf dimension, D and W). Most parameters were measured by the LI-6000, except Qabs, D, and W which were estimated following the methods by Nobel (1983) for Qabs and Taylor (1975) for D and W. Leaf temperature (TL) and transpiration rate are the calculated output. These outputs are transferred to the leaf photosynthesis submodel to calculate AP at the calculated leaf temperature. Two different photosynthesis submodels were used. One is the formula originally provided by Charles-Edwards (1981), which does not consider the potential effects of mesophyll cell morphology. In the original model, the biochemical process of photosynthesis was expressed as;

$$P = \frac{aI(k_1 C_i - k_2 O)}{aI + k_1 C_i} - R_d \quad (1)$$

and the physical process of photosynthesis was expressed as;

$$C_i = \frac{g_m g_s C - (g_m + g_s) P}{g_m g_s} \quad (2)$$

where P is the net rate of photosynthesis per unit leaf area ( $\text{g/m}^2$ ), a is the light utilization efficiency ( $\text{g/J}$ ), I is the photosynthetic irradiance ( $\text{W/m}^2$ ),  $k_1$  is the carboxylation efficiency ( $\text{m/s}$ ),  $k_2$  is the oxygenation efficiency ( $\text{m/s}$ ),  $C_i$  is the internal  $\text{CO}_2$  concentration ( $\text{g/m}_3$ ), O is ambient oxygen concentration ( $\text{g/m}_3$ ),  $R_d$  is the dark respiration rate ( $\text{g m}^{-2} \text{s}^{-1}$ ), C

is ambient CO<sub>2</sub> concentration (g/m<sup>3</sup>), g<sub>m</sub> is the mesophyll conductance to CO<sub>2</sub> diffusion (m/s), and g<sub>s</sub> is the gas phase conductance to CO<sub>2</sub> diffusion including leaf boundary layer, stomatal, and intercellular air space (m/s). Eq (1) and (2) were combined to give the complete submodel for leaf photosynthesis, where no morphological effect was considered.

The other is the same formula but was modified to accommodate the potential effects of mesophyll cell morphology as suggested by Yun and Taylor (1988). Eq (2) was modified to accommodate the effects of cell surface area on CO<sub>2</sub> diffusion by redefining the g<sub>m</sub>. The g<sub>m</sub> was defined as the product of AR and the conductance per unit cell surface, g<sub>c</sub> (Nobel, 1977). Hence, Eq (2) becomes :

$$C_i = \frac{AR g_c g_s - (AR g_c + g_s) P}{AR g_c g_s} \quad (3)$$

Assuming an average value of g<sub>m</sub> 4.8 × 10<sup>-3</sup> m/s (Charles-Edwards, 1981) and an average value of AR 20 (observed in this experiment), the liquid phase diffusive conductance per unit cell surface, g<sub>c</sub>, was calculated as 2.4 × 10<sup>-4</sup> m/s.

Eq (1) was modified to accommodate potential relationships between cell volume and biochemical process. The cell volume per unit leaf area was incorporated into the equation as the ratio (VR/VR<sub>0</sub>), that influences the three reaction rates, k<sub>1</sub>, k<sub>2</sub>, and R<sub>d</sub>. Hence, Eq (1) was modified as :

$$P = \frac{aI(VR/VR_0 k_1 C_i - VR/VR_0 k_2 O)}{aI + VR/VR_0 k_1 C_i - VR/VR_0 R_d} \quad (4)$$

where VR<sub>0</sub>, the normal value of VR, was determined experimentally. Eq (3) and (4) were combined to give a complete photosynthesis submodel, where increase in cell surface area was assumed directly proportional to increase in mesophyll conductance to liquid phase diffusion of carbon dioxide, and increase in cell volume was thought to increase metabolic activities such as carboxylation, oxygenation, and dark respiration. Computed results from each model were compared with the observed values.

## RESULTS AND DISCUSSIONS

### Leaf Anatomy

The plants which were thinned and debranched

showed an increasing trend in AR and VR during the experiment period, even though the leaf was still expanding (Fig. 1). This trend contrasts with that of specific leaf weight found in field grown *Beta vulgaris* (Cole, 1975), where the specific leaf weight decreased as leaves expanded and increased as leaves matured. The thinned and debranched plants also showed consistently higher values for all the anatomical parameters (AR, VR, and H) than the control plants. The values of anatomical parameters for depodded plants were very similar to those for the control plants. Cell volume increased in the control and depodded plants, but there was little change in cell surface area and leaf thickness during this period.

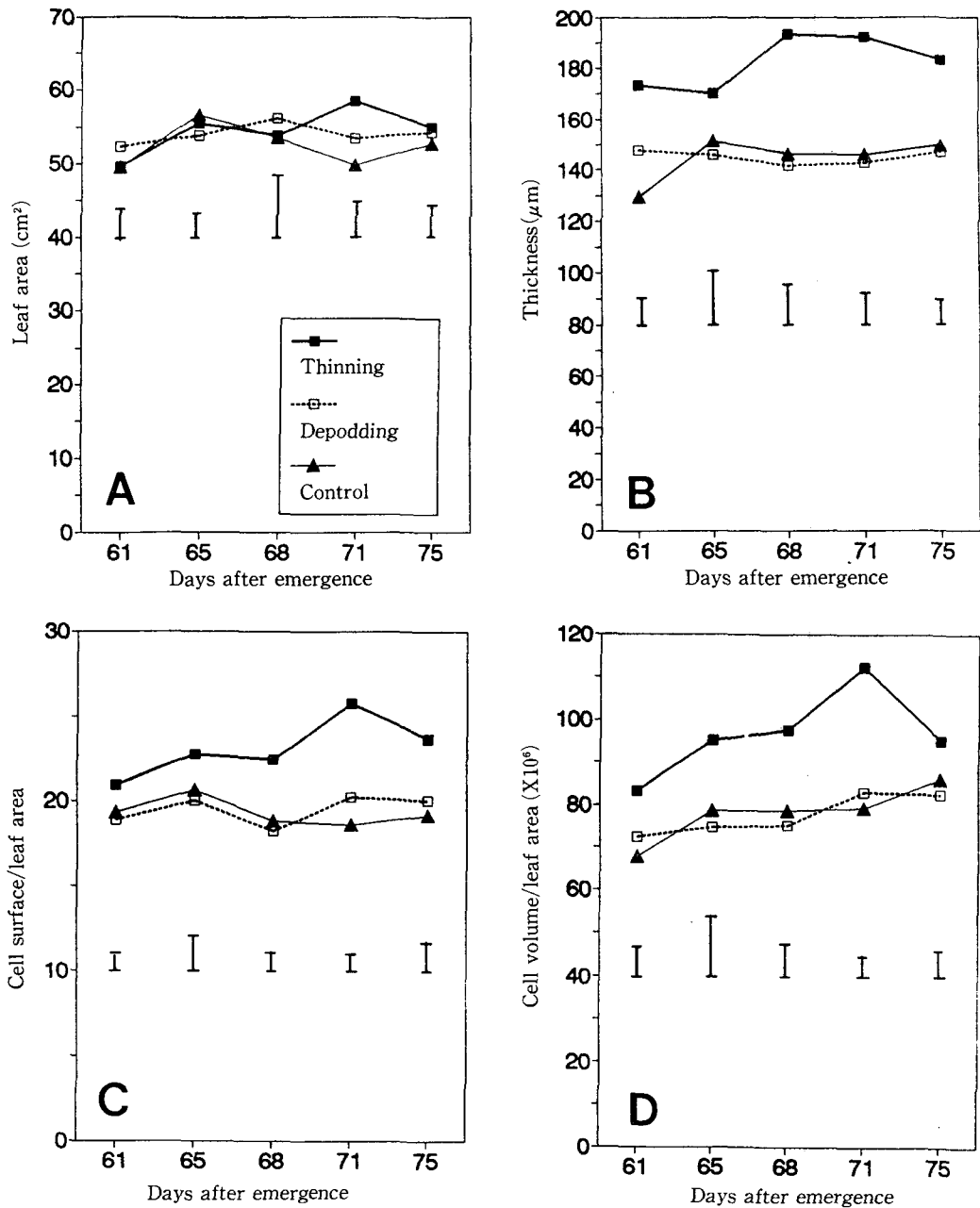
Positive correlations were found among leaf thickness (H), and VR (significant at p < 0.01). The correlation coefficients were : 0.62 between H and AR, 0.77 between H and VR, and were 0.65 between AR and VR. These values are much lower than those found in younger leaflets of chamber grown soybean plants (Yun and Taylor, 1988), and were anticipated from the greater variation found in field grown plant materials than in chamber grown ones.

### Transpiration

Transpiration rates were observed to range from 80 to 170 mg m<sup>-2</sup> s<sup>-1</sup> during the period. There was no significant difference in transpiration rates among treatments. Predicted values, which were computed by the model using the microclimatic and leaf data within the leaf chamber, were in general lower than the observed rates (Fig. 2). Underestimation of transpiration rates have been reported in other simulation studies (Young and Smith, 1982). Because the standard energy balance equation has been refined by many workers, and the transpiration rates calculated from it are believed to be accurate enough for most conditions, the accuracy of porometer reading was suspect in this study.

### Photosynthesis

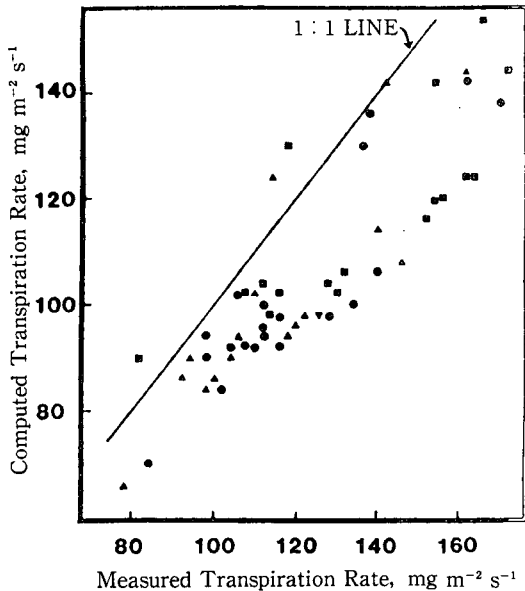
In this experiment, the mean values of AR and VR of the leaflets from the control plants were assigned to be normal values and they were 20 and 70, respectively. These values were used to modify the



**Fig. 1.** Changes in morphological and anatomical parameters for 10th node leaflets of field-grown 'Hodgson 78' soybean during early seed filling period. Points indicate the thinned and debranched (■), the depodded (□), and control (▲) plants. Vertical bars represent mean standard errors over treatments.

original photosynthesis formula to accommodate the potential effects of mesophyll morphology on CO<sub>2</sub> diffusion and the biochemical reactions as described in Model Computations section. Hence if a leaflet

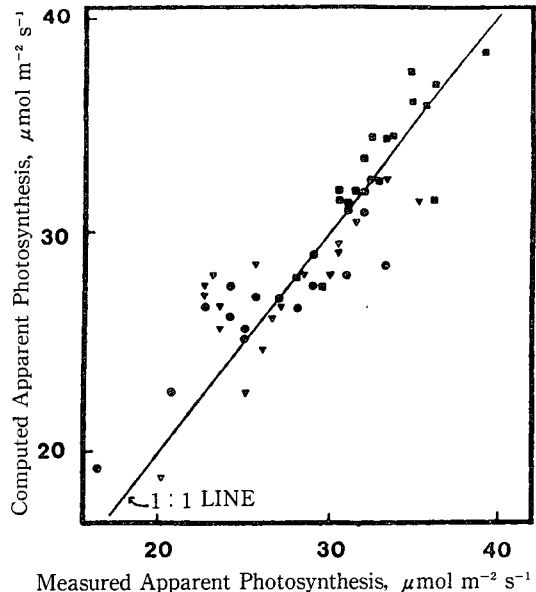
has just normal volume and surface area of mesophyll cells, the original photosynthesis formula and the modified one should compute the same rate of photosynthesis under the given environmental condi-



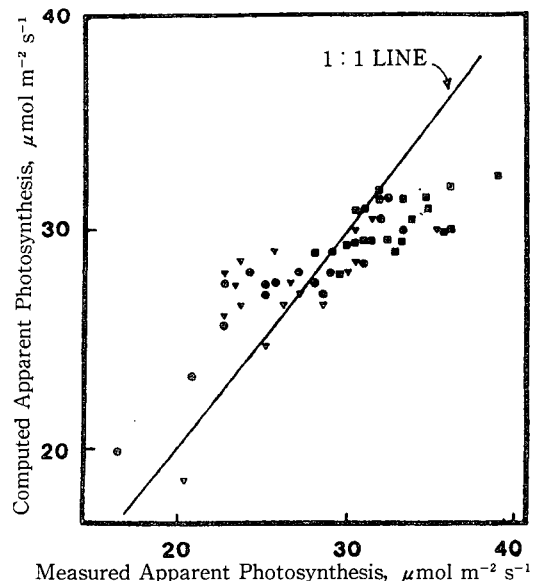
**Fig. 2.** Comparison of estimated transpiration rates by the model with the observed rates for 10th leaflets of 'Hodgson 78' soybean during early seed filling period. Points indicate the thinned and debranched (■), the depodded (▼), and control (●) plants.

tion.

The apparent photosynthesis values, calculated by both photosynthesis submodels, are plotted against the measured values (Fig. 3 and 4). Near 69% of the total variations in AP during this period was accounted for by the original model that considered no effect of morphological variations on photosynthetic variation during the same period (Table 1). The additional 10% could be attributed to the incorporation of the morphological effects in the photosynthesis submodel. The remaining 21%, which could not be explained by the model, might indicate a rough estimate for the magnitude of errors taking place in the experimental procedures. Sources of the error can be divided into three major procedures in the experiment: gas exchange measurement, microclimate measurement, and anatomical observation. The anatomical step contains possibly the greatest potential error, because only a few tiny sections of a leaflet were used for observations and they are not always an indicator for the whole leaflet. Indirect estimation of the anatomical parameters through the stereological method might introduce more uncer-



**Fig. 3.** Comparison of leaf photosynthetic rates by the "morphology" model with the observed rates for 10th node leaflets of 'Hodgson 78' soybean. Points indicate the thinned and debranched (■), the depodded (▼), and control (●) plants.



**Fig. 4.** Comparison of leaf photosynthetic rates computed by the original model without considering the morphology effects on photosynthesis with the observed rates for 10th node leaflets of 'Hodgson 78' soybean. Points indicate the thinned and debranched (■), the depodded (▼), and control (●) plants.

**Table 1.** Analysis of variance table for the model "observed AP=computed AP based on the modified photosynthesis model".

source	df	mean square	F-value	PR>F	r <sup>2</sup>	C.V.
Model	1	1.70993526	204.64	0.0001	0.788176	7.2775
Error	55	0.00835590				
Total	56					

**Table 2.** Analysis of variance table for the model "observed AP=computed AP based on the original photosynthesis model".

source	df	mean square	F-value	PR>F	r <sup>2</sup>	C.V.
Model	1	1.50391905	124.27	0.0001	0.693207	8.7581
Error	55	0.01210165				
Total	56					

tainty.

The modified model showed a consistent performance in predicting AP of the leaves from the plants treated differently, while the original model did not (Fig. 3 and 4). For example, the original model could barely account for 40% of total variations in the AP for the thinning and debranching treatment, even though it showed much better performance in the case of depodding or control plants. The modified model showed more than 70% of coverage in all three cases.

In summary, the results of this study showed a potential of improving predictability of leaf photosynthesis model by incorporating the mechanistic relationship between morphological variation and photosynthetic variation. Independent relationships should be pursued for other crop species by further investigations.

### 摘 要

콩잎의 광합성능력이 잎의 내부형태 변이와 관련되어 있는지 검토하기 위해 대두품종 'Hodgson 78'을 공시하여 포장실험을 수행하였다. 잎의 내부형태변이를 촉진시키기 위해 着萊始(R3 stage)에 속아주기(1m이랑당 26주에서 6.5주)와 결가지 치기를 통해 source활성 증대를, 계속적인 꼬투리 제거(절위당 한개의 꼬투리만 남김)를 통해 sink활성 감소를 시도하였다. 萊伸長期(R4 stage)로부터 3-4일 간격으로 5회에 걸쳐 제 10 절위 복엽의 중앙소엽을 대상으로 기체교환특성, 잎의 두께, 葉肉細胞의 體積 및 表面積, 그리고 주변 미기상변수를 측정하였다. 가설검증을 위해

기존의 광합성모형을 葉肉細胞의 表面積이 기체 확산과, 葉肉細胞의 體積이 생화학적 활성과 관련되도록 수정하였다. 實測 광합성속도의 변이가운데 79%는 이 수정된 모형에 의해 설명 가능하였으며, 엽내부형태의 영향을 무시한 기존의 광합성모형에 비해 평균 14.5%의 추정능력 향상을 확인할 수 있었다.

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