韓作誌(Korean J. Crop Sci.) 33(4) : 353~359(1988)

Leaf Photosynthesis as Influenced by Mesophyll Cell Volume and Surface Area in Chamber-Grown Soybean (*Glycine max*) Leaves

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중엽세포의 체적 및 표면적과 콩잎의 광합성 능력간 관계

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ABSTRACT

Variations in photosynthetic capacities of leaves differing in thickness were explained on the basis of relationships between gas exchange and internal leaf structure. The relative importance of gas diffusion and of biochemical processes as limiting for leaf photosynthesis was also determined. Mesophyll cell surface was considered to be the limiting internal site for gas diffusion, and cell volume to be indicative of the sink capacity for CO_2 fixation. Increases in cell surface area were assumed to reduce proportionately mesophyll resistance to the liquid phase diffusion of CO_2. Increased cell volume was thought to account for a proportional increase in reaction rates for carboxylation, oxygenation, and dark respiration. This assumption was tested using chamber-grown *Glycine max* (L.) Merr. cv. Amsoy plants. Plants were grown under 200, 400, and 600 μmol photons m^{-2} s^{-1} of PAR to induce development of various leaf thickness. Photosynthetic CO_2 uptake rates were measured on the 3rd and 4th trifoliolate leaves under 1000 μmol photons m^{-2} s^{-1} of PAR and at the air temperature of 28 C. A pseudo-mechanistic photosynthesis model was modified to accommodate the concept of cell surface area as well as both cell volume and surface area. Both versions were used to simulate leaf photosynthesis. Computations based on volume and surface area showed slightly better agreement with experimental data than did those based on the surface area only. This implies that any single factor, whether it is structural or metabolic, is not sufficient to explain the observed photosynthetic variations. The photosynthetic model utilized in this study was suitable for relating leaf thickness to leaf productivity.

INTRODUCTION

Variations in leaf photosynthetic capacity among genotypes have been studied in relation to anatomical and physiological leaf traits in some C3 crop species[18,7,13,9,24]. Numerous traits, reported to be correlated with photosynthetic capacity, may be evidence for co-limitation by multiple factors[8,9,24]. This implies that correlation techniques are not well suited for identifying the contributing factors.

Mathematical modeling, with mechanistic techniques, accommodates the complexity of the cause

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and effect relationship between the numerous potential factors and photosynthesis[6,21]. Unit volume of mesophyll cells, rather than unit leaf area, was considered as the smallest unit of photosynthetic machinery, and hence the base for calculation of photosynthesis in those models. This assumption is supported by a study with *Glycine max*(L.) where an increase of the mesophyll layer was primarily responsible for seasonal changes in leaf thickness (also in specific leaf weight), and the increase in photosynthesis after full expansion of the leaves coincided with post-expansion thickening[13,22].

Our study was conducted to delineate a method of expressing variations of leaf internal structure that influence the photosynthetic capacity of plants grown in differing environments. The model was formulated on theoretical and experimental bases, and observations were made for evaluation of computed responses.

METHODS AND MATERIALS

Theory Gas absorption may be influenced by both of the volume and surface area in most living organisms. If gas exchange were entirely a process of passive diffusion, the net amount of a substance exchanged would be determined by the contact area as expressed by Fick's first law. However, Russell[20] showed that the absorption of nutrients in root tissues varies closely with the volume. Size or volume of a living thing may represent the magnitude of capacity or activity of biochemical processes taking place inside of the organism. Absorption or uptake of CO_2 may be considered as dependent upon two factors: passive diffusion (surface area) and the integrated activity of all the photosynthetic apparatus (biochemical activity). If there is no significant quality variation among cells, the biochemical activity may be proportional to the volume, while the diffusive influx will be proportional to the surface area.

Design Soybean [*Glycine max* (L.) Merr. cv. Amsoy] seeds were planted in 15-cm diameter pots filled with an equal mixture (by volume) of Perlite, Canadian peat moss, and local farm soil (Webster loam; fineloamy, mixed, mesic Typic Haplaquoll). Plantings were thinned to one plant per pot where unifoliolate leaves were about to expand. Plants were grown in a reach-in chamber which was controlled at temperatures of 26 C in the light and 20 C in the dark with 16/8 day/night period. A mixture of cool-white fluorescent and incandescent lamps provided a PAR of 400 μmol m^{-2} s^{-1} at the top of the pots. The lights were positioned above a thermal barrier made of plexiglass. Plants were watered twice per day with modified Hoagland's solution[21]. Relative humidity (not controlled) was found to be near 60 % in the light and 90 % in the dark. When leaflets of the second trifoliolate leaf were completely separated from each other, 3 levels of PAR were used to induce variation in leaf thickness as was suggested by Bunce et al.[5] and Nobel and Hartsock[16]. The initial PAR treatments were 200, 400, and 600 μmol m^{-2} s^{-1} at the top of the plants. To maintain the quality of light, these different PAR levels were provided by adjusting the height of pots rather than by controlling the light source[10]. Three days after full expansion of the 4th trifoliolate leaf of the medium-light treated plants, pots were placed in the greenhouse with illumination by sunlight (about 1000 μmol m^{-2} s^{-1}).

After 1 h of equilibration to this environment, CO_2 exchange characteristics were measured on terminal leaflets of the 3rd and 4th trifoliolate leaf by use of a closed gas exchange measurement system(LI-6000, LiCor Inc., Lincoln, NE, USA). After the leaf area measurement, small samples of the same leaflets were collected with a razor blade (aboiding major veins) for use in anatomical measurements following preparation with TBA/paraffin series[1]. Leaf cross sections were photographed at 400x magnification on a high contrast B/W film (Kodak Technical Pan 2415). Mesophyll cell volume per unit leaf surface (VR) and surface area per unit leaf surface (AR) were estimated by the stereological method of

Parkhurst[17].

Model Computation Estimated values for the mesophyll cell volume and the surface area were incorporated into the pseudo-mechanistic leaf photosynthesis model of Charles-Edwards[6]. In the original model, the biochemical process of photosynthesis was expressed as :

$$P=\frac{a\ I(K_1\ C_i-K_2\ O)}{aI+K_1\ C_i}-R_d \quad \cdots\cdots (1)$$

and the physical process of photosynthesis was expressed as :

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

where P is the net rate of photosynthesis per unit leaf area (g/m^2), a is the light utilization efficiency (g/J), I is the photosynthetic irradiance (W/m^2), K_1 is the carboxylation efficiency (m/s), K_2 is the oxygenation efficiency (m/s), C_i is the internal CO_2 concentration (g/m^3), O is ambient oxygen concentration (g/m^3), R_d is the dark respiration rate ($g\ m^{-2}\ s^{-1}$), C is ambient CO_2 concentration (g/m^3), g_m is the mesophyll conductance to CO_2 diffusion (m/s), and g_s is the gas phase conductance to CO_2 diffusion including leaf boundary layer, stomatal, and intercellular air space (m/s).

The model was modified in two ways to enable comparison of the relative contribution of the anatomical parameters, AR and VR. First, Eq (2) was modified to accommodate the effects of cell surface area on CO_2 diffusion by redefining the g_m. The g_m was defined as the product of AR and the conductance per unit cell surface, g_c [15]. Hence, Eq (2) becomes :

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

and the complete photosynthesis model was formulated by combining Eqs (1) and (3). Assuming an average value of 4.8×10^{-3} m/s for g_m[6] and an average value of 14 for AR (observed in this experiment), the liquid phase diffusive conductance per unit cell surface, g_c, was calculated as 3.43×10^{-4} m/s. The range found for AR was 7 to 23. This model was formulated to demonstrate the relative effect of the diffusion process on photosynthesis under conditions of constant reaction rates (biochemical activity) during leaf thickening.

Second, 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 ratio (VR/VR_0), that influences the three reaction rates, K_1, K_2, and R_d. Hence, Eq (1) was modified as :

$$P=\frac{a\ I(VR/VR_0\ K_1\ C_i-VR/VR_0\ K_2\ O)}{a\ I+VR/VR_0\ K_1\ C_i}-VR/VR_0\ R_d \quad \cdots\cdots (4)$$

where VR_0, the average value of VR, was determined experimentaly to be 3×10^{-5}. Equations (3) and (4) were combined to give a photosynthesis model that considered the limiting effects of the cell volume, i.e., the biochemical process as well as those of the cell surface area representing the diffusion process. Both models were used to predict photosynthetic rates for leaves in the study. Computed results were compared to determine the relative contribution of each process (or anatomical parameter). According to the assumptions used, the differential AP between the two models should represent only the contribution of the change in biochemical activity.

RESULTS

Leaf Structure A linear relationship between the volume and surface area of mesophyll cells was found (Fig. 1). High-light grown leaves showed greater values. The different levels of irradiation used to induce different leaf thickening may cause alterations in leaf characteristics (other than the thickness). Any change in leaf characteristics, which can influence gas exchange, complicates interpretation of thickness effects on photosynthesis. If the frequency and the size of stomatal aperture is altered singificantly due to the irradiance treatment and as a consequence g_s is different among the treated plants, any observed difference in photosynthesis should be interpreted

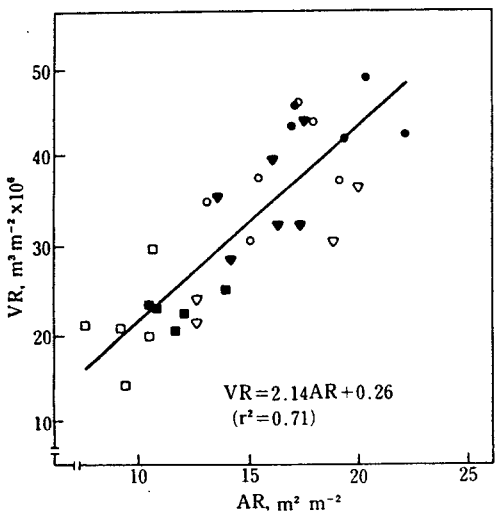

Fig. 1. Relationship between surface area and volume of mesophyll cells in the 3rd (open symbols) and the 4th (closed symbols) trifoliolates of *Glycine max* plants. Plants were grown under the PAR levels of 200(■ □), 400(▼ ▽), and 600(● ○) μmol m^{-2} s^{-1}.

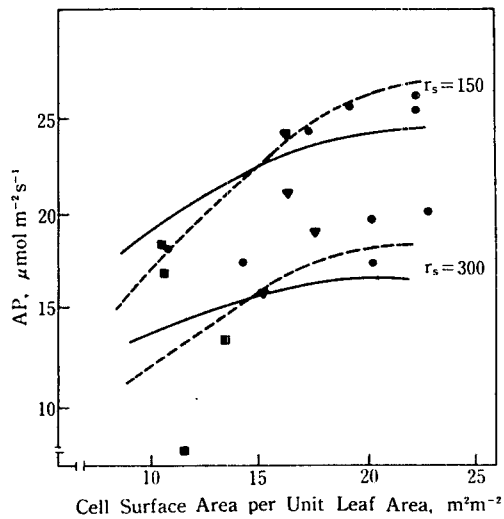

Fig. 2. Computed apparent photosynthesis for leaves of low resistance to the diffusion of CO_2 under high PAR (1000 μmol m^{-2} s^{-1}). Predicted AP using the ratio of leaf internal surface area to external area (solid line) is less responsive to factors associated with leaf thickness than is the combined surface area-cell volume model (dotted line). Points represent observations for low (■), moderate(▼), and high(●) light grown *Glycine max* plants.

with respect to both the thickness and g_s. Small, though significant differences in g_s were detected among treated plants (resistance values ranging from 239±56 s/m for high light grown to 199±41 s/m for low light grown plants). In this study, both low and high light grown leaves had sufficient stomatal area to achieve a conductance of 0.33 cm/s or higher (a resistance of 300 s/m or lower), which agrees with observations made by Taylor[23].

Photosynthesis Model validation was achieved by comparison of predicted rates of CO_2 uptake with rates measured in the greenhouse. Two values of leaf conductance were used in the computation to represent "typical" high and low g_s that were measured on the chamber-grown plants used in this study. The computation considering only the cell surface area with the maximum and minimum g_s encompasses most of the observations, although the model seems to be somewhat conservative (Fig. 2). The model computations that consider both the cell volume and the surface area, show a slight improvement based on "slope comparison". When we compared the slopes of the curves from the two models with the slope of linear regression for actual data points, the slope from the model that considered both the cell volume and the surface area was closer to that of the regression by 11% than the slope from the model that considered only the cell surface area. The model computations (upper and lower sets in Fig. 2) represent computations for the highest and lowest g_s (the lowest and highest resistance values, respectively) measured in the study. The model was utilized to delineate a range of values that would encompass the observed values. The actual points represent photosynthesis measurements at uncontrolled leaf diffusion resistances within the range of conditions modeled.

DISCUSSION

This result implies that the effects of leaf

thickness may arise primarily because of increased cell surface area, under the conditions used in this experiment. However, the effect of increased cell volume, which represents the increased sink activity, should not be neglected as it made an apparent contribution to net photosytnhesis beyond that attributed to cell surface area. Visual inspection of Fig. 2 suggests that the model that utilizes the area and volume [Eqs (3) and (4)] better agrees with observed values. The relatively smaller contribution attributed to modified cell volume may be explained by concurrent increase in oxygenation and dark respiration activities.

Most attempts directed toward improving leaf photosynthetic capacity of C_3 plants have involved the modification of leaf resistance to gas diffusion, based on a diffusion resistance scheme analogous to electric resistance networks. Two ways are possible to improve photosynthetic capacity by modification of that network. One is decreasing stomatal resistance and the other is decreasing the so-called "residual resistance". A cool desert annual *Camissonia claviformis* has been suggested as a typical example found in nature for its high apparent photosynthsis as a result of combination of both factors[12]. However, the term "residual resistance" could be misleading because only a part of the "residual resistance" has a similar nature relevant to the definition of diffusion resistance, and the remaining portion may be better represented by metabolic capacity. The metabolic capacity has no diffusion-like pathway implied. In this study, the former was represented by mesophyll surface area and the latter by cell volume, although little was known about the actual magnitude of either parameter.

Some workers have regarded any photosynthetic variation, except that by cell surface area, to be a result of variation in the quality of photosynthetic apparatus[11]. There is little reported evidence for dilution of photosynthetic apparatus within a cell during leaf thickening[9], while numerous workers reported more or less the same amount of photosynthetic apparatus on unit leaf volume basis[4,3,14]. Variations in the quality of photosynthetic machinery were neglected in this study. Sink activity per mesophyll cell was assumed to increase as the cell expands in both surface area and volume. Most, if not all, of the photosynthetic variations observed in this study were attributed to changes in these two anatomical features.

Although no sensitivity test for photosynthetic parameters was done, it was apparent, however, that any single factor, whethehr it is structural or metabolic, was not sufficient to fully explain observed photosynthetic variations. Interpretation of the simulated responses could be an artifact of the model used, dependent upon the values selected for either the reaction rate constants or for mesophyll resistance, or both. Depending on viewpoint, the mesophyll resistance could be more emphasized[15,19], or the biochemical process could be preferred[8,9]. From the performance of the model formulated in this study, a combination of the cell surface area and volume seems to serve as a practical indicator for variations in photosynthetic capacity among ecotypes. It will be necessary to further verify the extent to which genotypic variations in photosynthetic capacity are explained by these anatomical parameters.

摘 要

두께가 서로 다른 C_3 植物의 잎은 單位葉面積當 光合成 能力에 있어서도 차이가 나는바 잎의 內部構造와 氣體交換 사이의 관계를 바탕으로 그 원인을 究明하였다. 光合成의 2大 制限要因으로 氣體擴散과 生化學的 과정의 相對的인 重要度를 결정하기 위해 中葉細胞의 표면은 氣體擴散 抵抗의, 그리고 細胞의 체적은 炭素固定 能力의 지표로 假定하였다. 즉 細胞의 表面積이 증가하면 이산화탄소의 액상확산 저항이 減少하며 體積이 증대되면 carboxylation, oxygenation, 그리고 dark respiration 反應速度가 증가한다고 간주하였다. 이러한 槪念을 함축하는 光合成 模型을 작성하고 이 假說의 檢證을 위해 大豆 品種 Amsoy 잎을 利用한 實驗을 수행하였다. 生長調節室內에서 200, 400, 600 μmol photons m^{-2} s^{-1} PAR을 供給하여 서로 다른 두께의 잎을 準備하였으며 제3 및 4本葉에

대해 1,000 μmol photons m^{-2} s^{-1} PAR 및 28 ℃ 기온 環境下에서 이산화탄소 吸收速度를 측정한 결과 細胞의 體積과 表面積의 영향을 동시에 고려한 光合成 模型이 세포 표면적만을 고려한 경우 보다 實測値에 가까운 豫測値를 算出하였다. 이로 미루어 細胞의 表面積과 體積은 잎의 두께 및 그에 따른 光合成 能力의 예측에 적절한 變數로 간주된다.

LITERATURE CITED

1. Berlyn, G.P. and J.P. Miksche. 1976. Botanical microtechnique and cytochemistry. The Iowa State University Press, Ames, pp. 35-120.
2. Berry, W.L. 1978. Nutrition, containers, and media. *In* R.W. Langhans, ed, A growth chamber manual : environmental control for plants. Comstock Pub. Association, Ithaca, pp.117-135.
3. Björkman, O. 1981. Ecological adaptation of the photosynthetic apparatus. *In* G. Akoyunoglou, ed, Photosynthesis VI : photosynthesis and productivity, photosynthesis and environment. Balaban International Science Series, Philadelphia, pp.191-202.
4. Boardman, N.K. 1977. Comparative photosynthesis of sun and shade plants. Ann. Rev. Plant Physiol. 28 : 355-377.
5. Bunce, J.A., D.T. Patterson, M.M. Peet. and R.S. Alberte. 1977. Light acclimation during and after leaf expansion in soybean. Plant Physiol. 60 : 225-229.
6. Charles-Edwards, D.A. 1981. The mathematics of photosynthesis and productivity. Academic Press, New York, pp.13-54.
7. Dornhoff, G.M., and R.M. Shibles. 1976. Leaf morphology and anatomy in relation to CO_2 exchange rate of soybean leaves. Crop Sci. 16 : 377-381.
8. Farquhar, G.D., S. Von Caemmerer, and J. A. Berry. 1980. A biochemical model of photosynthetic CO_2 assimilation in leaves of C3 species. Planta 149 : 78-90.
9. Ford, D.M. 1984. Leaf physiology and anatomy of soybean genotypes differing in leaf photosynthetic ability. Ph.D thesis, Iowa State University, Ames.
10. Grace, J. 1983. Plant-atmosphere relationships. Chapman and Hall Ltd., London.
11. Longstreth, D.J., J.A. Balanos, and R.H. Goddard. 1985. Photosynthetic rate and mesophyll surface area in expanding leaves of *Alternanthera philoxeroides* grown at two light levels. Am. J. Bot. 72 : 14-19.
12. Longstreth, D.J., T.L. Hartsock, and P.S. Nobel. 1980. Mesophyll cell properties for some C3 and C4 species with high photosynthetic rates. Physiol. Plant. 48 : 494-498.
13. Lugg, D.G. and T.R. Sinclair. 1980. Seasonal changes in morphology and anatomy of field-grown soybean leaves. Crop Sci. 20 : 191-196.
14. McMillen, G.G. and J.H. McClendon. 1983. Dependence of photosynthetic rates on leaf density thickness in deciduous woody plants grown in sun and shade. Plant Physiol. 72 : 674-678.
15. Nobel, P.S. 1977. Internal leaf area and cellular CO_2 resistance : photosynthetic implications of variations with growth conditions and plant species. Physiol. Plant. 40 : 137-144.
16. Nobel, P.S. and T.L. Hartsock. 1981. Development of leaf thickness for *Plectranthus parviflorus* : influence of photosynthetically active radiation. Physiol. Plant. 51 : 163-166.
17. Parkhurst, D.F. 1982. Stereological methods for measuring internal leaf structure variables. Am. J. Bot. 69 : 31-39.
18. Pearce, R.B., G.E. Carlson, D.K. Barnes, and C.H. Hansen. 1969. Specific leaf weight and photosynthesis in alfalfa. Crop Sci. 9 : 423-426.
19. Raven, J.A. and S.M. Glidewell. 1981. Processes limiting photosynthetic conductance. *In* C.B. Johnson, ed, Physiological processes limiting plant productivity. Butterworth, London, pp.109-136.

20. Russell, R.S. 1977. Plant root system : the function and interaction with the soil. McGraw-Hill, London, pp.80-81.
21. Sharpe, P.J.H. 1983. Responses of photosynthesis and dark respiration to temperature. Ann. Bot. 52 : 325-343.
22. Sinclair, T.R. 1980. Leaf CER from post -flowering to senescence of field-grown soybean cultivars. Crop Sci. 20 : 196-200.
23. Taylor, S.E. 1971. Ecological implications of leaf morphology considered from the standpoint of energy relations and productivity. Ph.D thesis, Washington University, St. Louis.
24. Yun, J.I. and S.E. Taylor. 1986. Adaptive implications of leaf thickness for sun- and shade-grown *Abutilon theophrasti*. Ecology 67 : 1314-1318.