

Ratio of intercellular CO₂ concentration to stomatal conductance is a reflection of mesophyll efficiency

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Carbon assimilation rates (A) have been shown to be substrate limited associated with the stomatal conductance (G_s). However, a significant species as well as genotypic variation in intercellular CO₂ concentration (C_i) in the present study implies that mesophyll factors also play an important role in regulating A through its effect in determining the CO₂ concentration gradient. Efficiency of the mesophyll factors is often estimated by measuring the initial slope of CO₂ response curve (dA/dC_i). In this paper we provide experimental evidences to show that C_i at a given G_s is also a reflection of mesophyll efficiency. Steady state C_i levels are maintained by both G_s and the efficiency of the mesophyll to utilize the substrate CO₂. Therefore, at a given stomatal conductance, lower C_i should indicate a better mesophyll efficiency. The C_i/G_s ratio also showed an inverse relationship with dA/dC_i . Therefore, C_i/G_s ratio can be considered as a novel approach to estimate the mesophyll efficiency. We also noticed that the C_i/G_s ratios showed a significant inverse relationship with assimilation rate, suggesting the role of mesophyll factors in the regulation of photosynthesis. The implications of the C_i/G_s ratio are discussed in this paper.

ALTHOUGH essentially a biochemical process, photosynthesis is often regarded as a diffusive process also. The rate of diffusion of CO₂ is largely controlled by two factors. One, the stomatal conductance (G_s) and second the CO₂ concentration gradient between the carboxylation site and the ambient air. This CO₂ concentration gradient at a given G_s and C_a is established predominantly by the intercellular CO₂ concentration (C_i), which is a result of mesophyll efficiency. Therefore, the diffusive entry of CO₂ into the leaf is a reflection of intrinsic mesophyll capacity also.

A significant research effort has gone in to understand the implications of gas exchange measurements and to relate the gas exchange traits with biochemistry of photosynthesis¹. Gas exchange techniques have been used to quantify the relative stomatal and mesophyll limitations^{2,3}, carboxylation efficiency¹⁻³, limitations imposed by the photochemical and biochemical reactions⁴, specificity factor of RuBisCO (ref. 5).

It is evident from several studies that the assimilation rate (A) is substrate limited under non-stress condi-

tions^{2,6-8}, mostly in C₃ plants. However, at the prevailing C_a (340 ppm), the mesophyll factors establish the gradient for CO₂ diffusion and hence determine the rate of CO₂ entry. Further, the C_i , which is determined by the mesophyll factors, in turn has a feedback control over stomatal conductance⁹. Therefore, quantification of mesophyll factors assumes great importance.

The level of C_i that establishes the gradient for CO₂ entry is largely governed by the rate of CO₂ incorporation by the carboxylation process as well as the rate of its supply through stomata.

In view of this, we hypothesize that across species or across genotypes of a species, lower C_i/G_s ratio must be inversely related to A . To test this hypothesis, we measured gas exchange parameters in a few species, including both crop plants and forest tree species, and also in a few genotypes of sunflower and soybean.

The crop species and genotypes were raised in carbonized rubber containers (30 kg capacity) with adequate water and nutrients up to 30 days. Similarly, the forest tree seedlings were raised in poly bags (20 kg capacity) for one year and used for the study. The top fully expanded leaves were used to record the gas exchange traits and to develop the CO₂ response curves. All measurements were recorded during the morning time (9 to 11 AM) on a bright sunny day with 1500 μ moles m⁻² s⁻¹ light intensity. The vapour pressure deficit (VPD) was maintained around 12 to 15 mbars by altering the flow rate of dry air by the Air Supply Unit of Portable Photosynthesis System (LCA-2, ADC, London). Photosynthetic CO₂ response curves were made as detailed by Sheshshayee and coworkers¹⁰.

Gas exchange traits recorded are presented in Tables 1 to 4. The results indicate a significant variation A and G_s between the species as well as between certain genotypes of a given species. C_i also showed a significant species and genotypic variation.

To study the relationship between A and C_i/G_s , these two parameters were fitted into a best fit polynomial function (Figures 1 and 2). A significant inverse relationship was evident between A and C_i/G_s in all the plants. Similar inverse relationship was noticed even when a pooled regression analysis was carried out (Figure 3).

Table 1. Gas exchange parameters in a few crop plants*

Species	A	G_s	C_i	C_i/G_s	dA/dC_i
<i>Arachis hypogea</i>	21.46	542	269	0.505	0.140
<i>Helianthus annuus</i>	21.38	774	265	0.353	0.144
<i>Vigna unguiculata</i>	15.50	506	232	0.461	0.079
<i>Glycine max</i>	13.50	340	240	0.705	0.080
CD ($P=0.05$)	2.05	18	7	-	0.007

*Plants were grown in carbonized rubber containers for 35 days. Data were recorded on the top fully expanded leaves on a bright sunny day (light intensity was around 1500 μ mole m⁻² s⁻¹).
 $A = \mu$ moles m⁻² s⁻¹; $G_s =$ mmoles m⁻² s⁻¹; $C_i =$ ppm.

Table 2. Gas exchange parameters in a few forest tree species*

Species	A	G _s	C _i	C _i /G _s	dA/dC _i
<i>Terminalia bellarica</i>	3.78	167	271	1.05	0.042
<i>Madhuca indica</i>	5.13	223	272	1.22	0.049
<i>Delbergia latifolia</i>	7.96	362	279	0.77	0.072
<i>Swietenia mahagoni</i>	5.66	228	267	1.20	0.050
<i>Syzijium jambolana</i>	6.31	196	281	1.43	0.031
<i>Eucalyptus citriodora</i>	6.93	257	263	1.02	0.055
<i>Pterocarpus santalinous</i>	5.75	231	272	1.18	0.034
<i>Alternanthera</i> (Herb)	5.06	121	247	2.04	0.032
<i>Artocarpus heterophyllus</i>	5.13	199	278	1.40	0.029
<i>Acacia auriculiformis</i>	13.07	442	267	0.60	0.045
CD (P=0.05)	0.97	27	6	-	0.004

*One-year-old polybag-grown seedlings were selected. Gas exchange data were recorded on the top fully expanded leaves on a bright sunny day (light intensity was around 1500 μ mole m⁻² s⁻¹).
A = μ moles m⁻² s⁻¹; G_s = mmoles m⁻² s⁻¹; C_i = ppm.

Table 3. Genotypic variations in gas exchange parameters in sunflower (*Helianthus annuus*)*

Genotype	A	G _s	C _i	C _i /G _s
Acc 1610	27.2	1097	277	0.253
Acc 1599	27.2	848	263	0.310
Acc 1600	23.0	849	272	0.320
Acc 1648	22.6	963	276	0.287
Acc 1616	22.2	877	276	0.315
M 787-7-2	20.8	863	282	0.327
Acc 1630	20.2	621	262	0.422
62-B	19.6	814	277	0.340
339-B	16.3	438	244	0.557
CD (P=0.05)	2.37	21	9	

*Data were recorded on the top fully expanded leaves of 35-day-old plants (light intensity was around 1500 μ mole m⁻² s⁻¹).
A = μ moles m⁻² s⁻¹; G_s = mmoles m⁻² s⁻¹; C_i = ppm.

Table 4. Genotypic variations in gas exchange parameters in soybean (*Glycine max*)*

Genotype	A	G _s	C _i	C _i /G _s
IS-87-60	23.4	1025	279	0.270
Punjab 1	22.7	983	269	0.274
MACS-409	22.0	864	280	0.324
Seln-5	21.4	1016	284	0.279
Pusa-16	20.8	1035	285	0.275
JS-75-46	19.5	841	279	0.332
PK-1029	19.4	660	270	0.409
Pusa-20	18.8	714	269	0.377
JS-87-59	18.7	780	282	0.362
KB-92	17.0	671	281	0.419
DS-83-12-2	16.9	576	275	0.477
CD (P=0.05)	1.6	26	8	

*Data were recorded on the top fully expanded leaves of 35-day-old plants (light intensity was around 1500 μ mole m⁻² s⁻¹).
A = μ moles m⁻² s⁻¹; G_s = mmoles m⁻² s⁻¹; C_i = ppm.

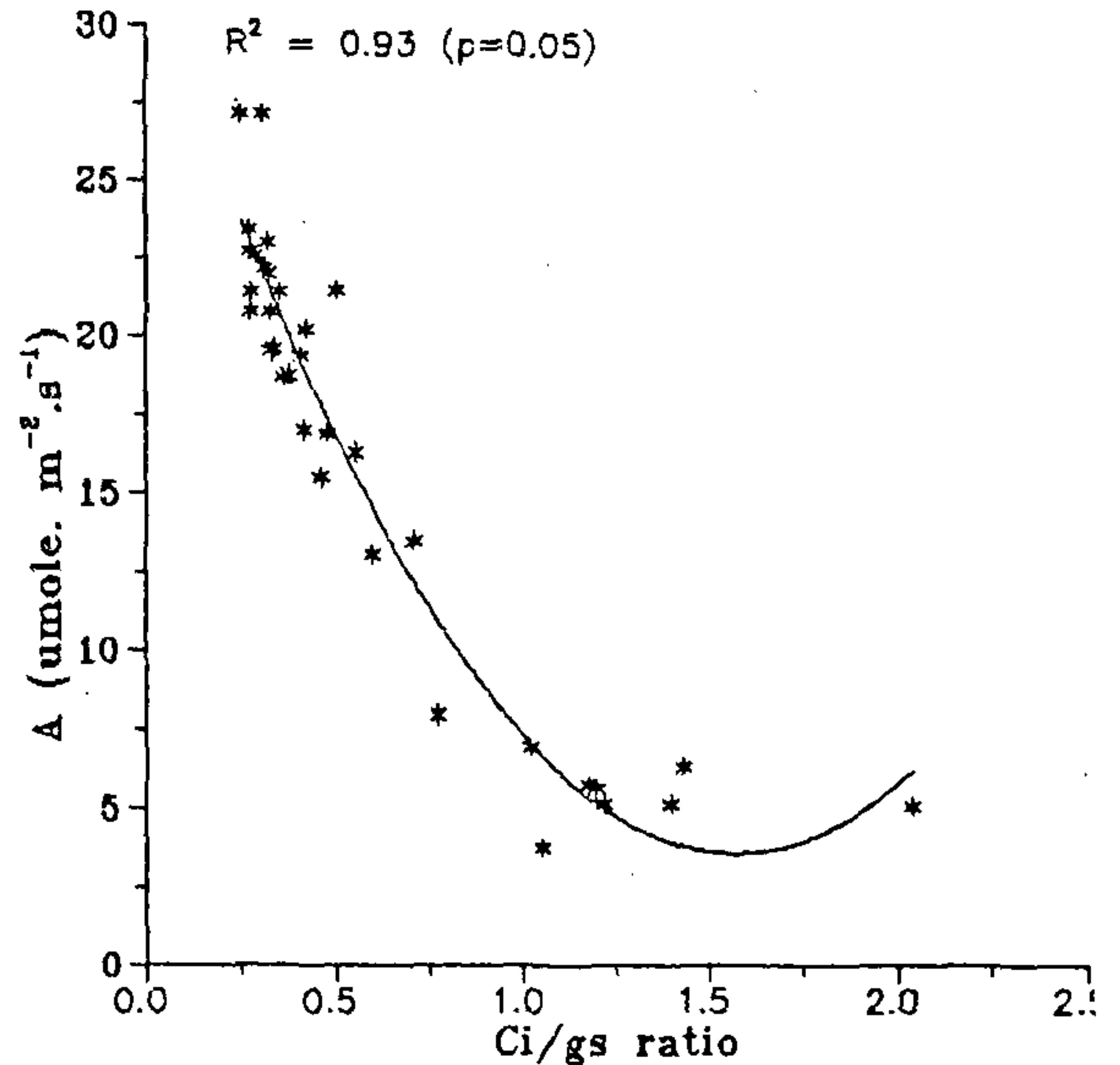


Figure 1. The relationship between the ratio of C_i/G_s and assimilation rate in a few species. Both crop plants and forest tree species were used. Data were recorded on the top fully expanded leaves on a bright sunny day.

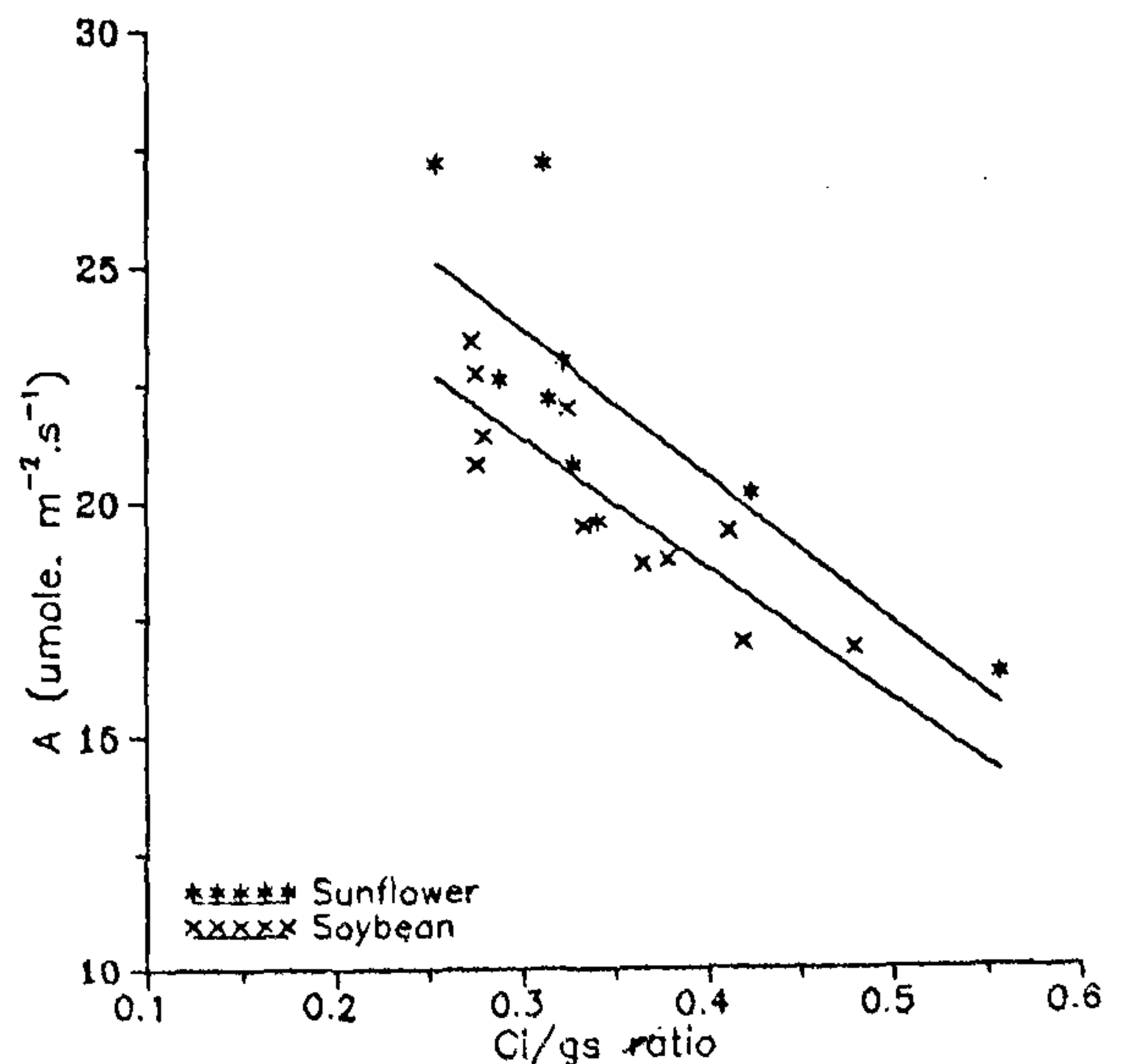


Figure 2. The relationship between the ratio of C_i/G_s and assimilation rate in a few genotypes of sunflower and soybean. Data were recorded on the top fully expanded leaves on a bright sunny day.

These plots clearly indicate that higher A is associated with lower C_i/G_s ratio.

Literature suggests that A is under the control of both stomatal and mesophyll factors. Though the stomatal conductance can be measured, we do not have a suitable technique to quantify the mesophyll conductance. However, there are a few approaches for approximately estimating the mesophyll factors. One such approach is the determination of the initial response of a A/C_i function. We attempted to compare the ratio of C_i/G_s with this frequently adopted estimate of carboxylation efficiency (dA/dC_i). A strong inverse relationship between C_i/G_s and dA/dC_i (Figure 4) offered further proof that C_i/G_s ratio can be used as a parameter to approximate the mesophyll efficiency of a leaf.

The entry of CO_2 into the leaf is mainly regulated by the stomatal conductance and concentration gradient between ambient air and intercellular space. At any given stomatal conductance, the intercellular CO_2 concentration determines the diffusion gradient for CO_2 transfer.

Under *in situ* conditions, higher carboxylation efficiency is always substrate CO_2 dependent. Availability of the substrate at the carboxylation site is directly under the control of stomatal conductance as well as the intrinsic mesophyll traits which determine the CO_2 diffusion gradient between air and chloroplast. Therefore, the entry of CO_2 is governed by both the stomatal and mesophyll factors.

A number of evidences suggest that G_s determines the availability of substrate CO_2 and hence a positive relationship between stomatal conductance and assimilation rates is possible^{11,12}. In confirmation, many recent studies also have shown that assimilation rates are limited by the stomatal factors under non-stress conditions^{3,6,7}.

To assess the relationship between A and G_s , we developed regression plots between them. These plots revealed a positive correlation between A and G_s ($r=0.87$), suggesting that assimilation rates are under the stomatal control. If stomatal conductance alone were to control assimilation, then one might expect least variations in C_i . However, our data revealed a significant variation in C_i both across species and genotypes within a species. This strongly implies that the mesophyll efficiency also influences variations in C_i , further suggesting that the variations in A are not merely due to a higher stomatal conductance that result in increased supply of CO_2 .

At any given stomatal conductance, the variations in assimilation rate should be dependent on the efficiency of the mesophyll to fix the available substrate CO_2 . If the mesophyll efficiency to utilize carbon is higher, the steady state C_i will be lower at that G_s .

When the ratio of C_i to G_s was plotted against A , a significant negative relationship was evident in both crop plants and forest tree species (Figure 1). A similar trend was also noticed among genotypes of soybean

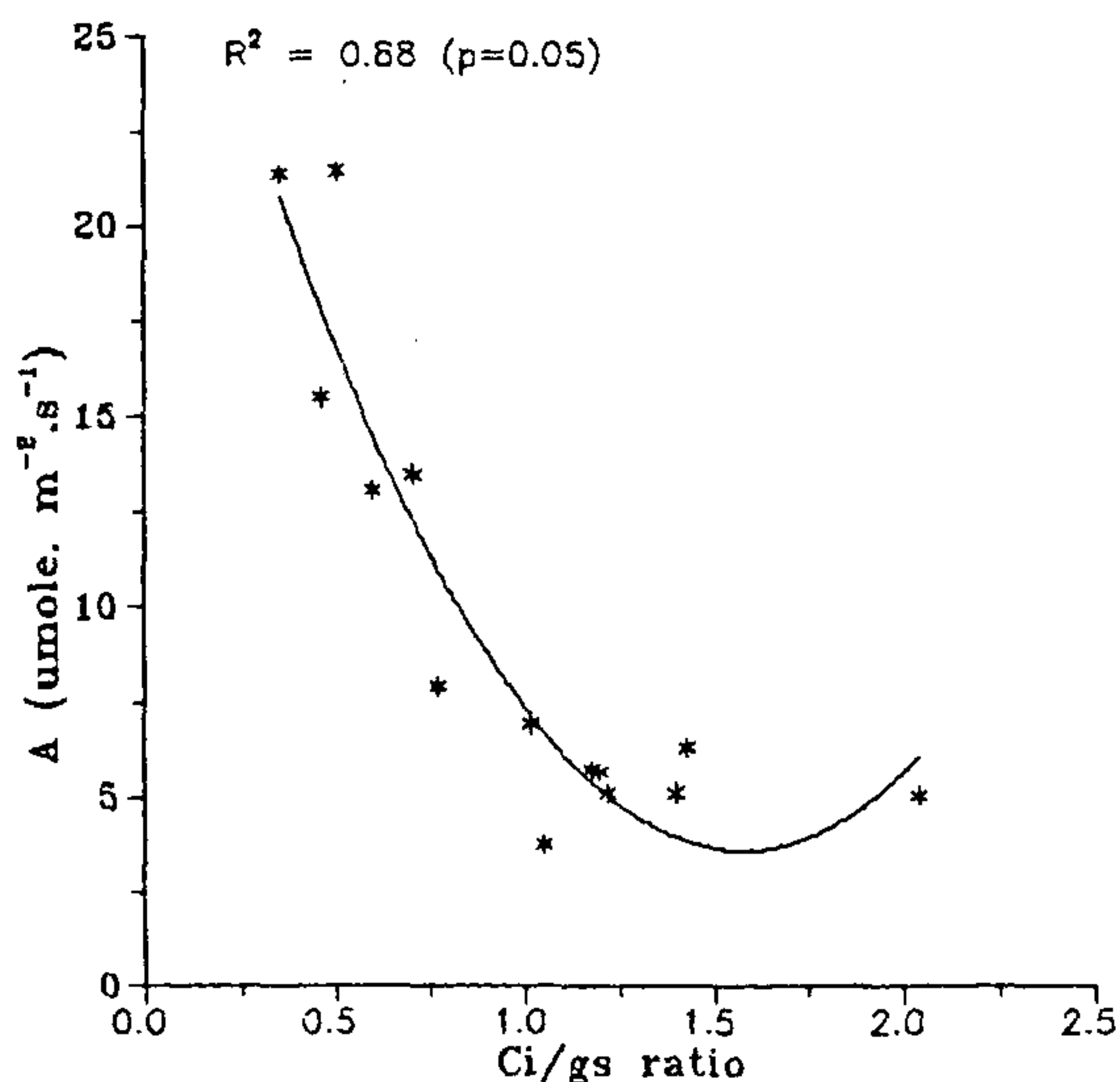


Figure 3. The relationship between the ratio of C_i/G_s and assimilation rate among the genotypes and species. Data points from Figures 1 and 2 are pooled.

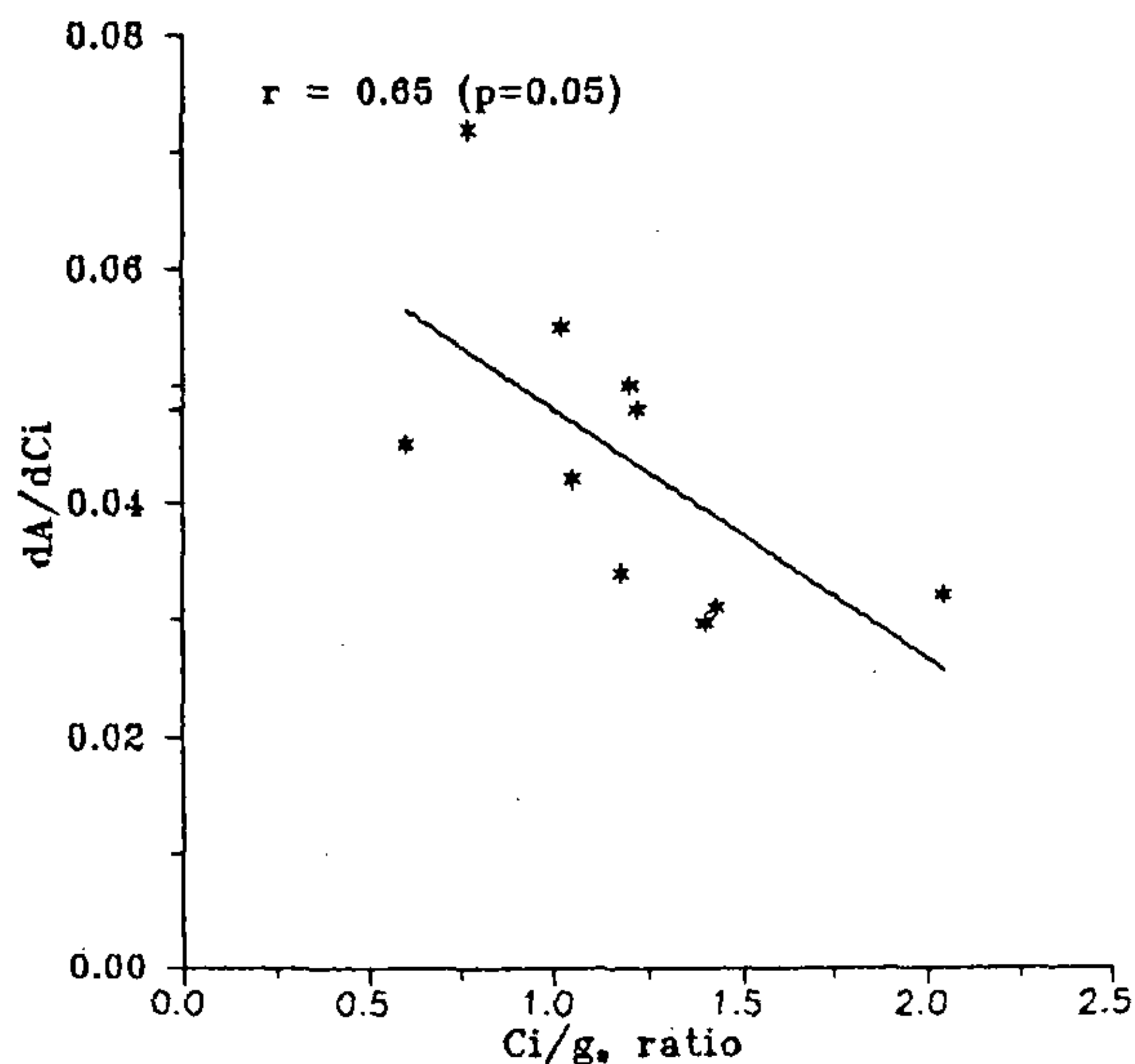


Figure 4. Relationship between carboxylation efficiency (dA/dC_i) and C_i/G_s ratio among a few species. CO_2 response curves were generated in all the species and their initial slope determined to arrive at the carboxylation efficiency.

and sunflower (Figure 2). This illustrates that assimilation rates are controlled by the mesophyll factors also and the proposed method can be conveniently used for rapid evaluation of variations in the mesophyll efficiency of a given leaf.

If higher carboxylation efficiency determines a better mesophyll factor, a leaf that shows higher dA/dC_i should have lower C_i/G_s ratio. A strong inverse relationship ($R^2 = 0.87$) clearly suggests that C_i/G_s does indeed reflect the mesophyll efficiency (Figure 4).

Since the proposed method only involves the measurements of gas exchange traits, estimation of mesophyll efficiency can be done relatively rapidly, especially while evaluating the germplasm lines. Further evaluation of the approach in relation to the carboxylation efficiency associated with RuBisCO has been carried out (Krishna Prasad *et al.* 1996, this issue).

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Regulation of carboxylation by RuBisCO content and its efficiency in sunflower and soybean

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In addition to the CO₂ diffusive processes, the mesophyll efficiency also determines the variability in carbon assimilation rates (A) of leaves. Mesophyll efficiency as calculated by the ratio of the intercellular

CO₂ concentration to the stomatal conductance (C_i/G_s) offers proof for the mesophyll control of photosynthesis. The mesophyll factors are, in turn, largely associated with the Ribulose 1,5-biphosphate carboxylase (RuBisCO) content and/or its efficiency of carboxylation. To evaluate the role of RuBisCO in determining the variability in mesophyll efficiency, we studied these parameters in a few genotypes of sunflower and soybean, showing significant genetic variability in gas exchange parameters and in RuBisCO content. The efficiency of RuBisCO was computed by the ratio of A per unit RuBisCO. A among soybean genotypes showed a positive relationship with RuBisCO content, while in sunflower genotypes it was related to the efficiency of RuBisCO. Its content in soybean genotypes and the efficiency among sunflower genotypes showed a strong relationship with the C_i/G_s ratio, reiterating the role of RuBisCO in regulating the mesophyll efficiency. We conclude that the mesophyll efficiency is regulated by RuBisCO content and/or its efficiency across the genotypes of sunflower and soybean.

A considerable genotypic variability in photosynthetic CO₂ assimilation rate per unit leaf area (A) has been reported in many crop species¹⁻⁵. But, exploiting this genetic variability for enhancing A, by conventional breeding approaches has met with little success^{6,7}. This is partly due to the complex genetic control and the lack of adequate knowledge of limiting factors of photosynthesis under a given environment.

Under optimum growing conditions, the realized assimilation rates are often a few degrees of magnitude less than the potential of chloroplasts^{8,9}. The primary limitation to achieve these potential assimilation rates is the lack of substrate CO₂ availability at the carboxylation site due to stomatal diffusive resistances for CO₂ (refs 10–14).

Apart from the stomatal factors, mesophyll also plays an important role in influencing the variability in A. A linear relationship between the carboxylation efficiency as computed by the initial slope of the CO₂ response curve (dA/dC_i) and assimilation rate suggests that A is controlled by the mesophyll (RuBisCO) factors^{9,15,16}. In an earlier paper, we reported that the ratio of the intercellular CO₂ concentration (C_i) to stomatal conductance (G_s) is a good reflection of the mesophyll efficiency for carboxylation¹⁷. A strong inverse relationship between A and C_i/G_s ratio suggests that A is controlled by the mesophyll efficiency.

At a given G_s , the fixation of CO₂ depends on the carboxylation associated with RuBP carboxylase/oxygenase (RuBisCO), the photochemical reactions of the lamellar system, and the regeneration of inorganic orthophosphate (P_i) associated with end product synthesis. Recent studies has shown that the assimilation rates at ambient CO₂ concentrations are not limited by photochemical reaction¹⁸⁻²⁰ or by P_i recycling^{21,22}.