Relationships between carbon dioxide exchange rate, photosynthetic area and biomass in pea

Hobbs, S. L. A.

Publisher's version / Version de l'éditeur:
https://doi.org/10.4141/cjps86-064
Canadian Journal of Plant Science, 66, 3, pp. 465-472, 1986-07-01

RELATIONSHIPS BETWEEN CARBON DIOXIDE EXCHANGE RATE, PHOTOSYNTHETIC AREA AND BIOMASS IN PEA

S. L. A. HOBBS

Plant Biotechnology Institute, National Research Council, Saskatoon, Saskatchewan S7N 0W9. *Contribution no. 25108, received 28 Nov. 1985, accepted 17 Jan. 1986.*

HOBBS, S. L. A. 1986. Relationships between carbon dioxide exchange rate, photosynthetic area and biomass in pea. Can. J. Plant Sci. **66**: 465–472.

Compensation between carbon dioxide exchange rate per unit photosynthetic area (CER) and total photosynthetic area (TPA) of a plant was examined in field-grown pea (*Pisum sativum* L.). Eight near-isogenic lines of cv. Alaska, representing all possible phenotypes of the genes *af* (leaflets transformed to tendrils), *st* (reduced stipule area) and *tl* (tendrils transformed to leaflets), were examined. The CER was measured on the leaflets (*AfAf*), tendrils (*afafTlTl*) or minute leaflets (*afaftltl*). The TPA was significantly reduced by the *st* gene in *AfAf* types (normal leaflets) with an apparently associated increase in CER. The *st* gene also significantly reduced the TPA in *afaf* types but there was no associated increase in CER. Tendrils had a lower CER than normal leaflets and comprised 22% of the TPA of the semi-leafless (*afafStStTlTl*) type. Crosses were made between a semi-leafless pea and four normal-leafed types previously selected for high or low CER. The CER means (normal leaflets) of the F_1 progeny showed variability which was related to parental values. This was also true for the CER means (tendrils) of the populations of semi-leafless F_2 segregants showing that genetic variability for CER can exist in tendrils. In the F_2, tendril CER was correlated negatively to stomatal resistance and positively to chlorophyll content and final shoot dry weight (biomass). Genetic improvement in CER may be important when a plant ideotype requires substantial reduction in TPA.

Key words: Photosynthesis, pea, chlorophyll content, stomatal resistance, *Pisum sativum*

[Rapports entre le rythme d'échange du CO_2, la surface photosynthétisante et la biomass du pois.]

Titre abrégé: Rythme d'échange du CO_2, surface photosynthétisante et biomasse du pois.

L'effet compensatoire entre le rythme d'échanges du CO_2 (CER) par unité de surface photosynthétisante et la surface photosynthétisante totale (TPA) d'une plante a été examiné dans une culture de pois (*Pisum sativum* L.). Huit lignées quasi-isogéniques du cv. Alaska, recouvrant tous les phénotypes possibles des gènes *af* (folioles transformées en vrilles), *st* (surface stipulaire réduite) et *tl* (vrilles transformées en folioles) ont été étudiées. La CER était mesurée sur les folioles (*AfAf*), *les vrilles* (*afafTlTl*) ou les mini-folioles (*afaftltl*). La TPA a été significativement réduite par la présence du gène *st* chez les types *AfAf* (folioles normales), avec un accroissement apparemment relié de la CER. Le gène *st* a également réduit de façon significative la TPA chez les types *afaf*, mais là sans accroissement de la CER. Les vrilles démontraient une CER moindre que les folioles normales et formaient 22% de la TPA chez les plantes de type semi-aphylle (*afafStStTlTl*). Des croissements on t été obtenus entre un pois semi-aphylle et quatre types à folioles normales auparavent sélectionnés sur la CER (basse ou élevée). Les CER moyennes (folioles normales) de la descendance F_1 ont révélé une variabilité qu'on a pu rattacher aux valeurs

parentales. Il en était de même pour les CER moyennes (vrilles) des populations de ségrégants F_2 semi-aphylles, ce qui montre que la variabilité génétique pour la CER peut exister dans les vrilles. Dans la F_2, la CER des vrilles fournissait une corrélation négative à la résistance stomatique et une corrélation positive à la teneur en chlorophylle et au poids sec final des pousses (biomasse). Un gain génétique en CER peut avoir son importance lorsqu'une plante requiert une baisse substantielle de la TPA.

Mots clés: Photosynthèse, pois, teneur en chlorophylle, résistance stomatique, *Pisum sativum*

Carbon dioxide exchange rate per unit leaf area (CER) is genetically variable in many crop plants, for example: pea, *Pisum sativum* L. (Mahon and Hobbs 1981; Hobbs and Mahon 1982); soybean, *Glycine max* L. (Merr.) (Buttery et al. 1981; Curtis et al. 1969); wheat, *Triticum aestivum* L. and related species (Evans and Dunstone 1970; Austin et al. 1982); alfalfa, *Medicago sativa* L. (Pearce et al. 1969); and corn, *Zea mays* L. (Crosbie et al. 1978). However, although photosynthesis is one of the primary processes of plant growth, Evans (1983) points out that there is little evidence of a correlation between genetic improvement of CER and increased yield. This may be caused in part by a negative relationship between total leaf area and CER in some crop plants, due to compensation between them.

In crop ideotypes, reduced leaf area is often considered desirable, for example, to allow for increased planting densities or a higher harvest index. This would lead to a compensatory increase in CER. However, if the genetic maximum for CER is not sufficient to compensate for the leaf area loss, total photosynthate production would be reduced. Under such circumstances it may be expected that genetic improvement in CER could be of great importance (Evans 1983).

In pea, attempts have been made to redesign the crop, to reduce lodging and vegetative structures, using genes that affect the total leaf area of a plant (Harvey 1972; Hedley et al. 1983). The *af* gene converts leaflets into tendrils, the *st* gene reduces the size of the stipules and the *tl* gene produces no tendrils but five or six pairs of leaflets per leaf. However, crop growth rates and early relative growth rates appear lower in leafless (*afafstst*) than in normal peas which may result in low plant biomass and economic yield (Hedley et al. 1983). This reduced dry matter accumulation may be due to decreased total photosynthate per plant (Harvey and Goodwin 1978; Hedley et al. 1983; Pyke and Hedley 1983) and it has been shown that photosynthesis at each node (stipule + leaflets or stipule + tendrils) is lower in *afaf* than in *AfAf* types (Guillon et al. 1982). Increasing the area of photosynthetic tissue in *afaf* types by increasing stipule size might lead to an increase in the amount of total photosynthate but may not produce a crop with the required improvement in standing ability (Hedley et al. 1983). Increasing CER might be an alternative method of increasing total available photosynthate (Hobbs and Mahon 1985b). CER is genetically variable in normal leaf tissue in peas and is simply inherited (Mahon and Hobbs 1981; Hobbs and Mahon 1982). However, such genetic variability has not been demonstrated in tendrils nor has it been shown that variable CER under circumstances of reduced photosynthetic area is associated with differences in plant yield.

Using genetic variability for leaf area and CER in peas, the purpose of this research was: (1) to investigate CER in the field in near-isogenic lines that differ in leaf area; (2) to determine whether tendrils could show genetic variability in CER, as do normal leaflets; and (3) to determine whether

genetic variability in CER was related to biomass in plants with reduced photosynthetic area.

MATERIALS AND METHODS

Plant Material

EXPERIMENT 1. Eight genotypes were used, displaying all possible phenotypes of the *af, st* and *tl* genes backcrossed into cv. Alaska. These were obtained from Dr. F. Muehlbauer, USDA-ARS, Washington State University, Pullman, Wash. 99164. The eight leaflet and stipule phenotypes are shown in Fig. 1.

EXPERIMENT 2. Single seed descent material from genotypes selected for high (PI 269810 and Trapper) and low (PI 244253 and PI 269770) CER (Hobbs and Mahon 1985b) were crossed with a semileafless genotype, *afafStStTlTl*, obtained from Dr. S. Ali-Khan, Agriculture Canada, Morden, Man. R0G 1J0. The F_1 was grown in the field in 1984 to produce F_2 seed.

Experimental Design

EXPERIMENT 1. Material was grown in the field at Saskatoon during the summer of 1984. For each genotype, eight seeds were planted in a row with 15 cm between seeds. There was 1 m between genotypes in a row and 1 m between rows. Each genotype was represented in a complete row and the experimental design was a randomized complete block with four replications.

When the plants were well established, one plant in each genotype in each block was marked and all CER measurements were made on these plants. The CER was measured once a week for 3 wk starting 40 d after planting.

EXPERIMENT 2. F_1 hybrids and the nonrecurrent parents were grown in the field at Saskatoon in 1984 and F_2 material in 1985. Three seeds of each F_1 and parent were sown in a row 60 cm apart with 1 m between different F_1 or parents. The experimental design was a randomized complete block with four replications. After establishment, one plant of each F_1 or parent in each block was marked. The CER was measured on these marked plants once a week for 6 wk beginning 29 d after planting.

In 1985, each F_2 population was planted as a 10-m row with 0.25 m between seeds in the row and 1 m between rows. After establishment, four plants within a row that were *afaf* were marked for measurement of CER. The experimental design was a complete randomized block with four replications. The CER was measured once a week for 6 wk beginning 33 d from planting.

Measurements

Leaf, stipule and tendril area measurements were made using a Li-Cor model LI-3000 portable area meter. The CER measurements for exp. 1 and for the F_1 and parental types in exp. 2 were made using a portable infra-red gas analyzer (Mahon and Hobbs 1981). The CER and stomatal resistance were measured simultaneously on the F_2 of exp. 2 using a Li-Cor model 6000 Portable Photosynthesis System. The tissue measured depended on the phenotype of the plant. In exp. 1, individual leaflets were measured in *AfAf* types, tendrils in *afafTlTl* types and multiple minute leaflets in *afaftltl* types. In exp. 2, individual leaflets were measured in the parents and F_1 and tendrils in the F_2. Photosynthetic areas of tendrils were corrected by $\pi/2$ (Harvey and Goodwin 1978). The material measured for CER was also dried and weighed so that data could be expressed on a dry weight basis. In 1984, however, dry weights from 1 wk were lost due to a fire and so only two sets of data were available for analysis on this basis. Chlorophyll measurements were made on the stipules of the F_2 plants and assayed by the method outlined in Hobbs and Mahon (1985a).

In exp. 1, one plant from each genotype in each block was harvested each week and total photosynthetic area was measured. In exp. 2, the marked F_2 plants were collected at the end of the season and air dried for total shoot weight (biomass) determination.

Statistical Analysis

Nested analyses of variance were used with time as subtreatments. Homogeneity of variances was tested using Bartlett's test and data were transformed to natural log before analysis when necessary. Duncan's multiple range test was used to compare means. For the F_2 material in exp. 2, correlations between characters were calculated on population means (r; $df = 2$) and means for individual plants (r_i; $df = 62$). Combined correlations (r_c; $df = 52$) were calculated by testing the correlation coefficients from within F_2 populations for homogeneity, and then pooling them (Steel and Torrie 1960).

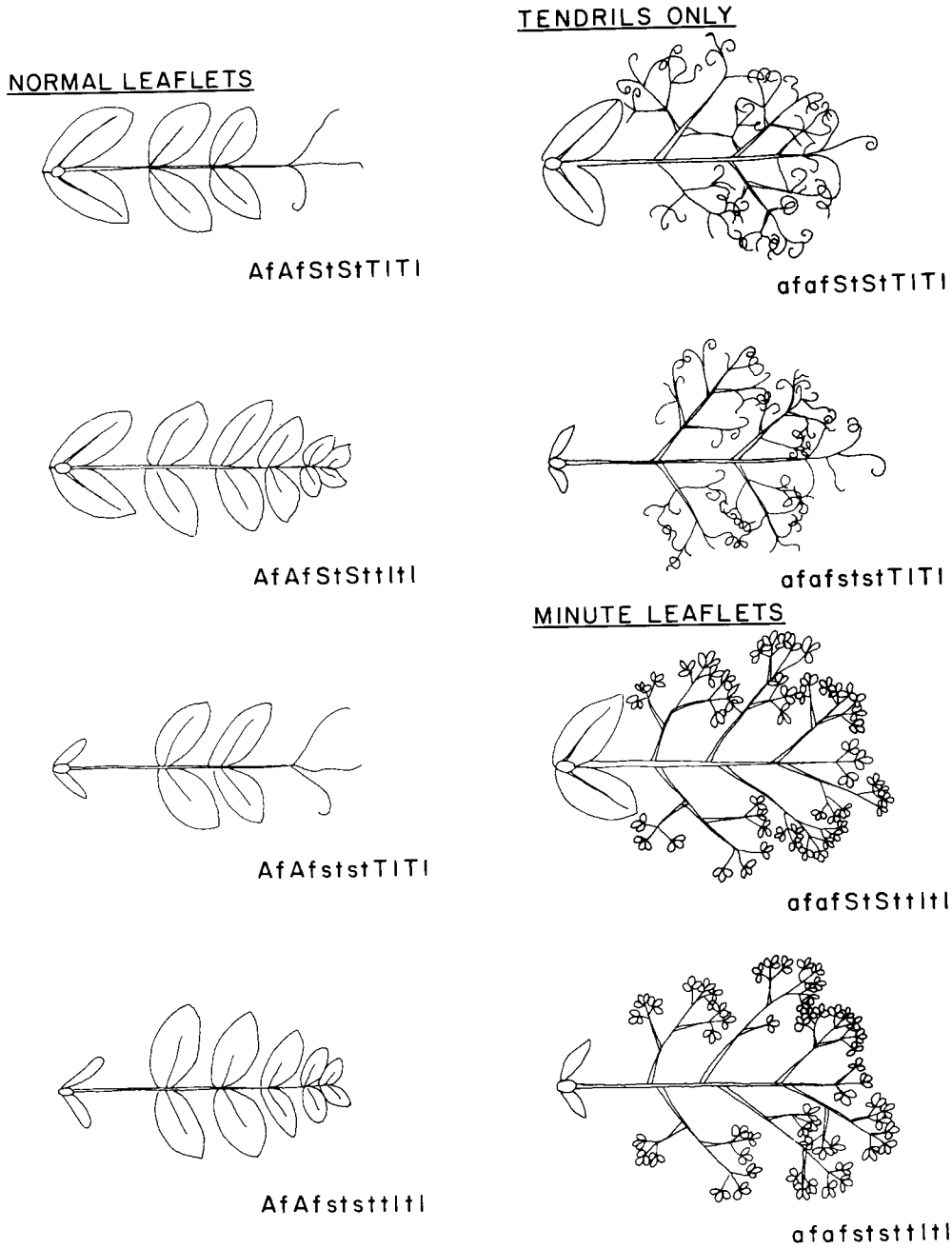

Fig. 1. Leaf and stipule phenotypes of *af*, *st* and *tl* combinations.

RESULTS AND DISCUSSION

Results from exp. 1 are given in Table 1. In plant types with normal leaflets, the homozygous *st* gene reduced total photosynthetic area and there was an apparently associated increase in CER cm^{-2} in the leaflets. In those plant types with either tendrils only or with minute leaflets, the homozygous *st* gene also reduced total photosynthetic area but there was no associated increase in CER cm^{-2} in either type of tissue. The homozygous *af* gene significantly reduced total photosynthetic area in all types (except *afafStStTlTl*); however, this reduction was not accompanied by tendril CER being higher than corresponding leaflet CER. The homozygous *tl* gene did not lead to a significant increase in total photosynthetic area.

The increase in CER of normal leaflets which accompanies the reduction in total photosynthetic area by the *st* gene (Table 1) supports the theory that there is some compensation between CER and leaf area (Evans 1983). This is also supported by the CER of stipules being higher in *afaf* plants than in *AfAf* ones (Guillon et al. 1982). That tendril CER was not similarly affected by total photosynthetic area variation in *afafTlTl* plants (Table 1) indicates either that the tendrils cannot vary in CER or that the tendril CER was already at its maximum rate in the *StSt* type. Such would also be the case in plants with minute leaflets (Table 1).

There was no increase in CER cm^{-2} when total photosynthetic area was reduced by the homozygous *af* gene (Table 1) which is in contrast to the results of Harvey and Goodwin (1978) who found that tendrils in *afafstst* types had a higher CER cm^{-2} than corresponding normal leaflets. However, in that report a different genotype was used which was grown under controlled, well-watered conditions. CER comparisons between the different types of photosynthetic tissue measured are difficult to make due to the very different architecture of the tissue (Lafond and Evans 1981) and the difficulties inherent in calculating photosynthetic area in tendrils (Harvey and Goodwin 1978). However, in this experiment, the differences between types were similar when expressed on a per gram weight basis (Table 1), despite the reduced precision of these calculations due to accidental loss of material. Similar difficulties arise when comparing CER in minute leaflets and so this material cannot be directly compared to either normal leaflet or tendril measurements.

In the field-grown Alaska genotype,

Table 1. CER and photosynthetic area means for Alaska peas with *af*, *st* and *tl* genes in different combinations

	CER nmoles sec^{-1}		Total photosynthetic area	Total photosynthetic area as tendrils
	(cm^{-2})	(g^{-1})	(cm^2)	(%)
Normal leaflets				
AfAfStStTlTl	1.32*b*	302*ab*	895*a*	3
AfAfStSttltl	1.32*b*	265*ab*	975*a*	0
AfAfststTlTl	1.78*a*	352*a*	575*bc*	6
AfAfststtltl	1.50*ab*	355*a*	606*bc*	0
Tendrils only				
afafStStTlTl	0.87*c*	88*d*	753*ab*	22
afafststTlTl	0.80*c*	102*d*	138*d*	74
Minute leaflets				
afafStSttltl	1.29*b*	156*cd*	438*c*	0
afafststtltl	1.35*b*	178*bc*	190*d*	0

a–d Means within a column followed by the same letter are not different at the 5% level according to Duncan's multiple range test.

however, tendrils have a lower CER than normal leaflets, either per unit photosynthetic area or per gram (Table 1). As the tendrils account for 22% of the total photosynthetic area in *afafStStTlTl* but only 3% in *AfAfStStTlTl* (Table 1) it is possible that the former type has a reduced total photosynthate availability despite its total photosynthetic area not being significantly lower (Table 1). Experiment 2 was therefore concerned with the genetic manipulation of CER in this type and any resulting effect on biomass.

The CER means of genotypes selected for high or low expression of this character and of the F_1 progeny from crosses between these genotypes and semileafless material are given in Table 2. These show that the parental genotypes differed as expected and also that the F_1 from crosses involving the high parents had higher CER means than those from crosses involving the low ones. As this parental and F_1 material had normal leaflets, it was not possible to compare CER values with the semileafless parent where only tendrils could be measured. However, assuming a simple additive model for the inheritance of CER (Mahon and Hobbs 1981; Hobbs and Mahon 1982) the semileafless parent should have a CER equal to $(F_1 - P)2 + P$ (where P is the mean parental CER and F_1 the mean F_1 CER). These calculations for each cross are given in Table 2 and have a mean of 1.55 nmol cm^{-2} s^{-1}, which is not high when compared with the highest parents.

Measurements on F_2 plants were made only on the tendrils of semileafless (*afaf*) types. The F_2 populations differed in tendril CER whether expressed per square centimetre or per gram (Table 3). The F_2 progeny from high CER parents (PI 269810 and Trapper) exhibited a higher mean CER than those from low parents (PI 244253 and PI 269770). Stomatal resistance and chlorophyll content also differed among populations (Table 3).

Correlations between characters were fairly constant no matter how calculated (Table 4). Shoot dry weight at the end of the season was positively correlated with CER whether calculated on a per unit photosynthetic area or on a per gram basis. CER was also positively correlated with chlorophyll content and negatively with stomatal resistance.

There are two indications that the semileafless types may be restricted by total photosynthate availability. First, tendrils can vary in CER (Table 3). Therefore, the absence of an increase in CER between *afafStStTlTl* and *afafststTlTl* (Table 1), even though the latter had only about 20% of the total photosynthetic area, indicates that the tendrils must have been at their maximum genetic potential for CER in the *StSt* type. There was no compensation between photosynthetic area and CER in this case. Second, although the semileafless type (*afafStStTlTl*) had a total photosynthetic area that was not significantly less than the

Table 2. CER means for nonrecurrent parents and F_1 populations from crosses with a semileafless type and the calculated mean CER of recurrent parent

Nonrecurrent parent	Parent (P)	F_1	$(F_1\text{-}P)2 + P$
	(nmoles cm^{-2} sec^{-1})		
PI 244253	1.43*bc*	1.45*b*	1.48
PI 269770	0.94*d*	1.19*c*	1.44
PI 269810	1.87*a*	1.83*a*	1.77
Trapper	1.92*a*	1.71*a*	1.50

a–d Means followed by the same letter are not different at the 5% level according to Duncan's multiple range test.

Table 3. Means of CER, stomatal resistance and chlorophyll content for semileafless F_2 populations

Parent	CER nmoles sec^{-1} (cm^{-2})	CER nmoles sec^{-1} (g^{-1})	Stomatal resistance (s cm^{-1})	Chlorophyll content (μg cm^{-2})
PI 244253	0.63*b*	79*b*	3.01*a*	36.8*a*
PI 269770	0.59*b*	72*b*	3.36*a*	33.6*b*
PI 269810	0.72*a*	99*a*	2.57*b*	32.7*b*
Trapper	0.78*a*	104*a*	2.22*b*	37.4*a*

a,b Means within a column followed by the same letter are not different at the 5% level according to Duncan's multiple range test.

Table 4. Correlations between CER and other characters in semileafless F_2 populations

	r†	r_i‡	r_c§
CER cm^{-2}: shoot dry weight	0.525	0.415**	0.424**
CER g^{-1}: shoot dry weight	0.473	0.350**	0.388**
CER cm^{-2}: chlorophyll content	0.338	0.294*	0.268*
CER cm^{-2}: stomatal resistance	−0.998**	−0.756**	−0.712**

†Calculated on population means; df = 2.
‡Calculated on individual plants; df = 62.
§Calculated by pooling correlation coefficients from individual plants within populations (df = 52).
*,** Significant at the 0.05 and 0.01 levels of probability, respectively.

normal-leafed type (*AfAfStStTlTl*), 22% of this area was made up of tendrils, which were apparently photosynthetically inferior to leaflets (Table 1). It may be expected, therefore, that variability in CER would be associated with variability in total plant biomass and this was found in exp. 2 (Table 4).

The frequency of stomates on tendrils is only 50% of the mean values of stomates on the upper and lower surface of leaflets (Harvey 1972) and the chlorophyll content of tendrils is often lower than that found in leaflets (Lafond and Evans 1981). Therefore, increasing photosynthesis per unit area of photosynthetic tissue, through increasing chlorophyll content or reducing resistance to gas exchange, might lead to an increase in CER in the leafless or semileafless pea. Previous work showed that the genetic variability in CER of normal leaflets was related to variability in chlorophyll content, and stomatal resistance (Mahon et al. 1983; Hobbs and Mahon 1985b). In this work, the chlorophyll was measured on the stipules and the stomatal resistance was measured with a different instrument from that used in previous reports. Nevertheless, CER variability in the tendrils was correlated with these characters (Table 4).

The data presented here show that genetic variability in CER of normal leaflets can be introduced into tendrils in semileafless peas and that such CER variability is related to total shoot dry weight at the end of the season. This indicates that there may be potential for the improvement of CER in ideotypes that involve a drastic reduction in photosynthetic area. The use of leafless, semileafless or rogue (Pyke and Hedley 1984) phenotypes may be examples of such ideotypes in peas.

ACKNOWLEDGMENTS

I thank Ms. Catherine DeLong, and several summer students employed through the NRCC Summer Student Program for their technical assistance. The help of the Crop Development Centre of the University of Saskatchewan in tending field crops is also gratefully acknowledged.

Austin, R. B., Morgan, C. L., Ford, M. A. and Bhagwat, S. G. 1982. Flag leaf photosynthesis of *Triticum aestivum* and related diploid and tetraploid species. Ann. Bot. **49**: 177–189.

Buttery, B. R., Buzzell, R. I. and Findlay, W. I. 1981. Relationships among photosynthetic rate, bean yield and other characters in field-grown cultivars of soybean. Can. J. Plant Sci. **61**: 191–198.

Crosbie, T. M., Mock, J. J. and Pearce, R. B. 1978. Inheritance of photosynthesis in a diallel among eight maize inbred lines from Iowa stiff stalk synthetic. Euphytica **27**: 657–664.

Curtis, P. E., Ogren, W. L. and Hageman, R. H. 1969. Varietal effects in soybean photosynthesis and photorespiration. Crop Sci. **9**: 323–327.

Evans, L. T. 1983. Raising the yield potential: by selection or design. Pages 371–389 *in* T. Kosuge, C. P. Meredith, and A. Hollaender, eds. Genetic engineering of plants: an agricultural perspective. Plenum Press, New York and London.

Evans, L. T. and Dunstone, R. L. 1970. Some physiological aspects of evolution in wheat. Aust. J. Biol. Sci. **23**: 725–741.

Guillon, P., Cherbuin, A., Moutot, F., Cousin, R. and Jolivet, E. 1982. Effet de la mutation afila sur les caracteristiques photosynthetiques du Pois (*Pisum sativum* L.). C. R. Acad. Sci., ser. III. **t294**: 231–234.

Harvey, D. M. 1972. Carbon dioxide photoassimilation in normal-leaved and mutant forms of *Pisum sativum* L. Ann. Bot. **36**: 981–991.

Harvey, D. M. and Goodwin, J. 1978. The photosynthetic net carbon dioxide exchange potential in convential and 'leafless' phenotypes of *Pisum sativum* L. in relation to foliage area, dry matter production and seed yield. Ann. Bot. **42**: 1091–1098.

Hedley, C. L., Ambrose, M. J. and Pyke, K. A. 1983. Designing an improved pea crop plant. Pages 52–57 *in* R. Thompson and R. Casey, eds. World crops. Production, utilization, description, Volume 8. Perspectives for peas and lupins as protein crops. Martinus Nijhoff, The Hague, The Netherlands.

Hobbs, S. L. A. and Mahon, J. D. 1982. Variation, heritability, and relationship to yield of physiological characters in peas. Crop Sci. **22**: 773–779.

Hobbs, S. L. A. and Mahon, J. D. 1985a. Inheritance of chlorophyll content, ribulose-1,5-biophosphate carboxylase activity, and stomatal resistance in peas. Crop Sci. **25**: 1031–1034.

Hobbs, S. L. A. and Mahon, J. D. 1985b. Genetic, environmental and interactive components of photosynthesis in peas. Pages 307–315 *in* P. Hebblethwaite, M. Heath, and T. Dawkins, eds. The pea crop — a basis for improvement. Butterworth and Co. Ltd., London.

Lafond, G. and Evans, L. E. 1981. A comparative study of conventional, leafless and semileafless phenotypes of peas: photosynthetic CO_2 fixation in vitro. Can. J. Plant Sci. **61**: 665–671.

Mahon, J. D. and Hobbs, S. L. A. 1981. Selection of peas for photosynthetic CO_2 exchange rate under field conditions. Crop Sci. **21**: 616–621.

Mahon, J. D. and Hobbs, S. L. A. and Salminen, S. O. 1983. Characteristics of pea leaves and their relationships to photosynthetic CO_2 exchange in the field. Can. J. Bot. **61**: 3283–3292.

Pearce, R. B., Carlson, G. E., Barnes, D. K., Hart, R. H. and Hanson, C. H. 1969. Specific leaf weight and photosynthesis in alfalfa. Crop Sci. **9**: 423–426.

Pyke, K. A. and Hedley, C. L. 1983. Growth and photosynthesis of mutant pea seedlings. Ann. Bot. **52**: 719–724.

Pyke, K. A. and Hedley, C. L. 1984. Aspects of the rogue phenotype of peas. Plant Sci. Lett. **35**: 87–90.

Steele, R. G. D. and Torrie, J. H. 1960. Principles and procedures of statistics. McGraw-Hill, New York.