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Nuclear DNA regulates the level of ribulose 1,5-bisphosphate carboxylase oxygenase in *Medicago sativa* L.

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Summary. The response to selection for leaf proteins was studied during three selection cycles. Selection for high total nitrogen content showed 75% heritability, and the levels of both ribulose 1,5-bisphosphate carboxylase oxygenase (Rubisco) and cytoplasmic protein were strongly under nuclear DNA control. High and low protein content were correlated with chloroplast area. Although the amounts of nuclear DNA were similar, the ratio of Rubisco/DNA and chlorophyll/DNA changed during the selection process. It can be concluded that the levels of Rubisco achieved in mature plants of *M. sativa* are under nuclear DNA control. The possible involvement of small subunit (SSU) genes in controlling these levels is discussed.

Key words: Ribulose 1,5 – Bisphosphate carboxylase oxygenase – Small subunit – Nuclear control of Rubisco – *Medicago*

Introduction

Ribulose 1,5 bisphosphate carboxylase oxygenase (Rubisco) is responsible for catalysing the initial step of carbon fixation in photosynthesis. Rubisco consists of eight large subunits (LSU) and eight small subunits (SSU) as found by Kawashima and Wildman (1970). Genetic studies showed that LSU polypeptides are inherited maternally (Chan and Wildman 1972) while SSU polypeptides are inherited in a Mendelian manner through nuclear DNA (Kawashima and Wildman 1972).

Cobb and Wellburn (1976) suggested that the chloroplast is the site of assembly of both LSU and SSU polypeptides. Ellis et al. (1978) postulated that the level of Rubisco is controlled by the levels of nuclear-coded polypeptides. In contrast, Hallier et al. (1978) have suggested that the SSU is being controlled by synthesis of LSU. Li et al. (1983) considered that the SSU is a signal from nucleus to chloroplast for controlling biosynthesis of LSU. Light-induced increase of Rubisco is controlled by mRNA level for the SSU in seedlings of *Pisum sativum* L. (Sasaki et al. 1981).

In *Medicago sativa* L. the leaf proteins consist largely of Rubisco and cytoplasmic proteins (Daday and Whitecross 1985); 56% of this leaf protein is Rubisco and heterogeneity in SSU has been demonstrated (Daday et al. 1986). The predicted response to selection of leaf proteins has been measured by heritability studies in *M. sativa.* Reported heritability values for Rubisco are 33% (Heinrichs 1972), 28% (Miltimore et al. 1974) and 20% (Gutek et al. 1976). The cytoplasmic fraction had heritabilities of 19 and 27%, while total soluble protein varied between 23 and 31% during two consecutive years (Gutek et al. 1976). The estimated predicted heritability for the nitrogen content of herbage was 56% (Lamprecht 1965).

The amount of nuclear DNA in a cell is known to affect the amount of the cell components. The influence of polyploidization on buffer-soluble protein, chlorophyll, and DNA was examined in isogenic diploid-tetraploid and tetraploid-octoploid sets of *M. sativa* plants (Meyers et al. 1982). Tetraploids had an 80% higher level of buffer-soluble protein than comparable diploids and had essentially twice the fresh weight of Rubisco, chlorophyll, and DNA when compared on a per leaf basis. Ratios of Rubisco to DNA and of chlorophyll to DNA were similar across ploidy levels of both isogenic sets, suggesting that the cellular content of chlorophyll and Rubisco increased proportionately with the amount of DNA per cell.

It is known from observations on spinach that during leaf development chloroplast number per cell, chloroplast size and the chlorophyll content of chloroplasts increase, while the DNA content of chloroplasts decreases (Possingham and Saurer 1969; Scott and Possingham 1980). Although some plastid division occurs in darkness, chloroplast division is, in general, light stimulated (Possingham and Smith 1972). However, low light intensities suppress the division of spinach chloroplasts while stimulating chloroplast growth (Possingham 1973). Temperature also affects the growth and replication of spinach chloroplasts. At low temperature (12 °C) large chloroplasts are formed and 25 °C appears to be optimal for division (Possingham and Smith 1972). Mineral deficiencies markedly affect chloroplast division as the numbers of chloroplasts per cell are reduced in leaves deficient in iron, manganese or nitrogen (Possingham 1970).

This investigation was aimed at determining whether the level of Rubisco is controlled principally by nuclear or chloroplast DNA. The correlation between the level of Rubisco synthesis and chloroplast size was examined. The ratios of the amount of Rubisco and chlorophyll relative to the amount of nuclear DNA were also estimated. The possible role of control of SSU gene expression in relation to the level of Rubisco is discussed.

Materials and methods

Plant material and culture

M. sativa cv. 'Hunter River' is a commercial cultivar from South Australia. Plants of this cultivar were grown in 30 cm pots containing standard greenhouse soil under natural light at 1,200–1,500 uE, m^{-2} s^{-1}, in an airconditioned greenhouse with the temperature maintained between 20° and 25 °C. Samples of plants were collected for analysis when plants reached the green bud stage.

Nitrogen assays

Nitrogen determinations were carried out with a Kjeldahl automatic analyser (Williams and Twine 1967).

Plant selection for nitrogen content

The basic population consisted of randomly selected plants of the HR ('Hunter River') genotype. The mean nitrogen yield was based on the analysis of 100 plants. Selected high-nitrogen- yielding individual plants were then intercrossed and the progenies of 10 plants formed the population for the first generation of "High" selection. In the same way the ten low-nitrogen-yielding plants of the basic population were intercrossed and their progenies formed the first generation of the "Low" selection. Both total and non-protein nitrogen were determined.

Isolation of Rubisco and cytoplasmic proteins by Sepharose 6B column chromatography

Leaf samples (10 g) were frozen in liquid nitrogen and finely chopped in an Omnimix homogeniser. The chopped material was then shaken for 20 min in 20 ml of 25 mM Tris-HCl (pH 6.8) containing 50 mM NaCl, 1 mM $MgCl_2$, 0.5 mM EDTA and 40 mM 2-mercaptoethanol in an ice bath. The resulting homogenate was then squeezed through two layers of Miracloth (Chicopee Mills Inc.). The filtrate was centrifuged at 20,000 g for 30 min and the supernatant was recentrifuged at 108,000 g for 75 min. Five ml of the resulting supernatant was applied to a Sepharose 6B column (1.5 × 86 cm). Fractionation was carried out at 4 °C using 20 mM Tris-HCl, pH 6.8, containing 50 mM NaCl and 0.02% sodium azide. The flow rate was 0.1 ml min^{-1} and each eluted fraction had a volume of 2.5 ml. The fractions rich in Rubisco were further purified by DE-52 column chromatography with an NaCl gradient (Kawashima and Wildman 1970). The sample was dialysed against one litre of 0.05 M Tris-HCl, pH 7.6, containing 0.01 M NaCl and 0.5 mM EDTA. The dry matter of Rubisco and cytoplasmic proteins were determined.

Chloroplast number per cell, plastid area and cell number

The measurements were carried out according to Possingham and Smith (1972).

Determination of cell area

The cell preparations were photographed, the film printed, and the circumference of each cell was measured on prints by means of a planimeter wheel attached to a programmed computer (Hewlett Packard 9845) which then estimated the area of each cell. The cell area of a genotype was taken as the mean of 24 measurements.

Estimation of nuclear DNA amounts

DNA amounts of individual nuclei from mature leaves of 3 high and 3 low protein lines of *M. sativa* were measured relative to prophase nuclei from root tips of *Pisum sativum* cv. 'Massey Gem'. Relative values were determined by microspectrofluorimetry of separated cells stained with DAPI (4,6-diamidino-2-phenyl indole) using the methods of Lawrence and Possingham (1986). Slides were prepared with *M. sativa* leaf cells on one end and *P. sativum* root cells on the other, and for each comparison, a total of 30 nuclei of *M. sativa* and 30 of *P. sativum* were measured. The majority of nuclei in the mature *M. sativa* leaves were of a uniform size, and it was these nuclei that were measured; elongate nuclei from vascular tissue and occasional larger endopolyploid nuclei were excluded from the measurements. Background readings were found to be less than 1% of nuclear values, and were therefore not included. Relative values were converted to absolute amounts using the value of 19.46 pg for 4C prophase nuclei (Bennett and Smith 1976).

Statistics

The standard analysis of variance was used in most calculations. The realized heritability was determined as follows

$h^2 = R/S$

where h^2 = realised heritability, R = response to selection and S = selection differential.

The selection response was calculated from the means of the nitrogen content of two consecutive generations, and the selection differential was obtained from the analysis of variance (Falconer 1964).

Radioimmune assay

The Rubisco determination of the "high" and "low" genotypes were carried out by radioimmune assay, according to Collatz et al. (1979).

Results

Genetic control of leaf protein content

The mean total nitrogen yields from three selection cycles and the hybrid generation are presented in Fig. 1. The mean nitrogen content of the base population was 2.55 mg/100 mg dry matter and increased to 4.08 mg in the High selection and to 3.20 mg in the Low selection of the third generation. The increase in the mean High selection of third generation over the base population was 60%. In general, the High selections increased significantly, and while the Low selection also increased,

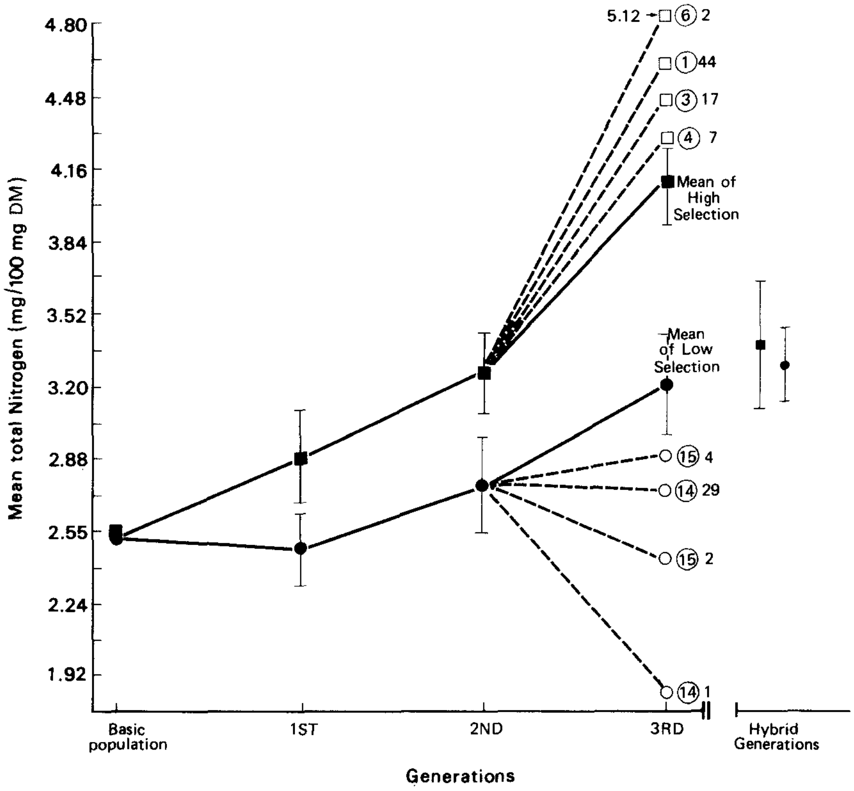

Fig. 1. Response to selection for high and low nitrogen content in *M. sativa*. Closed symbols, mean nitrogen content for each generation of the High (■) and Low (●) selections. Open symbols, nitrogen content of individual plants of the High (□) and Low (○) selections at the third generation. (6) 2, 144 etc., reference numbers of individual plants

after an initial decrease, it did so at a significantly lower rate. Among individual plants of the third generation which were assayed (the extremes of the range) were plant (6)2 with 5.12 mg nitrogen and plant (14)1 with 1.84 mg nitrogen. Progenies of the reciprocal crosses derived from extreme High × Low and Low × High selection showed very similar mean nitrogen contents: 3.41 and 3.28 mg, respectively. The mean nitrogen contents of H × L and L × H hybrid generations showed no significant difference ($t = 12$) and their values were similar to the means of the parental nitrogen contents. These results indicate that the increased synthesis of the two major protein fractions Rubisco and cytoplasmic proteins are under nuclear DNA control.

Estimates of realized heritability were obtained from the results of all three generations. These estimates are the regression coefficients obtained from the regression of response (mean nitrogen content) on the cumulative selection differential (Falconer 1964) for both High and Low nitrogen lines. The estimates for heritability obtained by this procedure were:

High selection 0.747 ± 0.132 or 75%, and Low selection -0.256 ± 0.158 or -26%.

There was a good response to high-nitrogen selection. In contrast, selection for low nitrogen showed only a slight gain on the basis of the mean figures. However, extreme 'low' nitrogen genotypes like plant (14)1 still indicate a possibility of further decrease in nitrogen content (Fig. 1).

Differences in the total nitrogen content of individual plants of *M. sativa* could be due either to protein nitrogen and/or non-protein nitrogen. The proportion of non-protein nitrogen was therefore determined in 'high' and 'low' nitrogen plants. In a separate experiment a considerable difference was again found between the means of the total nitrogen of 'high' and 'low' nitrogen plants; (3.44 and 2.39 mg/100 mg DM) and the difference was statistically significant ($t = 4.33^*$). The means of non-protein nitrogen in both 'high' (21.6%) and 'low' (22.7%) genotypes were not statistically different ($t = 0.57$). The total nitrogen increase of 'high' compared to 'low' genotypes was largely due to increased protein nitrogen content. Therefore, the 'high' and 'low' nitrogen genotypes will be described as 'high' and 'low' protein genotypes during the remainder of this article.

Changes in protein fractions during the selection experiments were investigated. When isolated leaf proteins were fractionated and the ratio of isolated Rubisco relative to cytoplasmic protein of the 'high' and 'low' protein genotypes calculated (four genotypes of the High and Low selections from the third, selection cycle were included in this comparison) there was considerable variation in the ratio of the two protein groups within the 'high' and 'low' protein genotypes. No sta-

Table 1. Relationship between 'high' and 'low' protein genotypes and chloroplast characteristics

Protein genotype	Protein (%)	Plastid no./cell	Plastid area (μm^2)	Cell no./ 3 mm disc	Cell area ($mm^2 \times 10^{-4}$)
'High'					
(1) 44	22.8	29.8	34.38	45,203	12.27
(3) 17	22.0	29.8	26.65	54,817	7.75
(4) 7	21.2	29.5	32.05	57,630	6.80
(6) 2	25.1	28.3	30.93	62,761	6.78
Mean	22.8	29.35	31.00	55,102	8.33
'Low'					
(14) 1	9.3	29.7	12.69	55,598	6.47
(14) 9	17.0	29.5	13.80	48,594	6.21
(14) 29	13.1	28.8	12.84	57,859	6.49
(15) 4	13.9	27.8	11.13	51,692	5.87
Mean	13.3	28.95	12.61	53,435	6.26

tistical difference ($t = 1.0$) was found between the mean values (5.58 and 4.74) of the two selections. High and low protein selection did not appear to change the ratio of Rubisco and cytoplasmic proteins in the cells of *M. sativa*. In the regulation of protein synthesis, this balance in the proportion of proteins may well have some biological significance.

Cell and chloroplast morphology

The relationship between protein content of cells and cellular morphology of 'high' and 'low' protein genotypes was examined.

Measurements of protein content, plastid number per cell, plastid area, number of cells per 3 mm disc and cell area of four 'high' and four 'low' protein genotypes are presented in Table 1. The mean percent protein of the 'high' genotypes was 22.8% and that of the 'low' genotypes was 13.3. No significant difference was found between the mean measurements of plastid number per cell, cell number per 3 mm disc, and cell area. In contrast, the mean plastid area of the 'high' genotypes was 31.00 μm^2 and that of the 'low' genotypes was 12.61 μm^2, with the difference being highly significant ($P < 0.001$).

Large plastids from cells of a 'high' protein genotype are shown in Fig. 2a. The plastids occupy the entire space of the cells. In contrast, the cells of 'low' protein genotypes (Fig. 2b) have smaller plastids and there are considerable gaps between the plastids and the cell walls. These results suggest a correlation between protein levels and plastid size, as plastids are the location of assembly of the large and small subunits.

Further investigations concerned the effect of developmental stage on plastid and cell morphology of 'high' and 'low' protein genotypes. It was concluded that differences in plastid area of 'high' and 'low' protein genotypes are expressed at both early and late stages of development. The correlation between plastid area and protein content applies to segregating population as well.

Relationships between Rubisco, chlorophyll and nuclear DNA in amounts

Measurements of non-protein nitrogen and the ratio between Rubisco and cytoplasmic proteins predicted that different levels of Rubisco are found in 'high' and 'low' protein genotypes. The difference was measured directly by radioimmune assay, with 5.96 mg of Rubisco extracted from 1 g of leaves of the 'high' protein genotypes, and significantly less, 3.09 mg ($t = 3.90^{**}$) from the 'low' protein genotypes (Table 2). The difference can also be expressed in the quantity of Rubisco per cm^2 in both selection lines (93.65 and 48.70 μg, $t = 4.33^{**}$). In a similar comparison, the chlorophyll in 1 g of fresh leaves was 4.71 and 1.41 mg, and chlorophyll per cm^2 was 83.32 and 23.77 μg and significantly different ($t = 5.34^{*}$ and 6.67^{**}) in both genotypes. No differences were found between the ratio of chlorophyll a and b (means 3.07 and 2.91; $t = 0.8$, N.S.) between the two types.

Nuclear DNA amounts of 7.3 pg and 6.5 pg were measured for the most frequent nuclear size in mature leaves of the high and low protein genotypes respectively (Table 3). The values are in close agreement with a previous estimate of 7.0 pg for the 4C nuclear DNA amount of *M. sativa* root tip (Bennett and Smith 1976), and suggest that the majority of nuclei in mature leaves are in the same endopolyploid 4C state. The 10% difference between mean nuclear DNA amounts of the two protein genotypes was statistically significant ($t = 2.82^{*}$), but insufficient to directly explain the large differences in Rubisco and chlorophyll synthesis in terms of altered nuclear ploidy levels. A difference of at least 100% would be expected if the high and low protein genotypes differed in ploidy level.

Table 2. Mean quantities of the extracted Rubisco and chlorophyl of 'high' and 'low' protein genotypes

Genotypes	mg Rubisco g fresh leaves	µg Rubisco cm^2	% Rubisco of DM	mg chlorophyll/g fresh leaves	mg chlorophyll/cm^2
'High' protein genotpyes	5.96±0.51	93.65±7.18	3.05±0.38	4.706±0.55	83.32±7.60
'Low' protein genotypes	3.09±0.41	48.70±5.82	1.70±0.24	1.412±0.22	23.77±3.63

Table 3. DNA, Rubisco, and chlorophyll content and ratios of 'high' and 'low' protein genotypes

	Protein genotypes	
	'high'	'low'
DNA (pg cell^{-1})	7.3	6.53
Rubisco (pg cell^{-1})	124.7	64.8
Chlorophyll (pg cell^{-1})	98.6	29.6
Rubisco/DNA	17.1	9.9
Chlorophyll/DNA	13.5	4.5

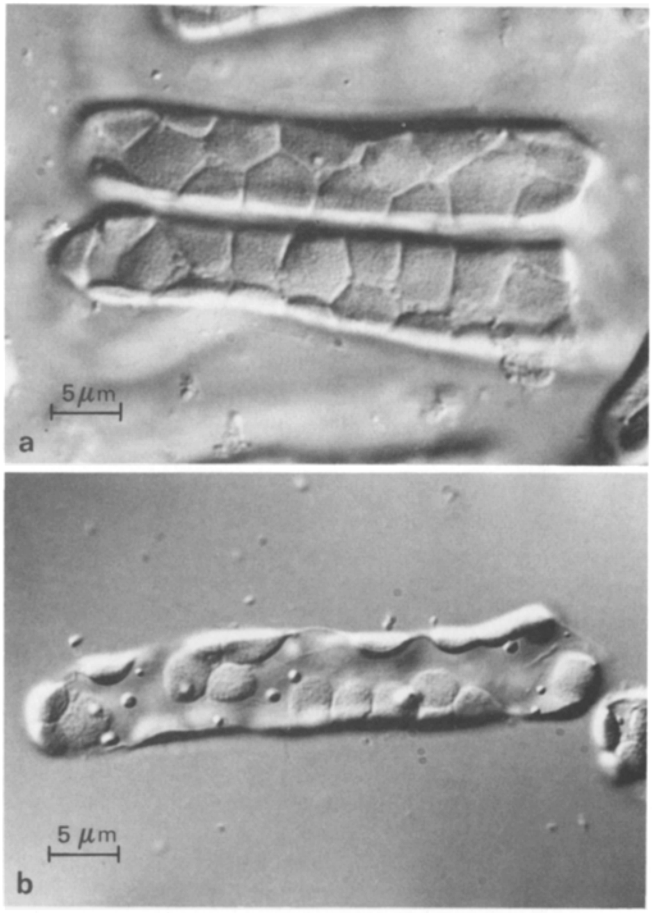

Fig. 2. **a** Chloroplasts in cell from a 'high' protein genotype; **b** chloroplasts in cell from a 'low' protein genotype

It is evident that the expressed levels of both Rubisco and chlorophyll are susceptible to selection pressure. Larger chloroplast size also appears to be correlated with higher levels of Rubisco.

Discussion

The significant finding of this investigation is that the level of Rubisco is both under nuclear DNA control and correlated with plastid size.

In the present study an impressive realised heritability of 75% was achieved which is considerably greater than values predicted from previous heritability studies on Rubisco and cytoplasmic proteins in *M. sativa* (Heinrichs 1972; Miltimore et al. 1974; Gutek et al. 1976; Lamprecht 1965). These calculations of predicted heritability were based on early generation selection which disregarded the curvilinear nature of the response to selection. This response is very apparent in the present experiments: as seen in Fig. 1 the means of the Low selection also showed a lesser but still significant increase, which is reflected in the curvilinear shape of the response. Compared to the High selection response this Low selection response was unexpected. It is tentatively suggested (FE Binet, pers. commun.) that the negative response from 2.68 mg N (2nd generation) to 3.20 mg N (3rd generation) was at least partly due to natural selection. This implies that Low selection is contrary to balanced interaction between protein level and photosynthetic ability. It is admitted that this conjecture requires further experiments for its corroboration. Of course natural selection is likely to be less effective with respect to a few not well balanced genotypes: the presence of such extremes may well acount for the few low and very low protein-content-plants which invariably appear in low selected generations (e.g. [14]1, [15]2 etc. in Fig. 1).

A difference of 100% was found in the level of Rubisco between genotypes (Table 2) in spite of similar amounts of nuclear DNA (Table 3). In contrast, other studies have found that the ratios of Rubisco to DNA and of chlorophyll to DNA were similar across ploidy levels, suggesting that cellular content of chlorophyll and Rubisco increase proportionally with the amount of DNA per cell in *M. sativa* (Meyers et al. 1982), in *Triticum* (Dean and Leech 1982) and also in *Lolium perenne* L. (Rejda et al. 1981). These results suggest that genetic manipulation of nuclear DNA is more important than the ploidy stage of the species as far as the regulation of level of Rubisco is concerned.

While the present investigation was concerned mainly with total Rubisco levels, other studies have demonstrated the importance of balanced amounts of SSU and LSU for efficient Rubisco activity. Andrews and Ballment (1983) showed that activity of SSU-depleted Rubisco from *Synechococcus* was proportional to residual SSU attached to the LSU octomer and that activity could be enhanced by the addition of further isolated SSU. They concluded that LSU could be catalytically competent only if SSU was bound to it, indicating an essential, if indirect, role of SSU in Rubisco activity. In vivo the amount of SSU may influence the total activity of Rubisco. It is not unreasonable to suggest that nuclear factors which control the amount of SSU produced would affect the level of fully functional Rubisco produced in the chloroplast.

Although the amount of nuclear DNA was similar both in the present work and that of Meyers et al. (1982), the quantity of Rubisco and chlorophyll was markedly different due to the different cultural conditions, different time of harvesting and stage of plant growth.

As the SSU genes are located in the nuclear DNA, it is reasonable to suggest that SSU gene products are regulating the level of Rubisco. The sequencing of DNA encoding the precursor of the SSU of *Pisum sativum* showed that the molecule consists of 123 amino acids and has a considerable homology with that of spinach: 89 residues were the same in both species (Bedbrook et al. 1980). The estimated number of genes for SSU in *Petunia* has been estimated to be between 4 and 12 (Dunsmuir et al. 1983). Gene expression in *Petunia* has been shown to vary considerably: one SSU gene accounted for 47% of the total, five others between 2 and 23% each, while expression of the remainder was undetectable. The same study revealed considerable variation, up to 500-fold, in the levels of expression in cells of different organs (Dean et al. 1985). Further supporting evidence of nuclear DNA control of Rubisco is provided by the identification of homologous chromosome group four as the site of major control of Rubisco levels in *Triticum aestivum* (Jellings et al. 1983). The basis of variability of SSU polypeptide composition was due to gene mutation in *Lycopersicum* (Cammaerts et al. 1981). Therefore, the frequent occurrence of heterogeneity of SSU polypeptides within species, as in *Nicotiana* (Wildman 1979) and *Medicago* (Daday et al. 1986), is not surprising. As mutation of SSU genes does occur, it is quite likely that genetic variability for levels of synthesis occurs within a random population and can be detected by selection experiments. Finally, the genetic analysis of nuclear control of Rubisco synthesis in developed plants of *M. sativa* is in full agreement with the results of Tobin et al. (1980), Sasaki et al. (1981) and Gallagher and Ellis (1982), who showed that light-induced increase of Rubisco is controlled by mRNA level for SSU.

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References

Andrews TJ, Ballment B (1983) The function of the small subunits of ribulose bisphosphate carboxylase EC-4.1.1.39. J Biol Chem 258:7514–7518

Bedbrook JR, Smith SM, Ellis RJ (1980) Molecular cloning and sequencing of cDNA encoding the precursor to the small subunit of chloroplast ribulose-1,5-bisphosphate carboxylase. Nature 287:692–697

Bennett MD, Smith JB (1976) Nuclear DNA amounts in angiosperms. Philos Trans R Soc London, Ser B 274:222–274

Cammaerts D, Jacobs M (1981) Genetic control of small subunits polypeptides of Fraction 1 protein in the genus *Lycopersicum.* Plant Sci Lett 22:147–154

Chan PH, Wildman SG (1972) Chloroplast DNA codes for the primary structure of the large subunit of Fraction 1 protein. Biochim Biophys Acta 277:677–680

Cobb AH, Wellburn AR (1976) Polypeptide binding to plastid envelopes during chloroplast development. Planta 129:127–131

Collatz GJ, Badger M, Smith C, Berry JA (1979) A radio immune assay for RuP_2 carboxylase protein. Annu Rep Dir Dept Plant Biol 78:171–178

Daday HV, Whitecross MI (1985) Heterogeneity of alkaline small subnits of ribulose-1,5 bisphosphate carboxylase-oxygenase from *Medicago sativa* and *M. falcata.* Plant Cell Rep 4:212–215

Daday HV, Whitecross MI, Shaw DC (1986) One large subunit of ribulose 1,5-bisphosphate carboxylase oxygenase in *Medicago, Spinacia* and *Nicotiana.* Theor Appl Genet 71:708–715

Dean C, Van den Elzen P, Tamaki S, Dunsmuir P, Bedbrook J (1985) Differential expression of the eight genes of the petunia ribulose bisphosphate carboxylase small subunit multi-gene family. EMBO J 4:3055–3061

Dean C, Leech RM (1982) Genome expression during normal leaf development. 2. Direct correlation between ribulose bisphosphate carboxylase content and nuclear ploidy in a polyploid series of wheat. Plant Physiol 70:1605–1608

Dunsmuir P, Smith S, Bedbrook J (1983) A number of different nuclear genes for the small subunit of RuBPCase are transcribed in petunia. Nucleic Acids Res 11:4177–4183

Ellis RJ, Highfield PE, Silverthorne J (1978) The synthesis of chloroplast proteins by subcellular systems. In: Hall DO, Coombs J, Goodwin TW (eds) Photosynthesis 77. Proc 4th Congr Photosynth. Biochem Soc, London, pp 497–506

Falconer DS (1964) Introduction to quantitative genetics. Oliver and Boyd, Edinburgh London, p 365

Gallagher TF, Ellis RJ (1982) Light stimulated transcription of genes for 2 chloroplast polypeptides in isolated pea *Pisum sativum* cultivar 'Feltham'-First leaf nuclei. EMBO J 1:1493–1498

Gutek LH, Goplen BP, Howarth RE (1976) Heritability of soluble protein in alfalfa. Crop Sci 16:199–201

Hallier UW, Schmitt JM, Heber U, Chaianova SS, Volodarky AD (1978) Ribulose-1,5-bisphosphate carboxylase-deficient plastome of *Oenothera.* Biochim Biophys Acta 504:67–83

Heinrichs DH (1972) Genetic results from diallel of eight high and eight low plants for fraction 1 (18S) protein content. Rep 23rd Alfalfa Improv Conf, pp 21–22

Jellings AJ, Leese BM, Leech RM (1983) Location of chromosomal control of ribulose bisphosphate carboxylase EC-4.1.1.39. Amounts in wheat *Triticum aestivum* cultivar 'Chinese-Spring'. Mol Gen Genet 192:272–274

Kawashima N, Wildman SG (1970) A model of the subunit structure of Fraction I protein. Biochem Biophys Res Commun 41:1463–1468

Kawashima N, Wildman SG (1972) Studies on Fraction 1 protein. 4. Mode of inheritance of primary structure in relation to whether chloroplast or nuclear DNA contains the code for chloroplast protein. Biochim Biophys Acta 262:42–49

Lamprecht MP, Stevens JM, Laubscher FX (1965) Genetiese variabiliteit in sud-afrikaanse lusern (*Medicaga sativa* L.). S Afr Tydskr Landbouwet 8:3–20

Lawrence ME, Possingham JV (1986) Direct measurement of femtogram amounts of DNA in cells and chloroplasts by quantitative microspectrofluoremetry. J Histochem Cytochem 34:761–768

Li LR, Sisson VA, Kung SD (1983) Relationship between the kinetic properties and the small subunit composition of *Nicotiana* ribulose 1,5-bisphosphate carboxylase. Plant Physiol 71:404–408

Meyer SP, Nichols SL, Bear GR, Molin WM (1982) Ploidy effects in isogenic populations of alfalfa. Plant Physiol 70:1704–1709

Miltimore JE, Goplen BP, Majak W, Howarth RE (1974) Variability of fraction 1 protein and heritability estimates for fraction 1 protein and total phenolic constituents in alfalfa. Agron J 66:384–386

Possingham JV (1970) Some effects of mineral nutrient deficiencies on the chloroplasts of higher plants. In: 6th Int Coll Plant Anal Fertil Probe (ISHS). Tel Aviv, Israel, pp 155–165

Possingham JV (1973) Effect of light quality on chloroplast replication in spinach. J Exp Bot 24:1247–1260

Possingham JV, Saurer W (1969) Changes in chloroplast number per cell during leaf development in spinach. Planta 86:186–194

Possingham JV, Smith JW (1972) Factors affecting chloroplast replications in spinach. J Exp Bot 23:1050–1059

Rejda JM, Johal S, Chollet R (1981) Enzymic and physicochemical characterization of ribulose 1,5-bisphosphate carboxylase oxygenase EC-4.1.1.39 from diploid and tetraploid cultivars of perennial rye grass *Lolium perenne*. Arch Biochem Biophys 210:617–624

Sasaki Y, Ishiye M, Sakihama T, Kamikubo T (1981) Light induced increase of mRNA activity coding for the small subunit of ribulose-1,5-bisphosphate carboxylase. J Biol Chem 256:2315–2320

Scott NS, Possingham JV (1980) Chloroplast DNA in expanding spinach leaves. J Exp Bot 31:1081–1091

Tobin EM, Suttie J (1980) Light effects on the synthesis of ribulose-1,5-bisphosphate carboxylase in *Lemna gibba* L. G-3. Plant Physiol 65:641–647

Wildman SG (1979) Aspects of fraction 1 protein evolution. Arch Biochem Biophys 196:598–610

Williams CH, Twine JR (1976) Determination of nitrogen, sodium, calcium and magnesium of plant material by automatic analysis. Div Plant Ind Tech Paper No 24, CSIRO, Australia