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Regulation of Ribulose-1,5-Bisphosphate Carboxylase Expression in Second Leaves of Maize Seedlings from Low and High Yield Populations[1]

Herminia Loza-Tavera, Eleazar Martínez-Barajas, and Estela Sánchez-de-Jiménez*

Biochemistry Department, Facultad de Química, Universidad Nacional Autónoma de México, México, D.F. 04510

ABSTRACT

Ribulose-1,5-bisphosphate carboxylase oxygenase (EC 4.1.1.39) (Rubisco) activity, Rubisco-protein, and Rubisco large and small subunit gene (*rbc*L and *rbc*S) transcripts were measured at seven stages of development in the second leaf of maize (*Zea mays* L.) seedlings belonging to low and high yield populations. During the three early stages of development, when the leaf has not yet expanded, it was determined that increments in Rubisco-activity were caused by increases in Rubisco-protein and its mRNAs. Afterward, the *rbc*S level decreased sharply down to nondetectable levels at the seventh stage, when the leaf was at the beginning of senescence. As a contrast, *rbc*L transcript decreased slowly and Rubisco-protein accumulated up to the fifth stage, when the leaf reached its maximum expansion. A slight decrease in Rubisco-protein was then observed. These results suggest that at early stages of development Rubisco-activity and Rubisco-protein are regulated mainly at the transcriptional level. At the later phase the regulation seems to be at other biochemical levels. Neither Rubisco activity nor Rubisco-protein showed correlation with yield for both maize populations at this stage of development. Slightly higher levels of both transcripts were observed in the high yield population.

Rubisco,[2] the first enzyme in the Calvin-Benson cycle, carboxylates RuBP to produce two 3-PGA molecules. This is one of the most abundant and best studied plant proteins. It is a chloroplastic enzyme that in higher plants is made of eight chains each of two kinds of polypeptides (12): the small subunit (mol wt 14kD) codified in the nuclear genome by a multigene family (*rbc*S genes) and the large subunit (mol wt 55 kD) codified as one copy per chloroplast genome (*rbc*L gene). The number of genes per cell is between 3 and 10 for *rbc*S and between 100 and 2000 or even more for *rbc*L depending on the species (2). The small subunit is synthesized in cytoplasmic ribosomes as a precursor that is transported to the chloroplast. There, the precursor is processed before being assembled to the large subunit synthesized in the chloroplastic ribosomes (12).

This complex mechanism has raised questions regarding the possible coordination between Rubisco activity, Rubisco-protein and its levels of mRNAs, during leaf development or in response to light changes. Prioul and Reyss (23) found rapid changes in total activity and in *rbc*S-mRNA content without significant modification of the contents of Rubisco-protein or *rbc*L mRNA in tobacco leaves transferred from low to high light conditions in adults and seedlings. In the same way, Nelson *et al.* (15) found high levels of *rbc*S mRNA are not related to small subunit polypeptide amounts in the second leaf of light-grown maize seedlings sampled 3 to 7 DAS.

Several regulation levels have been proposed to account for changes in Rubisco activity at different stages of development. Transcriptional control has been proposed as the main regulatory level for *rbc*S gene expression (11). Although gene dosage and transcriptional regulation are determinant in the first expression of *rbc*L gene (26), posttranscriptional mechanisms have been invoked to control the rapid changes observed when leaves are exposed to different light environments (16). Furthermore, some metabolites such as 2-carboxyarabinitol 1-phosphate (CA1P) or RuBP (5) and Rubisco activase (22) have been found to regulate Rubisco activity without changes in Rubisco protein.

Within this frame of reference, we are interested in analyzing whether Rubisco activity, Rubisco-protein, and its mRNA levels change coordinately during the development of the second leaf in maize (*Zea mays* L.) seedlings from two populations: the original one and a selected high-yield population. This study is relevant because in the high-yield population, Rubisco activity was previously found to be higher during the grain filling period (8). A similar correlation has been observed between Rubisco activity and high yield in several C_3 species such as *Hordeum vulgare* (3), *Triticum aestivum* (14), and *Vigna radiata* (6). Therefore, it has been suggested that Rubisco activity could be used as a biochemical marker for high yield plant selection (3, 6, 14). This would be of great advantage if differences in Rubisco activity could be observed between high and low yield plants at early stages of development.

MATERIALS AND METHODS

Plant Material

Groups of 50 seedlings of maize (*Zea mays* L.) var Zacatecas 58 original (Z_0) and the improved population for higher

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[2] Abbreviations: Rubisco (ribulose-1,5-bisphosphate carboxylase oxygenase (EC 4.1.1.39); RuBP, ribulose 1,5-bisphosphate; 3-PGA, 3-phosphoglyceric acid; *rbc*L, Rubisco large subunit gene; *rbc*S, Rubisco small subunit gene; Z_0, Zacatecas 58, original population; Z_{18}, Zacatecas 58 SM-18, high yield population; DAS, days after sowing; kbp, kilobase pairs.

grain yield obtained after 18 cycles of stratified mass selection (Z_{18}) were grown in soil pots under greenhouse conditions in Mexico City. The seeds were provided by Dr. José Molina-Galán, (Centro de Genética, Colegio de Postgraduados, Montecillo, México). The second leaves from 7 to 12 developing seedlings belonging to both populations were harvested at seven different stages of development ranging from rolled leaf (stage 1 = 6 DAS) to fully expanded leaf, when signs of senescence can be observed (stage 7 = 17 DAS) (Fig. 1). For mRNA analysis a previous stage was taken (stage 0). Since duration for achieving a stage of development varies as a function of temperature and sunlight exposure, the description of results and discussion are based on stages instead of days after sowing. The samples were collected approximately at the same hour in the morning to avoid fluctuations in enzyme levels due to diurnal activation of Rubisco.

All determinations except transcript assessment were made in individual leaves. For dry weight determination, leaves were put in an oven at 60°C until constant weight was achieved. For transcript determinations, leaves were pooled, frozen in liquid nitrogen, and stored at −70°C until use.

Chl was measured by the Bruinsma method (1). The samples weighed between 0.1 to 0.2 g. Protein was measured by the Peterson method (19).

Leaf Extraction

Leaf samples (0.2–0.4 g) were ground in a mortar with 1 mL of extraction buffer (0.1 M Hepes-NaOH [pH 7.8], 0.25 mM Na_2EDTA, 5 mM DTT, 0.1 M sodium ascorbate, 0.35 mM sodium diethyldithiocarbamate), and centrifuged 6 min at 12,000*g* at 0°C in a Beckman microfuge (21). The supernatant was used for Rubisco-carboxylase activity assay, total protein and Rubisco-protein determinations.

Rubisco Assay

Rubisco-carboxylase activity was measured by the $^{14}CO_2$ fixation method (21). Five μL of crude leaf extract were used as the source of enzyme. The assay system contained 38 mM of [^{14}C]NaH-CO_3 (100 μCi/mmol, Amersham), 1.53 mM of ribulose 1,5 biphosphate-tetrasodium salt in a final volume of 65 μL of assay buffer (0.1 M Hepes-NaOH [pH 8.2], 20 mM $MgCl_2$, 5 mM DTT). Previous activation of the enzyme extract by 10 mM $MgCl_2$ and 15 mM $NaHCO_3$ was performed at 30°C for 9.5 min as reported by Randall *et al.* (24). The radioactivity incorporated in acid stable products was measured using 5 mL of Bray's scintillation liquid in a Packard scintillation counter (Mimaxi β series 400).

Immunoelectrophoretic Quantification of Rubisco-protein

Rubisco was purified from spinach leaves according to the method of Wishnick and Lane (30). The enzyme purity was tested by electrophoresis. One band (at 500 kD) and two bands (at 55 and 14 kD) were observed in native and denatured gel stained with Coomassie blue, respectively. This protein preparation was used to induce antibodies against the holoenzyme by standard techniques in 1.5 kg young rabbits. The serum was prepared from whole blood by allowing the latter to stand at 4°C overnight. This serum was used as source of Rubisco antibodies for the immunoassay (7). Rocket immunoelectrophoresis gels were made by dissolving agarose (5% weight/volume) in buffer (Tris-HCl 30 mM, glycine 15

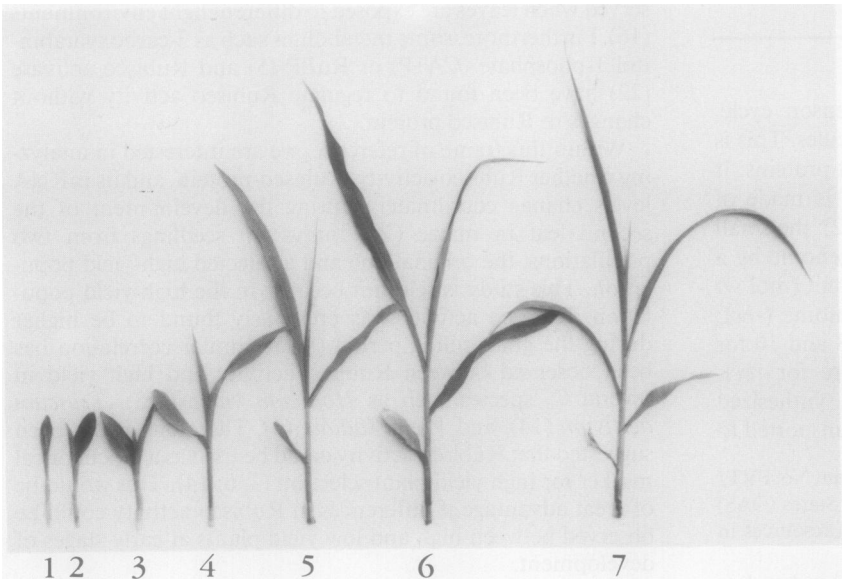

Figure 1. Stages of development used for screening Rubisco-activity, Rubisco-protein, and its mRNAs in the second leaf of maize seedlings. First stage: the first leaf does not have the ligula exposed; second leaf is rolled. Approximately 6 DAS. Second stage: the first leaf shows the ligula but it is not fully exposed; second leaf is rolled at the base but opened at the tip. Approximately 7 DAS. Third stage: the first leaf has the exposed ligula while in the second leaf, the ligula is at the level of the first one but it is not exposed; tip of the third leaf can be observed. Approximately 8 DAS. Fourth stage: first and second leaves have exposed ligulae; third leaf is expanded up to the middle; tip of the fourth leaf is observed. Approximately 10 DAS. Fifth stage: first and second leaves have exposed ligulae; third leaf has almost completely expanded but the ligula is not exposed; fourth leaf is half-way expanded. Approximately 12 DAS. Sixth stage: first, second, and third leaves have exposed ligulae; fourth leaf is almost completely expanded; fifth leaf shows tip; first leaf shows signs of senescence at the tip. Approximately 15 DAS. Seventh stage: first to fourth leaves have exposed ligulae; fifth leaf is almost fully expanded; sixth leaf shows tip; first leaf is complete senescent; second leaf shows signs of senescence at the tip. Approximately 17 DAS.

mM [pH 9.0]) and serum from Rubisco immunized rabbits at 50°C. Five μL of each maize leaf extract were applied to the gels for assay in a horizontal LKB immunoelectrophoresis apparatus (2117 Multiphor) and run overnight (4°C:80 V:5 mA). Standards were made with Rubisco spinach as controls. A linear correlation was found between the amount of Rubisco and rocket length in the 1 to 5 μg of Rubisco-protein range. Two standards were used for each run.

RNA Extraction

One and a half grams of frozen tissue were ground in a mortar, 4.5 mL of buffer (100 mM Tris-HCl [pH 8.6], 25 mM EDTA, 25 mM EGTA, 4 M guanidium thiocyanate, 2% (w/v) Sarkosyl, 100 mM β-mercaptoethanol), and 2.25 mL of STE (10 mM NaCl, 10 mM Tris, 1 mM EDTA) saturated phenol were added. The slurry was shaken vigorously for 5 min, then 2.25 mL of chloroform:isoamyl alcohol (24:1, v/v) were added and shaken again. The mixture was centrifuged to separate the aqueous phase which was then repeatedly extracted until there was no protein in the interphase. The nucleic acids were precipitated from the recovered aqueous phase by adding 0.6 volume of isopropanol and freezing at −20°C overnight (27).

To remove polysaccharides, RNA pellets were rinsed twice with 70% ethanol, vacuum dried, and resuspended in 0.9 mL of 0.1 M sodium acetate (pH 8.0) at 0°C. The polysaccharides were precipitated by adding ethanol up to 10% and keeping the sample at 0°C for 10 min. After centrifugation at 10,000*g* for 15 min at 0°C, the supernatant was transferred to a clean tube. There, the nucleic acids were precipitated by adding one volume isopropanol and freezing at −20°C for 2 h. The pellets were rinsed twice with 70% ethanol, vacuum dried, resuspended in sterile deionized distilled water, and stored at −70° C (Herrera-Estrella, 1987 personal communication). $A_{260} = 1$ was taken as 40 μg/mL RNA.

RNA Electrophoresis and Northern Blot Analysis

Thirty micrograms of total RNA were denatured in 50% (v/v) formamide, 2.2 M formaldehyde, 1× phosphate buffer (10× = 100 mM sodium phosphate [pH 7.0] and 10 mM EDTA [pH 8.0]) at 65°C for 10 min and applied to a 1.2% (w/v) agarose gel with 2.6 M formaldehyde. The running was 1× phosphate buffer in a horizontal electrophoresis apparatus. The gels were stained with 10 μg/mL ethidium bromide for 1 min and destained in distilled water until visualization of RNA was achieved. Densitometry of enlarged negatives of gel photographs was used to confirm the RNA content per lane. A GS 300 transmittance/reflectance scanning densitometer (Hoefer Scientific Instruments) and a LKB 2200 recording integrator were used. The fractionated RNA was blotted onto Nytram membranes (Schleicher and Shuell) and fixed to the filter by UV treatment using a model TM 36 Chromato-vue shortwave transilluminator (U.V.P. Inc.) during 7.5 min. The membranes were soaked at 65°C for 15 min in 10 mM Tris-HCl (pH 8.0), 2 mM EDTA, and 0.1% (w/v) SDS to remove unbound RNA.

The membranes were prehybridized for 2 h at 65°C in a sealed bag with 0.15 mL of prehybridization solution (0.5 M sodium phosphate buffer pH 7.2, 1% (w/v) BSA, 1% (w/v) SDS, and 100 μg/mL heat denatured salmon sperm DNA) per cm^2 of membrane, with slow constant shaking. ^{32}P-Labeled DNA probes were heat denatured in boiling water for 10 min and added to the bag containing membranes. After hybridization for 24 h at 65°C, the membranes were washed as follows: (a) 5 min at room temperature with 2× SSC (1× SSC = 0.15 M NaCl, 0.015 M sodium citrate [pH 7.0]), (b) 30 min at 65°C with 2× SSC, 1% (w/v) SDS, and (c) 30 min at room temperature with 0.1× SSC. They were then dried and exposed to x-ray films (X-Omat S, Kodak) with intensifying screens (DuPont) at −70°C. Spot intensities in autoradiograms were estimated by densitometry and normalized based in total RNA applied on each lane. The exposure time of the autoradiograms was optimized to avoid underestimation of the strongest hybridization signals.

Hybridization Probes

The plasmid pZmc460 contains the maize chloroplast DNA fragment Bam 9 of 4.9 kbp inserted into the Bam H1 site of pBR322. The Bam 9 fragment contains the gene for the large subunit of Rubisco (*rbc*L) (1.6 kbp) and the 5′ end of the divergent transcript encoding β and ε subunits of CF_1. This plasmid was used to transform *Escherichia coli* SF8 strain (10). The plasmid SS37 was constructed in pUC13; it contains a 0.7 kbp insert encoding the small subunit of Rubisco (*rbc*S) from maize. Both plasmids were kindly provided by Dr. Jean Lukens of the Laboratory of Biological Sciences, Harvard University. The cloned insert with the *rbc*L gene was isolated by digesting pZmc460 with Bam H1 and Sma1 to produce a 3.48 kbp DNA fragment from which most of the CF_1 genes were absent (the remainder was less than 0.4 kbp). The coding *rbc*S insert was isolated by digesting SS37 with Bam H1 and Eco R1. The DNA fragments were separated by gel electrophoresis in TBE buffer (89 mM Tris-base, 89 mM boric acid, and 2 mM EDTA) and 1% (w/v) of a low-melting-temperature agarose gel. The fragments were isolated by cutting the bands and placing the gel slices in a boiling water bath for 7 min. The inserts were labeled with [α-^{32}P]dCTP (180–230 TBq $mmol^{-1}$, DuPont/NEN) by using a random-primer extension labelling system (DuPont/NEN). The specific activities reached were between 1.44×10^8 and 1.75×10^8 cpm/μg DNA. The labeled probes were purified by the spun-column procedure (10) and heated in a boiling water bath for 10 min prior to hybridization.

Statistical Analysis

All the parameters except transcripts were analyzed by the *t*-test.

RESULTS

Specific stages of development were defined for the two populations of maize seedlings (Z_0 and Z_{18}). All the measurements were performed in the second leaf at the stated stages of development (Fig. 1). Fresh and dry weight reached a maximum at the fifth stage in the Z_{18} population and at the sixth in the Z_0 population. Afterward, the Z_{18} population kept a constant weight, whereas the Z_0 population showed a large

weight decrease in the last stage. The increases in fresh weight and dry weight were around 5 times and 4 times the initial values, respectively. At the end of the period Z_{18} had larger leaves than the Z_0 population (Fig. 2, a and b). Statistical analysis confirmed the significance of these differences between the two populations ($P < 0.05$).

Chl levels behave similarly to dry weight: it increased during the leaf development reaching the maximum value between the fifth and sixth stage and decreased slowly at the end of development (Fig. 3a). Increases of 8 to 9 times the initial values were observed at the maximum development. However, no differences were observed between the populations.

Protein per fresh weight, on the other hand, displayed a continuous decrease from the first to the third stage, remaining approximately constant thereafter (Fig. 3b). Although there were no differences between populations, it is interesting to point out that the Z_{18} population showed lower amounts of protein than Z_0 during almost the whole period studied.

Rubisco-protein per g of fresh weight increased to a maximum around the fifth stage, where 5 to 6 times the initial values were found. Subsequently, it decreased slowly and at the last three stages, the Z_0 population showed 28 to 32% more Rubisco-protein than Z_{18} (Fig. 4a). In spite of these differences, the amounts of Rubisco-protein per total protein were very similar for both populations (Fig. 4b). Rubisco-protein comprised 3 to 7% of total protein at the first stage of leaf development and reached between 47 and 55% at the maximum growth stage. Three phases with different rates in the accumulation of Rubisco-protein could be observed during the development of the second leaf: from the first to the third stage, from the third to the fifth stage, and from the fifth to the seventh stage. The accumulation rates values were approximately 12.5, 3.8, and 0.6% of Rubisco-protein accumulated per day of development for each phase (Fig. 4b).

Rubisco activity also increased drastically from the beginning until the fourth stage of the second leaf development (Fig. 5). Afterward, the activity fluctuated around the maximal value up to the last stage. At the last two developmental stages, the Z_{18} population showed higher activity than Z_0 even though it possesses lower Rubisco-protein contents (Fig. 4b).

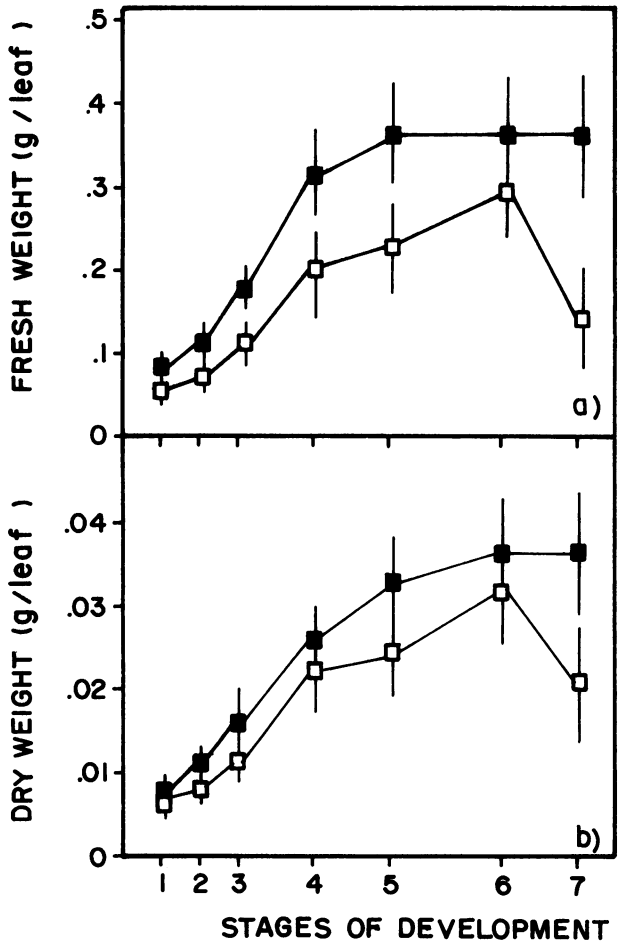

Figure 2. Fresh weight (a) and dry weight (b) of the second leaf of maize seedlings. Seven to 12 leaves from each population were weighed individually. The mean values are shown. Bars represent standard deviations. Dry weight was obtained by heating the leaves at 60° C in an oven until constant weight was reached. (□), Z_0 original population; (■), Z_{18} high-yield population.

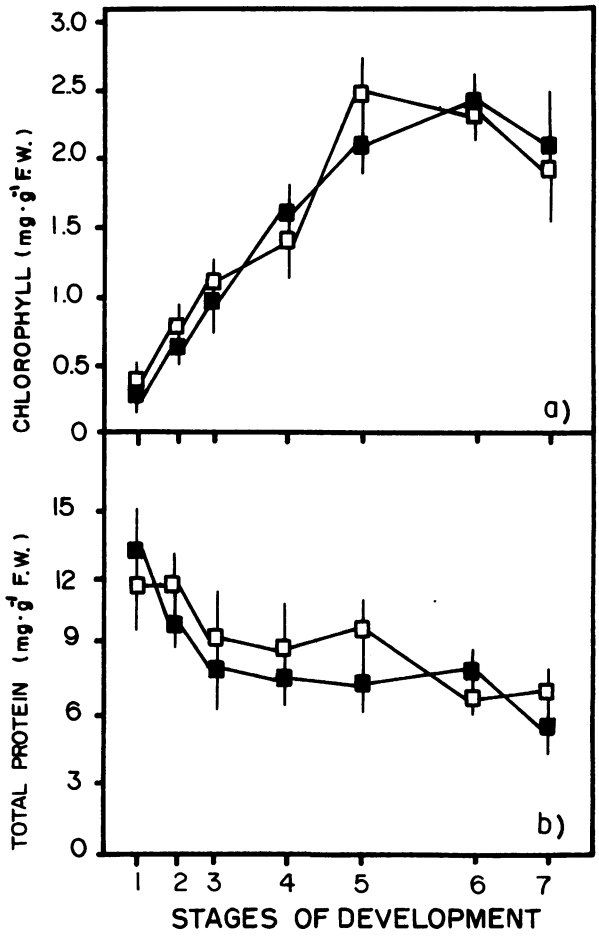

Figure 3. Chl (a) and total protein per g of fresh weight (b). Points are the mean values of 7 to 12 measurements; bars represent standard deviations. Chl was measured by the Bruinsma method (1) and total protein by the Peterson method (19). (□), Z_0 original population; (■), Z_{18} high-yield population.

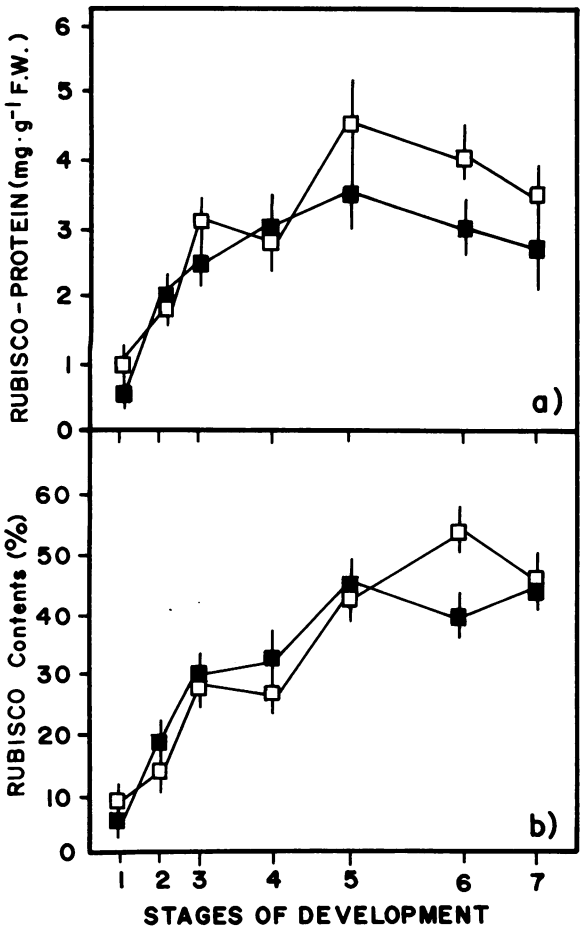

Figure 4. Rubisco-protein expressed either per gram of fresh weight (a) or per total protein (b). Measurements were done throughout the development of the second leaf of maize seedlings. Rubisco-protein was quantified by rocket immunoelectrophoresis against the holoenzyme antibody. Spinach Rubisco-protein was used as a standard. Each point represents the mean value of five determinations; bars are standard deviations. Rubisco contents (b) represent Rubisco-protein/total protein × 100. (□), Z_0 original population; (■), Z_{18} high-yield population.

The specific activities per mg of Rubisco-protein are presented in Table I. There was not a constant value for this parameter throughout the leaf development, nor were constant differences between the two populations. The mean specific activity for Z_{18} (257 nmol CO_2 fixed·min^{-1}·mg^{-1} of Rubisco) however, was higher than for Z_0 (191 nmol CO_2 fixed·min^{-1}·mg^{-1} of Rubisco).

The maximum values of Rubisco-carboxylase specific activity are in the same order of magnitude as those reported for maize seedlings: 0.22 μmol CO_2 fixed·min^{-1}·mg^{-1} protein, assayed in crude extracts (25).

Northern hybridization analyses were made with *rbc*S and *rbc*L probes to assess the amounts of transcript in the samples at each stage of development. The *rbc*L transcripts increased fivefold with respect to the initial values, reaching a maximum at the third stage of development in the two populations. They then decreased, reaching in the seventh stage, 5 and 22% of the maximum level in Z_0 and Z_{18}, respectively. Except for the first stage, the amounts of Z_{18} *rbc*L transcripts were higher than for Z_0, throughout the studied period, although the maximum value was almost the same (Fig. 6, a and b). In each autoradiogram lane two hybridization bands were observed, probably corresponding to the two *rbc*L transcripts reported for maize (13) (Fig. 6a, top). The *rbc*S transcripts increased up to 20- and 14-fold the initial values for Z_{18} and Z_0, respectively. The maximum levels reached were at the third and fourth stage of development in each case. The *rbc*S transcripts decreased sharply at the fifth stage, becoming almost undetectable at the sixth and absent at the seventh stage. The maximum amounts of *rbc*S transcripts were slightly higher for Z_{18} than for Z_0 population (Fig. 6, a and c).

DISCUSSION

Several biochemical and physiological parameters were measured during the development of the second leaf of seedlings from two populations of the same maize variety (Zacatecas 58) with different grain yield capacity: low grain producer (Zacatecas 58 Original, Z_0) and high-yield producer (Zacatecas 58 SM-18, Z_{18}). We were interested in determining whether Rubisco carboxylase activity, Rubisco-protein and its mRNAs were changing in a concerted fashion during the early leaf development in these populations. The knowledge of this matter is important since it has been proposed, in several C_3 species, that Rubisco activity might be a biochemical marker for detecting high yield plants (3, 6, 14). It has also been shown that during the grain filling period the high yield population (Z_{18}) has a higher Rubisco activity than the low yield producer (Z_0) (8).

Regulation of Rubisco Genes Expression during the Second Leaf Development

Changes in Rubisco activity roughly correlated with changes in Rubisco-protein. The variations observed in specific activity based on Rubisco-protein throughout leaf development (Table I) showed that the activation state of the enzyme was not the same during the whole life of the leaf. Similar results have been reported when chloroplast senescence and degradation of Rubisco in wheat have been studied (18). A wide range of Rubisco specific activity (0.12–0.25 μmol CO_2 fixed·min^{-1}·mg^{-1} of Rubisco) close to our values was detected.

The amount of Rubisco-protein found in the present study (55% of the total soluble protein (Fig. 4b) is similar to that reported by Makino *et al.* (9) in rice varieties using a different approach.

As to the coordination between Rubisco-protein and its levels of *rbc*L and *rbc*S transcripts, we found that the Rubisco-protein increments observed at the first three stages of development were supported by the fast increases in the levels of *rbc*L and *rbc*S transcripts. Beyond the third stage, however, the fast decrease in *rbc*S mRNA and even the slow decrease in *rbc*L mRNA, did not mirror the accumulation in Rubisco-protein observed from the third to the fifth stage. From these data we suggest that the rate of Rubisco-protein synthesis is different before and after the third stage of leaf development,

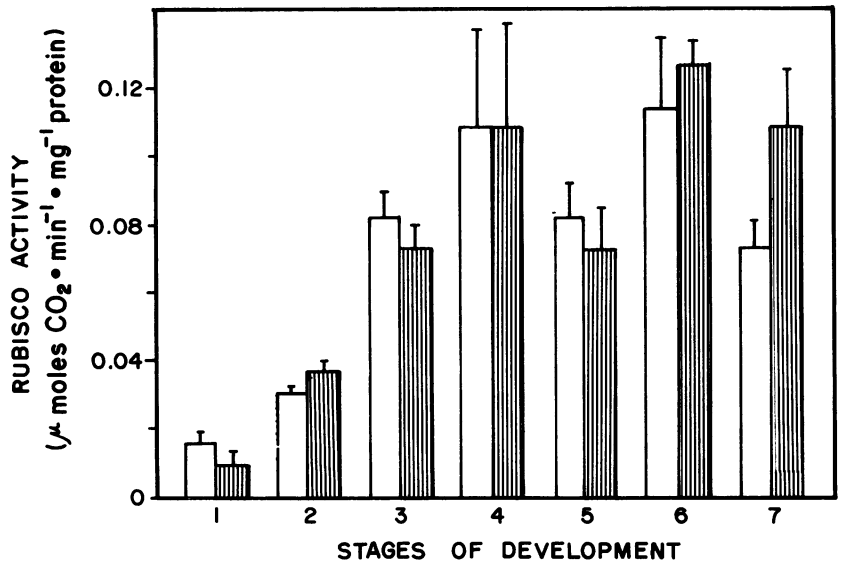

Figure 5. Rubisco-carboxylase activity per total protein (specific activity). Rubisco-carboxylase activity was measured in a crude extract by the $^{14}CO_2$ fixation method (21). The enzyme was incubated before the assay with 10 mM $MgCl_2$ and 15 mM $NaHCO_3$ during 9.5 min at 30° C. The incubated enzyme was added to the reaction mixtures and the reactions were stopped 30, 60, and 90 s later. (□), Z_0 original population; (▥), Z_{18} high-yield population. Vertical bars represent standard deviations.

Table I. *Rubisco Specific Activity Based on Rubisco-Protein for the Original Population (Z_0) and the High Yield Population (Z_{18}) of Maize, during the Development of the Second Leaf*

Stages of Leaf Development	Specific Activity			
	Z_0 (average ±σ)		Z_{18} (average ±σ)	
	nmol·min⁻¹·mg⁻¹ Rubisco			
1	215	±15	367	±83
2	190	±10	194	±96
3	265	±50	243	±16
4	380	±120	269	±39
5	185	±15	157	±16
6	205	±30	333	±31
7	160	±30	234	±24
Mean values	191	±104	257	±74

since abundance of Rubisco mRNAs changes. However, this assumes that the Rubisco-protein degradation velocity is the same throughout the leaf development. This is also consistent with the different Rubisco-protein accumulation slopes observed in the study (Fig. 4b).

If we assume that after the fifth stage little or no Rubisco-protein is synthesized, because there are low amounts or no *rbc*S mRNAs, the high stability of Rubisco-protein must account for the amounts observed at the last stages of leaf development (Figs. 4b and 6, a and c). A previous report (4) has shown that the Rubisco-protein half-life in maize leaves measured by ^3H_2O method is approximately 6.21 d. Although we did not measure the turnover of Rubisco-protein, estimations from our results agree with this value, since slight decreases in Rubisco-protein were observed during the last three stages, lasting approximately 5 d (from the fifth to the seventh stage, Fig. 4b).

Changes in *rbc*L and *rbc*S transcript levels were roughly coordinated. Both transcripts increased simultaneously until their maxima were reached at the third stage (the Z_0 population had its *rbc*S maximum at the fourth stage). Subsequently, both mRNAs decreased differently: *rbc*S declines more sharply than *rbc*L. In the last stage no *rbc*S transcripts were found, while *rbc*L transcripts are still detected. This behavior also has been observed in the pericarp of tomato fruit after pollination, where the levels of both transcripts increased during 15 d and then decreased. At the ripe fruit stage, *rbc*S was not detectable, while low levels in *rbc*L transcripts were still observed (20). Furthermore, from the data in Figure 6 it is clear that *rbc*S transcripts showed more extensive changes in level than the *rbc*L transcripts (14- and 20-fold for *rbc*S transcripts and fivefold for *rbc*L transcripts) (Fig. 6, b and c). Increases in *rbc*L and *rbc*S transcripts have been reported for tomato (29), mustard (17), and maize (15, 28) during the greening. In those cases, the increases also were greater for *rbc*S transcripts. The differences in the behavior of *rbc*S and *rbc*L transcripts suggest that *rbc*S gene expression could be the limiting factor for Rubisco accumulation, since the levels of its transcripts are more extensively controlled.

Overall, during early leaf development there is a strong correlation between Rubisco activity, Rubisco-protein, and Rubisco transcripts. This suggests that the Rubisco increases observed were the result of transcriptional regulation mechanisms. The presence of almost constant amounts of Rubisco protein, not coincidental with reduction in the levels of *rbc*S and *rbc*L transcripts, suggests that during the last stages of development, Rubisco activity depends on the large stability of Rubisco-protein (4), as well as on the internal pools of CO_2, Mg^{2+}, RuBP (5), and/or Rubisco activase (22).

Differences between Z_0 and Z_{18} Populations

Although we expected that Rubisco could be a biochemical marker for yield selection at the seedling stage, most of the parameters studied showed similar values for both maize populations. It is interesting, however, that the mean value for specific activity (activity per mg of Rubisco-protein) was 39% higher for Z_{18} population than for Z_0 (Table I). In addition, higher amounts of *rbc*L and *rbc*S transcripts were also found in Z_{18} populations. Although there might be several

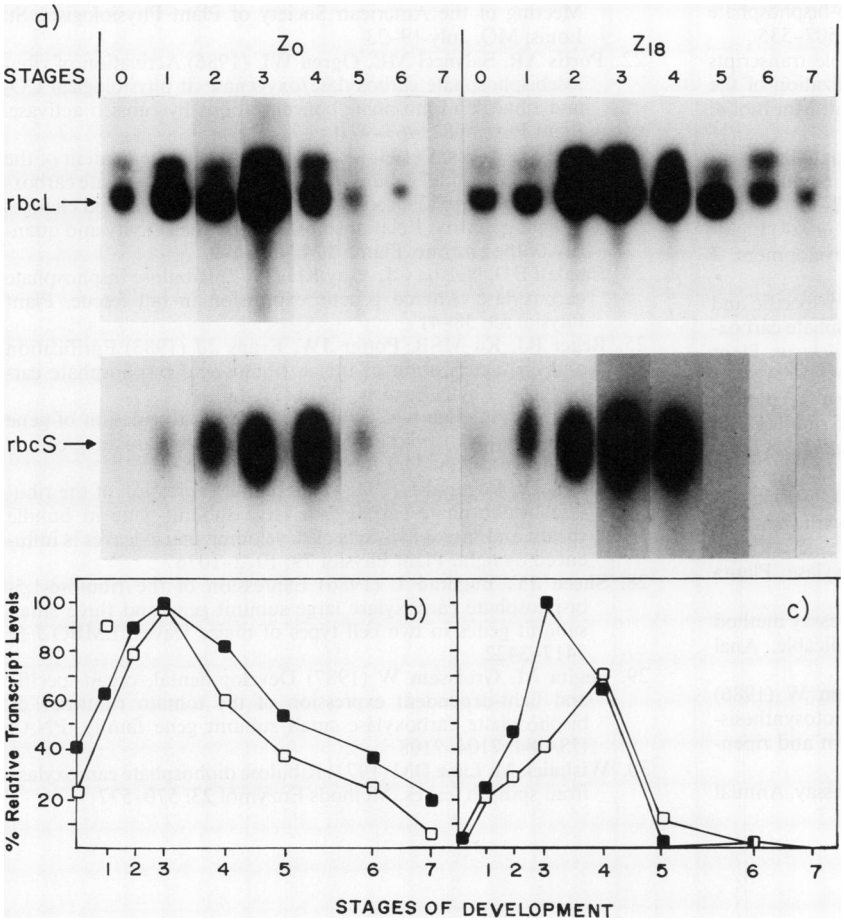

Figure 6. Northern hybridizations of *rbc*L and *rbc*S transcripts during the second leaf development of maize seedlings. (a), Autoradiograms show spots for *rbc*L and *rbc*S transcripts for both populations. Overexposed autoradiograms are presented to display the lightest spots. (b), Densitometric quantification of *rbc*L transcripts; (c), densitometric quantification of *rbc*S transcripts. The quantifications were made in properly exposed autoradiograms. Spots were scanned by a densitometer and normalized against the total RNA measured in enlarged negatives of the gel photographs. Estimation was made taking as 100% the darker spot in each autoradiogram. Stage 0: coleoptile is still covering the leaves. (□), Z_0 original population; (■), Z_{18} high-yield population.

explanations, the results suggest that the Z_{18} population could have larger rates of Rubisco-protein turnover and this positively relates to higher integrity of the protein. A higher specific activity per Rubisco-protein might then be expected. Experiments concerning the stability of the enzyme are presently underway in our laboratory. No clear differences in Rubisco activity were found between Z_0 and Z_{18} populations at the seedling stage, as opposed to the differences found during the grain filling period (8). This might be due to modifications on the signals regulating photosynthate demand and/or partitioning that are associated with plant development (*e.g.* seedling growth versus grain filling). From the data presented here, it can then be concluded that Rubisco activity by itself is not a suitable biochemical marker for selecting maize plants of high grain yield at the seedling stage. A combination of several parameters might be required to perform precise selection at this stage of plant development.

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