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Expression of plastid-encoded photosynthetic genes during chloroplast or chromoplast differentiation in *Cucurbitae pepo* L. fruits

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Abstract

The objective of the study was to determine the patterns of expression of two photosynthetic genes *rbc*L and *psb*A, during chloroplast and chromoplast differentiation in fruit tissues of three *Cucurbitae pepo* L. cultivars: Early Prolific, Foodhook Zucchini and Bicolor Gourds. In two Early Prolific isogenic lines, $YYBB$ and $YYB^{+}B^{+}$, the steady-state amounts of *rbc*L and *psb*A transcripts increased with fruit development upto 14 days post-pollination. The $YYB^{+}B^{+}$ line in which chloroplast differentiates into chromoplast at about pollination, did not show significantly higher amounts of both transcripts compared to $YYBB$, in which chromoplast develops early prior to pollination. In the Bicolor Gourds, in which the chromoplast and chloroplast containing tissues lie in juxtaposition on the same fruit, showed little differences in *rbc*L and *psb*A transcripts between the two tissues, if any the chromoplast containing tissue contained more of both transcripts than the chloroplast containing tissue. In Fordhook Zucchini fruits, where the chloroplast containing tissue developed early prior to pollination and was maintained, the steady-state amounts of *rbc*L transcripts increased to a maximum at 3 days post-pollination and levelled at 14 and 21 days post-pollination. In contrast, in Fordhook Zucchini fruits, the *psb*A transcript increased gradually up to 21 days post-pollination. In Fordhook Zucchini, the apparent ratios of *psb*A transcripts versus *rbc*L transcripts ranged from 2.5 to 3.9, at day 3 to 21 post-pollination, while in Bicolor Gourds were 2.9 and 4.5 at days 14 and 21 post-pollination. The two photosynthetic genes, *psb*A and *rbc*L were developmentally regulated and differentially expressed. However, their expression in chloroplast containing fruit tissues was not higher than in the chromoplast containing fruit tissues.

Keywords: *Cucurbitae pepo L*; *rbc*L; *psb*A; Gene expression; Fruit chloroplast and chromoplast

1. Introduction

Plant cells contain a unique group of organelles collectively called plastids. These organelles, which include proplastids, etioplasts, chromoplasts and chloroplasts are sites of many agricultural important processes. For example, chloroplasts are important for photosynthesis, amyloplasts for starch synthesis, while chromoplasts contain vitamin A precursors of many fruits and vegetables. Other important biological pathways including lipid and amino acid synthesis are partially compartmentalized in the plastids. Understanding plastid physiology and molecular biology is therefore pivotal to future improvement in agricultural productivity.

Plastid molecular biology has been investigated both extensively and intensively. Plastid related research has involved four key areas: determination of ultrastructural and biochemical changes during plastid biogenesis (Boyer, 1989; Camara and Brandgeon, 1981; Lim, 1990; Rosso, 1968; Scaffer, 1982; Spurr and Harris, 1968), development of physical and genetic maps of plastid genome (Hunt et al. 1986; Lim, 1990, Phillips, 1985), (partial and complete) sequencing of plastid genomes (Hiratsuka et al., 1989; Ohyama et al., 1986; Shinozaki et al., 1986; Shinozaki and Sunguira, 1982; Zurawski et al., 1982), and assays of plastid transcripts and translation products (Deng and Gruissem, 1987; Inamine et al., 1985; Jolly et al., 1981; Klein and Mullet,

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1986; Klein, 1991; Mullet, 1988; Mullet et al., 1985). Among the plastid genes the *rbc*L gene encoding the large subunit of ribulose-1,5-bisphosphate carboxylase/oxygenase and related Rubisco enzymatic activity has been widely studied (Berry et al., 1986, 1988, 1990; Edmondson et al., 1990; Gutteridge and Julien, 1989; Inamine et al., 1985; Jolly et al., 1981), followed by the *psb*A gene encoding the 32 kd photosystem II quinone binding protein (Fromm et al., 1985; Gamble and Mullet, 1989; Gruissem and Zurawski, 1985a,b).

Although literature relating to plastid molecular biology is voluminous, most previous workers focused on the study of chloroplasts (in leaf tissues), or differentiation of proplastids into chloroplasts, perhaps because of the central role played by leaf chloroplasts in photosynthesis. Relatively few studies have focused on developmentally controlled differentiation of proplastids and chloroplasts into chromoplasts. The later studies have mainly been limited to two systems, namely plastids of tomato fruits (Bathgate et al., 1958; Phillips, 1985; Piechulla et al., 1985; Richards et al., 1991) and pepper (Spurr and Harris, 1968; Gounaris and Price, 1987; Kuntz et al., 1989).

Mature green fruits are capable of assimilating CO_2 photosynthetically mainly in the external pericarp where the highest photosynthetic activity has been recorded (Bean and Todd, 1959; Fromm et al., 1985; Willmer and Johnson; 1976). The photosynthetic activities of these fruits suggest the presence of fully functional components of photosynthetic apparatus and the possible significance of these related processes for fruits. It has been suggested that the CO_2 evolved during respiration in fruits is refixed and recycled through the process of photosynthesis (Bean and Todd, 1959; Thomson et al., 1967; Tilney-Basset, 1989; Vu et al., 1985). Green tomato fruits contain chloroplasts that show typical ultrastructural features of leaf chloroplasts, namely the grana, which contain components involved in the light reaction and the stroma, where the Calvin cycle enzymes are localized (Bathgate et al., 1958). Though there is considerable information with regard to the composition, function and structural organization of the main polypeptide components of leaf thylakoids, little is known about the structure, stoichiometry of components and organization of fruit chloroplasts (Vianstein et al., 1994).

Several isogenic lines of *Cucurbitae pepo* L. have been developed that provide an excellent system for studying plastid/nuclear gene regulation during plastid biogenesis in fruits and in leaves (Boyer, 1989). For example, in the variety of Bicolor Gourds, the fruit chromoplast and chloroplast containing tissues develop in juxtaposition on the same fruit under identical nuclear genetic background. In Fordhook Zucchini fruits the proplastids develop into chloroplasts early in fruit development. In the Early Prolific isogenic line (YYB^+B^+), the proplastids develop into chloroplasts and later transform into chromoplasts, while in the isogenic line *YYBB*, the proplastids develop directly into chromoplasts. Each of these plastid developmental pathways could be of value in answering different biological questions.

Foundation studies in the development of squash as a system for studying the control of plastid gene expression have included three areas: development of the above-mentioned varieties and establishment of the gene control mechanisms of plastid interconversion (Boyer, 1989; Scaffer, 1982); development of the physical and genetic map of squash chloroplast DNA, isolation and comparison of chromoplast and chloroplast specific proteins in fruit tissues (Lim, 1990; Lim et al., 1990). In the current study we report unusual increased levels of *psb*A and *rbc*L transcripts in fruit chromoplast containing tissues of *Cucurbitae pepo* L. during ripening and slightly higher quantities of both transcripts in chromoplast than in the chloroplast containing tissues.

2. Materials and methods

2.1. Plant materials

Two isogenic lines of Early Prolific cultivars, *YYBB* and YYB^+B^+, were grown in pots under natural environmental conditions during summer at Pennsylvania State University. The plant growth media consisted of peat:perlite:soil (3:2:1). The plants were later fertilized with nitrogen:phosphorus:potassium (20:20:20) as appropriately required. Fresh ovary/fruit tissues were harvested from the *YYBB* and the YYB^+B^+ at −2 (pre-pollination), 0 (at pollination), 7, 10 and 14 days (post-pollination). The experiment was repeated and fruits harvested at −2, 0, 3, 14 and 21 days pre- and post-pollination. The amount of irradiation during the time of harvest was between 1400 and 1500 μmol m^{-2} s^{-1}. The Fordhook Zucchini plants were grown in the greenhouse at Pennsylvania State University during spring. The fruits were harvested at −2, 0, 3, 14 and 21 days at pre- and post-pollination and young leaves (15 cm in diameter) were concurrently harvested. The light intensity in the greenhouse was about 250–300 μmol m^{-2} s^{-1}. The Bicolor Gourds were grown at the Pennsylvania State University Horticulture farm at Rock Springs during summer. Fruits were harvested at 14 and 21 days post-pollination. Young leaves (15 cm diameter) were concurrently harvested. The harvested tissues from the three squash varieties were placed on ice in a cooler box and transported to the laboratory. Pericarp tissues from ovaries/fruits were scrapped off and immediately frozen in liquid nitrogen and stored at −70 °C until the time of total RNA extraction. Young leaves were concurrently frozen in liquid nitrogen and stored at −70 °C.

2.2. Extraction of total RNA

Total RNA was isolated by selective LiCl precipitation as described by Piechulla et al. (1986). The frozen tissue was first ground in a prechilled mortar and pestle under liquid nitrogen, and resuspended in 50 ml homogenization buffer (0.35 M sorbitol, 50 mM Tris–HCl pH 8.0, 25 mM EDTA, 15 mM 2-mercaptoethanol, 2 mM dithiotheital, 0.1% (w/v) polyvinlpyrolidone, 5 mM aurintricarboxylic acid). One-tenth lysis buffer (5% (w/v) sodium lauryl sarcosinate, 50 mM Tris–HCI pH 8.0, 25 mM EDTA) was added and the homogenate incubated for 5–15 min at room temperature. After lysis an equal volume of phenol:chloroform:isoamyl alcohol (25:24:1) was added to the homogenate and mixed thoroughly using a pasteur pipette. The mixed homogenate was centrifuged at 10,000×*g* for 15 min to remove the protein. The phenol/chloroform extraction was repeated 3–4 times depending on the protein content in the tissue. Finally, the top aqueous phase was recovered and 1/10 volume of 5 M ammonium acetate (pH 5.2) and 2 volumes absolute ethanol was added to the suspension and stored −70 °C for 1 h or at −20 °C overnight, to precipitate the total nucleic acids. It was observed that when RNA was precipitated from the total nucleic acids directly, the resultant RNA product was difficult to dissolve. This problem was circumvented by redissolving the total nucleic acid pellet into TE buffer (10 mM Tris, 1 mM EDTA) and adding 1/4 volumes of 10 M ammonium acetate, followed by centrifugation at 8000×*g* for 30 min. The supernatant was saved and the white pellet of polyuronic acid was discarded as described by Richards et al. (1991). Ten molar LiCl was added to the collected supernatant (to give a final concentration of 2.5 M LiCl), in order to selectively precipitate high molecular weight RNA. The precipitated RNA was pelleted by centrifugation at 10,000×*g* for 1 h. The RNA pellet was vacuum dried, resuspended in appropriate volume of diethylpyrocarbonate (DEPC) treated water and stored in aliquots at −70 °C.

2.3. RNA gel electrophoresis and northern blotting

Equal amounts of 4.5 and 7 μg of total RNA from each sample were denatured and fractionated on 1.2% (w/v) agarose-formaldehyde gels according to the procedure described by Sambrook et al. (1990). The loaded RNA samples were adjusted to such an extent that the 28S cytoplasmic rRNA bands as scanned by densitometry on photographic negatives, were equal for each stage. The RNA samples were fractionated for 12–16 h (at 17 mA, 20 volts hr) under a hood. The buffer was mixed regularly to maintain constant pH.

At the end of the run, the gel was stained with ethidium bromide (0.5 μg/ml in 0.1 M ammonium acetate) for 45–60 min, and later destained in DEPC water for at least 2 h. A transparent ruler was aligned with the gel and photographed by ultraviolet illumination. The photograph was used to measure the distance from the loading wells to each of the RNA bands. The $\log_{10}$ of the sizes of the RNA fragments were plotted against the distance migrated. The resulting curve was used to calculate the sizes of RNA species detected by hybridization after blotting (Richards et al., 1991).

The fractionated RNAs were transferred to nylon membrane by capillary method as described by Sambrook et al. (1990). The blotted RNA was immobilized by UV irradiation for 30 s at 6 wm^{-2}.

2.4. Labelling of probes and hybridisation

The 0.7 kb *Pst*I fragment *rbc*L gene specific probe isolated from pZMBIA (Gatenby et al., 1981) and the *psb*A specific gene probe from Cyanobacteria (Gingrich et al., 1988), were provided by Drs. Gatenby and Donald Bryant, respectively. The probes were ^{32}P labelled by the random primer labelling procedure. Typically, 50 ng of DNA probes were labelled to a specific activities of 10^5–10^8 counts per minute (cpm)/μg DNA. Unincorporated precursor dNTPS were removed from the radiolabelled by DNA Quick Spin Columns (G-50 sephadex).

Blots were prehybridized at 55 °C (for heterologous probes) or 65 °C (for homologous probes) in 0.5 M $NaHP0_4$, 7% (w/v) sodium dodecyl sulphate (SDS) for at least 40 min prior to hybridization. The purified probes were denatured in a boiling water bath for 5 min and immediately chilled on ice. Hybridization was performed overnight at 55 °C (for heterologous probes) and 65 °C (for homologous probes). The hybridized blots were washed once in preheated (to 55 °C for heterologous probes, and 65 0 °C for homologous probes) in 2.5% (v/v) SEN (2.5% (w/v) SDS, 1mM EDTA and 40 mM Na phosphate) for 10 min, and twice in preheated 0.5% (w/v) SEN (0.5% (w/v) SDS, 1mM EDTA and 40 mM Na phosphate) for 20 min each turn. The blots were sealed in plastic bags and autoradiographed by exposing them to X-ray film (Kodak XAR-2) at −70 °C with intensifying screens.

2.5. Data analysis

Autoradiogram X-ray films of the transcripts were scanned to determine levels of transcripts. Scans for *YYBB* representing the first variable and YYB^+B^+ the second variable, were determined at days −2, 0, 7, 10 and 14 to give the first set results and repeated in another experiment at days −2, 0, 3, 14 and 21. Both experiments showed a similar trends in the variation in steady-state amounts *psb*A and *rbc*L transcripts. However, the results from the second experiment are not reported in this paper. Comparisons of levels of *psb*A

and *rbc*L and apparent ratios *psb*A versus *rbc*L transcripts in Fordhook Zucchini were determined at days −2, 0, 3, 14 and 21 pre-and post-pollination. Apparent ratios of *psb*A and *rbc*L transcript were computed using data obtained from densitometric scan readings of transcripts and weighted based on levels of transcripts in the leaf tissues. In Bicolor Gourds the steady-state amounts of *rbc*L and *psb*A transcripts were determined on data derived from the same fruit at days 14 and 21 post-pollination.

3. Results

3.1. Variation in rbcL and psbA transcripts in Early Prolific fruits

Fig. 1 depicts the developmental form of plastids in four varieties of squash under study at different stages of fruit development. Steady-state levels of *rbc*L transcripts increased with fruit development from undetected amounts at 2 days before pollination (−2 days) to detectable amounts at pollination (0 days), and continued to increase at 7, 10 and 14 days post-pollination (Figs. 2a and b). During the differentiation of chloroplasts into chromoplasts at day 0 (Fig. 1), YYB^+B^+ did not produce more *rbc*L transcripts compared to *YYBB* (Fig. 2a and b).

Steady-state levels of *psb*A transcripts increased with fruit development from undetected amounts at −2 days to detectable levels at days, 0, 7, 10 and 14 (Fig. 3a and b). During differentiation of chloroplasts into chromoplasts in the YYB^+B^+ (at day 0) (Fig. 1), no significant differences were detected between the *YYBB* and the YYB^+B^+ lines in the steady-state amounts of *psbA* transcripts (Fig. 3a and b).

3.2. Variation in rbcL and psbA transcripts in Fordhook Zucchini

Steady-state levels of *rbc*L transcripts increased with fruit development from −2 days before pollination, levelled at days 3, 14 and slightly elevated at 21 days, post-pollination (Fig. 4a). The amounts of steady-state *rbc*L transcripts in leaf chloroplast containing tissues exceeded the amounts observed in the fruit chloroplast containing tissues in range of 8.6 times to 25 times at 3 and −2 days post- and pre-pollination, respectively (Table 1).

The steady-state amounts of *psb*A transcripts increased with fruit development from day −2 and remained stable at days 3, 14 and 21 (Fig. 4b). The amounts of *psb*A observed in the leaf chloroplast containing tissues exceeded the amounts observed in the fruit chloroplast containing tissues by a range of 2.8–58.8 times at 21 and −2 days, respectively (Table 1).

In Fordhook Zucchini, the amounts of *psb*A transcripts were lower than the amounts of *rbc*L at days −2 and 0 giving apparent ratios of 0.4 and 0.6, respectively. In contrast, at days 3, 14, and 21 fruits the apparent ratios of *psb*A versus of *rbc*L transcripts were ranged from 2.5 to 3.9 (Table 1), indicating that the expression of the two genes did not change in parallel.

3.3. Variation in rbcL and psbA transcripts in Bicolor Gourds

The amounts of steady-state *rbc*L transcripts in the fruit chromoplast containing tissues exceeded the quantities obtained in the fruit chloroplast containing tissues harvested at 14 and 21 days post-pollination, by 1.4 and 2 times, respectively (Fig. 5a; Table 2). Levels of *rbc*L transcripts in the leaf chloroplast containing tissues were in the range of 11.6–28.3 times higher than the amounts obtained in fruit chromoplast and chloroplast containing tissues at days 14 and 21, respectively (Table 2).

Levels of steady-state *psb*A transcripts in the fruit chromoplast containing tissues exceeded the amounts in the fruit chloroplast containing tissues by 1.2 and 2.4 times at days 14 and 21, respectively (Fig. 5b; Table 2). Amounts of *psb*A transcripts in the leaf chloroplast containing tissues were in the range of 4–8 times higher

Varieties	Days pre-Pollination	At pollination	Days post-pollination				
	-2	0	3	7	10	14	21
YYBB isogenic line	⊖	⊖	⊖	⊖	⊖	⊖	⊖
YYB^+B^+ isogenic line	⊕	⊕	⊖	⊖	⊖	⊖	⊖
Ford Hook Zucchini	⊕	⊕	⊕	⊕	⊕	⊕	⊕
Bicolor gourds:							
Yellow portion	⊖	⊖	⊖	⊖	⊖	⊖	⊖
Green portion	⊕	⊕	⊕	⊕	⊕	⊕	⊕

⊖: chromoplast containing tissue, ⊕: chloroplast containing tissue

Fig. 1. Changes in plastid types in fruit tissues of *Cucurbitae pepo* L. varieties with development.

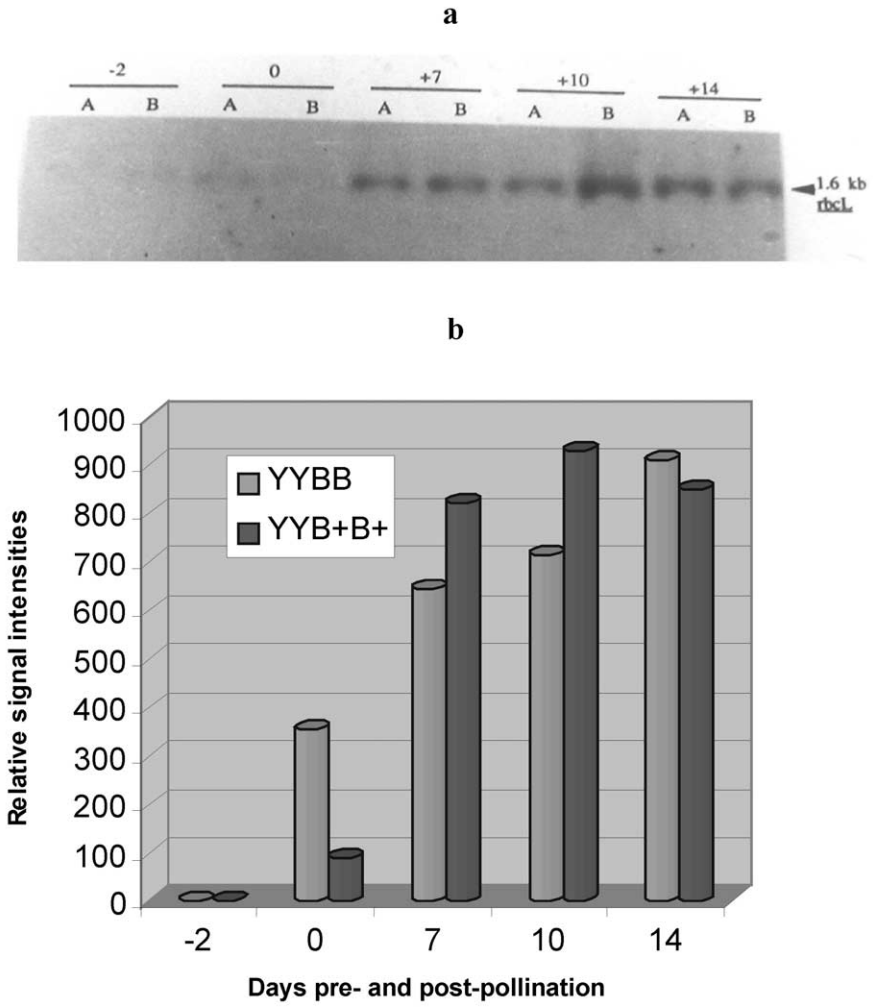

Fig. 2. (a) Northern blot analysis of the variation in steady-state amounts of *rbc*L transcripts in Early Prolific ovaries/fruits harvested at −2, 0, 7, 10, 14 days pre- and post- pollination. A: isogenic line *YYBB*, B: isogenic line $YYB^{+}B^{+}$. Seven micrograms of total RNA was loaded in each lane. The *rbc*L probe was ^{32}P-labelled to a specific activity of about 1.2×10^{8} cpm/g DNA. The blot was washed with high stringency (once with SEN for 10 min and twice in 0.5% (v/v) SEN for 20 min each) and exposed for 7 days with a screen at −70 °C. (b) Variation in the steady-state levels of the *rbc*L transcripts (in relative units based on densitometric scan of the blot in (a), normalized based on the intensities of the 28S cytoplasmic rRNA.

than the amounts obtained in fruit chloroplast/chromoplast containing tissues at days 14 and 21 (Table 2). The apparent ratios of *psb*A versus *rbc*L transcripts in Bicolor Gourd fruits were in the range of 2.9–4.5, implying that the expression of the two genes were not relatively constant at days 14 and 21 (Table 2).

4. Discussion

4.1. Developmental variation in rbcL transcripts

Two similar patterns of *rbc*L gene expression were observed in the Early Prolific and in the Fordhook Zucchini varieties of squash. In the Early Prolific the amounts of steady-state *rbc*L transcripts increased with fruit development up to 14 days post-pollination without a decline, while in Fordhook Zucchini fruits the *rbc*L transcripts reached a maximum early at 3 days and levelled out at days 14 and 21 post-pollination. The developmental regulation of the *rbc*L transcripts observed in the Fordhook Zucchini fruits was quite similar to that reported in chloroplast containing systems such as amaranth (Berry et al., 1986; Deng and Gruissem, 1987; Nikolau and Klessig, 1986), in spinach leaves (Deng and Gruissem, 1987) and in barley leaves (Mullet and Klein, 1987). In the four systems, Zucchini, spinach, barley and amaranth, the expression of the *rbc*L gene increased reaching a maximum, before declining or remaining stable. In spinach the developmental regulation of *rbc*L gene could be enhanced by the stability of the transcript rather than the rate of transcription per se (Deng and Gruissem, 1987).

In Early Prolific chromoplasts, unlike in the Fordhook Zucchini, the amounts of steady-state levels of the *rbc*L transcripts increased with fruit development and

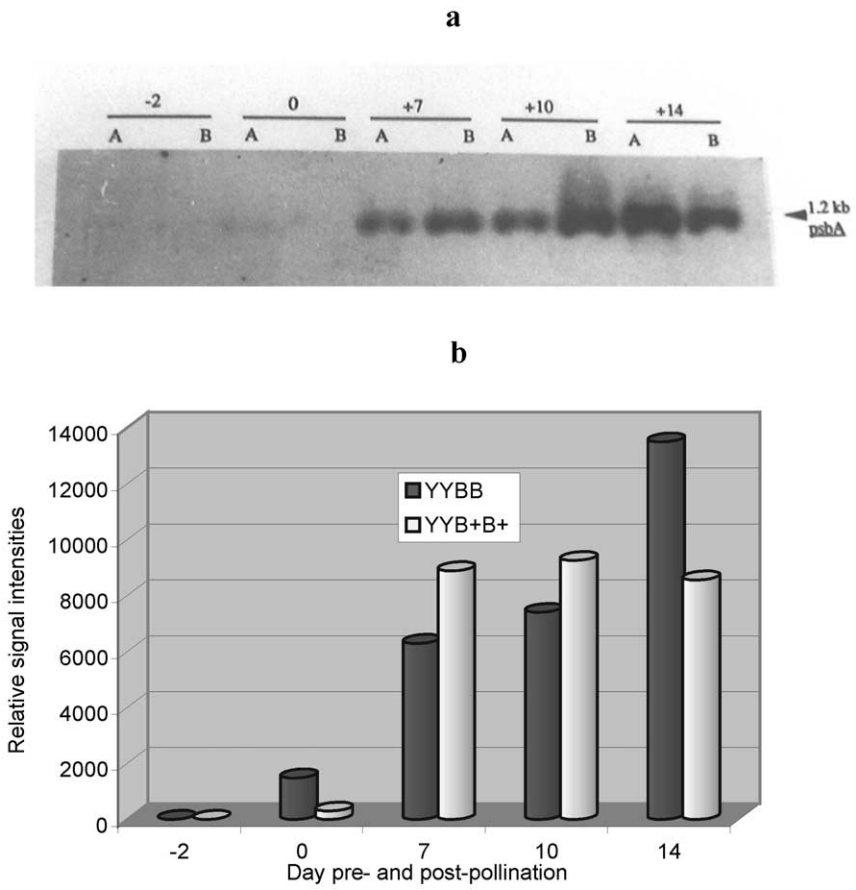

Fig. 3. (a) Northern blot analysis of the variation in steady-state amounts of *psb*A transcripts in Early Prolific ovaries/fruits harvested at −2, 0, 7, 10, 14 days pre- and post- pollination. A: isogenic line *YYBB*, B: isogenic line $YYB^{+}B^{+}$. Seven micrograms of total RNA was loaded in each lane. The *psb*A probe was ^{32}P-labelled to a specific activity of about 1.2×10^{8} cpm/μg DNA. The blot was washed with high stringency (once with SEN for 10 min and twice in 0.5% (v/v) SEN for 20 min each) and exposed for 7 days with a screen at −70 °C. (b) Variation in the steady-state levels of the *psb*A transcripts (in relative units based on densitometric scan of the blot in (a), normalization based on the intensities of the 28S cytoplasmic rRNA.

reached a maximum later, at days 10 days post-pollination. Developmental changes in the *rbc*L transcripts of ripening fruits have been reported in tomato and pepper. In developing tomato fruits, the *rbc*L transcripts were detected at day 7, culminated at days 11–19 and dropped drastically at days 31 and 35 (Piechulla et al., 1987). In contrast, in two tomato cultivars, the amounts of *rbc*L transcripts remained stable during ripening (Richards et al., 1991). Similarly, in ripening pepper and in maturing sunflower petals, the amounts of steady-state *rbc*L mRNAs remained constant during fruit ripening and flower petals maturation (Kuntz et al., 1989). Whereas results from the previous study obtained in tomato, pepper and sunflower unequivocally confirm the presence of *rbc*L transcripts in the chromoplasts, these levels could be attributed to *rbc*L mRNAs previously transcribed in the chloroplasts being maintained in the chromoplasts after transformation, since the levels either drastically dropped or were maintained constant during ripening. However, the results obtained in the Early Prolific especially the *YYBB* line confirm that the *rbc*L gene was transcribed in the chromoplast tissues of fruits and the elevated levels in developing fruits were either due to increased transcription rate or stability of the *rbc*L gene transcripts in the chromoplasts or both.

4.2. Developmental variation in psbA transcripts

The amounts of the *psb*A transcripts increased with fruit developmental in both the Fordhook Zucchini and Early Prolific fruits. Developmental regulations of the plastid genes have been documented in spinach and in barley (Deng and Gruissem, 1987; Deng et al., 1987). In spinach the differential high expression of psbA gene has been attributed to enhanced RNA stability of the transcript (Deng and Gruissem, 1987). The *psbA* gene and other chloroplast genes have been reported to have inverted repeats (IRs) in the 3′ region which can form stem and loop structures. The IR has been reported to contribute to the transcript stability (Deng and Gruissem, 1987). Kuntz et al. (1989), reported a similar gradual elevation in the levels of *psb*A transcripts in the chloroplasts during pepper maturation and in maturing

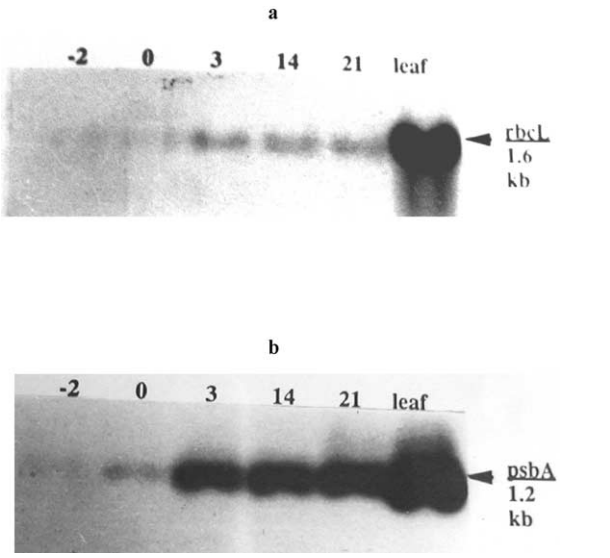

Fig. 4. (a and b) Northern blot analysis of the variation in steady-state amounts of *rbc*L and *psb*A transcripts in Fordhook Zucchini fruits and leaf tissues harvested at −2, 0, 3, 14 and 21 days pre- and post-pollination. Seven micrograms of total RNA was loaded in each lane. *rbc*L and *psb*A probe was ^{32}P-labelled by to a specific activity of about 1.2×10^{8} cpm/μg DNA. The blot was washed with high stringency (once with SEN for 10 min and twice in 0.5% (v/v) SEN for 20 min each) and exposed for one day with a screen at −70 °C.

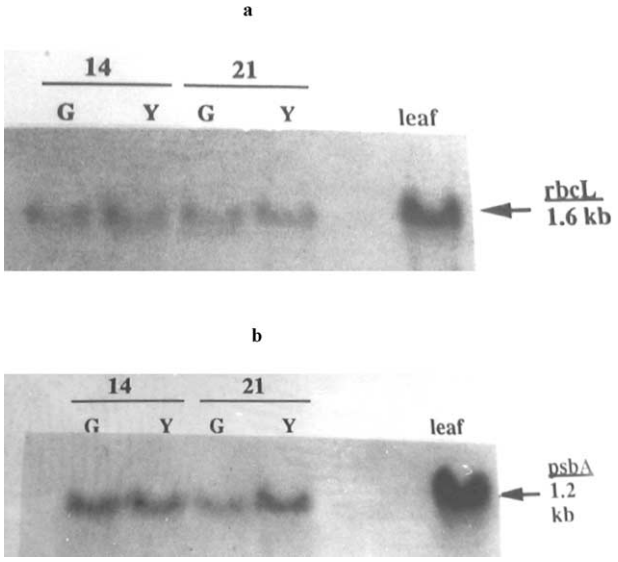

Fig. 5. (a and b) Northern blot analysis of the variation in steady-state amounts of *rbc*L and *psb*A transcripts in Bicolor Gourds fruits and leaf tissues harvested at 14 and 21 days pre- and post-pollination. Four and a half grams of total RNA was loaded in each lane. The *rbc*L probe was ^{32}P-labelled. The blot was washed with high stringency (once with SEN for 10 min and twice in 0.5% (v/v) SEN for 20 min each) and exposed for one day with a screen at −70 °C. G: green chloroplast containing tissue taken from the same fruit as Y. Y: the chloroplast containing tissues taken from the same fruit as G.

sunflower petals. In a run-on assay they reported transcription of the *psb*A gene in the chromoplasts and chloroplasts of bell pepper fruits. However, the transcription of the *psb*A gene followed the variation in the steady-state amounts of the transcript, suggesting that transcription was the primary control point for the levels of the *psb*A transcripts in the chromoplasts.

Lack of correspondence between the *psb*A *and rbc*L genes and the different plastid types observed in the Early Prolific fruits (chromoplasts versus chloroplasts) suggest that the two processes, namely the *rbc*L gene expression and plastid differentiation, were regulated independently of one another. The timing of the appearance of the chloroplasts and the chromoplasts is controlled by the *B* and the *Y* genes but not the structure or the content of the plastids (Boyer, 1989; Lim, 1990; Scaffer, 1982). In addition, Shiffriss (1981) hypothesized that the *B* gene acts as the timer which with the *Y* gene controls the blockage or the breakdown of chlorophyll synthesis. Results from the current study support the view that the *B* and the *Y* genes are timer genes for development.

4.3. Similarities in rbcL and psbA transcripts between the Early Prolific isogenic lines

The *YYBB* and the $YYB^{+}B^{+}$ isogenic lines had approximately comparable amounts of both the *rbc*L and the *psb*A transcripts. In contrast to this observation, plastid transcripts have been reported to differ between different cultivars of the same species (Piechulla et al., 1985; Richards et al., 1991). For example in the tomato cultivar (Vent), the levels of *psb*A and *rbc*L

Table 1
Apparent ratios of steady-state amounts of *psb*A transcripts compared to *rbc*L transcripts in fruit and leaf chloroplast containing tissues of Fordhook Zucchini

Days pre-post-pollination	Plastid type	Amounts of *rbc*L transcripts	Ratio of *rbc*L in leaves/fruits	Amounts of *psb*A transcripts	Ratio of *psb*A in leaves/fruits	Apparent ratio of *psb*A/*rbc*L in fruits
−2	Cl	1651	25.0	549	58.8	0.4
0	Cl	2890	14.2	1420	22.7	0.6
3	Cl	4790	8.6	9267	3.5	2.5
14	Cl	3840	10.8	9460	3.4	3.4
21	Cl	3840	10.8	11,721	2.8	3.9
Leaves	Cl	41, 296		32, 290		

The *psb*A *and rbc*L signals were scanned with a densitometer. Cl: fruit and leaf chloroplast containing tissues.

Table 2
Relative amounts of amounts of steady-state *rbc*L *and psb*A transcripts in chromoplast and chloroplast tissues of Bicolor Gourds fruits and leaves

Tissue type	Plastid type	Relative *rbc*L signal[a]	Ratio of *rbc*L in Cm/Cl	Ratio of *rbc*L in leaves/fruits	Relative *psb*A signal[a]	Ratio of *psb*A in Cm/Cl	Ratio of *psb*A in leaves/fruits	Apparent ratio of *psb*A /rbcL in fruits
Fruit at 14 days	Cl	3250		16.7	5110		4.8	3.5
	Cm	4670	1.4	11.6	6189	1.2	4.0	2.9
Fruit at 21 days	Cl	1910		28.3	3063		8.0	3.6
	Cm	3870	2.0	13.9	7999	2.4	3.1	4.5
Leaves	Cl	54,000			24, 524			

[a] The *rbc*L signals were scanned with a densitometer. Cm: fruit chromoplast containing tissues; Cl: fruit and leaf chloroplast containing tissues.

transcripts decreased with fruit development, while in another cultivar (Firstmore), the levels of *psb*A transcripts remained constant while the *rbc*L decreased (Piechulla et al., 1985, 1986). In another study, the levels of *psb*A mRNAs remained constant in tomato cultivar Firstmore and decreased in Count II (Richards et al., 1991). The two studied Early Prolific lines have distinct plastid structures up to 21 days post-pollination and thereafter the chromoplasts are identical in structure and carotenoid composition (Scaffer, 1982; Lim, 1990). The only discrepancy was that the YYB^+B^+ fruits grew slightly slower than the *YYBB* (Scaffer, 1982).

4.4. Relative abundance of psbA and rbcL transcripts in the Fordhook Zucchini and Bicolor Gourds

The apparent levels of the *psb*A transcripts were consistently higher than the *rbc*L in fruits and leaf tissues of the three squash varieties studied. In spinach the *psb*A gene was relatively more expressed than the *rbc*L and the *atp*B genes (Deng et al., 1987). In spinach the higher expression of the *psb*A was attributed to the strong promoter of the gene. Sequences of the promoter region of the *psb*A gene in wheat and six dicots closely resembled the prokaryotic-35 and -10 consensus elements (Hanley-Bowdoin and Chua, 1988).

5. Conclusions

The steady-state levels of the *psb*A and *rbc*L transcripts in the three varieties of squash studied: Early Prolific, Fordhook Zucchini and Bicolor Gourds ovaries/fruits were regulated by fruit developmental patterns. The *psb*A gene was apparently several times more highly expressed than the *rbc*L gene in the fruit chromoplast and chloroplast containing tissues of Bicolor Gourds. The expression of *rbc*L gene in YYB^+B^+ and *YYBB* isogenic lines did not significantly differ from one another during fruit development. Similarly, the two isogenic lines did not differ from one another in the expression of *psb*A gene during fruit development. Therefore, the presence of fruit chloroplast containing tissue in early developmental stages of the YYB^+B^+ isogenic line compared to chromoplast containing tissue in *YYBB* did not cause significant differences in the expression of the two photosynthetic genes between the two lines. Even more surprisingly, in Bicolor Gourds fruits the steady-state levels of the *psb*A and *rbc*L transcripts in the fruit chromoplast containing tissues slightly exceeded the amounts obtained in the fruit chloroplast containing tissues in the same fruit.

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