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BRIEF COMMUNICATION

Irradiation-regulated differential accumulation of chloroplast-encoded transcripts in mature leaves of *Populus deltoides*

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Abstract

Steady state transcript levels of *psbA*, *rbcL*, *atpB*, and *psbN* genes accumulated differentially in mature leaves of a field-grown tree *Populus deltoides* during natural diurnal cycle and in dark adaptation followed by irradiation. The *rbcL* transcript accumulated independent of irradiation under diurnal conditions, which demonstrated a correlation with the phenomenon of midday depression in photosynthesis. The *psbA* and *atpB* transcripts accumulated more in dark whereas *psbN* accumulated more in light. Diurnal rhythm of gene expression in mature chloroplasts was independent of development related changes.

Additional key words: dark adaptation; diurnal cycle; midday depression; Northern analysis; ribulose-1,5-bisphosphate carboxylase/oxygenase.

In higher plants, the expression of chloroplast genes and steady states of their transcripts fluctuate with developmental stages (Deng and Gruissem 1987, Dixit *et al*. 2002), diurnal variations and light-dark (L-D) cycles (Piechulla 1988, Kapoor *et al.* 1994, Reddy *et al*. 2000, Trivedi *et al*. 2000). Most of the studies on chloroplast gene expression have been related to differential accumulation of transcripts and polypeptides during L-induced greening of etiolated seedlings and biogenesis of chloroplasts (Kapoor *et al*. 1994, Mullet 1994). Hence, these studies may not provide conclusive information on the gene expression in a mature chloroplast under field conditions. We examined the steady state levels of chloroplast transcripts in attached mature leaves of field grown *Populus deltoides* under diurnal and variable irradiation in order to correlate it with photosynthetic behaviour of the plant. We chose four genes encoding polypeptides associated with different complexes and functions, namely *psbA* encoding the Q_B binding thylakoid membrane protein of photosystem 2 (PS2), *rbcL* encoding the large subunit of stromal ribulose-1,5-bisphosphate carboxylase/oxygenase (RuBPCO), *atpB* encoding the β-subunit of plastid ATP synthase, and *psbN* encoding the 3.8-kDa protein of PS2.

The study was done with field grown *Populus deltoides* cv. Stoneville, clone D121, in May–June (sunrise 05:00–05:30, sunset 18:30–19:00) when the photosynthetic photon flux density (PPFD>1 200 μmol m^{-2} s^{-1}) saturates around 09:00 and the atmospheric CO_2 concentration varies between 345–360 cm^3 m^{-3}. Mature green leaves were harvested and immediately frozen in liquid nitrogen after every 4 h during the 24-h period starting from 01:00 h. For examining L-D influenced changes, the plant was covered with thick layers of black cloth for 24 h (starting at 13:00 on one day to 13:00 next day). The leaves were harvested after the dark exposure (DD) for 12 h and after exposing dark-adapted leaves to natural daylight for two hours (DD2L). Time points at 13:00 and 01:00 h were considered as L and D, respectively, in this experiment. Total RNA from each sample was isolated following the procedure as described by MacDonald *et al*. (1987). Twenty μg of total RNA from each sample was electrophoresed on a 1.2 % denaturating formaldehyde-MOPS-agarose gel followed by transfer onto a hybond membrane (*Amersham Pharmacia*). Hybridization of blot was carried out using radio-labelled homologous gene probes prepared by the random priming method. The 448 bps amplified region of *psbA* (accession no. X78204), 304 bps amplified region of *psbN* (accession no. Y13328), 707 bps amplified region of *atpB*, 468 bps

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amplified region of *rbcL* (accession no. AF133677), and a 275 bps fragment from 23S rDNA (accession no. AY029747) from *P. deltoides* were used as probes. Prehybridization, hybridization, and washing of blots were performed as described by Sambrook *et al*. (1989). Blots were exposed to X-ray films for 3 to 5 d followed by analysis in *UltroScan XL* (*LKB Pharmacia*) densitometer.

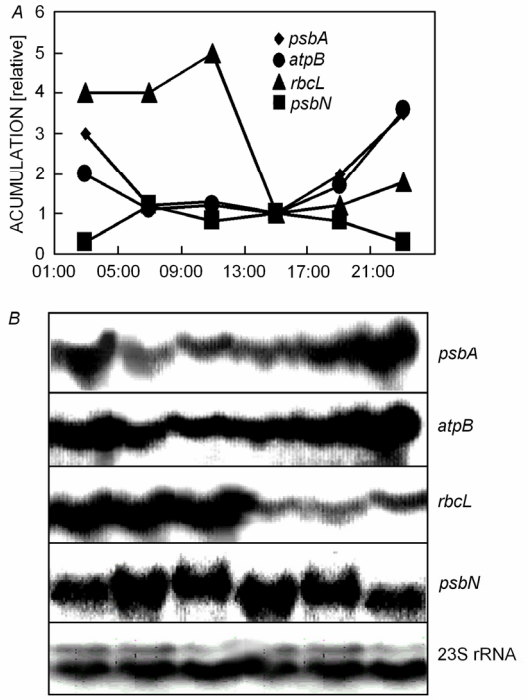

Fig. 1. Northern hybridization analyses of *psbA, atpB*, *rbcL,* and *psbN* transcripts during diurnal cycle. (*A*) Line graph showing variations in the steady state amounts of the four transcripts during the diurnal cycle. (*B*) Autoradiograms showing differential accumulation of *psbA, atpB*, *rbcL, psbN*, and *23S rDNA* transcripts during the diurnal cycle. Northern blot was probed with 23rDNA to confirm equal loading.

The changes in steady state levels of different transcripts during different time points in a day are shown in Fig. 1. Steady state accumulation of *psbA* and *atpB* transcripts followed a more or less similar pattern. These transcripts accumulated more in D (between 21:00 and 01:00) than in L (05:00 to 17:00). An approximately 2-fold increase was observed between 17:00–21:00, *i.e*. before and after the sunset. But the second set of experiment revealed a reverse effect of irradiation. There was an actual increase in the amounts of *psbA* and *atpB* transcripts in D-adapted leaves, which further increased with leaf irradiation (Fig. 2). Irradiation-induced increase in the rate of transcription of several photosynthetic genes has been demonstrated by Kapoor *et al*. (1994). The low levels of transcript pool in day for these two genes were probably due to rapid post-transcriptional processing or usage, which might result in a lower accumulation and not due to low rates of transcription.

The *rbcL* transcript accumulation showed a different pattern independent of diurnal condition. The transcript levels remained more or less constant between 01:00–09:00 h (Fig. 1). There was a steep decline after that with minimum at 13:00 h, *i.e*. at the time of midday depression in photosynthesis. The accumulation pattern of transcripts and polypeptides of chloroplast encoded PS2 genes was probably not associated with midday depression in photosynthesis (Trivedi *et al*. 2000). However, the steep decline in *rbcL* transcript level at the time of midday depression of photosynthesis suggests that *rbcL* gene expression might play a role in midday depression. This is the first study demonstrating the expression of any photosynthetic gene to be correlated with midday depression in photosynthesis. Though there was some increase after sunset, it was not as large as observed in the cases of *psbA* and *atpB*. In contrast to *psbA* and *atpB* transcript pool, *rbcL* transcript level did not show decrease between 21:00–01:00 but rather an increase. Similar to *psbA* and *atpB*, the amount of *rbcL* transcript also increased in dark adapted leaves which further increased after irradiation.

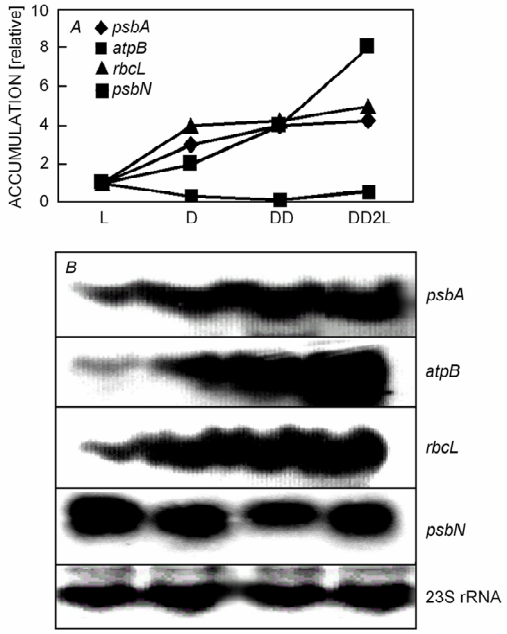

Fig. 2. Northern hybridization analyses of *psbA, atpB*, *rbcL*, and *psbN* transcripts under experimental light/dark (L/D) conditions. (*A*) Line graph showing variations in the steady state levels of the four transcripts under experimental L/D. (*B*) Autoradiograms showing differential accumulation of *psbA, atpB*, *rbcL, psbN,* and *23S rDNA* transcripts under experimental L/D. Northern blot was probed with 23rDNA to confirm equal loading. DD = dark adapted, DD2L = dark adapted + 2 h of daylight.

Even though the *psbN* gene codes a polypeptide associated with PS2, as does *psbA*, it showed an entirely different pattern of accumulation. The *psbN* transcript accumulation was higher in L than in D. There was an approximately 3-fold increase in accumulation during the

L hours (05:00–17:00) than D hours (21:00–01:00) of the day, with negligible fluctuations within these hours. Unlike the other three transcripts, the *psbN* transcript level had reduced to one-fifth during the 24-h D adaptation (DD). Irradiation of dark-adapted leaves (DD2L) resulted in about 3-fold increase in the *psbN* transcript pool.

The increase in accumulation of *psbA*, *rbcL*, and *atpB* transcripts after 24 h of extended D suggests that the transcription of these genes continues even in the dark. The higher steady state level of *psbN* transcript in L than in D suggests that transcription rate and/or stability of this transcript is higher in L than in D. The extended D adaptation caused a decrease in *psbN* transcript pool, which increased after irradiation. This suggests that transcription rate of *psbN* is directly dependent on the availability of radiant energy. Hence we demonstrate that chloroplastic gene expression in mature and field-grown leaves is highly variable amongst themselves. Though number of genes may follow rhythmicity due to diurnal variation, their adaptation to D and/or transient irradiation may alter the rhythm, which could be linked to variations incurred in post-transcriptional processing.

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