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Regulation of plastid gene expression by high temperature during light induced chloroplast development

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Abstract

Effect of high temperature on the plastid gene expression during the light induced chloroplast development in etiolated seedlings was determined by Northern hybridisation using cloned DNA fragments of wheat chloroplast genome. Based on their response to high temperature, plastid genes were grouped into three categories: (*1*) plastid genes whose expression was not affected by high temperature (genes for rRNA, ribosomal proteins, tRNAs, and some genes coding for putative NADH dehydrogenase); (*2*) plastid genes whose expression increased at high temperature (genes coding for α-subunit of RNA polymerase and some unidentified transcripts, and (*3*) plastid genes whose expression decreased at high temperature (genes coding for proteins involved in photosynthetic process). Loss of a number of primary transcripts originating from operons consisting of genes that code for proteins involved in the photosynthetic process was observed. The expression of all the light inducible plastid genes was inhibited suggesting that the light inducibility property was lost at high temperature.

Additional key words: biogenesis; photosynthesis; wheat; *Triticum aestivum*.

Introduction

High temperature exerts a pronounced effect on the growth of plants (Berry and Björkman 1980). There is unequivocal evidence that prior to the impairment of other cell functions, chloroplast functions are irreversibly damaged (Berry and Björkman 1980). High temperature induces marked inhibition in photosynthesis due to disruption of the functional integrity of photosystem 2, PS2 (Mamedov *et al.* 1993, Singh and Singhal 1999), loss of chlorophyll, Chl (Smillie *et al.* 1978, Thomas and Ortiz 1995), and non-development of plastid ribosomes (Feierabend and Mikus 1977). Among these, loss of photosynthetic activity is most sensitive and has been studied in detail (Mamedov *et al.* 1993, Singh and Singhal 1999).

Photosynthesis, together with other plastid functions, requires the products from both chloroplast and nucleus. The complete nucleotide sequence of chloroplast DNA from various organisms revealed the existence of *ca.* 130 genes. About half of these are involved in plastid protein synthesis and about 30 code for subunits of the four supramolecular complexes involved in photosynthetic process. Other proteins required for various chloroplast functions are synthesised in cytoplasm and transported to chloroplast.

The synthesis of nuclear-coded chloroplast proteins is co-ordinated with the protein synthesis in chloroplasts. Understanding of this co-ordination between chloroplast and nucleus is therefore essential to explain the overall maintenance of chloroplasts. Different strategies including mutants, inhibitors of protein synthesis, and high temperature induced loss of 70S plastid ribosomes have been used to understand this process (reviewed in Feierabend and Berberich 1991). High temperature induced loss of 70S plastid ribosome offers a unique experimental approach as it specifically eliminates plastid translation and hence is an ideal system to study interactions between chloroplast and nucleus.

Induction of ribosome-deficient plastids and Chl bleaching in light-grown plants occurs after their several-hour treatment with high temperature (Falk *et al.* 1993). Contrary to light-grown plants, dark-grown plants contain

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Abbreviations: Chl, chlorophyll; PS2, photosystem 2; SDS, sodium dodecyl sulphate.
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etioplasts and lack most of the plastid functions. Irradiation of etiolated seedlings leads to the conversion of etioplasts into chloroplasts and activation of a number of plastid functions including rapid accumulation of Chl, photosynthetic membranes and associated proteins, activation of plastid transcription, and the increase of abundance plastid transcripts (Mullet 1988, Gruissem 1989, Baumgartner *et al.* 1993). The analysis of light-induced chloroplast development in etiolated seedlings therefore offers an excellent system to study the co-ordination between chloroplast and nucleus. Our previous study showed that irradiation of etiolated seedlings at 25 °C resulted in the decrease of Chl *a/b* ratio, and in accumulation of Chl and PS2-related proteins. However, none of these events were observed during irradiation of etiolated seedlings at 38 °C suggesting that light-induced development of chloroplast was inhibited by high temperature (Singh and Singhal, unpublished). It was our interest, therefore, to study the expression of plastid genes during chloroplast development at this restricted temperature.

Materials and methods

Plant growth: Wheat seeds (*Triticum aestivum* L. cv. HD-2329) were pre-soaked in distilled water for 12 h and then germinated on moist germination paper in dark at 25 °C for 5 d in controlled environmental chamber. Thereafter, etiolated seedlings were transferred to a plant growth chamber irradiated by 50 μmol(photon) m^{-2} s^{-1} maintained at either 25 (for 8 h) or 38 °C (for 4 and 8 h). After completion of this treatment, the primary leaves were harvested and immediately frozen in liquid nitrogen. Harvesting of etiolated seedlings was done under dim green radiation.

RNA isolation and Northern blotting: Total RNA from the seedlings was isolated essentially as described by Choniczynski and Sacchi (1987). RNA concentration was determined spectrophotometrically at 260 nm using an extinction coefficient of 25 m^{-3} kg^{-1}. 10 μg of total RNAs was used for Northern blotting. Total RNA was denatured, fractionated on 1.2 % agarose-formaldehyde gels, and blotted to nylon membrane by the capillary transfer method as described in Sambrook *et al.* (1989). Blots were stained with the methylene blue to mark the standard RNA molecular mass marker (*Promega Co.*). All prehybridisation and hybridisation reactions were carried out as described in Sambrook *et al.* (1989). Washing was carried out twice in buffer containing 2×SSC, 0.1 % SDS for 5 min followed by washing twice in buffer containing 0.1×SSC, 0.1 % SDS for 10 min at 68 °C.

DNA probe preparation: The cloned DNA fragment of wheat chloroplast genome in pBR322 was kind gift of Dr. E. Kellog. DNA fragments covering almost the complete chloroplast genome from various plasmids were digested with appropriate restriction enzymes and purified using low melting agarose as described in Sambrook *et al.* (1989). Purified DNA fragments were radio-labelled with a nick translation kit (*Promega Co.*). Some of the DNA fragments (B18, B20, B21, and B25) were radio-labelled using Random primer labelling kit (*Promega Co.*).

Results

Location of genes on various DNA fragments of wheat chloroplast genome: Fig. 1*A* shows the location of various fragments on the wheat chloroplast genome (after Bowman *et al.* 1981). Most plastid genes are organised into polycistronic transcription units. The sequence analysis of the chloroplast genome from various organisms has revealed that the arrangement of genes within these transcription units is highly conserved although transcription units are rearranged in some plant species (Palmer and Thompson 1982). Comparison of the known location of plastid genes in wheat suggests similar arrangement of genes as in rice plastid genome. Besides, plastid genome size of wheat and rice is similar in size (135 kb) and a 20 kb inversion found in maize and wheat has also occurred in rice chloroplast genome (Quigley and Weil 1985, Hiratsuka *et al.* 1989). The nucleotide sequence of wheat chloroplast gene coding for the α-subunit of RNA polymerase (*rpo*A) is largely contained within the B20 fragments (Hird *et al.* 1989). The various genes on different fragments of wheat chloroplast genome have been assigned following walking upstream and downstream of *rpo*A gene on rice chloroplast genome. This yielded the similar location of genes on the fragments of wheat chloroplast genome which have been characterised separately (see legend of Fig. 1*B*).

Effect of high temperature on plastid gene expression was studied using various cloned DNA fragments of wheat chloroplast genome. Based on their response, plastid genes have been categorised into three groups:

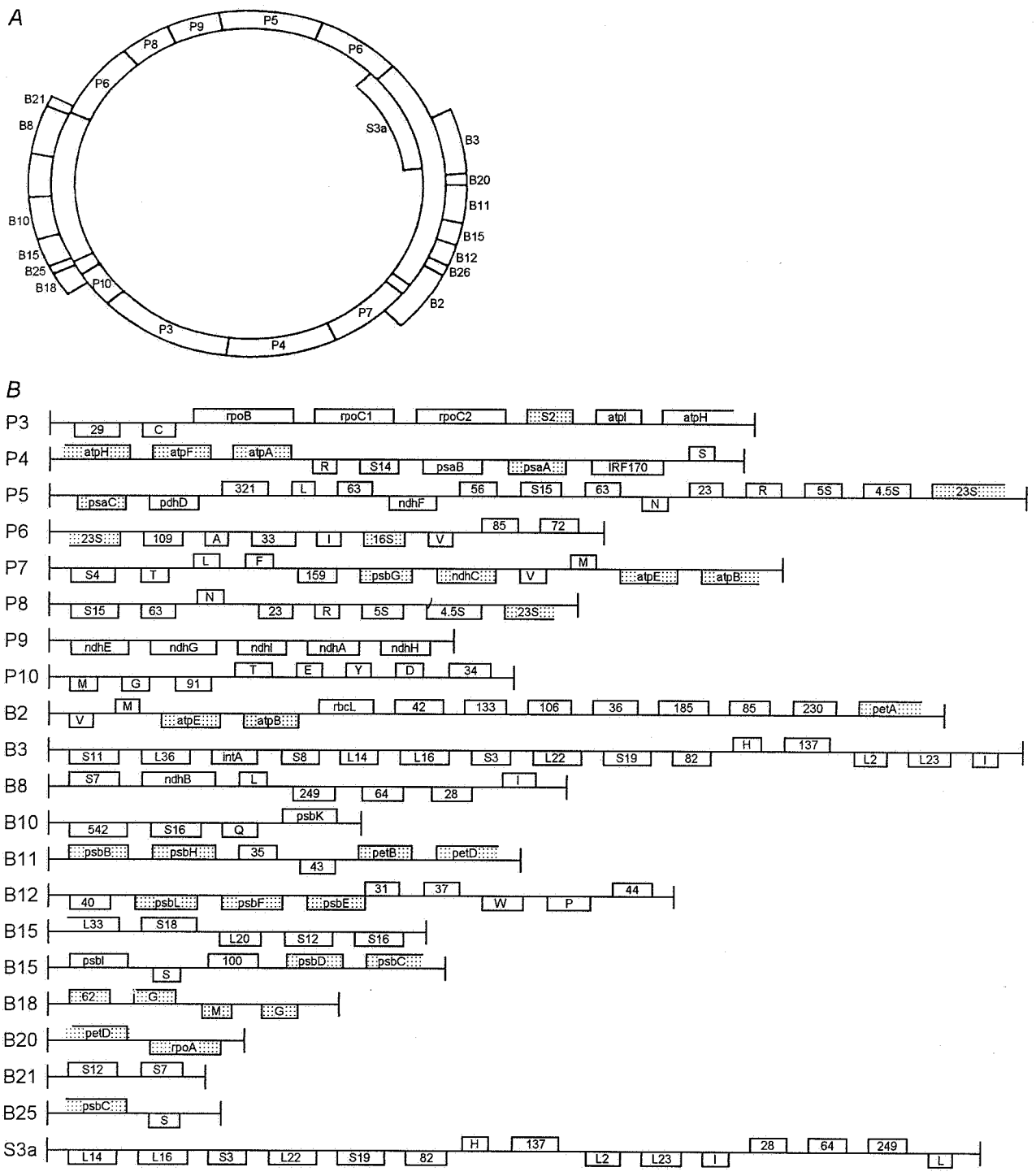

Fig. 1. Map of wheat chloroplast genome. (*A*) Localisation of various DNA fragments on wheat chloroplast genome. (*B*) Localisation of genes on the DNA fragments of chloroplast genome (for detail see text). The plastid genes localised in wheat, *i.g.*, 16S, 23S, *rbc*L (Bowman *et al.* 1981); *atp*F (Bird *et al.* 1985); *atp*H, *atp*A, *atp*β, *atp*ε (Howe *et al.* 1985); *psb*E-F-L (Webber *et al.* 1989); *psb*H (Hird *et al.* 1986); *psb*B, *psb*C, *psb*D, *psa*A, *pet*A, *pet*B, *pet*D (Courtice *et al.* 1985); *psa*C (Dunn and Gray 1988); *rpo*A (Hird *et al.* 1989); S2 (Höglund and Gray 1987); *psb*G, *ndh*C (Nixon *et al.* 1989), and ORF62, *trn*G, *trn*M, *trn*G (Quigley and Weil 1985) have been shaded. Unfinished rectangle at the end of fragments indicates that gene is also present on other fragment. The numerical num-bers denote the number of codons in an open reading frame. tRNAs carrying different amino acids are represented by assigning the one letter name of amino acid carried by the respective tRNA. The genes coding for proteins of small and large subunit of ribosome are given by the polypeptide number preceded by letter L for Large subunit and S for Small subunit.

(*1*) Plastid genes whose expression decreased at high temperature

***Psb*A**: *psb*A mRNA is expressed monocistronically in higher plants. The Northern blot analysis of *psb*A mRNA is shown in Fig. 2*A*. In etioplasts, a single transcript of 1.3 kb was detected, the concentration of which increased following irradiation of etiolated seedlings at 25 °C. However, *psb*A mRNA failed to accumulate when etiolated seedlings were irradiated at 38 °C.

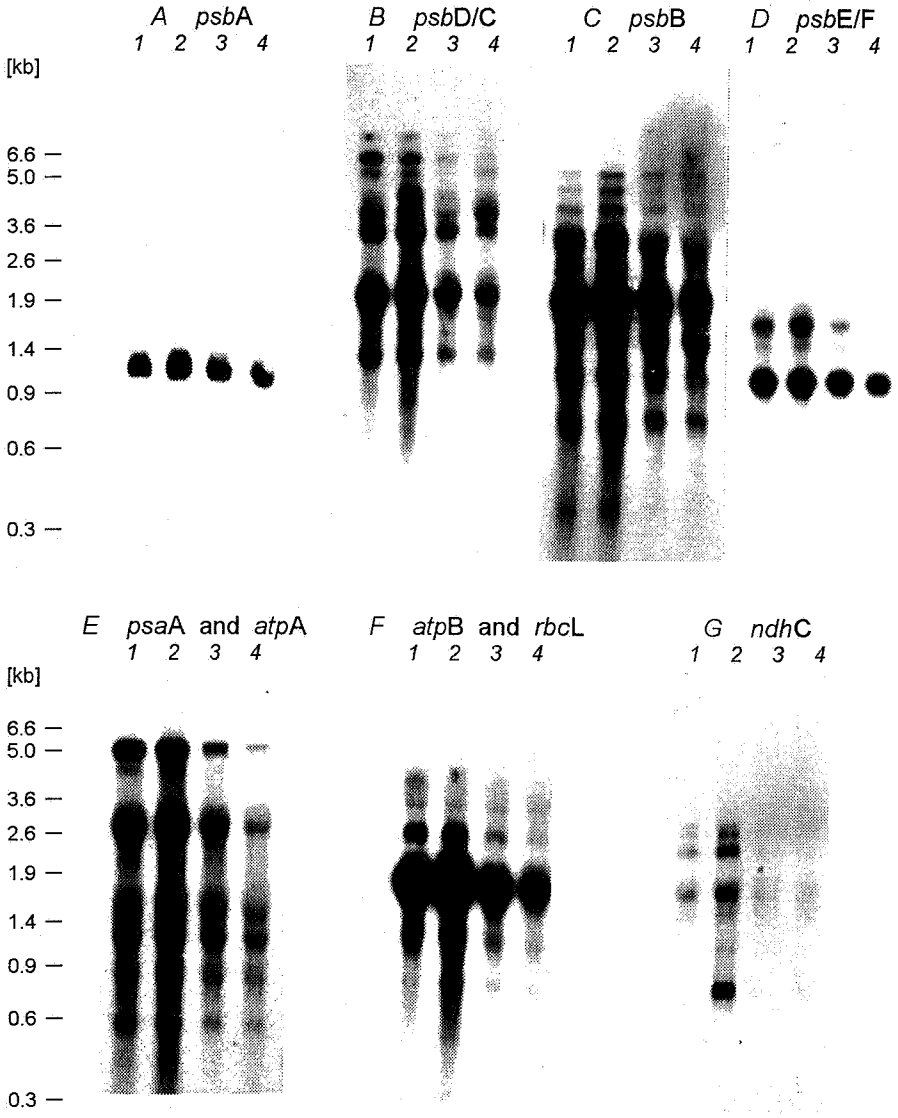

Fig. 2. Expression of plastid genes. Total RNAs isolated from dark grown seedlings (lane *1*), dark grown seedlings irradiated (50 μmol m^{-2} s^{-1}) at 25 °C for 8 h (lane *2*), dark grown seedlings irradiated at 38 °C for 4 h (lane *3*) and 8 h (lane *4*) were fractionated on 1.2 % formaldehyde-agarose gel. RNAs were transferred on nylon membrane by capillary transfer method and subjected to hybridisation with *respective* fragments gene: (*A*) *psb*A, (*B*) *psb*D/C, (*C*) *psb*B, (*D*) *psb*E/F, (*E*) *psa*A and *atp*A, (*F*) *atp*B and *rbc*L, and (*G*) *ndh*C.

***psb*D/C operon**: Analysis of transcripts originating from *psb*D/C operons revealed a total of eight transcripts of 7.5, 5.9, 5.0, 3.9, 3.4, 1.9, 1.5, and 1.3 kb in the etiolated seedlings (Fig. 2*B*). When etiolated seedlings were irradiated at 25 °C, a novel transcript of 4.4 kb appeared and the 3.4 kb transcript level increased. Light-induced accumulation of 4.4 and 3.4 kb has been shown by Sexton *et al.* (1990). However, when etiolated seedlings were irradiated at 38 °C, the various transcripts originating from *psb*D/C operon showed complex behaviour. As such, the level of transcripts of 7.5, 5.9, 3.4, 1.9, 1.5, and 1.3 kb decreased during irradiation of etiolated seedlings at 38 °C. Transcripts of 5.0 and 3.9 kb showed mixed behaviour; initially they disappeared, however, longer period of high temperature treatment resulted in the reappearance of these transcripts. Besides it, the novel light-

induced transcripts were also sensitive to high temperature, and after 4 h of treatment both the 4.4 and 3.4 kb transcripts were lost.

***psb*B operon**: Analysis of transcripts originating from *psb*B operon revealed a total number of 10 transcripts of size 5.4, 4.7, 3.9, 3.2, 2.5, 1.9, 1.6, 1.0, 0.75, and 0.4 kb in the etiolated seedlings (Fig. 2*C*). Irradiation of etiolated plants at 25 °C caused overall increase in the level of all transcripts, however, no new transcripts were detected. When the etiolated seedlings were irradiated at 38 °C, transcripts of 5.4, 4.7, 3.9, 3.2, and 2.5 kb disappeared. More drastic disappearance was observed in the case of 4.7, 3.2, and 2.5 kb transcripts.

***psb*E operon**: The *psb*E operon is localised on B12 fragment in wheat plastid genome (Fig. 1*B*). Few ORFs are also present. Northern blot analysis of RNAs isolated from etiolated seedlings using B12 fragments revealed two transcripts of 1.1 and 1.7 kb and one minor transcript of 1.5 kb (Fig. 2*D*). Irradiation of etiolated seedling at 25 °C led to increase in 1.7 kb transcript level, however, no effect of irradiation on 1.1 kb transcript was observed. When the irradiation was carried out at 38 °C, the level of

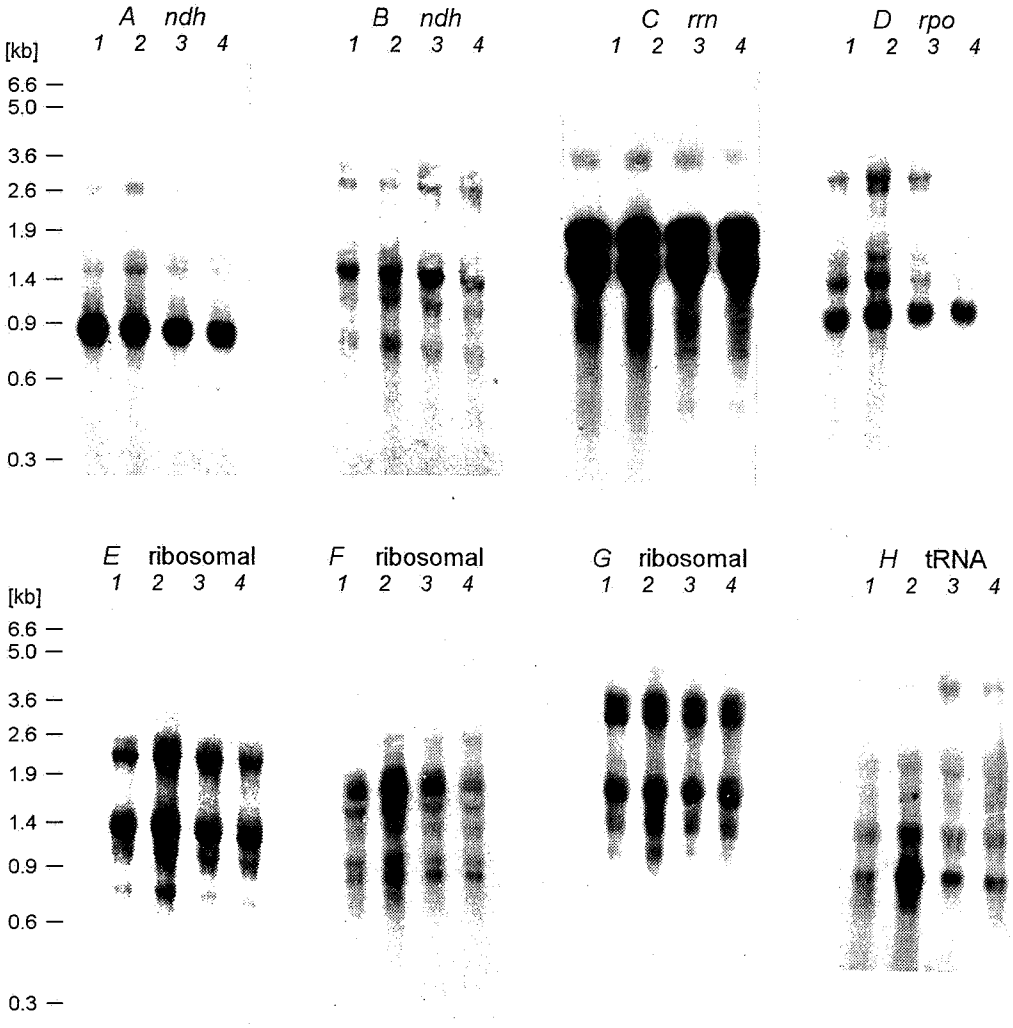

Fig. 3. Expression of plastid genes. Experimental details are the same as in Fig. 2. (*A, B*) *ndh* genes, (*C*) *rrn* genes, (*D*) *rpo* genes, (*E, F, G*) ribosomal genes, (*H*) tRNA genes.

the transcripts decreased as compared to control.

***psa*A and *atp*A operons**: Both these operons are localised on P4 fragment in wheat plastid genome (Fig. 1*B*). The Northern blot analysis of RNAs isolated from etiolated seedlings using this fragment revealed six transcripts of 5.2, 2.7, 1.6, 1.2, 0.9, and 0.6 kb (Fig. 2*E*). Minor transcripts of 4.7 and 0.7 kb were also detected in the etiolated seedlings. Level of all six major transcripts slightly increased during irradiation of etiolated seedlings at 25 °C, however, no new transcript appeared from either operon. It could be possible that appearance of a new transcript originating from one operon is compensated by decreased content/disappearance of a transcript from another operon. When etiolated seedlings were irradiated at

38 °C, transcripts of 5.0 and 2.7 kb were the most affected as compared to the other ones, and after 8 h of irradiation at 38 °C these transcripts were lost. The concentration of smaller transcripts did not change compared to control, suggesting that these transcripts were not affected by high temperature.

Both operons are expected to produce primary transcripts of 5.2 kb. Therefore the results of Fig. 2*E* suggested that the primary transcripts originating from both the operons were affected by high temperature. The transcript of 2.7 kb may belong to spliced RNA that covers *atp*IHF. A 2.6 kb transcript in pea may cover *atp*IHF (Hudson *et al.* 1987).

***atp*B operon and *rbc*L**: In wheat plastid genome, *atp*B and *atp*E are located on B2 fragment along with *rbc*L, *pet*A, and several ORFs (Fig. 1*B*). The Northern blot analysis of RNAs isolated from etiolated seedlings using B2 fragment revealed the presence of three major transcripts of 2.6, 1.8, and 1.3 kb and some minor transcripts of size greater than 2.6 kb (Fig. 2*F*). When the etiolated seedlings were irradiated at 25 °C, the level of all three transcripts increased. Besides, a novel transcript of 0.8 kb appeared during irradiation of etiolated seedlings at 25 °C. Transcript of 0.8 kb was also detected with the P7 fragment (Fig. 2*G*). *atp*E gene was also present on P7 fragment (Fig. 1*B*), suggesting that the 0.8 kb transcript could be the mature *atp*E transcript. When the etiolated seedlings were irradiated at 38 °C, the concentration of transcripts of 2.6 and 0.8 kb decreased and after 8 h of high temperature treatment, these transcripts were lost. Level of 1.8 kb transcript, which is the mature transcript of *rbc*L, also decreased at 38 °C. There was no change in the level of minor transcript of 3.4 kb upon irradiation of etiolated seedlings either at 25 or 38 °C.

***ndh* genes**: Various *ndh* genes are localised on P7, P9, and B8 fragments (Fig. 1*B*). Northern blot analysis of RNAs isolated from etiolated seedlings using P7 fragment revealed the presence of three transcripts of 2.8, 2.4, and 1.8 kb (Fig. 2*G*). Irradiation of etiolated seedlings at 25 °C led to increase in the level of all the transcripts. Besides, a new transcript of 0.8 kb and two minor transcripts of 1.6 and 1.2 kb were detected in irradiated seedlings. Transcription of all genes originating from this fragment was sensitive to high temperature.

(*2*) Plastid genes whose expression was not affected at high temperature

***ndh* genes**: The Northern hybridisation of RNAs isolated from etiolated seedlings using P9 fragment showed one major transcript of 0.9 kb and two minor transcripts of 2.6 and 1.5 kb (Fig. 3*A*). Irradiation of etioplast seedlings either at 25 or 38 °C had no effect on these transcripts. Northern blot analysis of RNAs isolated from etiolated seedlings using B8 fragment revealed four transcripts of 2.6, 1.5, 1.1, and 0.9 kb (Fig. 3*B*). The level of these transcripts was not affected either by irradiation or high temperature.

***rrn* operon**: In wheat plastid genome, the *rrn* operon is spread over P5, P6, and P8 fragments (Fig. 1*B*). Northern blot analysis of RNAs isolated from etiolated seedlings using P5 and P8 fragments gave identical transcripts pattern, which was different from the transcripts pattern obtained with P6 fragment. The transcript level of rRNA did not change when the dark grown seedlings were irradiated either at 25 or 38 °C as compared to etiolated seedlings (Fig. 3*C*).

***rpo* operon**: In wheat plastid genome, operon consisting of *rpo*B-*rpo*C-*rpo*C1 is present in P3 fragment (Fig. 1*B*). Northern blot analysis of RNAs isolated from etiolated seedlings using P3 fragments revealed four transcripts of 2.8, 1.6, 1.4, and 1.0 kb (Fig. 3*D*). A new transcript of 2.6 kb appeared while the level of other transcripts slightly increased during irradiation of etiolated seedlings at 25 °C. When the dark grown seedlings were irradiated at 38 °C, the level of all the transcripts except 1.0 kb decreased.

Genes coding for ribosomal proteins: Chloroplast genome codes for nearly 20 proteins of the small and large subunits of the ribosome. In wheat plastid genome, S3a and B3 fragments contain majority of the genes coding for ribosomal proteins whereas the rest of these genes is scattered throughout the plastid genome and is expressed with the genes coding for other plastid proteins. Northern blot analysis of RNAs isolated from etiolated seedlings using B3 and S3a fragments revealed identical pattern. The Northern blot with S3a fragment is shown in Fig. 3*E*: Four major transcripts of size 2.5, 1.5, 1.2, and 0.8 kb were detected. Irradiation of etiolated seedlings either at 25 or 38 °C did not affect the level of any of these transcripts.

B15 is another fragment that carries genes for three ribosomal proteins (Fig. 1*B*). Northern blot analysis of RNAs isolated from etiolated seedlings using B15 fragment revealed the presence of four transcripts of 1.8, 1.6, 1.0, and 0.9 kb (Fig. 3*F*). Irradiation of etiolated seedlings at 25 °C led to slight increase in the level of all the transcripts. However, there was no change in the transcripts level as compared to control when irradiation of etiolated seedlings was carried at 38 °C.

B21 fragment also carries genes for ribosomal proteins (Fig. 1*B*). Northern blot analysis of RNAs isolated from etiolated seedlings using B21 fragment revealed five major transcripts of 3.8, 3.4, 1.8, 1.4, and 1.0 kb (Fig. 3*G*). The transcript level did not change after irradiating etiolated seedlings either at 25 or 38 °C.

Genes for tRNAs: All the required tRNAs for the chloroplast protein biosynthesis are present in plastid genome. Some of the tRNAs are clustered and expressed together while others are expressed along with the protein-coding genes. The clustered tRNAs are present on P7, P10, and

B18 fragments. The pattern of transcripts observed with P10 and B18 was identical. Northern blot analysis of RNAs isolated from etiolated seedlings using P10

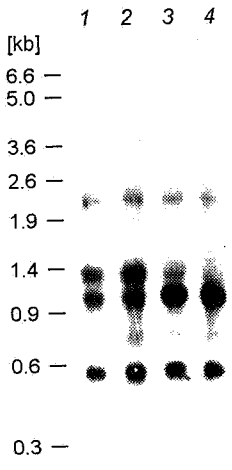

Fig. 4. Expression of *rpo*A gene. Experimental details are the same as in Fig. 2.

fragment revealed two transcripts of 1.4 and 0.9 kb and several minor transcripts (Fig. 3*H*). After irradiating etioplast seedlings at 25 °C, the level of all the transcripts except for 0.9 kb did not change. The level of 0.9 kb transcript increased dramatically after irradiation at 25 °C. When the etiolated seedling was irradiated at 38 °C, this transcript level did not increase as compared to etiolated seedlings. The accumulation of 0.9 kb is both irradiation dependent and temperature sensitive, a result also found with the expression of various light-inducible genes. The level of other transcripts did not change in response to high temperature. However, the transcript level of 3.2 kb increased at 38 °C.

(*3*) Expression of gene whose expression increased at high temperature

*rpo*A gene gave complex pattern of transcripts in the etiolated seedlings. The Northern blot analysis of RNAs isolated from etiolated seedlings revealed four major transcripts of 2.4, 1.4, 1.2, and 0.6 kb (Fig. 4). When the seedlings were irradiated at 25 °C, there was no change in the level of transcripts. However, when dark grown plants were irradiated at 38 °C, transcript level of 1.4 kb decreased. There was no change in the level of 2.4 and 0.6 kb transcripts at high temperature. The level of 1.2 kb transcript, which is the mature transcript of *rpo*A gene, increased at 38 °C.

Discussion

Exposure of etiolated seedlings to high temperature (38 °C) for short periods leads to the development of some of the characteristics observed in heat-bleached plants. Chl and PS2-related proteins fail to accumulate during exposure of etiolated seedlings to heat (Singh and Singhal, unpublished). However, the transcript level of rRNAs is not affected during temperature treatment of etiolated seedlings. Processed rRNA transcripts are present in very low amount in the heat-treated seedlings (Feierabend and Berberich 1991).

Determination of steady state level of transcripts of plastid genes during high temperature treatment of etiolated seedlings showed mixed results in our case. Expression of most of the plastid genes, whose expression is not affected by chloroplast development, was not affected by high temperature. These include genes coding for ribosomal proteins, rRNA, tRNA, and some genes coding for the putative NADH dehydrogenase subunits. However, expression of genes coding for the photosynthetic proteins was affected by high temperature. The expression of these genes is regulated during light-induced chloroplast development (Mullet 1988, Gruissem 1989, Gruissem and Tonkyn 1993).

Transcript level of some genes increased with high temperature. The most significant increase was observed for transcript of *rpo*A gene coding for subunit of chloroplast RNA polymerase. Increased transcription of genes coding for subunits of RNA polymerase was shown in high temperature induced ribosome-deficient chloroplasts by Hess *et al.* (1993). However, the expression of the other three genes coding for the subunits of RNA polymerase was not affected by high temperature in our experimental conditions.

A number of transcripts originating from light-inducible genes failed to accumulate during irradiation of etiolated seedlings at high temperature. These genes require factors for transcription (Wada *et al.* 1994, Kim and Mullet 1995). Besides, a number of transcripts of these genes require additional proteins for their stability (Gruissem and Tonkyn 1993, Memon *et al.* 1996). Thus synthesis/function of these proteins may be negatively affected at high temperature which in turn leads to the loss of transcripts. Analysis of stroma proteins by 2-D gel electrophoresis isolated from etiolated seedlings and seedlings irradiated at either 25 or 38 °C showed that a number of proteins which accumulate at 25 °C are absent in seedlings irradiated at 38 °C (Singh and Singhal, unpublished).

The molecular events associated with heat bleaching have not been elucidated yet, although the loss of transcripts coding for CP43 and CP47 proteins of the PS2 complex is one of the earliest events in the bleaching process. In the present study using etiolated seedlings we showed that the expression of most of plastid genes cod-

ing for proteins involved in photosynthetic process is affected by high temperature. Mechanism of this differential regulation of plastid genes at high temperature is at present not known. Two different possibilities might explain this differential regulation. First, there could be two sets of RNA polymerases (one transcribing specifically tRNA, rRNA, and ribosomal genes whereas the other one involved in the expression of remaining plastid genes) and high temperature could only affect the latter type of RNA polymerase. Two types of RNA polymerase are present in plastids (Igloi and Kössel 1992). The second possibility may be that the function of nuclear-coded proteins required for transcription and/or stability of plastid transcripts is affected at high temperature.

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