Degradation of chloroplast DNA during natural senescence of maple leaves

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The fate of chloroplast DNA (cpDNA) during plastid development and conversion between various plastid types is still not very well understood. This is especially true for the cpDNA found in plastids of naturally senescing leaves. Here, we describe changes in plastid nucleoid structure accompanied with cpDNA degradation occurring during natural senescence of the free-growing deciduous woody species Acer pseudoplatanus L. Natural senescence was investigated using three types of senescing leaves: green (G), yellow-green (YG) and yellow (Y). The extent of senescence was evaluated at the level of photosynthetic pigment degradation, accumulation of starch and plastid ultrastructure. Determination of cpDNA amount was carried out by in planta visualization with 4,6-diamidino-2-phenylindole, by Southern hybridization, and by dot-blot using an rbcL gene probe. During natural senescence, plastid nucleoids undergo structural rearrangements accompanied by an almost complete loss of cpDNA. Furthermore, senescence-associated protein components exhibiting strong binding to an ~10 kbp rbcL-containing cpDNA fragment were identified. This interaction might be important for rbcL expression and Rubisco degradation during the course of natural senescence in trees.

Keywords: deciduous plants, nucleases, nucleoids, quantification and arrangement of plastid DNA, rubisco regulation.

Introduction

Age-dependent senescence is a genetically programmed series of molecular events that leads to cell death and leaf abscission (Lim et al. 2007). The loss of soluble proteins, chlorophylls, RNA and starch is generally considered a criterion for leaf yellowing and senescence (Kirk and Tilney-Bassett 1978). It has been postulated that degradation of chloroplast DNA (cpDNA) precedes loss of proteins and yellowing associated with the onset of senescence (Sodmergen et al. 1989). A series of subsequent reports (Rowan et al. 2004, 2009, Li et al. 2006, Zoschke et al. 2007, Shaver et al. 2008) has initiated a controversy as to whether the gradual reduction of cpDNA during plant development occurs in all plant species. Recently, Zheng et al. (2011) demonstrated that cpDNA content drops as soon as the seedling leaves begin to green, before plastids mature and long before leaf senescence. Chloroplast DNA degradation associated with leaf ageing has also been documented for a number of other species (Sodmergen et al. 1989, 1991, Rowan et al. 2004, Shaver et al. 2006) and it appears that the demise of cpDNA is a common event in chloroplast development (Rowan and Bendich 2009).

As in prokaryotes, regions in the chloroplast stroma containing DNA molecules are not enclosed by membranes. Instead, multiple copies of cpDNA are packed into discrete structures called chloroplast nucleoids. The forces which cause condensation of the DNA into nucleoids are poorly understood. Data from...
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et al. 2000) which degrades Rubisco (Kato et al. 2004), in a process aimed at remobilization of nitrogen during senescence (Kato et al. 2005). Additional DNA compaction in nucleoids is achieved by an abundant nucleoid protein of 70 kDa, identified as sulfite reductase (SiR) (a ferredoxin-dependent enzyme that participates in sulphur assimilation for cysteine and methionine biosynthesis) (Sekeine et al. 2002, 2007, Chi-Ham et al. 2002). Apparently, SiR also regulates the transcriptional activity of chloroplast nucleoid through changes in DNA compaction. It is also possible that SiR acts as a redox-regulated architectural transcription factor (Sekeine et al. 2002).

Senescing chloroplasts undergo massive ultrastructural changes which include reduction of the thylakoid membrane system, degradation of proteins, accumulation of lipid conglomerates, and increase in the number and size of plastoglobuli. Such late stages of chloroplast differentiation are known as gerontoplasts (Sitte 1977, Kutik 1998). The processes of gerontoplast formation are accompanied by remobilization of nitrogen and carbon necessary for filling of seeds, and expansion of tubers and roots (Gregersen et al. 2008). Rubisco is the major source of the reduced nitrogen in photosynthetically active cells of leaves; therefore, its regulated degradation is of utmost importance for senescing plants (Gregersen et al. 2008). Various proteases have been implicated in this process (Martinez et al. 2008, Izumi et al. 2010); however, very little is known about factors influencing rbcL transcriptional regulation during senescence.

In this work, we investigate changes in the content of cpDNA during natural senescence of the free-growing deciduous woody species Acer pseudoplatanus L. We also identify senescence-related proteinaceous component(s) exhibiting strong binding to an ~10 kbp fragment of cpDNA containing the rbcL gene.

Materials and methods

Plant material

Three different types of maple (A. pseudoplatanus L.) leaves: green (G), yellow-green (YG) and yellow (Y) were harvested in September and October of 2007 and 2011, from three different 30-year-old trees (global positioning system coordinates 45°33′31.33″N, 18°41′6.37″E and 45°51′16″N, 15°57′16″E). Leaves were sampled at different dates and pools were made from each time-point and for each leaf type. Sampling of sun-adapted leaves was done from all sides of the canopy and from the height of up to 2.5 m. Five replicates were taken for every analysis. In all measurements leaf tissue was used after the main veins were removed.

Meteorological data

Average climatic parameters for the weather station Osijek in 2007 were: August: temperature 22.2 °C, precipitation...
45.0 mm m⁻², insolation 268.6 h; September: temperature 14.5 °C, precipitation 65.2 mm m⁻², insolation 185.1 h; October: temperature 10.3 °C, precipitation 92.5 mm m⁻², insolation 117.8 h; November: temperature 4.0 °C, precipitation 65.2 mm m⁻², insolation 137.5 h; December: temperature 4.3 °C, precipitation 0.7 mm m⁻², insolation 55.1 h.

Analysis of photosynthetic pigments

For determination of chlorophyll a (chl a) and chlorophyll b (chl b) as well as for total carotenoid determination, leaf tissue was macerated into a fine powder with liquid nitrogen using a pestle and mortar. Fine tissue powder was extracted with cold anhydrous acetone (ρ = 0.79 kg l⁻¹) and the absorbance was measured at different wavelengths (661.6 and 644.8 nm). The concentrations of chl a, chl b and total carotenoids were calculated according to Lichtenthaler (1987).

Light and electron microscopy

For light microscopy studies maple leaves were cut into small pieces and fixed for 24 h in 1% glutaraldehyde in 0.05 M phosphate buffer (pH 6.8). Plant tissue was then dehydrated in an alcohol series: 2-methoxyethanol, ethanol, n-propanol and n-butanol, and embedded in methacrylate resin (Historesin, Leica Microsystems GmbH, Wetzlar, Germany). Three-micrometre-thick sections were stained with Lugol reagent for microscopic observation the cover glass was gently pressed against the sample. Due to the presence of a thick cuticle, plastid nucleoids were visible in situ in cells proximal to the place where the leaf was cut, as well as in the plastids that were released on the specimen slide. All investigations were carried out with a Zeiss Axiosvert 35 (Carl Zeiss AG, Göttingen, Germany) epifluorescence microscope equipped with phase contrast and differential interference contrast optics. An HBO 100 mercury lamp, G365, FT 395, and LP 420 filters (all from Carl Zeiss AG, Göttingen, Germany) were used. Number of nucleoids per plastid was determined by direct counting while varying the focal plane of the microscope. Images were taken by using a digital camera (Pixera Corporation, San Jose, CA, USA).

Dot-blot rbcL DNA analysis

Total nucleic acids (TNA) were isolated according to Lepeduš et al. (2008). Frozen leaf tissue (0.5 g) was ground to a powder in liquid nitrogen with the addition of 4 ml CTAB (cetyltrimethyl-ammonium bromide) extraction buffer (3% CTAB, 1 M Tris, 1.4 M NaCl, 20 mM EDTA, pH 8.0 adjusted with HCl, and 0.2% 2-mercaptoethanol–freshly added to the extraction buffer just prior to TNA extraction). The homogenate was incubated in a 12-ml plastic test tube at 65 °C for 25 min. The aqueous phase was recovered after the extraction with an equal volume of chloroform and centrifugation at 16,000 g. The nucleic acids were precipitated with an equal volume of isopropanol. The pellet was collected by centrifugation, washed once with 70% ethanol and air-dried (~1/2 h at room temperature) and resuspended in 100 µl of sterile distilled water.

Total nucleic acids (50 ng) isolated from green leaves were used to amplify polymerase chain reaction (PCR) fragments of 199 bp of the rbcL gene, using the primers 5′-ACATGGACAGCTGTTGGGAC-3′, 5′-GCGGGCTTTAACCACCAAT-3′, and of 285 bp of the ndhF gene using the primers 5′-AAGAAACATACCGAATGG-3′, 5′-ATTGGCAAGAATTCTAC-3′. Primers were designed on the basis of the Acer rubrum L. cultivar Minnesota Flame ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit (rbcL) gene (DQ459381). Primers for NADH dehydrogenase subunit F (ndhF) were designed on the basis of the multiple sequence alignment of ndhF genes from Acer komarovii Pojark. (HM008565), Acer mormonense Hayata (HM008564), Acer tschonoskii Maxim. (HM008561), Acer tegmentosum Maxim. (HM008560), Acer rufinerve Siebold & Zucc. (HM008559), Acer pensylvanicum L. (HM008558), Acer morillonense Koidz. (HM008553), Acer crataegifolium Siebold & Zucc. (HM008548), Acer caudatifolium Hayata (HM008546), Acer davisii Franch. (HM008551), Acer chienii Hu & W.C. Cheng (HM008547), Acer sikkimense Miq. (HM008556), Acer pectinatum Wall. ex G. Nicholson (HM008562), Acer forrestii Diels (HM008554), Acer maximowiczii Pax (HM008563), Acer laxiflorum Pax (HM008555) and Acer micranthum Siebold & Zucc. (HM008552). Polymerase chain reaction conditions were as follows: 5’ denaturation at 94 °C, 35 cycles (30° 94 °C; 1° 51 °C; 30° 72 °C), 7° 72 °C. The reaction was performed in a total volume of 50 µl containing 5 µl 10x PCR buffer, 1× DIG DNA Labeling Mix (Roche, Mannheim, Germany) 1.5 mM MgCl₂, 2.5 U AmpliTaq polymerase (Applied Biosystems, Foster City,
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CA, USA) and 0.2 µM of each primer. Purification of the PCR products was carried out using a Qiaquick PCR purification kit (Qiagen, Hilden, Germany), according to the manufacturer’s instructions. Successful incorporation of digoxigenin (DIG)-labelled dUTP was confirmed with a band-shift electrophoresis assay. For DNA dot-blot analysis, TNA from green (G), yellow-green (YG) and yellow (Y) leaves was isolated as described above. RNA was digested with RNase, according to the manufacturer’s instructions (Promega, Sunnyvale, CA, USA) and 2 µg of each DNA sample in 6x SSC (0.9 M NaCl, 0.09 M Na citrate) was applied to a positively charged nylon membrane (Roche), and hybridized with 20 ng ml⁻¹ of labelled probe at 45 °C, according to the manufacturer’s instructions (Roche). Plasmid ptz57 DNA (200 ng) was included in the experiment as a negative control. Stringency of washing was: 2x SSC, 0.1% sodium dodecyl sulphate (SDS) for 5 min at room temperature, performed twice; 0.5x SSC, 0.1% SDS for 15 min at 66 °C, performed twice. Detection of specifically bound probe was performed by using a commercial DIG DNA Labelling and Detection Kit (Roche). Developed blots were scanned with a flatbed scanner and quantified by using Kodak 1D software.

Southern analysis of the samples

For Southern blot analysis, RNA was removed from TNA as described above. Ten micrograms of total DNA (totDNA) was digested with 30 U of KpnI (Roche) overnight at 37 °C. For protein gel retardation experiments, proteins were digested prior to KpnI digestion by the addition of protease K to a final concentration of 1 mg ml⁻¹, incubation for 1 h at 37 °C, and subsequent inactivation of the protease K at 85 °C for 30 min. Total DNA fragments were separated on 0.8% agarose slab gels in 0.5x Tris/borate/EDTA buffer. Samples were loaded on the gel in duplicate; one half was used for visualization of the samples by ethidium bromide and the other for Southern transfer. Transfer onto positively charged nylon membrane (Roche) was performed by using a standard capillary blotting procedure with 20x SSC. Following transfer, membranes were probed with rbcL and ndhF probes, as described for dot-blot. Stringency of washing was the same as for DNA dot-blot.

Sodium dodecyl sulphate polyacrylamide gel electrophoresis

Total nucleic acid samples were treated with DNase (Promega) and RNase (Promega) to remove DNA and RNA. Samples were mixed with corresponding volumes of denaturing 0.2 M Tris-HCl, pH 6.8 buffer containing 8% SDS, 6.2% dithiothreitol, 40% glycerol and 0.01% bromphenol blue (Laemmli 1970). The extracts were boiled for 2 min. Constant protein amounts (10 µg total protein per lane) were resolved by SDS-PAGE (10% w/v of polyacrylamide) and subsequently stained with silver nitrate according to Blum et al. (1987).

Data analysis

Data were analysed by one-way analysis of variance with five replicates from every leaf type. The mean values were compared using the least significant difference test. Differences were considered significant at P ≤ 0.05. All statistical analyses were done with Statistica 7.1. software (StatSoft, Inc., Tulsa, OK, USA, 2005).

Results

To establish that the observed yellowing of leaves can be attributed to natural senescence and was not caused by water deficiency, physical damage, or some other biotic or abiotic stressors, we investigated typical biological parameters associated with natural senescence (Lim et al. 2007). First, starch degradation in intact leaf tissue was monitored by using staining with Lugol’s iodine. Healthy G leaves and YG leaves contained clearly detectable amounts of assimilatory starch (Figure 1a and b), whereas starch granules were not detectable in Y leaves (Figure 1c). This demonstrates that the processes of carbohydrate remobilization associated with natural senescence have been entirely completed in the Y tissues. Secondly, plastid ultrastructure was investigated by using electron microscopy. Chloroplasts of G leaves were normally developed and contain an undamaged thylakoid membrane system (Figure 1d). Large starch granules and electron-dense plastoglobules were detected in every investigated chloroplast. Plastids found in YG leaves had clearly deteriorated ultrastructure, with an almost undetectable thylakoid membrane system (Figure 1e). Accumulation of plastoglobules was enhanced and their content was apparently altered, as judged by their diminished electron density. Accumulated assimilatory starch occupied large areas of plastid cross sections, indicating that carbohydrate remobilization processes have not yet been initiated at the YG stage of senescence. The ultrastructure of Y leaves revealed swollen plastids with grossly altered ultrastructure (Figure 1f). No photosynthetic membranes could be detected, which correlates with the almost complete absence of photosynthetic activity previously found in Y maple leaves (Lepeduš et al. 2008, 2010). Large plastoglobules with electron-translucent content occupy the entire interior of these plastids. Most likely, plastoglobules contain less, or different, osmiophilic lipids. The complete absence of starch was confirmed in every investigated plastid. Third, we addressed the accumulation of photosynthetic pigments (Figure 2). Degradation of chlorophylls and retention of carotenoids is considered to be a typical sign of natural senescence (Lim et al. 2007). A large, statistically significant, reduction in chl a and chl b was detected in YG and Y leaves. Degradation of chl b was more pronounced, leading to an increase in the chl a/chl b ratio in YG and Y leaves. Damage and degradation of
photosystem II antenna and relative stability of photosystem I could explain the observed changes. As expected, carotenoids were more resilient to degradation, which could be attributed to their protective roles (Lichtenthaler 2007).

Our next aim was to investigate and quantify the extent of cpDNA degradation during the processes of natural leaf senescence of deciduous woody species living in a free natural environment. The chosen species of maple (A. pseudoplatanus) is a widely distributed tree species which could serve as a model plant for these investigations. The number, size and distribution of plastid nucleoids were studied by using staining with the sensitive fluorochrome DAPI in planta (Figure 3a). This experimental setting enabled us to study the fate of cpDNA in various stages of senescence without disturbing the cellular and plastid structure.

Figure 1. In situ localization of starch in leaf tissue sections. The accumulation of starch (indicated by an arrowhead) was found in G (a) and YG (b) leaves. Total disappearance of starch is visible in Y leaves (c). Bar = 20 µm. Insets depict representative leaves from all three investigated types. Electronmicrographs of plastids found in G (d), YG (e) and Y (f) leaves. Complete degradation of plastid fine structure is represented. Bar = 1 µm.

Figure 2. Photosynthetic pigments content in G, YG and Y leaves of maple (A. pseudoplatanus). Chl a, chlorophyll a; Chl b, chlorophyll b; Chl a+b, total chlorophyll; Car, total carotenoids. The measurements were performed in five replicas. Significant differences between G, YG, and Y leaves for each parameter were designated by different letters (a, b and c) placed on the top of the columns. Differences were considered significant at \( P \leq 0.05 \). Error bars represent standard deviation.
contained on average seven evenly distributed nucleoids which started to aggregate during the transition to YG phase, where on average only three nucleoids could be found (Figure 3b). Additionally, YG plastids contained luminous foci of red fluorescence, most likely representing droplets of chlorophyll degradation products (Figure 3a). In Y leaves, very little or no nucleoids could be detected, indicating significant degradation of cpDNA (Figure 3a and b). To further substantiate these findings, we have isolated totDNA from leaf tissue and subjected it to dot-blot analysis using the \( rbcL \) gene probe. \( rbcL \) is represented on the cpDNA in a single copy and can thus serve as a quantitative indicator of cpDNA amount. This approach corroborated extensive reduction of cpDNA in Y leaves (Figure 4a). Next, we have digested totDNA with KpnI and subjected it to electrophoretic separation, followed by Southern blotting and final detection with the \( rbcL \) gene probe (Figure 4b). To our surprise, a ~10 kbp DNA fragment containing the \( rbcL \) gene isolated from G leaves showed marked gel retardation and an electrophoretic mobility shift in YG and Y material (Figure 4b, lanes 2 and 3). These findings led us to the conclusion that the onset of natural leaf senescence in maple is accompanied by a strong binding of a certain factor or factors to the \( rbcL \)-containing region of the cpDNA.

**Figure 3.** Epifluorescence photomicrographs of plastid nucleoids stained with DAPI in planta (a). Nucleoid compaction is clearly visible in plastids from YG leaves. Images depict both plastids within leaf tissue and those released from the cells. Number of nucleoids was determined by direct counting under the varying focal plane of the microscope (b). Ninety-eight per cent in G, 70% in YG, and 2% in Y of observed plastids contained DAPI-stained nucleoids. Only those plastids that showed some DAPI-stained nucleoids were scored. One hundred plastids of each leaf type were investigated. Chart represents average number of nucleoids per plastid with minimum and maximum values. Significant differences between G, YG and Y leaves were designated by different letters (a, b and c) placed on the top of the columns. Differences were considered significant at \( P \leq 0.05 \). Almost complete degradation of cpDNA in nucleoids from Y leaves is depicted.

**Figure 4.** Detection of \( rbcL \) gene on dot-blots of totDNA (a). Reduction of hybridization signal is quantified by densitometry. NC, negative control (plasmid ptz57 DNA). Strength of the hybridization signal is represented as percentage of the signal found in the G sample. Southern hybridization with \( rbcL \) gene probe of totDNA digested with KpnI before (b) and after protease (c) treatment. Gel retardation of \( rbcL \)-containing fragment is lost upon protein digestion. Efficiency of KpnI digestion is represented on the gels stained with ethidium bromide (left). Hybridization signal in samples from G (1), YG (2) and Y (3) isolates. On the left part of the blot 0.25 µg of totDNA was loaded, while on the right 0.5 µg of totDNA was loaded. As a control, Southern hybridization with \( ndhF \) gene probe of totDNA digested with KpnI before (left portion of the right panel) and after (right portion of the right panel) was performed (d). M7, SPPI DNA digested with EcoRI; M, \( \lambda \) bacteriophage DNA digested with HindIII; M, 1-kb DNA leader molecular length standard. Sizes of selected fragments in base pairs (far left).
We speculated that the factor is a protein whose interaction with the \textit{rbcl}, or neighbouring DNA regions, is important for the process of natural senescence. To demonstrate the proteinaceous nature of the observed factor, we have subjected totDNA to additional digestion with the proteinase K. Subsequent blotting and detection with \textit{rbcl} probe revealed the disappearance of gel retardation of the \textit{rbcl}-containing DNA fragment (Figure 4c, lanes 2 and 3), thus proving that the gel mobility shift was caused by protein–cpDNA interactions. In order to show that gel retardation is specific only for a cpDNA fragment harbouring the \textit{rbcl} gene and that other cpDNA fragments are not retarded in mobility by proteins, we have performed Southern hybridization with the chloroplast \textit{ndhF} gene (Figure 4d). In cpDNA \textit{ndhF} is also present in a single copy. In all analysed chloroplast genomes found in the GenBank, \textit{ndhF} is distal from \textit{rbcl} and should be present on a different DNA fragment generated by the KpnI digestion. Neither native nor proteinase K digested totDNA showed retardation of the \textit{ndhF}-containing cpDNA fragment (Figure 4d, lanes 2 and 3), thus demonstrating that protein is binding to the \textit{rbcl} cpDNA region and not to the \textit{ndhF} region.

To study the protein constituents of TNA isolates, we have subjected them to the SDS-PAGE analysis (Figure 5). Prior to denaturation and gel loading, DNA and RNA in the isolates from all three leaf types were removed by respective DNase and RNase treatments. On separation and silver staining, prominent bands could be detected at ~110, 27 and 24 kDa. These components most likely represent major TNA interaction proteins whose presence in all isolates is most likely not associated with natural senescence. Numerous minor components could also be detected in all three samples. Minor changes in their pattern are noticeable. We therefore concluded that the factor responsible for gel mobility shift of the ~10 kbp \textit{rbcl}-containing cpDNA fragment from YG and Y samples is a low-abundance, regulatory-type, component. Further identification of this protein will require isolation of a transcriptionally active chromosome or at least plastid DNA preparations.

**Discussion**

The accumulation and the dynamics of cpDNA during the course of plant development are still poorly understood and quantitative data describing these processes are scarce. This is especially so for studies describing these patterns in free-living plants exposed to natural environmental factors. In this study, we have addressed the fate of cpDNA during the natural senescence of the deciduous woody species \textit{A. pseudoplatanus}. Similar studies have been performed in laboratory model systems (Rowan et al. 2004, 2009, Li et al. 2006, Zoschke et al. 2007, Shaver et al. 2008), ultimately leading to a controversy over whether degradation of cpDNA is part of the integrated system of plant development. Plastid genomes are highly redundant and compacted in several nucleoids within chloroplasts (Rauwolf et al. 2010). The exact conformation of cpDNA in nucleoids is also a matter of debate (Bendich 2004). Apparently, besides circular forms, cpDNA can be found in linear forms representing structures involved in recombination-dependent DNA replication (Shaver et al. 2008).

Regulated senescence and the controlled breakdown of proteins and nutrients needed for re-mobilization to other organs are crucial for winter survival of deciduous woody species (Krupinska 2007). The fate of cpDNA during these processes is entirely unknown. In this work, we clearly demonstrate that cpDNA is degraded to almost completion during yellowing of maple leaves, and that certain protein factors bind to the \textit{rbcl}-containing segment in a senescence-dependent fashion. We however have to point out that leaves were harvested starting from the mature green stage, not an early stage at which the leaves were expanding. Consequently, we do not know whether the level of cpDNA in G leaves is the highest attained during development. The decline in cpDNA seen in Figure 3b could have begun at a stage prior to the stage at which the G leaves were harvested. We provide several lines of evidence that cpDNA degradation occurs in natural environmental conditions. 4,6-Diamidino-2-phenylindole...
staining in planta enabled visualization of the reduction without disturbing cellular and chloroplast structure. This has been one of the major objections in similar studies performed in laboratory conditions and in model plants (see Rowan and Bendich 2009 and references therein). Discrepancy between our results and observations that cpDNA is not degraded during senescence in laboratory conditions (Li et al. 2006) might be a consequence of different light intensities used in those studies; also Li et al. (2006) performed their experiments on different plant species. Our model system is entirely natural, relying on natural sunlight exposure and varying climatic conditions. Apart from DNA dot-blot analysis, we show separation of cpDNA restriction fragments and specific mobility shift of the ~10 kbp rbcL-containing fragment. Strong protein binding to this segment isolated from YG and Y leaves could possibly be linked to the control of rbcL expression, akin to the CND41-negative regulation of chloroplast gene expression (Nakano et al. 1997). Interestingly, CND41 also acts as a protease degrading Rubisco (Murakami et al. 2000, Kato et al. 2004, 2005, Diaz et al. 2008). Three scenarios leading to marked mobility shift of the 10 kbp fragment could be envisaged. A protein binds to the regulatory elements found on this stretch of DNA and causes retardation. Binding occurs close to the site of KpnI cleavage and prevents restriction, which leads to the extension of the fragment. And lastly, DNA is strongly compacted by architectural factors which render some restriction sites inaccessible. Strong compaction of nucleoids during leaf yellowing has also been demonstrated in other studies (Fulgori and Ljubešić 1992).

In conclusion, cpDNA decline during maturation and senescence of maple occurs under natural environmental conditions during late summer and autumn. Compaction and reduction in the number of plastid nucleoids are associated with the onset of natural senescence. Protein factors strongly bind to the rbcL-containing region of cpDNA already at early stages of senescence, and remain bound until final cpDNA degradation. Further studies are needed to identify minor protein components involved in this interaction, as well as in the processes of cpDNA condensation.

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Conflict of interest

None declared.

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