The pattern of amyloplast DNA accumulation during wheat endosperm development

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Abstract. The accumulation of amyloplast DNA during endosperm development was studied in two cultivars of spring wheat, *Triticum aestivum* L. ‘Chinese Spring’ (CS) and ‘Spica’, small and relatively larger-grained cultivars, respectively. Endosperms were isolated between 9 and 45 days post anthesis (dpa) and the amyloplast DNA content of endosperm nucleic-acid extracts was measured by quantitative hybridisation with a homologous chloroplast-DNA probe. The endosperm cells of CS and Spica accumulated amyloplast DNA during development in a similar way. In both cultivars there was a large increase in the amount of plastid DNA (ptDNA) per endosperm between 9 and about 15 dpa, after which there was no further increase. Because nuclear DNA continued to accumulate until 24 dpa, the percentage contribution of amyloplast DNA to total DNA fluctuated in both cultivars during development, reaching maxima at 12 dpa of about 1.00% and 0.85%, and dropping to apparently constant levels of 0.60% and 0.52% in CS and Spica, respectively, by 24 dpa. In both cultivars, the average number of ptDNA copies per amyloplast was calculated to increase from about 10 copies at 9 dpa to about 50 copies in the mature amyloplasts at 31 dpa. However, the heavier endosperms of Spica contain more cells than those of CS and the varieties therefore differed in the amount of ptDNA that accumulated per endosperm: Spica endosperms accumulated 110 ng of ptDNA by 15 dpa, compared with only 85 ng in CS. The apparent accumulation of ptDNA copies in wheat amyloplasts during endosperm development contrasts with the decline in chloroplast-DNA copies in wheat chloroplasts during leaf development.

Key words: Amyloplast DNA – DNA accumulation – Endosperm development – *Triticum aestivum*.

Introduction

Nothing is known about the role of plastid DNA (ptDNA) in organelles other than chloroplasts. However, other plastids also have roles of direct agronomic importance, for example amyloplasts, which in wheat (*Triticum aestivum*) contain starch granules that contribute 70–80% of the crop’s yield.

Comparison of the cultivar Spica with Chinese Spring (CS) has shown that the higher final mean grain weight of Spica (Chojecki et al. 1986a) results from larger and 31% more A-type starch granules per endosperm (Chojecki et al. 1986c). This paper describes experiments to measure endosperm amyloplast DNA during grain development in these cultivars, a parameter which has as yet received little attention. It was of interest to see whether, and to what levels, ptDNA accumulates in the developing wheat endosperm, and whether there is any difference in the pattern of increase in CS and Spica that could be related to the known grain-size difference.

Studies on amyloplast DNA in sycamore have used intact amyloplasts as the source of ptDNA (Macherel et al. 1985). In wheat however, the isolation of intact amyloplasts from endosperm is difficult (Rijven 1984), and is in any case feasible only during very early stages of development before B-type granule initiation, when the plastids contain a single A-type granule only. Using the method described here, however, it is possible to determine
indirectly the DNA content of amyloplasts at different stages of endosperm development. This was achieved by estimating the percentage contribution of wheat amyloplast DNA to total DNA in endosperm total-nucleic-acid extracts by hybridising with a wheat chloroplast-DNA (ctDNA) probe.

**Materials and methods**

*Collection of endosperms.* Developing grains for the determination of starch-granule numbers, total DNA content and ptDNA levels, were collected from field-grown Chinese Spring and Spica wheat (*Triticum aestivum* L.) plants at intervals between 9 and 45 days post anthesis (dpa). Endosperms were dissected from embryo and pericarp tissues of grains taken from basal and second florets of five central spikelets. Removal of the inner green pericarp, which contains green plastids in the cross-cell layer, ensured the elimination of grain ctDNA. For starch-granule analyses, five endosperms from each of three ears were pooled, and the hydrolysed DNA was estimated with 10% perchloric acid at 90 °C. Supernatants from each of three ears were fixed and stored at 4 °C in a solution of 3:1 ethanol:acetic acid. Endosperms for total DNA estimation (12 from each of three ears) and those for DNA extraction (approx. 100), were frozen in liquid nitrogen following dissection, and stored at −70 °C.

*Isolation of endosperm and leaf total nucleic acid.* The method used was that described by Bowman (1986) for the isolation of total nucleic acid from leaves. Each extract was made from 50 mg of freeze-dried endosperm or leaf powder, and the concentration of total DNA in the extracts was estimated by the diphenylamine reaction of Burton (1956), as modified by Giles and Myers (1965).

Total-nucleic-acid samples were digested with restriction enzymes as described by the suppliers of the enzyme (Bethesda Research Laboratories, Paisley, UK). Agarose gel electrophoresis, the transfer of DNA to nitrocellulose and subsequent hybridisation to *32*P-labelled probes were as described earlier (Bowman 1986). For quantitative hybridisation, total-nucleic-acid samples were denatured and applied to nitrocellulose (using the dot-blot conditions of Kafatos et al. 1979) also as described in Bowman (1986). To estimate the quantity of probe hybridised to each sample, the region containing the sample was located by autoradiography and the radioactivity in the excised segments measured by Cerenkov counting under optimised conditions.

*Assay of endosperm total DNA.* Frozen endosperms were thawed and treated in a manner modified from that described by Smillie and Krotkov (1960). To extract soluble nucleotides and other potentially interfering substances, single endosperms were crushed in 1 ml of ice-cold 10% trichloroacetic acid (TCA) and kept on ice for 15 min. After centrifugation the TCA treatment was repeated on the pellets. Nucleic acids were solubilised and extracted from the tissue pellets by two 15-min treatments with 10% perchloric acid at 90 °C. Supernatants from each extraction were pooled, and the hydrolysed DNA was estimated as for the extracts of isolated nucleic acids.

*Estimation of endosperm starch-granule numbers.* Endosperms were prepared for starch-granule counting as described by Chojecki et al. (1986c). Numbers of A-type granules in single-endosperm extracts were determined using a Coulter Counter model TAI (Coulter Electronics, Harpenden, Herts., UK). The machine registered the total number of particles in a preset volume of sample, and recorded the total volume of particles in 16 size channels. Using values for the percentage volumes accumulated in each channel, the appropriate mean channel-volume size, and the total particle count, the number of particles in each channel was calculated. The number of A-type granules in an endosperm extract was calculated by summing the numbers registering in channels 9–16, that is, particles above 8 μm in diameter.

**Results**

*Homology between wheat endosperm ptDNA and wheat ctDNA.* The contribution of wheat ptDNA to the total DNA in endosperm nucleic-acid samples was estimated by quantitative hybridisation. The amount of a wheat ctDNA fragment hybridising with ptDNA in the endosperm samples was compared with the amount hybridising to a total-nucleic-acid sample taken from a wheat leaf of known ctDNA content (Bowman 1986). For such an assay to be valid, the ctDNA fragment must be homologous with the endosperm ptDNA fragment. Also, because hybridisation to unfraccionated samples is to be interpreted as hybridisation to ptDNA alone, the probe ctDNA fragment must be shown to hybridise only to the ptDNA in the samples. As it is known that plant nuclear DNA (nDNA) and mitochondrial DNA (mtDNA) have sequence homology with ctDNA (e.g. Whisson and Scott 1985), it must be demonstrated that there is no significant hybridisation with nDNA or mtDNA in the extracts, or with any contaminating DNA, such as plasmid DNA. Finally, since hybridisation must be quantitative, contaminants of endosperm nucleic-acid extracts, such as starch, should not physically interfere with the immobilisation of the nucleic acids on nitrocellulose.

The general homology between ctDNA and endosperm ptDNA in wheat was demonstrated by the identical hybridisation patterns obtained using ctDNA probes pTacB (32–21), pTacB (18–24) and pTacB (2–15) (Bowman and Dyer 1986) with BamHI, PstI and SalI digests of leaf and endosperm total-nucleic-acid extracts. These probes cover about 40% of the plastome. On this basis the two genomes were indistinguishable. The recombinant plasmid pTacP7, containing the 8.1-kbp wheat ctDNA PstI fragment P7 (Bowman and Dyer 1986), was chosen as the probe for quantitative hybridisation, and the homology between the ctDNA and the endosperm ptDNA in the particular region of the genome containing P7 is demonstrated in Fig. 1. In track c, containing total leaf nucleic acid, the bands characteristic of PstI-digested wheat ctDNA are visible, and the position of band P7 is shown. Comparable bands are not evident in the PstI-digested endosperm nucleic acid (track b) because the proportion of ptDNA is too