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Genome Expression during Normal Leaf Development[1]

2. DIRECT CORRELATION BETWEEN RIBULOSE BISPHOSPHATE CARBOXYLASE CONTENT AND NUCLEAR PLOIDY IN A POLYPLOID SERIES OF WHEAT

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ABSTRACT

The quantitative relationships between ribulose bisphosphate carboxylase, nuclear ploidy, and plastid DNA content were examined in the nonisogenic polyploid series *Triticum monococcum* (2×), *Triticum dicoccum* (4×), and *Triticum aestivum* (6×). Ribulose bisphosphate carboxylase per mesophyll cell increased in step with each increase in nuclear ploidy so the ratios of ribulose bisphosphate carboxylase per mesophyll cell (picograms) to nuclear DNA per mesophyll cell (picograms) were almost identical in the three species. Ribulose bisphosphate carboxylase per plastid was 14.1, 14.7, and 16.8 picograms in the 2×, 4×, and 6× ploidy levels, respectively. Plastid area in these three species decreased with increasing nuclear ploidy so the concentration of ribulose bisphosphate carboxylase in the plastoids was 60% higher in the hexaploid compared to the diploid species. DNA levels per plastid were 64 and 67 femtograms for the diploid and tetraploid species, respectively, but were 40% less in the plastids of the hexaploid species. These relationships are discussed in terms of cellular and plastid control of ribulose bisphosphate carboxylase content.

Increased nuclear ploidy levels in cereals and other plants are frequently correlated with changes in several cellular and chloroplast features (5–8, 11, 12, 16, 19, 24–26). One chloroplast protein which has received attention in this respect is RuBPCase.[2] Although earlier reports suggested that the affinity of RuBPCase for CO_2 increases with an increase in nuclear ploidy (14, 21), later more-detailed examinations have failed to confirm this correlation (15, 18). In recent work on a ploidy series of *Festuca arundinacea* (tall fescue) (15), it was found that the amount of RuBPCase, expressed as a concentration per leaf section, increased with ploidy from 4× to 8×. The changes were small but were also correlated with changes in net photosynthetic rate. If this relationship is found to be general for other species, the control of the synthesis of the amount of RuBPCase assumes a new importance. One current hypothesis proposes that the small subunit controls the intrachloroplast synthesis of the large subunit (2, 13). Experimental support for this hypothesis has come from our recent demonstration (10) that the two subunits undergo coordinated, simultaneous synthesis in young wheat leaves (*Triticum aestivum*) and that changes in the synthesis of the subunits can be accounted for by changes in their translatable mRNAs at each stage of development.

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[2] Abbreviation: RuBPCase, ribulose bisphosphate carboxylase/oxygenase (EC 4.1.1.39).

The next question concerns the role of gene dosage in determining amounts of RuBPCase. There is limited experimental evidence for gene dosage effects on the synthesis of specific proteins in higher plants (15, 19, 25, 26) although such effects are well documented in *Drosophila* in which the phenomenon is so well understood that dosage relationships have been utilized to map the chromosomal locations of genes which encode for specific enzymic activities (23).

The availability of a polyploid series in the genus *Triticum* offered us an opportunity to examine the effect of gene dosage on the synthesis of RuBPCase. However, there is a great danger that significant correlations may not be observed if the chosen basis for comparisons is inappropriate. We have become aware in our studies on RuBPCase (9, 10) that comparative studies of different species are much more informative if the comparisons are made between similar cell populations or numbers of organelles rather than in terms of leaf area or leaf section. The present studies were undertaken to measure RuPBCase content in a ploidy series of wheat to establish whether (a) a quantitative relationship exists between nuclear ploidy and RuBPCase content on a cellular or plastid level, and (b) the number of genome copies per plastid influences the RuBPCase content per plastid.

In this work, we used the nonisogenic polyploid series of the *Triticum* genus, *Triticum monococcum* (2× AA), *Triticum dicoccum* (4× AABB), and *T. aestivum* (6× AABBDD). These species were chosen because of their commercial importance and because the methods for measuring RuBPCase content in cells and plastids and DNA content per plastid had been successfully developed in our laboratory with *T. aestivum* (4, 9). In addition, the choice of this particular series also allows any specific effects of the addition of the BB and the DD genomes to be examined.

MATERIALS AND METHODS

Plant Material. Grains of *Triticum monococcum* and *Triticum dicoccum* were obtained from the Plant Breeding Institute, Maris Lane, Trumpington, Cambridge, England. Grains of *Triticum aestivum* cv Maris Dove were obtained from the National Seed Development Organisation, Newton Hall, Cambridge. The seeds were soaked in running tap water at 20°C for 1 h and then surface sterilized in NaOCl solution (13% free chlorine). After being soaked for an additional 16 h, the seeds were sown in Levington Universal Compost (Fisons U.K.) at a depth of 1 cm and were grown at 70% RH using a photoperiod of 16 h at 20°C with a 5°C night depression. The light intensity at the level of the seedlings measured with a solarimeter (Kipp and Zonen) was 4.0 mw cm^{-2}. The seedlings were harvested when their first leaves were fully expanded. This was 8 d from sowing for *T. monococcum* and 7 d from sowing for *T. dicoccum* and *T. aestivum*. The average lengths of the first leaves of the three species *T. monococcum* (2×), *T. dicoccum* (4×), and *T. aestivum* (6×) were 15, 18, and 11 cm, respectively. All comparative measurements were made using leaf

sections 12–13, 15–16, and 8–9 cm from the leaf bases of the three species, respectively. These leaf sections represented parts of the first leaves at approximately the same developmental stage.

Mesophyll Cell Number, Plastid Number per Cell, and Cell and Plastid Area Estimations. Mesophyll cell number/leaf section was determined as in Dean and Leech (9). Plastid numbers and areas were determined in separated wheat cells which were separated by shaking leaf sections, fixed for 30 min in 3.5% glutaraldehyde, in 0.1 M EDTA (pH 9.0) at 60°C for 3 h (based on Possingham and Smith [20]). The sections were tapped gently on a slide, and the plastids were counted using a Zeiss photomicroscope fitted with Nomarski differential interference optics. To ensure that the plastid counts were performed on a sample of cells representative of the whole population, the average cell area of the counted cells was compared with that of a very much larger sample of cells. Plastids were counted in twenty cells whose average area was not significantly different from the average of a sample of 100 cells from the same leaf. Cell and plastid areas were measured from photographs of the separated wheat cells using a 9864A Hewlett-Packard digitizer linked to a model 30 Hewlett-Packard calculator. Only cells and plastids whose major axes were sharply in focus were measured.

RuBPCase and Total Protein Estimations. These were measured as described by Dean and Leech (9).

DNA per Plastid Estimations. These were determined by the method of Boffey and Leech (4). DNA per plastid has previously been shown to decrease as plastids mature (4). Because of the different growth characteristics of the leaves of the different *Triticum* species, the leaf regions from which mature chloroplasts can be isolated are different in the different species. We have established that the final 'minimal' mature value of DNA per plastid is found in cells in leaf sections taken 9 cm above the leaf base for the first leaves of *T. monococcum*, 12 cm above the leaf base for the first leaves of *T. dicoccum*, and 6 cm above the leaf base for the first leaves of *T. aestivum*. The DNA specific fluorochrome DAPI (4',6-diamidino-2-phenylindole) was used to check that the chloroplasts from *T. monococcum* and *T. dicoccum* judged to be intact by phase contrast microscopy were resistant to DNAse action as found previously for *T. aestivum* by Boffey and Leech (4). The values for nuclear DNA per cell for the three species, *T. monococcum*, *T. dicoccum*, and *T. aestivum* were taken from the comprehensive survey of nuclear DNA values determined by microdensitometry by Bennett and Smith at the P.B.I., Cambridge (3) (the suppliers of the *T. monococcum* and *T. dicoccum* grains used in this study). The same standard was used for the determination of nuclear DNA per cell in each of the three *Triticum* species. We have measured the value of DNA per nucleus in the first leaves of *T. aestivum* using the microdensitometric method of Bennett and Smith (3) and obtained similar values (J. R. Ellis, A. J. Jellings, and R. M. Leech, in preparation). The DNA content for isolated nuclei from *T. aestivum* leaves was also found to be very similar to the value obtained for nuclear DNA per cell obtained by microdensitometry.

RESULTS

The RuBPCase content per leaf section increased with increasing nuclear ploidy by 14% between the 2× and 4× ploidy levels and 8% between the 4× and 6× levels (Table I). However, there is a large decrease in the mesophyll cell number per leaf section as ploidy increases (Table I), in part due to the large changes in mesophyll cell area (Table I), so the relative increase in RuBPCase per cell with ploidy is much larger than its relative increase per leaf section. Indeed, the RuBPCase content per mesophyll cell increases in step with each increase in nuclear ploidy level (Table I). The closest correlation is the constant ratio of RuBPCase per mesophyll cell to nuclear DNA per mesophyll cell.

Because RuBPCase content and plastid number both increase with increasing ploidy, the question of whether RuBPCase per plastid is constant in these species was asked. As can be seen from the results in Table II, the diploid and tetraploid species have 14.1 and 14.7 pg RuBPCase/plastid, respectively, the hexaploid species has a similar but slightly higher value of 16.8 pg, but this is not significantly different from the tetraploid and diploid species. In contrast to the close correlations between RuBPCase content and ploidy found at the cellular and organelle level, the results in Table II show that the amount of RuBPCase expressed as a percentage of the total protein does not change with ploidy.

The relationships of DNA per plastid, and chloroplast DNA per cell to RuBPCase content were next determined to see if there is an additional relationship between chloroplast DNA amount and RuBPCase content. The DNA per plastid values for the diploid and tetraploid wheats are 6.4 and 6.7 g × 10^{-14}, respectively (Table III). The DNA per plastid values in the hexaploid wheat are 3.8 g × 10^{-14}, only 60% of the amount in the diploids. These represent the final 'mature' levels of DNA per plastid in the three species. The DNA per plastid values are converted to plastid DNA per cell by multiplying by the plastid number per cell in Table III.

Table I. *Calculations of RuBPCase per Mesophyll Cell and as a Ratio with Nuclear DNA in the Mature Leaf Sections of the First Leaves of Three Wheat Species*

All measurements were made on the leaf sections 12–13, 15–16, and 8–9 cm from the leaf bases in *T. monococcum*, *T. dicoccum* and *T. aestivum*, respectively. The values for RuBPCase estimations are mean ± SE for two measurements, the values for mesophyll cell area are mean ± SE for 100 measurements.

	T. monococcum (2×)	*T. dicoccum* (4×)	*T. aestivum* (6×)
RuBPCase/leaf section (μg)	93.2 ± 0.45	108 ± 12.6	117.5 ± 4.1
Mesophyll cell no./ leaf section × 10^{-4}	12.2	7.12	5.24
Mesophyll cell area (μm^2)	1,670 ± 44	3,062 ± 77	3,650 ± 89
RuBPCase/mesophyll cell (pg)	764	1517	2242
Nuclear DNA/cell (pg)[a]	12.4	24.2	34.6
RuBPCase/ cell:nDNA/cell	62	63	65

[a] Nuclear DNA values (see "Materials and Methods") are taken from Bennett and Smith (3).

Table II. *Calculation of RuBPCase per Plastid and as a Percentage of Total Protein in the Mature Leaf Sections of the First Leaves of Three Wheat Species*

All measurements were made on the leaf sections 12–13, 15–16, and 8–9 cm from the leaf bases in *T. monococcum*, *T. dicoccum* and *T. aestivum*, respectively. Values for total protein estimation are mean ± SE for two measurements. Values for plastid number/cell are mean ± SE for 17 cells. Values for RuBPCase/mesophyll cell and RuBPCase/leaf section needed for the calculation of RuBPCase/plastid and percentage of RuBPCase are taken from Table I.

	T. monococcum (2×)	*T. dicoccum* (4×)	*T. aestivum* (6×)
Plastid no./cell	54 ± 3	103 ± 5	133 ± 6
RuBPCase/plastid (pg)	14.1	14.7	16.8
Protein/leaf section (μg)	223.7 ± 13.6	307 ± 9	297.2 ± 7.9
% RuBPCase of total protein	41.7	35.2	39.5

Table III. *Calculation of Plastid DNA/Cell and Plastid DNA as a Percentage of Total Cellular DNA in the First Leaves of Three Wheat Species*

Values for plastid number/cell used to calculate plastid DNA/cell are taken from Table II. Values of nuclear DNA/cell from (3), used to calculate plastid DNA as a percentage of total DNA are taken from Table I. Total DNA is calculated to be nuclear DNA plus plastid DNA (ignoring the small contribution from mitochondrial DNA).

	T. monococcum (2×)	*T. dicoccum* (4×)	*T. aestivum* (6×)
DNA/plastid (pg × 100)	6.4	6.7	3.8
Plastid DNA/cell (pg)	3.46	6.9	5.05
Plastid DNA/% of total DNA	22	22	13
Plastid RuBPCase: plastid DNA	220	219	442

Table IV. *Calculation of RuBPCase and DNA per μm^2 of Plastid in the First Leaves of Three Wheat Species*

Values of projected plastid area are mean ± SE for 70 measurements. Values expressed per μm^2 give an estimate of 'concentration' in the plastids.

	T. monococcum (2×)	*T. dicoccum* (4×)	*T. aestivum* (6×)
Projected plastid area (μm^2)	42 ± 1	37 ± 1	31 ± 0.5
RuBPCase/plastid area (pg × 100/μm^2)	33.6	39.7	54.2
Plastid DNA/plastid area (fg × 10/μm^2)	15.2	18.2	12.3

When these values are calculated as a percentage of the total cellular DNA (nDNA per cell plus chloroplast DNA per cell), the values for the diploid and tetraploid are 22%, and for the hexaploid 13%. These values are within the range found previously in wheat, pea, and spinach (4, 17, 22). Table III also shows the RuBPCase:plastid DNA ratio through the ploidy series. The ratio is very similar in the diploid and tetraploid but 2-fold higher in the hexaploid, the larger ratio resulting from both increased RuBPCase levels and decreased DNA levels in the plastids.

When considering RuBPCase and DNA concentrations in chloroplasts, changes in plastid size in addition to plastid number must be taken into account. Table IV shows the average plastid area in the three species decreases as the ploidy level increases. If it is assumed that the chloroplast shapes in the three species are similar, then the RuBPCase and DNA content expressed per μm^2 of plastid will give approximate concentrations. It is clear that the increase in RuBPCase 'concentration' is even greater than was apparent when considering pg RuBPCase per plastid. The hexaploid has an almost 2-fold higher concentration of RuBPCase than the diploid. When the plastid DNA content is expressed as concentration, it is clearly shown that the differences in plastid DNA concentration between ploidies are relatively small compared with differences in DNA amount per plastid (Table III).

DISCUSSION

The most striking relationship found during these investigations of the three wheat species is the constant ratio of RuBPCase per mesophyll cell to nuclear DNA per mesophyll cell (Table I). This very important relationship is masked when the RuBPCase content in the three species is compared per leaf section. The strict quantitative relationship also does not hold for the RuBPCase amount per plastid nor for concentration per plastid as might have been expected. These results suggest that increased nuclear DNA content leads to greater synthesis of the small subunit of RuBPCase through an increased gene dosage which in turn leads to increased levels of RuBPCase in the mesophyll cells. If this is the case, it could be predicted that the plastid genome has the capacity to respond to an increase in the synthesis of the small subunit with an increase in the synthesis of the large subunit. Dean and Leech (10) have already shown that the two subunits undergo coordinated simultaneous changes in synthesis in young wheat leaves. Further, the extremely constant ratio between nuclear DNA and RuBPCase per cell strongly suggests that the amount of the small subunit is the limiting and therefore controlling factor in the synthesis of RuBPCase. This is in line with the previous suggestion that the small subunit may act as a positive control signal for the synthesis of the large subunit in the plastid (2, 13). The hexaploid wheat plastids contain the highest amount of RuBPCase and the lowest amount of plastid DNA, indicating that much of the plastid DNA in the diploid and tetraploid species may be redundant in terms of genes for the large subunit. It also seems that the plastid alone may have little control over its RuBPCase content because there is no quantitative relationship between plastid DNA amount (genome copies) and RuBPCase amount or RuBPCase concentration per plastid.

The close quantitative parallelism in the ratios between RuBPCase per mesophyll cell and nuclear DNA per mesophyll cell for all three *Triticum* species also suggests that the expression of the small subunit gene in the AA, BB, and DD genomes is very similar.

The results presented in this paper clearly indicate that the cellular content of RuBPCase in these wheat species is largely under nuclear control. The plastid DNA may alter RuBPCase concentration in the plastid by influencing chloroplast size.

The demonstration of the nuclear control of RuBPCase amount raises the possibility of increasing the RuBPCase amount in developing leaf cells by genetic manipulation of the number of small subunit genes in the nuclear DNA. Plants of this type would also provide valuable material in which to examine the extent to which RuBPCase amount influences photosynthetic capacity and photosynthetic performance in leaves.

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