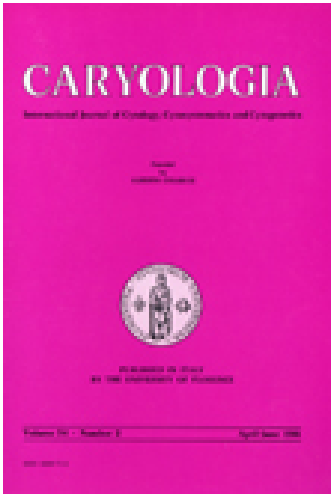

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Changes in Histones/DNA Ratio in Scutellum Nuclei During Ageing of Triticum Durum Caryopses

A.M. Innocenti[a] & M.B. Bitonti[a]

[a] Department of Ecology, University of Calabria, Cosenza, Italy.

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CHANGES IN HISTONES/DNA RATIO IN SCUTELLUM NUCLEI DURING AGEING OF *TRITICUM DURUM* CARYOPSES

A.M. INNOCENTI and M.B. BITONTI

Department of Ecology, University of Calabria, Cosenza, Italy.

SUMMARY — Columnar epithelium and parenchyma cells of the scutellum of *Triticum durum* caryopses show different quiescence models: while epithelium cells display a DNA content per nucleus of 2C, the parenchyma cells undergo poliploidization. Furthermore, in old seeds, the distribution of the histones/DNA ratio shifts much more in parenchyma cells than in columnar epithelium cells. It may thus be concluded that different tissues of the same organ (scutellum) are affected in a different way by damaging effect of ageing factors.

INTRODUCTION

When the aged seeds fail to germinate they loose some metabolic functions (GALLESCHI and FLORIS 1978) but they are still able to perform certain synthetic processes (BARKER and BRAY 1972; SEN *et al.* 1975). Therefore, during storage, not all the seed tissues are affected by the damaging effect of ageing in the same way. Also in *Triticum durum* seeds which had just lost their viability, INNOCENTI and FLORIS (1979) provided evidence for the persistence, in the embryo first node and in the scutellum, of DNA, RNA and protein synthetic activity, similar to that of young embryos. On the contrary, other embryo areas as shoot and root apexes, leaf primordia and coleoptiles reduced or lost their metabolic activity as soon as the seeds fail to germinate.

The study of the localization of the last activity in the embryo of aged non-germinating seeds raises some questions regarding the nuclear changes produced in different tissues of seeds subjected to prolonged storage.

As the histones/DNA ratio changes during ageing and appears to be higher in root meristematic nuclei of old caryopses (INNOCENTI and BITONTI 1979) our purpose in the present work is to decide whether similar changes in histones/DNA ratio take place also in scutellum nuclei of aged seeds.

MATERIALS AND METHODS

Caryopses of *Triticum durum* cv. Cappelli, harvested in 1971 and in 1977 and stored in glass containers under laboratory conditions were used in 1978. At this time the old seeds from crop 1971 just lost their germination ability. The young seeds of the 1977 harvest, with 95% germination, were utilized as control.

The caryopses were fixed in 10% neutral formalin, and the excised embryos washed overnight. Some embryos embedded in wax were sectioned at 15 μm and mounted, in sequence, on glass slides. Sections on slides were hydrolysed in 1N HCl at 60° C for 20 minutes, stained with Schiff's reagent, and mounted in Canada balsam. In other samples one scutellum was separated from an old embryo and another from a young embryo. The ephitelial layer covering the scutellum surface was kept apart from the parenchyma cells beneath the epithelium.

a) Columnar epithelium cells from young and old embryos were squashed separately on one and same slide.

b) Parenchyma cells from young and old embryos were squashed separately on one and same slide.

Trials a) and b) were repeated three times.

The squashes were stained with the Feulgen method, substituting 1N trichloroacetic acid (TCA) for 1N hydrochloric acid (HCl) in both the hydrolysis (20 minutes) and the Schiff reagent, respectively. After three passages in SO_2 water and dehydration, the slides were mounted in Canada balsam. Measurements of DNA content in individual nuclei were carried out with a microscope photometer Zeiss 01, following the procedure of McLEISH and SUNDERLAND (1961). The nuclei scanned for Feulgen-DNA were thereafter scanned for alkaline fast-green stainable histones after restaining the slides according to the method of ALFERT and GESCHWIND (1953) as modified by BLOCH and GODMAN (1954). The double measurements of DNA and histones in the same nuclei were made possible by locating the individual nuclei in microphotographs. Nuclei were measured at 565 nm for Feulgen and at 635 nm for fast-green determinations.

RESULTS

Within the mature caryopsis of *Triticum durum* the embryo is appressed to the endosperm by its cotyledon, the scutellum. Longitudinal sections of the embryo show that a single cell layer epithelium covers the scutellum over its entire surface. The cells of this tissue appear elongated and contain small nuclei. Beneath the epithelium, irregularly shaped parenchyma cells with large nuclei are present (diagram in Fig. 1).

DNA.— The relative amount of DNA per nucleus as estimated by Feulgen cytophotometry demonstrates that the cells of scutellum epithelium have

only 2C (G_1) nuclei (Fig. 2) while in parenchyma cells, the DNA content varies among the cytological zones: showing 2C; 4C; 8C and 16C nuclei (Fig. 2). It is worth noting that the amount of DNA per cell was significantly

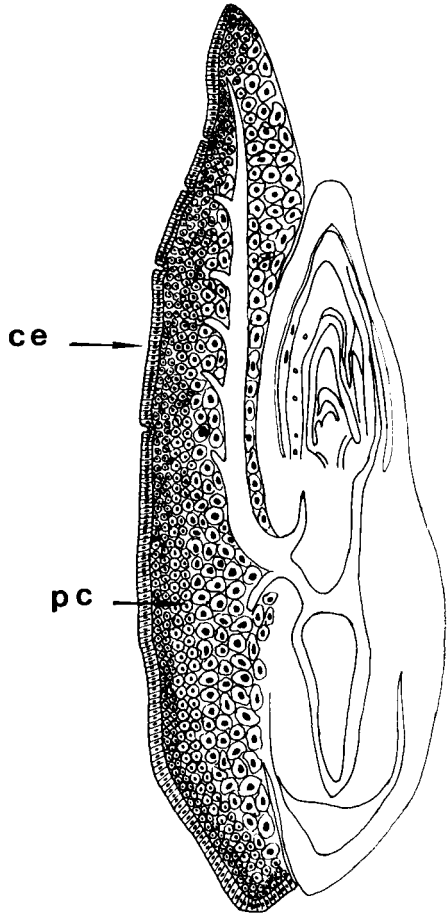

Fig. 1. — Longitudinal section through a *Triticum durum* embryo showing the tissues of the scutellum. c.e.=columnar epithelium; p.c. = parenchyma cells.

higher in the inner zone of scutellum just close to the embryo axis, while it was lower close to the epithelium layer, as shown in the diagram of Fig. 1.

Histones/DNA ratio. — In order to have a general picture of the phenomenon we have given in Figs. 3 and 4 the distributions of the histones over DNA ratio for the total data collected in the three different trials. It is easily seen that these distributions show a shift between young and old seeds which is more evident in the parenchyma then in the columnar epithelium

cells. Actually by evaluating the Student's t for each trial (see Table 1) it may be shown that the difference between the averages of the histones over DNA ratios for young and old seeds are significant at the 1‰ CL in all the

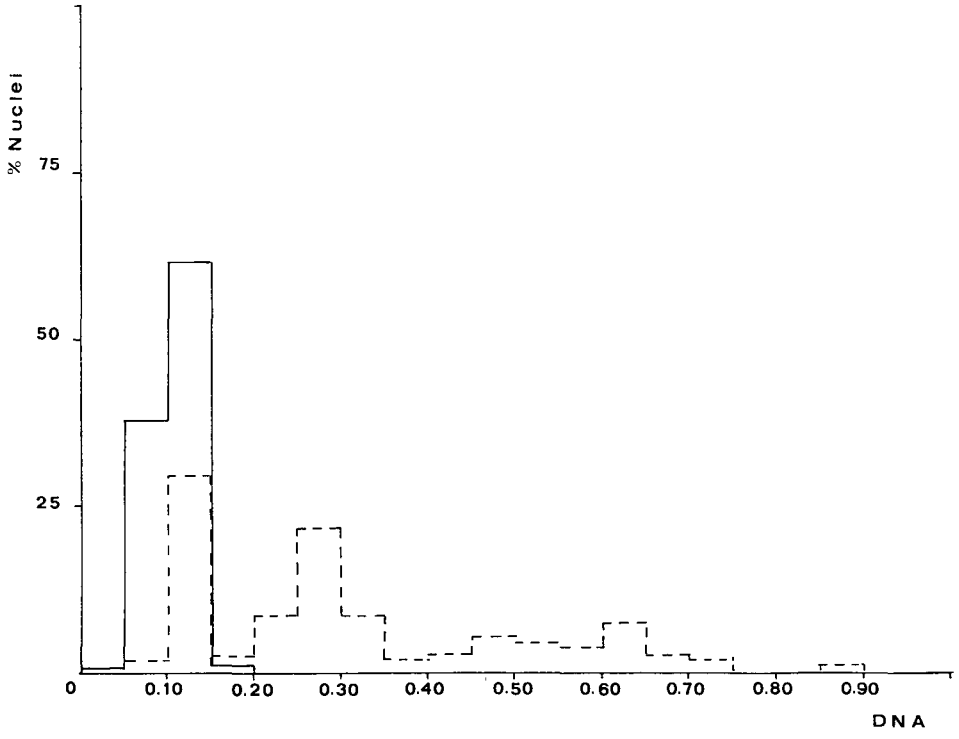

Fig. 2. — DNA amount of individual nuclei of parenchyma cells of the *Triticum durum* scutellum (broken line histograms)and of columnar epithelium cells (whole-drawn line histograms). Ordinate: percentage of nuclei; abscissa: DNA amount in arbitrary units as estimated by Feulgen cytophotometry.

three cases for the parenchyma cells and only in one case (case C) for the columnar epithelium cells.

A much more quantitative view of this phenomenon may be given by defining an ageing parameter

$$Ap = \frac{O - Y}{\sqrt{\sigma_o^2 + \sigma_y^2}}$$

where O and Y are the average histones over DNA ratios for the old (O) and young (Y) seeds respectively, and σ_o and σ_y are the standard errors of the corresponding distributions. It is clear that such a parameter represents

an absolute measure of the shifts in the distributions since these shifts are opportunely normalized to the spreads of the distributions. Note however that the Ap definition is identical to the usual definition of Student's *t*, when the numbers of old and young nuclei are equal. The values calculated for

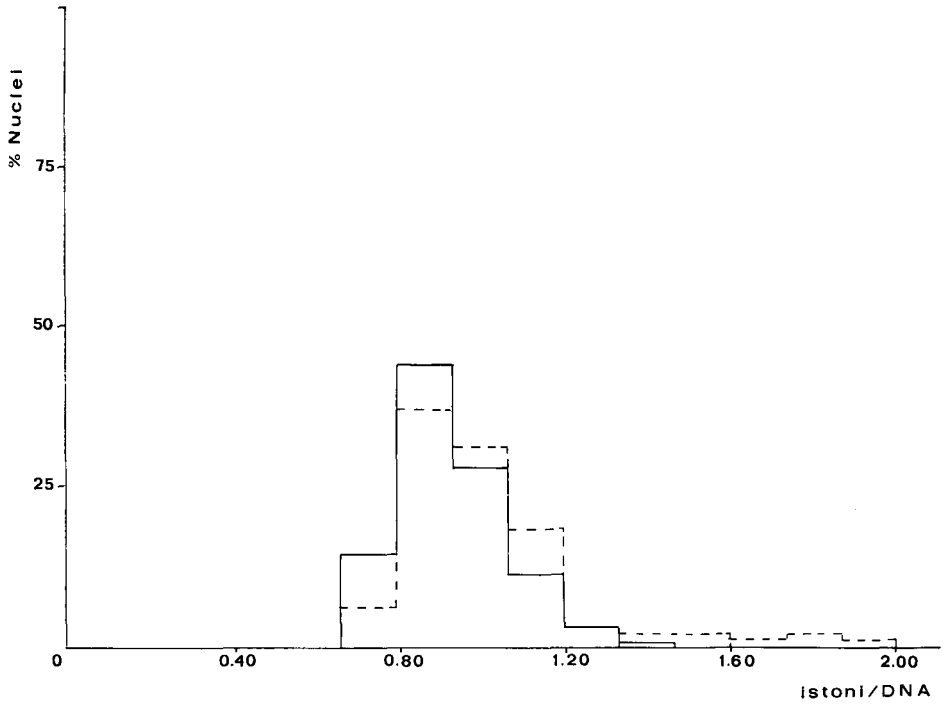

Fig. 3. — Frequency of histones/DNA ratio in columnar epithelium nuclei of young caryopses of *Triticum durum*, crop 1977 (whole-drawn line histograms), and of old caryopses of *Triticum durum*, crop 1971 (broken line histograms).

this ageing parameter Ap, are given in the last column of Table 1 where it may be seen that this parameter is much greater for the trials relative to the parenchyma than for the trials relative to the epithelium cells, thus indicating that the parenchyma cells are more sensitive to the ageing factors than the epithelium cells.

DISCUSSION

The epithelium and parenchyma cells of the scutellum of *Triticum durum* show quite different characters both morphologically (ESAU 1977) and physiologically (NOMURA, KONO and AKAZAWA 1969).

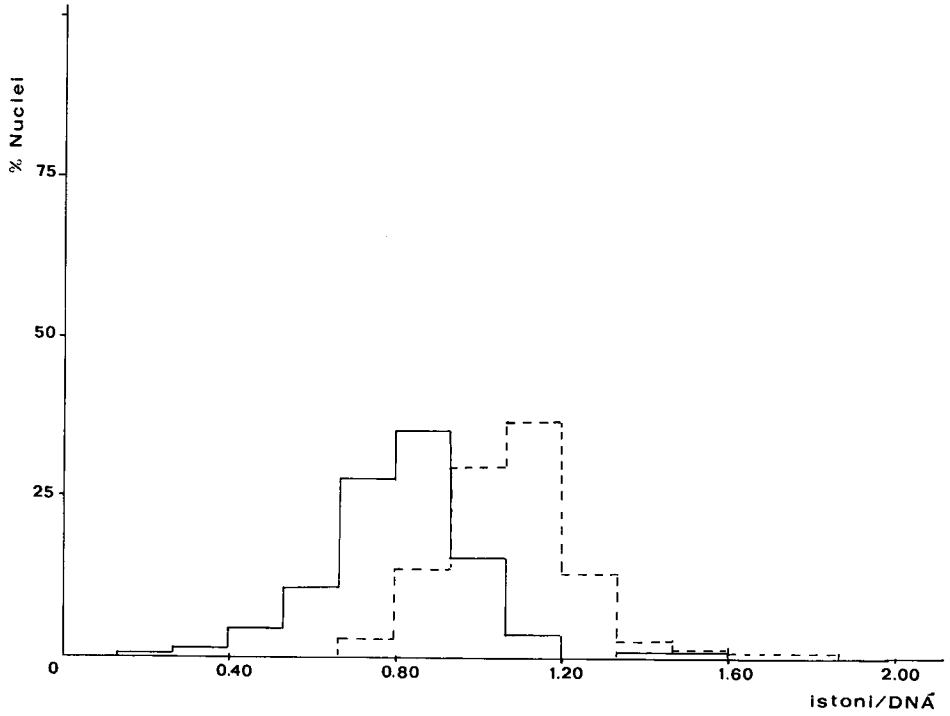

Fig. 4. — Frequency of histones/DNA ratio in parenchyma nuclei of scutellum of young caryopses of *Triticum durum*, crop 1977 (whole-drawn line histograms), and of old caryopses of *Triticum durum*, crop 1971 (broken line histograms).

Our cytophotometric analyses demonstrate that also cytological differences occur between these tissues. Thus epithelium cells show only 2C nuclei (in G_1 period) while parenchyma cells undergo polyploidization. The higher values are close to the embryo axis, in line with D'AMATO's data in plant cotyledons (1952).

Comparing the two scutellum tissues epithelium and parenchyma in young versus old seeds, we see clearly that the ratio histones/DNA varies in different ways: in both tissues, the ratio is higher in old than in young cells, and this difference is much more pronounced in the parenchyma tissue.

Since during seed storage the histones/DNA ratio of meristematic tissues, which lose their metabolic activity (INNOCENTI and FLORIS 1979) appears higher than in young seeds (INNOCENTI and BITONTI 1979), it seems worthy of note that the scutellum tissues of aged non-germinating seeds show a metabolic activity similar to that of young seeds (INNOCENTI and FLORIS 1979). Besides, if we analyze the histones/DNA ratio in epithelium cells of the scutellum in young and old seeds we see that the ratio does not change

TABLE 1 - *Histones/DNA ratio in nuclei of the scutellum from young (1977 harvest) and old (1971 harvest) caryopses of « Triticum durum ».*

	Test	Year	Histones/DNA	n. nuclei analyzed	t	Degrees of freedom	Ageing parameter
Columnar epithelium	A	1971	0.99 ± 0.03	40	1.39	99	1.39
		1977	0.94 ± 0.02	61			
	B	1971	0.95 ± 0.03	38	0.68	81	0.83
		1977	0.92 ± 0.02	45			
	C	1971	1.05 ± 0.02	47	4.74	73	4.24
		1977	0.93 ± 0.02	28			
Parenchyma	A	1971	1.10 ± 0.02	49	8.77	124	9.71
		1977	0.75 ± 0.03	77			
	B	1971	1.11 ± 0.02	46	5.42	90	4.99
		1977	0.93 ± 0.03	46			
	C	1971	1.05 ± 0.03	52	9.36	131	7.59
		1977	0.81 ± 0.01	81			

much with time. Instead in the nuclei of parenchyma cells a clear shift was found in the ratio distribution of the old seeds.

At this point we might conclude that the ageing process acts in a different way not only between single embryo zones (INNOCENTI and FLORIS 1979) but even between tissues of the same organ (epithelium and parenchyma of scutellum).

Apparently it is sufficient that only few tissues lose their metabolic activity (GALLESCHI and FLORIS 1978; INNOCENTI and FLORIS 1979) to bring the life functions of the whole organism in jeopardy.

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