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HISTONES/DNA RATIO IN YOUNG AND OLD ROOT MERISTEMS OF *TRITICUM DURUM* CARYOPSES *

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INTRODUCTION

Seed subjected to prolonged storage reduce progressively their capacity to resume germination when imbibed. Some Authors demonstrated that during seed ageing toxic and mutagenic substances were produced and accumulated (D'AMATO and HOFFMAN-OSTENHOF 1956; SAX 1962; HARRISON 1966) causing an increase in both mutations and chromosome aberrations (BIASUTTI and OWEN 1956; CORSI and AVANZI 1969; KAUL 1969; INNOCENTI and AVANZI 1971; FLORIS and ANGUILLESI 1974). Other Authors claim that also genetic changes (MEDVEDEV 1967; BERJAK and VILLIERS 1972*a*; ROBERTS 1972) and physiological damage, (ROBERTS, ABDALLA and OWEN 1967; FLORIS 1970; BERJAK and VILLIERS 1973) were caused by the ageing process.

In *Triticum durum* seeds a negative relation between nuclear damage and the frequency of germinated caryopses was observed throughout the storage period. This relation suggests that the same mechanism might be responsible for both chromosome breakage and reduced germination (INNOCENTI and AVANZI 1971). It has also been found that ageing of *Triticum durum* caryopses affects not only the mitotic cycle time, but also the relative duration of its phases (TAGLIASACCHI and VOCATURO 1977).

When metabolic changes became conspicuous they did not allow the aged caryopses of *Triticum durum* to germinate. Under these conditions, numerous physiological functions are lost (GALLESCHI and FLORIS 1978) even though some cells may still be capable of some synthetic functions.

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Indeed not all the embryo-zones are subjected to the same damaging effect of ageing: some zones loose their metabolic activity as soon as the aged seeds fail to germinate while other zones still mantain their metabolic activity (INNOCENTI and FLORIS 1979).

Preliminary observations on old seeds of *Triticum durum* indicated that the histones/DNA ratio in the presynthetic 2C nuclei of root meristems is higher in the older than in the younger seeds. Besides, 2C meristematic nuclei from the older seeds showed a nuclear area smaller than 2C nuclei from the younger seeds (INNOCENTI and BITONTI 1977). Since, however, these results might be due to a different maturation degree of caryopses at the time of harvest, we decided to repeat our analyses at the time when the caryopses of *Triticum durum* considered « young » in the experiment of INNOCENTI and BITONTI (1977) become aged and fail to germinate. Thus we are able to make two kinds of comparison:

i) comparison of 2C meristematic nuclei between young and old caryopses taken from different crops and analyzed at the same time;

ii) comparison of 2C meristematic nuclei between young and old caryopses taken from the very same crop and analyzed at different times.

It was expected that the two comparisons would help to clarify whether or not the detected changes in histones/DNA ratio, were really related to the ageing process. In fact, in the first comparison (i) both technical mishops and probable seasonal physiological changes of seeds are excluded, whereas in the second comparison the different starting conditions of the seeds at the harvest time (maturation degree, etc...) are excluded.

MATERIAL AND METHODS

Young an old caryopses of *Triticum durum* cv. Cappelli were stored in glass containers under laboratory conditions and used for two separate experiments:

1) In 1972, cytophotometric measurements of DNA and histone content were made in the presynthetic (2C) nuclei from the primary root meristems of young caryopses 1971 harvest, with 100% germination and on the 2C meristematic nuclei from the primary root of old caryopses 1968 harvest which had just lost their germination ability. The histones/DNA ratio was calculated in the nuclei of young and old meristems.

2) In 1978, a comparison was made between the histones/DNA ratio, in 2C root meristematic nuclei from young caryopses 1977 harvest, 100% of germination, and the histones/DNA ratio in 2C root meristematic nuclei from old caryopses 1971 harvest, which had just lost their germination ability.

The caryopses were fixed in 10% neutral formalin and washed overnight. One radicle (primary root) meristem from an old caryopses and another from a young caryopses were squashed separately on one and the same slide. The

squashes were stained with the Feulgen method, substituting 1N trichloracetic acid (TCA) for 1N hydrocloric acid (HC1), 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 Mc LEISH and SUNDERLAND (1961). The nuclei scanned for Feulgen-DNA were thereafter scanned for alkaline fast-green stainable histones, after restaining the slides, according the method of ALFERT and GESCHWIND (1953) as modified by BLOCH and GODMAN (1954). The double measurement of DNA and histones in the same nuclei was made possible by locating the individual nuclei in microphotographs. Nuclei were measured at 565 nm for Feulgen and 635 nm for fast-green determinations.

The areas (in μm^2) of each nucleus used for the cytophotometric analysis was calculated. Measurements of nuclear diameters were made using a Zeiss ocular micrometer.

RESULTS AND CONCLUSIONS

As seen in Table 1, the histones/DNA ratio is higher in 2C meristematic nuclei of old caryopses 1968 harvest than in 2C meristematic nuclei of young caryopses 1971 harvest (Table 1A). Also the cytophotometric comparison between the 2C nuclei of old caryopses 1971 harvest and the 2C nuclei of young caryopses 1977 harvest clearly confirms that histones/DNA ratio is higher in the nuclei of old root meristems (Table 1B).

In Fig. 1, the distribution of 2C nuclei of root meristems of young and old caryopses according to the histones/DNA ratio is recorded. As seen in Fig. 1A, the nuclei of old caryopses are distributed prevalently in

TABLE 1

Histones/DNA ratio in the presynthetic nuclei (2C) from young (y) and old (o) root meristems of Triticum durum caryopses.
A: comparison between young caryopses harvested in 1971 and old caryopses harvested in 1968;
B: comparison between young caryopses harvested in 1977 and old caryopses harvested in 1971.

	A		B	
Test	Year	Histones/DNA	Year	Histones/DNA
1	1968 o	0.89 ± 0.03	1971 o	0.84 ± 0.02
	1971 y	0.57 ± 0.02	1977 y	0.72 ± 0.04
2	1968 o	0.95 ± 0.03	1971 o	0.90 ± 0.01
	1971 y	0.59 ± 0.01	1977 y	0.70 ± 0.02
3	1968 o	0.93 ± 0.02	1971 o	0.80 ± 0.01
	1971 y	0.56 ± 0.02	1977 y	0.74 ± 0.02

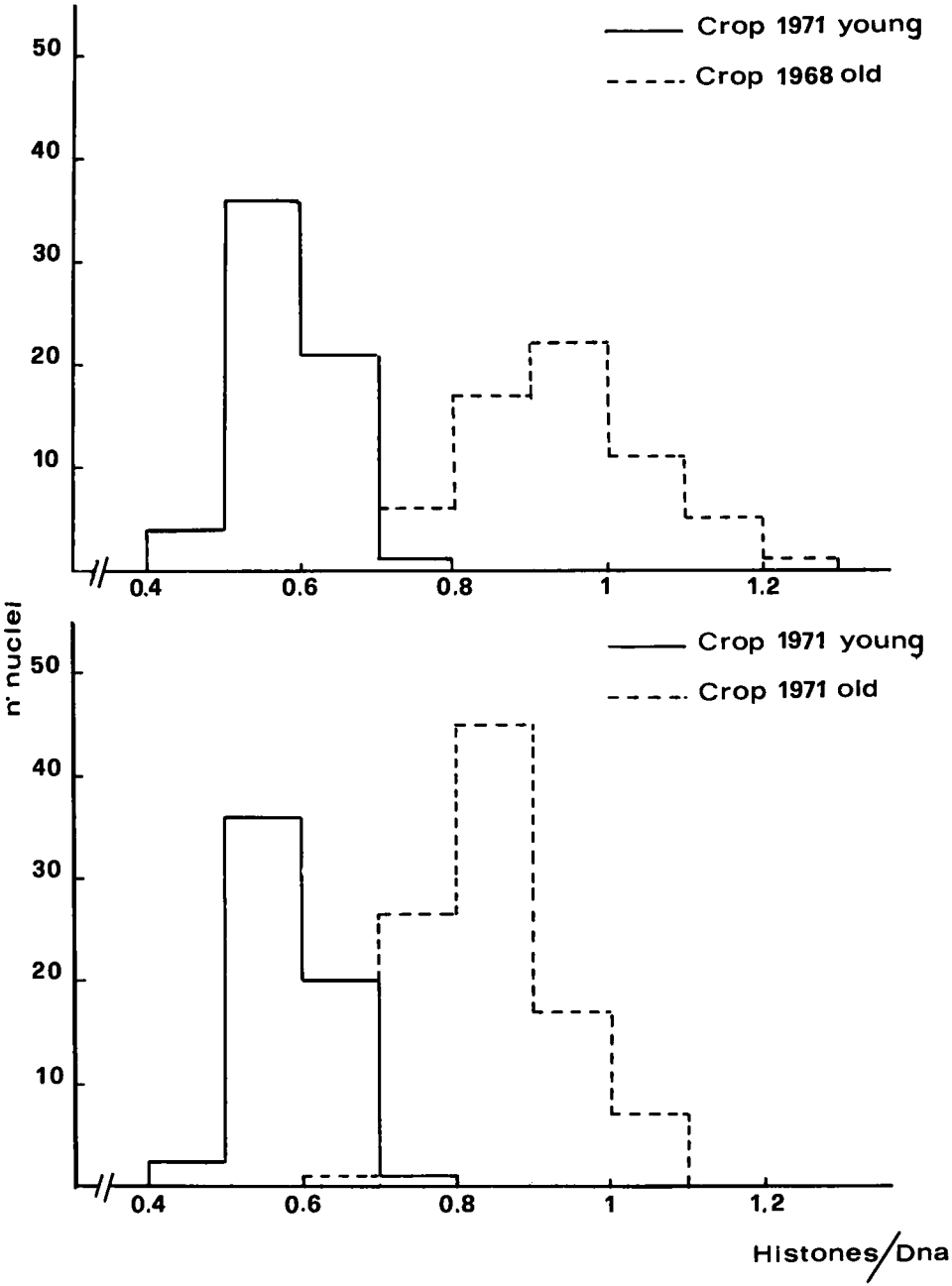

Fig. 1A. — Frequency of histones/DNA ratios in 2C root meristematic nuclei of young caryopses of *T. durum*, crop 1971 (entire line histograms) and of old caryopses of *T. durum*, crop 1968 (broken line histograms) analyzed ot the same time.
Fig. 1B. — Frequency of histones/DNA ratios in 2C root meristematic nuclei of young (entire line histograms) and old (broken line histograms) caryopses of *T. durum* taken from the very same crop (1971) and analyzed at different times.

the higher classes of ratios while nuclei of young caryopses show the higher frequency in the lower classes of histones/DNA ratios. Fig. 1B shows that 2C meristematic nuclei of young and old seeds from the same crop (1971) also display apparent differences in histones/DNA ratio distribution: the nuclei of old caryopses are distributed in the higher classes of ratios, while the nuclei of the young caryopses are distributed in the lower classes of ratios.

Comparing the data in Figs. 1A and 1B, it also appears that, in both cases the ratio distribution shows a wider scatter in the old root meristems than in the young ones. By comparing the average values of histone content per nucleus with the average values of nuclear areas in 2C nuclei from young and old root meristems, we have found an exponential regression represented by the equation $A = 96.2e^{-7.1Hs}$ where A represents the areas measured in μm^2 and Hs represents the histone content in arbitrary units (Fig. 2). The correlation coefficient for such regression was found to be 0.97.

The above results indicate a distinctive behaviour in the amount of fast-green stainable histones between 2C meristematic nuclei from young and old caryopses.

On the basis of the results reported in Fig. 1A and 1B, we may assume that the detected increase of histones/DNA ratio is related to seed ageing. In this connection we might recall that also in root meristems of old caryopses of *Haynaldia villosa* we have found an histone content per nucleus higher than in root meristems of young caryopses (INNOCENTI and BITONTI, unpublished data). These observations suggest that the detected increase of DNA bound histones might be a general aspect of ageing in caryopses of various species. In accordance with our knowledge of the function of histones (BONNER *et al.* 1968) the high histone content per nucleus in root meristematic tissues of old caryopses which have lost germinability may indicate that during prolonged storage a complete repression of DNA genetic activities occurs. Indeed the root apex, as the shoot apex, coleoptyle and leaf primordia in embryos from aged non-viable seeds of *Triticum durum* loose their synthetic activities as soon as aged caryopses fail to germinate (INNOCENTI and FLORIS 1979).

Worthy of remark is the decrease in nuclear areas during the storage period (Fig. 2). The observed effect might be a response to a gradual decrease in water content in the nucleus or to a loss of nuclear non-histone proteins which seem to influence both nuclear volume (ALFERT 1952) and DNA activation (FRENSTER 1965; PAUL and MALCOM 1976; PEDERSON 1974*a*, 1974*b*). We might, however, also think that some changes in nuclear DNA bound histones might be responsible for the reduced nuclear volume. If so, the decreased nuclear volume in 2C meristematic nuclei from

old caryopses might be the result of qualitative changes in nuclear histones. In this respect, we recall that: i) a larger amount of DNA bound lysine-rich histones contracts the chromatin as demonstrated in methaphase chromosomes (MIRSKY, BURDICK, DAVIDSON and LITTAU 1968); ii) according

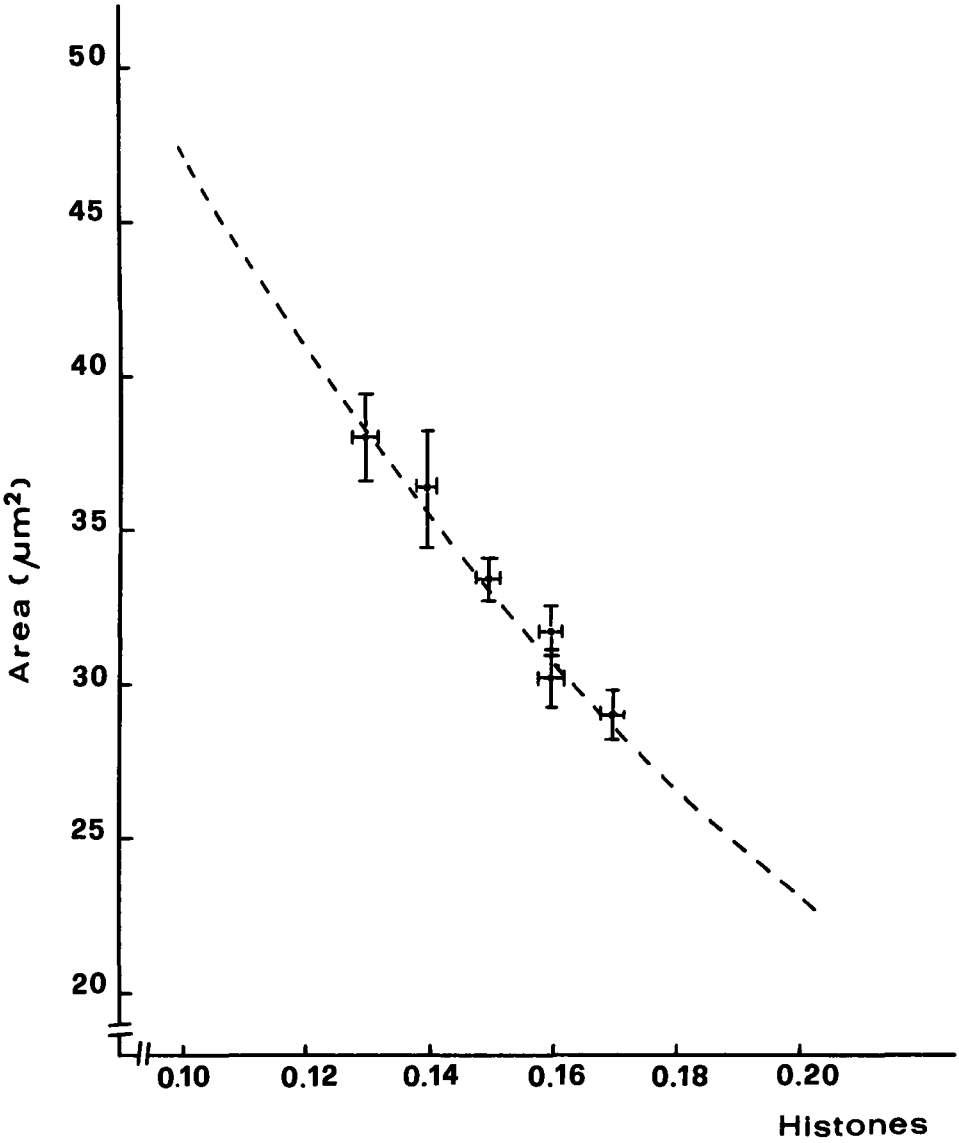

Fig. 2. — Average nuclear area vs. average nuclear histones content in 2C root meristematic nuclei of *T. durum* caryopses. The broken line represents the best exponential fit, the correlation coefficient being 0.97.

to PEDERSON (1978) histone 1 might determine the chromatin fiber folding pattern; iii) histone acetylation and phosphorylation may actually have more to do with chromatin folding and packing as exemplified by chromosome coiling during mitosis (GURLEY *et al.* 1974; BRADBURY *et al.* 1974*a*, 1974*b*) or the progressive condensation of chromatin that occurs during avian erythroid cell maturation (RUIZ-CARRILLO *et al.* 1975). Hypothesis iii) seems to be the least probable, because histone acetylation and phosphorylation were found during cell physiological activity (mitosis, cell differentiation etc.), whereas in this work, quiescent seeds with a very low (latent) metabolism were used. Anyhow, the detected differences in histones/DNA ratio between young and old seeds of *Triticum durum*, whether due to quantitative or qualitative variations of DNA-bound histones, suggest an alteration of DNA regulation pattern which occurs during seed storage.

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SUMMARY

The histones/DNA ratio has been calculated in the 2C nuclei from the primary root meristems of young and old caryopses of *Triticum durum*. In a first experiment young and old caryopses taken from different crops were considered in one and the same trial, at the same time. In a second experiment young and old caryopses taken from the very same crop were considered in different trials at different times. In both cases 2C meristematic nuclei of old caryopses show a higher amount of DNA bound histones and smaller average nuclear area as compared to 2C meristematic nuclei of young caryopses. The possible significance of this variations in histones/DNA ratio and in nuclear areas detected in the study is discussed.