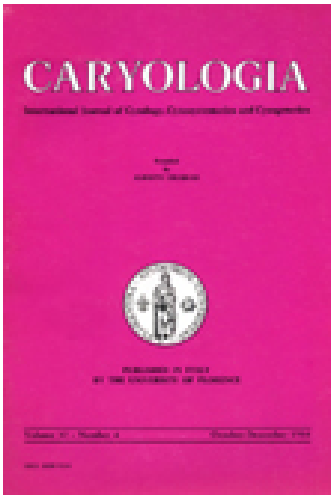


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# Variation of Histone/DNA Ratio in the Embryonic Areas of Triticum Durum Aged Seeds

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

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

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# VARIATION OF HISTONE/DNA RATIO IN THE EMBRYONIC AREAS OF *TRITICUM DURUM* AGED SEEDS

A. M. INNOCENTI and M. B. BITONTI
Department of Ecology, University of Calabria, Cosenza, Italy

SUMMARY — The histone/DNA ratio has been estimated independently for each embryonic area in young and old caryopses of *Triticum durum*. As the ratio is already quite different in the single embryo areas of over dormant young seeds, it can be suggested that nuclear conditions at ripening time influence the response of the cell populations which undergo senescence. Furthermore, when old seeds are considered, it can be clearly observed that the single areas of the embryo exibit specific increases in the histone/DNA ratio. These observations are discussed in terms of a heterogeneity in the ageing process and loss of metabolic activity and regulatory control in the embryos of aged seeds.



## INTRODUCTION

Some tissues in aged seeds are damaged less than others during storage and some of their metabolic activities are retained (BARKER and BRAY 1972; SEN and OSBORNE 1977; INNOCENTI and FLORIS 1979). It has in fact been found that when portions of these tissues from aged non-germinating seeds are placed in *in vitro* culture, they produce not only undifferentiated calli but also plantlets (INNOCENTI *et al.* 1983). Furthermore, it has been shown that during the ageing process the histone/DNA ratio of these tissues remains practically unchanged (INNOCENTI *et al.* 1983) whilst in others significant increases are to be observed (INNOCENTI and BITONTI 1979, 1981).

In the present paper we aim to give a general picture of the variations occurring in the amount of basic proteins bound to DNA in single embryonic areas of aged seeds.

In order to identify possible differences in the tissues of over-dormant seeds and to estimate the variations produced in the single areas during storage we analyze:

i) the histone/DNA ratio for each single area of young *Triticum durum* caryopses (100% germinated seeds);

ii) the histone/DNA ratio for the same areas in embryos which have lost their germinative vigour.

## MATERIALS AND METHODS

Old non-germinating caryopses of *Triticum durum* harvested in 1974 and stored in glass containers at room temperature were cheked against young caryopses harvested in 1979.

The caryopses were fixed in 10% neutral formalin for 6 hours and the embryos washed overnight. For cytophotometric analyses portions of the embryo from both old and young caryopses were excised under a dissecting microscope and each single area was separately squashed on the same slide. The embryo areas considered were: a) the root apex; b) the coleoptile; c) the leaf primordia; d) the scutellum, parenchima and columnar epithelium; e) the first node. For each embryo area analyzed at least six samples were taken.

The slides were stained with the Feulgen method with 1N trichloracetic acid (TCA) substituted for 1N hydrocloric acid (HCl) both during hydrolysis (20 minutes) and in the Schiff reagent. The slides were washed in $SO_2$ water, dehydrated and then mounted in Canada balsam.

The DNA contents in individual nuclei were measured by means of an automatic scanning microscope photometer Zeiss 01, following the procedure proposed by McLeish and Sunderland (1961). The slides were then restained according to the Alfert and Geschwind method (1953) as modified by Block and Godman (1954) and the nuclei already scanned for Feulgen-DNA were then scanned for alkaline fast-green stainable histones.

The two measurements for DNA and histone were obtained from individual nuclei located by means of microphotographs. These nuclei were measured at 565 and 635 nm for Feulgen and fast-green determinations respectively.

## RESULTS

In order to provide a general picture of the changes produced in the nuclear components during seed storage, the histone/DNA ratios for the single embryonic areas of young (crop 1979) and old (crop 1974) seeds were considered. Fig. 1 shows significant variations in the histone/DNA ratios for the single embryonic areas. In particular, the ratio exibits increased values in practically all areas of the embryo though not always at the same degree; the only exception is the first node where the histone/DNA ratios are practically the same as in young and old seeds.

From Fig. 1 and Table 1 it is seen that:

i) the histone/DNA ratio differs in the single embryo areas of young seeds;

ii) the detected changes are specific to each area.

The last column of Table 1 also reports the «ageing parameter» $Ap = \frac{O - Y}{\sqrt{\sigma_o^2 + \sigma_y^2}}$, where O and Y are the average histones over DNA

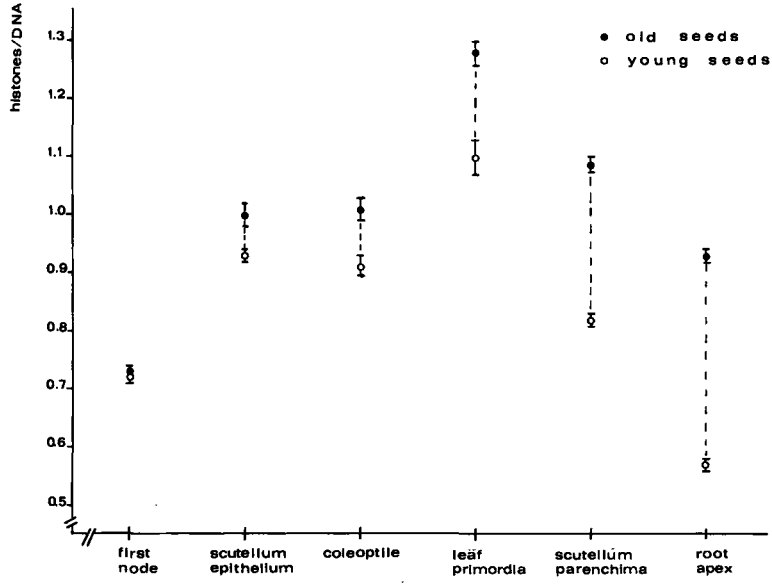


Fig. 1

ratios for the old (O) and young (Y) seeds respectively and σo and σy are the standard errors of the corresponding distribution, as defined by INNOCENTI and BITONTI (1981). It can be clearly seen that this parameter is much greater for the root apex and shifts towards progressively lower values in scutellum parenchima nuclei, leaf primordia, coleoptile, scutellum columnar epithelium; it reaches its lowest value in the nuclei of the first node.

TABLE 1. — *Histone/DNA ratio in different embryo areas of young caryopses harvested in 1979 and old caryopses harvested in 1971.*

| Embryonic areas | Young seeds | | | Old seeds | | | Ageing parameter |
|---|---|---|---|---|---|---|---|
| | n. of seeds | n. of nuclei | histone/DNA | n. of seeds | n. of nuclei | histone/DNA | |
| First node | 3 | 118 | 0.72 ± 0.01 | 3 | 126 | 0.73 ± 0.01 | 0.71 |
| Scutellum colum. epith. | 3 | 134 | 0.93 ± 0.01 | 3 | 125 | 1.00 ± 0.02 | 3.13 |
| Coleoptile | 3 | 92 | 0.91 ± 0.02 | 3 | 95 | 1.01 ± 0.02 | 3.54 |
| Leaf primordia | 3 | 152 | 1.10 ± 0.03 | 3 | 204 | 1.28 ± 0.02 | 4.99 |
| Scutellum parenchima | 3 | 201 | 0.82 ± 0.01 | 3 | 147 | 1.09 ± 0.01 | 12.07 |
| Root apex | 3 | 60 | 0.57 ± 0.01 | 3 | 60 | 0.93 ± 0.02 | 16.10 |

DISCUSSION

Ageing in seeds is a progressive phenomenon characterized by the deterioration of several metabolic processes which ultimately ends in a loss of germinative vigour and viability (GOSH *et al.* 1981).

The general problem of senescence in individual cells and organisme is quite complex. Though there is obviously a correlation between the ageing of cells and the senescence of the whole organism, there is no evidence to indicate a relationship of cause and effect between them (ROSEN 1978). Despite the apparently unlimited number of possible mechanisms which can be invoked, it is clear that senescence shows heterogeneity in both cells (ROSEN 1978) and tissues (INNOCENTI and FLORIS 1979).

Since in our experiments the histone/DNA ratio already proved different for single embryonic areas in young seeds (Fig. 1, Table I) it might be suggested that the nuclear conditions at ripening time can in some way influence the response of cell populations to the ageing gactors.

If we now consider the shift in the histone/DNA ratio in old seeds we see that it is greatest for the root apex and progressively smaller for the scutellum parenchima, leaf primordia, coleoptile, scutellum epithelium and first node (Fig. 1, Table I). This indicates that the root apex is the area of the embryo most sensitive to ageing.

In this connection it is worth noting that in *Hordeum vulgare* seeds subjected to thermic shock, the protein pattern shows changes which are larger in the root tip than in other zones of the seed (MARMIROLI *et al.* 1983).

If we now compare the present data with the metabolic picture of old non-germinating *durum* wheat seeds, as described by INNOCENTI and FLORIS (1979), it is clear that the greatest decrease in metabolic activity is found in the root apex which is exactly where the largest increase was detected in the histone/DNA ratio. Concerning the other embryonic areas, our results show that the greatest increases in the histone/DNA ratio occurs in those tissues showing the highest loss in RNA metabolic activity (INNOCENTI and FLORIS 1979). The only apparent discrepancy between the metabolic and cytophotometric data concerns the scutellum. INNOCENTI and FLORIS (1979) found that metabolic activity is very high in the scutellum of old seeds but they considered the epithelium and parenchima together.

The present experimental data, on the other hand, shows a smaller shift in the histone/DNA ratio for the columnar epithelium than for the parenchima cells. This agrees with results from the literature which suggest that metabolic activity is primarily located in the nuclei of the columnar epithelium cells (AKAZAWA and MIYATA 1982; INNOCENTI and BITONTI 1981).

It is known that the ageing of the embryos involves biochemical lesions which compromise cell activity at both transcriptional and translational levels (DELL'AQUILA *et al.* 1978; BROCKLEHURST and FRASER 1980) and bring about a

loss in the regulatory control of the embryo (BERJAK and VILLIERS 1972; GRILLI *et al.* 1982). As the histonic proteins play an important role in regulating the transcriptional activity of DNA (HUANG and BONNER 1962; GEORGIEV 1969) our results are of particular interest because they show specific changes in the histone/DNA ratio for each area of an embryo subjected to storage. These changes compromise the genetic activity of DNA and the viability of the whole seed.

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